CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

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PHARMACOLOGY REVIEW(S)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER: **22-433**

Sequence number	Center Receipt Date
010	January 29, 2010
021	April 30, 2010
033	June 11, 2010
079	March 31, 2011

PRODUCT: **Brilinta® (ticagrelor)**

INTENDED CLINICAL POPULATION: **patients with unstable angina, non-ST elevation myocardial infarction or ST elevation myocardial infarction**

SPONSOR: **Astra Zeneca**

DOCUMENTS REVIEWED: **Electronic**

REVIEW DIVISION: **Division of Cardiovascular and Renal Products**

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Reference ID: 2937847

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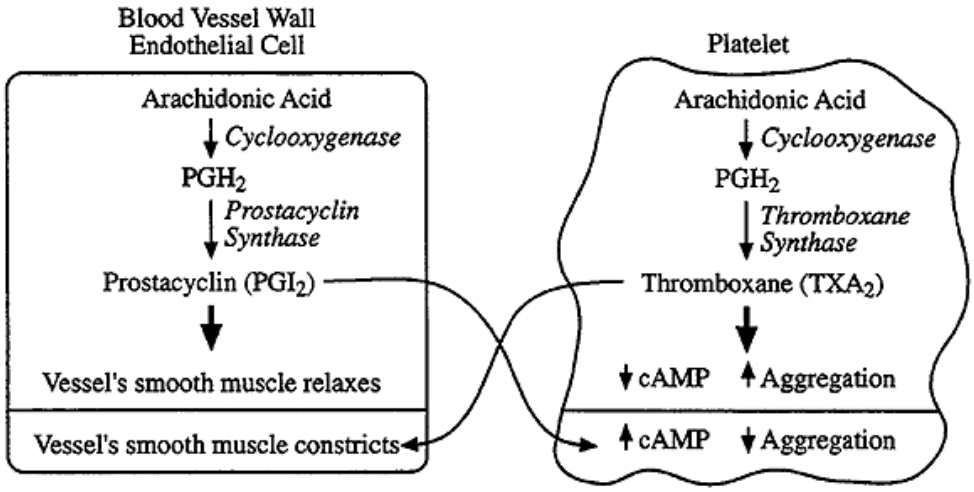
EXECUTIVE SUMMARY

The sponsor proposes that ticagrelor plus a low dose of aspirin is beneficial while increasing doses of aspirin in combination with ticagrelor produce adverse effects. The sponsor uses this hypothesis to explain ticagrelor's reversal of clinical performance compared to clopidogrel in North America (PLATO trial). The sponsor adds that ticagrelor, having nearly complete $P2Y_{12}$ blockade does not show the added anti-aggregatory benefit from aspirin that is seen with clopidogrel. The partial and variable $P2Y_{12}$ blockade seen with clopidogrel is stated to derive more benefit from aspirin with less display of adverse effects. This hypothesis distinguishes between aspirin-mediated effects in platelets versus in endothelial cells in the vessel wall; that is, in platelets, aspirin's inhibition of COX-1 causes a decrease in thromboxane A_2 formation, decreasing platelet aggregation. However, at higher doses, aspirin also blocks the COX-2– mediated production of the vasodilator prostacyclin, causing an increase in vascular resistance.



Ref: Google Images

The sponsor investigated $P2Y_{12}$ biology and secondary pharmacology of ticagrelor compared to clopidogrel and prasugrel as well as the possible interaction of the $P2Y_{12}$ antagonists with aspirin. For purposes of brevity, only those studies designed to test the sponsor's hypothesis will be discussed in this summary.

Using platelet rich plasma (PRP) from healthy human volunteers, the sponsor compared ticagrelor (AZ119) to prasugrel active metabolite (AZ117) using the IC_{50} and IC_{90} for platelet aggregation for each drug. According to the sponsor, the pharmacological properties of prasugrel active metabolite (PAM) can be considered identical to those of clopidogrel active metabolite (CAM). The conditions also included the addition of aspirin using maximal concentrations expected after ingestion of 75-80 mg aspirin (low dose, ASA_{LD}) or 325 mg aspirin (high dose, ASA_{HD}). The conditions used are summarized in the reviewer's table below.

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Reviewer's Summary of In Vitro Platelet Testing Conditions

Drug tested	Combinations tested		
Ticagrelor (AZ119)	Ticagrelor IC_{50}	Ticag IC_{50} +ASA_{LD}	Ticag IC_{50}+ASA_{HD}
	Ticagrelor IC_{90}	Ticag IC_{90} +ASA_{LD}	Ticag IC_{90}+ASA_{HD}
Prasugrel active metabolite (AZ117)	Prasugrel, IC_{50}	Pras IC_{50} +ASA_{LD}	Pras IC_{50}+ASA_{HD}
	Prasugrel, IC_{90}	Pras IC_{90} +ASA_{LD}	Pras IC_{90} +ASA_{HD}
ASA_{LD}	Tested alone		
ASA_{HD}	Tested alone		

Under the conditions of the study, the addition of either low dose (30μM) or high dose (100μM) aspirin to either ticagrelor or prasugrel active metabolite added little to no anti-aggregatory effect. The minor differences that were apparent were primarily found at the low concentration of ticagrelor only in the presence of specific aggregation stimuli.

The sponsor then examined effects of AZ117 and AZ119 on platelet production of thromboxane A_2 (TXA_2) stimulated by arachidonic acid. As expected, aspirin inhibited platelet aggregation and TXA_2 production in response to arachidonic acid. AZ117 and AZ119 also caused concentration-dependent inhibition of platelet aggregation and TXA_2 production. This may support the premise of $P2Y_{12}$ receptors being involved in more than one mechanism for platelet activation. Another interpretation may come from the basic receptor binding studies conducted by the sponsor which showed both AZ117 and AZ119 interact with the prostanoid TXA_2 receptor, but at 1000-fold higher concentratons.

The sponsor used a mechanical injury model of arterial thrombosis in the anesthetized dog to study ticagrelor ±ASA and clopidogrel ±ASA. This study was conducted in two discrete portions using the same in-life methodology. The first part of the study was submitted as a complete report. The data from the additional animals that were subsequently studied was added to the original data and a new analysis performed. Both study reports are analyzed within this review. The most salient parts of these reports will be presented here.

After a period of baseline determination, either ticagrelor or clopidogrel was added at different concentrations to produce submaximal or maximal inhibition of $P2Y_{12}$ receptors. One hour after administration of the first drug, a high dose of aspirin (50 mg/kg compared to the human dose of 5 mg/kg or 325 mg ASA given to a 65 kg patient) was also given. Blood flow through the mechanically damaged femoral artery was used as a measure of anti-thrombosis activity *in vivo.* Vascular resistance in the undamaged femoral artery was used as a measure of aspirin inhibiting the production of prostacyclin in the vascular wall. Platelet aggregation was evaluated *ex vivo*. Systemic PGI_2 (i.e., prostacyclin) and TXA_2 production were evaluated by measuring urine levels of 2,3-dinor-6-keto-PGF1α (a PGI_2 metabolite) and 11-dehydro-TXB_2 (a TXA_2 metabolite).

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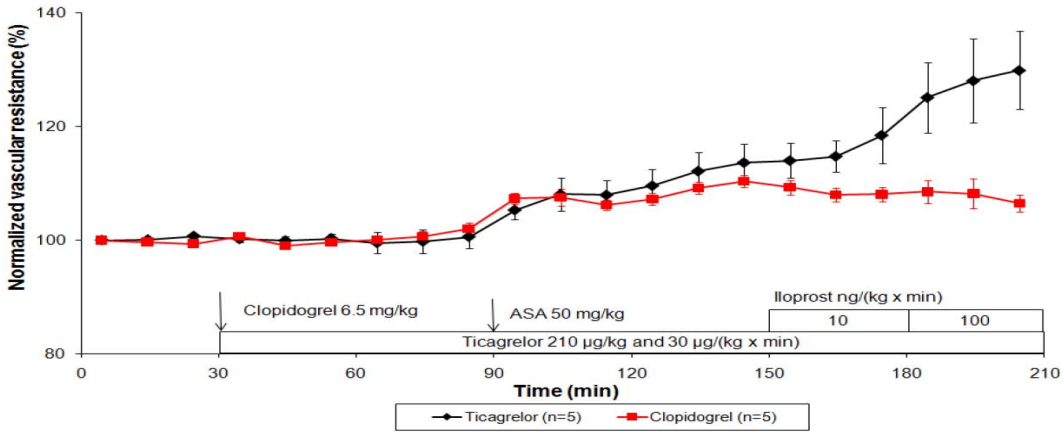
In the first report, it was stated that in the presence of maximal $P2Y_{12}$ inhibition, the addition of aspirin did not alter the observed *in vivo* effects but did produce some additional *ex vivo* inhibition of platelet aggregation induced by arachidonic acid and collagen. In the injured femoral artery, under conditions of maximal $P2Y_{12}$ inhibition, aspirin administered in addition to either ticagrelor or clopidogrel, caused a decrease in normalized vascular resistance (defined as mean aortic pressure divided by the femoral artery flow and normalized by expression as percentage change from the first 10 minutes of the vehicle infusion), due to inhibition of platelet aggregation and thrombus formation. However, in the uninjured control femoral artery, under the same conditions of drug administration, the normalized vascular resistance appeared to increase, theoretically due to the inhibition of prostacyclin production. However, this effect appeared after infusion of the prostacyclin analog iloprost (sponsor's figure 7 shown below). The difference in normalized vascular resistance after the infusion of iloprost is paradoxical as iloprost causes vasodilation, inhibits platelet aggregation and is used clinically for treatment of pulmonary arterial hypertension and various vascular conditions where vasodilation and/ or greater perfusion are required. When asked in a telecom if they had any explanation for the iloprost results, the sponsor replied that the work was entirely exploratory and no explanation could be provided.

Figure 7 **Normalized vascular resistance in the control leg at maximal $P2Y_{12}$ inhibition alone and in combinations**



Normalized vascular resistance in the control leg at maximal $P2Y_{12}$ inhibition by ticagrelor (♦) and clopidogrel (■) alone and in combination with ASA or ASA plus iloprost. Data is expressed as mean ± SEM of 5 separate experiments performed in 5 dogs.

At sub-maximal $P2Y_{12}$ inhibition mean normalized vascular resistance increased 30 minutes after aspirin infusion but the differences between clopidogrel and ticagrelor do

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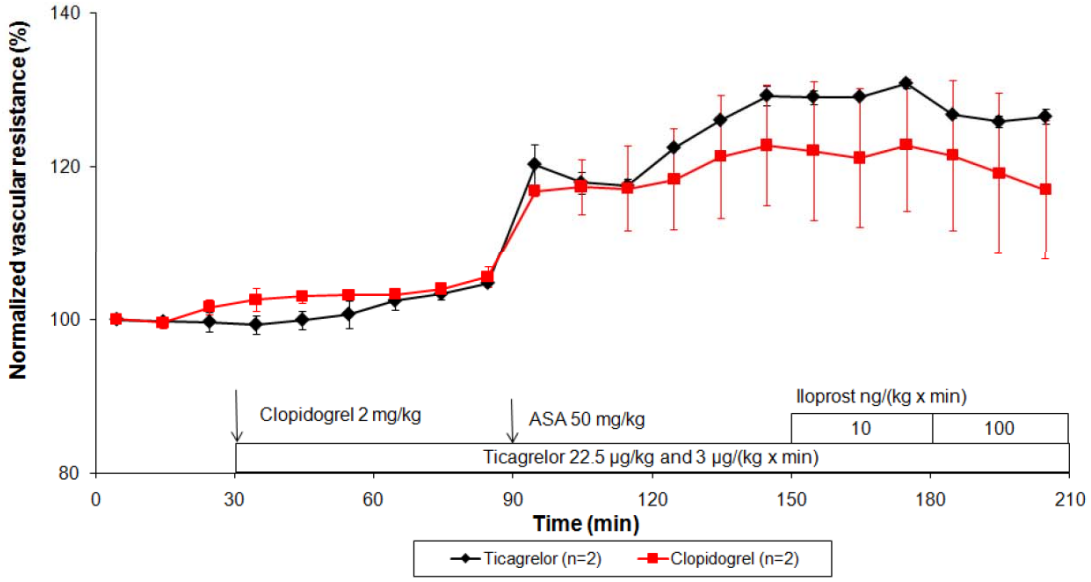
not achieve statistical significance (sponsor's figure 8, shown below).The addition of iloprost in this situation does not appear to affect the results.

Figure 8 **Normalized vascular resistance in the control leg at sub-maximal $P2Y_{12}$ inhibition alone and in combinations**



Normalized vascular resistance in the control leg at sub-maximal $P2Y_{12}$ inhibition by ticagrelor (♦) and clopidogrel (■) alone and in combination with ASA or ASA plus iloprost. Data is expressed as mean ± SEM of 2 separate experiments performed in 2 dogs.

The translation into humans from the dog study in general and the first report in particular presents other questions. The reported aspirin level is approximately 9X lower than what is reported for a single dose in humans while the level of salicylates is almost 4 times higher than what is reported for humans. It has proved very difficult to find published values for steady state plasma levels of aspirin used for prophylaxis in the cardiovascular population.

Dog plasma values after a single dose of aspirin, 50 mg/kg	Human plasma[1] values after a single dose of aspirin, 320 mg
$AUC_{0-\tau}$ ASA = 3.50 µmol/L= 0.63µg/ml	$AUC_{0-\tau}$ ASA = 3 µg/ml
$AUC_{0-\tau}$ SA = 547 µmol/L = 75.5 µg/ml	$AUC_{0-\tau}$ SA = 19 µg/ml

The molecular weight for ASA is listed as 180.15 g/mol(MSDS sheet) and for SA is 138.12 g/mol. [1]The human plasma values were taken from Bonati et al., J Lab Clin Med.1984.Jun;103(6):869-77. [2] Sharma et al Drug Dev Res 1989. 17:219-226.

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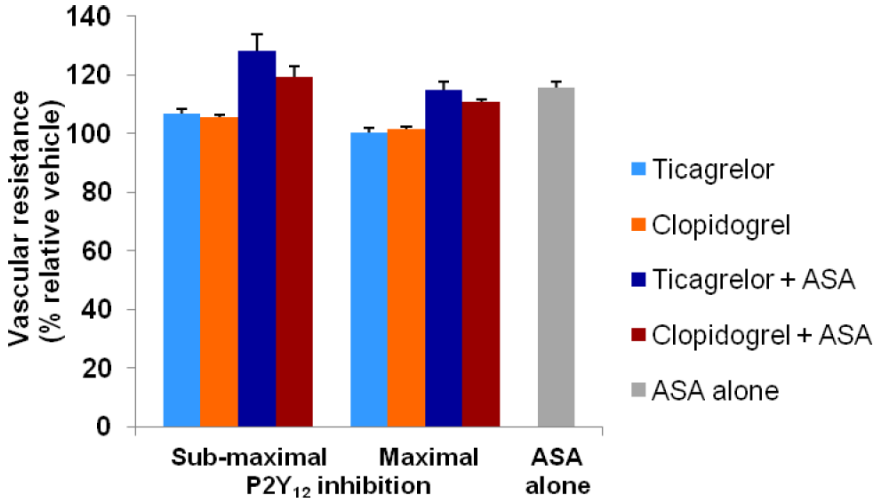
The sponsor subsequently submitted another version of this report. One dog was added to each of the ticagrelor and clopidogrel maximal effect groups, 4 dogs were added to the submaximal effect groups and a new aspirin-treatment only group was added. No concurrent controls were examined. The report again cited difficulty in interpreting the iloprost results, in that the iloprost data was omitted from the graphs but raw data was included in the appendices.

The sponsor provided graphical demonstrations of "normalized vascular resistance", i.e., percentage change relative to vehicle. As shown in the sponsor's graph, adding aspirin caused a greater difference in this parameter under conditions of submaximal $P2Y_{12}$ inhibition compared to maximal $P2Y_{12}$ inhibition. The sponsor noted that the increase in normalized vascular resistance at submaximal $P2Y_{12}$ inhibition was of the same magnitude as after administration of vehicle in the aspirin alone group.

Figure 6 Vascular resistance at "steady-state"



Data for the second period of mono and combination therapy "steady state" expressed as % relative the first 10 min of the vehicle period which was set to 100%, mean ± SEM of 6 separate experiments performed in 6 dogs.

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The reviewer analyzed the data in the appendices of the sponsor's report to examine the components of vascular resistance and the variability of the experimental system. Femoral artery flow (ml/min) showed no significant differences between aspirin alone, ticagrelor alone followed by the addition of aspirin or clopidogrel alone followed by the addition of aspirin under conditions of either maximal or sub-maximal $P2Y_{12}$ inhibition. Mean vascular resistance showed trends, but, there was no indication of what level of change was biologically significant.

Using different analytical techniques in the second study, the sponsor was able to report urinary PGI_2 metabolite values of substantially lower variability than seen in the first report. As expressed in the sponsor's hypothesis, the increase in measured PGI_2 in the ticagrelor group relative to baseline should presage a decrease in vascular resistance. By the same hypothesis, the apparent decreases in PGI_2 production in the clopidogrel and aspirin groups relative to baseline would be expected to cause increases in vascular resistance. This was not reflected in the reviewer's analysis of femoral artery flow or mean vascular resistance.

PGI_2 production *in vivo* (ng/mg creatinine): mean values for maximal $P2Y_{12}$ inhibition

Time interval(minutes)	Ticagrelor (n=1)	Clopidogrel (n=1)	Aspirin (n=6)
0-30	5.7	7.6	10.2±1.1
60-90	7.7	7.9	11.1±1.2
120-150	6.6 (+16%)	4.7 (-38%)	7.1±0.3 (-30%)

Numbers in parentheses ( ) are the percent change from baseline.

If vascular resistance is tied to PGI_2 in this model system, then under conditions of sub-maximal $P2Y_{12}$ inhibition, the decrease in vascular resistance should be similar for ticagrelor and clopidogrel and of a greater magnitude than the decrease seen in vascular resistance for aspirin alone. It is not clear what level of change in vascular resistance in this system is biologically meaningful and it's not clear what level of change in PGI_2 as measured by this assay is biologically meaningful.

PGI_2 production *in vivo* (ng/mg creatinine): mean±sem values for sub- maximal $P2Y_{12}$ inhibition

Time interval (minutes)	Ticagrelor (n=4)	Clopidogrel (n=4)	Aspirin (n=6)
0-30	10±1.5	14.1±4.1	10.2±1.1
60-90	9.0±1.2	13.2±3.5	11.1±1.2
120-150	5.3±0.5 (-47%)	7.6±1.6 (-46%)	7.1±0.3 (-30%)

Numbers in parentheses ( ) are the percent change from baseline.

The sponsor provided an opinion piece, "Aspirin and the Cardiovascular System in the Presence of $P2Y_{12}$ Receptor Antagonists" written by their consultant Tim Warner, and two Astra Zeneca employees. The hypotheses were re-stated with minimal discussion of additional data from the published literature, thus contributing little to understanding the mechanism.

Something not noted in the mechanistic studies, but pertinent, is previous examination of the relative affinity of ticagrelor, clopidogrel and prasugrel for the COX-1 and COX-2

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enzyme isoforms. AR-C13313XX, another major metabolite (b) (4) in the drug product, has not been evaluated for this activity.

Reviewer's Summary of Reported COX Activities

drug	% inhibition		Report #
	COX-1	COX-2	
Ticagrelor(AZ11939728-015)	-19% (agonist)	1%	0951SY
Ticagrelor metabolite (AZ11879328)	-8%	8%	0952SY
clopidogrel	11%	8%	0928SY
Prasugrel active metabolite	7%	16%	092SY

Conclusion

The sponsor hypothesized that the efficacy of aspirin for prophylaxis of platelet hyperaggregability depends upon selective blockade of thromboxane A_2 synthesis by platelets, sparing the production of the vasodilator PGI_2 by vascular endothelial cells. This hypothesis was originally stated in 1982 when Vane, Bengt, and Samuellsson accepted the Nobel Prize in Medicine for their work with prostaglandins (1). A more recent statement can be found in Goodman and Gilman's 10th edition (Chapters 26, 27 and 55: (2)). This idea has been explored in the scientific literature for several decades. Some investigators feel that the clinical confirmation of this hypothesis was demonstrated with the increased cardiovascular events produced by COX-2 inhibitors (5). Recent work indicates that the concept of balance between the two pathways is simplistic and does not take into account more recently described effects of prostanoids (3, 4).

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From: Grosser et al. 2006. J Clin Invest 116(1):4-15.

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From: Grosser et al. 2006. J Clin Invest 116(1):4-15.

The sponsor compares ticagrelor and prasugrel active metabolite alone and in combination with aspirin. The in vitro platelet results indicated that the addition of either low dose (30 μM) or high dose (120 μM) aspirin enhanced the platelet inhibitory effect when P2Y12 inhibition was partial, but not when P2Y12 inhibition was complete. Further, no additional platelet inhibition was seen under either partial or complete P2Y12 inhibition when aspirin was increased from 30 μM to 120 μM. This suggests that there is no difference in effect between the two drugs at the level of the platelet.

A study conducted in anesthetized dogs attempts to show vascular effects secondary to inhibition of prostacyclin production. The study showed no difference between ticagrelor + ASA compared to clopidogrel + ASA or ASA alone when absolute blood flow is examined. Published studies for other drugs, including aspirin, have confirmed the inhibition of vascular PGI_2 by demonstrating elimination of vasodilation in response to locally administered arachidonic acid. For vasoactive drugs, a challenge by arachidonic acid produces small but measurable difference in vasodilation or blood flow (6, 7). For

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femoral arterial studies, femoral artery pressure is measured as a crosscheck for establishing that flow is due to vasodilation (8). The present dog study did not include a local vasoreactivity challenge, measurement of femoral artery pressure or concurrent controls. When the inherent variability of the test system is added to the lack of controls, challenges, and essential measurements, it becomes difficult to interpret the study with confidence.

The effects of ticagrelor, clopidogrel carboxylic acid and prasugrel active metabolite on platelets both with and without aspirin have been relatively well characterized in these studies. There is no characterization of what, if anything, these drugs do at the level of the vascular wall and no exploration of possible direct effects on vascular endothelium.

While the sponsor has examined the possible effect of aspirin on ticagrelor's pharmacokinetics, the possible effect of ticagrelor on aspirin's pharmacokinetics has not been examined. Ticagrelor and its two major metabolites are highly protein bound. The sponsor states that it is not known to which plasma proteins the metabolites bind. It remains a possibility that simple displacement of salicylates from plasma proteins increases free or unbound levels of aspirin that counterbalance the therapeutic effects of ticagrelor. This possibility could be assessed experimentally with standard protein binding and competitive displacement studies.

The sponsor has also examined *in vitro* the effect of ticagrelor and one of the metabolites, AR-C124910XX on COX-1 and COX-2. There was no information provided on the major metabolite AR-C133913XX (b) (4) It may be informative to examine the affinity for COX-1 and COX-2 when ASA is combined with ticagrelor or the major metabolites.

In summary, there is no clear explanation why aspirin's proposed inhibition of endothelial prostacyclin is able to outweigh ticagrelor's, but not clopidogrel's, beneficial effects of TXA_2 inhibition, platelet inhibition, and interactions with phosphodiesterase isoforms.

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References

1. Vane JR. 1983. Nobel lecture. Adventures and excursion in bioassay: the stepping stones to prostacyclin. **Postgrad Med J**. Dec;59(698):743-58.
2. Goodman & Gilman's The Pharmacological Basis of Therapeutics, 10th ed. JG Hardman and LL Limbird editors. McGraw-Hill, New York, 2001.
3. Grosser et al. 2006. **J Clin Invest** 116(1):4-15.
4. Salinas G, UC Rangasetty, BF Uretsky, Y Birnbaum. 2007. **J Cardiovasc Pharmacol Ther.** 12(2):98-111.
5. Gleim S, Z Kasza, K Martin, J Hwa. 2009. **Curr Ather Reports.** 11:227-235.
6. Hennan JK, J Huang, TD Barrett, EM Driscoll, DE Willens, AM Park, LJ Crofford, BR Lucchesi. 2001; **Circulation**:104:820-825.
7. Hintze TH and G Kaley. 1977. **Circulation Re**s. 40: 313-320.
8. Lie M, OM Sejersted, F Kiil. 1970. **Circulation Res.** Vol XXVII:727-737.

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DRUG HISTORY AND INFORMATION

NDA number: 22,43
Sponsor and/or agent: AstraZeneca

Manufacturers for drug substance:

Manufacturer	Substance
AstraZeneca AB Gärtunavägen S-151 85 Södertälje, Sweden	ticagrelor
(b) (4)	ticagrelor

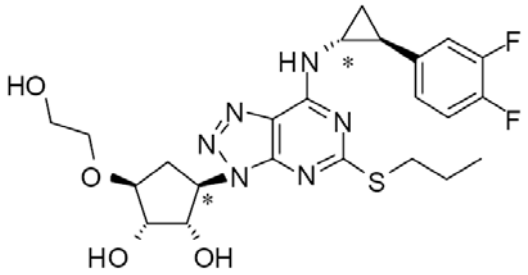
Reviewer name: Elizabeth Hausner, D.V.M.
Division name: Cardiovascular and Renal Products
Review completion date: April 25, 2011

Drug:

Trade name: Brilinta®
Generic name: ticagrelor
Code name: AZD6140, AR-C126532XX

Chemical name: (1S,2S,3R,5S)-3-[7-{[(1R,2S)-2-(3,4-Difluorophenyl)cyclopropyl]amino}-5- (propylthio)-3H-[1,2,3]triazolo[4,5- d] pyrimidin-3-yl]-5-(2- hydroxyethoxy)cyclopentane-1,2-diol

CAS registry number: 274693-27-5
Molecular formula/molecular weight: $C_{23}H_{28}F_2N_6O_4S$/522.57
Structure:



Relevant INDs/NDAs/DMFs: IND 65,808
Drug class: platelet $P2Y_{12}$ receptor inhibitor

Intended clinical population: Patients with acute coronary syndrome (ACS) who are managed medically or to be managed invasively with percutaneous coronary intervention (with or without stent) and/or coronary artery bypass grafting (CABG).

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Studies Reviewed

Report to Astra Zeneca on *in vitro* work performed at the [redacted] (b) (4) on the effects of P2Y12 antagonists on platelet function

Effect of Aspirin pretreatment on ticagrelor inhibition of 2Me-S-ADP-induced human $P2Y_{12}$ receptor signalling, *in vitro*

Ticagrelor and AZ11702105, effect alone and in combination with aspirin on LTB4 production by the 5-LO pathway in human whole blood, *in vitro*

Ticagrelor and Clopidogrel, effects on arterial thrombosis, vascular resistance and *ex vivo* platelet function alone and in combination with a high dose acetylsalicylic acid in the anaesthetised dog, *in vivo*

Pharmacokinetic study of aspirin (ASA), salicylic acid (SA), ticagrelor, AR-C124910XX and carboxylic acid metabolite of clopidogrel after oral administration of ASA alone or in combination with oral ticagrelor or intravenous clopidogrel in the dog study AZM090928-01

Ticagrelor and its metabolites AZ11879328 and AZ11879477: *in vitro* assessment of inhibition of platelet aggregation. AZM100303-03

Analysis of the effects of ticagrelor (AZ11939728) and prasugrel active metabolite (AZ11702105) on platelet responses *in vitro*

Ticagrelor and its metabolites AZ11879328 and AZ11879477, displacement of [^{125}I]AZ11931285 radio-ligand binding and inhibition of 2Me-S-ADP-induced human $P2Y_{12}$ receptor signalling, *in vitro*

Studies given minimal review

AZ11702105:Selectivity screening in radioligand binding, enzyme and electrophysiological assays in vitro. *in vitro* radioligand binding, enzyme and electrophysiological assays to assess the pharmacological activity of AZ11702105 (the active metabolite of Prasugrel) in a panel enzymes, receptors, transporters and ion channels. Not directly related to the mechanistic hypothesis

0928SY AZ11696405: Selectivity Screening in Radioligand Binding, Enzyme and Electrophysiological Assays in vitro *in vitro* radioligand binding, enzyme and electrophysiological assays to assess the pharmacological activity of AZ11696405 (Clopidogrel carboxylic acid) in a panel of enzymes, receptors, transporters and ion channels. Not directly related to mechanistic hypothesis

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0951SY Ticagrelor: Selectivity screening in radioligand binding, enzyme and electrophysiological assays in vitro. Reviewed during the first NDA cycle.

0952SY Ticagrelor Metabolite, AZ11879328-003: Selectivity Screening in Radioligand Binding, Enzyme and Electrophysiological Assays in vitro Reviewed during the first NDA cycle.

Evaluation of induction potential of CYP1A2, CYP2B6 and CYP3A4 by acetylsalicylic acid and salicylic acid in the human hepatoma cell line HepaRG Clinical pharmacology report

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Report to Astra Zeneca on *in vitro* work performed at the (b) (4) **on the effects of P2Y12 antagonists on platelet function**

Study location: (b) (4)

These *in vitro* studies examined the possibility that inhibition of the platelet $P2Y_{12}$ receptor may engage additional platelet receptors. The sponsor compared ticagrelor, prasugrel active metabolite (PAM) and clopidogrel active metabolite (CAM) in human platelet rich plasma (PRP). The sponsor examined:
1. IP receptor using the IP agonist iloprost (the principal biological agonist is PGI2).
2. EP3 receptors using the EP3 agonist sulprostone (the principal biological agonist is PGE2).
3. EP4 receptors using an unspecified agonist (principal biological agonists are PGE1 and PGE2)
4. EP2 receptor using an unspecified agonist (principal biological agonists are PGE1 and PGE2)

Platelet aggregation was measured by platelet counting instead of light aggregometry or impedance aggregometry. The report noted that this choice was based upon the greater rapidity of platelet counting.

The agonist chosen for platelet aggregation was the thromboxane A_2(TXA_2) mimetic U46619 and or the thrombin receptor activating peptide TRAP6. ADP was rejected for the experiments because of the high effectiveness with which $P2Y_{12}$ antagonists inhibit ADP-induced platelet function.

The sponsor then modulated conditions to minimize the ADP or adenosine produced within the PRP from contributing to aggregation as follows:

1. ± apyrase, an enzyme that removes ADP
2. ± adenosine deaminase (ADA), an enzyme that removes adenosine
3. ± both 1 and 2
4. ± phosphocreatine (CP)/creatine phosphokinase (CPK), which converts ADP to ATP

The studies demonstrated that under the conditions used the agonists that act at the IP and EP, EP2, EP3 and EP4 receptors are able to modulate platelet aggregation induced by the thrombin receptor activating peptide (TRAP-6) in the presence of $P2Y_{12}$ receptor antagonists and under conditions where ADP and adenosine have been removed. This does not provide direct evidentiary support for the sponsor's aspirin hypothesis but does provide evidence that platelet aggregation can be modulated by various platelet agonists in the presence of a $P2Y_{12}$ receptor antagonist.

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Reviewer's Summary of Experiments

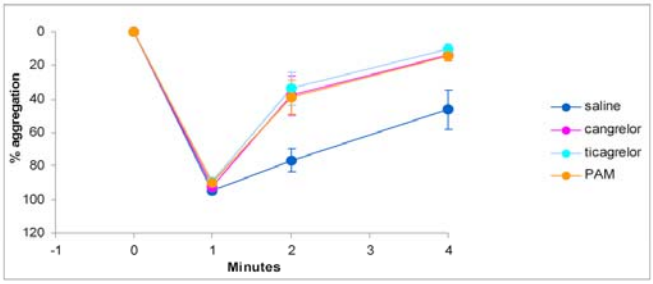
PRP pre-incubated with	Agents to remove ADP + adenosine	Receptor targeted	Receptor specific compounds	Stimulus for aggregation
1μM cangrelor 10μM ticagrelor 10μM PAM	ADA + ADA pyrase CP+CPK CP+CPK+ADA	$P2Y_{12}$	No others	TRAP 6
	ADA + ADA pyrase CP+CPK CP+CPK+ADA	$P2Y_{12}$	No others	1μM U46619
1μM cangrelor 10μM ticagrelor 10μM PAM	ADA + apyrase	IP	0.1, 0.3, 1, 3, 10nM iloprost	TRAP 6
		EP3	0.01, 0.03, 0.1, 0.3, 1μM sulprostone	TRAP 6
		EP3	3μM DG041, 1μM sulprostone, 3μMDG041+ 1μMsulprostone	TRAP 6
		EP4	0.01, 0.03, 0.1, 0.3, 1μM unspecified EP4 agonist	TRAP 6
1μM cangrelor 10μM ticagrelor 10μM PAM	ADA + apyrase	EP2	1, 3,10μM unspecified EP2 agonist	TRAP 6
1μM cangrelor 10μM ticagrelor 10μM PAM	ADA + apyrase	EP2, EP3, EP4 *	0.1, 0.3, 1, 3, 10μM PGE2	TRAP 6

PAM= prasugrel active metabolite; iloprost= IP receptor agonist; sulprostone = EP3 receptor agonist; DG041= EP3 receptor agonist
* The effect of PGE on platelets was evaluated as a balance of the effects at EP2, EP3 and EP4 receptors.

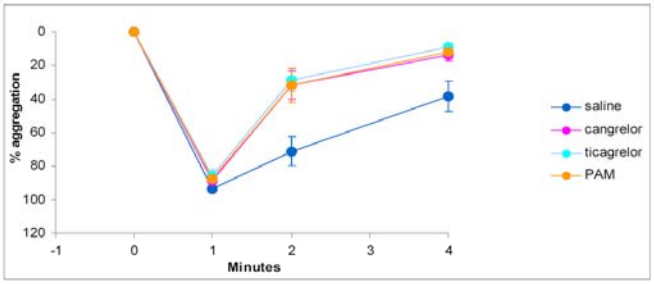
The sponsor also examined the vasodilator-stimulated phosphoprotein phosphorylation (VASP-P). VASP is an intracellular actin-regulatory protein that acts as a substrate of both cAMP and cGMP-dependent protein kinases and can be used to measure signal transduction processes following $P2Y_{12}$ stimulation and the reversal by $P2Y_{12}$ antagonists (Thromb Haemost 2006;96:767-73). The basis for the current evaluation of VASP was due to the observation that cangrelor added alone to PRP induced a concentration dependent increase in VASP-P. Cangrelor added to PRP in the presence of ADP caused a very large increase in VASP-P. The sponsor sought to determine if this phenomenon was due to small amounts of adenosine being formed in the PRP and what are the effects of the $P2Y_{12}$ antagonists on VASP-P in the presence of agents that act at IP, EP3, EP4 and EP2 receptors.

In light of the sponsor's hypothesis, the effects or lack of effects with respect to iloprost are of special interest and the sponsors' graphs for these assays are shown below. Cangrelor, ticagrelor and prasugrel active metabolite show essentially the same behavior.

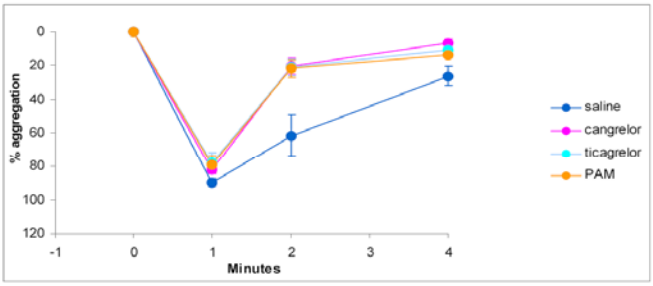
Reference ID: 2937847



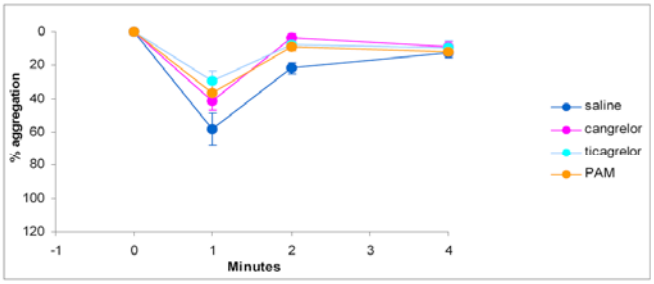
a) 0nM iloprost



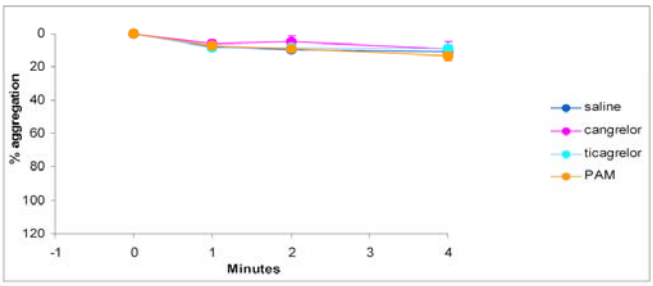
b) 0.1nM iloprost



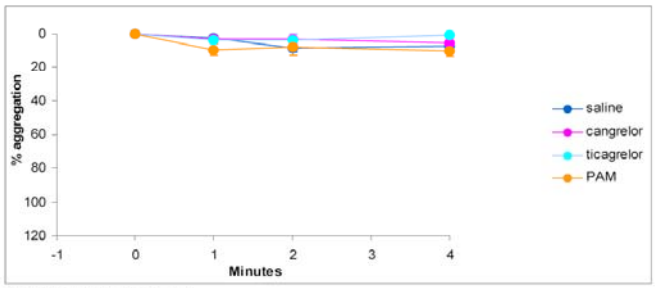
c) 0.3nM iloprost



d) 1nM iloprost

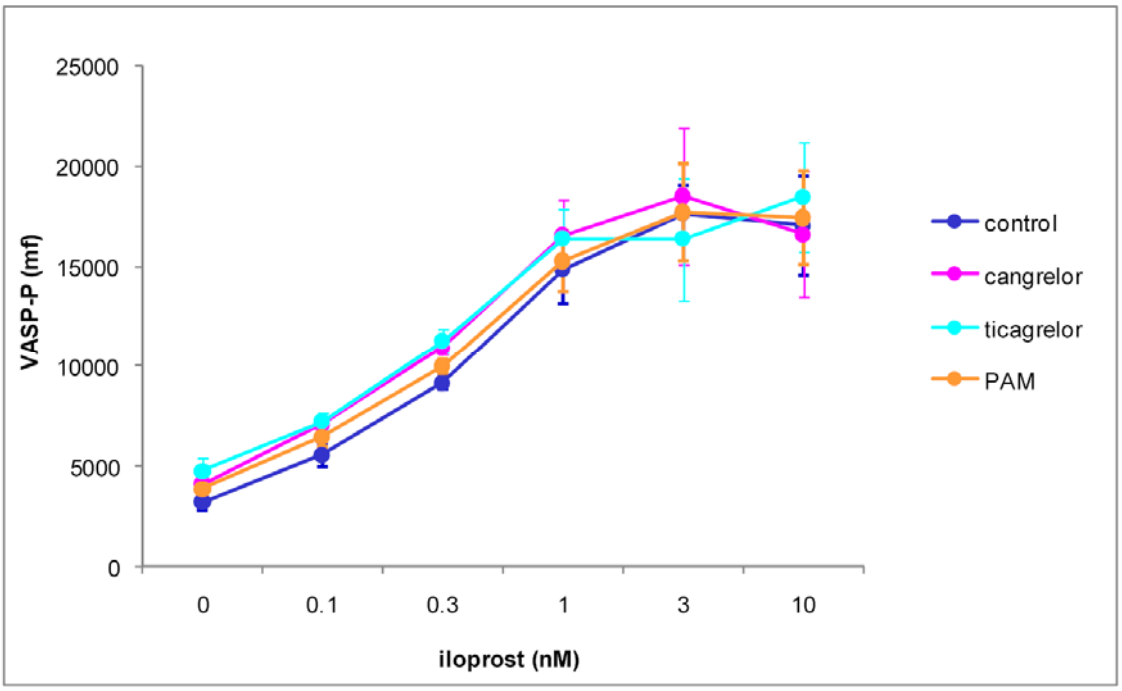


e) 3nM iloprost



f) 10nM iloprost

Reference ID: 2937847



The results of these studies do not provide evidentiary support for the aspirin hypothesis.

Effect of aspirin pretreatment on ticagrelor inhibition of 2Me-S-ADP-induced human $P2Y_{12}$ receptor signaling, *in vitro*

Study number AZM090928-03, AZM080407-03
Report number: AZMR10072
Study location: Astra Zeneca R&D, Mölndal, Sweden

Possible interactions of aspirin with the $P2Y_{12}$ receptor were studied by measuring displacement of 2Me-S-ADP (stable ADP analogue) from the $P2Y_{12}$ receptor by ticagrelor. ^{35}S-GTPgS binding to the membranes was used as a measure of $P2Y_{12}$ signaling.

Arachidonic acid-induced human platelet aggregation in platelet rich plasma was used to confirm activity of the aspirin formulation.

Aspirin was added to the membrane mixture at final assay concentrations of 0.1, 0.5, 1.0 or 5.0 mmol/l and pre-incubated for either 60 or 90 minutes at room temperature. Ticagrelor was tested over the concentration range 0.0002 to 10 µmol/l .

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The radiolabeled ligand ^{35}S-GTPλS was diluted to a final assay concentration that gives >80% maximal $P2Y_{12}$ signalling response.

Ticagrelor or DMSO , ^{35}S-GTP λS/2Me-S-ADP and $hP2Y_{12}$ membranes (with or without aspirin pre-incubation) were added to a 96-well plate. The preparations were incubated at 30°C for 45 minutes then transferred to a filter for harvesting. The dried filters were counted using a microbeta counter. Each concentration was tested in 2-4 replicates per experiment.

Results

Aspirin-mediated inhibition of aggregation in PRP could be detected, confirming activity of the preparation.

Ticagrelor inhibited $P2Y_{12}$ signalling in a concentration-dependent manner. The IC_{50} was determined to be 0.022±0.001 µM (mean±SD of two experiments) in the absence of aspirin pre-incubation. Pre-incubation of the $P2Y_{12}$ receptor with aspirin over the test concentration range did not change the IC_{50} value for ticagrelor.

Table 1 **Inhibition of 2Me-S-ADP-induced $P2Y_{12}$ signalling by ticagrelor after pre-incubation with different aspirin concentrations. Individual IC_{50} values**

Aspirin (mmol/L)	IC50 (µmol/L) Exp 2009-09-30	Exp 2009-10-07
0	0.021	0.022
0.1	0.018	0.024
0.5	0.018	
1.0	0.021	0.023
5.0		0.019

The results indicated an apparent lack of detectable effect of aspirin on ^{35}S-GTP binding in PRP in the presence of ticagrelor. It might have been useful to examine the effect of aspirin by itself in the radio-labeled binding assay. However, given the data presented, the sponsor concluded that pre-incubation of the $P2Y_{12}$ receptor with aspirin (0.1 to 5 mmol/l) had no effect on the inhibition of 2Me-S-ADP-induced $P2Y_{12}$ signalling by ticagrelor.

Reference ID: 2937847

Comparison of results of receptor binding, purified enzyme interaction and electrophysiological assays for AZ11702105 (prasugrel active metabolite, PAM) and AZ11696405 (Clopidogrel carboxylic acid, CCA)

Study Numbers 0926Y PAM
0928Y CCA
0952SY ticagrelor metabolite
0951SY ticagrelor

The sponsor determined the receptor binding profiles for PAM and CAM.

Table 1 **Effect of AZ11702105 in *in vitro* radioligand binding and enzyme assays: % inhibition at 10 µM**

Target[a]	% inhibition
UDP Glucuronosyltransferase, UGT1A1	75
Lipoxygenase 15-LO (rabbit)	52

[a] human except where noted

Table 2 **Effect of AZ11702105 in electrophysiological assays**

Channel	Active / Inactive	IC_{50} (µM)	Top test concentration (µM)	% inhibition	n
$Ca_v1.2$	I	Inactive at 31.6	31.6	7.1 ± 4.0	6-8
$hNa_v1.5$	I	Inactive at 31.6	31.6	-4.4 ± 2.4	2-8
$hK_v4.3$ / hKChIP2.2	I	Inactive at 31.6	31.6	-1.2 ± 4.1	6-8
$hK_v7.1$ / hKCNE1	I	Inactive at 31.6	31.6	14.9 ± 5.0	6-8
$hCa_v3.2$	I	Inactive at 31.6	31.6	6.1 ± 4.4	2-5
$hK_v1.5$	I	Inactive at 31.6	31.6	4.8 ± 1.4	10-14
hHCN4	I	Inactive at 31.6	31.6	5.6 ± 1.2	6-8

Reference ID: 2937847

In addition to the interactions cited by the report, the following were also noted by this reviewer:

Additional findings for PAM

Target	% interaction
Nitric oxide synthase	28
Opiate δ	44
Prostanoid thromboxane A2	26

No significant receptor or enzyme interactions were reported for CCA. Activity was reported for one of the ionic channels evaluated.

Table 1 Effect of AZ11696405 in electrophysiological assays

Channel	Active / Inactive	IC_{50} (μM)	Top test concentration (μM)	% inhibition	n
$Ca_v1.2$	I	Inactive at 31.6	31.6	13.8 ± 2.5	5-8
$hNa_v1.5$	I	Inactive at 31.6	31.6	2.9 ± 3.5	5-8
$hK_v4.3$ / hKChIP2.2	I	Inactive at 31.6	31.6	2.5 ± 1.9	6-8
$hK_v7.1$ / hKCNE1	A	>31.6	31.6	36.1 ± 4.4	7-8
$hCa_v3.2$	I	Inactive at 31.6	31.6	7.1 ± 1.8	2-6
$hK_v1.5$	I	Inactive at 31.6	31.6	5.9 ± 3.2	8-12
hHCN4	I	Inactive at 31.6	31.6	3.2 ± 2.0	4-8

Reference ID: 2937847

From the NDA review (on file in DARRTS), ticagrelor (AR-C126532XX) showed more interactions than either CCA or PAM.

Table 8. Sponsor's Summary of Significant Receptor Interactions

Detectable activities of AR-C126532XX in a number of binding and enzyme assays performed *in vitro* at (b) (4)

Assay	Concentration tested (μM)	% Inhibition (IC_{50})
Adenosine A_3	10	94
Phospholipase C	10	71 (IC_{50} = 8.4 μM)
P59fyn tyrosine kinase	10 Inactive on retest @ 1-10 μM	56
Platelet activating factor	10	55 (IC_{50} = 9.4 μM)
A_{2b} Adenylyl cyclase	Inactive @ 1-10 μM	
5-lipoxygenase	30	83
Phosphodiesterase 1	100	78
Phosphodiesterase 2	100	70
Phosphodiesterase 3	100	73
Phosphodiesterase 4	100	79
Phosphodiesterase 5	100	97
Ca2+/calmodulin dependent PKII	100	91
Phospholipase A_2 (pancreatic)	300	87
Cyclooxygenase	300	53
Leukotriene C_4 synthetase	1000	66

Further examination of the interaction with phosphodiesterases (PDE) 1, 3, 4 and 5 showed that AZD6140 exhibited weak (IC50 = 10μM) interaction with PDE1, 3, and 4. The IC50 for PDE5 was reported as 0.482μM.

Reference ID: 2937847

Ticagrelor's active metabolite, AR-C124910XX (AZ11879328-003) was also examined in the same test systems. A summary and comparison table from the NDA review is shown below.

Table 11. Reviewer's Summary of Recent Radioligand Binding Assays for AZD6140 and Metabolite AR-C124910XX

Radioligand assays %inhibition at 10μM			
target	Positive control	AZD 6140	AR-C124910XX (AZ11879328-003)
Phosphodiesterase PDE3	Cilostamide	41	49
Phosphodiesterase PDE4	Ro 20-1724	66 (IC_{50} 3.9μM)	56 (IC_{50} 10.6μM)
Protein tyrosine kinase insulin receptor	Tryphostin 47	34	30%
Adenosine A1 (human)	R(-)-PIA	25	11
Adenosine A2 (human)	CGS-21680	17	49
Adenosine A3 (human)	IB-MECA	98 (IC_{50} 0.113μM)	103 (IC_{50} 0.196μM)
Androgen receptor	testosterone	30	30
Calcium channel L-type	diltiazem	29	31
Estrogen receptor ERα (human)	diethylstilbesterol	6	1
Glucocorticoid (human)	dexamethasone	17	-5
Histamine H2 (human)	tiotidine	-30	-70
Peripheral benzodiazepine (rat)	diazepam	40	36
Progesterone (rat)	progesterone	37	25
Prostanoid thromboxane A2 (human)	ICI-192,605	41	33
Serotonin 5HT2B (human)	ketanserin	52(IC_{50} 8.21μM)	99 (IC_{50} 2.42)
Somatostatin SST4 (human)	Somatostatin T4	1	30
Adenosine transporter (guinea pig)	Nitrobenzyl -thioinosine	84 (IC_{50} 2.21μM)	40
Norepinephrine transporter (human)	desipramine	66 (IC_{50} 4.52μM)	24

Reference ID: 2937847

Table 12. Reviewer's Summary of Recent Biochemical Assays for AZD6140 and ARC124910XX

Biochemical Assays % inhibition at 10μM			
target	Reference compound	AZD6140	AR-C124910XX (AZ11879328-003)
Deacetylase,sirtuin SIRT2, human	nicotinamide	60 (IC_{50} 8.64μM)	60(IC_{50} 11.8μM)
Lipoxygenase 5-LO, human	NDGA	98 (IC_{50} 1.58 μM)	99(IC_{50} 1.59μM)
Peptidase CTSL2 (cathepsin L2) human		75 (IC_{50} 4.56μM)	57 (IC_{50} 3.4μM)
Peptidase, CTSS (cathepsin S) human		53 (IC_{50} 2.63μM)	86 (IC_{50} 3.32μM)
Phosphodiesterase PDE10A1 human	papaverine	70 (IC_{50} 3.85μM)	60 (IC_{50} 5.07μM)
PDE5 human	zaprinast	77 (IC_{50} 0.379μM)	82 (IC_{50} 0.382μM)
PDE6 bovine	zaprinast	52(IC_{50} 2.36 μM)	56 (IC_{50} 8.91μM)
Phospholipase PLC [ba]	manoalide	55 (IC_{50} 7.46 μM)	53(IC_{50} 8.14μM)
UDP glucuronysyltransferase (UGT1A1) human	17β-estradiol (IC_{50} 15 μM)	60 (IC_{50} 1 μM)	84(IC_{50} 0.759μM)
Human dopamine transporter †	GBR-12909	95 (IC_{50} 0.169μM)	91(IC_{50} 0.757μM)
Inhibition of agonist-stimulated IP1 formation			IC_{50} 15.8μM
[Ba] bacillus cereus			

Another major metabolite is AR-C133913XX (AZ11879477). The relative potency of this metabolite for the $P2Y_{12}$ receptor compared to ticagrelor and the active metabolite (AZ11879328) is shown in the reviewer's table below.

Potency of ticagrelor and its metabolites in the $P2Y_{12}$ receptor radio-ligand binding and signalling assays

	[^{125}I]-AZ11931285 binding assay		**[^{35}S]-GTPgS assay**	
Compound name	**IC_{50} (nmol/L)**	**N**	**IC_{50} (nmol/L)**	**N**
ticagrelor	11±8 (mean±SD)	63	66±28 (mean±SD)	98
AZ11879328	7 and 6	2	36 and 34	2
AZ11879477	2500	1	10000	1

Reference ID: 2937847

Ticagrelor and AZ11702105, effect alone and in combination with aspirin on LTB4 production by the 5-LO pathway in human whole blood, *in vitro*
Study number: AZM090617-05
Report number: AZMR10091
Study location: Astra Zeneca R&D, Molndal, Sweden

Human whole blood 5-lipoxygenase (5-LO) assays were performed to determine 1) whether the $P2Y_{12}$ receptor antagonists ticagrelor (AZ11939728) and prasugrel active metabolite (PAM; AZ11702105) can inhibit leukotriene B4 (LTB4) production, and 2) to investigate whether adding aspirin alone or in combination with ticagrelor or PAM can affect shunting of arachidonic acid substrate to LTB4 production.

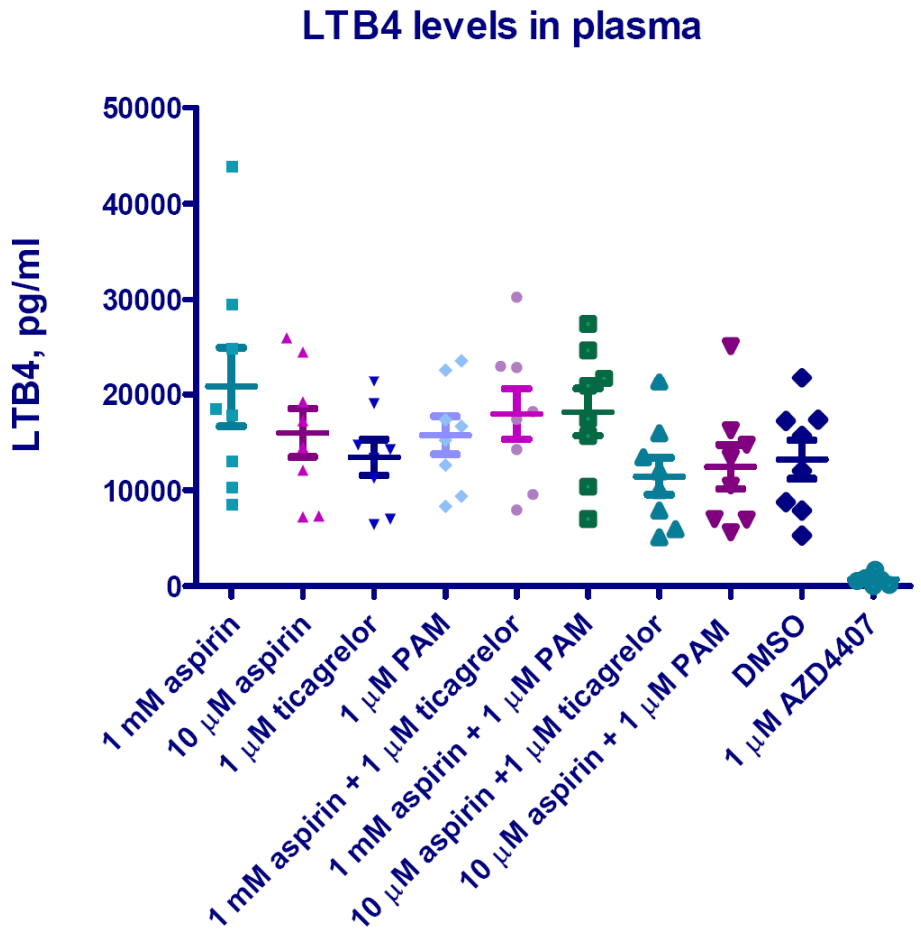
When assayed in blood from two healthy donors, both AZ11939728 and AZ11702105 dose dependently inhibited LTB4 production with a maximum inhibition of 45% and 53% respectively at 30 µM. Since neither compound significantly inhibited LTB4 production above 50%, IC_{50} values were recorded as >30 µM. Based on this data, neither compound is likely to inhibit LTB4 production at plasma levels reached in the clinic.

Aspirin was added to blood from 8 different donors at 10 µM or 1 mM to simulate low and high dose aspirin exposure. When 5-LO assays were subsequently performed, LTB4 levels were dose-dependently elevated when compared with blood incubated without aspirin for each donor (p=0.037). However, when AZ11939728 or AZ11702105 were added at 1 µM in combination with low or high dose aspirin, LTB4 levels were not significantly different compared to blood incubated only with aspirin. Therefore this data does not support the concept that ticagrelor or PAM can affect arachidonic acid shunting in the presence of aspirin. As the sponsor states:

> Arachidonic acid shunting to different biosynthetic pathways in the presence of aspirin would not in itself account for any potential interaction between aspirin dose and ticagrelor. However, in a [redacted] (b) (4) selectivity screen, ticagrelor was found to inhibit 5-LO activity up to 53% in an *in vitro* assay when tested at 30 µM. If this translated to an effect in blood, this could mean that arachidonic acid shunting to the 5-LO pathway could be affected by ticagrelor.

