SCIENTIFIC DISCUSSION

This module reflects the initial scientific discussion for the approval of Angiox. This scientific discussion has been updated until 1 September 2004. For information on changes after this date please refer to module 8.

1. Introduction

Bivalirudin is an anticoagulant intended for parenteral use during invasive intravascular procedures such as percutaneous coronary intervention (PCI), including percutaneous transluminal coronary angioplasty (PTCA) procedures like angioplasty and balloon angioplasty and PTCA with stenting.

Patients with ischaemic heart disease are treated with medical therapy, PCI and CABG. The number of PCI performed in Europe now exceeds 500,000 per year. The technique involves positioning of a balloon within the coronary artery, which is then inflated to distend the occlusion. The ensuing damage to the arterial wall releases tissue factor, causes platelet adhesion and aggregation and starts the clotting cascade.

Currently, unfractionated heparin with GPIIb/IIIa inhibitor and adjuvant treatment with aspirin, clopidogrel is regarded as standard care in patients undergoing PCI. Despite this treatment approximately 5-11% develop ischaemic events with 4-10% having clinically major bleed.

Bivalirudin is a direct thrombin inhibitor with linear pharmacokinetics and dynamics. It is effective against fluid phase and clot bound thrombin and is alleged to inhibit protease-activated receptor (PAR) mediated activation of platelets.

Bivalirudin is a single chain of 20 amino-acid polypeptide based on the structure of hirudin. It is a direct thrombin inhibitor that binds reversibly to thrombin at both the active site and the substrate recognition site. Bivalirudin inhibits all thrombin-catalysed or -induced reactions, including fibrin formation, activation of coagulation factors V, VIII, and XIII, activation of protein C and platelet aggregation. Bivalirudin is highly selective for thrombin with an inhibitory constant (Ki) of 2.3 nM, and does not require a co-factor.

The claimed indication is for use as an "anticoagulant in patients undergoing percutaneous intervention (PCI)". The recommended dose is an intravenous bolus of 0.75 mg/kg followed immediately by an intravenous infusion at a rate of 1.75 mg/kg/h for at least the duration of the procedure. The infusion may be continued for up to 4 hours post-PCI as clinically warranted.
An additional 0.3 $mg \cdot kg^{-1}$ bolus dose of bivalirudin may also be administered during the procedure to reach a target activated clotting time (ACT) of greater than or equal to 225 seconds. The dose may need to be reduced in patients with renal impairment. A physician experienced in coronary intervention procedures should administer Angiox.

2. Quality aspects

Introduction

The proposed market dosage form is a lyophilised powder presented in a 10 ml single use sterile Type I glass vial, closed with a bromobutyl rubber stopper. Each vial nominally contains 250 mg of bivalirudin together with the bulking agent mannitol.

Immediately prior to use the lyophile is reconstituted with 5 ml Water for Injections to yield a concentrate of 50 mg/ml. This is then further diluted in 50 ml of either 5% dextrose in water or 0.9% sodium chloride to give a final concentration of 5 mg/ml. The resulting solution is intended for intravenous injection or infusion.

Drug Substance

The drug substance bivalirudin is a trifluoracetate salt of a synthetic 20 amino acid peptide with an average molecular weight of 2180.3 (anhydrous free base). It is an amorphous powder with lowest solubility around its isoelectric point about 3.6.

Manufacture

The manufacturing process of bivalirudin has evolved over time from a solid phase method via a pilot liquid phase process that was subsequently scaled up and further optimized. Further modification resulted in the current process.

An organic chemical process in solution-phase involving the synthesis and the condensation of two key intermediates to finally obtain the fully assembled peptide obtains Bivalirudin. Purification and isolation processes for bivalirudin drug substance involve the use of reverse phase preparative chromatography by two buffer systems (neutral and acidic), desolventization of acetonitrile by thin-film evaporation and a final filtration and lyophilisation step.

In comparison to the earlier manufacturing processes of the active substance, the formation of impurities which are difficult to remove during chromatographic purification (e.g. $[(D)Phe]^{12}$ and $[Asp]^{9}$) are minimised with the current process.

Only batches manufactured with the earlier processes have been used in clinical trials. However, equivalence has been demonstrated. Also, the specifications of the drug substance have been tightened.

Characterisation

The chemical structure of bivalirudin peptide has been elucidated by a number of standard methods including Amino acid analysis, Counter ion characterization, Elemental analysis, Mass spectrometric analysis (both fast atom bombardment and collision-induced dissociation techniques), Amino acid sequence analysis by automated Edman degradation, Analysis of enantiomeric purity of amino acids (after deuterolysis of the peptide, 2-dimensional NMR analysis, UV spectral analysis and Peptide mapping (after enzymatic digestion).

Physicochemical characterization of bivalirudin includes studies of hygroscopicity, solubility, decomposition and secondary structure.

It can be concluded that bivalirudin is a well-characterized peptide, and that the structure has been conclusively established.

Impurities

Following a comparison of the initial pilot scale and commercial synthetic processes, several HPLC methods were developed for impurity analysis. These methods were based on more advanced technologies and allowed improved resolution of impurities.