Reference ID: 2937847

Figure 2 **LTB_4 levels in blood from 8 different donors pre-incubated with aspirin ± ticagrelor or PAM and stimulated with calcium ionophore.**



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Analysis of the effects of ticagrelor (AZ11939728) and prasugrel active metabolite (AZ11702105) on platelet responses *in vitro*

Blood was obtained from an unspecified number of healthy volunteers of unspecified sex and platelet rich plasma was isolated. Aggregation of platelets in PRP was measured by light aggregometry, using agonists of ADP, arachidonic acid (AA), epinephrine, TRAP 6 and U46619 (a stable thromboxane A_2 mimetic). At the end of monitoring for PRP aggregation, cyclooxygenase activity was halted by the addition of diclofenac. Thomboxane B_2 (TXB_2) in the supernatant was measured as a marker of TXA_2 formation.
Ticagrelor was referred to as AZ119 and the prasugrel active metabolite was referred to as AZ117 in the report. According to the sponsor, the pharmacological properties of prasugrel active metabolite (PAM) can be considered identical to those of clopidogrel active metabolite (CAM).

Determination of Concentrations for Definitive Studies

ADP-induced aggregation

AZ117 and AZ119 caused concentration dependent inhibition of ADP-induced aggregation. ADP was tested over the concentrations 0.625-10μM. Aspirin at concentrations up to 100μM did not cause a significant effect upon aggregation.

U46619 (TBxA2)-induced aggregation

AZ117 and AZ119 caused concentration dependent inhibition of U46619-induced aggregation while aspirin was without effect.

Arachidonic acid –induced aggregation

AZ117, AZ119 and aspirin each produced concentration dependent inhibition of arachidonic acid-induced platelet aggregation.

Based on the above trials, the sponsor selected concentrations of AZ117 of 2 μM (approximate IC_{50}) and 10 μM (approximate IC_{90}) for use in combination with aspirin (30μM, approximately the peak levels after ingestion of 75mg of aspirin).

When AZ117 was tested at concentrations of 0.5μM, 1μM, 2μM or 10 μM, aspirin did not provide additional anti-aggregation against

ADP
TRAP-6
U46619

Reference ID: 2937847

Aspirin did enhance the anti-aggregatory effect of AZ117 (0.5μM, 1μM, 2μM) against collagen and epinephrine-stimulated aggregation but did not increase the efficacy of 10 μM AZ117.

AZ117 at 2 and 10 μM caused concentration related inhibition of TXA_2 production accompanying arachidonic acid and collagen induced aggregation. Aspirin by itself and in combination with AZ117 caused complete inhibition of TXA_2 production.

The initial experiments identified AZ119 concentrations of 0.3 μM and 3 μM as the approximate IC_{50} and IC_{90} respectively for ADP induced platelet aggregation. The results for these studies were similar to the results for AZ117. The addition of 30 μM aspirin to the IC_{50} and IC_{90} concentrations of AZ119 did not increase the anti-aggregatory efficacy against ADP, TRAP-6 or U46619 –induced platelet aggregation. The two higher concentrations of AZ119 inhibited production of TXA2 that accompanied arachidonic acid and collagen induced aggregation.

To study whether greater concentrations of aspirin could produce additional inhibition of aggregation, AZ117 (2 μM and 10 μM) and AZ119 (0.3μM and 3 μM) were tested ±aspirin 120 μM, to mimic the effects of a 300 mg aspirin dose. These experiments produced similar results to the preceeding experiments. Aspirin provided additional platelet inhibition at the lower concentrations of AZ117 and AZ119 against collagen and epinephrine –induced aggregation but provided little additional effect to the higher concentrations of these drugs (see sponsor's figures below).

Taken together, these results indicated that the addition of either low dose (30 μM) or high dose (120 μM) aspirin enhanced the platelet inhibitory effect when $P2Y_{12}$ inhibition was partial, but not when $P2Y_{12}$ inhibition was complete. Further, no additional platelet inhibition was seen under either partial or complete $P2Y_{12}$ inhibition when aspirin was increased from 30 μM to 120 μM.

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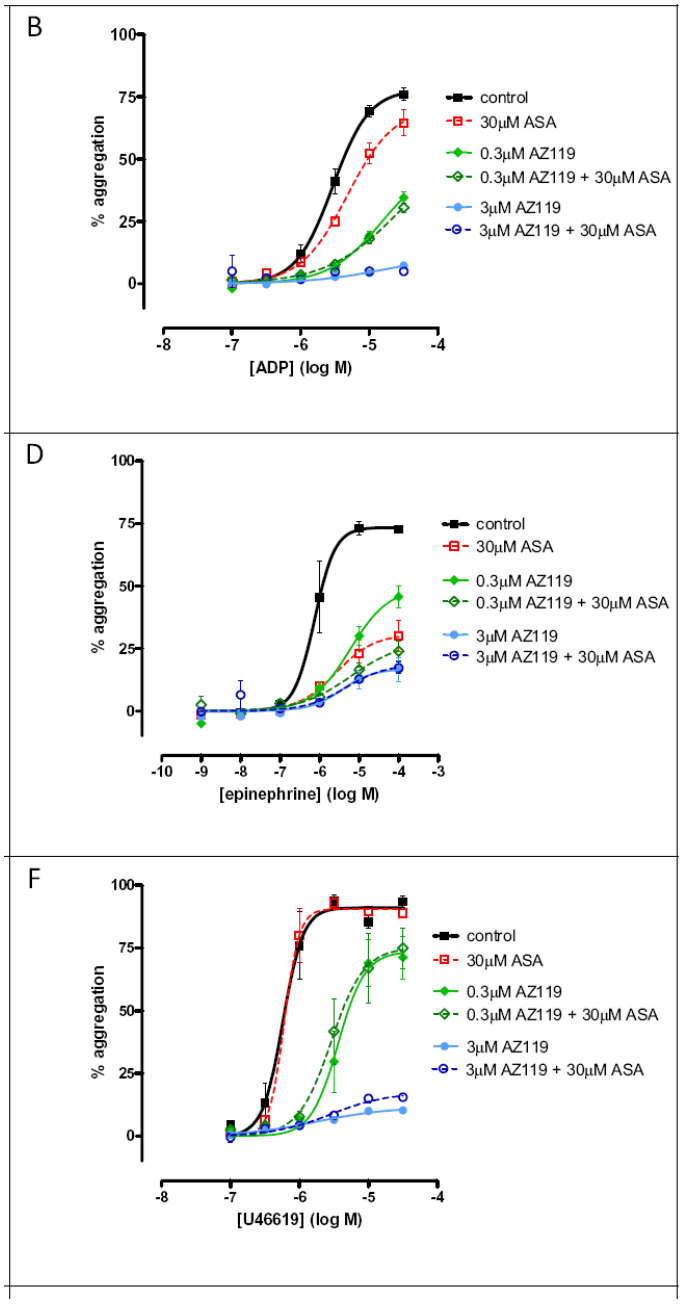

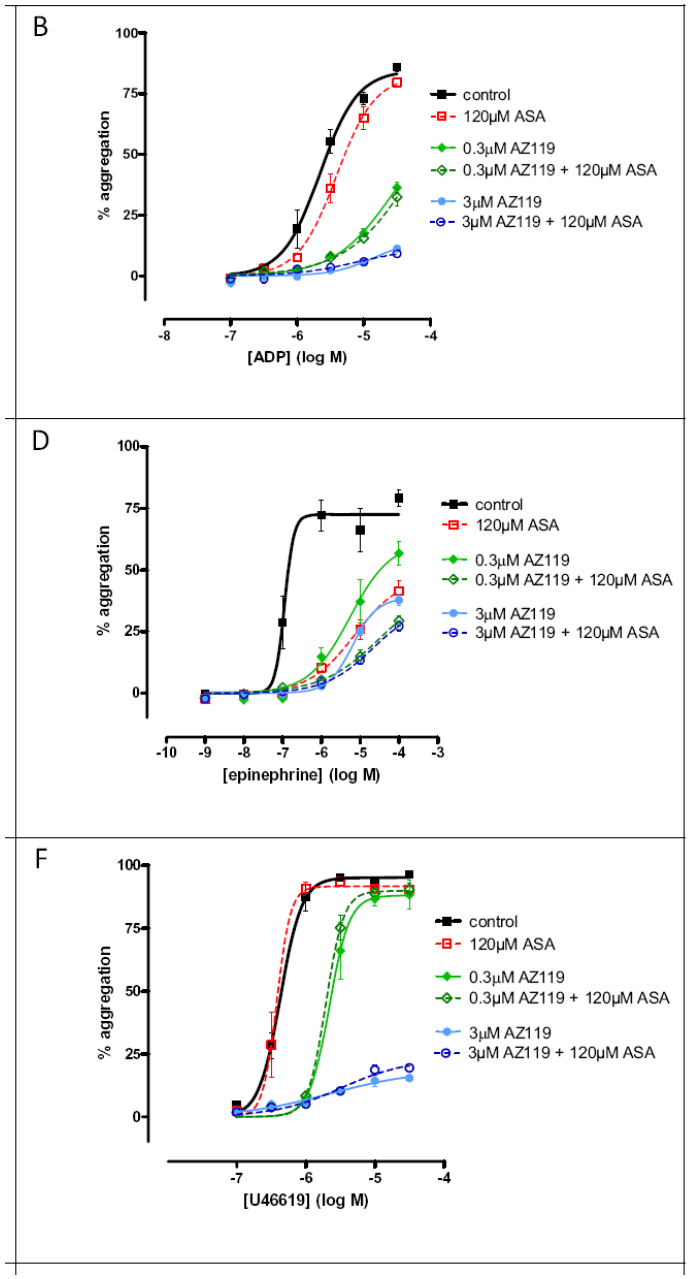

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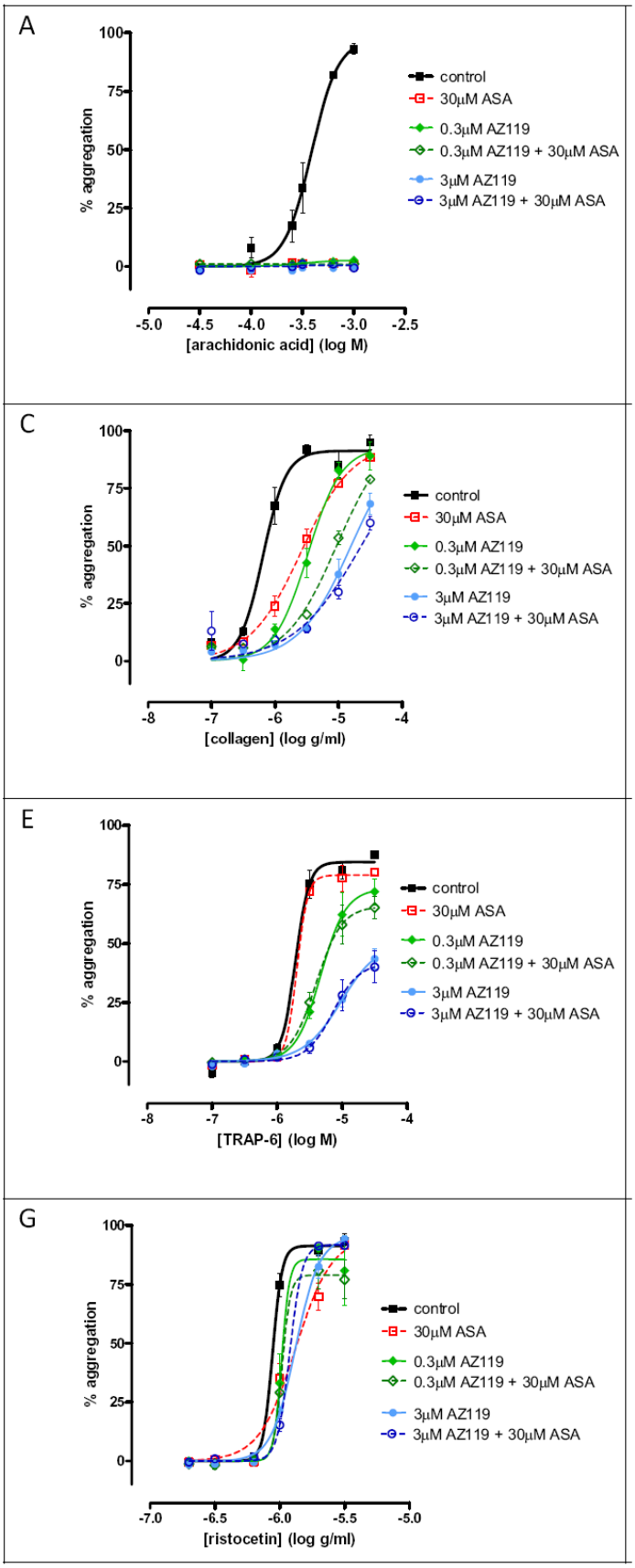

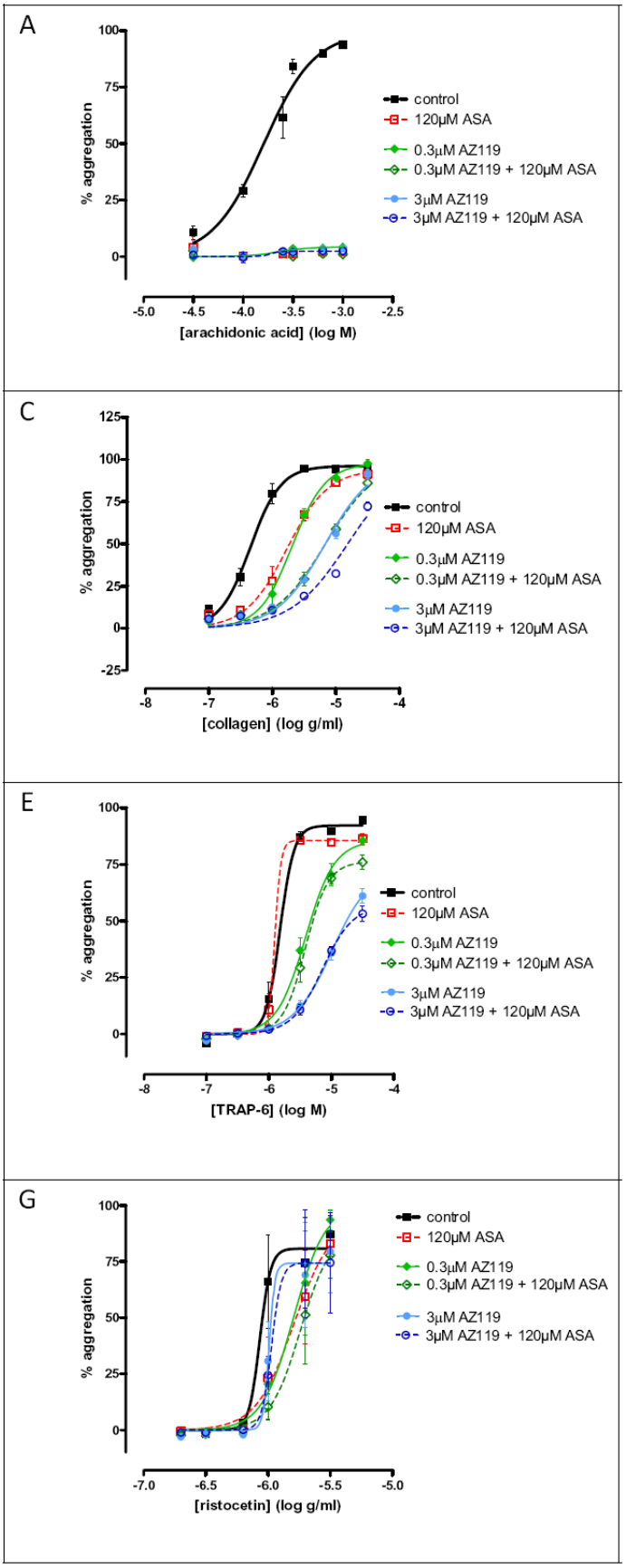

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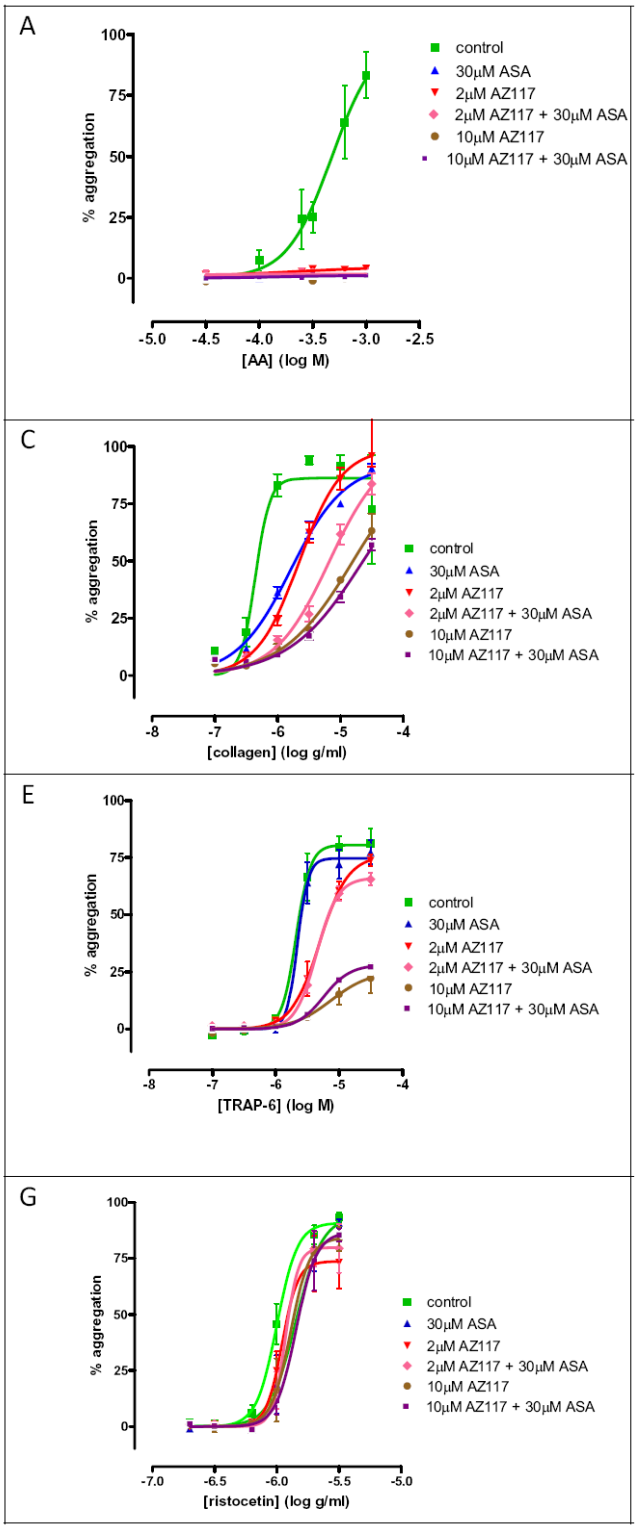

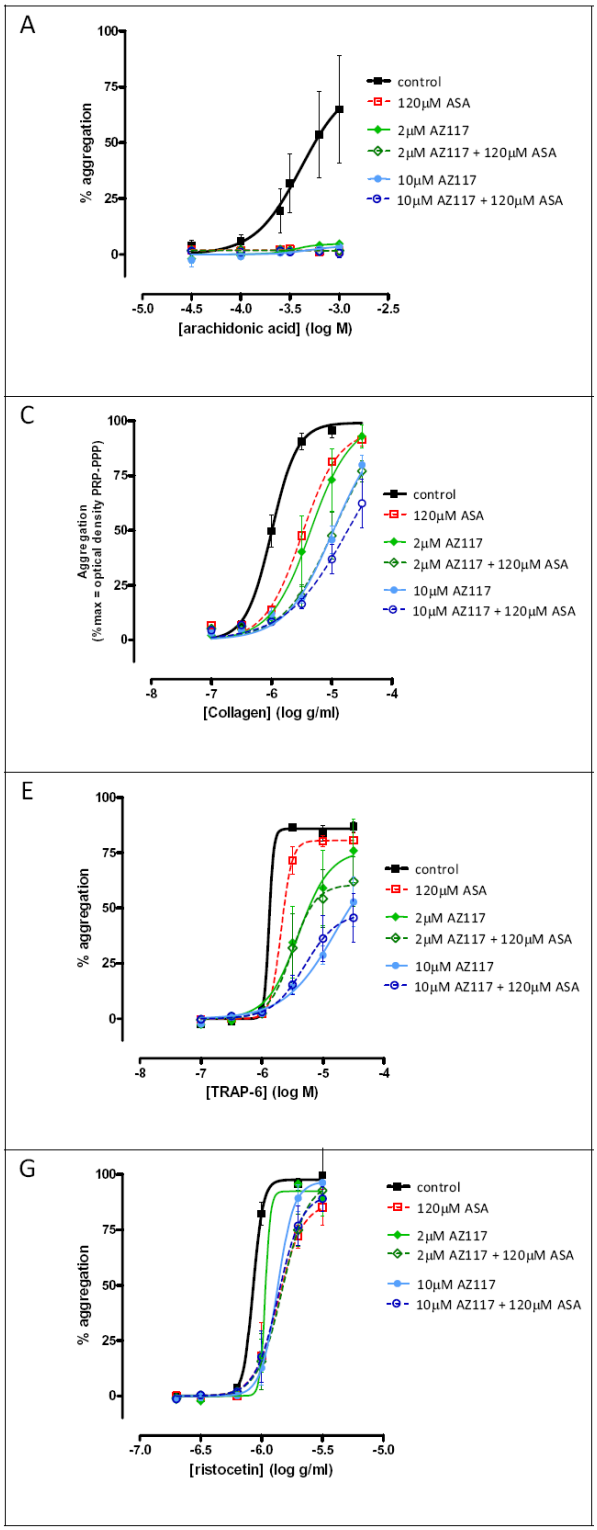

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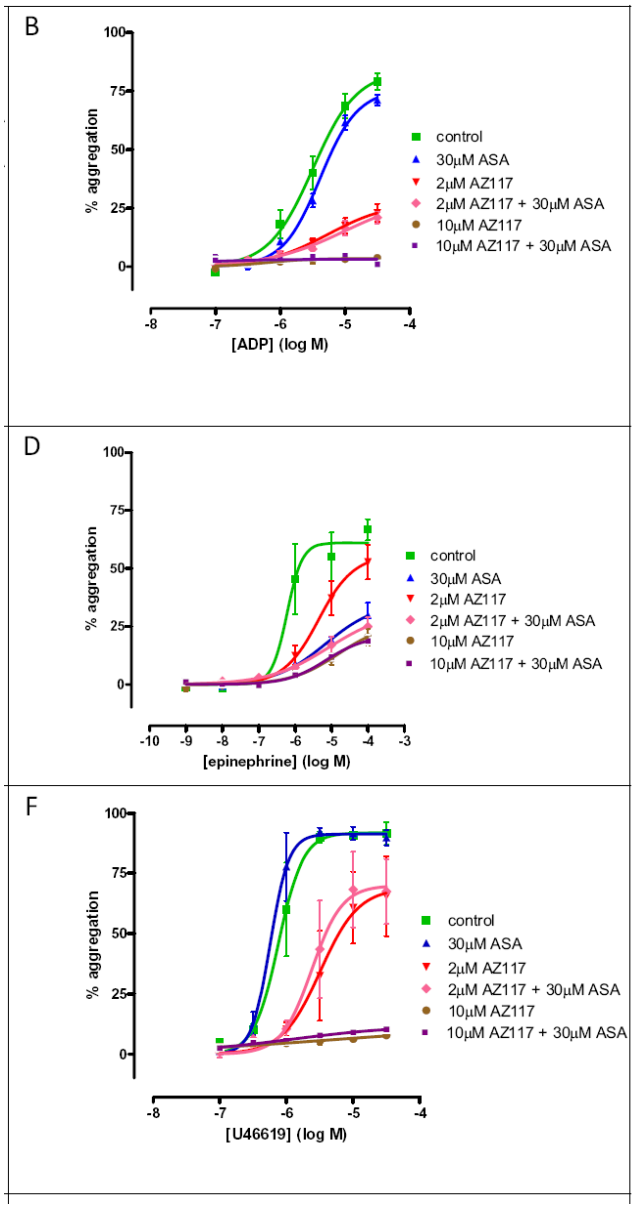
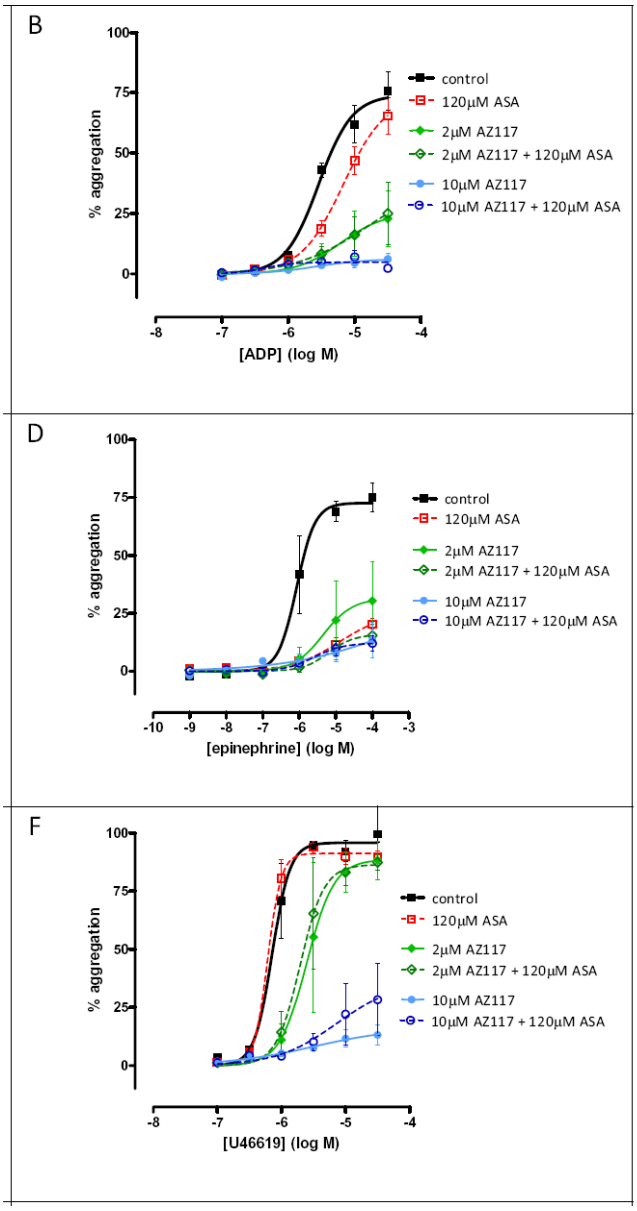
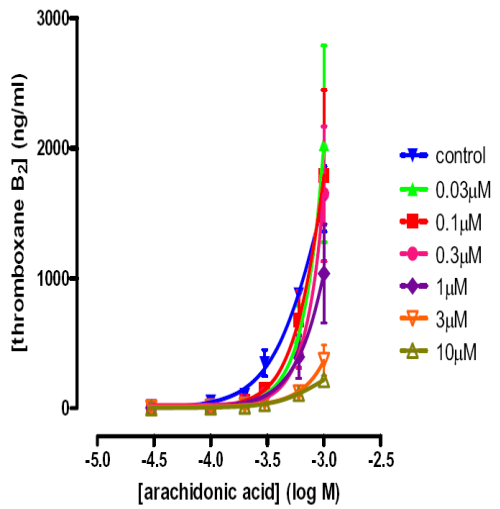
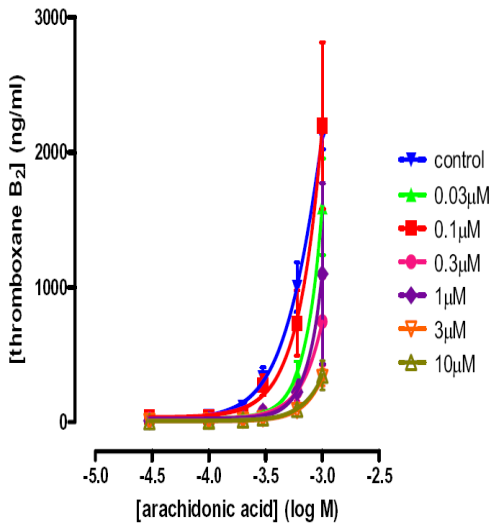

B
% aggregation
[ADP] (log M)
control
30μM ASA
2μM AZ117
2μM AZ117 + 30μM ASA
10μM AZ117
10μM AZ117 + 30μM ASA
D
% aggregation
[epinephrine] (log M)
control
30μM ASA
2μM AZ117
2μM AZ117 + 30μM ASA
10μM AZ117
10μM AZ117 + 30μM ASA
F
% aggregation
[U46619] (log M)
control
30μM ASA
2μM AZ117
2μM AZ117 + 30μM ASA
10μM AZ117
10μM AZ117 + 30μM ASA

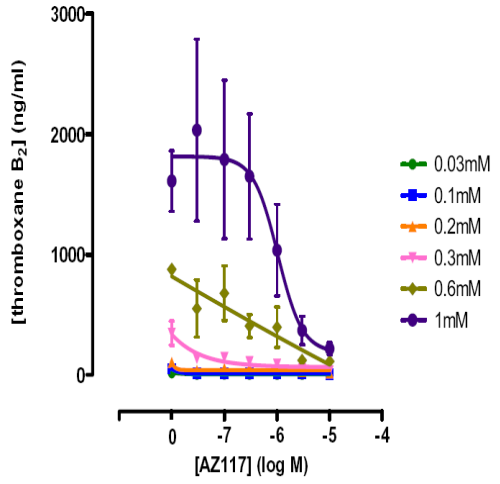

B
% aggregation
[ADP] (log M)
control
120μM ASA
2μM AZ117
2μM AZ117 + 120μM ASA
10μM AZ117
10μM AZ117 + 120μM ASA
D
% aggregation
[epinephrine] (log M)
control
120μM ASA
2μM AZ117
2μM AZ117 + 120μM ASA
10μM AZ117
10μM AZ117 + 120μM ASA
F
% aggregation
[U46619] (log M)
control
120μM ASA
2μM AZ117
2μM AZ117 + 120μM ASA
10μM AZ117
10μM AZ117 + 120μM ASA

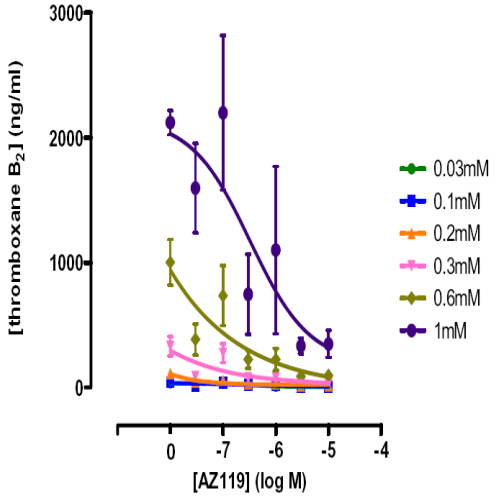
The sponsor then examined effects of AZ117 and AZ119 on platelet production of thromboxane A_2 stimulated by arachidonic acid. As expected, aspirin inhibited platelet aggregation and TXA_2 production in response to arachidonic acid. AZ117 and AZ119 also inhibited platelet aggregation and TXA_2.

Concentration response curves for the inhibition by AZ117 or AZ119 of platelet produced thromboxane A_2 (TXA_2 measured as TXB_2) induced by arachidonic acid are shown below. The upper panel shows effects of arachidonic acid response curve of increasing concentrations of AZ117 and AZ119 (0.03µM – 10µM). The lower panels show effects of AZ117 or AZ119 against TXA_2 production induced by arachidonic acid (0.03-1 mM)









Reference ID: 2937847

Ticagrelor and Clopidogrel, effects on arterial thrombosis, vascular resistance and *ex vivo* platelet function alone and in combination with a high dose acetylsalicylic acid in the anaesthetised dog, *in vivo* First Report Submitted

Study number: AZM091112-02
Report number: AZMR10115
Study location: Astra Zeneca, Molndal, Sweden

A modified Folts model of arterial thrombosis in the anesthetized dog was used to examine the effect of ticagrelor or clopidogrel alone and in combination with acetylsalicylic acid (ASA).

Five male dogs for ticagrelor and 5 male dogs for clopidogrel were used to evaluate the effect of maximal $P2Y_{12}$ inhibition alone or in combination with high dose ASA (50 mg/kg.min). An additional four experiments using 2 male dogs for ticagrelor and 2 male dogs for clopidogrel were performed to examine the effect of submaximal $P2Y_{12}$ inhibition alone or combined with high dose ASA.

Anesthesia was induced and maintained with pentobarbital. The dogs were then instrumented. The femoral arteries were exposed and both were fitted with flow probes. A variable occluder was used to produce a platelet-dependent thrombus and flow reduction in one femoral artery referred to as the stenosed artery. The undamaged femoral artery was referred to as the control. Following a 30 minute period for establishment of baseline measurements, either ticagrelor or clopidogrel were administered. Ticagrelor was given as a one minute bolus infusion followed by a continuous infusion. Clopidogrel was given as one minute boluses only due to the irreversible mode of action. A 60 minute observation period followed. Aspirin was then given intravenously, also as a 1 minute bolus, due to the irreversible mode of action. Another observation period followed the administration of aspirin. The vasodilator iloprost was given at two different dose levels for the last two 30 minute periods in an attempt to reverse the effects of aspirin. The sponsor found the iloprost data difficult to interpret and felt that methodological issues required further refinement. They did not discuss the results of the iloprost administration.

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Table 1 **Dose and time scheme for ticagrelor. Maximal $P2Y_{12}$ inhibition level**

Time (min)	Treatment	Dose (µg/(kg x min))
0-30	5% mannitol	
30-31	Ticagrelor	210
31-210	Ticagrelor	30
90-91	ASA	50000
150-180	Iloprost	0.01
180-210	Iloprost	0.1

Table 2 **Dose and time scheme for clopidogrel. Maximal $P2Y_{12}$ inhibition level**

Time (min)	Treatment	Dose (µg/(kg x min))
0-30	5% mannitol	
30-31	Clopidogrel	6500
90-91	ASA	50000
150-180	Iloprost	0.01
180-210	Iloprost	0.1

Table 3 **Dose and time scheme for ticagrelor. Sub-maximal $P2Y_{12}$ inhibition level**

Time (min)	Treatment	Dose (µg/(kg x min))
0-30	5% mannitol	
30-31	Ticagrelor	22.5
31-210	Ticagrelor	3
90-91	ASA	50000
150-180	Iloprost	0.01
180-210	Iloprost	0.1

Table 4 **Dose and time scheme for clopidogrel. Sub-maximal $P2Y_{12}$ inhibition level**

Time (min)	Treatment	Dose (µg/(kg x min))
0-30	5% mannitol	
30-31	Clopidogrel	2000
90-91	ASA	50000
150-180	Iloprost	0.01
180-210	Iloprost	0.1

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Ex vivo analysis

Blood for evaluation of *ex vivo* platelet aggregation, TXA_2 formation, drug plasma concentration and platelet count was collected 20 minutes into each observation period. Blood was also drawn before the start and end of the experiment for determination of pH, blood gases, electrolytes, hemoglobin (Hb), glucose and lactate.

Urine for evaluation of metabolites of TXA_2 and PGI_2 was collected for each 30 minute period. The results were expressed as picograms of 2,3-dinor-6-keto-prostaglandin F1α or 11-dehydro-TXB_2 per mg of creatinine.

Agonist-induced platelet aggregation ex vivo

Platelet aggregation ex vivo was evaluated by impedance aggregometry in diluted (whole blood diluted with saline 1:1) whole blood using the Multiplate® device. Platelet agonists used were ADP, AA and collagen.

Vascular Resistance

The sponsor calculated vascular resistance as: (mean aortic pressure)/ (femoral artery flow). This is a variation of the formula

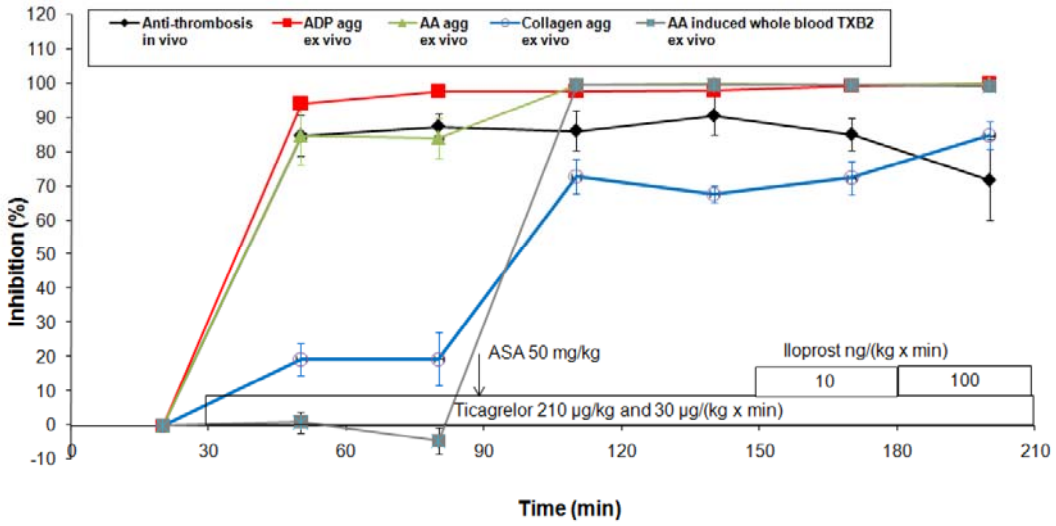
SVR= MAP/ CO x 80 in units of (dyne*sec)/cm^5
SVR= systemic vascular resistance
MAP= mean arterial pressure
CO= cardiac output
(www.cvphysiology.com/blood%20pressure/BP021.htm)

Reference ID: 2937847

Results

The addition of aspirin only increased the percent inhibition of collagen-induced platelet aggregation under conditions of maximal $P2Y_{12}$ inhibition from ticagrelor, but not that seen with ADP-induced platelet aggregation. The *in vivo* anti-thrombosis effect showed a slight decrease with the addition of iloprost.

Figure 1 ***In vivo* anti-thrombotic and *ex vivo* anti-platelet effects of maximal $P2Y_{12}$ inhibition by ticagrelor alone and in combinations**

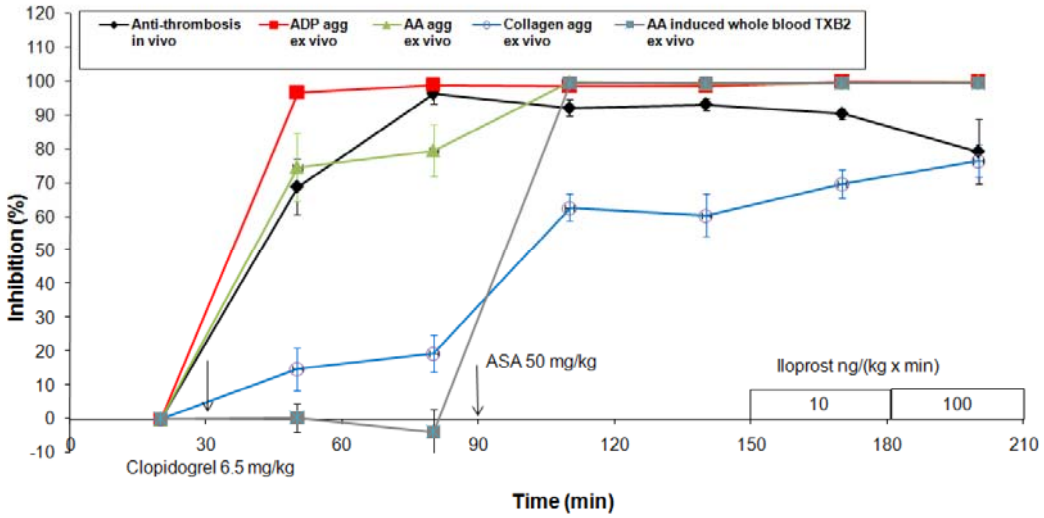


In vivo anti-thrombotic and *ex vivo* anti-platelet effects of maximal $P2Y_{12}$ inhibition by ticagrelor alone and in combination with ASA or ASA plus iloprost. Percent inhibition data is expressed as mean ± SEM of 5 separate experiments performed in 5 dogs.

Reference ID: 2937847

The addition of aspirin only increased the percent inhibition of collagen-induced platelet aggregation under conditions of maximal $P2Y_{12}$ inhibition from clopidogrel, but not that seen with ADP-induced platelet aggregation. The *in vivo* anti-thrombosis effect showed a slight decrease with the addition of iloprost.

Figure 2 ***In vivo* anti-thrombotic and *ex vivo* anti-platelet effects of maximal $P2Y_{12}$ inhibition by clopidogrel alone and in combinations**



In vivo anti-thrombotic and *ex vivo* anti-platelet effects of maximal $P2Y_{12}$ inhibition by clopidogrel alone and in combination with ASA or ASA plus iloprost. Percent inhibition data is expressed as mean ± SEM of 5 separate experiments performed in 5 dogs.

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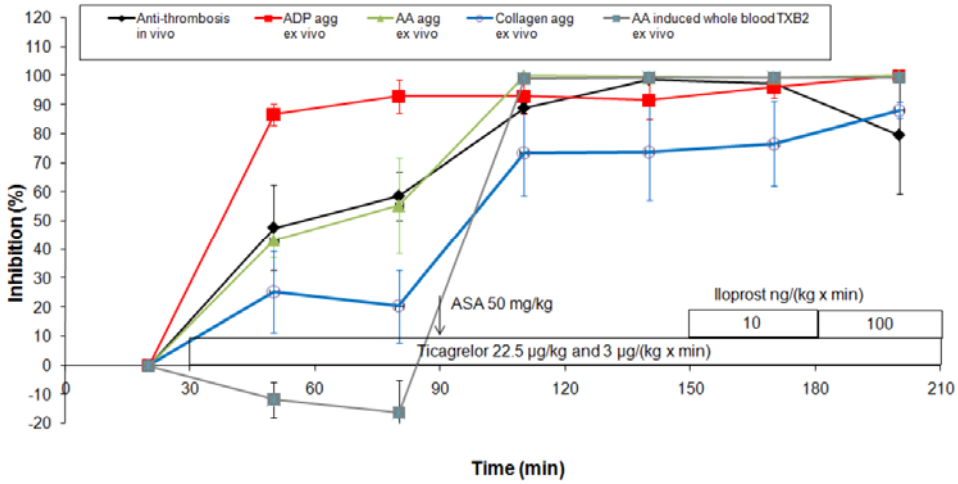
In the case of submaximal inhibition of $P2Y_{12}$ by either clopidogrel or ticagrelor, the addition of aspirin did produce some increase in the measured anti-thrombotic and anti-platelet effects induced by AA or collagen.

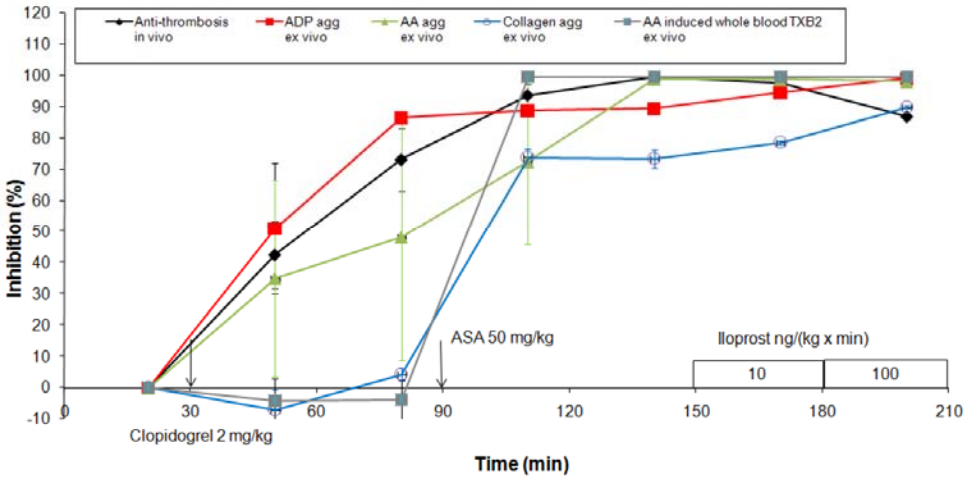
Figure 3 ***In vivo* anti-thrombotic and *ex vivo* anti-platelet effects of sub-maximal $P2Y_{12}$ inhibition by ticagrelor alone and in combinations**



In vivo anti-thrombotic and *ex vivo* anti-platelet effects of sub-maximal $P2Y_{12}$ inhibition by ticagrelor alone and in combination with ASA or ASA plus iloprost. Percent inhibition data is expressed as mean ± SEM of 2 separate experiments performed in 2 dogs.

Reference ID: 2937847

Figure 4 ***In vivo* anti-thrombotic and *ex vivo* anti-platelet effects of sub-maximal $P2Y_{12}$ inhibition by clopidogrel alone and in combinations**

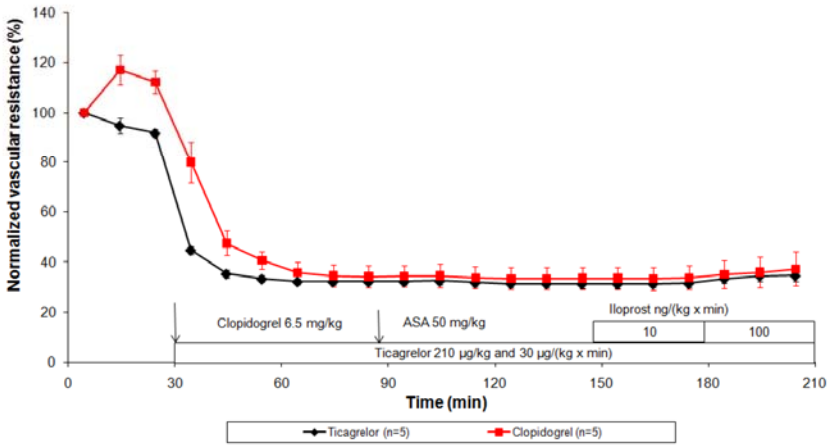


In vivo anti-thrombotic and *ex vivo* anti-platelet effects of sub-maximal P2Y12 inhibition by clopidogrel alone and in combination with ASA or ASA plus iloprost. Percent inhibition data is expressed as mean ± SEM of 2 separate experiments performed in 2 dogs.

Vascular resistance

Vascular resistance, expressed as a normalized percentage of the vehicle infusion value, decreased in the injured leg. This demonstrated the anti-thrombotic effect of the test articles.

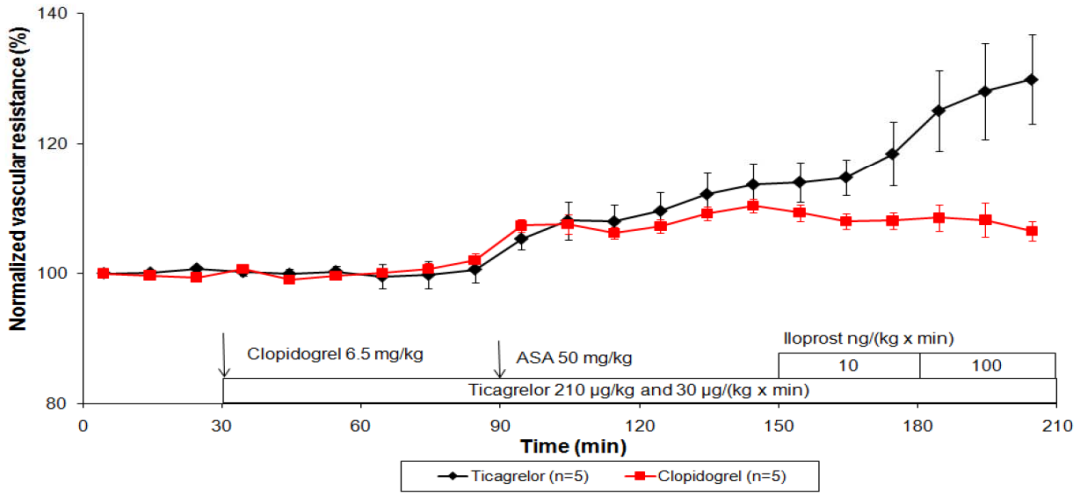
Figure 5 **Normalized vascular resistance in the stenosed/damaged leg at maximal $P2Y_{12}$ inhibition alone and in combinations**



Reference ID: 2937847

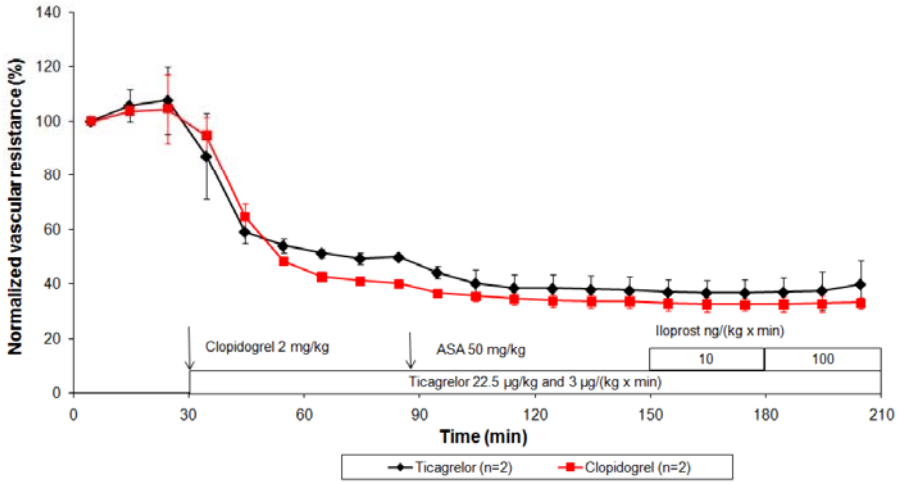
At maximal $P2Y_{12}$ inhibition, the increase in vascular resistance in the control leg (no damage to the femoral artery, sponsor's figures 7 and 8) was greater in the ticagrelor group than the clopidogrel group. However, the increase does not appear until the infusion of iloprost, a stable prostacyclin analogue, that theoretically should have reduced vascular resistance. The sponsor was asked in a telecon (April 5, 2011, email in Appendix 1) if they had any hypotheses for the iloprost results. The sponsor replied that the work was entirely exploratory and provided no explanation for the data.

Figure 7 **Normalized vascular resistance in the control leg at maximal $P2Y_{12}$ inhibition alone and in combinations**



At submaximal $P2Y_{12}$ inhibtion, there was no difference in effect on normalized vascular resistance between ticagrelor and clopidogrel in the injured leg.

Figure 6 **Normalized vascular resistance in the stenosed/damaged leg at sub-maximal $P2Y_{12}$ inhibition alone and in combinations**



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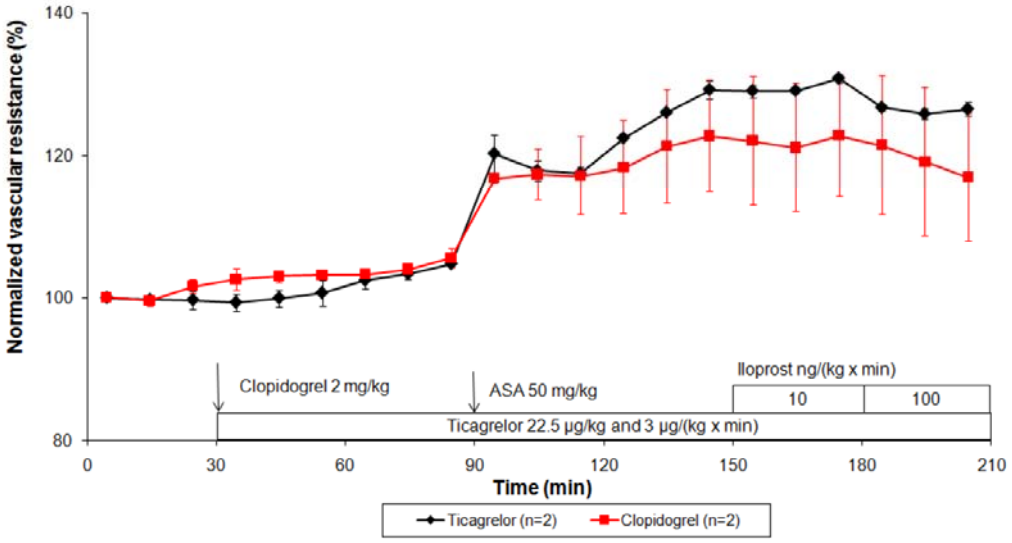
In the uninjured legs, normalized vascular resistance showed a statistically non-significant separation of mean values. The infusion of iloprost made no apparent difference on the considered parameter.

Figure 8 **Normalized vascular resistance in the control leg at sub-maximal $P2Y_{12}$ inhibition alone and in combinations**



The determination of blood pH, blood gases, electrolytes, hemoglobin (Hb), glucose and lactate indicated only the changes to be expected for anesthetized animals. There were no obvious differences between the treatment groups.

The analysis of urinary metabolites showed a high degree of inter-individual variability. The mean values of 11-dehydro-TXB_2, normalized for creatinine excretion, showed an increase when aspirin was administered to the dogs. Also, 11-dehydro-TXB_2 decreased in the presence of prostaglandins, as might be expected. This held for ticagrelor and clopidogrel under conditions of both maximal and submaximal $P2Y_{12}$ inhibition.

Reviewer's Summary of Mean Urinary Metabolite Levels

11-dehydro-TXB_2 pg/mg U-Creatinine						
Treatment period	Maximal $P2Y_{12}$ inhibition N=5				Submaximal $P2Y_{12}$ inhibition n=2	
	Ticagrelor		clopidogrel		Ticagrelor mean	Clopidogrel mean
	mean	range	mean	range		
Vehicle	110	38-228	90	45-215	694	185
Drug	86	41-131	164	24-367	75	110
Drug	115	26-351	83	15-206	211	193
Drug+ASA	1004	555-1436	1134	878-1676	1060	1158
Drug+ASA+ Iloprost	395	93-862	453	266-806	319	402

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Inter-individual variability was apparent in the data for the mean 2, 3-dinor-6-keto-PGF1α concentrations normalized for creatinine excretion. It is difficult to perceive any pattern in the data.

Reviewer's Summary of Mean Urinary Metabolite Levels

2,3-dinor-6-keto-PGF1α pg/mg U-Creatinine						
Treatment period	Maximal $P2Y_{12}$ inhibition N=5				Submaximal $P2Y_{12}$ inhibition n=2	
	Ticagrelor		clopidogrel		Ticagrelor	clopidogrel
	mean	range	mean	range	mean	mean
Vehicle	2877	685-6002	4392	869-14617	10267	5664
Drug	2577	269-6157	5337	339-15950	1143	1094
Drug	2771	290-6911	2905	383-8908	2808	2616
Drug+ASA	3344	2717-4588	5905	2442-9222	4762	5858
Drug+ASA+ Iloprost	3688	1213-8137	3212	1500-5797	2201	2767

Ticagrelor, Clopidogrel and High Dose Acetylsalicylic Acid, Effects on Arterial Thrombosis, Vascular Resistance and *ex vivo* Platelet Function Alone and in Combinations in the Anesthetized Dog *in vivo* Second Report Submitted

Report number: AZMR10239
Study number: AZM091112-02
Study location: Astra Zeneca, Mölndal, Sweden

This was submitted as an extension of study AZM091112-02 and an addendum to report AZMR 10115. The sponsor has re-submitted the entire report with some new /different presentations of the data.

The sponsor examined aspirin alone in the modified Folt's model using the *in vivo* methodology described above in the first report. The sponsor also studied an additional 1 dog for each of the clopidogrel and ticagrelor maximum $P2Y_{12}$ inhibition groups. Four dogs were added to each of the submaximal $P2Y_{12}$ groups. With the additional animals studied in this work, there are a total of 6 dogs in each of the ticagrelor and clopidogrel groups.

Six male Beagles were used to evaluate the effects of high dose aspirin (ASA) alone at 50 mg/kg. The experiments consisted of several 30-minute periods. Each experiment was

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started with a vehicle period followed by a dose of aspirin. Two dose levels of iloprost were added at the end of all experiments (at 150 and 180 minutes). This is summarized in the sponsor's table below.