One related substance detected by the new neutral gradient HPLC method was characterized as D-Phe^{12}-bivalirudin. In this method, D-Phe^{12}-bivalirudin co-elutes with other minor impurities, e.g. di-Gly-bivalirudin (the so-called NGIP, neutral gradient impurity peak). When this mixture was first identified, D-Phe^{12}-bivalirudin was the major impurity. A separate acid gradient assay was developed to quantitate D-Phe^{12}-bivalirudin, which later turned out to also contain di-gly bivalirudin. The neutral gradient method therefore gives the total peak area of the three bivalirudin diastereomers (as the NGIP peak) while the acid gradient HPLC method is used to accurately quantify the levels of D-Phe^{12}-bivalirudin and the quantities of the other, co-eluting minor impurities and related substances.

Retrospective testing of historical material confirmed that this second and minor impurity di-Gly-bivalirudin has been present at relatively low and consistent levels also in material manufactured according to the old processes. The levels of both impurities are therefore described as a combined impurity by a relative retention time.

During the development of the current synthetic process, one of the coupling reactions, which were found to be responsible for the formation of the D-Phe^{12}-bivalirudin, was succesfully modified to reduce the impurity level.

A further impurity of importance is Asp^{9}-bivalirudin, which is also reflected in the specfication. Asp^{9}-bivalirudin is both a process impurity and degradant of bivalirudin drug substance.

Both impurities (D-Phe12- and Asp9-) have been investigated in pre-clinical studies. And it was concluded that no toxicological effects are associated with either impurity. Furthermore, it has been demonstrated that the D-Phe12-bivalirudin impurity is pharmacologically inactive.

The other major group of impurities eluting in the the neutral gradient system have been shown to be peptide breakdown products produced during the post purification desolventization and drying process - the levels of these are consistent and are independent of the synthesis process.

Specification

Testing of the active substance is conventional and includes Characteristics (appearance) and Identity (MS, TLC, amino acids relative composition), water and TFA acid content. Tests for bivalirudin characterize both the peptide (HPLC testing using two solvent systems to confirm the levels of specific and non specific impurities as well as the purity of bivalirudin) and the non-peptide components (water, trifluoroacetate, residual solvents and residual palladium).

The product is adequately assayed by the use of quantitative HPLC against a reference standard and the use of quantitative amino acid analysis to determine peptide content. Rather to provide historical continuity, a biological assay (thrombin binding activity) of the well-characterised petide is also included.

Impurities are controlled by HPLC methods as described above. The [Asp^{9}]- bivalirudin analog content increases with the water content. This is controlled in the specifications and adequate packaging of the active substance is guaranteed.

All test method has been appropriately validated. Batch analytical data have confirmed that the impurity profile of drug substance is consistent irrespective of the route of synthesis. It is is well defined for a peptide product and consistent over a range of batches.

Container

Hygroscopicity studies have shown that, as expected for a peptide, bivalirudin drug substance is slightly hygroscopic. Long-term stability studies have shown that this is not a practical problem provided it is adequately packaged for storage. To ensure this, Bivalirudin drug substance is packed and stored in polyethylene multi-layered polyethylene heat-sealed bags. The multi-layered bag is then packed into an aluminium foil layer and sealed. The foil laminate pouch acts as an impermeable moisture barrier.

Stability

Stability studies on material manufactured using the current manufacturing route have been carried out on one development batch and on three validation batches, stored in the same quality of packaging as used for bulk storage. Up to 24 month data have been provided from samples stored under ICH storage conditions including 25°C/60%RH, 5°C in darkness, -20°C in darkness, 40°C/75%RH and under light at 20°C. Analytical methods are as above.

No changes are seen to appearance, the level of bacterial endotoxins and microbial contamination under all storage conditions. At -20°C and +5°C no change is seen over the storage timeline to purity, impurity profile or assay. At elevated temperatures +25°C and +40°C and/or light a significant degree of degradation was observed.

Batch analytical data indicate that moisture levels on drug substance release do not increase significantly after 12 months storage. Based on the current data, the recommended storage conditions of -20°C to -10°C in the dark with a retest date of 12 months is acceptable.

Drug Product

Each 10 mL vial provides 250 mg of bivalirudin in a mannitol-bulked, lyophilized formulation. The product is presented in single-use glass vials with rubber stoppers.

Pharmaceutical Development

The development of the current (lyophilised) formulation began with a solution formulation that was stored frozen. A further solution formulation was developed that required refrigeration only which finally led to the development of the current (lyophilised) formulation.

The formulation proposed for commercialization is simple and unremarkable and was based upon the considerations of pharmaceutical properties of the active ingredient, chemical stability, intended clinical use and commercial considerations. The proposed formulation, 50-mg/mL bivalirudin at pH 5.5 in 2.5% mannitol solution, was found to be robust during laboratory scale lyophilization testing.

Moisture content and solubility have been identified as important considerations for the successful formulation of an amorphous lyophilised drug substance. In aqueous solutions, pH is expected to influence bivalirudin solubility while it should be unaffected by parameters such as temperature and the addition of mannitol.

The excipients are commonly used for parenteral products. Mannitol was chosen to provide a product that would be approximately isotonic upon reconstitution. Nitrogen is used as the headspace gas of the filled vial to provide an inert atmosphere for storage. Poor bioavalibility of peptide-based products also supports their formulation for direct intravenous administration. A manufacturing filling overage of 10% is used during manufacture to ensure reaching of the target concentration. Exposure to a standard autoclave cycle resulted in loss of 90% bivalirudin indicating that aseptic filling would be required.

Two formulations of finished product (frozen liquid formulation and the lyophilized) have been used during clinical development. Bioequivalence between the two formulations was confirmed in in two pharmacokinetic/pharmacodynamic studies.

Compatibility data on reconstituted product in sterile water and intravenous solution has shown the solutions to be chemically stable. In addition, eighty-seven out of ninety six intravenous drugs tested were compatible with bivalirudin. Products, which are found to be incompatible, have been listed in the SPC.

Manufacture of the product

The manufacture of bivalirudin proceeds through the two main stages preparation of the bulk drug solution (compounding of the drug substance, and filtration prior to aseptic filling) and sterile filling of vials followed by lyophilization and sealing. The sterilization conditions for vials and stoppers and automatic machine parts comply to the Ph Eur standard conditions with margin and are considered satisfactory. In process controls include the following critical tests: appearance; assay by UV; density; pH and bioburden.

The drug product manufacturing process has been validated by three non-consecutive batches, one at 38% of commercial scale and two at 100% of commercial scale. An satisfactory explanation for not validating three successive batches has been provided (due to limited expiry of bivalirudin). The evaluation has been thoroughly carried out and has involved each of the stages of the process i.e. formulation, post-filtration, filling and lyophilisation. In addition, considerable experience has been gained at the intended site of manufacture with the production of over thirty batches of material.

The excipients are standard and are controlled in accordance with their respective current Ph Eur monographs. Exemplary Certificates of Analysis have been provided. None of the raw materials are of human or animal origin.

Specifications

Specifcation include identity (UV and HPLC), biological activity (inhibition of thrombin), purity (HPLC), strength (UV). Additional tests of product quality, including particulates, pH, sterility, and uniformity of dosage units are carried out. Tests to assure product sterility and microbial contamination (endotoxin) comply with the Ph.Eur.

It is considered that the control tests described for the finished product specification contain standard tests and limits that are relevant for a product that is intended for parenteral administration.

The levels of D-Phe^{12}-bivalirudin are greatly reduced in drug product containing drug substance manufactured using the modified synthetic process. Drug product therefore contains only a residual quantity of this impurity plus a small amount of the second minor impurity, di-gly-bivalirudin.

Stability

Eighteen months real-time data at 5°C and 25°C/60%RH and 6 months accelerated data at 40°C/75%RH are provided on three batches of drug product manufactured using drug substance produced by the most recent process stored in the marketing pack. Three-year real-time data and 6 months accelerated data are also provided on three batches containing drug substance manufactured using the previous process.

Compendial tests are carried out according to USP requirements. However; the sterility test has been modified to meet both Ph Eur and USP requirements. Although it would be preferable to have all testing conducted to comply with the Ph Eur, overall this is not considered to have any impact on the interpretation of the results as critical non-compendial test methodologies are identical to those used for release testing in the EU. Moreover, it is considered that that the relevant compendial USP tests, applied consistently can be used to provide evidence of adequate stability**.**

For product containing active substance, which was manufactured using the recent process, the results at all, storage conditions are seen to be well within the specification limits. Overall the data support the stability equivalence between drug products manufactured with bivalirudin manufactured using either synthetic processes or the recommended storage conditions of up to 25°C for a maximum of 36 months is acceptable.

Furthermore, it was shown that following reconstitution with 5mL water for injections to a concentration of 50 mg/mL that the diluted material was stable for at least 24 hours at between 2-8°C. Subsequent dilution in dextrose or saline, and storage in plastic infusion bags to final concentrations ranging from 0.5 mg/mL to 5 mg/mL showed that the product remained unchanged for up to 48 hours at 25°C. No evidence was seen of adsorption of the material onto infusion bag materials.

The overall storage conditions as stated in detail in the SPC are therefore supported.

Discussion

Impurities

As is common with synthetic peptides purified by reverse faze preparative HPLC, most of the impurities are deletions, additions or optical enantiomers of the parent peptide and are grouped around the foot of the major bivalirudin peak. The levels of these impurities have decreased with manufacturing experience and as described above, in the most recent process the level of D-Phe^{12}-bivalirudin are significantly reduced.

Manufacturing experience to date indicates that the levels of the combined impurities in the NGIP in bivalirudin drug substance are below 0.5% and as such, there is a justification for removing D-Phe^{12}-bivalirudin as a named impurity from the specification. In view of the fact that manufacturing experience with the current route is still limited, a limit for D-Phe^{12} bivalirudin on the drug substance specification is retained, and, in order to control the levels of this and the di-Gly derivative, describe both impurities on the release specification for drug substance manufactured by the modified synthetic route by their relative retention time. A conserative limit for the combined impurity is imposed.

A number of additional impurities at levels of less than 0.5% (including di-Gly bivalirudin), and a range of break down sequences have also been identified and characterized. These products do not feature on the release specification and this is acceptable since the ICH guideline on impurities does not apply to peptide-based drug products with respect to impurity qualification levels.

In summary it can be noted that as a result of the improvements to the manufacturing process for the drug substance the specification for the drug substance has been tightened. This in turn has led to a tightening of the specification for the drug product. Process validation has been provided for commercial scale batches. The stability data shows a stable drug product.

3. Non-clinical aspects

GLP

Although most of the safety pharmacology studies were made before it became mandatory for these to be performed in accordance with GLP, the Applicant states that they were carried out under GLP conditions and only lack Quality Assurance (QA) involvement. All toxicity studies were carried out in compliance with GLP.