Table 3 ASA alone, Dose and time scheme

Time (min)	Treatment	Dose (µg/(kg x min))
0-210	5% mannitol	0.01 mL/(kg x min)
90-91	ASA	50000
150-180	Iloprost	0.01
180-210	Iloprost	0.1

The sponsor offers the following definitions for the work:

Both $P2Y_{12}$ antagonists were studied at two doses corresponding to two effect levels of $P2Y_{12}$ inhibition. The doses administered were set by their anti-thrombotic effect:

1) "Maximal $P2Y_{12}$ inhibition": complete anti-thrombotic effect *in vivo* (abolition of CFR) and nearly 100% inhibition of *ex vivo* ADP-induced platelet aggregation

2) "Sub-maximal $P2Y_{12}$ inhibition": incomplete anti-thrombotic effect *in vivo* (CFR attenuated but still present) and approximately 80% inhibition of *ex vivo* ADP-induced platelet aggregation

Vascular Resistance

The sponsor calculated vascular resistance as (mean aortic pressure)/ (femoral artery flow). This is a variation of the formula

SVR= MAP/ CO x 80 in units of (dyne*sec)/cm^5
SVR= systemic vascular resistance
MAP= mean arterial pressure
CO= cardiac output
(www.cvphysiology.com/blood%20pressure/BP021.htm)

PGI_2 production in vivo

The sponsor used the urinary levels of the PGI_2 metabolite 2,3-dinor-6-keto-prostaglandin F1α as a time-integrated index of PGI_2 biosynthesis and therefore systemic COX-2 activity. The measured values were normalized against the vehicle period which was considered 0% inhibition.

Urine was collected for each 30 minute period but only analyzed for the second 30 minute period on mono or combination therapy which the sponsor referred to as the "steady state."

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PGI_2 production was analyzed by different methods than used in the original work. The new methods included [redacted] (b) (4) derivitization and LC-MS analysis. The coefficient of variability of the assay was not provided.

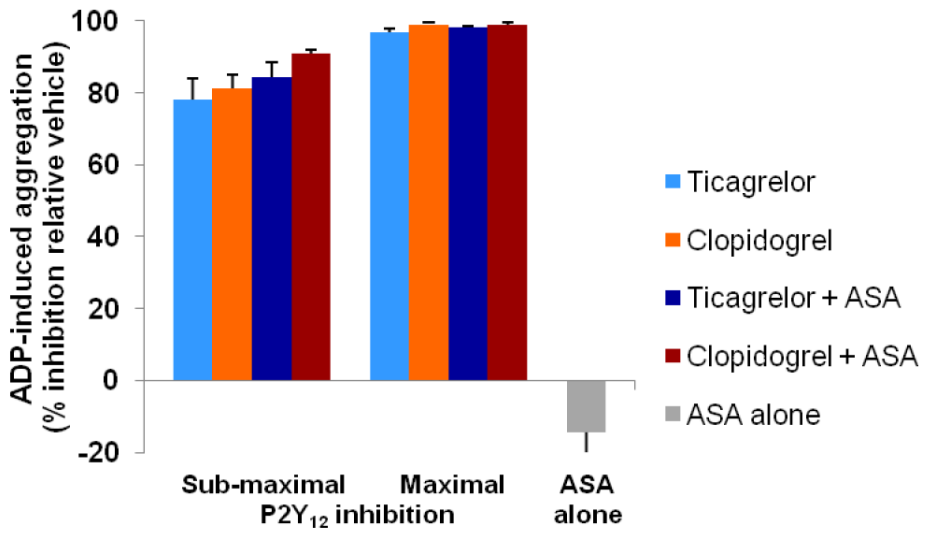
Ex vivo platelet aggregation

Blood for ex vivo analysis was collected 20 minutes into each 30 minute period. Platelet aggregation was assessed by impedance aggregometry with agonists of ADP, AA, and collagen.

Results

Adding aspirin to clopidogrel or ticagrelor at maximal $P2Y_{12}$ inhibition made little difference in the inhibition of ADP-induced aggregation. There was some additional efficacy when aspirin was added to submaximal concentrations of ticagrelor and clopidogrel. The sponsor presented Figure 2 to show the difference in platelet inhibition between sub-maximal versus maximal $P2Y_{12}$ inhibition.

Figure 2 Inhibition of ADP-induced aggregation at "steady-state"



Inhibition of platelet aggregation for the second period of mono and combination therapy "steady state" expressed as % inhibition relative vehicle which was set to 0%, mean ± SEM.

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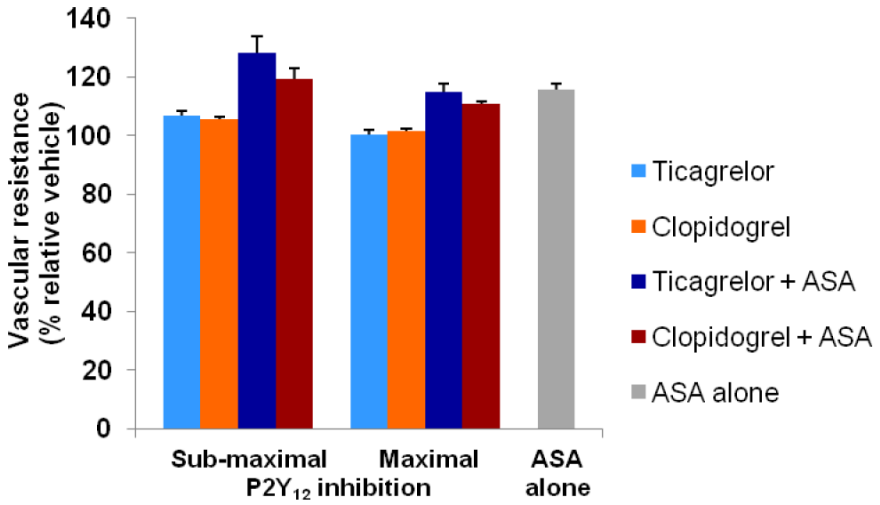
The sponsor provided graphical demonstrations of "normalized vascular resistance", i.e., percentage change from the vehicle period. As shown in the sponsor's graph, adding aspirin causes a greater increase under conditions of submaximal $P2Y_{12}$ inhibition than maximal $P2Y_{12}$ inhibition.

Figure 6 Vascular resistance at "steady-state"

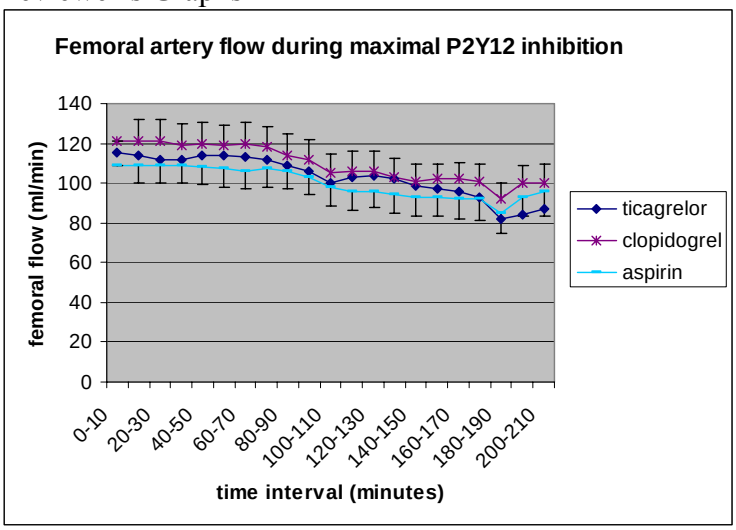


Data for the second period of mono and combination therapy "steady state" expressed as % relative the first 10 min of the vehicle period which was set to 100%, mean ± SEM of 6 separate experiments performed in 6 dogs.

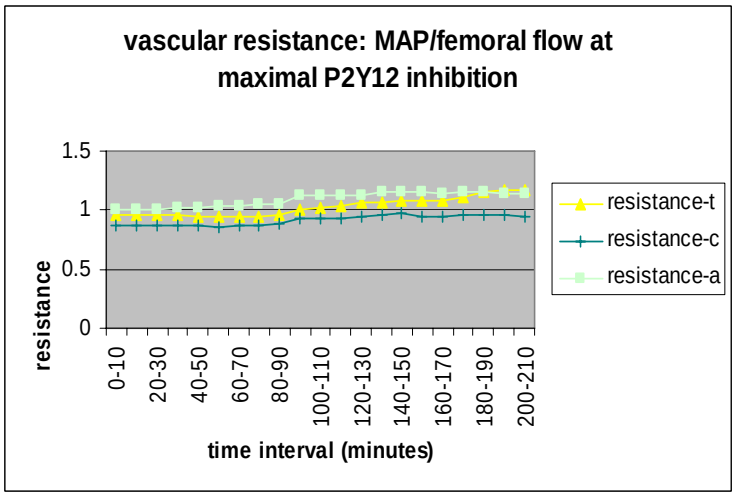
Reference ID: 2937847

The reviewer analyzed the data in the appendices of the sponsor's report to generate the following presentations of the components of vascular resistance and examine the variability of the model system.

Reviewer's Graphs



Time line
0-30 minutes: vehicle infused for dogs receiving ticagrelor or clopidogrel
30 minutes: clopidogrel or ticagrelor infused
0-90 minutes: vehicle infused for dogs receiving spirin
90 minutes: aspirin added
150 minutes: iloprost infused
180 minutes: iloprost infused



Key: Resistance-t = values calculated for ticagrelor
Resistance-c = values calculated for clopidogrel
Resistance-a = values calculated for aspirin

Error bars were not added to the vascular resistance graph to allow for observation of any trends in mean values.

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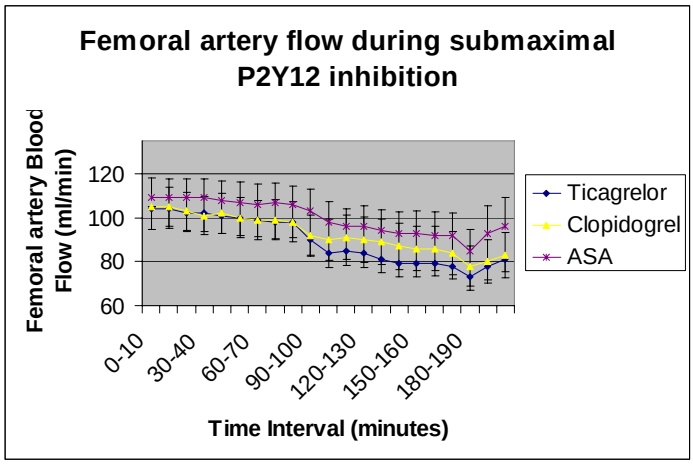
Based on the measured PGI_2 values, a decrease in vascular resistance would be expected in the ticagrelor group. The apparent decrease in PGI2 production in the clopidogrel and aspirin groups would be expected to cause increases in vascular resistance. This is not reflected in the graphs.

PGI_2 production *in vivo* (ng/mg creatinine): mean values for maximal $P2Y_{12}$ inhibition

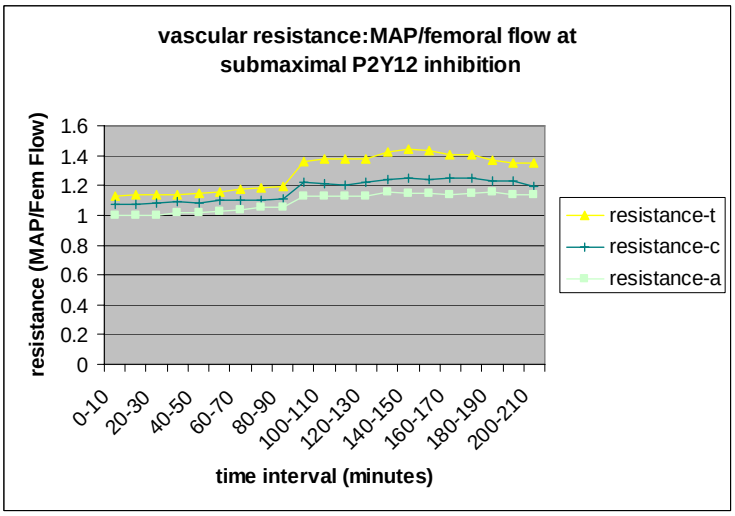
Time interval(minutes)	Ticagrelor (n=1)	Clopidogrel (n=1)	Aspirin (n=6)
0-30	5.7	7.6	10.2±1.1
60-90	7.7	7.9	11.1±1.2
120-150	6.6 (+16%)	4.7 (-38%)	7.1±0.3 (-30%)

Numbers in parentheses ( ) are the percent change from baseline.

The reviewer then examined the component data for normalized vascular resistance under conditions of submaximal $P2Y_{12}$ inhibition.



Time line
0-30 minutes: vehicle infused for dogs receiving ticagrelor or clopidogrel
0-90 minutes: vehicle infused for dogs receiving spirin
30 minutes: clopidogrel or ticagrelor infused
90 minutes: aspirin added
150 minutes: iloprost infused
180 minutes: iloprost infused



Key: Resistance-t = values calculated for ticagrelor
Resistance-c = values calculated for clopidogrel
Resistance-a = values calculated for aspirin
Error bars were not added to the above graph to allow for demonstration of any trends in the mean values.

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Based on the reported values for PGI_2, one would expect similar changes in vascular resistance in the clopidogrel and ticagrelor groups. It is not clear what level of change in vascular resistance in this system is biologically meaningful. It is not clear what level of change in PGI2 as measured by this assay is biologically meaningful.

PGI_2 production *in vivo* (ng/mg creatinine): mean±sem values for sub- maximal $P2Y_{12}$ inhibition

Time interval (minutes)	Ticagrelor (n=4)	Clopidogrel (n=4)	Aspirin (n=6)
0-30	10±1.5	14.1±4.1	10.2±1.1
60-90	9.0±1.2	13.2±3.5	11.1±1.2
120-150	5.3±0.5 (-47%)	7.6±1.6 (-46%)	7.1±0.3 (-30%)

Numbers in parentheses ( ) are the percent change from baseline.

If vascular resistance is tied to PGI_2, then under conditions of sub-maximal $P2Y_{12}$ inhibition, the change in vascular resistance should be similar for ticagrelor and clopidogrel and greater than that for aspirin alone.

The sponsor was asked in a telecon (April 4, 2011) to provide the coefficient of variability for the PGI_2 assay. The email answer is shown below:

> The CV% for the measurement of the PGI_2 metabolite in urine, 2,3-dinor-6-keto-prostglandin F_{1a}, based upon data from 3 standard curves. The largest CV% is obtained close to the limit of quantification and is 17%. However, most samples showed levels that were at a part of the standard curve that had a CV% between 2.2% and 12.3%.

These are acceptable levels of variability for a urinary assay. The email may be found in Appendix 1.

The sponsor's graph below demonstrates the percentage change in *in vivo* PGI_2 production.

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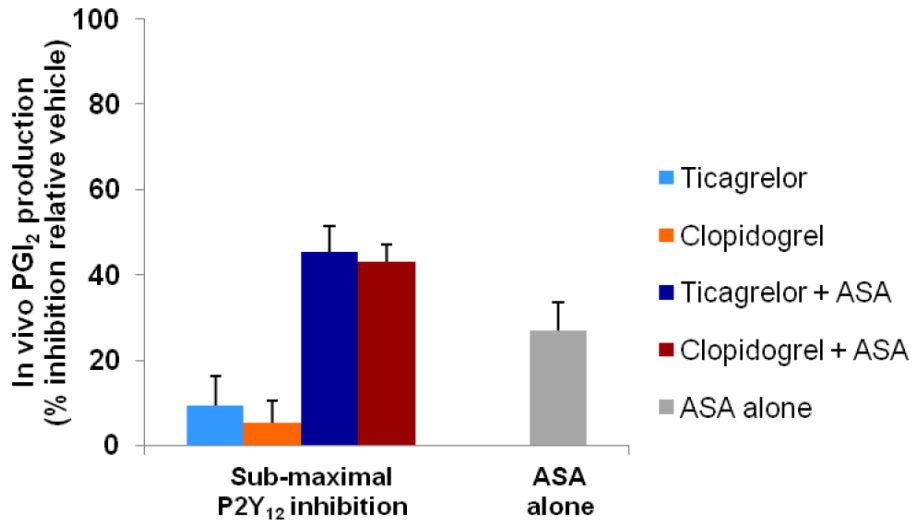
Figure 7 Inhibition of *in vivo* PGI_2 production at "steady-state"



Data for the second period of mono and combination therapy "steady state" expressed as % relative vehicle period which was set to 0%, mean ± SEM of 6 separate experiments performed in 6 dogs.

There is no concurrent group of animals treated with vehicle only for the duration of the study period. Therefore it is unclear what the variability and cardiovascular stability of the model itself is given the prolonged period of anesthesia. Some indication of the biological variability may be obtained from the mean arterial pressure (MAP), reported in mm Hg.

MAP (mm Hg) mean ±sem (numbers in parentheses are the range)

Time interval (minutes)	Ticagrelor		Clopidogrel		ASA	Vehicle
	Maximal $P2Y_{12}$ inhibition	Submaximal $P2Y_{12}$ inhibition	Maximal $P2Y_{12}$ inhibition	Submaximal $P2Y_{12}$ inhibition	50 mg/kg	Historical control
0-10	110±1.8 (104-117)	117±1.7 (111-122)	105±2.3 (98-113)	112±2.0 (106-119)	109±3.0 (97-118)	105±2.17 (99-113)
10-20	109±1.6 (103-115)	118±1.6 (110-122)	105±2.1 (99-112)	112±2.0 (106-119)	110±2.2 (103-116)	105±2.17 (99-113)
20-30	108±1.3 (104-113)	116±1.8 (110-120)	105±2.0 (101-112)	111±1.9 (104-117)	110±1.9 (104-117)	104±2.37 (97-112)

Note: the time period for which the MAP values are shown is the vehicle period when no drug has been administered. The historical control values were taken on the sponsor's recommendation (email received April 7, 2011 and confirmed in second email received April 7, 2011) from study number 25773, "The effects of intravenous infusion of AZD6140 on arterial thrombosis and hemostasis in the anesthetized dog: comparison with AR-H076866XX and clopidogrel."

The animals that the sponsor recommended as historical control data (study number 25773, "The effects of intravenous infusion of AZD6140 on arterial thrombosis and hemostasis in the anesthetized dog: comparison with AR-H076866XX and clopidogrel.") were used in the same modified Folt's model as described in the current study. The methods of anesthesia and maintenance were the same between the historical and current studies, including infusion of the same fluids for the vehicle.

Reference ID: 2937847

Femoral artery flow (ml/min) mean ±sem (numbers in parentheses are the range)

Time interval (minutes)	Ticagrelor		Clopidogrel		ASA	Vehicle
	Maximal $P2Y_{12}$ inhibition	Submaximal $P2Y_{12}$ inhibition	Maximal $P2Y_{12}$ inhibition	Submaximal $P2Y_{12}$ inhibition	50 mg/kg	Historical control
0-10	115±9.06 (93-130)	104±9.13 (68-124)	121±10.88 (89-162)	105±8.70 (81-141)	109±9.04 (82-150)	110±9.30 (81-139)
10-20	114±9.05 (92-129)	104±8.78 (68-123)	121±10.68 (89-161)	105±8.61 (82-141)	109±8.55 (87-149)	110±9.58 (80-139)
20-30	112±9.24 (90-152)	102±8.45 (67-118)	121±10.54 (87-160)	103±8.86 (86-140)	109±8.66 (88-150)	109±9.97 (78-139)

Note: the time period for which the MAP values are shown is the vehicle period when no drug has been administered. The historical control values were taken on the sponsor's recommendation (email received April 7, 2011 and confirmed in second email received April 7, 2011) from study number 25773, "The effects of intravenous infusion of AZD6140 on arterial thrombosis and hemostasis in the anesthetized dog: comparison with AR-H076866XX and clopidogrel."

Under the conditions of the study, aspirin added to sub-maximal $P2Y_{12}$ inhibition contributes additional anti-thrombotic and anti-platelet effect. Added to maximal $P2Y_{12}$ inhibition, aspirin did not provide additional benefit.

Anti-thrombotic effect (% inhibition relative to vehicle): mean±sem

	Ticagrelor		Clopidogrel		ASA
	Max	Sub-max	max	Sub-max	
Vehicle	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Tic/clo	87.8±4.6	47.0±9.1	69.7±6.3	36.1±4.2	1.7±1.6
Tic/clo	100±0.0	67.5±6.5	100±0.0	69.7±4.6	3.4±2.6
Tic/clo +ASA	100±0.0	94.9±2.0	100±0.0	96.7±1.8	88.3±9.1
Tic/clo+ ASA	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0

Inhibition of platelet TXA_2 production (% inhibition relative to vehicle): mean±sem

	Ticagrelor		Clopidogrel		ASA
	Max	Sub-max	max	Sub-max	
Vehicle	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Tic/clo	0.9±2.5	-12.7±2.7	0.3±3.5	-0.3±2.2	1.2±5.3
Tic/clo	-6.4±3.7	13.0±3.7	-2.7±5.7	-5.2±2.5	-0.6±4.8
Tic/clo +ASA	99.6±0.1	99.4±0.1	99.4±0.0	99.2±0.4	99.6±0.1
Tic/clo+ ASA	99.5±0.1	99.5±0.1	99.4±0.1	99.6 ±0.0	99.6±0.1

Pharmacokinetic study of aspirin (ASA), salicylic acid (SA), ticagrelor, AR-C124910XX and carboxylic acid metabolite of clopidogrel after oral administration

Reference ID: 2937847

of ASA alone or in combination with oral ticagrelor or intravenous clopidogrel in the dog study AZM090928-01

Study number: AZM090928-01
Study location: Astra Zeneca, R&D Molndal, Sweden

Fasted male dogs received a single oral dose of 5 mg/kg ASA alone or in combination with either 15 mg/kg ticagrelor (given orally) or 3 mg/kg clopidogrel (given intravenously). The sponsor then determined the plasma concentrations of ASA, SA, ticagrelor, AR-C124910XX and the carboxylic acid metabolite of clopidogrel.

Aspirin given by itself had a half life of approximately 20 minutes. The Cmax of the metabolite, salicylate, was reported at 1 hour after administration with a half life of 2-3 hours. Given in combination with either ticagrelor or clopidogrel, there were slight variations in the PK parameters. The sample size for this study is small enough that the results should be interpreted cautiously. It is also unfortunate that these doses bear no relation to the doses used in the Folts model study. The dose of aspirin is 1/10th that used in the pharmacology study and the route of administration of ticagrelor is different in the two studies.

Summary of pharmacokinetic parameters of ASA and SA in male dogs following oral dosing with ASA alone or in combination with oral dosing of ticagrelor or with intravenous dosing of clopidogrel, geometric mean (range)

Compounds of administration[a]	n	C_{max} (µmol/L)	t_{max}[b] (h)	$AUC_{(0-t)}$ (h*µmol/L)	AUC (h*µmol/L)	$t_{1/2}$ (h)
Pharmacokinetics of ASA						
ASA	4	5.40 (3.95-6.83)	0.63 (0.25-1)	3.50 (3.07-3.75)	NC	0.29 (0.26-0.34)
ASA + ticagrelor	4[c]	3.64 (2.18-6.87)	1.5 (0.75-2)	5.20 (4.94-5.70)	NC	NC [0.30]
ASA + clopidogrel	4	7.07 (5.66-9.94)	0.5 (0.25-0.75)	4.68 (4.07-5.27)	NC	0.39 (0.38-0.40)
Pharmacokinetics of SA						
ASA	4	105 (79.5-128)	1 (1-2)	NC	547 (441-730)	2.5 (1.8-3.2)
ASA + ticagrelor	4	101 (75.8-122)	3 (2-3)	NC	580 (470-699)	1.9 (1.5-2.8)
ASA + clopidogrel	4	105 (92.7-120)	2 (1-2)	NC	567 (473-729)	2.1 (1.9-2.6)

[a] ASA (5 mg/kg) and ticagrelor (15 mg/kg) were administered orally and clopidogrel (3 mg/kg) intravenously (clopidogrel at 30 min after administration of ASA).
[b] Median (range).
[c] n=1 for $t_{1/2\lambda z}$.
Individual values in square brackets.
NC Not calculated

Reference ID: 2937847

Summary of pharmacokinetic parameters of ticagrelor and AR-C124910XX in male dogs following oral dosing with ASA and ticagrelor, geometric mean (range)

Compounds of administration[a]	n	C_{max} (µmol/L)	t_{max}[b] (h)	AUC (h*µmol/L)	$t_{1/2\lambda z}$
Pharmacokinetics of ticagrelor					
ASA + ticagrelor	4	7.80 (4.28-14.0)	2.5 (0.75-3)	43.3 (25.3-86.4)	4.0 (3.6-4.6)
Pharmacokinetics of AR-C124910XX					
ASA + ticagrelor	4	0.351 (0.196-0.603)	2.5 (2-3)	2.60 (1.17-4.76)	4.2 (3.4-5.1)

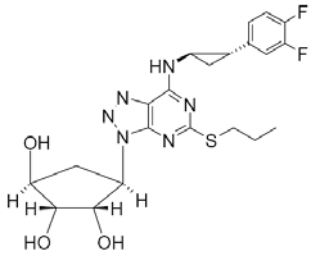
[a] ASA (5 mg/kg) and ticagrelor (15 mg/kg) were administered orally.
[b] Median (range).

Ticagrelor and its metabolites AZ11879328 and AZ11879477: *in vitro* assessment of inhibition of platelet aggregation. AZM100303-03

This study compared the inhibition of platelet aggregation by ticagrelor and its two active metabolites in human, rat and marmoset blood. Impedance aggregometry was used for the evaluation.

AZ11879328 was previously coded as AR-C124910XX.

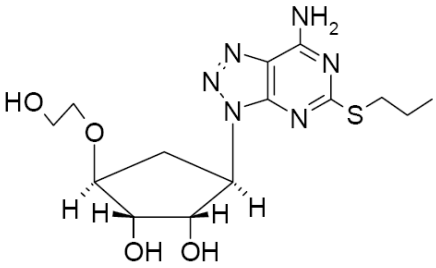
AR-C124910XX



AZ11879477 was previously coded as AR-C133913XX.

Reference ID: 2937847

AR-C133913XX



Ticagrelor produced a concentration-dependent inhibition of 6.5 µM ADP-induced platelet aggregation in human, rat and marmoset blood. Similar potency was found for AZ11879328 in human, rat and marmoset. AZ11879477 was significantly less potent than ticagrelor and AZ11879328 in all three species. The IC_{50} values for each of these compounds is summarized in the sponsor's table below.

Table 1 **Potency of ticagrelor AZ11879328 and AZ11879477 in human, rat and marmoset**

	Mean IC_{50}-values (µM)		
	Human	**Rat**	**Marmoset**
AZ11939728	0.24	0.13	0.12
AZ11879328	0.17	0.06	0.06
AZ11879477	>10	>10	1.91

Ticagrelor and its metabolites AZ11879328 and AZ11879477, displacement of [^{125}I]AZ11931285 radio-ligand binding and inhibition of 2Me-S-ADP-induced human $P2Y_{12}$ receptor signaling, *in vitro*

The present study evaluated the potency of ticagrelor and its metabolites AZ11879328 (formerly AR-C124910) and AZ11879477 (formerly AR-C133913) in a radio-ligand binding assay and a functional receptor signaling assay using CHO K1 cells transfected with the human $P2Y_{12}$ receptor.

The [^{35}S]GTPλS assay evaluates functional G-protein-coupled receptor signaling after agonist stimulation as the membrane bound [^{35}S]GTPλS accumulates with increased signaling. The compound 2Me-S-ADP is a stable ADP analogue. [^{125}I]AZ11931285 was undefined, but it is assumed that this is a $P2Y_{12}$ antagonist.

Ticagrelor, AZ11879328 and AZ11879477 produced concentration-dependent inhibition of both $P2Y_{12}$ receptor radio-ligand binding and 2Me-S-ADP-induced receptor signalling. The IC_{50} values are summarized in the sponsor's table below.

Reference ID: 2937847

Table 1 **Potency of ticagrelor and its metabolites in the $P2Y_{12}$ receptor radio-ligand binding and signalling assays**

	[^{125}I]-AZ11931285 binding assay		[^{35}S]-GTPgS assay	
Compound name	**IC_{50} (nmol/L)**	**N**	**IC_{50} (nmol/L)**	**N**
ticagrelor	11±8 (mean±SD)	63	66±28 (mean±SD)	98
AZ11879328	7 and 6	2	36 and 34	2
AZ11879477	2500	1	10000	1

It is concluded that AZ11879328 is at least as potent as ticagrelor as an antagonist against the human $P2Y_{12}$ receptor whereas AZ11879477 is >100-fold less potent.

Appendix I: Email correspondence

Variability of PGI_2 assay

Reference ID: 2937847

From: Monteleone, Michael V.
Sent: Friday, April 08, 2011 4:22 PM
To: Hausner, Elizabeth A; Papoian, Thomas
Subject: FW: NDA 22-433 - Certificate of Variability
FYI - from AZ

Mike Monteleone
Regulatory Health Project Manager
Division of Cardiovascular and Renal Products
michael.monteleone@fda.hhs.gov
p:(301) 796-1952
f:(301) 796-9838

From: Gigger, Emery [mailto:Emery.Gigger@astrazeneca.com]
Sent: Friday, April 08, 2011 3:56 PM
To: Monteleone, Michael V.
Cc: Prosser, Judith
Subject: NDA 22-433 - Certificate of Variability

Hi Mike,

The following certificate of variability use in the expansion study for PGI is being provided in follow-up to Dr. Hausner request during the April 4th tcon.

The CV% for the measurement of the PGI_2 metabolite in urine, 2,3-dinor-6-keto-prostglandin F_{1a}, based upon data from 3 standard curves. The largest CV% is obtained close to the limit of quantification and is 17%. However, most samples showed levels that were at a part of the standard curve that had a CV% between 2.2% and 12.3%.

Regards,
Emery

Emery V. Gigger
Director, Cardiovascular (CV)
Regulatory Affairs

AstraZeneca Pharmaceuticals LP
Regulatory Affairs
1800 Concord Pike, C1C-716, Wilmington, DE 19803
Tel.: +1 302.885.4048
Mobile: + 1 610.570.9508
Fax: +1 302.886.4894
emery.gigger@astrazeneca.com

Informal telecon regarding mechanism of action

Reference ID: 2937847

From: Monteleone, Michael V.
Sent: Monday, April 11, 2011 11:35 AM
To: Hausner, Elizabeth A
Subject: April 5 Tcon

Dr. Hausner -

The participants on our April 5, 2011 Tcon with AZ about the ASA mechanism and ticagrelor were:

From FDA:
Elizabeth Hausner
Thomas Papoian
Melanie Blank
Michael Monteleone

From AZ:
Greg Christoph, PhD, Senior Project Director, Toxicological Sciences
Simon Clowes, BSc (Hons), Global Product Vice President
Jonathan C. Fox, MD, PhD, Vice President, Clinical Therapeutic Area, Cardiovascular and Gastrointestinal Diseases
Emery Gigger, Regulatory Affairs Director
Alex Gold, MD, Executive Director, Clinical Development (tentative)
Sven Nylander, PhD, Discovery Project Leader
Judith Prosser, Associate, Regulatory Affairs Director
Hans van Giezen, PhD, Innovative Medicines Project Director
Mary Whealy, Global Regulatory Affairs Director

Mike Monteleone
Regulatory Health Project Manager
Division of Cardiovascular and Renal Products
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Historical Control Email

Reference ID: 2937847

Mike Monteleone
Regulatory Health Project Manager
Division of Cardiovascular and Renal Products
michael.monteleone@fda.hhs.gov
p:(301) 796-1952
f:(301) 796-9838

From: Gigger, Emery [mailto:Emery.Gigger@astrazeneca.com]
Sent: Wednesday, April 06, 2011 4:59 PM
To: Monteleone, Michael V.
Cc: Prosser, Judith
Subject: NDA 22-433 - Dr. Hausner Tcon Follow-up Request for Information

Hi Mike,

Please find attached study 2629-01 (25773) entitled, "AZD6140, The effects of Intravenous Infusion of AZD6140 on Arterial Thrombosis and Haemostasis in the Anaesthetized Dog: a Comparison with AR-H076866XX and Clopidogrel" submitted with the original NDA, 13 November 2009, Module 4.2.1.1 Primary Pharmacodynamics and provided here for easy of review, which was requested by Dr. Hausner during the tcon yesterday in response to her first question regarding the stability of the dogs under anesthesia.

Also please find attached a publication entitled, "Armstrong PC et al. In the presence of strong P2Y(12) receptor blockade, aspirin provides little additional inhibition of platelet aggregation". *J Thromb Haemost*. 2011 (previously e-mail March 4th), in referenced Dr. Hausner's comment regarding hemodynamic cardiovascular stability of the animals overtime of their aortic blood pressure.

As always, please call if you have any questions.

Regards,
Emery

Emery V. Gigger
Director, Cardiovascular (CV)
Regulatory Affairs

AstraZeneca Pharmaceuticals LP
Regulatory Affairs

Reference ID: 2937847

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

ELIZABETH A HAUSNER
04/25/2011

THOMAS PAPOIAN
04/25/2011
I concur.

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
NDA: 22-433
Submission receipt date: Nov. 16, 2009
Drug: Brilinta (ticagrelor)
Sponsor: Astra Zeneca
Indication: Antithrombotic therapy in subjects with acute coronary syndrome
Reviewing Division: Division of Cardiovascular and Renal Products

Comments: The pharm/tox reviewer and supervisor found the nonclinical information submitted for ticagrelor to be sufficient to support the use indicated above.

The primary reviewer for this NDA initially made two recommendations. First that a receptor binding profile was needed for one of the two main metabolites, AR-C133913XX (b) (4) Second, that the sponsor wished to qualify an impurity (b) (4) above recommended levels without supporting data. The reviewer recommended that the impurity should either be controlled at levels recommended for a genotoxic impurity or assays should be conducted to demonstrate the lack of genetic toxicity.

I discussed the first issue with the reviewer who informed me that this issue was resolved. The sponsor submitted bacterial reverse mutation assay data to address the second issue. These data were reviewed and the impurity is now considered adequately qualified at the proposed limits.

The reviewer proposes pregnancy category C for the labeling. This appears appropriate because some adverse effects were observed in rat and rabbit offspring in reproductive and developmental toxicity studies.

The carcinogenicity of ticagrelor was evaluated in two-year studies in mice and rats. The Executive Carcinogenicity Assessment Committee found these studies to be acceptable. The committee concluded that there were no drug-related tumors in mice. There was some evidence of drug related tumors in rats. There was a statistically significant increase in hepatocellular adenoma, uterine adenocarcinoma and uterine squamous cell carcinoma in female rats. The sponsor suggested that the uterine tumors may be due to a decrease in prolactin. However, the committee did not find the evidence for this mechanism convincing. The committee did note that the relevance of the liver tumors to humans is not clear.

This drug moiety is a $P2Y_{12}$ ADP receptor antagonist that inhibits platelet aggregation. There are several other approved members of this pharmacologic class. The proposed Established Pharmacologic Class for ticagrelor is $P2Y_{12}$

platelet inhibitor. This is consistent with the term used in labelling for the other members of this class.

Conclusion:
I concur with the Division pharm/tox conclusion that the nonclinical data support approval of this NDA.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

PAUL C BROWN
12/15/2010

Review and Evaluation of Toxicology Data

NDA 22433

Sponsor's Sequence Number And DARRTS number	Date of submission	Type of submission
0045	July 23, 2010	Ames assay for impurity

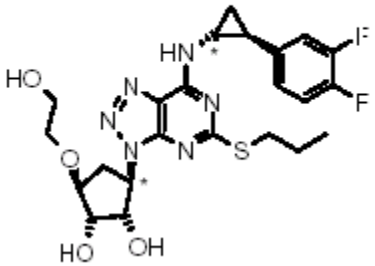
Sponsor: AstraZeneca, LP
Manufacturer for drug substance : AstraZeneca

Reviewer name: Elizabeth Hausner, D.V.M.
Division name: DCRP
Review completion date: August 10, 2010

Drug:

Trade name: Brilinta®
Generic name: ticagrelor
Code name: AZD6140 (formerly AR-C126532XX)
Chemical name: (1S,2S,3R,5S)-3-[7-{[(1R,2S0-2-(3,4-Difluorophenyl)cyclopropyl]amino}-5-(propylthio)-3H-1,2,3-triazol[4,5-d]pyrimidin-3-yl]-5-(2-hydroxyethoxy)cyclopentane-1,2-diol
CAS registry number: 274693-27-5
Mole file number:
Molecular formula/molecular weight: $C_{23}H_{28}F_2N_6O_4S$ 522.57
Structure:

Figure 2.0-1 Structure of AZD6140:



Related applications: IND65808. Clopidogrel (approved NDA 20839) and Prasugrel (NDA 22307) are other drugs of the same mechanistic class .
Drug class: ADP receptor antagonist, specifically $P2Y_{12}(P_{2T})$ antagonist

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CONCLUSION

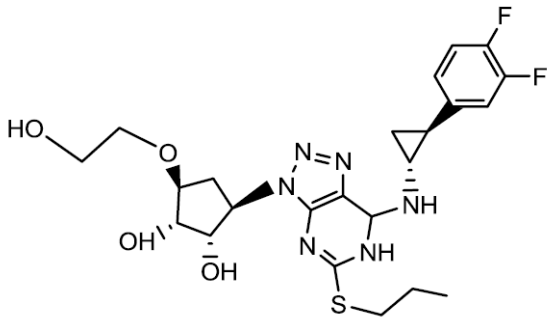
The impurity (b) (4) has been qualified for the sponsor's proposed specification limit.

BACKGROUND

This study was submitted in response to the Division's request for data to support the qualification of the impurity (b) (4) at the sponsor's proposed specification of (b) (4)

When the structure of this impurity was submitted to the ICSAS group for a computational toxicology consult, the (b) (4) caused a structural alert for genetic toxicity. The sponsor was asked to generate data to examine this alert.

Ticagrelor



(b) (4)

Genetic Toxicity Evaluation using a Bacterial Reverse Mutation Test

Study number: 2568BV
Study location: Safety assessment UK, Astra Zeneca R&D Alderley, Alderley Park, England
GLP: Statement included
QA: yes

Experimental Start Date: July 15, 2010

Test article: (b) (4) batch 05-knrk522-49, purity 98.4% by an unspecified method At this time, the stability of the test article has not been confirmed. A certificate of analysis was not provided, stability of the test solutions, homogeneity, and measured concentration of the test article were not provided. The report notes that "…the sample of the test article remaining from the test has not yet been re-analysed[sic] to confirm identity but this does not prejudice the validity of the study."

Vehicle: DMSO

Bacterial strains used:
Salmonella typhimurium LT2 TA1535, TA1537, TA98, TA100
E.coli WP2 *uvrA/*pKM101
Metabolic activation: S9 fraction from Aroclor 1254-treated rat livers. Metabolic Capacity was demonstrated by "key enzyme assays[unspecified]" and the ability to activate reference agents to bacterial mutagens.
Positive controls: summarized in the sponsor's table shown below.

Sponsor's Summary of Positive Controls

Positive controls				
Positive control chemical	**Solvent**	**Dose per plate**	**Bacterial strain**	**Metabolic activation**
		µg		
Sodium azide	water	0.5	TA1535	-
		0.5	TA100	-
2-Nitrofluorene	DMSO	0.5	TA98	-
9-Aminoacridine.HCl	water	50	TA1537	-
Potassium dichromate	water	25	*E. coli uvrA*/pKM101	-
2-Aminoanthracene	DMSO	2	TA1535	+
		2	TA100	+
		2	TA98	+
		2	TA1537	+
		20	*E. coli uvrA*/pKM101	+

The assay was conducted by the plate incorporation method.

The concentrations tested were:

0.5, 1.6, 5, 16, 50, 160, 500, 1600, 5000µg/plate

Plates were scored with a Colony Counter and an in-house validated analysis program. If the mean incidence of revertant colonies was ≤20 or if cytotoxicity or precipitate interfered with image analysis, all of the plates in that particular group were scored manually and the data entered into the analysis program.

The sponsor specified the criteria for acceptance of solvent controls and positive controls. Criteria for acceptance of the assay overall and a positive result were also specified.

Results

The positive controls for all strains produced appropriate responses. Toxicity to the colonies from the test article was not reported.
Both ± S9, precipitation was reported for all bacterial strains at concentrations ≥500 µg/plate. At concentrations ≥1600µg/plate the bacterial lawn was reported to be obscured by precipitate. There was no increase in revertants apparent in the data as presented, either with or without metabolic activation. The sponsor's summary tables are shown below.

[redacted] (b) (4): Plate incorporation method – without metabolic activation

Plate incorporation assay - without metabolic activation
Study Number : 2568BV
Experiment : 2568BV 01 Start date : 2010-07-15 End : 2010-07-18
Test compound : [redacted] (b) (4) Batch: 05-knrk522-49
Solvent : Dimethyl sulphoxide
Bacterial strain and number of revertant colonies per plate

(Dose/plate) µg	µmol	TA1535 Mean		SD	TA1537 Mean		SD	TA98 Mean		SD	TA100 Mean		SD	E.coli uvrA/pKM101 Mean		SD
Solvent control 1		18		2	11		3	28		7	118		8	169		10
0.5	0.000886	14		1	12		4	34		7	119		11	155		5
1.6	0.00283	16		1	12		3	27		6	117		13	153		10
5	0.00886	18		3	14		2	24		3	112		6	148		3
16	0.0283	17		2	11		2	28		4	111		11	161		6
50	0.0886	15		1	11		1	28		6	116		5	157		9
160	0.283	16		1	12		2	28		6	113		21	174		19
500	0.886	17	p	2	11	p	1	24	p	6	122	p	7	175	p	10
1600	2.83	14	lp	3	11	lp	1	27	lp	5	114	lp	5	161	lp	13
5000	8.86	13	lp	2	12	lp	3	26	lp	6	118	lp	16	156	lp	19
Positive control 1		508		15	278		66	231		7	599		16	830		71

Pos. controls 1:
TA1535:Sodium azide 0.5 µg, TA1537:9-Aminoacridine.HCl 50 µg, TA98:2-Nitrofluorene 0.5 µg, TA100:Sodium azide 0.5 µg,
E.coli uvrA/pKM101:Potassium dichromate 25 µg
p = Precipitate formed on the plate l = Lawn obscured by precipitate

[redacted] (b) (4): Plate incorporation method – with metabolic activation

Plate incorporation assay - with metabolic activation
Study Number : 2568BV
Experiment : 2568BV 01 Start date : 2010-07-15 End : 2010-07-18
Test compound : [redacted] (b) (4) Batch: 05-knrk522-49
Solvent : Dimethyl sulphoxide
Bacterial strain and number of revertant colonies per plate

(Dose/plate) µg	µmol	TA1535 Mean		SD	TA1537 Mean		SD	TA98 Mean		SD	TA100 Mean		SD	E.coli uvrA/pKM101 Mean		SD
Solvent control 1		17		1	13		1	34		8	123		8	201		10
0.5	0.000886	15		2	15		2	37		8	131		11	206		25
1.6	0.00283	18		3	15		2	34		3	128		8	187		31
5	0.00886	17		3	16		3	39		4	130		20	206		23
16	0.0283	14		2	16		2	32		5	135		4	218		6
50	0.0886	16		2	15		3	31		2	140		7	214		11
160	0.283	14		1	13		2	32		4	123		7	189		32
500	0.886	17	p	1	13	p	1	33	p	7	104	p	2	162	p	8
1600	2.83	15	lp	2	11	lp	2	34	lp	5	128	lp	29	166	lp	10
5000	8.86	13	lp	2	12	lp	2	26	lp	5	109	lp	1	178	lp	29
Positive control 1		233		13	223		28	1355		104	1599		96	2077		89

Pos. controls 1:
TA1535:2-Aminoanthracene 2 µg, TA1537:2-Aminoanthracene 2 µg, TA98:2-Aminoanthracene 2 µg, TA100:2-Aminoanthracene 2 µg,
E.coli uvrA/pKM101:2-Aminoanthracene 20 µg
p = Precipitate formed on the plate l = Lawn obscured by precipitate

Summary:

As it stands, there does not appear to be a test article –induced increase in revertants. The chemical analysis will be necessary to confirm this interpretation and was requested from the sponsor via email.

The first chemical analysis form provided contained discrepancies from the limited information provided in the study. This was brought to the attention of the sponsor who then provided a second chemical analysis form. These are attached as Appendices1 and 2 respectively.

Because the analysis forms did not contain stability data, the FDA review chemist was asked if he could provide any information. This email exchange is provided as Appendix 3. Overall, the FDA review chemist gave the opinion that (b) (4) is stable under the conditions in which it has been tested.

The sponsor tested this impurity somewhat unusually in that (b) (4) was not added in fractional percentages to the parent compound. Instead, (b) (4) was tested by itself, as a "pure" impurity, up to saturated concentrations. Under the conditions of the assay the impurity did not cause an increase in revertants. While this is only one genotoxicity assay out of the usual battery, it is a reasonably convincing negative result.

Appendix 1: Chemical Analysis Form with Discrepancies

Multiple Samples Results Report for Sodertalje

(b) (4)

Request Information: AR1004062

Compound	AZD6140 other
Project	D5130
Requested By	(b) (4)
Start Of Analysis	28-Jun-10
Additional Information	

Sample Information

Sample Id	Batch Number	Sample Status	Approved By	Approved Date	Sample Description	Checking Required
S10008468	05-knrk522-59	Approved	(b) (4)	06-Jul-10	(b) (4)	No

ASSAY BY NMR

Sample Id	Batch Number	Determination	Results
S10008468	05-knrk522-59	Result	(b) (4)
		Units	
		Method ID	
		Balance	
		Instrument	
		Notebook Reference	
		Comments	

REL ORG IMP BY LC

Sample Id	Batch Number	Determination	Results
S10008468	05-knrk522-59	Total Impurities	(b) (4)
		Units	
		Method ID	
		Balance	
		Instrument	
		Notebook Reference	
		Comments	

Attachment Tables

Request Attachment Description	Number	Status
NMR assay	3	Active
Chromatographic purity	2	Active
Raw data sheet	1	Active

Sample id	Sample Attachment Description	Number	Status

Appendix 2. Second Chemical Analysis Form

(b) (4)

Multiple Samples Results Report for Sodertalje

Request Information: AR1003651

Compound	AZD6140 other
Project	D5130
Requested By	(b) (4)
Start Of Analysis	03-Jun-10
Additional Information	Two samples: 1) fraction 9-14, 2) fraction 15-21

Sample Information

Sample Id	Batch Number	Sample Status	Approved By	Approved Date	Sample Description	Checking Required
S10007665	05-knrk522-49	Approved	(b) (4)	29-Jun-10	(b) (4)	No

ASSAY BY NMR

Sample Id	Batch Number	Determination	Results
S10007665	05-knrk522-49	Result	(b) (4)
		Units	
		Method ID	
		Balance	
		Instrument	
		Notebook Reference	
		Comments	

ID BY MS

Sample Id	Batch Number	Determination	Results
S10007665	05-knrk522-49	Identity Confirmed?	(b) (4)
		Method ID	
		Balance	
		Instrument	
		Notebook Reference	
		Comments	

Appendix 2. Second Chemical Analysis Form, continued

REL ORG IMP BY LC

Sample Id	Batch Number	Determination	Results
S10007665	05-knrk522-49	Total Impurities	(b) (4)
		Units	
		Method ID	
		Balance	
		Instrument	
		Notebook Reference	
		Comments	

Attachment Tables

Request Attachment Description	Number	Status
LC-renhet	7	Active
NMR	6	Active
Rådatablad	5	Active
AR1003651 structure	4	Active
AR1003651 MS results	3	Active
AR1003651 MS rådata blad	2	Active

Report produced on 29-Jun-10 7:24 by (b) (4) Page 1

Request Information: AR1003651

AR1003651 MS method	1	Active

Sample id	Sample Attachment Description	Number	Status

Appendix 3. FDA Email Regarding Stability of Impurity [redacted] (b) (4)

From: Tele, Chhagan
Sent: Tuesday, August 03, 2010 9:53 AM
To: Hausner, Elizabeth A
Subject: RE: Ames Assay
It remained constant (storage of primary stability batches: initial [redacted] (b) (4), after for 12 months at long term and 6 months at accelerated conditions [redacted] (b) (4)).

From: Hausner, Elizabeth A
Sent: Tuesday, August 03, 2010 9:45 AM
To: Tele, Chhagan
Subject: RE: Ames Assay

Did it decrease in concentration over that storage period?

From: Tele, Chhagan
Sent: Tuesday, August 03, 2010 9:44 AM
To: Hausner, Elizabeth A
Subject: RE: Ames Assay

Hi Elizabeth,

Stability data on impurity [redacted] (b) (4) itself is not provided by the applicant. It did not increase on stability (remained constant [redacted] (b) (4) in DS primary batches 26-01, 32-01, and 33-01) on storage for 12 months at long term and 6 months at accelerated conditions . [redacted] (b) (4) is the [redacted] (b) (4) to ticagrelor, formed by [redacted] (b) (4)

Chhagan

From: Hausner, Elizabeth A
Sent: Tuesday, August 03, 2010 9:13 AM
To: Tele, Chhagan
Cc: Monteleone, Michael V.
Subject: FW: Ames Assay

Chhagan,
attached is a chemical analysis form for the impurity recently tested in the Ames assay. Do you have any stability data for this chemical or can you give your opinion as to how stable or unstable you think it maybe?
Elizabeth

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22433	ORIG-1	ASTRAZENECA LP	AZD6140

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

ELIZABETH A HAUSNER
08/10/2010

MURIEL J SAULNIER
08/10/2010

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	22433
Supporting document/s:	000
Applicant's letter date:	November 18, 2009
CDER stamp date:	November 18, 2009
Product:	Brilinta® (ticagrelor)
Indication:	To reduce the rate of thrombotic events in patients with Acute Coronary Syndrome who are to be managed medically or managed invasively with percutaneous coronary intervention and/or coronary artery by-pass grafting
Applicant:	Astra Zeneca
Review Division:	Cardiovascular and Renal Products
Reviewer:	Elizabeth Hausner, D.V.M.
Supervisor/Team Leader:	Patricia Harlow, Ph.D.
Division Director:	Norman Stockbridge, M.D., Ph.D.
Project Manager:	Michael Monteleone

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1 Executive Summary

1.1 Recommendations

1.1.1 Approvability: From the non-clinical perspective, this NDA is approvable for the purpose of preventing platelet aggregation. The toxicities that were identified in the non-clinical stages have been shown to be not clinically significant or satisfactory safety margins have been demonstrated.

1.1.2 Additional Non Clinical Recommendations: 1) Receptor binding profile is needed for one of the two main metabolites, AR-C133913XX [redacted] (b) (4) 2) The sponsor wishes to qualify the impurity [redacted] (b) (4) above recommended levels without supporting data. The impurity should either be controlled at levels recommended for a genotoxic impurity or assays should be conducted to demonstrate the lack of genetic toxicity.

1.1.3 Labeling

Section 8.1: Pregnancy, Teratogenic effects:
The section that reads

[redacted] (b) (4)

Should be modified to read

[redacted] (b) (4)

(b) (4)

Section 13.1: Carcinogenesis, Mutagenesis, Impairment of Fertility

The paragraph that reads

(b) (4)

Should be modified to read:

Uterine carcinomas, uterine adenocarcinomas and hepatocellular adenomas were seen in female rats at doses of 180 mg/kg/day (29 fold the maximally recommended dose of 90 mg bid per day on the basis of AUC comparison).

Section 13.1: The paragraph that reads

(b) (4)

Should be modified to read:

Ticagrelor did not demonstrate genotoxicity when tested in the Ames assay, mouse lymphoma assay and the rat micronucleus test. The active O-demethylated metabolite did not demonstrate genotoxicity in the Ames assay and mouse lymphoma assay.

1.2 Brief Discussion of Nonclinical Findings

The non-clinical studies have shown *in vitro*, *ex vivo* and *in vivo* that AZD6140 (ticagrelor) inhibits $P2Y_{12}$-mediated platelet effects in a reversible manner. Studies using washed human platelets showed inhibition of ADP-induced platelet aggregation with an IC_{50} of 13 nM. *Ex vivo* studies using platelet rich human plasma showed that AZD6140 inhibits ADP-induced platelet aggregation with an IC_{50} of 398 nM. *In vitro* receptor binding studies also indicated a 94% inhibition of the adenosine A3 receptor at 10µM. Assessment of the human adenosine A3 receptor transfected into human embryonic kidney cells (HEK) showed an IC_{50} of 0.3µM, suggesting that there may be effects for both the $P2Y_{12}$ and A_3 receptors at equivalent plasma concentrations. Ticagrelor also shows affinity for the phosphodiesterase 5 (PDE5) enzyme with an IC_{50} for inhibition of 0.482µM. The steady state plasma Cmax in humans is 687ng/ml (1.3µM) for 100 mg, b.i.d.,making it possible that the PDE5 effect will be clinically significant. The sponsor presented data for dissociation of ^{3}H-AZD6140 from rh$P2Y_{12}$ expressed on CHO-K1 cells. Reversibility of binding is also supported by the *in vivo* pharmacology studies showing a decreasing anti-aggregatory effect over hours rather than days.

Of the metabolites identified *in vitro* using hepatocytes from rats, mice, dogs, marmosets and humans, the two main *in vivo* metabolites of ticagrelor were identified as AR-C124910XX, the O-deethylated metabolite, and AR-C133913XX, formed by the parent drug losing the difluorophenyl-cyclopropyl group. These metabolites have been demonstrated to occur at adequate levels in the non-clinical species used for toxicology. AR-C124910XX has been shown to inhibit specific radioligand binding (P_{2T} antagonist AR-C119608XX) to human washed platelets (Ki value, 2.5 nM) and inhibits ADP-induced platelet aggregation in human platelet rich plasma (IC_{50} = 126 nM). This metabolite has been separately tested in the Ames assay and the mouse lymphoma assay. No signals for genotoxicity were apparent. AR-C124910XX has also been quantified in several toxicology studies, including the 2 year rodent carcinogenicity studies where up to 24X the human exposure based on AUC was achieved in female rats. The sponsor designates AR-C1333913XX as inactive. This metabolite has an IC_{50} value of approximately 3µM for the $P2Y_{12}$ receptor, making it less active than AZD6140 and AR-C124910XX but not inactive. Receptor binding profiles were presented for AR-C124910XX, showing similar affinity for the adenosine A3 receptor as the parent drug. Receptor binding information has not been submitted for AR-C133913XX.

The toxicology was assessed in mice, rats, rabbits and marmosets. The sponsor determined that the ratio of AUC values for AR-C124910XX and ticagrelor in the marmoset was 48%, compared to the human ratio of 35%. The sponsor considered the marmoset profile to be preferable to the dog AR-C124910XX: ticagrelor ratio of 9% for determination of the toxicological profile.

The non-clinical toxicology studies indicated the target organs of toxicity to be the gastrointestinal tract (dogs, rats, marmosets), liver (rats), bone marrow (rats, marmosets), immune system (marmosets, rats), adrenal (rodents) and endocrine system (mice and female rats).

Pulmonary function changes of increased respiratory rate (up to 20% of pre-dose baseline, $p<0.01$), peak inspiratory flow(up to 35% of pre-dose baseline, n.s.) and expiration time (decreased by up to

20% from pre-dose baseline, p<0.01)were identified in the safety pharmacology studies in rats. These findings were apparent in clinical trials as dyspnea.

Across species, the hematology findings were relatively consistent in showing minor blood loss with evidence of regeneration. Mice showed signs of regenerative anemia including increased red cell distribution width (16% compared to control, p<0.001), increased reticulocytes (36% compared to control, p<0.01) from doses ≥250 mg/kg. Rats at doses ≥180 mg/kg showed increased red cell distribution width (12%, n.s. compared to the control group) and reticulocytes (26%, p<0.05, compared to the control group). Marmosets at doses ≥50 mg/kg showed increased reticulocyte counts of 56% compared to the controls (not marked as significant). At the marmoset high dose of 200 mg/kg, hemoglobin and red cell counts were decreased compared to the control group (13% and 10% respectively). In each species, these changes did not always persist for the full duration of a study, and were not equally apparent in both sexes, suggesting adaptation or differences in sensitivity.

Gastrointestinal irritation showed different manifestations in different species and may have been the root of the blood loss. Rats given doses of ticagrelor ≥300 mg/kg/day showed gastric erosions, squamous hyperplasia, and inflammation. Marmosets given >10 mg/kg/day showed enteritis with mucosal atrophy, ulceration and hemorrhage. Dogs in a dose-ranging study showed vomiting and loose feces at all doses tested, with no NOAEL identified. The lowest dose tested in this sole canine toxicology study was 50 mg/kg. Gastrointestinal irritation has not manifested itself in clinical trials and may have been a function of the non-clinical formulation.