Pharmacology

Primary pharmacodynamics

Bivalirudin is a direct thrombin inhibitor that was designed using the leech anticoagulant protein hirudin as a model. It comprises a COOH-terminal region, which binds to the anion binding exosite through a dodecapeptide sequence based on residues 53-64 of hirudin, and a 4 amino acid NH_2-terminal region that binds to the active catalytic site, separated by 4 glycine residues. Unlike hirudin, which binds so tightly to thrombin that its action is essentially irreversible, bivalirudin is a reversible inhibitor. Bivalirudin was shown to be a mixed competitive/non-competitive thrombin inhibitor with a K_i value of 2.3 nM.

The kinetic data for the interaction between bivalirudin and thrombin are consistent with a 4-step mechanism. The first step, binding of the C-terminal region to the anion-binding site, is followed by an intramolecular conformational change, the rate-limiting step. The N-terminal portion of bivalirudin then binds very rapidly to the catalytic site and a second intramolecular conformational change occurs. These steps stabilise the enzyme-inhibitor complex 400-fold.

Bivalirudin is a highly specific thrombin inhibitor and causes a dose-dependent prolongation of clotting time as assayed by 3 different methods, namely thrombin (TT), Activated partial

thromboplastin time (APPT), and prothrombin time (PT). APTT is considered to be the most useful estimation for assessment of bivalirudin activity. The dose-response relationship for prolongation of APTT is close to linear at bivalirudin concentrations of ≥ 100 $ng \cdot mL^{-1}$.

There is considerable inter-species variation in the efficacy of bivalirudin as a thrombin inhibitor. In a dose-response study, the order of sensitivity for bivalirudin inhibition was mouse >rat>human>cynomolgus monkey>cow>pig>dog>rabbit. At the concentrations required for prolongation of APTT to 300% of baseline, the species closest to man was the cynomolgus monkey.

Bivalirudin inhibits thrombin-induced platelet aggregation and α-granule secretion. However, the IC_{50} for α-granule secretion is approximately 3.5 times greater than that for aggregation. Bivalirudin activity is specifically directed against thrombin. It does not appear to interfere with normal platelet function.

Bivalirudin inhibits the cleavage of fibrinogen by clot-bound thrombin as effectively as by free thrombin, but has no obvious intrinsic effect on the fibrinolytic system.

The effects of bivalirudin on preformed thrombi and thrombus formation were investigated in several species. In a rat model, bivalirudin potentiated tPa-induced thrombolysis of an artificially established thrombus in the aorta. In combination with tPA, bivalirudin, but not a similar dose of recombinant hirudin (rHIR) or heparin, significantly reduced time to re-establishment of blood flow. Anticoagulant treatment significantly reduced re-thrombosis in comparison with control, but although all 3 anticoagulants increased the duration of patency, bivalirudin was significantly more effective than heparin.

Bivalirudin was also more effective than heparin in reducing incidence of repeated thrombosis after clot formation and dislodgement in injury and stenosis damaged carotid artery in the pig.

Bivalirudin itself is slowly hydrolysed by thrombin at the Arg_3-Pro_4 bond that lies at the scissional point of the catalytic site.

Secondary pharmacodynamics

No specific studies have been performed, which is considered acceptable since bivalirudin is highly specific and does not appear to inactivate serine proteases other than thrombin.
Although it inhibits platelet aggregation, bivalirudin does not increase platelet reactivity, as it appears able to inhibit clot-bound thrombin and consequently the secondary and primary mediated pathways for thrombin activation.

Safety pharmacology

The only effects of any consequence observed in the safety pharmacology screening studies were on the cardiovascular system in rats and dogs; bivalirudin, 1 or 5 $mg \cdot kg^{-1}$ *iv* caused a dose- related increase (~30% at the higher dose) in both systolic and diastolic blood pressure in the rat. A dose of 5 $mg \cdot kg^{-1}$, given as an *iv* infusion over 15 minutes increased blood pressure parameters in the dog, with the maximal effect observed at 4 minutes. However, as discussed later, no cardiovascular effects were seen in the baboon or in cynomolgus monkeys in the repeated dose toxicity studies.

There was no evidence of adverse effect on the CNS or ANS, or on the gastrointestinal tract or renal function at pharmacologically active doses.

Bivalirudin did not cause anaphylaxis in the guinea pig and there were no indications of immunological effects in the repeated dose toxicity studies.

Pharmacodynamic drug interactions

There are no indications of adverse interactions with aspirin, warfarin, urokinase or streptokinase although, not unexpectedly, bivalirudin may potentiate the anticoagulant effects of warfarin and aspirin.

Pharmacokinetics

Methods of analysis

Although bivalirudin is intended for *iv* administration only, absorption following subcutaneous (*sc*) administration was also investigated to validate this route for the reproductive studies.

The pharmacokinetic studies generally used radioactive bivalirudin labelled with ^{3}H and/or ^{14}C in different parts of the peptide and an enzyme-linked immunoassay (ELISA) that detected the parent compound.

Th ELISA method detects bivalirudin by its ability to inhibit the binding of a specific monoclonal antibody to a bovine serum albumin (BSA)-peptide conjugate bound to the solid phase. The accuracy of the ELISA assay is not very high (± 20%).

Validation was only reported in one study, where recovery was 96 to 110 % over the range of 300 to 900 ng·mL^{-1}.

The ^{3}H and ^{14}C labelled peptides were synthesised from labelled and unlabelled amino acids by a combination of automated and manual solid phase procedures. The ^{3}H label was placed in the proline$_2$ amino acid to trace the N-terminal DPh-Pro moiety. In the initial rat study the ^{14}C label was placed in the tetra-glycine spacer only. In all other studies, the ^{14}C label was distributed along the remaining 18 C-terminal residues.

Absorption - Bioavailability

Pharmacokinetics after a single bolus dose

Species	**Rat**			**Rabbit**		
Sex	**Males**			**Males**		
Administration Route	***s.c***	***s.c***	***iv***	***sc***	***sc***	***iv***
Dose (mg·kg^{-1})	**50**	**250**	**50**	**50**	**250**	**50**
Max. Plasma level (µg·mL^{-1})						
Approx.2 min			381.0			1,036.9
15 min	29.8	72.3	65.4	21.5	75.1	620.6
30 min	37.7	81.8	34.0	16.4	78.8	339.8
1h	17.7	67.3	17.3	10.7	111.8	95.9
12h	1.8	0.9	0.4	619	3.3	BLQ
24 h	<50	0.2	0.6	BLQ	BLQ	BLQ
Coagulation						
Evident	15 min	15min	≤ 2 min	15min	15min	≤ 2 min
Maximal	30 min	1h	≤ 2 min	30 min	2 h	≤ 2 min
Elevated	>2<4 h	>4 h	≤ 30 min	6 h	24 h	6 h

BLQ = Below level of quantification

Pharmacokinetics after 4 h continuous infusion

Species	**Rat**			**Dog**		**Cynomolgus Monkey**	
Sex	**Males & Females**			**Females**		**Females**	
Dose (mg·kg^{-1})	1.04	3.12	10.42	6.8	6.8	6.9	42.5
Cmax (ng·mL^{-1})	2,468	4,525	19,484	2223	2053	2707	23750
Tmax (h)	1	1	1	2	2	2.5	4
AUC (ng.h/mL)	6970	11,772	60,431			8480	93159
$t_{1/2}$, (h)	2.2		0.4			0.7	0.7

Pharmacokinetics: absorption after repeated doses

Species	**Cynomolgus Monkey**			
Sex	**M**	**M**	**F**	**F**
Method of Administration	***iv***	***sc***	***iv***	***sc***
Duration, days	**7**	**7**	**7**	**7**
Dose ($mg \cdot kg^{-1} \cdot day^{-1}$)	**4**	**4**	**4**	**4**
AUC, h.ng/mL Day 1				
Day 7	12,046	11,469	8,528	4,909
	11,439	dead	9,491	2,636
Cmax (ng/mL) Day 1				
Day 7	37,118	3,382	21,572	1,831
	24,102	dead	23,036	1,635
Tmax (min) Day 1				
Day 7	2	120	2	30
	2	dead	2	30

Distribution

Two tissue distribution studies were performed in rats using ^{3}H /^{14}C labelled bivalirudin. In the first study, the dose used was $3 mg \cdot kg^{-1}$ and tissue levels were assayed at 24 h. The only tissue containing measurable levels of ^{3}H was the kidney.

In the second study, the ^{14}C label was uniformly incorporated throughout the 18 residue C-terminal peptide. The dose was 10 $mg \cdot kg^{-1}$ and tissues were assayed at 3 time points. At the first sampling time, the only tissue with a higher concentration of ^{3}H than plasma was the kidney and very little remained by 6 h after dosing. In contrast, ^{14}C was rapidly taken up into skeletal muscle, bone skin and kidney and by 6 h was distributed to skeletal muscle, skin and bone with a smaller amount in liver and spleen. At 24 h, the concentrations in skeletal muscle and bone were still high, while those in other tissues had declined and small quantities had appeared in kidney and small intestine.

The distribution of the N-terminal non-physiological amino acid D-phenylalanine was investigated in the rat using bivalirudin labelled with ^{3}H in this position. It was rapidly cleared from blood and initially widely distributed. Within 6 h after administration of an *IV* bolus dose of 1 $mg \cdot kg^{-1}$, tissue levels were all <1.5% of the dose.

Binding to plasma proteins were examined by 2 methods, equilibrium dialysis across a membrane and gel filtration, using ^{125}I- labelled bivalirudin. In the absence of plasma, equilibration across the dialysis membrane was reached in 2 h. The gel chromatography elution profile of ^{125}I- labelled bivalirudin was also unchanged in the presence of human plasma, peak radioactivity being found at 11.0 mL.

Human plasma causes a volume-dependent inhibition of thrombin-catalysed hydrolysis of a chromogenic substrate by thrombin. However, bivalirudin caused dose-related inhibition in the presence of 5, 10 and 25 % human plasma. Bivalirudin's biological activity does not, therefore, appear to be affected by the presence of plasma proteins.

Binding of ^{125}I- labelled bivalirudin to human erythrocytes was investigated using gel-filtration chromatography and sucrose-layer centrifugation. Binding would appear to be non-specific, involving a high number of low affinity-sites.

In the study using the N-terminal dipeptide, whole blood and plasma levels of ^{3}H activity declined in parallel, indicating that the cleaved dipeptide does not bind to or penetrate erythrocytes.