Liver effects in rats occurred at doses ≥80 mg/kg and included indications of altered function or damage evidenced by decreased triglycerides (67%, p<0.001), increased AST (20%, p<0.001) or ALP (31%, p<0.001) when compared to the control groups. Centrilobular hypertrophy was inconsistently reported (mice ≥250 mg/kg/day; rats ≥180 mg/kg). Liver effects in marmosets were inconsistent. One marmoset study showed a 45% decrease in triglycerides (n.s.) and a 167% (not marked as significant) increase in AST compared to control at a dose of 200 mg/kg. Another marmoset study showed localized, chronic active inflammation in the liver at 20 and 100 mg/kg. The sponsor attributed this to trauma. Rats and mice were the most sensitive species to the liver effects with essentially no margin of safety between the AUC values at the NOAEL and the human AUC values. Liver effects have not been apparent in the clinical trials.

Immune system effects such as splenic lymphoid depletion, thymic atrophy and bone marrow atrophy were reported for rats given doses ≥300 mg/kg/day. Marmosets showed lymphocytolysis of the thymic cortex, follicular atrophy of lymph nodes, splenic lymphoid atrophy and bone marrow hemorrhage at doses ≥1000 mg/kg/day, which was also associated with unscheduled mortality. Some lymphoid atrophy was reported in marmosets for doses of ≥20 mg/kg/day. In both the rat and the marmoset, there is a question as to how much the general debility associated with the high doses contributed to the immune system effects. Interpretation of the marmoset data was made difficult by the extraordinary fragility of these animals in the toxicology studies. There was no margin of safety between the AUC for the NOAEL in marmosets and the human AUC and approximately 5 fold

margin of safety for the rat NOAEL compared to the human AUC. Immunological effects have not been apparent in the clinical trials.

Foamy alveolar macrophages were reported in the lungs of rats at doses ≥180 mg/kg/day. These were described as minimal changes. Similar changes were not reported for marmosets.

The sponsor evaluated fertility of males at the end of the 6-month toxicology study. In addition to the usual behavioral parameters in rats, the sponsor also evaluated sperm motility, morphology and number in these animals. The main study animals did not show histologic effects on the testes or epididymides. There were no apparent effects on male fertility up to and including the high dose of 180 mg/kg. The sponsor did not indicate why they performed the additional, more extensive evaluation.

Embryo-fetal development (Segment II reproductive toxicology) studies showed affects on the liver and the skeletal systems in both rats and rabbits. Doses of ≥ 63 mg/kg in rabbits caused delayed development of the gallbladder and incomplete ossification of the hyoid, pubis and sternebrae. Doses of ≥100 mg/kg in rats caused supernumerary liver lobes and incomplete ossification of the parietal bone, sternebrae, misshapen/misaligned sternebrae, displaced articulation of the pelvis, and supernumerary ribs.

The pre- and post-natal development (Segment III) study in rats indicated that exposure to ticagrelor in late gestation or during lactation also affected development. Pinna unfolding was delayed at doses ≥10mg/kg, i.e., all drug-treated groups in this study. Some 18% of the high dose (HD) pups ($p<0.01$) compared to the control group showed delay in this parameter. Eye opening was delayed in all drug-treated groups also (approximately 50% of the HD group, $p<0.01$). Incisor eruption was delayed only in the HD group of 180 mg/kg (approximately 44% of the group compared to the control, $p<0.01$).

Adrenal effects included increased weight with associated histologic hypertrophy in mice (≥750 mg/kg). Rats given doses of ≥80 mg/kg showed cortical vacuolation or cortical inflammatory foci. It is unclear if the adrenal effects were direct drug effects, secondary to the general debilitation or related to the endocrine effects.

There were reported drug-related effects on the reproductive organs of both sexes. In male mice, doses of ≥250 mg/kg caused seminiferous epithelial degeneration of the testes. Female mice had an absence of corpora lutea at doses ≥750 mg/kg. In rats, endocrine effects were manifested as dose-related decreases in regular estrus cycles at doses ≥10 mg/kg. The relatively non-specific finding of irregular estrus cycles became more important in light of the carcinogenicity study where female rats showed statistically significant incidences of uterine adenocarcinoma and uterine squamous cell carcinoma. The rat carcinogenicity study also demonstrated a significant decrease in female survival (Cox: $p=0.018$, Kruskal-Wallis: $p=0.0424$), possibly due to metastatic uterine neoplasia. Fourteen of the 31 HD females who died ahead of scheduled termination had (metastatic) uterine adenocarcinoma listed as the cause of death.

Table 1. Significant Tumor Findings for Female Rats

Reviewer's Summary of Significant Tumor Findings

Tumor name	CTR1	CTR2	Low	Med	High	P-value Exact method	P-value Trend test
Hepatocellular adenoma(females)	0/50	1/50	1/50	2/50	4/50	0.0104	0.0052
Uterine adenocarcinoma	6/50	4/49	5/50	6/50	21/50	0.0000	0.0000
Uterine squamous cell carcinoma	0	0	0	1/50	3/50	0.0060	0.0010

Statistical analysis from the CDER statistical reviewer's report (on file in DARRTS under IND65808

The sponsor conducted a series of *in vitro*, *ex vivo* and *in vivo* studies to determine if the postulated mechanism of testosterone excess was species specific. The sponsor did not elucidate the reasoning behind a testosterone hypothesis nor did they present human data to demonstrate a lack of the postulated hormonal imbalance in the species of interest. The Executive CAC did not find the overall weight of evidence for the mechanism to be convincing. The margin of safety however, is 29 fold the human AUC for AZD6140.

The following impurities and solvent were indicated by the review chemist, Chhagan Tele, Ph.D., as points of concern.

(b) (4)

(b) (4)

(b) (4)

(b) (4)

[redacted]

2 Drug Information

2.1 Drug

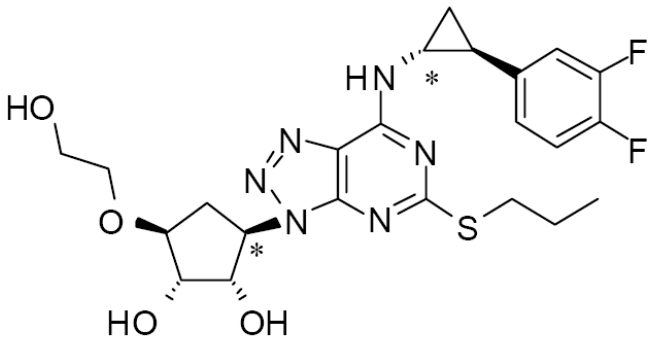
2.1.1 CAS Registry Number: 274693-27-5

2.1.2 Generic Name: ticagrelor

2.1.3 Code Name: AZD6140, AR-C126532XX

2.1.4 Chemical Name: (1S,2S,3R,5S)-3-[7-{[(1R,2S)-2-(3,4-Difluorophenyl)yclopropyl]amino}-5-(propylthio)-3H-[1,2,3]triazolo[4,5- d] pyrimidin-3-yl]-5-(2- hydroxyethoxy)cyclopentane-1,2-diol

2.1.6 Structure



2.1.5 Molecular Formula/Molecular Weight $C_{23}H_{28}F_2N_6O_4S$/522.5

2.1.7 Pharmacologic class: anti-platelet

2.2 Relevant IND/s, NDA/s, and DMF/s: IND 65808 (ticagrelor)

2.3 Clinical Formulation

Table 2. Sponsor's Clinical Formulation

component	quantity (mg/tablet)
(b) (4)	
ticagrelor (AZD6140)	90
mannitol	(b) (4)
dibasic calcium phosphate	
sodium starch glycolate	
hydroxypropyl cellulose	
magnesium stearate	
(b) (4) [a]	
(b) (4)	
(b) (4)	
Hypromellose[b] (b) (4)	(b) (4)
titanium dioxide	
talc	
polyethylene glycol 400	
ferric oxide yellow	
(b) (4) (b) (4)	

[b] the name hydroxypropyl methylcellulose is used throughout the NDA

2.3.1 Drug Formulation for Non-Clinical Studies: information provided in individual studies

2.3.2 Comments on Novel Excipients: N/A

2.3.3 Comments on Impurities/Degradants of Concern: (b) (4) and (b) (4). These are discussed in detail in the review.

2.4 Proposed Clinical Population and Dosing Regimen: Patients with acute coronary syndrome (ACS) who are managed medically or to be managed invasively with percutaneous coronary intervention (with or without stent) and/or coronary artery bypass grafting (CABG).

2.5 Regulatory Background

The IND for AZD6140 was initiated in 2003 after the completion of several Phase I studies conducted in Europe.

The embryo-fetal development studies contained in Amendment 24 to IND 65808 raised the question as to whether the drug caused ante-natal retinal folding. Published references found in MEDLINE indicated a role for the P2Y2 receptor in the blood-retinal barrier. Given this mechanistic support that the finding might be drug-related, this concern was communicated to the sponsor by telephone. After retinal effects were identified in a second study in rabbits, another teleconference was held with the sponsor, including the pathologist from the test facility (November 11, 2004, memo on file in DARRTS). The pathologist had conducted a lineage analysis of the animals used in the two studies and found a common ancestor for the animals in whom retinal folding was identified. Additional information also supported the identified phenomenon as an inherited genetic syndrome more strongly than a drug-induced developmental anomaly. This is detailed in the review for amendment 031, on file in DARRTS.

The sponsor notified us by email on April 24, 2007 of initial findings from the rodent carcinogenicity studies. The sponsor also outlined a plan to inform and re-consent all female clinical trial participants, inform all relevant health authorities and those involved with the management and oversight of the clinical trials. A series of discussions was held between the reviewer, Dr Salma Lemtouni (Medical Officer for this application at the time) and Dr Norman Stockbridge, Division Director, followed by discussions with the sponsor. These discussions are recorded in memos to the file for IND 65808, available in DARRTS.

The sponsor also conducted non-clinical studies to elucidate a possible hormonal mechanism for the tumor findings. The mechanistic studies were reviewed and presented to the Executive CAC at the same time as the presentation of the rodent study results. The Executive CAC did not agree with the sponsor's proposal that testosterone was responsible for the uterine tumors and also felt that the data generated did not preclude the possibility of clinical relevance. These results are recorded in the minutes of the meeting and are on file in DARRTS. Separate reviews are available in DARRTS for the carcinogenicity studies and the mechanistic studies.

December 16, 2009, the sponsor was contacted by email to assist in locating the receptor binding profile for the active metabolite, AR-C124910XX. The sponsor reported that the (b) (4) receptor binding screen was not done for AR-C124910XX or AR133913XX.

On May 18, 2010, Dr Blank, the medical safety reviewer, requested information from the sponsor pertaining to gynecomastia. The May 28th response to that information request included also a receptor binding profile for AR-C124910XX. At a face to face meeting with the sponsor on June7, 2010, the review team asked the sponsor what information they had regarding the metabolite AR-C133913XX to be able to refer to it as "inactive." At that time, the sponsor replied that they had several studies that had not been submitted but that they would do so soon.

During the NDA review, the sponsor was contacted several times for clarification of information regarding impurities. One issue was the changing of codes/designations for identification of various impurities. I sent an email request (late November, 2009) to the sponsor via the project

manager (Mike Monteleone) asking if the designations or codes for the impurities had changed. The sponsor replied that the codes had changed and sent a table, received by email on December 2, 2009. An additional table was sent December 4, 2009. The identity of various impurities was still unclear. A telephone discussion with the sponsor's director for CMC (Sweden), M. Monteleone and E. Gigger (Astra-Zeneca, Regulatory Affairs) was held to clarify the identity of various impurities.

Another discussion occurred in regard to (b) (4) an organic impurity for which no experimental data was provided. When submitted to FDA's Computational Toxicology, this chemical produced a positive signal for genotoxicity. The sponsor has objected to either controlling it at the level of a genotoxic impurity or providing data to demonstrate a lack of genotoxic potential. The sponsor's objections are based on (b) (4) (b) (4) the presence of this impurity in several batches of AZD6140 used in the non-clinical toxicology studies. However, the level of this impurity is listed in each batch as either (b) (4) The sponsor wishes to specify this at (b) (4) At the sponsor's request, AZD6140 was submitted to the Computational Toxicology evaluation for potential genetic toxicity. AZD6140 was assessed by the Informational and Computational Safety analysis Staff(ICSAS)as negative for genotoxic potential. The sponsor was requested to provide some genetic toxicity data to support the specification of (b) (4).

The reviewer requested via email (March 15, 2010) information from the sponsor pertaining to 1) the evaluation of impurities (b) (4) and 2) the canine metabolism of AZD6140. The sponsor's answer for (b) (4) was incomplete and not very helpful. A search of the reviews from the IND provided more pharmacokinetic information than the sponsor seemed to be aware of.

The sponsor requested a telecon, held June 11, 2010, to discuss their most recent mechanistic hypothesis. Because the sponsor's objective seems to be a statement in the label, it was recommended that they compile a very concise (1-2 pages) summary of their position for presentation to the Executive CAC.

3 Studies Submitted

3.1 Studies reviewed

The following studies were reviewed when submitted under the IND. Reviews are on file in DARRTS under IND 65, 808

Receptor Binding/Enzyme Activity

- The effect of AR-C126532XX in a functional assay for agonist/antagonist activity at the human transfected P2Y2 receptor in vitro
- The effects of AR-C126532XX in a functional assay for agonist/antagonist activity at the human P2X1 receptor transfected into cultured RLE cells in vitro
- The effect of AR-C126532XX in a functional assay for antagonist activity at the human P2X7 receptor in vitro
- The effect of AR-C126532XX in a functional assay for agonist/antagonist activity at the human transfected P2Y1 receptor in vitro
- The effect of AR-C126532XX in a functional assay for agonist/antagonist activity at the human transfected P2Y11 receptor in vitro
- The effect of AR-C126532XX on binding of [125I]MIP-1a to the human recombinant CCR1 receptor expressed in HEK-293 cells in vitro
- SC-103281: Activity of AR-C126532XX in a number of binding and enzyme assays in vitro conducted at (b) (4)
- SC-103281: Activity of AR-C126532XX in a number of binding and enzyme assays in vitro conducted at (b) (4)
- (b) (4) radioligand binding assays
- (b) (4) enzyme assays
- Characterization of receptor binding and kinetics of inhibition using hP2Y12 transfected CHO-K1 cells, *in vitro*
- Receptor kinetic studies with ^{3}H-AZD6140 using hP2Y$_{12}$ transfected CHO-K1 cells, *in vitro*

Safety Pharmacology

- Rat: neurobehavioral and motor activity observations after single oral dose
- Rat: motor coordination observations after single oral dose
- Rat: analgesic activity assessment after single oral dose
- Rat: assessment of effects on learning and memory after single oral dose
- Rat: assessment of anesthetic activity after single oral dose
- Rat: assessment of convulsive activity after single oral dose
- Rat: Evaluation of effects on respiration in unrestrained conscious male rats following single oral dose
- Rat: Evaluation of effect on conscious rats with oral administration of AZD6140 after IV pretreatment with an adenosine receptor antagonist
- Investigation of the respiratory effects of AZD6140, adenosine and DPSPX in rats
- Respiratory effects of intravenous adenosine in rats
- Rats: Gastrointestinal transit effects after a single oral dose
- Rats: Effects on renal function after a single oral dose
- Dog: Evaluation of hemodynamic effects following intraduodenal dosing in anestheized dogs
- Evaluation of effect on cardiac action potential in isolated canine purkinje fibers
- Effect on the hERG potassium channel

Pharmacology

- The effect of intravenous infusion of AR-C126532XX on arterial thrombosis and haemostasis in the anaesthetised dog: a comparison with clopidogrel and orbofiban
- The effects of intravenous infusion of AZD6140 on arterial thrombosis and hemostasis in the anesthetized dog: a comparison with AR-H076866XX and Clopidogrel

ADME

- Determination of AZD6140 and its Metabolite, AR-C124910XX in Rat, Dog, Marmoset, Rabbit, Cynomolgus Monkey, and Mouse Plasma by Liquid Chromatography – Atmospheric Pressure Chemical Ionization (APCI) Tandem Mass Spectrometry
- Validation of an analytical method for the determination of AZD6140 and AR-C124910XX in marmoset plasma using liquid chromatography with tandem mass spectrometric detection
- Validation of an analytical method for the determination of AZD6140 and AR-C124910XX in rat plasma using liquid chromatography with tandem mass spectrometric detection
- Validation of an analytical method for the determination of AZD6140 and AR-C124910XX in mouse plasma using liquid chromatography with tandem mass spectrometric detection
- AR-C126532XX: Pharmacokinetics of AR-C126532XX in the male rat after a 100 mg/kg oral dose
- Pharmacokinetics Study in Male and Female Rats and Marmosets Following Oral and Intravenous Administration of [^{14}C]-AZD6140
- Investigation of the oral bioavailability of AR-C126532XX in the male rat
- Investigation of IV formulations in the male rat
- Pharmacokinetics study in the rat, dog and marmoset
- Determination of the bioequivalence of AR-C12653XX when dosed as a suspension and as a tablet to the male Beagle
- Determination and pharmacokinetics of AR-C126532XX in plasma samples taken after oral dosing of AR-C126532XX and iv dosing of [^{3}H]AR-C126532XX to the male marmoset
- Plasma kinetics of AR-C126532XX following oral administration and [^{3}H]-AR-C126532XX following intravenous administration to the marmoset
- Pharmacokinetic study in Cynomologous monkeys:bioanalysis
- Pharmacokinetics of AZD6140 in the Cynomologous following oral administration
- *In vitro* binding of [^{3}H]-AR-C126532XX to the plasma proteins and the blood cells of rat, dog, marmoset, rabbit, mouse and man
- An *in vitro* study to assess the free fraction of AR-C133913XX in human, marmoset, rat, mouse and rabbit plasma
- Quantitative whole body autoradiography in the rat following intravenous administration of[^{3}H]-AR-C126532XX
- Quantitative whole body autoradiography in the rat following intravenous administration of[^{14}C]-AZD6140
- Quantitative whole body autoradiography in the rat following oral administration of[^{14}C]-AZD6140
- *In vitro* metabolism of [^{14}C]-AZD6140 in human and animal liver preparations
- Metabolite profiling (excretion) in samples taken following oral and intravenous administration of [^{3}H]AR-C126532XX to male rats
- Excretion balance and intravenous pharmacokinetic study of [^{3}H] AR-C126532XX in the male rat
- Biliary excretion balance of [^{3}H]AR-C126532XX in the male rat
- Disposition of [^{14}C]-AZD6140 in the rat following oral and intravenous administration

- Metabolite profiling (excretion) in samples taken following oral and intravenous administration of [^{3}H]AR-C126532XX to the male marmoset
- Characterization of *in vivo* [^{14}C]-AZD6140 metabolites in marmoset
- Disposition of[^{3}H]-AR-C126532XX in the male marmoset following oral and intravenous administration
- Disposition of [^{14}C]-AZD6140 in the marmoset following oral and intravenous administration
- Disposition of [^{14}C]-AZD6140 in the mouse following oral and intravenous administration

Toxicology

- Two week dose range finding study in mice with administration by gavage
- Thirteen week dose range finding study in mice with gavage administration
- One month oral toxicity study with assessment of recovery in the rat
- Three month oral toxicity study in rats
- 26 week toxicity study in rats including male fertility assessment followed by a 13 week recovery period (gavage administration)
- Oral dose ranging toxicity study in the non-pregnant rabbit
- Ascending dose oral toxicity study in the dog
- One month gavage toxicity study in the marmoset
- Three month oral gavage dose range finding study in the marmoset
- Three month oral gavage dose tolerability and dose range finding study in the marmoset
- Three month oral gavage toxicity study in the marmoset with a one month recovery period.
- Twelve month oral toxicity study in the marmoset
- Oral fertility and early embryonic development study in the female rat
- Oral dose range finding embryofetal development study in the rat
- Oral embryofetal development study in the rat
- Oral dose range finding embryofetal development study in the rabbit
- Oral embryofetal development study in the rabbit
- Oral retinal development study in the rabbit

Special Toxicology

- Local tissue tolerance study in the mouse
- Assessment of tolerance of the venous intima to an intravenous injection in the rat
- *In vitro* hemolysis, erythrocyte clumping and protein precipitation in human blood of AZD6140 formulation

Impurities

- 0660AR 1 month oral toxicity study in the rat
- AZD6140 + 0.1% (b) (4) Ames assay
- (b) (4) Ames assay
- (b) (4) in vitro cytogenetic assay in human lymphocytes
- (b) (4): Ames assay
- (b) (4) Mouse lymphoma TK assay

Genetic Toxicology

- AR-C125917XX, AR-C126532XX, AR-C126583XX: Assessment of mutagenic potential in a limited Ames assay
- AR-C126532XX: Ames test
- AR-C126532XX: mouse lymphoma TK assay
- AZD6140: Rat micronucleus assay after oral administration
- AR-C124910XX: Ames Test
- AR-C124910XX : mouse lymphoma Cell TK Assay

Carcinogenicity and studies pertaining to the proposed mechanism of carcinogenicity

- 104 Week Carcinogenicity Study in Mice with Administration by Gavage
- 104 Week Carcinogenicity Study in Rats with Administration by Gavage

- Analysis of AZ11879328 and AZD6140 in human estrogen receptor-alpha and -beta binding assays, in vitro
- The effect of AZD6140 on testosterone intrinsic clearance in female rat liver microsomes
- Evaluation of hepatic cytochrome P450 activity following administration of AR-C126532XX to the rat
- Investigative study to determine the effects of AZD6140 on the expression of genes involved in steroidogenesis
- Effects of AZD6140 on steroidogenesis in rat adrenal cell cultures
- Effects of AZD6140 on steroidogenesis in rat ovarian cell cultures
- Effects of AZD6140 on hepatic and uterine CYPs following oral dosing for 7 or 28 days in the female Wistar Hannover rat
- Three month time course study of reproductive endocrinology and pathology and CYP expression in the ovary, uterus, adrenal and liver in the female Wistar Hannover rat
- Review of the uterus, cervix, vagina and ovaries for the presence of histological changes in Studies 0660AR, SR99302, TPR3143 and 456930

Studies reviewed under the NDA

- Determination of the Free Fraction of AR-C124910XX and AR-C133913XX in Human, Rat, Mouse, Rabbit and Marmoset Plasma Using Tandem Mass Spectrometric Detection
- Partial Validation of Method No: BA-542: Determination of the Free Fraction of AR-C124910XX and AR-C133913XX in Human, Rat, Mouse, Rabbit and Marmoset Heparin Plasma Using LC-MS/MS
- Method No: YAT/083 (AstraZeneca Method No: BA-502-01) Partial Validation for Determination of AZD6140, AR-C124910XX and AR-C133913XX in Rabbit Heparin Plasma Using Liquid Chromatography with Tandem Mass Spectrometric Detection
- Method No. YAT/084 (AstraZeneca Method No. BA-503-01):
- Method No. YAT/085 (AstraZeneca Method No. BA-504-01): Partial Validation for Determination of AR-C133913XX in Mouse Heparin Plasma using Liquid Chromatography with Tandem Mass Spectrometric Detection
- Method No. YAT/086 (AstraZeneca Method No. BA-505-01): Partial Validation for the Determination of AR-C133913XX in Rat Heparin Plasma using Liquid Chromatography with Tandem Mass Spectrometric Detection

- 0952SY Metabolite AZ11879328-003 (formerly AR-C124910XX) receptor binding, enzyme and electrophysiological activity

- AA39254 Oral dose range finding pre- and post-natal development study in the rat
- Pre- and Post-natal Development Study in the Rat

- (b) (4) Bacterial Mutation Assay
- (b) (4) in vitro cytogenetic assay in human lymphocytes
- (b) (4) Maximum Tolerated Single Oral Dose Toxicity in the Rat
- (b) (4) Rat Micronucleus Test Following a Single Oral Dose

- (b) (4) Ames Assay
- (b) (4) Chromosome Aberrations in Cultured Human Peripheral Blood Lymphocytes
- (b) (4) Maximum Tolerated Oral Dose in the Rat
- (b) (4) Rat micronucleus evaluation after a single oral dose

- (b) (4) In Vitro Cytogenetic Assay in Human Lymphocytes
- (b) (4) Bacterial Mutation Assay

- (b) (4): Ames assay
- In vitro cytogenetic assay in human lymphocytes
- Induction of chromosome aberrations in cultured human peripheral blood lymphocytes
- Maximum Tolerated Single Oral Dose Toxicity Study in the Rat
- Rat Micronucleus Assay

- In Vitro Cytogenetic Assay in Human Lymphocytes for (b) (4)

- (b) (4) In vitro cytogenetic assay in human lymphocytes
- (b) (4) Bacterial Mutation Assay
- (b) (4) Chromosome Aberration Assay in Human Lymphocytes in vitro
- (b) (4) Ames Assay
- (b) (4) Bacterial Mutagenicity Assay
- (b) (4) Bacterial Mutagenicity Test

3.2 Studies Not Reviewed

Studies of longer duration for the species were available, therefore, a detailed, written review of these short duration studies was not considered necessary.

- Mouse single oral dose
- Rat single oral dose
- Marmoset oral MTD

Not reviewed as the primary reports had already been reviewed

- Determination of AZD6140 and AR-C124910XX in Marmoset Plasma, Using Protein Precipitation and Liquid Chromatography with Tandem Mass Spectrometric Detection
- Determination of AZD6140 and AR-C124910XX in Rat Plasma Using Protein Precipitation and Liquid Chromatography with Tandem Mass-Spectrometric Detection
- Determination of AZD6140 and AR-C124910XX in Mouse Plasma Using Protein Precipitation and Liquid Chromatography with Tandem Mass-Spectrometric Detection
- Determination of AZD6140, AR-C124910XX and AR-C133913XX in Rabbit Heparin Plasma, Using Protein Precipitation and Liquid Chromatography with Tandem Mass Spectrometric Detection
- BA-503-01 YAT/084/V01 Determination of AR-C133913XX in Marmoset Heparin plasma, Using Protein Precipitation and Liquid Chromatography with Tandem Mass Spectrometric Detection
- BA-504-01 YAT/085/V01 Determination of AR-C133913XX in Mouse Heparin Plasma, Using Protein Precipitation and Liquid Chromatography with Tandem Mass Spectrometric Detection
- BA-505-1 YAT/086/V01Determination of AR-C133913XX in Rat Heparin Plasma, Using Protein Precipitation and Liquid Chromatography with Tandem Mass Spectrometric Detection
- Partial Validation of an Analytical Method for the Determination of AR-C133913XX in Marmoset Heparin Plasma using Liquid Chromatography with Tandem Mass Spectrometric Detection

Preliminary, non-validated methods

- BA-577-01 Determination of AZD6140 and AR-C124910XX in Dog Lithium Heparin Plasma after Protein Precipitation using Liquid Chromatography with Mass Spectrometric Detection
- BA-582-01 Determination of AZD6140 and AR-C124910XX in Animal Lithium Heparin Plasma after Protein Precipitation using Liquid Chromatography with Mass Spectrometric Detection

Not reviewed as these are human plasma methodology

- Determination of AZD6140, AR-C124910XX and AR-C133913XX in Human Plasma, Using Protein Precipitation and Liquid Chromatography with Tandem Mass Spectrometric Detection
- Validation of AstraZeneca Method No BA-486-01: Analytical Method for Determination of AR-C133913XX in Human Heparin Plasma Using Liquid Chromatography with Tandem Mass Spectrometric Detection : Report Amendment
- Determination of the Unbound Fraction of AZD6140, AR-C124910XX and AR-C133913XX in Human Plasma Using Tandem Mass Spectrometric Detection
- Method No. YAT/095 (AstraZeneca Method No. BA-522-01): Validation of an Analytical Method for the Determination of the Unbound Fraction of AZD6140, AR-C124910XX and AR-C133913XX in K2-EDTA Human Plasma Using Tandem Mass Spectrometric Detection

Not reviewed as these have been reviewed by the Clinical Pharmacology reviewer (email communication)

- **I**nvestigations into the permeability properties of AR-C126532XX

- AZD6140: Investigation of P-glycoprotein-Mediated Transport of AZD6140 and its Active Metabolite AR-C124910 Using MDCK Cells Expressing Human MDR1 P-glycoprotein
- AZD6140: Effect of AZD6140 and its Metabolite AR-C124910 on P-glycoprotein-Mediated Transport of Digoxin

3.3 Previous Reviews Referenced

Reviews (available in DARRTS) for the following submission numbers to IND 65, 808

- 000: safety pharmacology, rat studies (1,3 months), marmoset (1,3 months),genetic toxicology for AZD6140 and AR-C124910XX, ADME
- 016: plasma levels in mated rats and rabbits, *in vitro* metabolism, *in vivo* metabolism in rats and marmosets
- 019:special protocol, 020, special protocol,021, 022
- 024: embryo-fetal development (Segment II) studies in rats and rabbits
- 031: (b) (4) genotoxicity, retinal development in the rabbit, studies in marmoset (3 month), mouse (13-week), and rat (26 week). Male rat fertility study included in 6-month rat toxicology.
- 055: (b) (4) qualification study, ADME studies
- 068: hERG assay, 12 month marmoset study
- 092 (107 and 115 included in this review) Pharmacology, ADME
- 099 Pharmacology, ADME
- 0119: memo to the file regarding (b) (4) genotoxicity
- Telecom minutes: discussion of clinical handling of impurities
- 092, 107, 115: receptor binding , pharmacology, phosphodiesterase assessment
- 168: initial carcinogenicity findings
- 226: Changes to the Informed Consent form and Investigator's Brochure recommended by Medical Officer and Pharm/Tox reviewer
- 358,399 Review of carcinogenicity reports
- 523,562,566,569: the sponsor's investigation into the mechanism of carcinogenicity contained in one review

4 Pharmacology

4.1 Primary Pharmacology

AZD6140 is an orally active $P2Y_{12}$ receptor antagonist that, in vitro, causes a concentration-dependent inhibition of ADP-induced platelet aggregation. The drug is a cyclo-pentyl-triazolo-pyrimidine that appears to be a reversible inhibitor of platelet aggregation. The following mechanistic information is provided to facilitate reading this review.

The P2Y receptors are G protein coupled receptors belonging to the family of purinergic P2 receptors, with multiple subtypes identified and cloned. $P2Y_{12}$ is an ADP receptor coupled to inhibition of adenyl cyclase via Gi protein.

ADP, stored in high concentration in platelets, contributes to platelet activation occurring both during protective hemostasis (i.e. formation of the initial platelet mono-layer) and during formation of occlusive platelet-rich thrombi (Jennings 2010). The transduction of the ADP signal involves its interaction with two platelet receptors, the $P2Y_1$ receptor (Gq protein) and the $P2Y_{12}$ receptor. The concomitant activation of both the Gq and Gi pathways by ADP is necessary to elicit normal platelet aggregation. The $P2Y_1$ ADP receptor is thought to be important for the initiation of platelet activation while the $P2Y_{12}$ ADP receptor is thought to be more important for stabilizing the formed platelet aggregates. Activation of these two receptors ultimately leads to the GPIIb/IIIa ("fibrinogen receptor") changing shape to the active conformation.

In addition to its role in ADP-induced platelet aggregation, $P2Y_{12}$ mediates: (i) the potentiation of platelet secretion induced by strong agonists,:(ii) the stabilization of thrombin-induced platelet aggregates; (iii) shear induced platelet aggregation; and (iv) the inhibition of the antiplatelet effects of the natural regulator of platelet function, prostacyclin. The $P2Y_{12}$ receptor is also found in the central nervous system (Burnstock 2009), gastrointestinal tract, carotid body, lung, bladder, and inner ear (Housley 1998).

Figure 1. Role of P2Y receptors in ADP-promoted platelet aggregation

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From Nicholas RA. 2001. Mol Pharmacol. Sep;60(3):416-420.

References

- Burnstock, G. 2009. Purines and sensory nerves. Handbook Exp Pharmacol 194:333-392.
- Cattaneo M. 2009. New P2Y12 blockers. J Thromb Haemost 7(Suppl 1):262-265
- Housley GD. 1998. Mol Neurobiol. 16(1): 21-48.
- Jennings LK. 2009. Mechanisms of platelet activation: Need for new strategies to protect against platelet –mediated atherothrombosis. Thromb Haemost 102:248-257.

Primary Pharmacology

The activity of AZD6140 for platelet receptors was determined by transfection of a single human platelet receptor into a cell type otherwise lacking the receptors. In addition to measuring displacement of ligands some of the assays also assessed effects on various intracellular ion levels or processes. Positive controls were reported infrequently and the transfections were described as "stable" in very few reports.

Table 3. Reviewer's Summary of Additional Receptor Assay Results for AZD6140

Receptor or subtype	Cell type used for receptor expression	Assay mechanism	Action of AZD6140 (Maximum concentration tested)
Platelet receptor subtypes			
$P2Y_2$	Jurkat **XX**	Concentration-response on UTP modulated intracellular calcium flux	Weak antagonist at 10μM Positive control (UTP) only for agonist activity.
$P2X_1$	RLE(rat liver epithelial cells) **S**	Concentration-response on ATP modulated intracellular calcium flux	No significant activity at 10μM Positive control (ATP) for agonist activity
$P2X_7$	THP*-1 cells **C**	Effect on BzATP-induced ethidium bromide uptake	No significant activity at 3 μM No positive control
$P2Y_1$	Jurkat **XX**	Effect on ADP-induced intracellular calcium fluxes	No significant activity at 10μM Positive control (ADP) for agonist activity
$P2Y_{11}$	Jurkat **XX**	Effect on induced intracellular calcium flux	No significant activity up to 100μM AR-C67085MX (P_{2T} receptor antagonist) was the reference compound
Other Receptors			
Human adenosine A3	HEK293 (human embryonic kidney cells) membranes	Displacement of [^{125}I]AB-MECA (a selective A3 ligand)	IC_{50} 0.3μM affinity for the A3 receptor Positive controls: NECA: non-selective A_1/A_2 agonist R-PIA, S-PIA: A1 agonists DPCPX: A1 antagonist
rh CCR1	HEK293 membranes	Displacement of [^{125}I]MIP-1α (macrophage inflammatory protein-1 α)	No displacement up to 100 μM No positive control listed
rh CCR2b	CHO (Chinese hamster ovary cell) **XX**	Displacement of [^{125}I]MCP-1 (monocyte chemoattractant protein-1)	No displacement up to 100μM No positive control listed
Human CCR3	HEK cells **XX**	Increased cytosolic free calcium induced by eotaxin	No significant activity up to 10μM

* no further definition of cell line provided
S=stably transfected, **C**=constitutively expressed, **XX=**no designation of transfection

The sponsor used patch clamp electrophysiology to assess AZD6140 at recombinant human (rh)$P2X_1$, rh$P2X_3$, rh$P2X_4$,rh$P2X_5$, rat $P2X_2$ and rat $P2X_{2/3}$ receptors stably transfected onto human embryonic kidney (HEK) cells. The drug was tested at concentrations of 1µM and 10µM for the ability to inhibit agonist-induced (ATP or α,β-MeATP) channel current. No positive control was reported. There were no significant effects against the receptors tested. A wider concentration range could have been tested, but the conclusion would remain that any effect would be considered weak. Positive controls would increase confidence in the study.

Drug activity related to proposed indication

In Vitro Pharmacology

A series of in vitro studies examined the inhibitory effect of AZD6140 on ADP-induced aggregation in washed human platelets, human platelet rich plasma and heparinized human plasma. In each report, AZD6140 produced nanomolar IC_{50} values. The findings overall are supportive of competitive and reversible inhibition. There were no in vitro studies pertaining to ADP-independent platelet aggregation.

One study showed complete inhibition of ADP-induced aggregation following 5 minutes of incubation of AZD6140 with washed human platelets. With longer periods of incubation (up to 90 minutes), the drug-induced inhibition was no longer complete. The results of this study caused the sponsor to examine the characteristics of receptor binding and inhibition of receptor signaling using CHO-K1 cells transfected with $P2Y_{12}$. Under the conditions of the study, AZD6140 did not displace [^{3}H]-ADP from the recombinant receptor. The drug did however displace [^{125}I]-AZ11931285, a synthetic $P2Y_{12}$ specific ligand, from the receptor.

The particular method of aggregometry used for analyses was not always specified in the reports for the washed human platelets. Optical aggregometry was implied for these studies and stated for the platelet rich plasma. Impedance aggregometry was used for the heparinized human blood. The results of the studies are summarized in the reviewer's table below.

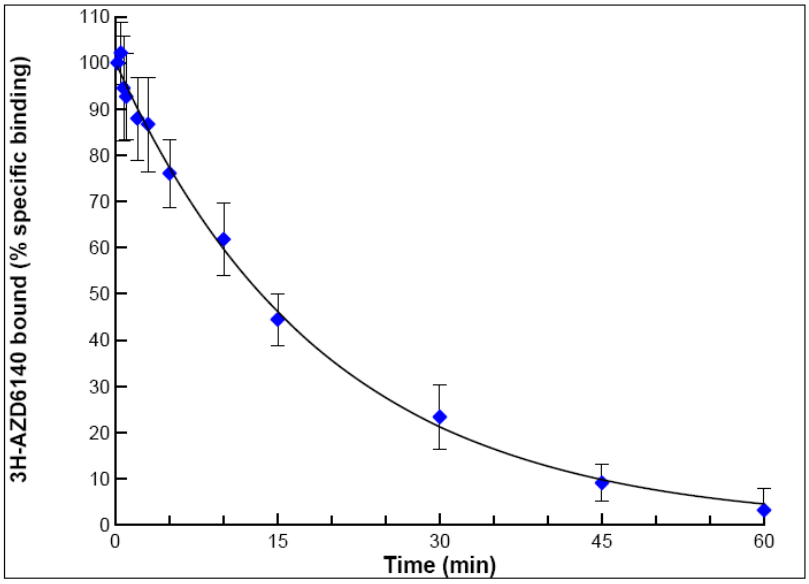
Table4. *In vitro* assessment of AZD6140 in human platelets.

Mechanism of assay	Comparator	Results
Studies done *in vitro* with washed human platelets		
Displacement of [^{125}I]AR-C98957XX (specific $P2Y_{12}$ receptor ligand) from P_{2T}	P_{2T} antagonist AR-C119608XX	AZD6140 displaced [^{125}I]AR-C98957XX Ki = 2.0nM (range tested 0.01-1000nM)
Inhibition of ADP-induced platelet aggregation	P_{2T} antagonist AR-C119608XX. 8SPT added to remove influence of P1-purinoceptors	AZD6140 inhibited ADP-induced platelet aggregation, IC50=13nM (range tested 0.1-1000nM)
Inhibition of ADP-induced platelet aggregation	AR-C67938MX (competitive P_{2T} antagonist)	Inhibition of ADP (0.3-300µM) by AZD6140 was complete after 5 minute incubation period for concentrations >30nM. Longer incubation periods showed less inhibition of response to ADP. Pre-incubation with AR-C67938 prior to addition of AZD6140 increased the maximum response to ADP (decreased the inhibition achieved by the test article)
Studies done *in vitro* with human platelet rich plasma		
Inhibition of ADP-induced platelet aggregation	P_{2T} antagonist AR-C119608XX.	IC50 398nM
Studies done *in vitro* with heparinized whole human blood		
Inhibition of ADP-induced platelet aggregation	None listed	IC50 58nM

Further in vitro assessment of receptor binding used rh$P2Y_{12}$ receptor expressed on CHO K1 cell membranes the $P2Y_{12}$ antagonist [^{125}I]-AZ11931285. AZ11931285 inhibited ADP- and 2-Me-S-ADP –induced activation of the recombinant human $P2Y_{12}$ receptor without displacing [^{3}H]-ADP. AZD6140 did not displace [^{3}H]-ADP, but did displace [^{125}I]-AZ11931285 from the recombinant receptor and antagonized both ADP and 2-Me-S-ADP mediated recombinant human $P2Y_{12}$ activation. The data indicates that AZD6140 interacts competitively with the $P2Y_{12}$ receptor rather than interacting with ADP.

Receptor kinetic studies were conducted using $hP2Y_{12}$ transfected (stability not reported) CHO-K1 cells. The K_d for AZD6140 was determined to be 10.5 nM, the K_{off} was determined to be $0.87x10^{-3}$ s^{-1} and the K_{on} for AZD6140 was $0.11x10^{-3}$ $nM^{-1}*s^{-1}$. This is depicted in figure 2.

Figure 2. Dissociation curve of ^{3}H-ticagrelor from CHO-K1 cells expressing $rhP2Y_{12}$



AR-C124910XX (active metabolite)

The O-deethylated metabolite, AR-C124910XX, has been shown to inhibit specific radioligand binding (P_{2T} antagonist AR-C119608XX) to human washed platelets (Ki value, 2.5 nM) and inhibits ADP-induced platelet aggregation in human platelet rich plasma (IC_{50} = 126 nM).

Non-Platelet $P2Y_{12}$ dependent effects

A study published by Wihlborg et al., 2004 demonstrated P2Y12 receptor mRNA expressed on human vascular smooth muscle cells (VSMC). Segments of internal mammary artery were exposed to 2-MeS-ADP. Contraction in response to the 2-MeS-ADP was defined as demonstration of functional $P2Y_{12}$ receptors in the VSMC Contraction was blocked by a selective $P2Y_{12}$ antagonist AR-C67085. The report does not provide any other information about the pharmacology of the reference compound. The clinical significance, if any, of this observation, is unclear.

***In Vivo* Pharmacology**

The non-clinical *in vivo* pharmacology was studied in the dog and rat. When metabolism in the different non-clinical species was assessed, the sponsor determined that the metabolism in dogs differed from humans in the ratio of parent drug to active metabolite. In humans, the active metabolite reaches an AUC_{0-24} value of approximately 35% that of the parent drug. In the dog, the active metabolite achieves an AUC value only 9% that of the parent level which the sponsor considered inappropriate for toxicological assessment but not the pharmacologic evaluation. There was no further explanation offered for this conclusion as to appropriate species for testing.

Oral administration of a single dose (0.8 mg/kg) of ticagrelor to the conscious male Beagle produced >90% inhibition of ADP-induced platelet aggregation measured *ex vivo*. The maximum effect was seen within 2 hours and was maintained to 6 hours. The IC_{90} for inhibition of ADP-induced aggregation ex vivo was estimated as ~65ng/ml.

Drug Comparisons and Pharmacodynamic Interactions

Several studies examined AZD6140 alone and in combination with other agents. It is not feasible to separate the study results into comparisons of single agents versus interaction analysis.

The first series of studies examined AZD6140 in combination with aspirin, a thrombin inhibitor, and fibrinolysis inhibitors. Beagles with surgically stenosed femoral arteries were used to examine platelet mediated anti-thrombotic effects as assessed by cyclic flow reduction (CFR) in the damaged artery. Effects on hemostasis were assessed by changes in bleeding time (BT). The results of these are summarized in the reviewer's table below.

Table 5. Reviewer's Summary of AZD6140 Alone, Comparisons to, and Interactions with Aspirin and Melagatran (thrombin inhibitor),

Drug or drug combination	model	Results	
		%inhibition ADP-induced aggregation	Comments
AZD6140	Conscious male Beagle	>90, ex vivo IC_{50} ~65ng/ml	Maximum effect within 2hours, maintained up to 6 hours
		Cyclic Flow Reduction (CFR)	Bleeding time (BT)
AZD6140+ Aspirin (oral pretreatment for 7 days)	Anesthetized dogs with stenosed femoral arteries	CFR ID_{50}= 0.41µg/kg/min IV	BT 6 min vs vehicle control , BT = 4 min (ns) at the CFR ID_{50}
AZD6140 ± melagatran 2754-01	Anesthetized dogs with stenosed femoral arteries	CFR ID_{50} ~ 2µg/kg/min IV	Vehicle= 2 min Ticagrelor ~3 min (2µg/kg/min) Melagatran = 2 min

Rat studies used ferric chloride to etch the carotid arteries. This model was used to evaluate whether DDAVP (deamino-Cys1, D-Arg8-vasopressin), aprotinin (anti-fibrinolytic) or the

combination of DDAVP and tranexamic acid (inhibitor of fibrinolysis) could decrease AZD6140-induced bleeding prolongation.

The intravenous administration of DDAVP, aprotinin or a combination of DDAVP and tranexamic acid during an infusion of AZD6140 did not significantly decrease the prolongation of bleeding time in this rat model.

The sponsor compared AZD6140 to a variety of drugs alone and in combinations. Orally (po) and intravenously (iv) administered AZD6140 was compared to clopidogrel, orbifiban active metabolite, and cangrelor. AZD6140 was tested in combination with other $P2Y_{12}$ inhibitors cangrelor and clopidogrel. For all these comparisons, Beagles with surgically stenosed femoral arteries were used to examine platelet mediated anti-thrombotic effects as assessed by cyclic flow reduction (CFR) in the damaged artery. Effects on hemostasis were assessed by changes in bleeding time (BT).

Intravenous infusion of AZD6140, clopidogrel or orbifiban active metabolite produced dose-related inhibition of CFR, prolongation of BT and *ex vivo* inhibition of ADP-induced platelet aggregation relative to baseline (percentage inhibition of CFR and ADP-induced aggregation, fold increase in BT). For each drug, decreased thrombosis was associated with complete or near complete inhibition of ADP-induced platelet aggregation (91±9% - 99±1%).

There was a non-statistically significant increase in the separation of the anti-thrombotic and anti-hemostatic effects for ticagrelor compared to clopidogrel. Both agents demonstrated better separation of effects than observed with the active metabolite of the GPIIb/IIIa antagonist orbofiban. The results are summarized in the sponsor's table shown below.

Table 6. Anti-thrombotic,anti-aggregatory and anti-hemostatic effects in the pentobarbitone anesthetized dog.

Table 4: Anti-thrombotic, anti-aggregatory and anti-haemostatic effects of AR-C126532XX, clopidogrel and orbofiban active metabolite in the pentobarbitone-anaesthetised dog, expressed as the geometric mean (95% confidence interval) dose producing 50% inhibition of cyclic flow reductions (CFR ID_{50}), 50% inhibition of ADP-induced platelet aggregation measured *ex vivo* (ADP ID_{50}) or a 3.5-fold increase in bleeding time ($BT_{3.5\text{-fold}}$).

$^{**}p < 0.01$ and $^{*}p < 0.05$ *cf* AR-C126532XX (n = 5 - 6).

Treatment Group	CFR ID_{50} Geometric Mean (95% confidence interval)	ADP ID_{50} Geometric Mean (95% confidence interval)	$BT_{3.5\text{-fold}}$ Geometric Mean (95% confidence interval)	Dose Ratio CFR ID_{50}:$BT_{3.5\text{-fold}}$ Geometric Mean (95% confidence interval)	Dose Ratio ADP ID_{50}:$BT_{3.5\text{-fold}}$ Geometric Mean (95% confidence interval)
AR-C126532XX (µg/kg/min iv) (n = 6)	0.96 (0.57 – 1.63)	0.65 (0.18 – 2.39)	13.5 (6.6 – 27.5)	14.0 (5.4 – 36.7)	17.5 (3.49 – 87.5)
Clopidogrel (µg/kg iv) (n = 5)	330[1] (172 - 649)	180[1] (56 - 548)	1910[1] (829 - 4390)	5.7 (2.9 – 11.1)	10.9 (4.4 – 27.4)
Orbofiban (µg/kg/min iv) (n = 5)	0.31* (0.15 – 0.63)	0.20 (0.05 – 0.77)	0.26** (0.17 – 0.39)	0.83** (0.41 – 1.67)	1.28** (0.32 – 5.1)

[1]Comparison with AR-C126532XX not valid due to different dosing protocol (see 3.7.2 'Dosage and Dose Groups')

A subsequent study to profile AZD6140 against clopidogrel in the femoral artery stenosis model indicated an increased separation between anti-thrombotic- and bleeding- effect for AZD6140 compared to the thienopyridine. AZD6140 was administered as a bolus followed by a continuous infusion while clopidogrel was administered as a bolus dose only.

Table 7. Effective dose of AZD6140 for 50% inhibition of thrombosis

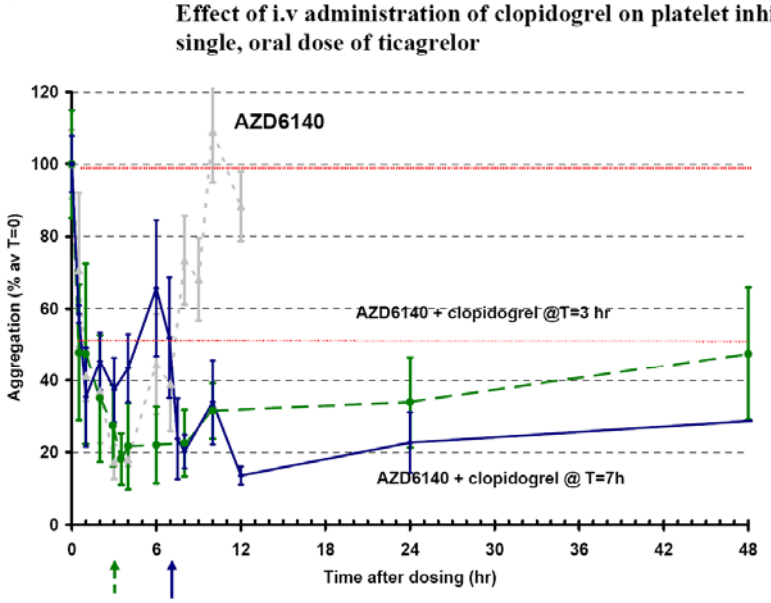
Drug	Thrombosis ED_{50}	Aggregation ED_{50}	Bleeding Time $ED_{BT:3.5}$	Fold increase in bleeding time at 100% anti-thrombotic effect
AZD6140	1.90 µg/kg/min	1.02µg/kg/min	>10µg kg/min	2.4
Clopidogrel	1.68 mg/kg	0.62 mg/kg	3.9 mg/kg	4.3*

*increase in bleeding time at 91% anti-thrombotic effect

A single oral dose of AZD6140 (0.8 mg/kg) was given to Beagles. This was followed by an intravenous dose of clopidogrel given either at maximum platelet inhibition from AZD6140 or when platelet function had partly recovered. Clopidogrel demonstrated greater activity (green line) over a longer

duration when given at 3 hours (maximal AZD effect) but greater short term activity given at 7 hours (blue line) after AZD6140's maximal effect.

Figure 3.

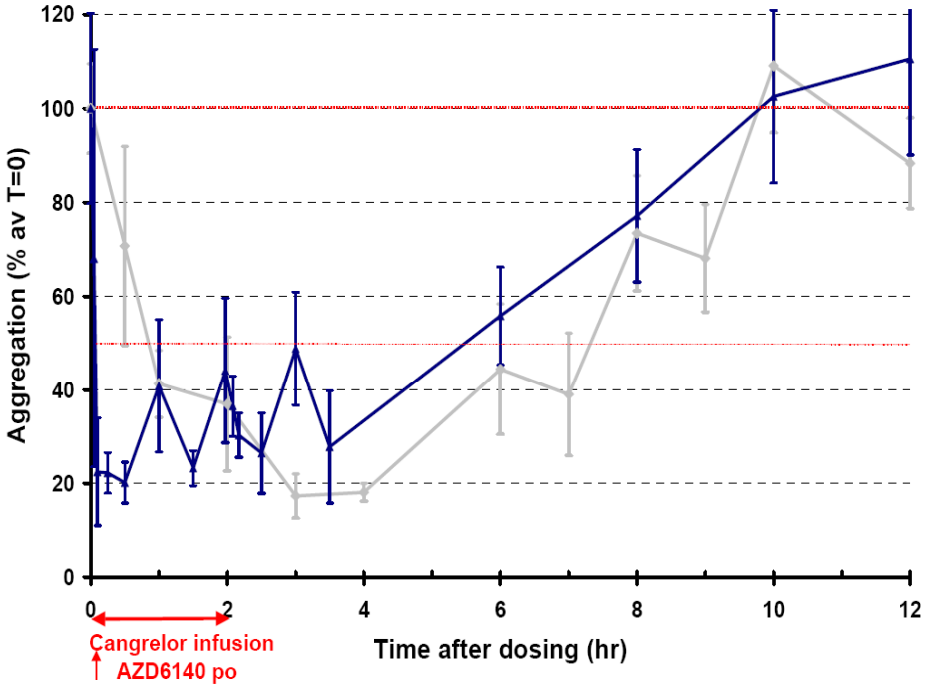


Ticagrelor (0.8 mg/kg) was dosed orally to dogs and ex vivo platelet function was measured using the whole blood impedance technology (grey dotted line). On separate occasions, the same dogs received orally ticagrelor, plus i.v. clopidogrel (3 mg/kg) either at max platelet inhibition (T=3 hr, green line) or at partially recovered of platelet function (T=7 hrs, blue line). Data are mean±sem, n=3.

Possible interactions with cangrelor were examined slightly differently. Beagles were given an intravenous infusion of cangrelor that began at the same time as the oral administration of ticagrelor (0.8 mg/kg). There was no statistically significant difference in degree of platelet aggregation between AZD6140 alone and co-administered with cangrelor. However, the intravenous administration produced a rapid onset of effect.

Figure 4

Effect of i.v administration of cangrelor on platelet inhibition after a single, oral dose of ticagrelor



Ticagrelor (0.8 mg/kg) was dosed orally to dogs and ex vivo platelet function was measured using the whole blood impedance technology (grey line). On a separate occasion, the same dogs received i.v. cangrelor (1 µg/kg/hr) plus orally ticagrelor , dosed at T=10 minutes (blue line). Data are mean±sem, n=3.

Secondary Pharmacology

Receptor binding

In a screen of activity against 125 radioligand binding and enzyme assays ([redacted] (b) (4) procedures), AZD6140 showed activity for the adenosine A3 receptor and the phosphodiesterase isoforms. The summary of those receptors for which there was significant activity is shown below.

Table 8. Sponsor's Summary of Significant Receptor Interactions

Detectable activities of AR-C126532XX in a number of binding and enzyme assays performed *in vitro* at (b) (4)

Assay	Concentration tested (µM)	% Inhibition (IC_{50})
Adenosine A_3	10	94
Phospholipase C	10	71 (IC_{50} = 8.4 µM)
P59fyn tyrosine kinase	10 Inactive on retest @ 1-10 µM	56
Platelet activating factor	10	55 (IC_{50} = 9.4 µM)
A_{2b} Adenylyl cyclase	Inactive @ 1-10 µM	
5-lipoxygenase	30	83
Phosphodiesterase 1	100	78
Phosphodiesterase 2	100	70
Phosphodiesterase 3	100	73
Phosphodiesterase 4	100	79
Phosphodiesterase 5	100	97
Ca2+/calmodulin dependent PKII	100	91
Phospholipase A_2 (pancreatic)	300	87
Cyclooxygenase	300	53
Leukotriene C_4 synthetase	1000	66

Further examination of the interaction with phosphodiesterases (PDE) 1, 3, 4 and 5 showed that AZD6140 exhibited weak ($IC_{50} \geq 10\mu M$) interaction with PDE1, 3, and 4. The IC_{50} for PDE5 was reported as 0.482µM.

Adenosine: in vitro

The respiratory effects identified in the safety pharmacology studies were manifested in the clinical studies as dyspnea, one of the common adverse effects reported. The sponsor thus examined possible mechanisms of this, including interactions of AZD6140 with the adenosine receptor subtypes. AZD6140 showed affinity for the human and rat A3 adenosine receptors. The sponsor also examined the effect of AZD6140 on the uptake of adenosine in cell lines from several

species: MDCK (a dog kidney cell line), MCF7 (human breast carcinoma), H4IIE (rat hepatoma cells). Dipyridamole, the reference agent, inhibited sodium-independent adenosine uptake in each of the cell lines. AZD6140 also demonstrated concentration-dependent inhibition of sodium-independent adenosine uptake in each of the cell lines, in the same order of magnitude as dipyridamole. When evaluated in washed human erythrocytes, AZD6140 inhibited adenosine uptake with an IC_{50} value of 100nM, approximately 10-fold less potent than dipyridamole in this assay system. The results are referred to as "from the original assays" because the sponsor repeated these assays in April of 2010. The repeated assays (results summarized under "Metabolites" section) are similar to the original results.

Table 9. Reviewer's Summary of the original *in vitro* assays for assessment of adenosine effects

Receptor	result	comment
Human A_1 adenosine	K_1 >10μM	Low affinity
Human A_{2A} adenosine	K_1 >10μM	Low affinity
Human A_{2B} adenosine	K_1 >10μM	Low affinity
Human A_3 adenosine	Ki = 0.104μM	Higher affinity
Rat A_1 adenosine	Not done	
Rat A_2 adenosine	Not done	
Rat A_3 adenosine (rat basilar artery)	EC50= 9.4μM	Weak agonist
Vagal C-fiber preparations, depolarized by adenosine via A_1, A_{2B}, A_3 receptors		
Rat vagal C-fibres	AZD6140 inhibited depolarization ± adenosine @10μM	
Guinea pig vagal C-fibres	AZD6140 inhibited depolarization ± adenosine @10μM	
Adenosine deaminase	Inhibition not apparent up to 10μM	
Human erythrocytes	Inhibition of adenosine uptake, IC_{50}=100 nM	Sponsor interpreted to indicate effect via sodium independent nucleoside transporter ENT-1
Canine MDCK	IC_{50}=34nM	
Rat H4IIE cells	IC_{50} = 104nM	
Human MCF7	IC_{50}= 61nM	

Adenosine: in vivo

One study in Beagles examined possible *in vivo* relevance of AZD6140's *in vitro* inhibition of adenosine uptake in erythrocytes. Coronary blood flow was measured after temporary occlusion of the left anterior descending artery (LAD) or direct infusion of adenosine into the LAD. Dogs (n=8 per group) were treated i.v. with vehicle or two doses of either AZD6140 or dipyridamole, an adenosine uptake inhibitor.

Blood flow after a 5 minute intra-coronary artery infusion of adenosine, 15 and 30 μg/min, before and after administration of AZD6140 or dipyridamole was increased compared with time-matched vehicle treated dogs. This is shown in the sponsor's table below.

The mean plasma levels of AZD6140 were 4.11±1.5µM (following treatment 1) and 13.42µM (following treatment 2). Both plasma levels were greater than the mean human Cmax (1.3µM) making it unlikely that this will be a clinically apparent effect.

Table10. Left anterior descending coronary artery blood flow increase after adenosine infusion 15µg/min (mean±SEM)

	Control period	Treatment 1 vehicle AZD6140, 30 µg/(kg x min) Dipyridamole, 0.17 µg/(kg x min)	Treatment 2 vehicle AZD6140, 100 µg/(kg x min) Dipyridamole, 0.5 µg/(kg x min)
Normalised blood flow rates (% of control period)			
Vehicle	100	98±10	110±7
AZD6140	100	114±10	150±19
Dipyridamole	100	160±2	193±17

Rh-GPR17

GPR17, a G-protein coupled receptor activated by both uracil nucleotides and cysteinyl-leukotrienes, is expressed mainly in brain, heart, and kidney tissue. Activation by the endogenous ligands leads to both adenyl cyclase inhibition and intracellular calcium increases (Ciana et al. 2006 EMBO Journal 25:4615-4627)

GPR17 is presumed to moderate secondary inflammatory responses as shown in a rat stroke model (Ciana P et al 2006). An *in vitro* study to investigate potential antagonistic properties of AZD6140 against rh-GPR17 showed inhibition of the receptor by ticagrelor with nano-molar potency against uracil nucleotides (IC_{50}=2 nM againstUDP-glucose induced activation) and cysteinyl-leukotriene (IC_{50}= 2nM against LTD4) (Abbracchio MP and Martini C 2009). The clinical significance, if any, is unclear.

AR-C124910XX (Active Metabolite)

During the NDA review, the sponsor changed the code for this metabolite to AZ11879328-003. Receptor binding, enzyme affinity and electrophysiological properties were studied in a different facility from where the original studies for the parent compound were conducted. The sponsor concurrently re-examined the receptor binding, enzyme affinity and electrophysiological properties of AZD6140.

AR-C124910XX showed ≥50% inhibition in 16 of the 332 *in vitro* receptor ligand and enzyme assays when tested at a concentration of 10µM. This metabolite also demonstrated some activity at 4 of the 7 human recombinant voltage-gated cardiac ion channels. Results are summarized in the reviewer's table below. The results for steroid hormone receptors are included in the summary table below due to the various questions pertaining to possible hormonal effects.

Table 11. Reviewer's Summary of Recent Radioligand Binding Assays for AZD6140 and Metabolite AR-C124910XX

Radioligand assays %inhibition at 10µM			
target	Positive control	AZD 6140	AR-C124910XX (AZ11879328-003)
Phosphodiesterase PDE3	Cilostamide	41	49
Phosphodiesterase PDE4	Ro 20-1724	66 (IC_{50} 3.9µM)	56 (IC_{50} 10.6µM)
Protein tyrosine kinase insulin receptor	Tryphostin 47	34	30%
Adenosine A1 (human)	R(-)-PIA	25	11
Adenosine A2 (human)	CGS-21680	17	49
Adenosine A3 (human)	IB-MECA	98 (IC_{50} 0.113µM)	103 (IC_{50} 0.196µM)
Androgen receptor	testosterone	30	30
Calcium channel L-type	diltiazem	29	31
Estrogen receptor ERα (human)	diethylstilbesterol	6	1
Glucocorticoid (human)	dexamethasone	17	-5
Histamine H2 (human)	tiotidine	-30	-70
Peripheral benzodiazepine (rat)	diazepam	40	36
Progesterone (rat)	progesterone	37	25
Prostanoid thromboxane A2 (human)	ICI-192,605	41	33
Serotonin 5HT2B (human)	ketanserin	52(IC_{50} 8.21µM)	99 (IC_{50} 2.42)
Somatostatin SST4 (human)	Somatostatin T4	1	30
Adenosine transporter (guinea pig)	Nitrobenzyl -thioinosine	84 (IC_{50} 2.21µM)	40
Norepinephrine transporter (human)	desipramine	66 (IC_{50} 4.52µM)	24

Table 12. Reviewer's Summary of Recent Biochemical Assays for AZD6140 and ARC124910XX

Biochemical Assays % inhibition at 10μM			
target	Reference compound	AZD6140	AR-C124910XX (AZ11879328-003)
Deacetylase,sirtuin SIRT2, human	nicotinamide	60 (IC_{50} 8.64μM)	60(IC_{50} 11.8μM)
Lipoxygenase 5-LO, human	NDGA	98 (IC_{50} 1.58 μM)	99(IC_{50} 1.59μM)
Peptidase CTSL2 (cathepsin L2) human		75 (IC_{50} 4.56μM)	57 (IC_{50} 3.4μM)
Peptidase, CTSS (cathepsin S) human		53 (IC_{50} 2.63μM)	86 (IC_{50} 3.32μM)
Phosphodiesterase PDE10A1 human	papaverine	70 (IC_{50} 3.85μM)	60 (IC_{50} 5.07μM)
PDE5 human	zaprinast	77 (IC_{50} 0.379μM)	82 (IC_{50} 0.382μM)
PDE6 bovine	zaprinast	52(IC_{50} 2.36 μM)	56 (IC_{50} 8.91μM)
Phospholipase PLC [ba]	manoalide	55 (IC_{50} 7.46 μM)	53(IC_{50} 8.14μM)
UDP glucuronysyltransferase (UGT1A1) human	17β-estradiol (IC_{50} 15 μM)	60 (IC_{50} 1 μM)	84(IC_{50} 0.759μM)
Human dopamine transporter †	GBR-12909	95 (IC_{50} 0.169μM)	91(IC_{50} 0.757μM)
Inhibition of agonist-stimulated IP1 formation			IC_{50} 15.8μM
[Ba] bacillus cereus			

Table 13. Reviewer's Summary of Recent Electrophysiological Assays for AZD6140 and AR-C124910XX

Electrophysiology Assays			
Target	Positive control	AZD6140	AR-C124910XX (AZ11879328-003)
hKv1.5	4-Aminopyridine	Inactive at 10μM	IC_{50}: 16.9 μM
hKv7.1/hKCNE1	XE991 Dihydrochloride	Inactive at 31.6μM	Inactive ≤32μM
$hCa_v1.2$	Verapamil HCl	IC_{50} >31.6μM	Inactive ≤32μM
hNav1.5	flecainide	IC_{50} 27.7μM	IC_{50}:21.6μM
hKv4.3/hKChiP2.2	flecainide	IC_{50} >31.6μM	26% inhibition at 32μM (highest conc. tested)
hCav3.2	Mibefradil	IC50 19.2μM	IC_{50}: 19.9μM
hHCN4	ZD7288*	Inactive at 31.6μM	Inactive ≤32μM
The electrophysiology was performed in Chinese Hamster Ovary (CHO) cells stably transfected with $hNa_v1.5$, $hK_v1.5$, $hK_v4.3$/hKChIP2.2, $hK_v7.1$/hKCNE1, hHCN4 or $Ca_v1.2$. Human embryonic kidney (HEK 293) cells were stably transfected with $hCa_v3.2$.			

AR-C133913XX (Major Metabolite)

During the NDA review, the sponsor changed the code for this metabolite to AZ11879477. Activity was assessed using membranes from CHOK1 cells transfected (stability not described) with the human $P2Y_{12}$ receptor. The sponsor used an undefined but apparently specific $P2Y_{12}$ radioligand, [^{125}I]-AZ11931285 to compare the relative affinity of AZD6140 , AZ11879328 (AR-C124910XX) and AZ11879477 (AR-C133913XX). AZD6140 and AR-C214910XX showed low nanomolar affinity for the $P2Y_{12}$ receptor. AR-C133913XX showed an order of magnitude less affinity, but was still active in the low micromolar range as summarized below.

Table 14.

Potency of ticagrelor and its metabolites in the $P2Y_{12}$ receptor radio-ligand binding and signalling assays

	[^{125}I]-AZ11931285 binding assay		[^{35}S]-GTPgS assay	
Compound name	**IC_{50} (nmol/L)**	**N**	**IC_{50} (nmol/L)**	**N**
ticagrelor	11±8 (mean±SD)	63	66±28 (mean±SD)	98
AZ11879328	7 and 6	2	36 and 34	2
AZ11879477	2500	1	10000	1

The sponsor also compared the effects of AZD6140, AZ11879328 and AZ11879477 on platelet aggregation in whole blood collected from humans (3 males, 1 female), rats and marmosets. ADP-induced aggregation was evaluated by impedance aggregometry. The sponsor reports using a low volume technique to decrease the amount of marmoset blood needed. A validation of this low volume technique was not provided nor was one referenced. Accepting the results at face value despite the lack of methodological validation, AZD6140 showed similar IC_{50} values in blood from all 3 species. AZ11879477 (formerly AR-C133913) showed low activity in humans and rats and greater activity in marmosets. The results are summarized in the sponsor's table.

Table 15

Potency of ticagrelor AZ11879328 and AZ11879477 in human, rat and marmoset

	Mean IC_{50}-values (µM)		
	Human	**Rat**	**Marmoset**
AZ11939728	0.24	0.13	0.12
AZ11879328	0.17	0.06	0.06
AZ11879477	>10	>10	1.91

AZ11939728 =AZD6140; AZ11879328=AR-C124910XX, AZ11879477=AR-C133913

4.3 Safety Pharmacology (detailed reviews are available in DARRTS primarily under the000 submission)

Neurological effects: There were no significant effects on overt neurological behavior, motor coordination, analgesic effect, anesthetic latency, or memory. There was a slight decrease in time of mean latency to chemically-induced seizures (31%, n.s. compared to the vehicle control group). At this point, the clinical database should be able to address the relevance or lack of relevance of this data.

Cardiovascular effects: There were no statistically significant effects on action potential parameters in canine Purkinje fibers at stimulation rates of 1 Hz and 0.33 Hz. AZD6140 inhibited the hERG channel with an IC_{50} of 1.72 μM.

Intraduodenal administration of 100 mg/kg AZD6140 to anesthetized dogs caused a mean 40% decrease in coronary resistance (n.s.) at 1.5 hours after dosing when compared to a vehicle control group. Doses of 1, 10 and 100 mg/kg caused decreases in mean arterial pressure (up to 20% change from baseline, n.s.) and diastolic arterial pressure (up to 25% change from baseline, n.s.) from 1-3 hours after administration. Systolic arterial pressure was also decreased (up to 15% from baseline, n.s.) at all 3 doses for the duration of monitoring. There was no *in vivo* effect on QT or QTc.

Renal effects: In a discrete study, single oral doses of 10 and 100 mg/kg given to saline loaded rats increased excretion of sodium and chloride, and increased pH. Urinary sodium excretion per mole of creatinine excreted was increased by 24% ($p<0.05$) and 35% ($p<0.01$) over control values at 10 mg/kg and 100 mg/kg respectively in the first 4 hour collection. At the high dose of 100 mg/kg chloride excretion was of necessity increased by 17% ($p<0.05$, normalized to creatinine excretion).The urine pH was also increased at the high dose (7.47 vs 7.00 in the vehicle control group, $p<0.05$). These effects were not apparent in the repeat dose toxicology studies. Given the usual variability of excreted urinary samples, the changes are of questionable biological significance. These effects were not apparent in the toxicology studies, but saline loading was not utilized in safety assessment.

Gastrointestinal effects: There were no effects on gastrointestinal transit time following single oral doses of 1 or 10 mg/kg to conscious rats. Gastrointestinal transit time was prolonged (29%) at the high dose of 100 mg/kg.

Abuse liability: not done

Pulmonary effects: Orally administered AZD6140 caused increases in respiratory rate (up to 20% of predose baseline, $p<0.01$), peak inspiratory flow (up to 35% of predose baseline, n.s.) and expiration time (decreased by up to 20% from predose baseline, $p<0.01$) at all doses. These effects were explored further once dyspnea was recognized in the clinical trials. The repeat dose toxicology studies did not report respiratory effects, but, were not designed to detect those effects.

The sponsor investigated the hypothesis that the dyspnea was due to an adenosine-based mechanism. In a mechanistic study (0789SR) AZD6140 again caused a dose-related increase in respiratory rate as well as significant increases in peak expiratory flow that were not

distinguishable between the doses. Other effects included increases in minute volume and decreased expiration time. Pre-treatment with DPSPX, an adenosine antagonist, seemed to prevent the effects. However, the vehicle for DPSPX, aqueous hydroxypropyl-cyclodextrin seemed to be just as effective in preventing the AZD6140 effects. The study did not clarify a possible relation to adenosine effects. Several additional studies were conducted. Comparator compounds of carbamoyl choline and theophylline were used in addition to adenosine. In several reports throughout the NDA, the sponsor comments on the structural similarity of AZD6140 to adenosine. It is known that the parent drug and the AR-C124910XX metabolite have affinity for the adenosine A3 receptor. Overall, it is not surprising that AZD6140 shows some pharmacodynamic similarity to adenosine. We have not examined the structural similarity of the two main metabolites to adenosine. However, at this point, there should be sufficient clinical data for this effect to inform dosing. The studies conducted to explore the respiratory effects are summarized in the reviewer's table below.

Table 16. Reviewer's Summary of Respiratory Studies

Report # and date	Dose and route of administration	Results and comments
SR99346-01 June, 2000	1, 10 and 100 mg/kg, p.o.	Original safety pharmacology study. AZD6140 at all doses caused ↑respiratory rate, peak inspiratory flow and expiration time
0789SR Dec, 2005	10, 100 mg/kg po	DPSPX, an adenosine antagonist was given at a no-observed-effect dose (0.3mg/kg, i.v). as a pre-treatment followed by AZD6140 at one of two doses. While the combination of AZD6140 and DPSPX caused some diminution of effect, the combination of the DPSPX vehicle and AZD6140 also produced a diminution of respiratory effects.
1266SR Dec, 2006	100 mg/kg p.o.	20% (n.s.) mean increase in respiratory rate and peak expiratory flow. Unclear if the effects are drug-related and not within the realm of normal variability.
1267SR Sept, 2007	100 mg/kg p.o.	Amendment to the study done comparing AZD6140 to DPSPX, clarifying plasma levels of drug
1207SR	Adenosine 0.05, 0.1, 0.25,0.5, 1,2 and 4 mg/kg i.v.	Infusion of 4 mg/kg adenosine caused a statistically significant increase in respiratory rate, tidal volume, minute volume, and peak inspiratory and expiratory flows. Inspiration and expiration times were significantly decreased. The lower doses did not produce discernible effects. When the doses of 2 and 4 mg/kg were repeated, the results for the high dose were similar to those of the initial study. The 2 mg/kg dose produced a statistically significant increase in respiratory rate, peak inspiratory flow and peak expiratory flow and statistically significant decreases in tidal volume and expiration time
1116sr (May 2006)	10, 100, 1000 mg/kg AZD6140 Theophylline as positive control	This was done to confirm the effects reported in the original safety pharmacology study. There was a statistically significant increase in respiratory rate, peak expiratory flow, and minute volume at 10 mg/kg AZD6140 that was not seen at 100 mg/kg. Expiration time was statistically significantly decreased at 10 mg/kg AZD6140 but not 100 mg/kg. When the study was repeated using the doses 100 and 1000 mg/kg AZD6140, there was a mild dose-related effect for increased minute volume, peak inspiratory flow, and peak expiratory flow.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Methods of Analysis: During the IND, studies were submitted for determination of AZD6140 and its main metabolite AR C124910XX in rat, dog, marmoset, rabbit, Cynomolgous and mouse plasma by LC- Atmospheric Pressure Chemical Ionization (APCI) tandem mass spectroscopy following partial protein precipitation (reviewed under IND submission # 055, review on file in DARRTS). The plasma samples were evaluated for interfering substances, effects of coagulants, stability of the internal standard, adsorption and light sensitivity, freeze thaw stability, room temperature matrix stability, blood collection stability, matrix container stability, hemolysis and long term frozen stability. This method had a lower limit of quantitation (LLOQ) of 5.00 ng/ml and an upper limit of quantitation of 500 ng/ml for both AZD6140 and AR-C124910XX. Diluting samples with plasma from drug-naïve animals extended the ULOQ to 400 µg/ml.

The sponsor submitted with the NDA methodology for determination of the free fraction of AR-C124910XX and AR-C133913XX in human, rat, mouse, rabbit and marmoset plasma using protein precipitation and tandem mass spectrometric analysis. Protein precipitation was used for all methods except BA-542 which used dilution of plasma (dialysis).

Absorption: AZD6140 was found to be absorbed dose-dependently at the lower doses (<20 mg/kg) and greater than dose-dependently at the higher doses (>20 mg/kg) used in the toxicology studies. In vivo oral bioavailability was determined in the rat (88%) and marmoset (37%). Bioavailability was not determined for the dog. Sex-related differences were apparent only in the rat, where females generally had greater exposure to ticagrelor than male rats. AZD-6140 and its active metabolite AR-C124910XX were both Pgp-substrates as well as weak inhibitors. The pharmacokinetic parameters are summarized in the sponsor's table reproduced below.

Table 17. Pharmacokinetic Parameters of Ticagrelor and AR-C124910XX after administration of Ticagrelor

species	Dose Mg/kg	Cl Ml/min/kg	V_{Dss} l/kg	$T_{1/2}$ hours	F%	AUC ng.hr/ml	Cmax ng/ml
Ticagrelor[1]							
human	100 mg b.i.d.	441 (L/h)	1.25	7		4455	687
rat	3,.i.v.	27	4.8	2.8		2090	
	20, p.o.			2.6	88	12900	1620
dog	5, p.o.			9.2		9020	1590
marmoset	3, i.v.	11	3.7	4.9		4870	
	20, p.o.			7.5	37	21600	1940
cynomolgous	5, p.o.			1.9		239[b]	62
AR-C124910XX[2]							
human	100 mg b.i.d of ticagrelor			8.5		899	135
rat	3,.i.v.[a]			4.1		314	55
	20, p.o.[a]			2.7		3890	390
dog	5, p.o.[a]			13		768	131
marmoset	3, i.v.[a]			7.4		520	44
	20, p.o.[a]			12		10400	538
cynomolgous	5, p.o.[a]			2.9		323[c]	66

[1] human values from I. Younis, Ph.D. Clinical Pharmacology reviewer
[2] AR-C124910XX values from CSR D5130C00008
[a] AZD6140
[b] AUC to last quantifiable timepoint 7hours
[c] AUC to last quantifiable timepoint 10 hours

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, approximately 3 to 4 times respectively the Vd reported for humans. Radiolabel studies in pigmented and non-pigmented male rats show wide distribution with greatest concentrations found in the liver followed by the adrenal gland, kidney, pituitary and thyroid gland. Lesser amounts have been reported for the lung, bone marrow, pancreas and spleen. Tissue levels following a single oral dose decrease to below quantifiable levels within ~48 hours in non-pigmented rats. There was little indication of melanin binding based on analysis of pigmented skin and uveal tract.

Following a single oral dose of [^{14}C]-ticagrelor to pregnant rats, radioactivity was found in the placenta, fetus, and milk. Milk samples displayed up to 23X higher levels of total radioactivity than maternal plasma during the entire sampling period. The radioactivity was cleared from milk at the same rate as total radioactivity from plasma.

Metabolism: Nineteen metabolites of AZD6140 have been identified in various in vitro testing systems and seem to be represented in the non-clinical species. AR-C124910XX and AR-C133913XX have been identified in each species as the major metabolites.

AR-C124910XX, identified as having pharmacologic activity similar to the parent drug, is formed by CYP3A4/5 and also metabolized by it. In humans, AR-C124910XX is present at approximately 30-35% the plasma AUC_{0-24} of the parent drug (personal communication from Islam Younis, Ph.D., Clinical Pharmacology reviewer). In rats, the AUC_{0-24} value for the active metabolite is up to 100% of the AUC_{0-24} value for the parent drug. In mice, the AUC_{0-24} values for the active metabolite typically exceed the values determined for the parent drug. The ratio of AR-C124910XX to AZD6140 was used as the basis of selecting the marmoset in preference to the dog for the toxicology studies. A summary of this ratio in different species is shown below.

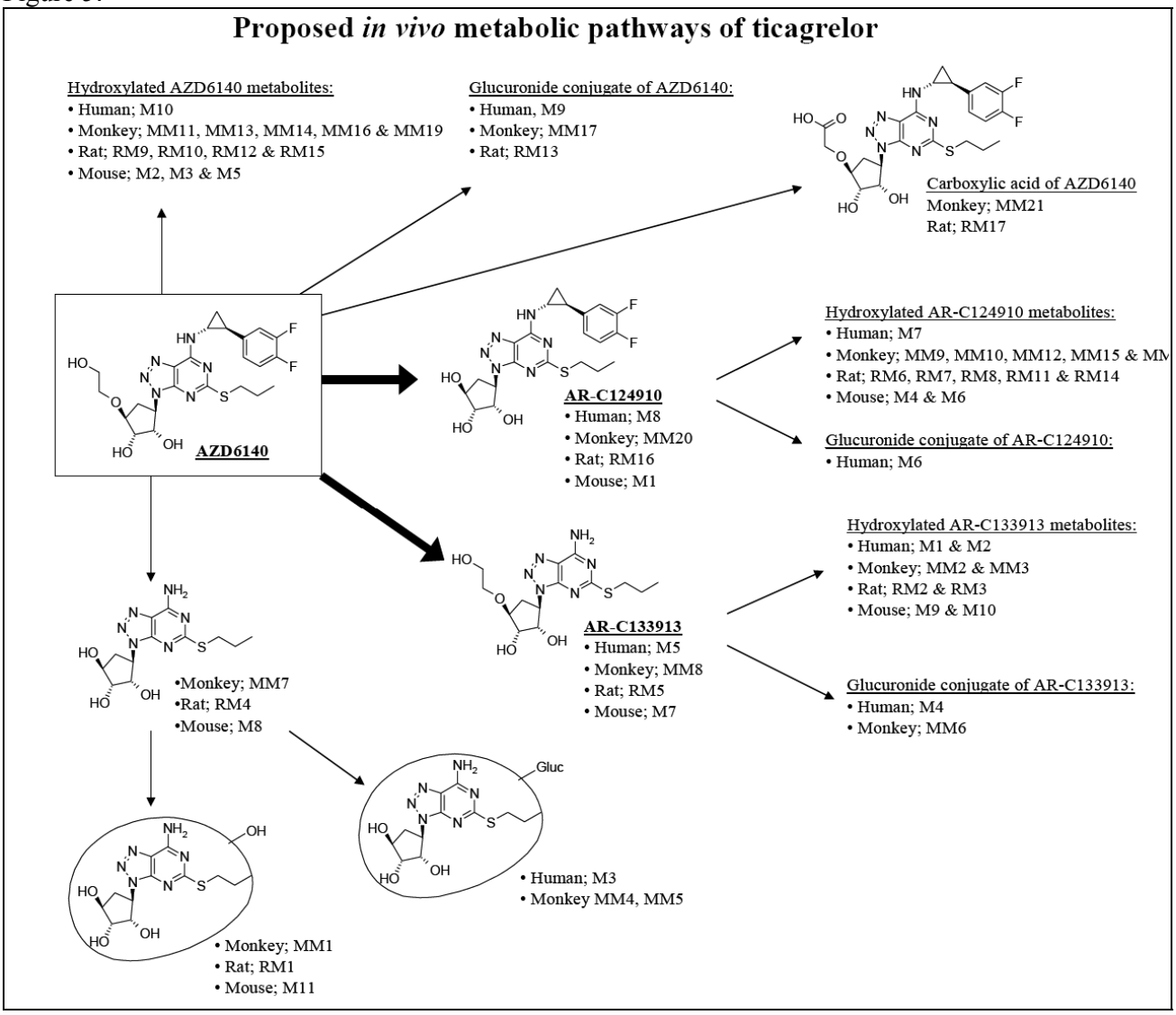
Table 18. Species ratios of AR-C124910XX $AUC_{(0-24)}$ to Ticagrelor $AUC_{(0-24)}$

species	Ratio of AR-C124910XX to Ticagrelor (ratio of AUC_{0-24})
human	30-35%
rat	30%
mouse	100%
dog	9%
marmoset	48%
cynomologous	135%

AR-C133913XX is formed by CYP3A4-mediated loss of the difluorophenyl-cyclopropyl group from ticagrelor. This metabolite has been listed (b) (4) in the drug substance.

The sponsor's proposed metabolic pathways for ticagrelor are shown below.

Figure 5.



Excretion: Excretion is predominantly fecal, independent of the route of administration, although modest amounts have been found in the urine. Experiments using bile duct-cannulated rats showed >70% of total radioactivity excreted in the bile within 24h of dosing. Fecal excretion

was >90% in the mouse, >80% in the rat, and approximately 60% in the marmoset. Urinary excretion was approximately 2% (mouse), 5% (rat) and 10% (marmoset) of the total radioactivity.

Ticagrelor and the metabolite AR-C124910XX were the major components found in feces 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. The sum of the excreted metabolite percentages shows that 85% of the administered material is accounted for in mice, 73% in humans, 55% in rats and 35% in marmosets. The disposition is not yet completely determined. A summary of excreted metabolites from the tested species is shown below.

Table 19. Reviewer's Modification of Sponsor's Comparison of Excreted Metabolites from Human, Rat, Mouse and Marmoset

sample	% of excreted dose									
	AZD 6140	Hydroxylated AR-C133913XX metabolites	HM3, MM4, MM5 (glucuronides of AZD6140 derivative))	Glucuronides of AR-C133913XX	AR-C133913XX	HM6: co-eluted with hydroxylated metabolitesAZD	Hydroxylated AR-C124910 metabolites[i]	AR-C124910XX	Glucuronides of AZD 6140	Hydroxylated AZD6140 metabolites
Human										
Urine po		2	2	7	9	1[h]			[h]	h
Feces po	27				3			22		
Mouse										
Urine po	2									
Urine iv	1									
Feces po	33				9[g]		12[j]	29		
Feces iv	34				6[g]		10[j]	24		
Rat										
Urine po					1					
Urine iv					1					
Feces po	41				6		1	6		
Feces iv	32				5			8		
Marmoset										
Urine po					3					
Urine iv		1		1	2					
Feces po	27				3			3		
Feces iv	22			2	2			5		

[a] AR-C124910XX
[b] AR-C133913XX
[f] Human: pooled faeces 0-48h; pooled urine 0-48h
[c] Mouse: pooled faeces 0-24h; pooled urine 0-48h
[d] Rat: pooled faeces 0-48h; pooled urine 0-48h
[e] Marmoset: pooled faeces 0-120h; pooled urine 0-120h
[f] Human: pooled faeces 0-48h; pooled urine 0-48h
[g] M7 co-eluted with M8 in mouse faeces
[h] HM6, HM9 and HM10 co-eluted in human
[i] structure not fully assigned in human but facile loss of H_2O and suggests β-OH
[j] M4 and M3 co-eluted in mouse faeces
– <1%
HM = human metabolites; M = mouse metabolites; RM = rat metabolite; MM = marmoset metabolite

Plasma Protein Binding AZD6140 and the active metabolite were both highly protein bound. The metabolite AR-C133913XX was less protein bound, approximately 50% in most of the tested species and up to 99% unbound in mice. The results are summarized in the reviewer's table below.

Table 20. Plasma Protein Binding of AZD6140, AR-C124910XX and AR-C133913XX in Mouse, Dog, Rabbit, Marmoset and Human

species	Unbound percentage of ticagrelor	Unbound percentage of AR-C124910XX	Unbound percentage of AR-C133913XX
Mouse	0.7	2.0	99
Dog	1.0	Not done	Not done
Rabbit	0.8	0.32	53
human	0.6	0.14	48
marmoset	0.9	0.37	48

Determination of the Free Fraction of AR-C124910XX and AR-C133913XX in Human, Rat, Mouse, Rabbit and Marmoset Plasma Using Tandem Mass Spectrometric Detection

Partial Validation of Method No. BA-542: Analytical Method For Determination of the Free Fraction of AR-C124910XX and AR-C133913XX in Human, Rat, Mouse, Rabbit and Marmoset Heparin Plasma Using LC-MS/MS

Study Number: YAT/079
Report number: BA-542-01
CRO reference number: YAT/079/V01
Experimental start date: August 17, 2007
Report dated March 25, 2008

Test article (analytical +internal standards)	batch	purity
AR-C124910XX	SN105687335	93%
AR-C133913XX	01-ktvs037-64	95.95%
(b) (4)	2743/164/1	99.67%

A method for determination of the free fraction and stability of AR-C124910XX and AR-C133913XX in rat, mouse, marmoset and human plasma was evaluated. This study compared plasma concentrations (protein bound) of the two metabolites to dialysate (free fraction) concentrations. The stability was assessed for approximately 26 hours of storage at 37°C. The determination of AR-C124910XX and AR-C133913XX from the plasma side of the dialysis cell (plasma retentate) used protein precipitation for the sample extraction followed by LC/MS/MS. The determination of AR-C124910XX and AR-C133913XX in dialysate used sample dilution, followed by LC/MS/MS.

The sponsor assessed selectivity of method, matrix effects, precision, and accuracy.

Selectivity: 3 replicates of plasma from each species were subject to equilibrium dialysis.
Dialysate was analyzed.
Control samples for both metabolites were required to have no peak evident or an area/height ≤20% of the mean peak area/ height for samples at the LLOQ

Matrix effects: 6 replicates of plasma samples from each species were subject to equilibrium dialysis. Internal standards were added and the samples analyzed. The mean accuracy of the observations was required to be within 85 – 115% of the nominal concentration.

For analysis of the dialysates, the following parameters were included:

- A set of calibration standards (one standard at each concentration level)
- Two replicates of each QC
- Dialysate samples resulting from the equilibrium dialysis of three replicates of control plasma from human, rat, mouse, rabbit and marmoset plasma, processed without internal standard.
- Matrix-related modification of ionization samples
- Precision of the equilibrium dialysis technique samples
- Two samples of phosphate buffered saline-acetonitrile, each run immediately after a sample at the upper limit of quantification
- A double blank
- A blank
- Sufficient samples of phosphate buffered saline, or other pre-designated samples, to make the total number of samples equal to 96

For analysis of the plasma from different species, the following parameters were included:

- A set of calibration standards (one standard at each concentration level)
- Two replicates of QCs at the LoQC and MeQC levels
- Six replicates of QCs at the HiQC level
- Six replicates of a HiQC sample maintained at nominal 37°C for approximately 26 hours
- Precision of the equilibrium dialysis technique samples
- A reagent blank
- A double blank

• A matrix blank

The lower limit of quantification was defined as the lowest concentration investigated using samples that gave acceptable precision (CV ≤20%) and accuracy (80-120%).

Table 21. Reviewer's Summary of Sample Volumes and Concentrations Ranges Studied

	AR-C124910XX		AR-C133913XX	
	Plasma retentate	dialysate	Plasma retentate	dialysate
For rat, rabbit, mouse, marmoset plasma				
Sample volume	100µl	75µl	100µl	75µl
Concentration range studied	5-5000ng/ml	0.25- 100ng/ml	5-2000ng/ml	10-1000ng/ml
Human plasma				
Sample volume	100µl	75µl	100 µl	75µl
Concentration range studied	2.5-2500 ng/ml	0.25- 100ng/ml	2-1000ng/ml	10-1000ng/ml

Results

The accuracy of the equilibrium dialysis technique, when measured by six replicates of one pooled source for each of the human, rat, mouse, rabbit or marmoset plasmas, met the pre-defined criteria for acceptance. The precision values obtained for AR-C133913XX in the rat, rabbit and marmoset experiments were outside the pre-defined acceptance criteria of 15% (for dialysate matrix-related modification of ionization experiments but ≤20%. It was postulated that this was due to non-specific binding of the analytes to dialysis membranes and the 96-well plate. To support this, the sponsor referenced Report YAT/095 (validation of this method in human plasma) as having demonstrated a high degree of non-specific binding of the analytes to plastic and glass containers when the analytes were present in aqueous environments.

The method was selective for AR-C124910XX and AR-C133913XX in dialysate produced from human, rat, mouse, rabbit and marmoset plasma. Any matrix-related effects within the dialysate were consistent between individuals.

AR-C124910XX and AR-C133913XX were observed to be stable in human, rat, mouse, rabbit and marmoset plasma when stored at nominal 37°C for approximately 26 hours.

Table 22. Summary of Results for AR-C124910XX

	Precision in dialysate	Precision in plasma retentate	dialysate		retentate	
			LLOQ	ULOQ	LLOQ	ULOQ
Rat	3.4	6.1	0.125 ng/ml	100 ng/ml	5ng/ml	5000ng/ml
Mouse	9.5	4.7	0.125 ng/ml	100 ng/ml	5ng/ml	5000 ng/ml
Rabbit	2.9	4.3	0.125 ng/ml	100 ng/ml	5ng/ml	5000 ng/ml
Marmoset	5.3	5.7	0.125 ng/ml	100 ng/ml	5ng/ml	5000 ng/ml
Human	9.4	3.7	0.125 ng/ml	100 ng/ml	2.5 ng/ml	2500 ng/ml

Table 23. Summary of Results for AR-C133913XX

	Precision in dialysate	Precision in plasma retentate	dialysate		retentate	
			LLOQ	ULOQ	LLOQ	ULOQ
Rat	17.0	11.2	5 ng/ml	1000 ng/ml	5ng/ml	2000 ng/ml
Mouse	16.7	10.5	5 ng/ml	1000 ng/ml	5ng/ml	2000 ng/ml
Rabbit	13.7	5.6	5 ng/ml	1000 ng/ml	5ng/ml	2000 ng/ml
Marmoset	13.0	3.0	5 ng/ml	1000 ng/ml	5ng/ml	2000 ng/ml
Human	9.9	3.7	5 ng/ml	1000 ng/ml	2.0 ng/ml	1000 ng/ml

YAT/083 Partial Validation of an Analytical Method for Determination of AZD6140, AR-C124910XX and AR-C133913XX in Rabbit Heparin Plasma Using Liquid Chromatography with Tandem Mass Spectrometric Detection ***April 5, 2007***

YAT/084 Partial Validation of an Analytical Method for the Determination of AR-C133913XX in Marmoset Heparin Plasma Using LC/MS/MS ***December 19, 2006***

YAT/085 Partial Validation of an Analytical Method for the Determination of AR-C133913XX in Mouse Heparin Plasma Using LC/MS/MS ***December 19, 2006***

YAT/086 Partial Validation of an Analytical Method for the Determination of AR-C133913XX in Rat Heparin Plasma Using LC/MS/MS ***December 19, 2006***

The studies listed above are adaptations of previously validated methodology [redacted] (b) (4) [redacted]) for human plasma samples. Similar concentration ranges of the analytes were examined across species. The studies were described as "partial" validation in that they did not examine animal plasma concurrently containing two different parent medications, e.g., aspirin and ticagrelor.

In the marmoset, mouse and rat studies, methodology for AR-C133913XX was assessed in plasma samples spiked also with AZD6140 and AR-C124910XX.

In each of the studies listed above, the methodology was evaluated for :

- Intra-run precision and accuracy for each analyte
- Selectivity, matrix-related modification of ionization and carryover
- Stability:
  - Subjected to >3 freeze-thaw cycles
  - After 24 hours storage at room temperature
  - When stored at -20° C

The sample volumes used and the concentration ranges studied are summarized in the reviewer's table below.

Table 24. Reviewer's Summary of Concentration Ranges Studied

Species	Sample volume	Concentration Range Studied		
		AZD6140	AR-C124910XX	AR-C133913XX
rabbit	25µl	5-5000 ng/ml	5-5000ng/ml	5-2000ng/ml
marmoset	25µl	Not done	Not done	5-2000 ng/ml
mouse	25µl			5-2000 ng/ml
rat	25µl			5-2000 ng/ml

Results

The precision and accuracy data met the pre-determined criteria.

Table 25. Reviewer's Summary of Methodology Results

		rabbit	marmoset	mouse	Rat
Intra-run Precision	AZD6140:	6-14%	Not assessed	Not assessed	Not assessed
	ARC124910XX:	6-17(at LLOQ)%	Not assessed	Not assessed	Not assessed
	AR-C133913XX	5-13%	5.4 -9.6%	6.4-11.8%	4.0-8.3%
Intra-run Accuracy	AZD6140:	88-110%	Not assessed	Not assessed	Not assessed
	ARC124910XX:	86-102%	Not assessed	Not assessed	Not assessed
	AR-C133913XX	89-102%	91.8-99.5%	98.7-109.0%	96.4-103.8%
Maximum # freeze-thaw cycles		3	3	3	3
Maximum storage period in plasma at room temperature		24 hours	24.5 hours	25.5 hours	27 hours
Maximum storage period in plasma at -20°C		48 days	78 days	76days	93

5.2 Toxicokinetics: Noted with the individual studies.

6 General Toxicology

6.1 Single-Dose Toxicity

Single oral doses of AZD6140 up to 2000 mg/kg were given to mice, rats, and dogs. Marmosets received single oral doses up to 1600 mg/kg. The rat micronucleus study used a dose of 2000 mg/kg.

Mice and rats given a single oral dose of 2000mg/kg showed no discernible clinical signs. No macroscopic findings were reported. Dogs were more sensitive to the gastrointestinal effects and showed vomiting and lose feces after a single oral dose of 50 mg/kg. Single oral doses up to 2000 mg/kg in dogs caused decreases in cholesterol, triglycerides, albumin and total protein. There were no macroscopic changes in any of these species after a single oral dose.

Marmosets tolerated the single exposure to 1600 mg/kg but died after repeated (18-21 doses) exposure to 2000 mg/kg. A repeat of this study with a high dose of 1000 mg/kg/day also caused unscheduled mortality within the first week of dosing. In a third marmoset study, a high dose of 200 mg/kg had to be discontinued after 8 doses caused unscheduled mortality.

Repeat-Dose Toxicity: Reviewed under the IND, reviews on file in DARRTS

These studies were reviewed under the IND as summarized in submissions 000, 016, 031, 068,358, 399, 523, 562, 566, and 569.

Toxicology was assessed in mice, rats, rabbits and marmosets. The metabolic differences between dogs and humans, primarily the amount of AR-C124910XX formed relative to AZD6140, described in Section 4.1, Primary Pharmacology, caused the sponsor to use marmosets as the non-rodent species instead of dogs. Pharmacokinetic studies showed measurable plasma levels of AZD6140, AR-C124910XX and AR-C133913XX in marmosets following oral administration. *In vitro* pharmacology studies showed that ticagrelor inhibits ADP-induced platelet aggregation in blood from marmosets with an IC_{50} value of 35nM (18.3 ng/ml). There was a reasonable expectation that oral administration of AZD6140 to marmosets in toxicology studies would produce pharmacodynamic effects.

Coagulation parameters were not monitored in all toxicology studies, only some of the marmoset studies. PT, APTT and platelet counts were not significantly affected except in the 12 month marmoset study. At 6 months, males in the high dose group of 200 mg/kg showed platelet counts increased by 63% ($p<0.01$) over the control group. At the same time point, all drug-treated females showed platelet counts that were significantly increased (up to 62%, $p<0.01$) over control. By the determination at 49 weeks, these patterns were no longer evident.

Non-clinical toxicology studies indicated the target organs of toxicity to be the gastrointestinal tract (dogs, rats, marmosets), liver (rats, marmosets), bone marrow (rats, marmosets), immune system (marmosets, rats), adrenal (rodents) and endocrine system (mice and female rats).

Across species, the hematology findings were relatively consistent in showing minor blood loss, i.e. subclinical bleeding, with evidence of regeneration. At doses of AZD6140 ≥250 mg/kg mice showed signs that included increased red cell distribution width (16% compared to control, $p<0.001$), increased reticulocytes(36% compared to control, $p<0.01$). Rats at doses ≥180mg/kg AZD6140 showed increased red cell distribution width (12%, n.s. compared to the control group) and reticulocytes (26%, $p<0.05$, compared to the control group). Marmosets at doses ≥50 mg/kg showed reticulocyte counts increased by 56% compared to the controls (not marked as significant). At the study high dose of 200 mg/kg, hemoglobin and red cell counts were decreased compared to the control group (13% and 10% respectively, n.s.). In each species, these changes did not always persist for the full duration of a study, and were not equally apparent in both sexes, suggesting adaptation or differences in sensitivity.

Gastrointestinal irritation showed different manifestations in different species. Rats given doses of ticagrelor ≥300 mg/kg/day showed gastric erosions, squamous hyperplasia, and inflammation. Marmosets given >10 mg/kg/day showed enteritis with mucosal atrophy, ulceration and hemorrhage. Dogs in a dose-ranging study showed vomiting and loose feces at all doses tested, with no NOAEL identified. The lowest dose tested in this sole canine toxicology study was 50 mg/kg.

Liver effects in rats occurred at doses ≥80 mg/kg and included indications of altered function or damage evidenced by decreased triglycerides (67%, $p<0.001$), increased AST (20%, $p<0.001$) or ALP (31%, $p<0.001$) when compared to the control groups. Centrilobular hypertrophy was inconsistently reported (mice ≥250 mg/kg/day; rats ≥180 mg/kg). Effects in marmosets were inconsistent. One marmoset study showed a 45% decrease in triglycerides (n.s.) and a 167% (not marked as significant) increase in AST compared to control at a dose of 200 mg/kg. Another marmoset study showed localized, chronic active inflammation in the liver at 20 and 100 mg/kg. The sponsor attributed this to trauma.

Immune system effects such as splenic lymphoid depletion, thymic atrophy and bone marrow atrophy were reported for rats given doses ≥300 mg/kg/day. Marmosets showed lymphocytolysis of the thymic cortex, follicular atrophy of lymph nodes, splenic lymphoid atrophy and bone marrow hemorrhage at doses ≥1000 mg/kg/day, also associated with unscheduled mortality. Some lymphoid atrophy was reported for doses of ≥20 mg/kg/day. In both the rat and the marmoset, there is a question as to how much the general debility associated with the high doses contributed to the immune system effects.

Foamy alveolar macrophages were reported in the lungs of rats at doses ≥180 mg/kg/day. These were described as minimal changes.

Genetic Toxicology

Both parent drug and the active metabolite have been examined for genetic toxicity in adequate studies with no signal apparent. The results are summarized in the reviewer's tables below.

Table 26. Reviewer's Summary of Genetic Toxicology Studies for AZD6140

Assay	Details	Concentrations tested	Results
Ames Assay	S. typhimurium strains TA1535, TA100, TA1537, TA98, TA102	3.3- 1000µg per plate	Negative ± S9. Range finding study. Precipitate and lawn reductions seen at 1000µg in all tester strains.
Ames Assay	S. typhimurium strains TA1535, TA100, TA102, TA98, TA1537	3.0- 3000 µg per plate	Negative ± S9. Precipitate reported at concentrations ≥300 µg/plate
In vitro mouse lymphoma assay	4hours+S9: 0.03-0.08 mmol/l ; 15.7-41.8 µg/ml 4 hours –S9: 0.01-0.03mmol/l ; 5.23-15.7µg/ml 24 hours –S9: 0.03-0.07mmol/l ; 15.7- 36.6µg/ml		4 hour incubation +S9 caused non-significant increase in mutants and increase in small colonies. 24 hour incubation –S9 showed an increase in mutant frequency that at one concentration was statistically significant ($p<0.05$)
Rat micronucleus assay	Wistar rats	Single oral dose of 2000 mg/kg	negative

Table 27. Reviewer's Summary of Genetic Toxicology Studies for AR-C124910XX

Assay	Details	Concentrations tested	Results
Ames Assay	S. typhimurium strains TA1535, TA100, TA102, TA98, TA1537	3.0- 3000 µg per plate	Negative ± S9. Precipitation reported for the highest concentration.
In vitro mouse lymphoma assay	preliminary(4 hours ± S9) and(24 hours –S9): 0.8-100 mmol/l ; 0.375 – 48.0mg/ml 4 hours +S9: 3-10mmol/l ; 1.44 – 4.80 mg/ml 4 hours –S9: 2-9 mmol/l ; 0.96- 4.32 mg/ml 24 hours +S9: 0.25-3.5 mmol/l ; 0.12-1.68mg/ml 4 hours –S9: 0.25-9mmol/l;0.12-4.32mg/ml		The 4 hour +S9 incubation showed a dose-related increase in mean mutation frequency and small colony count. This finding was not repeatable in a second assay.

7.2 Other Genetic Toxicity Studies

A plethora of genetic toxicology studies have been conducted for the numerous impurities that have appeared and been resolved in the [redacted] (b) (4) pathways for the drug. A few studies were reviewed under the IND (reviews available in DARRTS).The majority of studies were reviewed under

this NDA and are summarized in the reviewer's table shown below. The individual reviews may be found in the "Special Toxicology" section starting on page 79. As determined by Dr Tele, the review chemist for this NDA, the impurities of interest are (b) (4)

Table 28. Reviewer's Summary of Impurities and the Genetic Toxicity Studies Conducted for Them

(b) (4)

8 Carcinogenicity

The reviews of the carcinogenicity studies, sponsor's mechanistic studies, the summary package presented to the Executive CAC and the minutes of the Executive CAC meeting are on file in DARRTS under IND65808. The findings are briefly summarized here.

The sponsor conducted carcinogenicity studies in rats and mice, using study designs and doses that had received prior concurrence from the Executive CAC. The rat study used doses of 0, 20, 60, and 120 mg/kg/day (HD for males) and 180 mg/kg/day (HD for females). The mouse study used doses of 0, 50, 100 and 250 mg/kg/day.

The rat study demonstrated a significant decrease in female survival (Cox: $p=0.018$, Kruskal-Wallis: $p=0.0424$), possibly due to metastatic uterine neoplasia. Fourteen of the 31 HD females who died ahead of scheduled termination had uterine adenocarcinoma listed as the cause of death, based upon the pathologic criteria for metastasis. The increased incidence was statistically significant when compared to the combined controls ($p<0.001$). The major tumor findings were in females and included hepatocellular adenoma and uterine malignancies.

Table 29. Significant Tumor Findings for Rats

Reviewer's Summary of Significant Tumor Findings

Tumor name	CTR1	CTR2	Low	Med	High	P-value Exact method	P-value Trend test
Hepatocellular adenoma(females)	0/50	1/50	1/50	2/50	4/50	0.0104	0.0052
Uterine adenocarcinoma	6/50	4/49	5/50	6/50	21/50	0.0000	0.0000
Uterine squamous cell carcinoma	0	0	0	1/50	3/50	0.0060	0.0010

The statistical analysis is from the CDER statistical reviewer's report (on file in DARRTS u IND65808)

Table 30. Summary of Week 52 AUC Values for Ticagrelor and AR-C124910XX in Female Rats

Summary of Week 52 AUC values for ticagrelor and AR-C124910XX in female rats (Study 456993 (0508CR)) with margins to therapeutic human exposures

	Ticagrelor		AR-C124910XX		Combined	
Dose mg/kg/day	$AUC_{(0-24)}$ (µmol-h/L)	Exposure Margin[a]	$AUC_{(0-24)}$ (µmol-h/L)	Exposure Margin[b]	$AUC_{(0-24)}$ (µmol-h/L)	Exposure Margin[c]
20	31.3	1.5	9.33	1.2	40.6	1.4
60	163	8.0	39.4	4.9	202	7.1
180	595	29.3	195	24.3	790	27.9

[a] Exposure margins are calculated as the ratio of the animal exposures (AUC) listed in the table to the highest total daily ticagrelor AUC measured in humans following the 100 mg b.i.d. dose used in Study D5130C00008, converted to µmol-h/L by dividing by the molecular weight of ticagrelor and multiplying by 1000 (Day 28, 2X the 12-hour AUC of 5.3 µg·h/mL = 10.6/522.57 X 1000 = 20.3 µmol h/L total daily AUC). The 100 mg dose in study D5130C00008 is equivalent to the 90 mg dose formulated for use in PLATO.

[b] Exposure margins are calculated as the ratio of the animal exposures (AUC) listed in the table to the highest total daily AR-C124910XX AUC measured in humans following the 100 mg b.i.d. dose used in Study D5130C00008, converted to µmol-h/L by dividing by the molecular weight of AR-C124910XX and multiplying by 1000 (Day 28, 2X the 12-hour AUC of 1.9 µg·h/mL = 3.8/478 X 1000 = 8.0 µmol h/L total daily AUC). The 100 mg dose in study D5130C00008 is equivalent to the 90 mg dose formulated for use in PLATO.

[c] Combined Exposure margins were calculated as the rat:human ratio of the sum of ticagrelor and AR-C124910XX AUC values: (rat ticagrelor AUC + rat AR-C124910 AUC)/(human ticagrelor AUC + human AR-C124910XX AUC).

The sponsor identified several statistically significant findings in the mouse study. When CDER methods were used, the findings did not achieve statistical significance. All values fell within historical incidence ranges reported for control animals of the same strain.

Table 31.

Reviewer's Summary of CDER Statistical Findings for Female Mice

	Dose of AZD6140 (mg/kg)				P-value (Exact method)	P-value (Asymptotic method)
	Combined controls	50	100	250		
Bronchioalveolar adenoma	6	4	6	7	0.0867	0.0745
Broncioalveolar carcinoma	7	4	1	6	0.2801	0.2650
Follicular center cell lymphomas (Hematopoietic system)	11	12	12	6	0.6739	0.6658
Granulocytic leukemia	0	2	3	0	0.5234	0.5110

Table 32

Historical Control Tumor Incidences for Female Mice

Tumor	Historical incidence++	Total # organs #lesions	# studies using this diagnosis
		3143	
Bronchioloalveolar adenoma	1.67- 26.67%	299	49
Bronchioloalveolar carcinoma	0.77-18.37%	145	41
Follicular cell lymphomas	Not found in (b) (4) database		
		3192	
Granulocytic leukemia	0.77 -4.08%	7	5

++ spontaneous background lesions for mice of this strain and age at (b) (4) in 59 studies from 1987-2000.

Table 33. Mouse AUC_{0-24} Levels of AZD6140, Week 52

Mouse dose Mg/kg	Mouse AUC (µg h/ml)	Human AUC_{0-t} (µg.h/ml)	Ratio mouse/human exposure
50 males females	12.2 19.4	10.6	1.2X 1.8X
100 males Females	47.9 73.7		4.5X 6.9X
250 males females	174 224		16.4X 21.1X

*Human AUCτ , 2X the 12-hour AUC of 5.3µg.h/ml, following the 100 mg b.i.d. dose of AZD6140 in clinical study D5130C00008.

The sponsor then conducted *in vitro*, *ex vivo* and 2 additional *in vivo* studies to investigate the mechanism of the uterine tumors reported in the rat study. The studies examined steroid receptor binding, steroid metabolism in adrenal and ovarian cell cultures, aromatase inhibition, inhibition of testosterone metabolism, circulating hormone levels, CYP induction and inhibition (determined by protein and mRNA in liver, uterus and ovary).

Parent drug did not show significant affinity for estrogen, progesterone, testosterone, thyrotropin releasing hormone or vasopressin receptors. AR-C124910XX was not tested except for a fluorescence assay for estrogen receptor binding. The fluorescence assay was not acceptable due to lack of data for the positive controls and lack of documentation for the validity of the methodology. No information was presented for the other metabolites. While the parent drug did have several effects on steroid metabolism in cell cultures, it was not clear how, if at all, this translates to the *in vivo* situation.

This reviewer re-examined the fertility studies for evidence of hormonal imbalance. The male study (original review on file in DARRTS, sponsor sequence number 031) showed no findings of significance in the data as presented and nothing to suggest a disruption of hormonal balance. The most obvious effect in the female fertility study (reviewed under SDN 566) was a dose-related decrease in regular estrus cycles. There were no other findings of significance in the study as reported. Alterations in the duration and patterns of cyclicity are non-specific signs that may be related to a number of different causes.

This reviewer also re-examined the histopathology data from the 1, 3 and 6 month toxicology studies as well as the carcinogenicity study looking for the body weight changes, uterine weight changes and the histopathology changes typically associated with excess testosterone in a female rodent (Contraception July 2005. 72(1):77-80; J Steroid Biochem Molec Biol 1998.66(3):137-149.; Gynecol Obset Invest 1993; 36:29-33). These changes were not apparent in the data as presented.

(b) (4)

In a Response to Information Request on May 28, 2010, the sponsor has proposed a decrease in circulating prolactin as possible mechanism for the tumor findings. No plasma samples have been analyzed from either rats or humans to provide data for the evaluation of this hypothesis. It can't be said that the proposed metabolic effect is species specific for rodents without that final data.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

These studies were reviewed under the IND, amendment number 031 (male fertility study) and 122 (female fertility study, SDN 523). Reviews are on file in DARRTS.

Male fertility was assessed in rats who had received AZD6140 for 26 weeks. In addition to the usual parameters, the sponsor also evaluated sperm motility, total sperm count in the cauda epididymus and morphology. The sponsor did not explain the rationale for this extra level of analysis. There were no findings of significance in the data as presented up to and including the high dose of 180 mg/kg. The female fertility study showed an increased incidence of irregular duration of estrus cycles in drug-treated rats. This was observed at doses ≥10 mg/kg/day (no NOAEL identified)
.

Embryonic Fetal Development

These studies were reviewed under the IND, amendment numbers 024 and 031. The reviews are on file in DARRTS.

Embryo-fetal development studies (Segment II) were conducted in rats and rabbits. In both species, delayed development of liver and skeleton were seen. Rabbits also showed an increased incidence of retinal folding. Data was presented to support the retinal folding as a phenomenon inherited from a common ancestor, rather than drug related. In the rabbits, there was minimal maternal toxicity, primarily some weight gain compared to the control group. The rabbit HD of 63 mg/kg produced equivalent exposure to the human therapeutic dose based on comparison of AUC values. In rats, significant maternal toxicity (unscheduled mortality) was seen at the HD of 300 mg/kg (~7X the maximum proposed human therapeutic dose on the basis of surface area comparisons) but not at the MD of 100 mg/kg where the liver and skeletal effects were also seen.

Prenatal and Postnatal Development

Dose Ranging Study for the Prenatal and Postnatal Development Study

Study number: AA39254
Study location: In life at [redacted] (b) (4) bioanalytical at [redacted] (b) (4)
GLP: statement included
QA: yes
Experimental start date: December 13, 2006
Animals: Crl:WI(Han) Wistar rats
Test article: AZD6140, batch 06-009998AZ, purity 100%

Vehicle: 1% w/v sodium carboxymethylcellulose in 0.1% w/v polysorbate 80 in water for injection

Three groups of six mated female rats were given AZD6140 by daily oral gavage from Day 6 of gestation to day 7 of lactation.

Observations

Morbidity/mortality	Twice daily
Signs	Daily
Body weight	Days 0 and 6 of gestation then daily until Termination
Food consumption	days 0-6, 6-9, 9-15, 15-18, 18-20 of gestation then Days 1-4 and 4-7 of lactation
Pregnancy and parturition (F0 females)	duration of gestation abnormalities of delivery, nesting or nursing Behavior Number of implantation sites (ammonium sulphide)
Litter data	For each F1 litter, the following data were recorded: Number of male and female pups and viability External abnormalities Survival and weight on post natal days (PND) 1,4 And 7

Blood samples were collected from all dams and litters after dosing on PND 7 as shown in the sponsor's table shown below. All animals were euthanized following blood sampling. Samples were analyzed for plasma levels of AZD6140 using an LC-MS technique.