There were no investigations into possible placental transfer of bivalirudin, or its transfer into milk.

Metabolism

The differences in the fate of the ^{3}H in the N-terminal dipeptide and the ^{14}C in the rest of the molecule, suggest that the parent compound is metabolised early and extensively. The similarity of the data

whether the ^{14}C is placed in the glycine spacer only or evenly distributed along the 18-amino acid C-terminal portion indicates that the initial reaction is the hydrolysis of the N-terminal dipeptide. This is supported by the clear separation of the ^{3}H and ^{14}C activity by iso-electric focusing of urine from a rat treated with bivalirudin with the ^{14}C label in the glycine spacer. Bivalirudin is stable in citrated plasma from rat, cynomolgus monkey and man *in vitro* and, therefore, proteolysis must occur at an extravascular site. Published data for other small peptides suggest that this could be the kidney. A study in which the N-terminal dipeptide was administered indicated that this is metabolised in the kidneys.

The ^{14}C label was initially cleared from plasma in parallel with the ^{3}H label, but was widely distributed to tissues with prolonged maintenance of a low plasma level. There was an uptake into skeletal muscle and skin and a later appearance in the small intestine. These findings are consistent with breakdown of the peptide by non-specific proteinases with the fragments entering the amino acid pool.

Induction of cytochrome P_{450} liver enzymes was investigated in liver samples obtained from rats after 28 days continuous infusion of bivalirudin at doses up to 250 $mg \cdot kg^{-1} \cdot day^{-1}$. Total protein yield, total cytochrome P_{450} content and enzyme activities data revealed no biologically significant effects.

Excretion

Excretion of the breakdown products of bivalirudin is almost exclusively in the urine in rat and cynomolgus monkey. Excretion of ^{3}H activity was rapid, almost all within the first 4 h. Low recovery of ^{14}C is consistent with incorporation of the amino acids from the C-terminal peptide into newly synthesised protein.

The excretion of the N-terminal non-physiological amino acid D-phenylalanine was investigated in the rat using bivalirudin labelled with 3H in this position. The radioactivity was rapidly excreted in urine. Recovery from faeces was higher than for 3H label in the proline2 position. Total recovery was low and it was suggested that the radiolabel may have undergone proton exchange with body water and been exhaled through the lungs.

Pharmacokinetic drug interactions

Pharmacokinetic interactions were investigated in the cynomolgus monkey. Plasma levels of bivalirudin were not significantly altered by concomitant administration of urokinase. Prior administration of oral warfarin to give a steady state prolongation of prothrombin time did not significantly affect T_{max}, C_{max} or AUC of bivalirudin. The interaction between bivalirudin, aspirin and tPA was investigated in the rhesus monkey and compared with that of heparin. Aspirin, 40 mg, was given orally for 3 days before an infusion of tPA. Bivalirudin, 3 $mg \cdot kg^{-1} \cdot h^{-1}$ or heparin (100 U bolus, followed by 50 $U \cdot kg^{-1} \cdot h^{-1}$) was infused for 48 h starting 15 min before the end of the tPA infusion. Both bivalirudin and heparin increased plasma tPA levels and prolonged its half-life.

Other pharmacokinetic studies

Renal impairment
Partially nephrectomised rats were used as a model for renal impairment. Peak plasma levels and AUC for unchanged bivalirudin were increased and clearance was decreased. Recovery of the ^{3}H label from the normal kidney was comparable to controls, but that from the ischaemic kidney was reduced. Excretion of the radiolabel was also reduced but total excretion over 48h was comparable.

Comparison of Manufacturing Processes and Formulations

Changes in manufacturing process and formulation implemented during product development had no effect on the pharmacokinetic profile and pharmacodynamic activity of bivalirudin in animals. The material utilised in the first Phase III PTCA clinical trial was manufactured using pilot scale liquid phase synthesis and formulated as a frozen formulation. The material used in the second Phase III

study was manufactured for commercial use using commercial scale liquid phase synthesis and was formulated as a lyophile.

These formulations were administered to the monkey by continuous infusion for 14 days. The commercial scale product was kinetically similar to the pilot scale product, as indicated by the terminal half-lives, total body clearance, and mean residence time values. Pharmacological activity monitored as prolongation of aPTT measured during infusion on the first (Day 1) and last (Day 15) days of treatment indicated comparable effects of the two formulations.

Toxicology

Single dose toxicity
The acute toxicity of bivalirudin is low, with mice and rats tolerating single *iv* doses of up to 200 $mg \cdot kg^{-1}$ without any adverse effects. Cynomolgus monkeys tolerated increasing doses of 5 to 100 $mg \cdot kg^{-1}$ with only prolonged bleeding at the injection site.
Pilot studies revealed that the limiting toxicity would be an extension of the pharmacological effect.

Repeat dose toxicity
There were no adverse effects observed in rats administered an *iv* bolus of 36 $mg \cdot kg^{-1} \cdot day^{-1}$ for 28 days.