Table 34. Sponsor's Summary of Blood Sampling Schedule

Time after dosing	7 hours after dosing (Post-Natal Day 7)	24 hours after dosing (Post-Natal Day 8)
First 3 dams and litters per group	x	
Last 3 dams and litters per group		x

All dams and pups were given a macroscopic examination for abnormalities.

The sponsor's definitions of the following terms are shown:

Gestation index (%) = [(# of females with live pups)/ (#of pregnant animals)] x 100

Live birth index (%) = [(#pups born alive)/(#pups born)] x100

Viability index (%) = [(#pups alive day 4)/(# pups alive at birth)] x 100

Sex ratio (proportion of male pups) (%) = [(#males)/(#pups)] x 100

Results

There was no unscheduled mortality. There were no drug related clinical signs of any significance.

The samples of the dosing preparations were within ±5% of the target concentrations with one exception which was within 12% of the theoretical concentration.

Detectable levels of drug were found in all dams dosed with AZD6140. Detectable levels of drug were found in all MD and HD dams and none of the LD dams by 24 hours after dosing. The pups of all drug-treated dams had detectable levels of parent drug which at 24 hours exceeded the maternal exposure.

Table 35. Reviewer's summary of plasma AZD6140 levels (mean ±sd µmol/l)

Dose mg/kg/day	PND7 7 hours after dosing		PND8 24 hours after dosing	
	dams	pups	dams	pups
10	0.316±0.04 (n=3)	0.429±0.06 (n=3)	Not detected (n=3)	0.253±0.08 (n=3)
60	7.66 (n=2)	3.12 (n=2)	0.128±0.04 (n=3)	2.72±0.17 (n=3)
180	38.2±7.71 (n=3)	7.78±2.99 (n=3)	2.62 (n=2)	6.29 (n=2)

PND= post-natal day

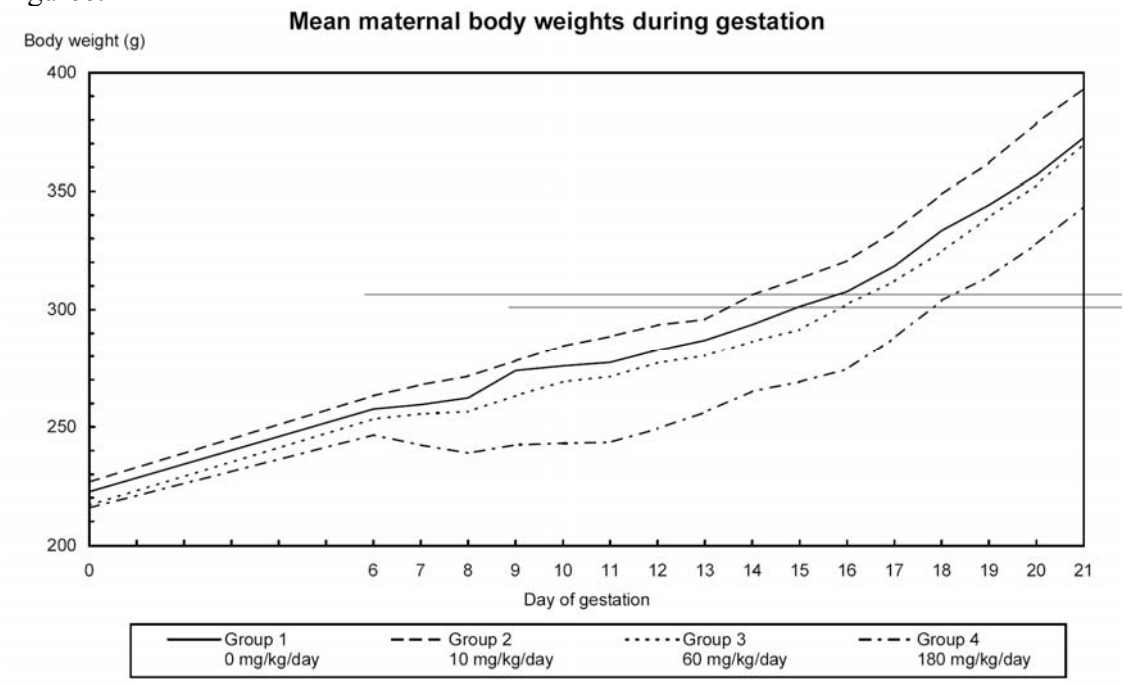
Body Weights

The high dose dams gained approximately 14% less than the control group, a difference that was not marked as significant. The sponsor's figure (shown below) indicates that the decrease in gain began shortly after initiation of drug exposure.

Table 36. Reviewer's summary of mean maternal body weight (g) (mean±sd) changes during gestation

Dose (mg/kg/day)	GD 6	GD21	Change from GD6-GD21
0	258±21	372±34	114
10	263±19	393±25	130
60	253±18	370±27	117
180	247±26	345±45	98 (14% < control)

Figure6.



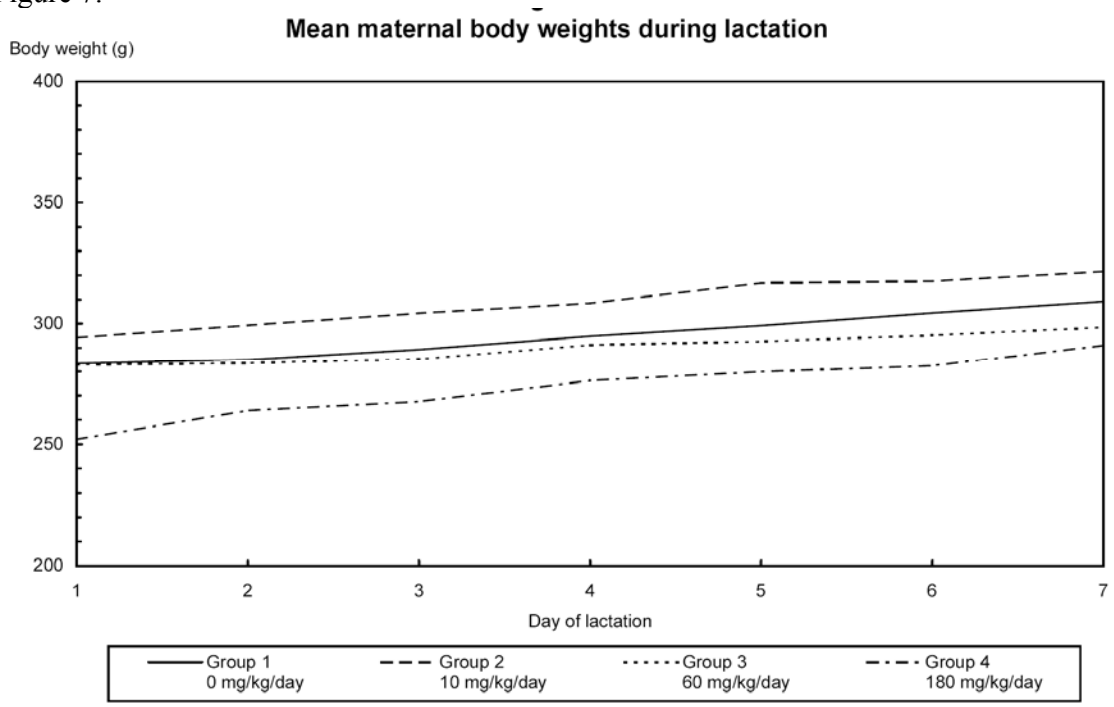
Maternal body weight changes during lactation

During lactation the high dose dams gained 50% more than the control group while the MD group gained approximately 50% less than the control group.

Table 37. Reviewer's summary of maternal body weight (g)(mean ±sd) changes during lactation

Dose (mg/kg/day)	LD1	LD7	Change from LD1 to LD7
0	283±26	309±29	26
10	294±18	322±17	28
60	283±19	299±17	16
180	252±36	291±31	39

Figure 7.



The female fertility index and number of females completing delivery were decreased at the MD and HD. The change in percentages was due to 1 out of 6 females in both dosing groups. Other parameters were apparently unaffected.

Table 38.

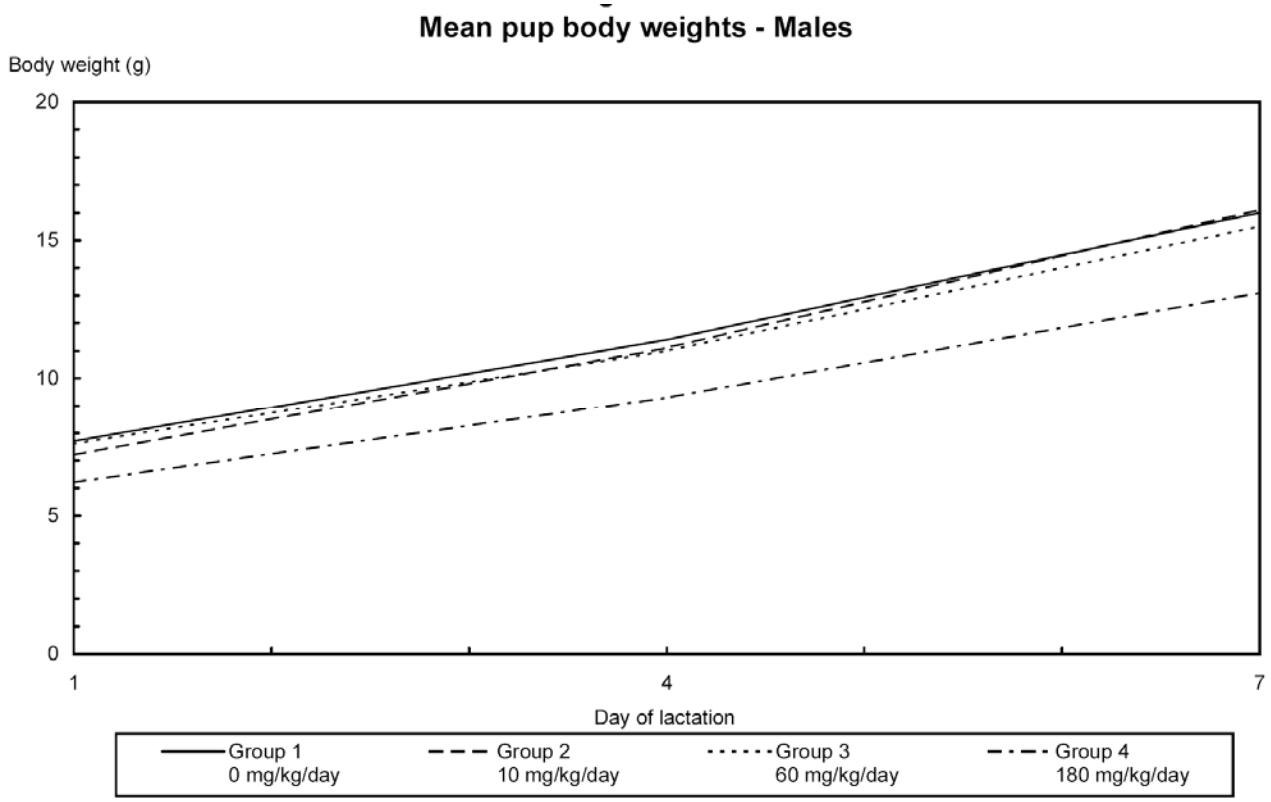
SUMMARY OF DELIVERY AND LITTER DATA

		Group 1 Control 0 mg/kg/day	Group 2 Low dose 10 mg/kg/day	Group 3 Intermed. dose 60 mg/kg/day	Group 4 High dose 180 mg/kg/day
Females on Study	N	6	6	6	6
Females Mated	N	6	6	6	6
Mating Index	%	100.0	100.0	100.0	100.0
Females Pregnant	N	6	6	5	5
Female Fertility Index	%	100.0	100.0	83.3	83.3
Females with Liveborn	N	6	6	5	5
Gestation Index	%	100.0	100.0	100.0	100.0
Females Completing Delivery	N	6	6	5	5
	%	100.0	100.0	83.3	83.3
with Stillborn Pups	N	1	2	0	0
	%	16.7	33.3	0.0	0.0
with all Stillborn	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Litters with Liveborn, but no Pups Alive					
day 4	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Duration of Gestation	MEAN	22.0	22.0	22.0	22.0
	S.D.	0.0	0.0	0.0	0.0
	N	6	6	5	5

Mean Pup Body Weights: Males

Male pups from the HD group were born at a lower mean body weight and gained at a somewhat slower rate than did pups from the other dose groups.

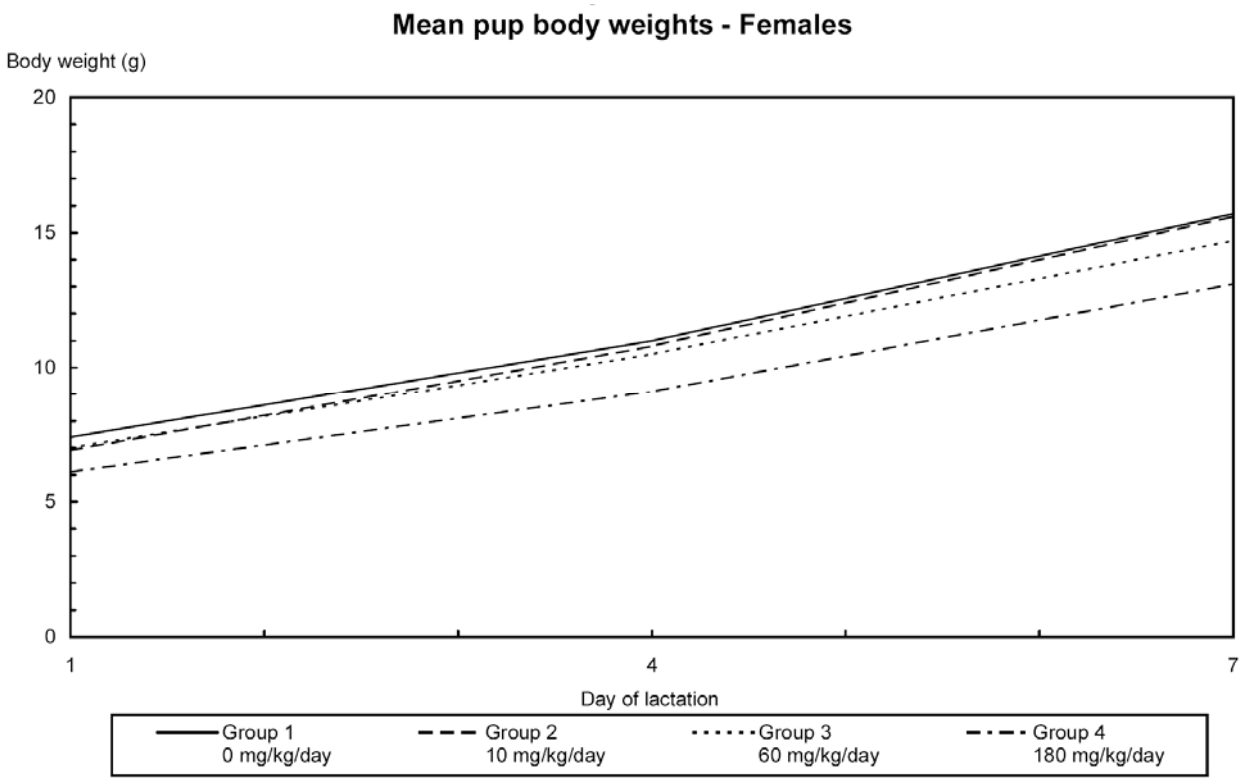
Figure 8



Mean Pup Body Weights: Females

The female pups of HD dams were born at a lower mean body weight than the other groups of pups and gained weight at a somewhat slower rate.

Figure 9.



Pup observations

Pale, thin, weak and/or cold pups were noted on post-natal days 4-7 in one HD litter. Two of these pups subsequently died (day not specified). A pup from another HD dam was pale post-natal days 1 - 4.

The mean pup weight at birth was lower in the HD group by comparison to the control by approximately 20%.

Pup necropsies did not reveal any significant lesions.

Table 39.

Study No.: AA39254 AZD6140 - DOSE RANGE-FINDING PRE- AND POST-NATAL DEVELOPMENT STUDY BY THE ORAL ROUTE (GAVAGE) IN THE RAT.

SUMMARY OF DELIVERY AND LITTER DATA

		Group 1 Control 0 mg/kg/day	Group 2 Low dose 10 mg/kg/day	Group 3 Intermed. dose 60 mg/kg/day	Group 4 High dose 180 mg/kg/day
Litters with Liveborn Pups	N	6	6	5	5
Pups Delivered (total)	N	62	79	57	61
	MEAN	10.3	13.2	11.4	12.2
	S.D.	2.1	1.6	1.5	1.3
Liveborn	N	61	77	57	61
Live Birth Index	%	98.4	97.5	100.0	100.0
Stillborn	N	1	2	0	0
	%	1.6	2.5	0.0	0.0
Liveborn, not culled prior to day 7	N	61	77	57	61
Pups Dying, Missing, and/or Cannibalized					
day 0	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
days 1-4	N	0	0	0	2
	%	0.0	0.0	0.0	3.3
days 5-21	N	0	0	0	1
	%	0.0	0.0	0.0	1.6
Pups Surviving 4 days	N	61	77	57	59
Viability Index	%	100.0	100.0	100.0	96.7
Pups Surviving 7 days	N	61	77	57	58
Lactation Index	%	100.0	100.0	100.0	98.3

The study achieved its purpose of determining doses that caused minimal maternal toxicity, produced systemic exposure in the pups and did not appreciably alter parturition or pup survival.

Pre and post-natal development oral gavage study in rats

Study number: AA41453
Study location: (b) (4)
Experimental start date: April 22, 2007
GLP: statement included
QA: yes
Vehicle: 1% w/v sodium carboxymethylcellulose in 0.1% w/v polysorbate 80
Test article: AZD6140, batch 06-009998 AZ, purity 100%
Animals: Wistar rats: Crl: WI(Han)

Groups of female rats (n=25) were given vehicle or AZD6140 at doses of 10, 60, or 180 mg/kg/day from GD6 to LD20.

Observations

Morbidity/mortality	twice daily
Signs	daily for F0 F1 animals received a full examination once a week
Body weights	weight for F0 females were recorded on GD 0, 6, 11, 15, 18 and 20 and LD 1, 4,7,10, 14, 17 and 21 F1 males: recorded weekly from time of selection to necropsy F1 females: weekly during the pre-mating and mating periods then on days 0,4,8 and 13 of gestation
Food consumption	F0 females only: recorded for GD 0-6, 6-11, 11-15, 15-18, 18-20 and LD1-4, 4-7, 7-10 10-14.
Pregnancy and parturition	F0 females only: Duration of gestation abnormalities of delivery, nesting or nursing behavior number of implantation sites (ammonium sulphide)
Litter data	number, sex and viability of pups

	external abnormalities of pups Number, weight, sex of pups alive on LD1, 4, 7, 14 and 21 Physical development: intra-litter onset and duration of pinna unfolding, incisor eruption, eye opening behavioral and functional development: surface righting reflex (LD8) gripping reflex (LD17) pupillary and auditory reflexes LD21 external and necropsy findings of dead pups
Lactation day 4:	Litters were culled to 8 pups where possible to have 4 males and 4 females per litter
LD21	After weaning F1 pups, one male and one female pup per litter were selected to give 25 males and 25 females per dose level for the behavioral tests and mating.
F1 ophthalmology	All retained pups in the vehicle control and HD groups were examined at 4 weeks of age. Adnexa, optic media and fundus were examined
Post weaning development and behavioral tests	
	F1 females examined daily from post-partum day 28 to detect vaginal opening. F1 males were examined daily from post-partum day 38 to detect balano-preputial skinfold opening. water maze: 5 and 6 weeks of age open field : 7 weeks of age
mating (F1)	Mated at approximately 11 weeks of age. One male and one female (avoiding siblings) were housed together for 1 week. Females were separated from males when mating had been confirmed by vaginal smears.

Necropsy	F0 females were necropsied after weaning of the F1 pups
	Mated F0 females failing to produce a viable litter on or after Day 26 post-coitum
	F1 pups not selected for further study were necropsied day 21 post-partum
	F1 males: euthanized after necropsy of the majority of F1 females
	F1 females who failed to mate were necropsied 13 days after end of mating period
	Culled pups were necropsied day 4 post-partum
	All adults were given a macroscopic examination.
Necropsy, F1 females	pregnancy status, Number of corpora lutea Intrauterine implantations Intrauterine deaths Early embryonic deaths (defined as only placenta visible at termination) Late fetal death (defined as both placenta and embryonic tissue visible at termination) The ammonium sulphide technique was used for all F1 uteruses
Organ weights	testes and epididymides of all F1 males

Sponsor's definitions

F0 Gestation index (in %): $\frac{\text{number of females with live pups}}{\text{number of pregnant females}} \times 100$

F0 Live birth index (in %): $\frac{\text{number of pups born alive}}{\text{number of pups born}} \times 100$

F0 Viability index (in %): $\frac{\text{number of pups alive on Day 4*}}{\text{number of pups alive at birth}} \times 100$

F0 Weaning (lactation) index (in %): $\frac{\text{number of pups alive on Day 21}}{\text{number of pups alive on Day 4*}} \times 100$

F1 Pre-coital interval (in days): $\frac{\text{sum of days until successful insemination}}{\text{number of inseminated females}}$

F1 Copulation index (in %): $\frac{\text{number of inseminated females}}{\text{number of paired females}} \times 100$

F1 Fertility index (in %): $\frac{\text{number of pregnant females}}{\text{number of inseminated females}} \times 100$

F1 Pre-implantation loss (in %): $\frac{\text{number of corpora lutea - number of implantations}}{\text{number of corpora lutea}} \times 100$

F1 Post implantation loss (in %): $\frac{\text{number of implantations - number of live embryos}}{\text{number of implantations}} \times 100$

Sex ratio (proportion of male pups in %): $\frac{\text{number of males}}{\text{number of pups}} \times 100$

Results

Formulation analysis: The analyses of dosing solutions were within ±6% of the nominal concentrations. The prepared formulations were within the targeted range.

Unscheduled mortality: One F0 LD female died during parturition after giving birth to 3 dead pups. Necropsy revealed 8 fetuses remaining in utero. There were no other unscheduled deaths during the gestation/parturition phase. All pups and one F1 male found dead were also necropsied. The report does not make clear on what day the F1 male died.

Signs: Four HD dams showed salivation on occasional days. This sporadic sign was not considered an adverse effect.

Maternal (F0) Body weights

The HD group lost weight in the interval between Days 6-11. The overall gestational weight gain in the HD group was significantly lower than in the other treatment groups. Food consumption was correspondingly decreased in the HD group.

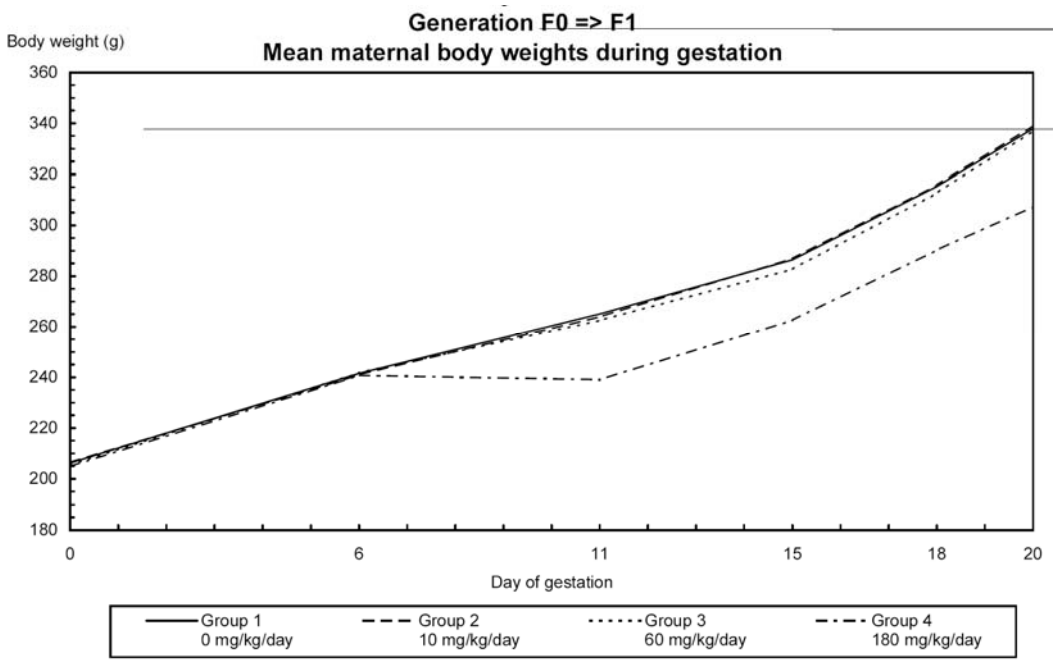
Table 40.

SUMMARY OF GESTATION BODY WEIGHTS (GRAMS)

		Group 1 Control 0 mg/kg/day	Group 2 Low dose 10 mg/kg/day	Group 3 Intermed. dose 60 mg/kg/day	Group 4 High dose 180 mg/kg/day
DAY 0	MEAN	206.2 d	206.4	205.4	204.8
	S.D.	11.4	12.8	13.1	13.0
	N	23	24	23	24
DAY 6	MEAN	241.5 d	241.0	241.8	240.7
	S.D.	14.5	14.7	16.1	15.9
	N	23	24	23	24
DAY 11	MEAN	265.1 d	263.9	262.5	239.0**
	S.D.	18.0	17.6	19.3	15.0
	N	23	24	23	24
DAY 15	MEAN	286.4 d	286.9	282.7	262.6**
	S.D.	19.4	19.7	22.0	19.3
	N	23	24	23	24
DAY 18	MEAN	315.3 d	315.8	312.7	290.3**
	S.D.	23.6	22.7	24.2	21.2
	N	23	24	23	24
DAY 20	MEAN	337.9 d	339.0	337.0	307.3**
	S.D.	28.4	25.0	28.1	25.9
	N	23	24	23	24

Statistical key: d=Anova/Dunnett test ** = p<0.01

Figure 10



Maternal body weight changes during lactation (post-partum)

The HD group gained weight at a greater rate post-partum than the other treatment groups. A reverse dose response in weight loss was apparent from Days 14-17: decreased weight loss was seen with increased dose of drug and a weight gain was seen at the HD.

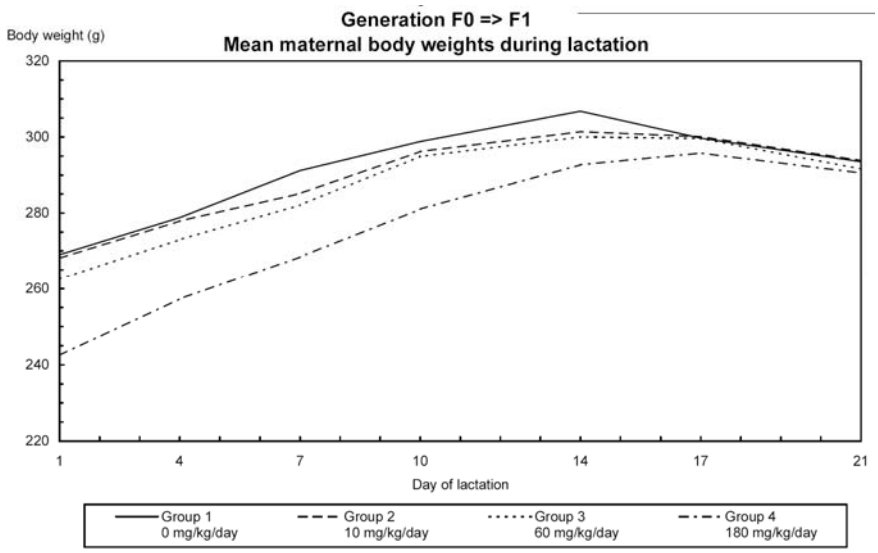
Table 41

SUMMARY OF BODY WEIGHT CHANGES POST-PARTUM (GRAMS)

		Group 1 Control 0 mg/kg/day	Group 2 Low dose 10 mg/kg/day	Group 3 Intermed. dose 60 mg/kg/day	Group 4 High dose 180 mg/kg/day
DAYS 1 TO 4	MEAN	9.8 d	9.7	10.5	14.8
	S.D.	6.9	8.4	6.4	8.7
	N	23	23	23	24
DAYS 4 TO 7	MEAN	12.4 d	7.3*	9.1	11.7
	S.D.	6.7	5.7	6.9	6.1
	N	23	23	23	22
DAYS 7 TO 10	MEAN	7.6 d	11.1	12.8*	12.6*
	S.D.	7.1	8.9	5.2	6.2
	N	23	23	23	22
DAYS 10 TO 14	MEAN	7.9 d	5.2	5.1	11.7
	S.D.	7.7	7.2	5.2	6.6
	N	23	23	23	22
DAYS 14 TO 17	MEAN	-7.1 d	-1.2*	-0.5**	3.1**
	S.D.	9.2	6.5	7.0	5.9
	N	23	23	23	22
DAYS 17 TO 21	MEAN	-6.2 d	-6.4	-7.9	-5.3
	S.D.	7.5	7.0	8.9	8.0
	N	23	23	23	22

Statistical key: d=Anova/Dunnett test * = p<0.05 ** = p<0.01

Figure 11.



Food consumption for the HD group post-partum was significantly less than the other groups, despite the increased rate of gain.

Table 42

SUMMARY OF FOOD CONSUMPTION POST-PARTUM (GRAMS/ANIMAL/DAY)

		Group 1 Control 0 mg/kg/day	Group 2 Low dose 10 mg/kg/day	Group 3 Intermed. dose 60 mg/kg/day	Group 4 High dose 180 mg/kg/day
DAYS 1 TO 4	MEAN	34.5 d	33.8	35.4	30.4*
	S.D.	5.2	5.0	5.0	6.1
	N	23	23	23	24
DAYS 4 TO 7	MEAN	46.0 d	44.2	43.2	39.9**
	S.D.	5.3	4.3	5.8	4.3
	N	23	23	23	22
DAYS 7 TO 10	MEAN	53.4 d	51.9	52.7	48.0**
	S.D.	7.1	6.0	4.8	5.1
	N	23	22	23	22
DAYS 10 TO 14	MEAN	59.2 d	58.4	57.5	51.5**
	S.D.	7.1	5.4	5.4	6.6
	N	23	22	23	22

Statistical key: d=Anova/Dunnett test * = p<0.05 ** = p<0.01

There were no signals of a drug-related effect in the delivery data. The sponsor's summary is shown below. By day 4, there were 2 HD litters out of 24 (8.3%) that no longer had living pups. No other dose group was similarly affected.

Table 43.

SUMMARY OF DELIVERY AND LITTER DATA

		Group 1 Control 0 mg/kg/day	Group 2 Low dose 10 mg/kg/day	Group 3 Intermed. dose 60 mg/kg/day	Group 4 High dose 180 mg/kg/day
Females on Study	N	25	25	25	25
Females Mated	N	25 f	25	25	25
Mating Index	%	100.0	100.0	100.0	100.0
Females Pregnant	N	23 f	24	23	24
Female Fertility Index	%	92.0	96.0	92.0	96.0
Females with Liveborn	N	23 f	23	23	24
Gestation Index	%	100.0	95.8	100.0	100.0
Females Completing Delivery	N	23 f	23	23	24
	%	92.0	92.0	92.0	96.0
with Stillborn Pups	N	0 f	1	1	1
	%	0.0	4.3	4.3	4.2
with all Stillborn	N	0 f	0	0	0
	%	0.0	0.0	0.0	0.0
Litters with Liveborn, but no Pups Alive					
day 4	N	0 f	0	0	2
	%	0.0	0.0	0.0	8.3
day 21	N	0 f	0	0	2
	%	0.0	0.0	0.0	8.3
Duration of Gestation	MEAN	22.0 d	21.8	21.9	22.0
	S.D.	0.2	0.4	0.4	0.4
	N	23	23	23	24

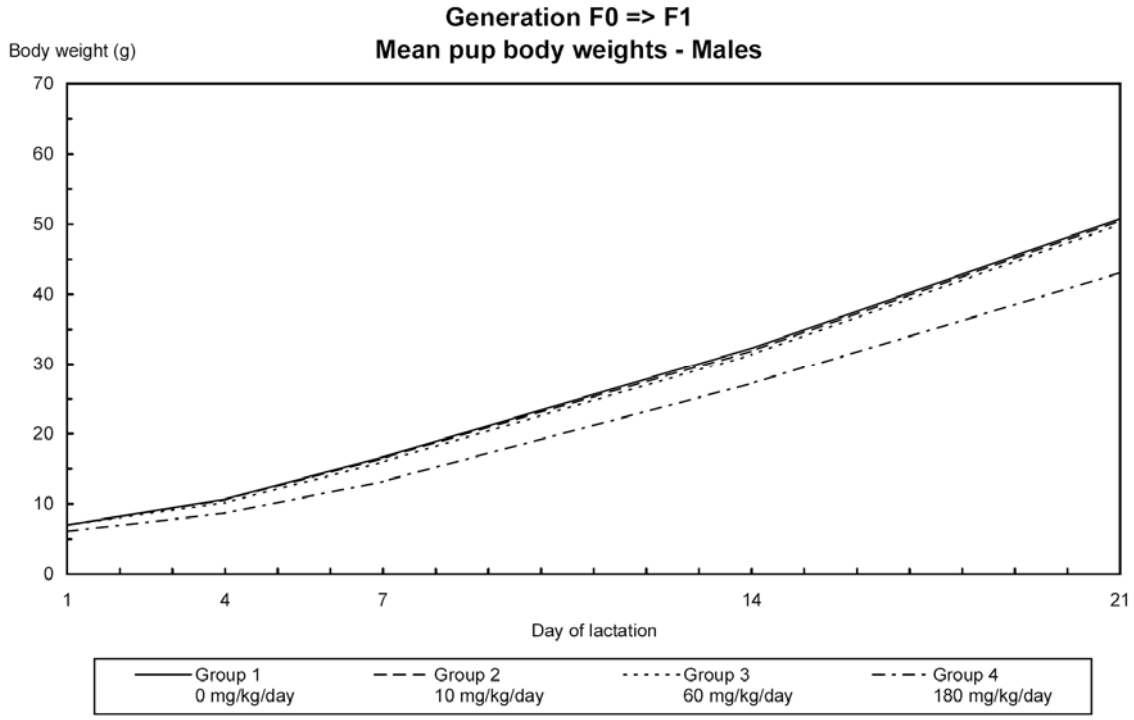
Statistical key: d=Anova/Dunnett test f=Chi2/Fisher Exact test

Corresponding to this, the pup data shows significantly decreased survival in the pups from HD dams with 22 out of 239 pups dying, missing or cannibalized in the first 4 days compared to 1 out 235 control pups ($p<0.01$) . None of the pups in the LD or MD groups were reported as affected.

Pup body weight: males

Males from HD dams weighed significantly less than the pups from other dose groups. By Day 4 post-partum the HD male pups weighed on average 20% ($p<0.01$) less than the control pups. By day 21, the HD male pups weighed on average 15% ($p<0.01$) less than the control pups.

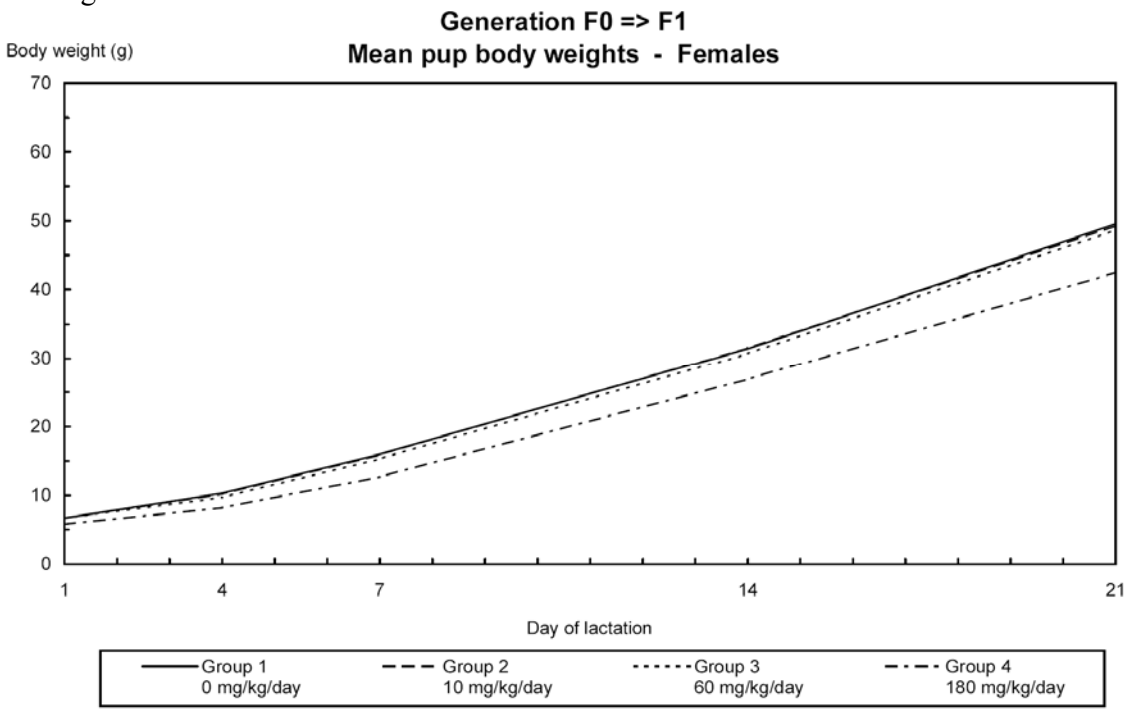
Figure 12



Pup body weight: females

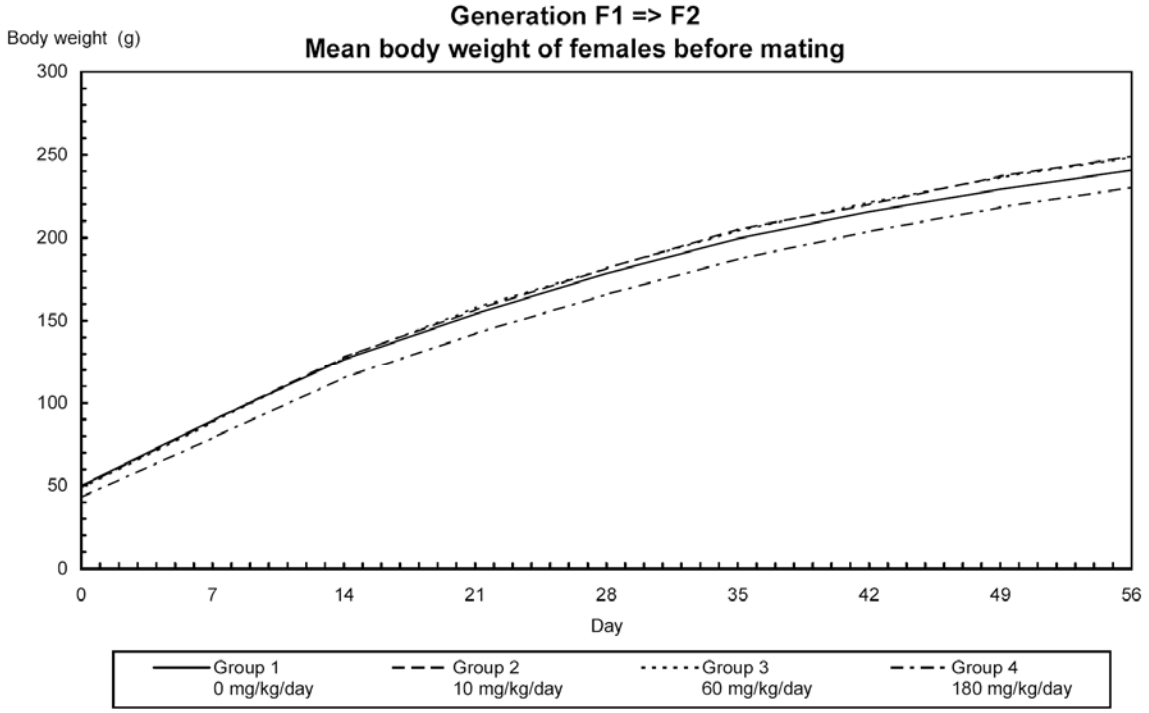
F1 females from HD dams weighed significantly less than pups from other dose groups. On post-natal day 4, the HD female pups weighed 20% (<0.01) less than the control group female pups. By day 21, the HD F1 female pups weighed on average 15% (p<0.01) less than the control female pups.

Figure 13



The last data point prior to mating of the F1 females was day 56. The HD females weighed on average 4% (n. s.) less than the control females. The sponsor's graph shows the mean body weights of F1 females.

Figure 14.



Reflex and Physical Development of F1

All pups from drug-treated dams showed dose-related delays in physical development. This was manifested in delays of pinna unfolding, incisor eruption, and eye opening. Surface righting reflex, gripping reflex, auditory reflex, papillary reflex were apparently unaffected. The sponsor's summary is shown below.

Table 44.

Summary of reflex and physical development

Group Treatment	1 0 mg/kg/day	2 10 mg/kg/day	3 60 mg/kg/day	4 180 mg/kg/day
PINNA UNFOLDING - % of pups positive:				
day 1 *post-partum*	0	0	3	0
day 2 *post-partum*	25	26	39	15
day 3 *post-partum*	94	87*	85**	77**
day 4 *post-partum*	100	100	100	99
day 5 *post-partum*	100			100
INCISOR ERUPTION - % of pups positive:				
day 7 *post-partum*	0	0	0	0
day 8 *post-partum*	0	2	0	0
day 9 *post-partum*	7	5	9	3
day 10 *post-partum*	22	27	30	11
day 11 *post-partum*	72	68	72	40**
day 12 *post-partum*	99	89	90	74
day 13 *post-partum*	100	97	99	96
day 14 *post-partum*		100	100	98
day 15 *post-partum*				100
EYE OPENING - % of pups positive:				
day 12 *post-partum*	1	1	3	0
day 13 *post-partum*	16	8	14	8
day 14 *post-partum*	76	60**	65	34**
day 15 *post-partum*	99	93	97	80
day 16 *post-partum*	100	100	100	99
day 17 *post-partum*				100

Sexual maturation: Both HD males and HD females achieved the outward physical signs associated with sexual maturation on the same days as their respective control groups. The HD female pups weighed on average 7% ($p<0.01$) less than the control female pups. The HD male pups weighed on average 9% ($p<0.01$) less than the control male pups.

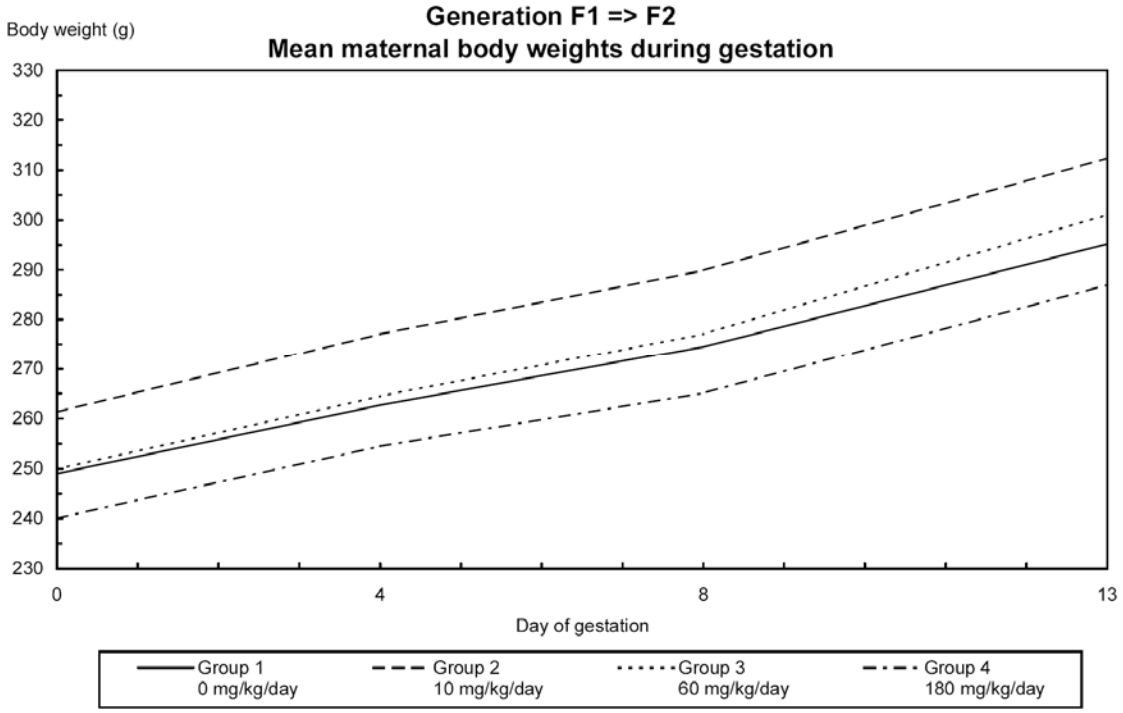
Watermaze Test: There were no findings of toxicological significance in the watermaze data.

Open Field Test: There were no findings of significance in the data presented.

Mating Performance and Fertility of the F1 Generations

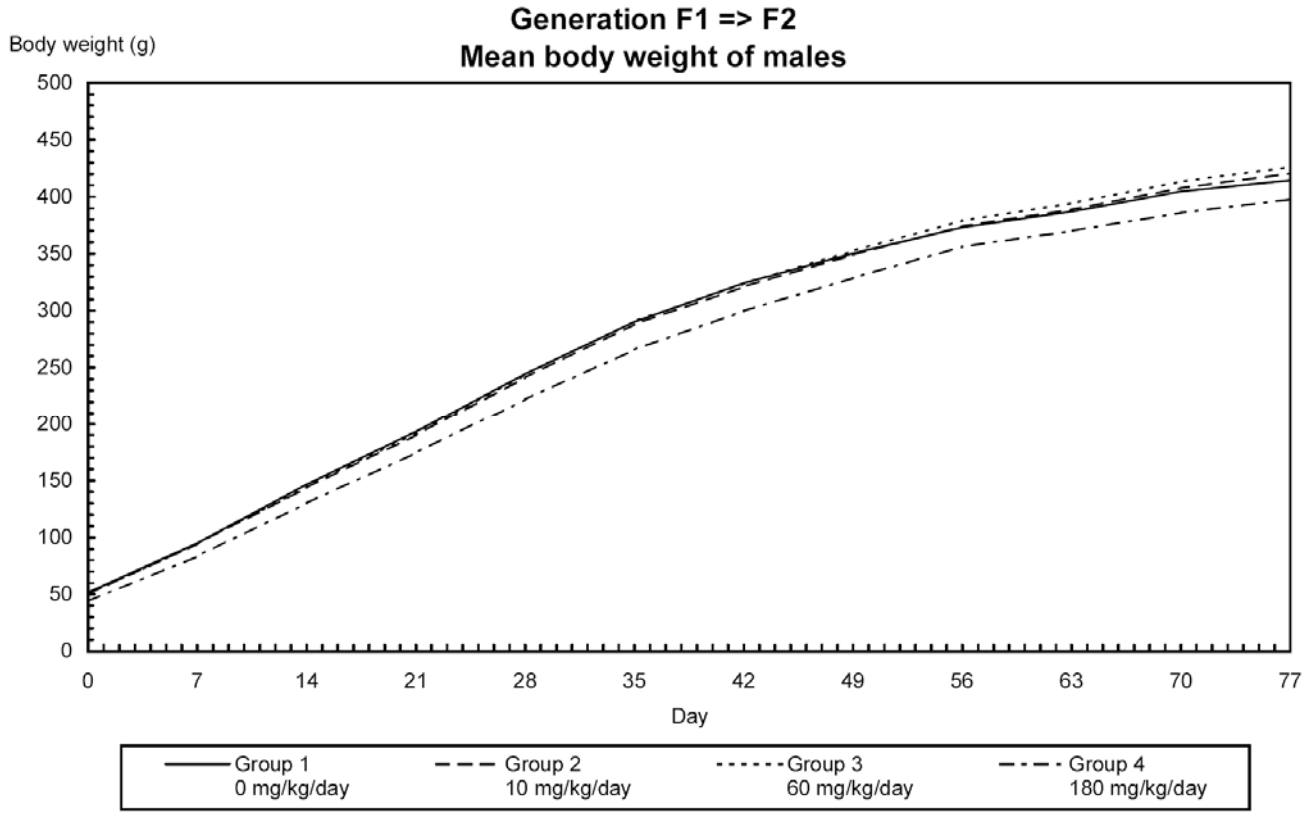
The HD F1 females started gestation at a lower mean body weight than the other groups. However, the weight gains for all the F1 groups (shown below) run in parallel lines, indicating similar rates of gain during gestation.

Figure 15



The HD males also began the mating period at a lower mean body weight than the control group. The last data point for the F1 males was Day 77 at which time the HD males weighed on average 4% (n.s.) less than the control group.

Figure 16



The F1 animals showed a slightly decreased fertility index. The sponsor's summary is shown below.

Table 45

Summary of mating performance and fertility
Generation F1=>F2

Group Treatment	1 0 mg/kg/day	2 10 mg/kg/day	3 60 mg/kg/day	4 180 mg/kg/day
Number of females:				
Paired	25	25	25	25
Inseminated	25	23	25	24
Pregnant	20	20	20	18
Pre-coital interval - days:				
MEAN	2.42	3.57	2.52	2.87
S.D.	1.02	3.49	2.40	3.20
N	24 (1)	23	25	23 (1)
Copulation index (%)	100	92	100	96
Fertility index (%)	80	87	80	75

(1) mating not detected for one pair of rats

The F1 HD group showed non-significantly lower numbers of corpora lutea, implantations and viable embryos falling within the historical control ranges.

Table 46. F1 Summary Data

SUMMARY OF CAESAREAN SECTION DATA

	Group 1 Control 0 mg/kg/day	Group 2 Low dose 10 mg/kg/day	Group 3 Intermed. dose 60 mg/kg/day	Group 4 High dose 180 mg/kg/day
Number of dams	20	20	20	17
Corpora Lutea				
per group	297	319	302	229
per dam: mean	15 d	16	15	13
st. dev.	2	3	3	3
Implantations				
per group	272 f	293	274	202
% of Corpora lutea	91.6	91.8	90.7	88.2
per dam: mean	14 d	15	14	12
st. dev.	3	3	2	3
Viable Embryos				
per group	253	274	260	190
per dam: mean	13 d	14	13	11
st. dev.	3	3	3	3
% of Implantations				
per group	93.0 f	93.5	94.9	94.1
per dam: mean	92 d	93	95	94
st. dev.	8	8	7	5
Postimplantation Loss				
total per group	19 f	19	14	12
% of implantations	7.0	6.5	5.1	5.9
per dam: mean	1 k	1	1	1
st. dev.	1	1	1	1
No. of dams affected	12	11	9	10
Preimplantation Loss				
per group	25 f	26	28	27
% of Corpora lutea	8.4	8.2	9.3	11.8
per dam: mean	1 k	1	1	2
st. dev.	1	1	2	1
No. of dams affected	15	13	13	13

Statistical key: d=Anova/Dunnett test f=Chi2/Fisher Exact test k=Kruskal-Wallis/Dunn test

Table 47.

Historical Control Data

Rat Wistar : Crl-WI

Caesarean data collected on, or close to, day 13 of gestation

Study	Year	Number of females				Number of corpora lutea		Number of uterine implants		Pre-implantation loss %		Post-implantation loss %		Live embryos	
		Paired	Mated	Pregnant	With live embryos	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
A4	2004	20	20	18	18	14.2	1.8	13.4	2.5	6.0	10.9	4.9	8.2	12.7	2.7
A5	2005	20	20	20	20	13.1	3.2	11.7	3.5	11.6	16.4	6.3	8.2	11.0	3.7
A6	2006	24	23	22	22	15.0	2.1	13.4	2.9	10.8	16.8	4.4	4.9	12.8	2.9
F6	2006	25	25	25	25	14.6	2.4	13.2	2.4	9.0	11.1	4.4	5.2	12.6	2.4
Total		*89*	*88*	*85*	*85*										
			99%	*97%*	*96%*										
Mean						*14.3*		*12.9*		*9.4*		*5.0*		*12.3*	
SD							*2.5*		*2.9*		*14.0*		*6.6*		*3.0*

Necropsy findings of F1 males: A small but statistically significant decrease in absolute testes weight was recorded for the HD males. However, this was not seen also in the normalized weight and is of doubtful, if any, biological significance.

A summary of ophthalmology findings or an ophthalmologist's report was not apparent in the report.

Special Toxicology: Impurities

(b) (4) Bacterial Mutation Assay

Study number: (b) (4)/YV6647
Study location: (b) (4)
Experimental start date: February 9, 2004
GLP: statement included
QA: statement included
Test article: (b) (4) batch reference 60165IL06, purity 99.6% by HPLC
Salmonella typhimurium strains used: TA1535, TA1537, TA98, TA100
Eschericia coli strains used: WP2(pKM101), WP2*uvr*A(pKM101)
Cultures were processed in triplicate.

Activation system: rat liver S9 from Sprague-Dawley rats treated with Phenobarbital/β-napthoflavone. The report did not specify how the activity was validated other than the use of controls

Positive controls:

+S9: Benzo[a]pyrene (BP), 2 aminoanthracene (2AA),
-S9: Acridine mutagen ICR191,daunomycin HCl(DR), N-Ethyl-N'-nitro-N-nitrosoguanidine (ENNG), mitomycin C (MMC), sodium azide (NaZ).

Formulation analysis was not performed, contrary to what is expected in a GLP study.

(b) (4) was initially assayed using the plate incorporation protocol over a concentration range of 100µg – 5000 µg per plate, ± S9. A pre-incubation protocol was used for the second phase of the study.

Under the conditions of this study, the test article did not produce an increase in revertants in any of the strains studied, either ± S9. A representative table of results is shown below. The positive controls produced appropriate responses. Negative control results fell within historical control ranges.

Table 48 (b) (4) Summary of Ames Results

Experiment: YV6647 Phase 1+

Date Plated: 09/02/2004
Date Counted: 12/02/2004

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA100	**Y12731/001**	5000µg	*210.7*	*11.0*	*1.4*	202, 207, 223
		2500µg	*213.7*	*28.9*	*1.4*	236, 224, 181
		1000µg	*205.0*	*7.5*	*1.3*	198, 213, 204
		500µg	*196.3*	*7.0*	*1.3*	189, 197, 203
		200µg	*169.7*	*13.9*	*1.1*	158, 166, 185
		100µg	*165.3*	*16.7*	*1.1*	175, 175, 146
	DMSO		*154.0*	*10.7*		162, 151, 142, 147, 168
TA1535	**Y12731/001**	5000µg	*13.3*	*2.5*	*1.1*	13, 11, 16
		2500µg	*17.7*	*4.5*	*1.5*	22, 13, 18
		1000µg	*19.7*	*6.5*	*1.7*	26, 13, 20
		500µg	*16.0*	*0.0*	*1.4*	16, 16, 16
		200µg	*11.3*	*4.0*	*1.0*	15, 12, 7
		100µg	*15.7*	*2.5*	*1.3*	13, 16, 18
	DMSO		*11.8*	*0.4*		11, 12, 12, 12, 12
TA1537	**Y12731/001**	5000µg	*15.3*	*7.8*	*0.6*	24, 13, 9
		2500µg	*17.3*	*2.5*	*0.7*	17, 20, 15
		1000µg	*22.3*	*9.2*	*0.9*	17, 33, 17
		500µg	*28.7*	*8.6*	*1.2*	27, 21, 38
		200µg	*25.7*	*5.5*	*1.0*	26, 31, 20
		100µg	*27.3*	*8.1*	*1.1*	18, 32, 32
	DMSO		*24.6*	*4.9*		27, 20, 32, 23, 21
TA98	**Y12731/001**	5000µg	*34.0*	*2.6*	*0.9*	32, 33, 37
		2500µg	*27.0*	*7.5*	*0.7*	20, 35, 26
		1000µg	*38.7*	*4.0*	*1.0*	43, 38, 35
		500µg	*29.7*	*7.0*	*0.8*	23, 29, 37
		200µg	*33.7*	*5.5*	*0.9*	39, 28, 34
		100µg	*36.7*	*8.5*	*1.0*	28, 37, 45
	DMSO		*37.0*	*7.6*		43, 29, 34, 47, 32
WP2 (pKM101)	**Y12731/001**	5000µg	*45.0*	*3.0*	*0.5*	48, 42, 45
		2500µg	*62.7*	*17.5*	*0.8*	48, 58, 82
		1000µg	*69.3*	*7.4*	*0.8*	72, 75, 61
		500µg	*67.3*	*4.9*	*0.8*	64, 73, 65
		200µg	*77.0*	*8.7*	*0.9*	83, 81, 67
		100µg	*76.3*	*7.1*	*0.9*	75, 70, 84
	DMSO		*83.2*	*5.0*		76, 89, 84, 86, 81
WP2 *uvr*A (pKM101)	**Y12731/001**	5000µg	*248.3*	*63.5*	*1.3*	250, 184, 311
		2500µg	*291.0*	*14.9*	*1.5*	280, 285, 308
		1000µg	*253.0*	*32.2*	*1.3*	283, 219, 257
		500µg	*219.0*	*5.6*	*1.1*	225, 214, 218
		200µg	*176.7*	*19.7*	*0.9*	189, 154, 187
		100µg	*201.3*	*8.4*	*1.0*	211, 196, 197
	DMSO		*193.6*	*21.9*		159, 185, 206, 206, 212

(b) (4) ***1225/*** (b) (4) ***in vitro cytogenetic assay in human lymphocytes.***
Study location: (b) (4)
Sponsor reference: AH/03/031
(b) (4) Study number: SV1225
GLP: statement included
QA: yes
Experiment started: January 13, 2004
Test article: (b) (4) batch 60165PIL06, purity 99.6% by HPLC
No analyses of stability, homogeneity or achieved concentration were carried out on the preparations of test substance, contrary to what is expected in a GLP study.

Vehicle: DMSO
Positive controls: mitomycin C and cyclophosphamide
S9: Rat liver derived. The report did not specify how the activity was validated other than via controls

(b) (4) was tested for clastogenic potential in an in vitro cytogenetic assay using pooled human lymphocytes from healthy male donors. Duplicate cultures were studied. In experiment 1, cultures were treated for 3 hours ±S9. In experiment 2, the cultures were treated for 3 hours +S9 and 20 hours –S9. The sponsor's summary table is reproduced below.

Table 49. Summary of Experimental Design

Experiment 1		Experiment 2	
+S9 mix 3 hr treatment	-S9 mix 3 hr treatment	+S9 mix 3 hr treatment	-S9 mix 20 hr treatment
500 µg/ml	500 µg/ml	1000µg/ml	200µg/ml
250 µg/ml	250 µg/ml	500µg/ml	100µg/ml
10 µg/ml	50 µg/ml	100µg/ml	50µg/ml

The sponsor investigated the effect of the test article on the pH and osmolality of the culture medium. Changes in these parameters have been shown to contribute to chromosomal aberrations in vitro.

The Quality Assurance statement lists slide coding. The report does not specify at what stage the coding occurred or the process.

For each experiment, both ±S9, duplicate cultures treated with (b) (4) at 3 concentrations were selected for analysis along with solvent and positive control cultures. Mitotic index was determined by examining 1000 lymphocytes per culture and calculating the percentage in metaphase. The slides were coded prior to aberration analysis and 100 cells in metaphase were selected from each culture for assessment of chromosomal damage. It is not at all clear how many slides were prepared per culture.

The criteria for negative and positive results were specified.

Results

Human lymphocytes incubated with (b) (4) showed a statistically significant increase in cells with chromosomal aberrations after 20 hours exposure only at the high dose in absence of metabolic activation. The sponsor's summary table is reproduced below:

Table 50. Mean Chromosomal Aberrations in the absence of metabolic activation

treatment		Mean% aberrant cells Excluding gaps	Mean % mitotic index
Experiment 1			
Solvent control	10 µl/ml	1.00	10.1
Mitomycin C	0.5 µg/ml	28.00**	7.0†
(b) (4)			
	500µg/ml	2.00	5.8
	250 µg/ml	2.50	6.1
	50µg/ml	2.50	9.8
Experiment 2			
Solvent control	10 µl/ml	3.00	9.8
Mitomycin C	0.2 µg/ml	56.00**	8.6†
(b) (4) 20 hours exposure			
	200µg/ml	14.67**	5.1
	100 µg/ml	4.00	8.2
	50µg/ml	5.50	8.7

**Statistically significant increase in the percentage of aberrant cells at $p<0.01$ by one-sided Fisher's exact test. †values for positive control determined from a single culture

Table 51. Historical control data for solvent controls

	+S9 mix	-S9 mix
Mean % aberrant cells excluding gaps	1.0±0.9 (n=241)	1.0±0.9 (n=267)
Range % aberrant cells	0.0 – 5.5	0.0- 4.0

Table 52. Mean Chromosomal Aberrations in the presence of metabolic activation

treatment		Mean% aberrant cells Excluding gaps	Mean % mitotic index
Experiment 1			
Solvent control	20 µl/ml	3.00	8.4
cyclophosphamide	50 µg/ml	40.00**	5.0†
(b) (4)			
	500µg/ml	3.50	3.6
	250 µg/ml	2.50	5.3
	50µg/ml	1.00	6.5
Experiment 2			
Solvent control	20 µl/ml	4.00	12.2
cyclophosphamide	50 µg/ml	52.00**	5.7†
(b) (4) 3 hour exposure			
	1000µg/ml	6.00	4.5
	500 µg/ml	6.50	9.5
	100µg/ml	2.00	11.2

**Statistically significant increase in the percentage of aberrant cells at $p<0.01$ by one-sided Fisher's exact test. †values for positive control determined from a single culture

The sponsor and the reviewer agree that under the conditions of the assay, a statistically significant increase, outside of the historical ranges, was observed in cultures treated for 20 hours in the absence of S9 mix.

(b) (4) Maximum Tolerated Single Oral Dose Toxicity in the Rat

Study number: 1402DR
Non GLP
Experimental start date: July 5, 2006
Test article: (b) (4) batch number and purity were unspecified.
Vehicle: unspecified.

This three page report did not specify analysis of formulation or determination of stability.
Two groups of 3 rats/group of unspecified strain were given single oral doses of either 51 or 85 mg/kg. The high dose group was apparently all male rats. The low dose group animals were of unspecified sex. No controls were used. Drug administration was followed by a 5 day observation period.

Two out of 3 of the HD rats were euthanized Day 1. One was found dead Day 2.

Two out of 3 of the LD animals had dark red adrenals and 2/3 had a slightly modular spleen with an "irregular" surface.

Of the two doses tested, 51 mg/kg permitted the three rats to survive to the end of the observation period but there were grossly observable changes in the spleen and adrenals.

(b) (4) Rat Micronucleus Test Following a Single Oral Dose

Report number: 1403 QR
GLP: statement included
QA: yes
Experimental start date: July 19, 2006
Test article: (b) (4) batch ACPS-G12, purity
Vehicle: 1% carboxymethylcellulose/ 0.1%Tween 80 in purified water

(b) (4) (b) (4) of AZD6140 that has been found in the final drug substance. Formulation analysis and stability determination were performed on samples of the test article.

(b) (4) was given at doses of 5, 25 and 50 mg/kg to groups of 7 male Han Wistar rats, approximately 9-10 weeks old. Two groups of 7 control rats were given the vehicle of 1% methylcellulose. Three rats were given the positive control of cyclophosphamide at 20 mg/kg.

Although it had been previously demonstrated that doses of 50 mg/kg could be tolerated for 5 days, excessive toxicity was seen in the present study and all animals in this dose group were euthanized were within 6 hours of dosing. The remaining animals were euthanized at 24 or 48 hours and bone marrow smears prepared. For each animal, 2000 immature erythrocytes (IE) were scored for the presence of micronucleated cells. The ratio of IE to mature erythrocytes was determined in 1000 cells as an estimate of bone marrow toxicity.

Results

The formulation analysis indicated that the content of test article in the dosing solutions was within ±5% of nominal. The results of the stability analysis were not located.

A statistically significant increase in number of micronucleated cells was reported for the highest dose analyzed. There was no significant change in the ratio of immature erythocytes to mature erythrocytes. The reviewer agrees with the sponsor's conclusion that 150 µmol/kg (25 mg/kg) of (b) (4) caused a statistically significant increase in micronucleated immature erythrocytes in the bone marrow of Han Wistar rats.

Table 53.

Mean incidence of micronucleated immature erythrocytes in rat bone marrow and percentages of immature erythrocytes

Group, Treatment and Dose level	Mean number of MIE in 2000 immature erythrocytes		Mean percentage of immature erythrocytes	
	24 hour	48 hour	24 hour	48 hour
1 Vehicle 10 ml/kg	1.3	ND	60.71	ND
2 (b) (4) 30 μmol/kg	1.0		63.59	
3 150 μmol/kg	3.9 **		62.77	
4 300 μmol/kg	ND	ND	ND	ND
5 Cyclophosphamide 72 μmol/kg	45.7 ***		44.43	

** $p<0.01$ Average square root transformation was performed. An ANOVA on transformed counts was carried out (5% level).
*** Fisher's Exact Test $p<0.001$.
ND Not Determined as the Group 4 animals were all terminated for welfare reasons 6 hours post dose

(b) (4) **Ames Assay**

Study Number: 1415BV
Study location: Astra Zeneca, Södertälje, Sweden
GLP: statement included
QA: yes
Experimental start date: May 25, 2007
Test article: (b) (4) batch 03-khds868-29, purity 89.9% by unspecified method. There was no discussion as to what comprised the other 11% of the test article.

Solvent: ethanol 99.7%
Activation system: rat liver S9. The activity was demonstrated by enzymatic assay and the ability to activate reference agents.
Bacterial strains used:
Salmonella typhimurium strains TA1535, TA100, TA98, TA1537
Escherichia coli WP2 *uvrA*/pKM101
Positive controls
-S9: sodium azide, 2-nitrofluorene, 9-aminoacridine, potassium dichromate
+S9: 2 aminoanthracene

The solvent control, test concentrations and positive controls were all tested in triplicate.
(b) (4) (b) (4) of AZD6140 that may be present in the drug substance.

(b) (4) was tested first using the plate incorporation method and subsequently using a liquid pre-incubation treatment. In both cases, the solvent used was ethanol 99.7% (V/V). Stability data showed minimal degradation over the course of the study. Formulation analysis was not performed due to lack of a validated analytical method.

Concentration ranges used:

Plate incorporation method 51.0 to 5100 µg (0.131 to 13.1 µmol) per plate
Liquid pre-incubation method 46.1 to 461 µg (0.118 to 1.18 µmol) per plate

Evidence of cytotoxicity as indicated by reductions in the growth of the background lawns or in the incidence of spontaneous revertant colonies was seen at the following concentrations in the plate incorporation assays:

-S9

≥510 µg/plate for strains TA1535, TA100 and TA1537,
≥1700 µg/plate for TA98
5100 µg/plate for E.coli uvrA/pKM101.

+S9

≥510 µg/ plate for strains TA1535, TA100 and TA1537,
≥1700 µg/plate for TA98
5100 µg/ plate for E.coli uvrA/pKM101.

In the pre-incubation assays, cytotoxicity was seen at the following concentrations:

-S9
≥ 46.1 µg /plate for all the Salmonella strains
≥323 µg/ plate for E.coli uvrA/pKM101

+S9
≥ 92.2 µg /plate for TA1535, TA100 and TA1537
≥ 184 µg /plate for TA98
323 µg/plate for E.coli uvrA/pKM101.

Under the conditions of the assay, an increase in revertants was not apparent.

Table 54

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Liquid pre-incubation test - without metabolic activation

Study Number : 1415BV
Experiment : 1415BV_L1 Start date : 2007-05-29 End : 2007-06-01
Test compound : (b) (4) Batch: 03-khds868-29
Solvent : ethanol 99.7 % (V/V)

(b) (4)

Bacterial strain and number of revertant colonies per plate

(Dose/plate) µg	µmol	TA1535 Mean		SD	TA100 Mean		SD	E.coli uvrA/pKM101 Mean		SD	TA98 Mean		SD	TA1537 Mean		SD
Solvent control		21		7	117		13	136		14	27		10	13		5
46.10	0.1180	15	t	9	50	t	12	140		2	31	t	3	9	t	2
92.20	0.2370	3	t	2	0	t	0	144		9	21	t	8	2	t	2
184.0	0.4730	0	t	1	0	t	0	118		12	15	t	5	1	t	2
323.0	0.8280	0	t	0	0	t	0	75	t	9	1	t	2	0	t	0
461.0	1.180	0	t	0	0	t	0	39	t	13	0	t	0	0	t	0
Positive control		326		35	458		19	832		56	300		36	382		10

Pos. controls TA1535:sodium azide 0.5 µg, TA100:sodium azide 0.5 µg, E.coli uvrA/pKM101:potassium dichromate 25 µg, TA98:2-nitrofluorene 0.5 µg, TA1537:9-aminoacridine 70 µg

t = Toxicity

Table 55

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Liquid pre-incubation test - with metabolic activation

Study Number : 1415BV
Experiment : 1415BV_L1 Start date : 2007-05-29 End : 2007-06-01
Test compound : (b) (4) Batch: 03-khds868-29
Solvent : ethanol 99.7 % (V/V)

(b) (4)

Bacterial strain and number of revertant colonies per plate

(Dose/plate) µg	µmol	TA1535 Mean		SD	TA100 Mean		SD	E.coli uvrA/pKM101 Mean		SD	TA98 Mean		SD	TA1537 Mean		SD
Solvent control		18		2	117		19	157		22	39		11	12		3
46.10	0.1180	22		4	144		8	165		16	39		6	13		4
92.20	0.2370	21	t	6	128	t	22	161		30	27		8	14	t	5
184.0	0.4730	13	t	3	74	t	7	124		19	24	t	6	7	t	1
323.0	0.8280	13	t	3	54	t	5	137	t	3	23	t	2	9	t	0
461.0	1.180	1	t	1	0	t	0	132	t	9	3	t	3	1	t	2
Positive control		134		12	592		24	735		53	871		41	63		6

(b) (4) Chromosome Aberrations in Cultured Human Peripheral Blood Lymphocytes

Study number: 1889/130
Reference number: 1419YV
Study location: (b) (4)
Experimental start date: unclear
GLP: statement included
QA: yes
Test article: (b) (4) batch 03-khds868-29, purity 89.92% by unspecified method
S9 : from Aroclor1254-induced rats. The activity was validated by the ability to convert known promutagens to bacterial mutagens and by assessment of alkoxyresorufin-O-dealkylase activities.
Positive controls:
-S9: 4-Nitroquinoline 1-oxide (NQO)
+S9: cyclophosphamide (CPA)
The report stated that "determinations of stability and characteristics of the test article were the responsibility of the Sponsor." Details of formulation analysis and stability determination were not located in the report.

Circulating lymphocytes were collected from the blood of healthy male volunteers. The design of the study and concentrations tested in the initial study are summarized in the sponsor's table below. Phytohemagglutinin (PHA) was used to stimulate the lymphocytes to divide.
Samples were analyzed in duplicate cultures at a minimum. Colchicine was used to arrest cells in metaphase.

Table 56

(b) (4) Concentrations Tested - Range-Finder

Cytotoxicity range-finder Concentration of treatment solution (mg/mL)	Final concentration (µg/mL)	Final concentration (µmol/mL)	Hours treatment + hours recovery 3+17 –S-9	3+17 +S-9	20+0 –S-9
0.9887	9.887	3.855	✓	✓	✓
1.412	14.12	5.505	✓	✓	✓
2.018	20.18	7.868	✓	✓	✓
2.882	28.82	11.24	✓	✓	✓
4.118	41.18	16.06	✓	✓	✓
5.882	58.82	22.93	✓	✓	✓
8.403	84.03	32.76	✓	✓	✓
12.00	120.0	46.79	✓	✓	✓
17.15	171.5	66.87	✓	✓	✓
24.50	245.0	95.53	✓	✓	✓
35.00	350.0	136.5	✓	✓	✓
50.00	500.0	195.0	✓	✓	✓

✓ Indicates concentration tested

Table 57

(b) (4) Concentrations Tested – Main Experiment

Main Experiment Concentration of treatment solution (mg/mL)	Final concentration (µg/mL)	Final concentration (µmol/mL)	Hours treatment + hours recovery 3+17 -S-9	3+17 +S-9	20+0 –S-9
0.800	8.000	3.119			✓
1.000	10.00	3.899			✓
1.200	12.00	4.679			✓
1.400	14.00	5.459			✓
1.600	16.00	6.238	✓	✓	✓
1.800	18.00	7.018			✓
2.000	20.00	7.798	✓	✓	✓
2.200	22.00	8.578			✓
2.400	24.00	9.358	✓	✓	✓
2.600	26.00	10.14			✓
2.800	28.00	10.92	✓	✓	✓
3.000	30.00	11.70			✓
3.200	32.00	12.48	✓	✓	
3.600	36.00	14.04	✓	✓	
4.000	40.00	15.60	✓	✓	
4.400	44.00	17.16	✓	✓	
4.800	48.00	18.72	✓	✓	
5.200	52.00	20.27	✓	✓	
5.600	56.00	21.83	✓	✓	
6.000	60.00	23.39	✓	✓	

✓ Indicates concentration tested

Table 58

(b) (4) Concentrations Tested – Main Experiment, Trial 2

Main Experiment, Trial 2 Concentration of treatment solution (mg/mL)	Final concentration (µg/mL)	Final concentration (µmol/mL)	Hours treatment + hours recovery 3+17 -S-9
1.000	10.00	3.899	✓
1.500	15.00	5.849	✓
2.000	20.00	7.798	✓
2.100	21.00	8.188	✓
2.200	22.00	8.578	✓
2.300	23.00	8.968	✓
2.400	24.00	9.358	✓
2.500	25.00	9.748	✓
2.600	26.00	10.14	✓
2.700	27.00	10.53	✓
2.800	28.00	10.92	✓
3.000	30.00	11.70	✓
3.500	35.00	13.65	✓
4.000	40.00	15.60	✓
5.000	50.00	19.50	✓

✓ Indicates concentration tested

Slides were examined uncoded to determine mitotic indices.

The slides selected for analysis were coded with randomly generated letters by an individual not associated with the scoring. Where possible, one hundred metaphases from each code were analyzed for chromosome aberrations. Where 10 cells with structural aberrations (excluding gaps) were noted on a slide, analysis may have been terminated. Only cells with 44 to 46 chromosomes were considered acceptable for analysis. Any cell with more than 46 chromosomes (that is, polyploid, hyperdiploid or endoreduplicated cells) observed during this evaluation was noted and recorded separately.

Under all conditions of testing, the (b) (4) treated cells showed increased chromosomal aberrations. At the higher concentrations used, the number of aberrations was similar to that of the positive controls.

Table 59

A1 3 hour treatment +S-9, 17 hour recovery (3+17), Main Experiment, Trial 1

Donor sex: male

Treatment (µg/mL)	Treatment (µmol/mL)	Replicate	Cells Scored	Cells with Aberrations Including Gaps	Cells with Aberrations Excluding Gaps	Significance §	MIH* (%)
Vehicle	Vehicle	A	100	3	0		
		B	100	1	0		
		Totals	200	4	0		-
Untreated	Untreated	A	100	0	0		
		B	100	2	2		
		Totals	200	2	2	NS	-
32.00	12.48	A	100	**13#**	**12#**		
		B	100	**12#**	**11#**		
		Totals	200	25	23	$p \leq 0.001$	3
36.00	14.04	A	70	**21#**	**20#**		
		B	60	**26#**	**20#**		
		Totals	130	47	40	$p \leq 0.001$	31
40.00	15.60	A	53	**22#**	**20#**		
		B	70	**22#**	**20#**		
		Totals	123	44	40	$p \leq 0.001$	55
CPA, 12.5	-	A	42	**21#**	**20#**		
		B	26	**20#**	**20#**		
		Totals	68	41	40	$p \leq 0.001$	

Binomial Dispersion Test $\chi^2 = 1.55$, NS
§ Statistical significance (Appendix E)

NS = not significant

*Mitotic inhibition (%) = [1 - (mean MI_T/mean MI_C)] x 100%

Table 60

A2 20 hour treatment -S-9, 0 hour recovery (20+0), Main Experiment

Donor sex: male

Treatment (µg/mL)	Treatment (µmol/mL)	Replicate	Cells Scored	Cells with Aberrations Including Gaps	Cells with Aberrations Excluding Gaps	Significance §	MIH* (%)
Vehicle	Vehicle	A	100	3	3		
		B	100	2	0		
		Totals	200	5	3		-
Untreated	Untreated	A	100	1	1		
		B	100	3	2		
		Totals	200	4	3	NS	-
12.00	4.679	A	100	**6#**	2		
		B	100	**6#**	3		
		Totals	200	12	5	NS	0
18.00	7.018	A	100	**9#**	**8#**		
		B	100	**11#**	**8#**		
		Totals	200	20	16	$p \leq 0.01$	15
22.00	8.578	A	100	**15#**	**13#**		
		B	100	**14#**	**12#**		
		Totals	200	29	25	$p \leq 0.001$	45
30.00	11.70	A	75	**18#**	**18#**		
		B	56	**20#**	**20#**		
		Totals	131	38	38	$p \leq 0.001$	59
NQO, 2.50	-	A	100	**17#**	**13#**		
		B	78	**23#**	**20#**		
		Totals	178	40	33	$p \leq 0.001$	

Binomial Dispersion Test χ^2= 5.43, NS
§ Statistical significance (Appendix E)
NS = not significant

*Mitotic inhibition (%) = [1 - (mean MI_T/mean MI_C)] x 100%

Table 61

A3 3 hour treatment -S-9, 17 hour recovery (3+17), Main Experiment, Trial 2

Donor sex: male

Treatment (µg/mL)	Treatment (µmol/mL)	Replicate	Cells Scored	Cells with Aberrations Including Gaps	Cells with Aberrations Excluding Gaps	Significance §	MIH* (%)
Vehicle	Vehicle	A	100	1	1		
		B	100	5	3		
		Totals	200	6	4		-
10.00	3.899	A	100	0	0		
		B	100	0	0		
		Totals	200	0	0	NS	8
15.00	5.849	A	100	1	1		
		B	100	1	1		
		Totals	200	2	2	NS	24
20.00	7.798	A	100	**7#**	**6#**		
		B	100	2	2		
		Totals	200	9	8	NS	33
23.00	8.968	A	87	**15#**	**15#**		
		B	100	**7#**	**7#**		
		Totals	187	22	22	$p \leq 0.001$	45
NQO, 2.50	-	A	94	**19#**	**19#**		
		B	100	**15#**	**15#**		
		Totals	194	34	34	$p \leq 0.001$	

Binomial Dispersion Test $\chi^2 = 7.80$, NS
§ Statistical significance (Appendix E)
NS = not significant

*Mitotic inhibition (%) = [1 - (mean MI_T/mean MI_C)] x 100%

(b) (4) Maximum Tolerated Oral Dose in the Rat

Study number: 1891DR
Non-GLP
Non-QA
Experimental start date: October 30, 2007
Test article: (b) (4) batch 03-khds868-48, purity 90.4%
Vehicle: 1% w/v carboxymethylcellulose and 0.1% w/v polysorbate 80 in water

(b) (4) (b) (4) of AZD6140. One or 3 rats (unspecified strain) per group received a single oral dose of (b) (4). No controls were used. The sponsor used the following study design:

Group 1: 300 mg/kg (b) (4)
Groups 2, 4, 5: 1000 mg/kg (b) (4)
Group3: 2000 mg/kg (b) (4)

The animals were given a single oral dose followed by a 7 day observation period. No formulation analysis was performed.

Results

2000 mg/kg: the single animal dosed was euthanized for welfare reasons within 2 hours after dosing. The animal showed ptyalism, decreased motor activity, increased depth of respiration, irregular respiration, and ptosis.

1000 mg/kg: all 3 animals showed decreased activity between 2 hours post dose and day 2. A single animal showed piloerection from 5 hours post-dose until day 3. Initial body weight losses (up to 9%) were also noted. Recovery of body weight was reported from day 4. The study ended with a net gain in weight.

300 mg/kg: the single animal dosed showed excessive chewing between 1 and 3 hours post dose. Body weight loss of <2% was reported for day 2 only.

The sponsor concluded that a single oral dose of 1000 mg/kg is the MTD.

(b) (4) Rat micronucleus evaluation after a single oral dose

Study number: 1885QR
Study location: Safety Assessment UK, Astra Zeneca R&D Alderley, Macclesfield, England
Experimental start date: November 27, 2007
GLP: statement included
QA: yes
Test article: (b) (4) 03-khds868-48, purity 90.4% by unspecified method
Vehicle: 0.1% w/v polysorbate 80 in purified water
Positive control: cyclophosphamide

(b) (4) was given to male HanWistar rats, 7/dose group, 10 weeks old at time of dosing. The doses are summarized in the table below. Formulation analysis was not performed, contrary to what is expected in a GLP study.

Table 62. Summary of Doses for Rat Micronucleus

dose	Time of sampling post dosing
0, 100, 500, 1000 mg/kg (b) (4)	24 hours
0, 1000 mg/kg (b) (4)	48 hours

At euthanasia, bone marrow smears were prepared. For each animal, 2000 immature erythrocytes (IE) were scored for the incidence of micronucleated cells (MIE) and the ratio of IE to mature erythrocytes (E) was determined in 1000 cells.

Three animals from the HD group were euthanized on day 1 for humane reasons. Adverse signs were seen from 1 hour post-dose and peaked from 6-24 hours after dosing.
The signs reported in the individual animal observations table included hunched posture, piloerection, decreased activity, pupils dilated, shallow respirations, splayed limbs, irregular respiration, ataxia, eyes partially closed (ptosis?). Signs were reported for 1/7 MD animals and 5/5 HD animals.
There was no apparent increase in the incidence of micronucleated immature erythrocytes as shown in the sponsor's table shown below.

Table 63.

Mean incidence of micronucleated immature erythrocytes in rat bone marrow and percentages of immature erythrocytes

Group, treatment and dose level	Mean number of MIE in 2000 immature erythrocytes		Mean percentage of immature erythrocytes	
	24 hour	48 hour	24 hour	48 hour
1 Vehicle 10 mL/kg	1.6	1.7	65.8	62.1
2 (b) (4) 100 mg/kg	1.1		62.5	
3 500 mg/kg	1.3		63.5	
4 1000 mg/kg	1.9	1.8[a]	59.0	61.6
5 Cyclophosphamide 20 mg/kg	37.3***		51.0	

*** Fisher's Exact Test p<0.001.
[a] Data based on a group size of 4 animals

Under the conditions of the study, there was no significant increase in the mean number of micronucleated immature erythrocytes. There was also no significant change in the mean percentage of immature erythrocytes.

(b) (4) In Vitro Cytogenetic Assay in Human Lymphocytes

Study number: (b) (4) 1226
Study location: AZ, Alderley Park Macclesfield, Cheshire, UK
GLP: statement included
QA: yes
Experimental start date: January 20, 2004
Test article: Ba 1, purity 99.3% by GC
Solvent: DMSO
Positive control: mitomycin C (- S9)
Cyclophosphamide (+ S9)
S9: from rat livers. The report does not detail how activity was assessed.

(b) (4) an unspecified impurity of AZD6140, was evaluated for its clastogenic potential in an *in vitro* cytogenetic assay using human lymphocytes ± S9. It is not clear why rat liver S9 was used instead of human S9. No analyses of stability, homogeneity or achieved concentration were carried out on either the test substances or the positive controls, contrary to what is expected in a GLP study.

In the first experiment, duplicate lymphocyte cultures from pooled blood from 2 healthy, non-smoking, females were treated for 3 hours ±S9.

In experiment 2, cultures from pooled blood were treated for 3 hours +S9 and 20 hours in the absence of S9. Treatment of the cultures started approximately 48 hours after culture initiation.

Solvent, untreated and positive controls were included in both experiments. Colcemid was added to the cultures approximately 2 hours prior to harvest.

The mitotic index was determined by examining 1000 lymphocytes per culture and calculating the percentage of cells in metaphase. For each experiment, both ±S9, duplicate cultures treated with (b) (4) at three concentrations were selected for chromosomal aberration analysis along with the appropriate control cultures. The slides were coded prior to analysis and 100 cells in metaphase were analyzed from each of the selected cultures for structural chromosome damage.

Table 64. Sponsor's Summary of Study Design for Chromosomal Aberrations

Experiment 1		Experiment 2	
+S9	-S9	+S9	-S9
3 hour treatment	3 hour treatment	3 hour treatment	20 hour treatment
5000 µg/ml	5000 µg/ml	5000 µg/ml	5000 µg/ml
2500 µg/ml	2500 µg/ml	2500 µg/ml	2500 µg/ml
500 µg/ml	500 µg/ml	500 µg/ml	1000 µg/ml

In the absence of S9, there was no observed increase in chromosomal aberrations. A slight but statistically significant increase in chromosomal aberrations was seen +S9 in one study but not both. The sponsor reports the results to be within the historical ranges.

Table 65

MEAN CHROMOSOMAL ABERRATIONS AND MITOTIC INDICES I
THE PRESENCE OF METABOLIC ACTIVATION (S9-MIX)

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Treatment		Mean % Aberrant Cells Excluding Gaps	Mean % Mitotic Index
Experiment 1			
Solvent Control	10µl/ml	0.50	10.6
Cyclophosphamide	50µg/ml	36.00**	7.1Δ
(b) (4)			
	5000µg/ml	4.00*	9.8
	2500µg/ml	2.00	11.4
	500µg/ml	1.50	10.1
Experiment 2			
Solvent Control	10µl/ml	5.00	8.6
Cyclophosphamide	50µg/ml	64.00**	6.0Δ
(b) (4)			
	5000µg/ml	1.50	7.2
	2500µg/ml	2.50	8.5
	500µg/ml	2.00	8.7

Table 66

APPENDIX 1 - HISTORICAL CONTROL DATA

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April 1995 – December 2002

Solvent controls

	68 hour sampling time	
	+S9-mix	-S9-mix
Mean % aberrant cells excluding gaps	1.0 ± 0.9 n = 241	1.0 ± 0.9 n = 267
Range (% aberrant cells excluding gaps)	0.0 – 5.5	0.0 – 4.0

(b) (4) **Bacterial Mutation Assay**
Report number: YV6645
Study location: Astra Zeneca, Macclesfield, Cheshire, UK
GLP: statement included
QA: Yes
Salmonella typhimurium strains used: TA1535, TA1537, TA98, TA100
Escherichia coli strains used: WP2(pKM101) and WP2*uvrA*(pKM101)
Positive controls:

+S9: 2-aminoanthracene (2AA) , Benzo[a]pyrene (BP)
-S9 : Acridine mutagen ICR191, daunomycin HCl (DR), N-ethyl-N'-nitro-N-nitrosoguanidine(ENNG), mitomycin C (MMC), sodium azide (NaZ)

Activation system: S9 from Phenobarbital/ β-napthoflavone-induced Sprague-Dawley rats. Other than the use of system controls, the report did not specify the manner of validating activity.
Test article: (b) (4) reference number Y12732/001, purity 99.3% by GC

(b) (4) was initially assayed using the plate incorporation protocol over a dose range of 100-5000µg per plate ±S9. Plates were processed in triplicate. The test substance was subsequently re-tested in all six strains over the same concentration range. The second assay was conducted using a pre-incubation protocol. The incubation period for each experiment was 3 days at 37° C. No analyses of stability, homogeneity or achieved concentrations of the test substances were performed.

Toxicity and precipitation were not indicated on the data tables. Under the conditions of the study there was no significant increase in revertants in any strain of bacteria.

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Table 67

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA100	Y12732/001	5000µg	163.7	29.7	1.1	198, 146, 147
		2500µg	162.0	18.6	1.1	174, 158, 154
		1000µg	162.3	14.6	1.1	178, 160, 149
		500µg	171.0	7.9	1.1	168, 165, 180
		200µg	165.3	23.9	1.1	147, 195, 154
		100µg	154.0	7.2	1.0	162, 152, 148
	DMSO		154.0	10.7		162, 151, 142, 147, 168
TA1535	Y12732/001	5000µg	11.0	2.0	0.9	13, 11, 9
		2500µg	12.7	4.7	1.1	11, 18, 9
		1000µg	14.7	4.2	1.2	18, 10, 16
		500µg	13.7	4.0	1.2	16, 16, 9
		200µg	13.7	7.0	1.2	13, 7, 21
		100µg	11.0	5.3	0.9	7, 9, 17
	DMSO		11.8	0.4		11, 12, 12, 12, 12
TA1537	Y12732/001	5000µg	19.3	8.0	0.8	27, 20, 11
		2500µg	25.7	3.8	1.0	24, 30, 23
		1000µg	25.3	4.6	1.0	28, 20, 28
		500µg	25.0	7.5	1.0	33, 18, 24
		200µg	19.0	1.7	0.8	20, 17, 20
		100µg	23.3	2.5	0.9	26, 23, 21
	DMSO		24.6	4.9		27, 20, 32, 23, 21
TA98	Y12732/001	5000µg	34.3	3.2	0.9	33, 38, 32
		2500µg	29.3	18.6	0.8	31, 10, 47
		1000µg	40.0	7.0	1.1	37, 48, 35
		500µg	27.3	7.1	0.7	26, 35, 21
		200µg	37.7	12.0	1.0	50, 37, 26
		100µg	30.0	4.4	0.8	28, 27, 35
	DMSO		37.0	7.6		43, 29, 34, 47, 32
WP2 (pKM101)	Y12732/001	5000µg	65.7	7.5	0.8	73, 66, 58
		2500µg	56.3	6.4	0.7	60, 60, 49
		1000µg	52.3	4.7	0.6	47, 56, 54
		500µg	78.7	5.1	0.9	73, 80, 83
		200µg	75.7	9.0	0.9	86, 71, 70
		100µg	68.3	10.2	0.8	80, 64, 61
	DMSO		83.2	5.0		76, 89, 84, 86, 81
WP2 uvrA (pKM101)	Y12732/001	5000µg	181.7	9.2	0.9	171, 187, 187
		2500µg	200.7	6.0	1.0	207, 195, 200
		1000µg	188.7	11.4	1.0	198, 192, 176
		500µg	200.3	9.0	1.0	191, 201, 209
		200µg	208.0	11.5	1.1	197, 207, 220
		100µg	186.3	9.8	1.0	192, 192, 175
	DMSO		193.6	21.9		159, 185, 206, 206, 212

Key to Plate Postfix Codes

Table 68 BEST AVAILABLE COPY

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA100	**Y12732/001**	5000µg	*119.3*	*10.1*	*0.7*	110, 118, 130
		2500µg	*118.0*	*15.0*	*0.6*	103, 118, 133
		1000µg	*132.0*	*6.6*	*0.7*	133, 138, 125
		500µg	*120.0*	*17.3*	*0.7*	100, 130, 130
		200µg	*123.3*	*11.7*	*0.7*	136, 113, 121
		100µg	*121.3*	*8.0*	*0.7*	122, 113, 129
	DMSO		*181.6*	*36.5*		206, 209, 189, 185, 119
WP2 (pKM101)	**Y12732/001**	5000µg	*40.7*	*10.2*	*1.1*	29, 48, 45
		2500µg	*47.0*	*6.6*	*1.3*	48, 40, 53
		1000µg	*41.0*	*7.2*	*1.1*	47, 43, 33
		500µg	*40.0*	*9.5*	*1.1*	29, 43, 48
		200µg	*40.0*	*7.2*	*1.1*	38, 48, 34
		100µg	*48.7*	*4.7*	*1.3*	45, 47, 54
	DMSO		*36.4*	*3.8*		38, 31, 39, 34, 40
WP2 uvrA (pKM101)	**Y12732/001**	5000µg	*161.0*	*15.9*	*1.1*	179, 155, 149
		2500µg	*173.3*	*18.8*	*1.2*	177, 190, 153
		1000µg	*131.7*	*4.2*	*0.9*	133, 127, 135
		500µg	*135.7*	*5.5*	*1.0*	141, 136, 130
		200µg	*159.7*	*25.1*	*1.1*	181, 166, 132
		100µg	*153.3*	*15.3*	*1.1*	165, 159, 136
	DMSO		*141.6*	*9.3*		154, 136, 147, 141, 130
TA100	**NaZ**	2µg	*399.0*	*89.6*	*2.2*	435, 465, 297
WP2 (pKM101)	**MMC**	1µg	*214.7*	*62.0*	*5.9*	260, 144, 240
WP2 uvrA (pKM101)	**ENNG**	1µg	*440.7*	*28.6*	*3.1*	468, 443, 411

Key to Positive Controls

NaZ	Sodium Azide
MMC	Mitomycin C
ENNG	N-Ethyl-N'-nitro-N-nitrosoguanidine

Key to Plate Postfix Codes

UL11 : Ames assay

Report number: 1416bv
Study location: Safety Assessment, Södertälje, Sweden
GLP: statement included
QA: yes
Experimental start date: June 5, 2007
Vehicle: ethanol 99.7% (v/v)
Test article: (b) (4), batch 48114, purity 96.6%
Positive controls:
-S9: sodium azide, 2-nitrofluorene, 9-aminoacridine, potassium dichromate
+S9: 2-aminoanthracene
Salmonella typhimurium LT2: strains TA1535, TA100, TA98 and TA1537
Eschericia coli strains used: WP2 strain uvrA/pKM101
Activation system: Aroclor 1254-induced rat liver (S9). Metabolic capacity was demonstrated by enzymatic assay and the ability to activate reference agents to bacterial mutagens.

(b) (4) AZD6140 that can be present in the drug substance of AZD6140. Formulation analysis was not performed for this study due to lack of a validated analytical method, contrary to what is expected in a GLP study.

Two separate plate incorporation tests were performed. The dose ranges used were:

Plate incorporation Test 1: 50.7 to 5070 µg (0.189 to 18.9 µmol) per plate

Plate incorporation Test 2: 0.508 to 50.8 µg (0.00190 to 0.190 µmol) per plate

Results

The test compound was cytotoxic to the bacteria as indicated by decreases in the growth of the background lawns or in the incidence of spontaneous revertant colonies.

First test:

-S9
no Salmonella bacteria survived in the absence of S9 mix at any concentration.
≥50.7 µg E.coli uvrA/pKM101 showed evidence of toxicity
≥507 µg no E.coli uvrA/pKM101 survived

+S9
≥169µg no Salmonella survived.
≥169µg E.coli uvrA/pKM101 showed evidence of toxicity

≥507 µg no E.coli uvrA/pKM101 survived

Second test:

-S9

16.9µg per plate toxicity in the E.coli uvrA/pKM101

≥ 5.08 µg per plate, toxicity seen for all other strains

+S9

50.8µg toxicity was seen for TA1535, TA100, TA1537 only

No toxicity reported for E.coli uvrA/pKM101 and TA98. Therefore higher concentrations could have been used.

Under the conditions of the assay, no consistent increase in revertants was seen.

Table 69

Table 6 Plate incorporation Test 2 - without metabolic activation

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Study Number : 1416BV
Start date : 2007-06-12 End : 2007-06-15
Test compound : (b) (4) Batch: 48114
Solvent : etanol 99.7 % (v/v)

(b) (4)

Bacterial strain and number of revertant colonies per plate

(Dose/plate) µg	µmol	TA1535 Mean		SD	TA100 Mean		SD	E.coli uvrA/pKM101 Mean		SD	TA98 Mean		SD	TA1537 Mean		SD
Solvent control		22		4	122		9	157		9	35		3	10		2
0.508	0.00190	25		5	120		5	167		3	34		4	10		1
1.69	0.00632	21		3	127		2	162		10	34		9	14		3
5.08	0.0190	18	t	4	100	t	13	122		7	38	t	8	8	t	1
16.9	0.0632	11	t	1	53	t	7	79	t	9	17	t	2	6	t	4
50.8	0.190	0	t	0	0	t	0	27	t	9	0	t	0	0	t	0
Positive control		412		16	492		40	992		9	406		32	950		50

Pos. controls TA1535:sodium azide 0.5 µg, TA100:sodium azide 0.5 µg, E.coli uvrA/pKM101:potassium dichromate 25 µg, TA98:2-nitrofluorene 0.5 µg, TA1537:9-aminoacridine 70 µg

t = Toxicity

Table 70

Table 7 Plate incorporation Test 2 - with metabolic activation

BEST AVAILABLE COPY

Study Number : 1416BV
Start date : 2007-06-12 End : 2007-06-15
Test compound : (b) (4) Batch: 48114
Solvent : etanol 99.7 % (v/v)

(b) (4)

Bacterial strain and number of revertant colonies per plate
(Dose/plate)

µg	µmol	TA1535 Mean	SD	TA100 Mean	SD	E.coli uvrA/pKM101 Mean	SD	TA98 Mean	SD	TA1537 Mean	SD
Solvent control		22	2	130	3	202	6	54	4	14	7
0.508	0.00190	24	2	160	21	198	16	46	2	15	2
1.69	0.00632	22	4	151	10	216	22	47	7	19	2
5.08	0.0190	19	4	106	9	181	15	41	6	11	3
16.9	0.0632	20	2	122	15	173	16	47	4	13	4
50.8	0.190	15 t	5	101 t	8	158	9	42	8	14 t	2
Positive control		260	28	2052	24	758	22	2131	96	337	47

Pos. controls TA1535:2-aminoanthracene 2 µg, TA100:2-aminoanthracene 2 µg, E.coli uvrA/pKM101:2-aminoanthracene 5 µg, TA98:2-aminoanthracene 2 µg, TA1537:2-aminoanthracene 5 µg
t = Toxicity

(b) (4) ***In vitro cytogenetic assay in human lymphocytes***

Study location: (b) (4)
Sponsor reference: AH/03/033
(b) (4) reference: (b) (4) 1227
GLP: statement included
QA: yes
Study date: initiated January 20, 2004
Test substance: (b) (4) batch Ba101/03, purity 99.0% by HPLC
Positive controls: mitomycin C, cyclophosphamide

(b) (4) was evaluated for clastogenic potential in an in vitro cytogenetic assay using human lymphocytes ±S9 from rats. Cultures were treated for 3 hours both ±S9. The sponsor's summary of conditions is reproduced below.

Table 71. Summary of (b) (4) concentrations tested

+S9 mix 3 hr treatment	-S9 mix 3 hr treatment
150 µg/ml	100µg/ml
100 µg/ml	50µg/ml
25µg/ml	10µg/ml

Treatment of the culture medium with (b) (4) up to 2681 µg/ml had no significant effect on osmolality. Significant changes in the pH of the medium were observed following treatment with (b) (4) at ≥1500µg/ml.
Changes in pH were not seen at the concentrations selected for chromosomal aberration analysis.

Results

There were statistically significant increases in aberration frequency both ±S9. The sponsor came to the conclusion that under the conditions of the assay, (b) (4) was clastogenic to cultured human lymphocytes both with and without the presence of the S9 mix. Both –S9 and +S9, mitotic index was reduced by more than 50%.

The sponsor's results are shown below.

Table 72. Mean Chromosomal Aberrations and Mitotic Indices in the Absence of Metabolic Activation (S9)

Treatment		Mean % Aberrant Cells Excluding Gaps	Mean % Mitotic Index
Solvent Control	10µl/ml	2.50	9.4
Mitomycin C (b) (4)	0.5µg/ml	40.00**	6.4Δ
	100µg/ml	13.33**	4.6
	50µg/ml	3.00	6.1
	10µg/ml	2.50	7.7

** Statistically significant increase in the percentage of aberrant cells at p<0.01 using Fisher's Exact Test (one-sided).

Δ Positive control mitotic index and % aberrant cells are determined from a single culture.

Table73. Mean Chromosomal Aberrations and Mitotic Indices in the Presence of Metabolic Activation (+S9)

Treatment		Mean % Aberrant Cells Excluding Gaps	Mean % Mitotic Index
Solvent Control	10µl/ml	0.50	7.6
Cyclophosphamide	50µg/ml	44.00**	4.7Δ
(b) (4)			
	150µg/ml	30.67**	3.2
	100µg/ml	2.50	5.3
	25µg/ml	1.00	6.0

** Statistically significant increase in the percentage of aberrant cells at p<0.01 using Fisher's Exact Test (one-sided).

Δ Positive control mitotic index and % aberrant cells are determined from a single culture.

(b) (4) Induction of chromosome aberrations in cultured human peripheral blood lymphocytes

Report number: 1889-131/1419YV
Study location: (b) (4)
Experimental start date: June 7, 2007
GLP: statement included
QA: yes
Test article: (b) (4), batch 48114, purity 96.6% by an unstated method
Vehicle: ethanol
Positive controls
-S9: 4-nitroquinoline l-oxide (NQO)
+S9: cyclophosphamide (CPA)
Activation system: Arochlor-induced rat liver S9. The S9 was checked for its ability to convert known pro-mutagens to bacterial mutagens and alkoxyresorufin-O-dealkylase activities.

(b) (4) was tested in an in vitro cytogenetics assay using duplicate human lymphocyte cultures prepared from the pooled blood of three male donors in a single experiment. Treatments were performed ±S9. The highest concentration used in the main experiment, 180.0 µg/mL, was determined in a preliminary cytotoxicity range-finding study.

In the main experiment, treatment was ±S-9 for 3 hours followed by a 17-hour recovery period prior to harvest (3+17) and continuous exposure to test article for 20 hours in the absence of S-9 (20+0). The test article concentrations for chromosome analysis were selected by evaluating the effect of (b) (4) on

mitotic index. Chromosome aberrations were analysed at three concentrations as shown below in the sponsor's summary.

Table 74

Chromosome aberration analysis - Main Experiment Summary

S-9	Treatment + recovery (hours)	Vehicle control	Concentration (µg/mL) (b) (4) [µmol/mL]	Percentage Cytotoxicity †
-	3+17	0[a]	2.000, 10.00, 30.00 [0.5362, 2.681, 8.044]	52%
+	3+17	0[a]	5.000, 10.00, 30.00 [1.341, 2.681, 8.044]	55%

[a] Vehicle control was Ethanol only
† At highest analysed concentration

The 20 hour exposure was conducted in a separate study, designated by the sponsor as Trial 2.

Table 75

Chromosome aberration analysis – Main Experiment, Trial 2 Summary

S-9	Treatment + recovery (hours)	Vehicle control	Concentration (µg/mL) (b) (4) [µmol/mL]	Percentage Cytotoxicity †
-	20+0	0[a]	2.500, 10.00, 20.00 [0.6703, 2.681, 5.362]	53%

[a] Vehicle control was Ethanol only
† At highest analysed concentration

Results

Precipitation was reported for concentrations ≥58.82 µg/ml for all treatment durations, ±S9.

There were sporadic increases in the frequency of structural aberrations under all conditions.

Table 76

A1 3 hour treatment -S-9, 17 hour recovery (3+17), Main Experiment

Donor sex: male

Treatment (µg/mL)	Treatment (µmol/mL)	Replicate	Cells Scored	Cells with Aberrations Including Gaps	Cells with Aberrations Excluding Gaps	MIH* (%)
Vehicle	Vehicle	A	100	1	1	
		B	100	0	0	
		Totals	200	1	1	-
2.000	0.5362	A	100	0	0	
		B	100	1	1	
		Totals	200	1	1	5
10.00	2.681	A	100	**6#**	**4#**	
		B	100	2	2	
		Totals	200	8	6	36
30.00	8.044	A	100	0	0	
		B	100	2	2	
		Totals	200	2	2	52
NQO, 2.50	-	A	100	**8#**	**8#**	
		B	53	**20#**	**20#**	
		Totals	153	28	28[a]	

Binomial Dispersion Test $\chi^2 = 4.72$, NS
[a] Statistical significance $p \leq 0.001$
NS = not significant

*Mitotic inhibition (%) = [1 - (mean MI_T/mean MI_C)] x 100%

(where T = treatment and C = negative control)

Highlighted numbers in bold exceed historical negative control range (Appendix F)

Table 77

A2 3 hour treatment +S-9, 17 hour recovery (3+17), Main Experiment
Donor sex: male

Treatment (µg/mL)	Treatment (µmol/mL)	Replicate	Cells Scored	Cells with Aberrations Including Gaps	Cells with Aberrations Excluding Gaps	Significance §	MIH* (%)
Vehicle	Vehicle	A	100	3	3		
		B	100	3	3		
		Totals	200	6	6		-
5.000	1.341	A	100	2	2		
		B	100	**5#**	**5#**		
		Totals	200	7	7	NS	6
10.00	2.681	A	100	**7#**	**6#**		
		B	100	4	**4#**		
		Totals	200	11	10	NS	22
30.00	8.044	A	100	**9#**	**8#**		
		B	100	**9#**	**9#**		
		Totals	200	18	17	$p \leq 0.01$	55
CPA, 12.5	-	A	37	**21#**	**20#**		
		B	39	**20#**	**20#**		
		Totals	76	41	40	$p \leq 0.001$	

Binomial Dispersion Test $\chi^2 = 1.82$, NS
§ Statistical significance (Appendix E)
NS = not significant

*Mitotic inhibition (%) = [1 - (mean MI_T/mean MI_C)] x 100%

(where T = treatment and C = negative control)

Highlighted numbers in bold exceed historical negative control range (Appendix F)

Table 78

A3 20 hour treatment -S-9, 0 hour recovery (20+0), Main Experiment, Trial 2

Donor sex: male

Treatment (µg/mL)	Treatment (µmol/mL)	Replicate	Cells Scored	Cells with Aberrations Including Gaps	Cells with Aberrations Excluding Gaps	MIH* (%)
Vehicle	Vehicle	A	100	3	2	
		B	100	2	1	
		Totals	200	5	3	-
2.500	0.6703	A	100	**4**	3	
		B	100	**6#**	3	
		Totals	200	10	6	4
10.00	2.681	A	100	**6#**	**6#**	
		B	100	2	2	
		Totals	200	8	8	17
20.00	5.362	A	91	**15#**	**12#**	
		B	100	**4#**	3	
		Totals	191	19	15	53
NQO, 2.50	-	A	33	**10#**	**10#**	
		B	1	0	0	
		Totals	34	10	10[a]	

Binomial Dispersion Test $\chi^2 = 9.25$, Not significant

[a] Statistical significance $p \leq 0.001$

*Mitotic inhibition (%) = [1 - (mean MI_T/mean MI_C)] x 100%

(where T = treatment and C = negative control)

Highlighted numbers in bold exceed historical negative control range (Appendix F)

It should be noted that due to unusually high toxicity a limited number of cells were scored from the positive control cultures, of the limited number of cells that were scored a clear positive response was observed, the experiment was therefore considered valid.

The sponsor concluded:

> It is concluded that (b) (4) induced chromosome aberrations in cultured human peripheral blood lymphocytes, when tested to its limit of cytotoxicity in the presence of a rat liver metabolic activation system (S-9). In the absence of S-9 (3 and 20 hour treatments) there were scattered single cultures with elevated frequencies of chromosome aberrations however the biological relevance of these results is questionable.

(b) (4) Maximum Tolerated Single Oral Dose Toxicity Study in the Rat

Study number1924DR, November 2007

This non-GLP study was used to determine doses to use in a micronucleus study for (b) (4) AZD6140. (b) (4) was formulated in 1% carboxymethylcellulose and 0.1% w/v polysorbate 80(aqueous). The batch number and purity of the test article were not specified. The number of animals used was not clearly specified but seems to be 3, given a single oral dose of 1000mg/kg. The rats were permitted to survive for an unspecified number of days for observation.

Two out of three animals showed piloerection and hunched posture. One showed decreased activity and altered fecal consistency. All animals lost 15% of their starting body weight up to days 4 or 5 days with an unspecified partial recovery of weight seen by the end of the study (duration unspecified). The essence of this 4-page report is that rats given a single oral dose of 1000 mg/kg of (b) (4) showed overt toxicity but survived for at least 5 days.

(b) (4) Rat Micronucleus Assay

Report number: 1886Q
Study location: Safety Assessment UK, Astra Zeneca R&D, Alderley, Macclesfield, England
GLP: statement included
QA: yes
Experimental start date: December 11, 2007
Test article: (b) (4) batch 48115, purity 98.8% as determined by unspecified method. It was stated that there is no analytical method available.
Vehicle: 1% w/v carboxymethylcellulose and 0.1% w/v polysorbate 80 in purified water.
Positive control: cyclophosphamide

(b) (4) was administered to groups of 7 male Wistar Han rats, approximately 10 weeks old at dosing. Groups were given doses of 0, 100, 500 and 1000 mg/kg (b) (4) and sampled for bone marrow analysis after 24 hours. Additional groups given 0 and 1000 mg/kg, the maximum tolerated dose, were sampled after 48 hours. The control rats were dosed with vehicle. A group of 3 rats was given the positive control.

Animals were euthanized 24 or 48 hours after dosing and bone marrow smears prepared, fixed and stained with acridine orange. For each animal, 2000 immature erythrocytes (IE) were scored for the incidence of micronucleated cells (MIE) and the ratio of IE to mature erythrocytes (E) was determined in 1000 cells.

The sponsor reported that signs were apparent in all high dose animals. Signs of pilo-erection, decreased activity, hunched posture, and partially closed eyes (ptosis?) were apparent from 3-6 hours after dosing in 9/14 animals and from 6-24 hours in 5/14 animals. One rat was euthanized at 6 hours after dosing for humane reasons.

There was no significant change in the mean number of micronucleated immature erythrocytes. At the high dose there was a non-significant decrease of approximately 6% in the mean percentage of immature erythrocytes. Under the conditions of the study (b) (4) did not increase the incidence of micronucleated immature erythrocytes in the bone marrow of Han Wistar rats.
Table 79

Mean incidence of micronucleated immature erythrocytes in rat bone marrow and percentages of immature erythrocytes

Group, treatment and dose level	Mean number of MIE in 2000 immature erythrocytes		Mean percentage of immature erythrocytes	
	24 hour	48 hour	24 hour	48 hour
1 Vehicle 10 mL/kg	1.1	1.4	66.9	64.0
2 (b) (4) 100 mg/kg	1.9		65.6	
3 500 mg/kg	2.0		63.0	
4 1000 mg/kg	1.3	2.0 [a]	57.0	58.6 [a]
5 Cyclophosphamide 20 mg/kg	43.0 ***		55.9	

*** Fisher's Exact Test p<0.001 [a] Data from 6 animals.

In Vitro Cytogenetic Assay in Human Lymphocytes for (b) (4)
Report number: (b) (4) 1381
Study location: (b) (4)
GLP: statement included
QA: yes
Experimental start date: October 3, 2006
Test article: (b) (4), batch 11706046, purity reported as >99% but not certified
Positive controls:
-S9: mitomycin C

Human blood samples were obtained on the day of the study from healthy, non-smoking, male volunteers. Approximately 48 hours after culture establishment, aliquots of the test substance, solvent control or positive control were added to duplicate human peripheral blood cultures in the absence of S9. Three concentrations of (b) (4) were tested: 250, 500 and 1000 µg/ml. The sampling time of 20 hours after the start of drug exposure correlated to 68 hours after culture initiation. Colcemid was added to the cultures 2 hours prior to harvest.

The effect of (b) (4) on the pH and osmolality of the culture medium was investigated, using single cultures containing medium only. The concentrations tested ranged from 16µg/ml to 1521 µg/ml. The pH and osmolality were not significantly affected. The solubility of the test substance in the treated blood cultures and in media only cultures was assessed by eye immediately after treatment and at the end of the treatment periods.

The statistically significant results in the sponsor's table below fall within the range of historical control values for the time period February 1996 – August 2005. The highest concentration tested (1521 µg/ml) was not evaluated due to the level of apparent cytotoxicity and the decrease in pH of the medium from 7.04 (solvent control) to 6.31.

Table 80.

Mean Chromosomal Aberrations And Mitotic Indices In The Absence Of Metabolic Activation (S9-Mix)

Treatment		Mean % Aberrant Cells Excluding Gaps	Mean % Mitotic Index
Experiment 1 – 20 hour treatment			
Solvent Control	10 µL/mL	1.00	7.0
Mitomycin C	0.2 µg/mL	40.00**	4.9Δ
(b) (4)			
	1000 µg/mL	4.50*	3.0
	500 µg/mL	4.50*	3.9
	250 µg/mL	1.50	6.2

* Statistically significant increase in the percentage of aberrant cells at p<0.05 using Fisher's Exact Test (one-sided).
** Statistically significant increase in the percentage of aberrant cells at p<0.01 using Fisher's Exact Test (one-sided).
Δ Positive control mitotic index and % aberrant cells are determined from a single culture.