Following 28 days of continuous *iv* infusion at 75 $mg \cdot kg^{-1} \cdot day^{-1}$, only the minimal effect of inflammation at the administration site (phlebitis/periphlebitis) was observed in rats. At a dose of 250 $mg \cdot kg^{-1} \cdot day^{-1}$, increased spleen weight in 3/20 animals was accompanied by lymphoid hyperplasia and the phlebitis/periphlebitis was exacerbated. These effects generally reversed over a 14-day recovery period. A dose of 1000 $mg \cdot kg^{-1} \cdot day^{-1}$ resulted in pale mucous membranes and apparent constipation in some male animals. Red blood cell parameters were decreased, adrenal and kidney weights increased, pale foci were seen in the kidneys and there was evidence of internal haemorrhage and splenomegaly, associated with extramedullary haematopoiesis. Treatment related deaths, possibly may due in part to haemorrhage, occurred at doses of ≥ 250 $mg \cdot kg^{-1} \cdot day^{-1}$.

In the cynomolgus monkey 14-day study, there was a minor decrease in red blood cell parameters at the highest dose of 150 $mg \cdot kg^{-1} \cdot day^{-1}$ and gross and microscopic evidence of haemorrhage in a wide variety of tissues in the males. One female died at this dose level, although the cause of death was not established.

After 28 days continuous infusion at 150 $mg \cdot kg^{-1} \cdot day^{-1}$, histological examination revealed focal/ multi-focal myocardial degeneration and/or necrosis associated with slight to mild haemorrhage in 2/6 animals. These lesions were not seen after a 14-day recovery period.

Studies using *sc* administration were also performed in rabbit and cynomolgus monkey. Repeated injection of 12.5 $mg \cdot kg^{-1} \cdot day^{-1}$ for 5 or 7 days produced mainly local effects in rabbits. Administration of 4, 12 and 36 $mg \cdot kg^{-1} \cdot day^{-1}$ for 28 days caused dose related bruising at the injection site and also at sites related to the restraint in cynomolgus monkey.

Toxicokinetics

Comparison of plasma levels and pharmacological effect

	Rat		**Cynomologous Monkey**	
Dose, mg/kg	Plasma level, µg/mL	APTT prolongation	Plasma level, µg/mL	APTT prolongation
40/45			2.8-4.0/4.4	2-fold/3-fold
75/100	7-9	2-fold		
150			10.5/13.4	5-7-fold
250	21	3-fold		
500	32	3-7-fold		
1000	30-40	3-7-fold		
2000	100-150	2-fold in males, slight in females		

Differences were found between the sensitivity of rat (less sensitive) and cynomologous monkey (sensitivity close to humans) to the anticoagulant action of bivalirudin. As expected, the rat has tolerated much higher doses of bivalirudin for 14 days with plasma levels about ten times above those levels considered in the therapeutic range, and no indication of toxicities different from those derived of anticoagulation were reported.

Genotoxicity

No positive results were obtained in 5 *in vitro* studies. Assays were carried out with and without metabolic activation by arochlor-induced rat S-9 mix except for DNA repair in rat hepatocytes which are capable of internal metabolic activation. Although a toxic level was not reached, each study employed the maximum recommended dose. Appropriate positive and negative controls were included in each study. Bivalirudin was degraded to a small extent in HAM's medium in the presence of S9, but this does not appear to have affected the interpretation of the cultured CHO cell mutagenicity study.

Bivalirudin did not increase the incidence of micronucleated polychromatic erythrocytes in bone marrow cells in rats at 24 hrs after 2 daily *sc* doses of 250 to 1,000 $mg \cdot kg^{-1}$. Plasma drug levels obtained in the range-finding study provided adequate evidence of exposure.

Carcinogenicity

No studies performed, which is considered acceptable in the light of the intended use of the product. Bivalirudin is intended for a short period of administration in the clinical setting. Bivalirudin will not be used for long-term administration or in children, and is not genotoxic and therefore it is not considered neccesary to perform any study.

Reproductive and developmental studies

Reproduction studies used the *sc* route of administration. The pharmacological effect of bivalirudin, and the potential bleeding, precluded administration during the peri-natal period.

In a combined fertility and development study, there was no effect on male fertility or mating behaviour up to a dose level of 500 $mg \cdot kg^{-1} \cdot day^{-1}$ administered for 28 days pre-mating. There were also no effects on female fertility, mating behaviour or implantation at this dose when administered from 15 days pre-mating to day 17 of gestation. Pre-implantation loss was, however, increased at 500 $mg \cdot kg^{-1} \cdot day^{-1}$.

In a developmental and peri- and post-natal study at doses of up to 150 $mg \cdot kg^{-1} \cdot day^{-1}$, there were no adverse physical or developmental effects (including reproductive function) in the F_1 generation. In a second study, dosing was recommenced during the lactation period (LD2 to LD20) and again there were no adverse effects on the F_1 generation at a maternal dose level of 150 $mg \cdot kg^{-1} \cdot day^{-1}$. No delayed adverse effects on parturition were seen in either study.

In studies covering the period of organogenesis in rats and rabbits there were no adverse effects on the offspring at doses up to 150 $mg \cdot kg^{-1} \cdot day^{-1}$. A toxic level was not reached in rabbits, although decreased body weight gain and food consumption were observed at doses of $\geq$ 75 $mg \cdot kg^{-1} \cdot day^{-1}$ in a pilot study.

Local tolerance

Three separate studies were made using *sc* administration in rabbits. Local irritation was minimal up to a dose level of 50 $mg \cdot kg^{-1}$.

Other toxicity studies

When incubated in human blood, bivalirudin did not cause haemolysis of red blood cells or plasma protein flocculation at a concentration of 10 $mg \cdot mL^{-1}$.