Table 81

Historical Values for Mean Chromosomal Aberrations for Vehicle Controls

Vehicle controls

	68 hour sampling time	
	+S9-mix	-S9-mix
Mean % aberrant cells excluding gaps	1.1 ± 1.0 n = 314	1.2 ± 1.0 n = 348
Range (% aberrant cells excluding gaps)	0.0 - 5.5	0.0 - 5.5

When gaps were included, there was one concentration where the percentage of aberrant cells fell within the range of positive controls. The mean mitotic activity was decreased by greater than 50% compared to the controls groups. The highest concentration evaluated also caused a decrease in pH of the medium from 7.04 (solvent control) to 6.63.

Table 82. Summary of Cell Aberrations

TREATMENT	CONCENTRATION	CULTURE	NO OF CELLS EXAMINED	NO OF CELLS WITH GIVEN ABERRATIONS: GAPS	CTB	CSB	CTE	CSE	OTH	MITOTIC INDEX	% ABERRANT CELLS (+GAPS)	% ABERRANT CELLS (-GAPS)
Dimethylsulphoxide	10 uL/mL	A	100	1	1					6.800	2.00	1.00
		B	100		1					7.200	1.00	1.00
Mitomycin C	0.2 ug/mL	B	25		4	5		1		4.900	40.00	40.00
(b) (4)	1000 ug/mL	A	100		5					3.300	5.00	5.00
		B	100		4					2.700	4.00	4.00
	500 ug/mL	A	100		6	1				3.600	7.00	7.00
		B	100		2					4.100	2.00	2.00
	250 ug/mL	A	100		2					6.100	2.00	2.00
		B	100		1					6.200	1.00	1.00

Under the conditions of the study the test article did not cause an increase in chromosomal aberrations in human lymphocytes.

(b) (4) In vitro cytogenetic assay in human lymphocytes

Report number: SV1355
Study location: (b) (4)
GLP: statement included
QA: yes
Experimental start date: March 6, 2006
Test article: (b) (4) batch 4, purity 99.8%, method of determination not listed
Positive controls: mitomycin C and cyclophosphamide
Solvent for positive control and test substance: deionised water
S9: prepared from male Sprague-Dawley rats treated with Phenobarbital and β-naphthoflavone.
The report did not specify how the activity of the S9 system was validated.

(b) (4) was tested for clastogenicity in vitro using human lymphocytes collected from 2 female donors. In experiment 1, cultures were treated for 3 hours both ±S9. In experiment 2, cultures were treated for 3 hours in the presence of S9 and 20 hours in the absence of S9. All cultures were harvested 68 hours after culture initiation.

In experiment 1, concentrations ≥262 in did not show sufficient number of metaphases for analysis. In experiment 2, concentrations > 150 µg/ml showed insufficient metaphases for analysis. The concentrations used for analysis based on the mitotic indices and the study designs are summarized in the sponsor's table below.

Table 83: Summary of Concentrations Tested

Experiment 1		Experiment 2	
+S9-mix 3 hour treatment	-S9-mix 3 hour treatment	+S9-mix 3 hour treatment	-S9-mix 20 hour treatment
131 µg/mL	131 µg/mL	150 µg/mL	50 µg/mL
66 µg/mL	66 µg/mL	100 µg/mL	25 µg/mL
33 µg/mL	33 µg/mL	75 µg/mL	13 µg/mL

The mitotic index was determined by examining 1000 lymphocytes per culture and calculating the percentage of cells in metaphase. The slides were coded prior to analysis and 100 metaphase cells were analyzed per culture for structural chromosomal damage.

A statistically significant increase in chromosomal aberrations was seen in the presence of S9.

Table 84

Mean Chromosomal Aberrations And Mitotic Indices In The Presence Of Metabolic Activation (S9-Mix)

Treatment		Mean % Aberrant Cells Excluding Gaps	Mean % Mitotic Index
Experiment 1 – 3 hour treatment			
Solvent Control	10 µL/mL	0.00	7.6
Cyclophosphamide (b) (4)	50 µg/mL	44.00**	3.5Δ
	131 µg/mL	2.00	2.3
	66 µg/mL	1.50	6.3
	33 µg/mL	2.00	7.2
Experiment 2 – 3 hour treatment			
Solvent Control	10 µL/mL	0.50	11.3
Cyclophosphamide (b) (4)	50 µg/mL	60.00**	5.6Δ
	150 µg/mL	4.00*	5.4
	100 µg/mL	3.00	6.4
	75 µg/mL	1.00	10.0

Statistically significant increase in the percentage of aberrant cells at $p<0.05$ using Fisher's Exact Test (one-sided).
Statistically significant increase in the percentage of aberrant cells at $p<0.01$ using Fisher's Exact Test (one-sided).
Positive control mitotic index and % aberrant cells are determined from a single culture.

The increases appear to fall within the range of historical controls.

Table 85

APPENDIX 1 Historical Control Data

February 1996 – August 2005

Vehicle controls

	68 hour sampling time	
	+S9-mix	-S9-mix
Mean % aberrant cells excluding gaps	1.1 ± 1.0 n = 314	1.2 ± 1.0 n = 348
Range (% aberrant cells excluding gaps)	0.0 - 5.5	0.0 - 4.5

(b) (4) Bacterial Mutation Assay

Report number: YV7312
Study location: (b) (4)
GLP: statement included
QA: yes
Experimental start date: February 28, 2006
Test article: (b) (4) batch 4, purity 99.8% by an unspecified method
Activation system: S9 from Phenobarbital/β-naphthoflavone induced Sprague-Dawley rats
The positive control substances were used to confirm the activity of the S-9 mix.
Bacterial strains used:
Salmonella typhimurium TA1535, TA1537, TA98, TA100
Escherichia coli: WP2 (pKM101) and WP2 *uvr*A (pKM101)
Positive controls:
+S9: benzo[a]pyrene (BP), 2-aminoanthracene (2AA),
-S9: Acridine mutagen ICR191 (ICR), daunorubicin HCl(DR), N-Ethyl-N'nitro-N-nitrosoguanidine (ENNG), Mitomycin C(MMC), sodium azide(NaZ)

Solvents: DMSO , water (MMC and NaZ)

(b) (4) was initially assayed with the plate incorporation method over the concentration range of 100-5000µg per plate ±S9. The re-test was conducted using the same concentration range. The +S9 portion of the re-test was done with a pre-incubation protocol. Cultures for each concentration were processed in triplicate.

Results
Precipitation was reported for all strains at concentrations ≥2500 µg both in the presence and absence of S9.
Under the conditions of the assay, the test substance did not cause an increase in revertants.

Table 86

(Plate Incorporation: +S9)

Experiment: YV7312:Phase 1+

Date Plated: 28/02/2006
Date Counted: 03/03/2006

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA100	**Y12511/002**	5000 µg	*95.0*	*5.3*	*0.7*	99 P, 89 P, 97 P
		2500 µg	*95.0*	*7.0*	*0.7*	87 P, 100 P, 98 P
		1000 µg	*103.7*	*22.6*	*0.7*	125, 80, 106
		500 µg	*134.3*	*11.1*	*0.9*	124, 133, 146
		200 µg	*137.7*	*17.8*	*1.0*	130, 125, 158
		100 µg	*135.3*	*7.6*	*0.9*	144, 130, 132
	DMSO		*143.0*	*15.2*		125, 136, 138, 152, 164
TA1535	**Y12511/002**	5000 µg	*4.3*	*0.6*	*0.6*	5 P, 4 P, 4 P
		2500 µg	*2.7*	*2.5*	*0.4*	3 P, 0 P, 5 P
		1000 µg	*4.7*	*0.6*	*0.6*	4, 5, 5
		500 µg	*9.0*	*2.0*	*1.2*	11, 9, 7
		200 µg	*5.7*	*1.2*	*0.8*	5, 5, 7
		100 µg	*8.0*	*1.7*	*1.1*	9, 6, 9
	DMSO		*7.4*	*1.5*		7, 7, 6, 7, 10
TA1537	**Y12511/002**	5000 µg	*3.7*	*0.6*	*0.4*	3 P, 4 P, 4 P
		2500 µg	*4.3*	*2.9*	*0.4*	6 P, 1 P, 6 P
		1000 µg	*6.0*	*1.0*	*0.6*	5, 6, 7
		500 µg	*10.0*	*2.6*	*1.0*	7, 11, 12
		200 µg	*9.0*	*3.0*	*0.9*	12, 9, 6
		100 µg	*10.7*	*2.1*	*1.0*	10, 9, 13
	DMSO		*10.2*	*2.6*		8, 9, 8, 13, 13
TA98	**Y12511/002**	5000 µg	*24.7*	*3.2*	*0.8*	27 P, 26 P, 21 P
		2500 µg	*16.0*	*1.7*	*0.5*	14 P, 17 P, 17 P
		1000 µg	*25.3*	*7.0*	*0.8*	32, 18, 26
		500 µg	*31.7*	*1.2*	*1.0*	33, 31, 31
		200 µg	*36.0*	*12.7*	*1.1*	45, 27, C
		100 µg	*25.7*	*8.1*	*0.8*	27, 17, 33
	DMSO		*31.6*	*3.4*		28, 28, 34, 33, 35
WP2 (pKM101)	**Y12511/002**	5000 µg	*33.7*	*8.1*	*0.7*	25 P, 35 P, 41 P
		2500 µg	*43.3*	*2.1*	*0.9*	44 P, 41 P, 45 P
		1000 µg	*49.0*	*11.3*	*1.0*	42, 62, 43
		500 µg	*62.0*	*3.0*	*1.2*	62, 59, 65
		200 µg	*63.3*	*13.2*	*1.3*	49, 66, 75
		100 µg	*56.7*	*7.4*	*1.1*	51, 65, 54
	DMSO		*50.0*	*10.2*		51, 39, 65, 42, 53
WP2 uvrA (pKM101)	**Y12511/002**	5000 µg	*143.7*	*5.5*	*0.9*	141 P, 150 P, 140 P
		2500 µg	*133.7*	*15.3*	*0.8*	117 P, 137 P, 147 P
		1000 µg	*158.7*	*5.1*	*1.0*	153, 163, 160
		500 µg	*170.0*	*24.3*	*1.1*	143, 190, 177
		200 µg	*196.7*	*18.0*	*1.2*	179, 196, 215
		100 µg	*180.3*	*8.5*	*1.1*	190, 177, 174
	DMSO		*158.2*	*22.1*		169, 147, 127, 185, 163

Key to Plate Postfix Codes

P	Precipitate
C	Contaminated

Table 87

(Plate Incorporation: -S9)

Experiment: YV7312:Phase 1-

Date Plated: 03/03/2006
Date Counted: 06/03/2006

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA100	**Y12511/002**	5000 µg	*86.0*	*7.2*	*0.7*	78 P, 92 P, 88 P
		2500 µg	*78.7*	*10.7*	*0.6*	67 P, 88 P, 81 P
		1000 µg	*85.0*	*5.6*	*0.7*	80, 91, 84
		500 µg	*118.0*	*5.3*	*0.9*	124, 116, 114
		200 µg	*129.3*	*16.1*	*1.0*	141, 136, 111
		100 µg	*125.3*	*15.7*	*1.0*	120, 143, 113
	DMSO		*128.0*	*15.2*		144, 104, 124, 133, 135
TA1535	**Y12511/002**	5000 µg	*3.0*	*1.7*	*0.3*	2 P, 2 P, 5 P
		2500 µg	*4.0*	*4.4*	*0.5*	1 P, 9 P, 2 P
		1000 µg	*7.0*	*4.4*	*0.8*	5, 12, 4
		500 µg	*8.3*	*2.3*	*0.9*	11, 7, 7
		200 µg	*10.0*	*3.0*	*1.1*	10, 13, 7
		100 µg	*8.7*	*2.5*	*1.0*	9, 11, 6
	DMSO		*8.8*	*2.7*		7, 10, 7, 13, 7
TA1537	**Y12511/002**	5000 µg	*4.7*	*3.1*	*0.3*	4 P, 2 P, 8 P
		2500 µg	*9.0*	*2.6*	*0.6*	6 P, 10 P, 11 P
		1000 µg	*11.3*	*5.1*	*0.7*	17, 7, 10
		500 µg	*9.7*	*4.0*	*0.6*	5, 12, 12
		200 µg	*14.0*	*2.6*	*0.9*	17, 13, 12
		100 µg	*16.7*	*1.5*	*1.1*	17, 15, 18
	DMSO		*15.4*	*4.6*		13, 17, 21, 9, 17
TA98	**Y12511/002**	5000 µg	*21.0*	*7.2*	*1.0*	29 P, 19 P, 15 P
		2500 µg	*20.0*	*0.0*	*1.0*	20 P, 20 P, 20 P
		1000 µg	*21.0*	*5.0*	*1.0*	21, 16, 26
		500 µg	*22.3*	*3.2*	*1.1*	21, 20, 26
		200 µg	*21.3*	*5.7*	*1.0*	15, 23, 26
		100 µg	*23.3*	*1.2*	*1.1*	22, 24, 24
	DMSO		*20.8*	*5.4*		16, 29, 20, 16, 23
WP2 (pKM101)	**Y12511/002**	5000 µg	*32.7*	*7.0*	*0.8*	32 P, 40 P, 26 P
		2500 µg	*24.0*	*4.6*	*0.6*	23 P, 20 P, 29 P
		1000 µg	*40.7*	*5.9*	*1.0*	34, 43, 45
		500 µg	*41.0*	*3.6*	*1.0*	44, 37, 42
		200 µg	*53.7*	*0.6*	*1.3*	54, 53, 54
		100 µg	*54.7*	*6.1*	*1.3*	48, 56, 60
	DMSO		*42.8*	*7.8*		48, 42, 53, 37, 34
WP2 uvrA (pKM101)	**Y12511/002**	5000 µg	*77.7*	*14.8*	*0.5*	65 P, 94 P, 74 P
		2500 µg	*106.0*	*30.0*	*0.6*	136 P, 106 P, 76 P
		1000 µg	*139.7*	*12.1*	*0.9*	151, 141, 127
		500 µg	*161.0*	*10.8*	*1.0*	173, 158, 152
		200 µg	*156.7*	*22.5*	*1.0*	131, 173, 166
		100 µg	*139.3*	*13.1*	*0.8*	135, 129, 154
	DMSO		*164.2*	*25.4*		193, 154, 126, 174, 174

Key to Plate Postfix Codes

P	Precipitate

(b) (4) Chromosome Aberration Assay in Human Lymphocytes in vitro

Project number: 2396/0012
Study location: (b) (4)
GLP: statement included
QA: yes
Test article: (b) (4) batch number 4, reference number Y12511/002. purity was not stated
Activation system: S9 from male Sprague-Dawley rats treated with phenobarbitone and β-naphthoflavone. Activity of the S9 was demonstrated by the use of positive controls.

Positive controls:
-S9: mitomycin C (MMC)
+S9: cyclophosphamide (CP)

Duplicate cultures of human lymphocytes treated with the test material as well as vehicle and positive controls were evaluated for chromosome aberrations. It was not specifically stated how many volunteers of what sex provided the cells for each experiment. Formulation analysis was not conducted.

- Experiment 1
  4 hours exposure: +S9 (2% final concentration), + 20-hour expression period
  4 hours: -S9 + 20-hour expression period.

  Table 88. Concentrations tested Experiment 1

Group	Final concentration of (b) (4) (µg/ml)
4(20)-hour without S9	0*, 16, 32*, 64*, 128*, 192, 256, MMC 0.4*
4(20)-hour with S9	0*, 16, 32*, 64*, 128*, 192, 256, CP 5*

  *dose levels selected for metaphase analysis

- Experiment 2
  4 hours exposure: +S9 was repeated using a 1% final S9 concentration, +20 hour expression
  24 hours exposure –S9

  Table 89. Concentrations tested Experiment 2

Group	Final concentration of (b) (4) (µg/ml)
24-hour without S9	0*, 4, 8, 16*, 32*, 48*, 64, MMC 0.2*
4(20)-hour with S9	0*, 16, 32*, 64*, 128*, 192, 256, CP 5*

  *dose levels selected for metaphase analysis

- Experiment 3
  a third experiment was performed using the same exposure conditions as Experiment 1 but with a modified test material dose range to get as close to 50% toxicity in both exposure groups.

  Table 90. Concentrations tested Experiment 3

Group	Final concentration of (b) (4) (µg/ml)
4(20)-hour without S9	0*, 32, 64*, 128*, 144, 160*, 176, MMC 0.4*
4(20)-hour with S9	0*, 32, 64, 128*, 144*, 160*, 176, CP 5*

  *dose levels selected for metaphase analysis

Colcemid was added to the cultures 2 hours prior to harvest.

A total of 2000 lymphocyte nuclei were counted. The number in metaphase were recorded and expressed as the mitotic index and as a percentage of the vehicle control value.

Where possible the first 100 consecutive well-spread metaphases from each culture were counted. When approximately 50% of cells showed aberrations, slide evaluation was terminated at 50 cells. If the cell had 44-48 chromosomes, any gaps, breaks or rearrangements were noted according to the simplified system of Savage (1976) recommended in the 1983 UKEMS guidelines for mutagenicity testing. Coded evaluation of the slides was implied in the statement that "Cells with chromosome aberrations were reviewed as necessary by a senior cytogeneticist prior to decoding the slides."

Results

The concentrations used in a preliminary toxicity test were in the range 16.41 - 4200 µg/ml. Precipitation and hemolysis were reported for concentrations ≥525µg/ml both in the 4 hour test article exposures (±S9) and in the 24 hour exposures. Hemolysis was also reported for concentrations ≥262.5µg/ml in the 4 hour (-S9) conditions.

Microscopic assessment of the slides prepared from the exposed cultures showed metaphase cells present at concentrations up to131.25 µg/ml in the 4(20)-hour exposures, both ± S9. The maximum concentration tested with metaphases present in the 24-hour continuous exposure was 32 µg/ml. The test material induced some evidence of toxicity in all of the exposure groups.

In experiment 1, 4 hours of treatment ±S9 produced no scorable metaphases at concentrations ≥192µg/ml. No precipitate of test material was noted. A concentration of 128 µg/ml produced scorable metaphases in both the presence and absence of S9 and was thus used as the maximum concentration for metaphase analysis.

Table 91.

Results of Chromosome Aberration Test - Experiment 1 Without Metabolic Activation (S9)

Treatment Group	Replicate	Mitotic Index (%)	Number of Cells Scored	Number of Aberrations						Total Number of Aberrations		Frequency of Aberrant Cells (%)	
				Gaps	Chromatid		Chromosome		Others				
					Breaks	Exchanges	Breaks	Exchanges	X	(+ Gaps)	(-Gaps)	(+Gaps)	(-Gaps)
Vehicle Control	A	4.05	100	0	0	0	0	0	0	0	0	0	0
	B	4.15	100	1	0	0	0	0	0	1	0	1	0
	Total	(100)	200	1	0	0	0	0	0	1	0	1 (0.5)	0 (0.0)
32 µg/ml	A	4.45	100	0	1	0	0	0	0	1	1	1	1
	B	5.50	100	0	0	0	0	0	0	0	0	0	0
	Total	(121)	200	0	1	0	0	0	0	1	1	1 (0.5)	1 (0.5)
64 µg/ml	A	4.10	100	0	0	0	0	0	0	0	0	0	0
	B	3.10	100	0	0	0	0	0	0	0	0	0	0
	Total	(88)	200	0	0	0	0	0	0	0	0	0 (0.0)	0 (0.0)
128 µg/ml	A	3.10	100	1	0	0	1	0	0	2	1	2	1
	B	3.50	100	0	0	0	0	0	0	0	0	0	0
	Total	(80)	200	1	0	0	1	0	0	2	1	2 (1.0)	1 (0.5)
Positive Control MMC 0.4 µg/ml	A	3.35	50[a]	0	12	10	5	1	0	28	28	20	20
	B	3.75	100	6	23	22	9	0	0	60	54	32	31
	Total	(87)	150	6	35	32	14	1	0	88	82	52 (34.7)	51*** (34.0)

Table 92

Results of Chromosome Aberration Test - Experiment 1 With Metabolic Activation (S9)

Treatment Group	Replicate	Mitotic Index (%)	Number of Cells Scored	Number of Aberrations						Total Number of Aberrations		Frequency of Aberrant Cells (%)	
				Gaps	Chromatid		Chromosome		Others				
					Breaks	Exchanges	Breaks	Exchanges	X	(+ Gaps)	(-Gaps)	(+Gaps)	(-Gaps)
Vehicle Control	A	4.00	100	0	0	0	0	0	0	0	0	0	0
	B	5.90	100	0	0	0	0	0	0	0	0	0	0
	Total	(100)	200	0	0	0	0	0	0	0	0	0 (0.0)	0 (0.0)
32 µg/ml	A	4.50	100	1	0	0	0	0	0	1	0	1	0
	B	4.65	100	0	0	0	0	0	0	0	0	0	0
	Total	(92)	200	1	0	0	0	0	0	1	0	1 (0.5)	0 (0.0)
64 µg/ml	A	4.00	100	1	0	0	3	0	0	4	3	3	3
	B	5.00	100	0	0	0	0	0	0	0	0	0	0
	Total	(91)	200	1	0	0	3	0	0	4	3	3 (1.5)	3 (1.5)
128 µg/ml	A	4.60	100	0	0	0	0	0	0	0	0	0	0
	B	4.30	100	0	0	0	0	0	0	0	0	0	0
	Total	(90)	200	0	0	0	0	0	0	0	0	0 (0.0)	0 (0.0)
Positive Control CP 5 µg/ml	A	0.85	50	13	15	8	0	0	0	36	23	23	18
	B	0.70	100	10	22	12	0	0	0	44	34	30	27
	Total	(16)	150	23	37	20	0	0	0	80	57	53 (35.3)	45*** (30.0)

Experiment 2 showed similar results to experiment 1. The concentrations used produced mitotic indices from 32-100% of control levels without S9 and from 75-82% in the presence of S9.

In the absence of metabolic activation, the third experiment showed essentially the same results as the first two experiments with mitotic indices 50-100% of control values. In the presence of S9 there was a minimal increase in frequency of aberrations.

Table 93

Results of Chromosome Aberration Test - Experiment 3 With Metabolic Activation (S9)

Treatment Group	Replicate	Mitotic Index (%)	Number of Cells Scored	Number of Aberrations						Total Number of Aberrations		Frequency of Aberrant Cells (%)	
				Gaps	Chromatid		Chromosome		Others				
					Breaks	Exchanges	Breaks	Exchanges	X	(+ Gaps)	(-Gaps)	(+Gaps)	(-Gaps)
Vehicle Control	A	5.70	100	0	2	0	0	0	0	2	2	2	2
	B	3.85	100	0	0	0	0	0	0	0	0	0	0
	Total	(100)	200	0	2	0	0	0	0	2	2	2 (1.0)	2 (1.0)
128 µg/ml	A	3.10	100	1	1	0	0	0	0	2	1	2	1
	B	3.80	100	1	0	0	4	0	0	5	4	4	3
	Total	(72)	200	2	1	0	4	0	0	7	5	6 (3.0)	4 (2.0)
144 µg/ml	A	2.65	100	0	0	1	0	0	0	1	1	1	1
	B	2.80	100	0	1	2	1	0	0	4	4	4	4
	Total	(57)	200	0	1	3	1	0	0	5	5	5 (2.5)	5 (2.5)
160 µg/ml	A	2.80	100	2	6	0	1	0	0	9	7	7	5
	B	1.70	100	0	0	0	0	0	0	0	0	0	0
	Total	(47)	200	2	6	0	1	0	0	9	7	7 (3.5)	5 (2.5)
Positive Control CP 5 µg/ml	A	1.70	100	8	11	2	3	0	0	24	16	18	12
	B	0.70	50	11	19	4	0	1	0	35	24	19	15
	Total	(25)	150	19	30	6	3	1	0	59	40	37 (24.7)	27*** (18.0)

The number of aberrations recorded falls within the historical control ranges, shown below. This is not likely to be a significant finding.

Table 94. Historical Control Ranges

Many experiments with human lymphocytes have established a range of aberration frequencies acceptable for control cultures. The current in-house historical aberration ranges are presented below:

	Pulse exposure –S9		Pulse exposure +S9 (1% S9)		24-Hour –S9		Pulse exposure +S9 (2% S9)	
	% cells with aberrations (-gaps)	% cells with polyploids	% cells with aberrations (-gaps)	% cells with polyploids	% cells with aberrations (-gaps)	% cells with polyploids	% cells with aberrations (-gaps)	% cells with polyploids
Minimum	0	0	0	0	0	0	0	0
Maximum	3	1	4	1.5	4.5	1	2.5	1.5
Mean	0.95	0.13	1.09	0.13	1.18	0.12	0.86	0.19
Standard Deviation	0.74	0.28	1.02	0.28	0.99	0.24	0.68	0.37
Number	47	47	47	47	43	43	40	40

(b) (4) **Ames Assay**
Study Code: (b) (4)-4064
Study location: (b) (4)
GLP: statement included
QA: yes
Experimental start date: February 13, 2008
Bacterial strains:
Salmonella typhimurium TA100, TA1535, TA98, TA1537
Escherichia coli WP2*uvr*A
Activation system: S9 from livers of male rats induced with phenobarbital and 5, 6-benzoflavone
The report did not specify any special procedures for validating activity of the S9 mix
Note: different lots of S9 were used for the confirmation versus main test.

Test article: (b) (4), lot number (b) (4), purity 99.4% by an unspecified method
Positive controls:
-S9: 2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide (AF-2)
Sodium azide
2-Methoxy-6-chloro-9-[3-(2-chloroethyl)-aminopropylamino]acridine 2HC1 (ICR-191)

+S9: 2-aminoanthracene (2AA)

This study was performed by the pre-incubation method, ±S9. Triplicate plates were used for the negative control group and duplicate plates per dose for the test substance concentrations and the positive control groups.
The dose-finding study showed bacterial growth inhibition at concentrations ≥1250µg/ml in all test strains ±S9. Precipitation of test material was not reported for any of the concentrations, either in the presence or absence of S9. The concentrations tested are summarized below:

Table 95. Summary of Concentrations Tested

Concentrations (µg/plate) used –S9	
TA100, TA1535, TA98, TA1537	1250, 625, 313, 156, 78.1, 39.1, 19.5
WP2*uvr*A	5000, 2500, 1250, 625, 313, 156, 78.1, 39.1
Concentrations (µg/plate) used +S9	
TA100, TA1535, TA98, TA1537	1250, 625, 313, 156, 78.1, 39.1
WP2*uvr*A	5000, 2500, 1250, 625, 313, 156, 78.1, 39.1

The dose-ranging study showed increases in revertants –S9 in all 5 bacterial strains. This is shown below.

Table 96. Number of Revertant Colonies: Dose Ranging Study (b) (4)

Test substance: (b) (4)

Test Period		From February 13, 2008 to February 15, 2008				
With(+)or without(-) S9 mix	Test substance dose (µg/plate)	Number of revertant colonies per plate				
		Base-pair substitution type			Frameshift type	
		TA100	TA1535	WP2*uvrA*	TA98	TA1537
-S9 mix	Negative control	128 94 122 (115)	11 15 17 (14)	18 31 27 (25)	19 21 21 (20)	10 20 11 (14)
	4.88	116 127 (122)	16 11 (14)	29 28 (29)	21 23 (22)	10 11 (11)
	19.5	163 152 (158)	16 19 (18)	26 34 (30)	25 28 (27)	16 12 (14)
	78.1	255 239 (247)	33 34 (34)	41 43 (42)	29 26 (28)	22 19 (21)
	313	581 571 (576)	81 75 (78)	107 94 (101)	40 26 (33)	25 25 (25)
	1250	0* 0* (0)	0* 0* (0)	135* 118* (127)	0* 0* (0)	0* 0* (0)
	5000	0* 0* (0)	0* 0* (0)	0* 0* (0)	0* 0* (0)	0* 0* (0)
+S9 mix	Negative control	147 126 117 (130)	11 10 11 (11)	27 22 27 (25)	30 44 36 (37)	30 34 42 (35)
	4.88	111 118 (115)	15 11 (13)	26 30 (28)	25 28 (27)	23 26 (25)
	19.5	158 134 (146)	11 5 (8)	38 20 (29)	37 44 (41)	33 38 (36)
	78.1	168 108 (138)	16 8 (12)	20 19 (20)	31 38 (35)	36 40 (38)
	313	214 239 (227)	19 12 (16)	49 38 (44)	36 34 (35)	36 30 (33)
	1250	106* 74* (90)	2* 3* (3)	49* 66* (58)	19* 22* (21)	21* 13* (17)
	5000	0* 0* (0)	0* 0* (0)	0* 0* (0)	0* 0* (0)	0* 0* (0)
Positive control -S9 mix	Chemical	AF-2	NaN_3	AF-2	AF-2	ICR-191
	Dose(µg/plate)	0.01	0.5	0.01	0.1	0.5
	Number of revertant colonies/plate	568 602 (585)	478 532 (505)	284 301 (293)	641 623 (632)	2083 2085 (2084)
Positive control +S9 mix	Chemical	2AA	2AA	2AA	2AA	2AA
	Dose(µg/plate)	1	2	10	0.5	2
	Number of revertant colonies/plate	785 747 (766)	242 247 (245)	711 754 (733)	267 287 (277)	254 249 (252)

The main study showed a repeat of these results, with almost 10-fold increase in revertants for TA100, TA1535 and WP2*uvr*A. A smaller increase in revertants was seen in TA100 +S9.

Table 97: (b) (4) Number of Revertants in Main Study

Test substance: (b) (4)

Test Period		From February 20, 2008 to February 22, 2008				
With(+)or without(-) S9 mix	Test substance dose (μg/plate)	Number of revertant colonies per plate				
		Base-pair substitution type			Frameshift type	
		TA100	TA1535	WP2*uvrA*	TA98	TA1537
-S9 mix	Negative control	107 108 124 (113)	13 13 16 (14)	22 25 36 (28)	19 28 29 (25)	8 6 10 (8)
	19.5	154 153 (154)	22 16 (19)	—	—	—
	39.1	190 181 (186)	25 28 (27)	59 37 (48)	25 31 (28)	8 12 (10)
	78.1	291 224 (258)	45 37 (41)	50 57 (54)	30 29 (30)	3 11 (7)
	156	341 368 (355)	59 63 (61)	103 76 (90)	36 43 (40)	10 8 (9)
	313	630 592 (611)	95 105 (100)	150 121 (136)	41 47 (44)	11 12 (12)
	625	1007 1047 (1027)	160* 124* (142)	252 228 (240)	47 29 (38)	5 7 (6)
	1250	0* 0* (0)	0* 64* (32)	217* 235* (226)	0* 0* (0)	0* 0* (0)
	2500	—	—	0* 0* (0)	—	—
	5000	—	—	0* 0* (0)	—	—
+S9 mix	Negative control	132 120 126 (126)	9 6 13 (9)	41 38 28 (36)	35 27 45 (36)	31 23 42 (32)
	39.1	123 117 (120)	11 18 (15)	38 31 (35)	42 25 (34)	25 29 (27)
	78.1	118 144 (131)	13 12 (13)	27 45 (36)	35 28 (32)	35 30 (33)
	156	168 145 (157)	14 15 (15)	45 38 (42)	31 43 (37)	20 19 (20)
	313	255 254 (255)	10 12 (11)	48 43 (46)	38 40 (39)	27 29 (28)
	625	513 482 (498)	17 18 (18)	95 99 (97)	35 35 (35)	28 21 (25)
	1250	83* 87* (85)	6* 0* (3)	82* 56* (69)	21* 23* (22)	7* 15* (11)
	2500	—	—	0* 0* (0)	—	—
	5000	—	—	0* 0* (0)	—	—
Positive control -S9 mix	Chemical	AF-2	NaN_3	AF-2	AF-2	ICR-191
	Dose(μg/plate)	0.01	0.5	0.01	0.1	0.5
	Number of revertant colonies/plate	520 526 (523)	497 477 (487)	293 276 (285)	702 682 (692)	2230 2171 (2201)
Positive control +S9 mix	Chemical	2AA	2AA	2AA	2AA	2AA
	Dose(μg/plate)	1	2	10	0.5	2
	Number of revertant colonies/plate	795 856 (826)	255 215 (235)	857 678 (768)	308 326 (317)	261 303 (282)

A repeat study was done to examine the results. It is not clear why WP2*uvr*A, TA98 and TA1537 were not evaluated. The results are shown below.

Table 98. Number of revertants in Repeat (b) (4) Study

Test substance: (b) (4)

Test Period		From February 22, 2008 to February 25, 2008				
With(+)or without(-) S9 mix	Test substance dose (μg/plate)	Number of revertant colonies per plate				
		Base-pair substitution type			Frameshift type	
		TA100	TA1535	WP2*uvrA*	TA98	TA1537
-S9 mix	Negative control	116 114 130 (120)	11 16 10 (12)	—	—	—
+S9 mix	Negative control	127 130 138 (132)	15 10 10 (12)	—	—	—
	39.1	94 111 (103)	11 12 (12)	—	—	—
	78.1	118 96 (107)	11 5 (8)	—	—	—
	156	117 138 (128)	6 7 (7)	—	—	—
	313	163 159 (161)	16 13 (15)	—	—	—
	625	285 285 (285)	25 21 (23)	—	—	—
	1250	0* 0* (0)	0* 1* (1)	—	—	—
Positive control -S9 mix	Chemical	AF-2	NaN_3	—	—	—
	Dose(μg/plate)	0.01	0.5	—	—	—
	Number of revertant colonies/plate	661 638 (650)	516 475 (496)	—	—	—
Positive control +S9 mix	Chemical	2AA	2AA	—	—	—
	Dose(μg/plate)	1	2	—	—	—
	Number of revertant colonies/plate	864 946 (905)	233 245 (239)	—	—	—

Even though the magnitude of increase was not as great as in the first two experiments, the numbers of revertants reported for TA100 were outside of historical control ranges, shown below.

Table 99. Historical control data HISTORICAL DATA (b) (4)

August, 2007 -January, 2008

Negative Control(Mean±3S.D.)

	-S9 mix					+S9 mix				
	TA100	TA1535	WP2*uvrA*	TA98	TA1537	TA100	TA1535	WP2*uvrA*	TA98	TA1537
Mean	120	12	29	23	11	129	11	34	34	27
S.D.	15	4	7	5	4	16	3	8	7	7
Upper Limit	165	24	50	38	23	177	20	58	55	48
Lower Limit	75	1	8	8	1	81	1	10	13	6

The reviewer agrees with the sponsor's conclusion that under the conditions of the study, the test article caused an increase in revertants. The main test showed an increase in strains TA100, TA1535 and WP*uvr*A with and without S9. It is not clear why WP2uvrA, TA98 and TA1537 were excluded from the confirmation study.

(b) (4) **Bacterial Mutagenicity Assay**

Report number: (b) (4) 4065
Study location: (b) (4)
GLP: statement included
QA: yes
Experimental start date: February 13, 2008
Test article: (b) (4), purity 99.9% by an unstated method, lot number (b) (4)
Solvent: DMSO

Metabolic activation: Rat liver S9 from phenobarbital and5,6-benzoflavone treated Sprague Dawley rats. The report did not specify the procedures used to validate activity.

Formulation analysis was not discussed.

Bacterial strains used:
Salmonella typhimurium TA100, TA1535, TA98, TA1537
Escherichia coli WP2uvrA
Positive controls:
-S9: 2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide (AF-2)
Sodium azide
2-Methoxy-6-chloro-9-[3-(2-chloroethyl)-aminopropylamino]acridine 2HC1 (ICR-191)

+S9: 2-aminoanthracene (2AA)

Using the pre-incubation method, duplicate plates for each concentration were assessed ±S9. The text of the report states that bacterial growth was inhibited at concentrations >313 µg/plate and that this finding established the concentrations tested in the definitive test as 9.77, 19.5, 39.1, 78.1, 156 and 313 µg/plate. Precipitation of the test material was not reported for plates either with or without S9.

Under the conditions of the study the test article did not cause an increase in revertants.

Table 100.

Test substance: (b) (4)

Test Period		From February 22, 2008 to February 25, 2008				
With(+)or without(-) S9 mix	Test substance dose (μg/plate)	Number of revertant colonies per plate				
		Base-pair substitution type			Frameshift type	
		TA100	TA1535	WP2*uvrA*	TA98	TA1537
-S9 mix	Negative control	116 114 130 (120)	11 16 10 (12)	38 38 36 (37)	19 31 26 (25)	5 14 11 (10)
	9.77	127 120 (124)	10 10 (10)	41 35 (38)	25 14 (20)	7 10 (9)
	19.5	108 144 (126)	8 7 (8)	34 31 (33)	19 20 (20)	11 11 (11)
	39.1	116 114 (115)	11 10 (11)	34 34 (34)	15 16 (16)	10 7 (9)
	78.1	111 70 (91)	4 2 (3)	27 28 (28)	14 11 (13)	14 5 (10)
	156	0* 39* (20)	0* 2* (1)	18* 17* (18)	6* 1* (4)	1* 3* (2)
	313	4* 55* (30)	0* 0* (0)	14* 14* (14)	2* 0* (1)	3* 5* (4)
+S9 mix	Negative control	127 130 138 (132)	15 10 10 (12)	47 62 54 (54)	36 35 31 (34)	29 18 26 (24)
	9.77	111 130 (121)	19 12 (16)	—	29 21 (25)	13 18 (16)
	19.5	121 123 (122)	10 7 (9)	—	36 23 (30)	30 22 (26)
	39.1	98 108 (103)	10 5 (8)	—	28 37 (33)	15 16 (16)
	78.1	98 94 (96)	14 11 (13)	—	25 30 (28)	20 21 (21)
	156	94 88 (91)	8 7 (8)	40 40 (40)	34 27 (31)	20 14 (17)
	313	88* 91* (90)	4* 1* (3)	45 40 (43)	15* 23* (19)	10* 11* (11)
	625	—	—	34 37 (36)	—	—
	1250	—	—	23 29 (26)	—	—
	2500	—	—	20* 14* (17)	—	—
	5000	—	—	16* 12* (14)	—	—
Positive control -S9 mix	Chemical	AF-2	NaN_3	AF-2	AF-2	ICR-191
	Dose(μg/plate)	0.01	0.5	0.01	0.1	0.5
	Number of revertant colonies/plate	661 638 (650)	516 475 (496)	310 354 (332)	506 574 (540)	1808 1615 (1712)
Positive control +S9 mix	Chemical	2AA	2AA	2AA	2AA	2AA
	Dose(μg/plate)	1	2	10	0.5	2
	Number of revertant colonies/plate	864 946 (905)	233 245 (239)	697 584 (641)	303 291 (297)	294 245 (270)

[Notes]
- ( ): The mean number of colonies per plate.
- *: Bacterial growth inhibition was observed.
- AF-2: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide
- NaN_3: Sodium azide
- ICR-191: 2-Methoxy-6-chloro-9-[3-(2-chloroethyl)-aminopropylamino]acridine·2HCl
- 2AA: 2-Aminoanthracene

(b) (4) Bacterial Mutagenicity Test

Report number: (b) (4)-4066
Study location: (b) (4)

GLP: statement included
QA: yes
Initiation of experiment: February 13, 2008
Test article: (b) (4) lot number (b) (4) 001M, purity 99.9% by unspecified method
Solvent: DMSO
Bacterial strains:
Salmonella typhimurium TA100, TA1535, TA98, TA1537
Escherichia coli WP2*uvr*A
Positive controls:
-S9: 2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide (AF-2)
Sodium azide
2-Methoxy-6-chloro-9-[3-(2-chloroethyl)-aminopropylamino]acridine 2HC1 (ICR-191)

+S9: 2-aminoanthracene (2AA)

Metabolic activation: rat liver S9 from male Sprague Dawley rats treated with Phenobarbital and 5,6-benzoflavone. The report did not specify procedures for validation of activity of the S9 mix.

Formulation analysis was not discussed.

The study was conducted using a pre-incubation method, ±S9. Duplicate plates were used per concentration of the test article. Bacterial growth inhibition was not noted for any dose in any of the bacterial strains. Precipitation of test substance was reported not to have occurred. The doses used in the definitive test were 313, 625, 1250, 2500 and 5000 µg/plate.

Under the conditions of the study the test article did not cause an increase in revertants.

Table 101. Ames Assay Results

Test substance: (b) (4)

Test Period		From February 20, 2008 to February 22, 2008				
With(+)or without(-) S9 mix	Test substance dose (µg/plate)	Number of revertant colonies per plate				
		Base-pair substitution type			Frameshift type	
		TA100	TA1535	WP2*uvrA*	TA98	TA1537
-S9 mix	Negative control	107 108 124 (113)	13 13 16 (14)	22 25 36 (28)	19 28 29 (25)	8 6 10 (8)
	313	120 111 (116)	15 15 (15)	25 23 (24)	31 30 (31)	12 15 (14)
	625	107 108 (108)	6 12 (9)	25 19 (22)	29 25 (27)	6 7 (7)
	1250	104 103 (104)	11 18 (15)	27 28 (28)	25 31 (28)	10 10 (10)
	2500	96 98 (97)	10 18 (14)	25 29 (27)	25 21 (23)	6 6 (6)
	5000	101 83 (92)	13 13 (13)	42 22 (32)	34 18 (26)	5 6 (6)
+S9 mix	Negative control	132 120 126 (126)	9 6 13 (9)	41 38 28 (36)	35 27 45 (36)	31 23 42 (32)
	313	99 115 (107)	11 11 (11)	31 26 (29)	31 25 (28)	45 30 (38)
	625	117 120 (119)	5 6 (6)	31 29 (30)	26 25 (26)	28 31 (30)
	1250	95 108 (102)	7 8 (8)	30 26 (28)	36 28 (32)	33 33 (33)
	2500	118 128 (123)	4 11 (8)	34 18 (26)	34 29 (32)	18 10 (14)
	5000	63 101 (82)	11 8 (10)	28 25 (27)	20 25 (23)	11 8 (10)
Positive control -S9 mix	Chemical	AF-2	NaN_3	AF-2	AF-2	ICR-191
	Dose(µg/plate)	0.01	0.5	0.01	0.1	0.5
	Number of revertant colonies/plate	520 526 (523)	497 477 (487)	293 276 (285)	702 682 (692)	2230 2171 (2201)
Positive control +S9 mix	Chemical	2AA	2AA	2AA	2AA	2AA
	Dose(µg/plate)	1	2	10	0.5	2
	Number of revertant colonies/plate	795 856 (826)	255 215 (235)	857 678 (768)	308 326 (317)	261 303 (282)

[Notes]

- ( ): The mean number of colonies per plate.
- AF-2: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide
- NaN_3: Sodium azide
- ICR-191: 2-Methoxy-6-chloro-9-[3-(2-chloroethyl)-aminopropylamino]acridine・2HCl
- 2AA: 2-Aminoanthracene

Summary of Impurities of Interest

The review chemist (Chhagan Tele, Ph.D.) identified the impurities of interest as (b) (4) (b) (4) and asked if the impurity (b) (4) was adequately qualified.

Summary for (b) (4)

The sponsor proposes a specification of (b) (4)
This impurity has been evaluated in the following studies (submitted to the IND under submission# 031. Review on file in DARRTS):

(b) (4)

This impurity is adequately characterized and the specification acceptable.

Summary for (b) (4)

(b) (4)

The sponsor replied to this question with *in vitro* data and excreted levels of (b) (4)

(b) (4) was in fact evaluated in more studies than the sponsor acknowledged. These were submitted to the IND under submission #016 (reviews on file in DARRTS).

(b) (4)

(b) (4)

(b) (4)

(b) (4)

human plasma and as such adequately represented in the toxicology studies. The specification is acceptable.

Summary for (b) (4)

has been given a specification limit of (b) (4), a level greater than the ICH qualification threshold of 0.15%. The sponsor asserts that this impurity is qualified as it has appeared in several batches, used in non-clinical testing.

The sponsor then goes on to calculate the exposure to the above impurities.

Table 105.

Table 13 Calculation of margins of safety for impurities in the ticagrelor drug substance above 0.15%

Impurity	Specification (%w/w)	Impurity concentration in qualification batch [1] (%w/w)	Impurity dose at LOEL[2] ($mg/m^2/day$)	Impurity dose at specification limit [3] ($mg/m^2/day$)	Safety margin [4]
(b) (4)					

[1] Batch 4855R/A

(b) (4)

A consult request was submitted to the Computational Toxicology Service for assessment of structural alerts for genotoxicity for (b) (4) The databases used were somewhat limited in data for this chemical structure and as such predicted a negative result in the ICH genotoxic assay battery. The review chemist provided his opinion that there were no structural alerts in the molecule. Therefore, the specification is acceptable.

Summary for (b) (4)

(b) (4)

The sponsor has controlled for this substance with a specification limit of (b) (4) consistent with the EMEA guidance for genotoxic impurities being controlled to <1.5 µg/day.

Summary for (b) (4)

(b) (4)

Table 106. Sponsor's Summary of (b) (4) in Non-Clinical Batches of AZD6140

	Concentrations (%w/w) of (b) (4) in ticagrelor batches used in repeat-dose studies			
Rat				
Study	**SR99302-01**	**0660AR**	**TPR3143**	**456930 (0400PR)**
Duration	1 month	1 month qualification	3 month	6 month
Batch	4358K/A	4855R/A*	4454L	4736N/A
(b) (4)				
Marmoset				
Study	**99228 (SR99228-01)**	**00019**	**505453 (0008FT)**	
Duration	1 month	3 month	6 month	
Batch	4358K/A	4511L/A	4736N/A	
(b) (4)				

*Spiked to achieve (b) (4) (b) (4) **Data not available.

Table 107. Sponsor's Summary Table

(b) (4)

[redacted]

Table 108 Reviewers Modification of Preceeding Table: Signs Associated with Doses of Impurity (b) (4)

(b) (4)

(b) (4)

When toxicology studies are performed in which the impurity is specifically added to the parent drug, one looks for augmentation of the usual effects or the presence of additional effects. Because of the incidental way in which this impurity is being qualified, it is not possible to do that.

(b) (4)

Summary for (b) (4)

(b) (4) The specification seems acceptable.

12. Integrated Summary and Safety Evaluation Part I

The tables below are compilations of plasma values for the no observed adverse effect levels for the non-clinical adverse effects. In some cases, a NOAEL was not identified and a LOAEL is included. The non-clinical AUC values used for these calculations were taken from the sponsor's CTD Toxicology Tabulated Summary.

Toxicity	Species	NOAEL (mg/kg) M/F	AUC_{0-24} µg.hr/ml	Safety margin based on AUC*
Liver function	Rat	20 (LOAEL)	26§	1.3
	Marmoset	100	232	11.3
	Mouse	50	30	1.5
Bleeding: ↓hb, hct, rbc count, ↑reticulocytes	Mouse	50	30	0.6
	Rat	60	97 §	4.7
	Marmoset	10	14	0.7
Adrenal	Rat	20	26§	1.3
	Mice	250	225	10.9
	Marmoset	50	61141	2911

*AUC in human: AUC_{0-12} 10.3 µg.hr/ml for 100 mg/day (equivalent in that study to 90 mg b.i.d).
2 x 10.3µg.hr/ml = 20.6 was used for the calculation of safety margins.

§ mean of male and female values

13. Integrated Summary and Safety Evaluation: Part II

The tables below are compilations of plasma values for the no observed adverse effect levels for the non-clinical adverse effects. In some cases, a NOAEL was not identified and a LOAEL is included. The non-clinical AUC values used for these calculations were taken from the sponsor's CTD Toxicology Tabulated Summary.

Toxicity	Species	NOAEL (mg/kg) M/F	AUC_{0-24} µg.hr/ml	Safety margin based on AUC*
Gastrointestinal	Rat	20	26 §	1.3
	Marmoset	10	14	0.7
	Dog	50	Not found	
	Mouse	250	225	10.9
Ovaries: ↓weight, interstitial cell hypertrophy, ↓corpora lutea	Mouse	250	225	10.9
Irregular estrus cycles	Rat	10	6.6	0.3
Bone marrow atrophy	Rat	80	155 §	7.5
	Marmoset	50	102	5.0
Immunotoxicity	Marmoset	20 (LOAEL)	27	1.3
	Rat	60	97§	4.7
Pulmonary (Foamy alveolar macrophages)	Rat	80	155 §	7.5
Seg II abnormalities and variants	Rabbits **	42	12 Cmax	
	Rats†	192µmol/kg (100 mg/kg)‡	6‡ Cmax	

*AUC in human: AUC_{0-12} 10.3 µg.hr/ml for 100 mg/day (equivalent in that study to 90 mg b.i.d).
2 x 10.3µg.hr/ml = 20.6 was used for the calculation of safety margins.
§ mean of male and female values
**gallbladders, incomplete ossification of hyoid, pubis, sternebrae
†additional liver lobe,incomplete ossification of parietal bone, displaced articulcation of pelvis, extra ribs, incomplete ossification of sternebrae, misshapen/misaligned sternebrae, calcaneus ossified
‡Cmax µg/ml as AUC was not determined

14 Appendix/Attachments

APPENDIX: Sponsor's Email answer to Request for Information

From: Gigger, Emery [mailto:Emery.Gigger@astrazeneca.com]
Sent: Friday, March 26, 2010 11:06 AM
To: Monteleone, Michael V.
Cc: Prosser, Judith
Subject: NDA 022433 IR received March 15, 2010

Hi Mike,

Please find below AZ responses to nonclinical questions received on March 15, 2010. As a reminded, the study report in response to question 2 was e-mailed on March 16, 2010.

We will submit an official correspondence through the exchange later.

Please note that Jude will be sending a log of the FDA NDA questions and responses this afternoon.

Please confirm receipt and contact me if you have any questions.

Kind regards,

Emery

FDA QUESTION 1:

(b) (4) . *Where can I find the quantification of this impurity/metabolite for each of the nonclinical species and for humans?*

AZ Response:

The designation for (b) (4) is (b) (4) As noted in the Nonclinical Pharmacokinetics Written summary, (b) (4)

(b) (4)

FDA QUESTION:

Where in the application can I find details about canine metabolism of AZD6140. I would like to know the details of the relative amounts of metabolites formed in dog compared to the human and non-human primate.

AZ Response:

Dogs were not pursued as the non-rodent species in the toxicity testing program base on the *in vivo* pharmacokinetics data (study SC-103120, Table 3, Section 2.6.4, Pharmacokinetics Written summary of the NDA) where it was shown that the exposure ratio between the active metabolite AR-C124910XX (O-demethylation) and ticagrelor was less in the dog compared to the marmoset monkey, rats and humans. This difference is quantitative, not qualitative. Therefore the marmoset was selected to ensure adequate margins for the metabolite in toxicity testing. Due to this no *in vivo* metabolism in the dog have been performed.

From: Monteleone, Michael V. [mailto:Michael.Monteleone@fda.hhs.gov]
Sent: Monday, March 15, 2010 10:19 AM
To: Gigger, Emery
Cc: Prosser, Judith
Subject: NDA 022433

Emery -

I have a question from the pharmacology reviewer of your NDA 022433:

Where in the application can I find details about canine metabolism of AZD6140. I would like to know the details of the relative amounts of metabolites formed in dog compared to the human and non-human primate.

Thank you-
-Mike Monteleone

Confidentiality Notice: This message is private and may contain confidential and proprietary information. If you have received this message in error, please notify us and remove it from your system and note that you must not copy, distribute or take any action in reliance on it. Any unauthorized use or disclosure of the contents of this message is not permitted and may be unlawful.

From: Monteleone, Michael V. [mailto:Michael.Monteleone@fda.hhs.gov]
Sent: Monday, March 15, 2010 12:00 PM
To: Gigger, Emery
Cc: Prosser, Judith
Subject: NDA 022433

Emery -

Another two questions from the pharmacology reviewer:

(b) (4)

Thanks-
-Mike

Mike Monteleone
Regulatory Health Project Manager
Division of Cardiovascular and Renal Products
Center for Drug Evaluation and Research
Food and Drug Administration
michael.monteleone@fda.hhs.gov
p:(301) 796-1952
f:(301) 796-9838

II. Computational Toxicology Reports

Consults were requested from the Computational Toxicology Group for AZD6140, the two main metabolites, AR-C124910XX and AR-C133913, and the impurities, [redacted] (b) (4) The consult requests for this NDA (from this reviewer) were designed to address specific data gaps and are summarized below:

1. What is the structural similarity of ticagrelor to drugs that modulate hormonal processes? (This report is in the form of an Excel spreadsheet and was converted to a pdf format to permit inclusion of relevant portions in this review.)
2. Please evaluate ticagrelor in the rodent carcinogenicity database and ICH S2 genetic toxicity battery.
3. What is the structural similarity of AR-C124910XX and AR-C133913 to drugs that modulate hormonal processes? (This report is in the form of an Excel spreadsheet and was converted to a pdf format to permit inclusion in this review.)
4. Please evaluate [redacted] (b) (4) in the rodent carcinogenicity database and ICH S2 genetic toxicity battery.
5. Please evaluate the two impurities, [redacted] (b) (4), in the genetic toxicology databases.

4 Pages Have Been Wittheld In Full As b(4) (CCI/TS) Immediately Following This Page

Computational Toxicology Assessment of AZD6140 (ticagrelor) for Genetic Toxicity and Carcinogenicity

Ticagrelor was evaluated by CDER/OPS/SRS/ICSAS for rodent carcinogenicity and genetic toxicity using four (quantitative) structure-activity relationship [(Q)SAR] computational toxicology software programs[1]. The results of the predictions from all four software programs were weighted equally and the analysis was optimized for sensitivity (minimizing false negatives) to reach the overall conclusions.

Rodent Carcinogenicity[2]

Software	Rat	Mouse
Derek for Windows	NSA	NSA
Leadscope	-	-
MC4PC	-	-
SciQSAR	-	-
Overall ICSAS Prediction	**-**	**-**

In considering the entire weight of evidence, ICSAS concludes that ticagrelor is predicted to be negative for both rat and mouse carcinogenicity.

Genetic Toxicity for Predicting ICH S2 Battery[2]

Software	*Salmonella* Mutagenicity	*E. coli* Mutagenicity	Mouse Lymphoma	*In Vitro* Chromosome Aberrations	*In Vivo* Micronucleus
Derek for Windows	NSA	NSA	NSA	NSA	NSA
Leadscope	-	NC	-	-	-
MC4PC	-	NC	-	NC	-
SciQSAR	-	N/A	+	-	-
Overall ICSAS Prediction	**-**	**NC**	**+**	**-**	**-**

In considering the entire weight of evidence, ICSAS concludes that ticagrelor is predicted to be negative for genetic toxicity in the ICH S2 battery.

Ticagrelor was evaluated by CDER/OPS/SRS/ICSAS for adverse hepatobiliary, cardiological, and renal/urinary tract effects in humans using the MultiCASE MC4PC and Leadscope Model Applier software programs. The results of the predictions from the software programs were weighted equally and the overall call was optimized for sensitivity (minimizing false negatives).

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[1] Derek for Windows 12, Leadscope Model Applier 1.3.2-7, MC4PC 2.1.0.11, and SciQSAR 2.2

[2] + = positive; − = negative; Eqv = equivocal; NSA = no structural alerts are identified by DfW; NC = test chemical features are not adequately represented in the model training data set, leading to a no call; N/A = no available model.

7 Pages Have Been Wittheld In Full As b(4) (CCI/TS) Immediately Following This Page

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22433	ORIG-1	ASTRAZENECA LP	AZD6140

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

ELIZABETH A HAUSNER
06/23/2010

PATRICIA P HARLOW
06/23/2010

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Number:22433 **Applicant: Astra-Zeneca** **Stamp Date: Nov 18, 2009**

Drug Name: ticagrelor **NDA Type: commercial**

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	x		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	x		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	x		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	x		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	x		
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	x		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	x		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	x		

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?		x	Mg/kg doses have been used. The sponsor does not specify the basis for multiples of human exposure that have been listed.
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)			This needs to be determined through review of the studies that have been submitted
11	Has the applicant addressed any abuse potential issues in the submission?			Not applicable
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			Not applicable

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? ___yes_____

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.
Determining the adequacy of qualification of the impurities has been hampered by the multiple codes used to identify each impurity. The multiple identification codes used created uncertainty as to whether the necessary substances had been studied. This has been discussed with the sponsor and the sponsor has produced a list of the report numbers addressing specific impurities with clarification of the identities. The adequacy of qualification of the impurities will now need to be determined in the review process.

Elizabeth Hausner, D.V.M. December 23, 2009
Reviewing Pharmacologist Date

Patricia P. Harlow, Ph.D. December 23, 2009
Team Leader/Supervisor Date

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22433	ORIG-1	ASTRAZENECA LP	AZD6140

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

ELIZABETH A HAUSNER
12/23/2009

PATRICIA P HARLOW
12/23/2009