The effect of 4 potential impurities, PB19-1, PB 19-2, PB-19-3 and PB 19-6 (D-PHE12 bivalirudin), was compared with those of the parent compound after a single *iv* bolus dose of 50 $mg \cdot kg^{-1}$ in rats. No adverse effects were observed with PB19-1, PB 19-2 and PB-19-3. The major impurity, PB19-6 administered at a dose of 36 $mg \cdot kg^{-1} \cdot day^{-1}$ for 14 days caused minimal lesions of reversible type in the liver and marginally exacerbated age-related changes in heart and kidney. Changes in the method of synthesis have apparently significantly reduced the potential for this compound to arise.

Administration of partially degraded bivalirudin, caused minor lesions in the liver, consistent with a response to a foreign protein, minimal biliary hyperplasia and slight exacerbation of the portal inflammation present in the controls. The same effects were present in the bivalirudin comparator group but have never been seen in other studies at higher doses.

The bivalirudin formulation was not pyrogenic in rabbits and did not cause systemic anaphylaxis in guinea pigs. No increase in antibody titres was seen in toxicity studies in cynomolgus monkeys after continuous *IV* infusion of up to 150 $mg \cdot kg^{-1} \cdot day^{-1}$ for 14 days or *sc* administration of up to 36 $mg \cdot kg^{-1} \cdot day^{-1}$ for 28 days.

Bivalirudin did not induce systemic anaphylaxis in guinea pigs. Also, anti-bivalirudin antibodies were not induced in the cynomologous monkey toxicity studies, and there is no pharmacokinetic or pharmacodynamic evidence of a neutralizing response. However, anti-bivalirudin antibody titres over pre-dose values at late stages (44 days after start of infusion) are not provided in these studies with cynomologous monkeys. According to these data, bivalirudin does not appear to elicit immunological responses or hypersensitivity reactions.

Ecotoxicity/environmental risk assessment

Ecotoxicity and environmental risk is not to be expected with bivalirudin.

Discussion on the non-clinical aspects

Pharmacology
The pharmacological activity of bivalirudin appears to reside solely in its inhibition of the enzymatic activity of thrombin. The anticoagulant effect should, therefore, be highly predictable and there should be no need for the frequent titration of dose as required with heparin. As it is a reversible inhibitor, normal coagulation is rapidly recovered after cessation of administration.

Bivalirudin is highly specific and does not appear to inactivate serine proteases other than thrombin. Although it inhibits platelet aggregation, bivalirudin does not increase platelet reactivity as it appears able to inhibit clot-bound thrombin and consequently the secondary and primary mediated pathways for thrombin activation.

Antithrombotic activity has been demonstrated *in vivo* in different models of venous and arterial thrombosis yet there are no animal models for PCI/PTCA. No relevant adverse effects on the major physiological systems studied were found for bivalirudin in safety pharmacology studies. The hypertensive response reported in rats and dogs is not due to inhibition of autonomic mediators, and was not found in primates. This difference can be reasonably explained by variation in the thrombin actions at the level of vascular tissues between species.

The specificity of thrombin as a serine protease appears to be due to its anion-binding site. Inter-species variations in the amino-acid sequence in these loops would affect binding affinity and consequently inhibitory activity. A similar interspecies variation in activity has been described for other hirudin-based peptides.

Bivalirudin did not induce liver P450 isoenzymes in rats, and therefore, metabolic interactions with other drugs that are subjected to hepatic biotransformation are not to be expected. Apart from reduction of tPA plasma clearance, bivalirudin does not appear to have an adverse interaction with warfarin, aspirin, urokinase or streptokinase when administered concurrently.

Pharmacokinetics
The Applicant has performed a number of studies concerning the pharmacokinetic profile of bivalirudin in a number of animal species.

The overall conclusions are that bivalirudin has a very short plasma half-life, the N-terminal dipeptide is rapidly hydrolysed and excreted in the urine, and the rest of the molecule is rapidly broken down and the amino acids incorporated into tissue proteins. However, the accuracy of the ELISA assay is not very high and the studies employed few animals. There was also considerable inter-individual variation in the cynomolgus monkey studies.

Bivalirudin is cleared rapidly from the plasma and consequently, its pharmacodynamic effects are readily reversible after cessation of administration, leading to a rapid recovery of normal coagulation. The metabolites of bivalirudin have not been specifically identified. However, the assumption made by the Applicant that the metabolites of bivalirudin are presumed to be small peptide fragments or individual amino acids, and therefore their characterisation in urine and tissues was not necessary, is considered acceptable. Renal clearance of bivalirudin is important and therefore its half-life is prolonged in renal failure.

Bivalirudin was synthesised by different procedures (initially by a solid- and later a liquid phase process) and was formulated in different ways (solid and early liquid phase bulk substance frozen) during its development process. The two formulations obtained during the development process showed to be bioequivalent in the non-clinical studies performed.

Bivalirudin does not induce its own metabolism and does not bind to plasma proteins or enter red blood cells. A small portion appears to bind in a non-specific fashion to low affinity sites on erythrocytes. Therefore, drug interactions derived from displacement of binding to plasma proteins are not to be expected from bivalirudin administration.

Bivalirudin is unlikely to affect the hepatic P450-mediated metabolism of concomitant therapies. There appears to be no relevant pharmacokinetic interaction when bivalirudin was co-administered with warfarin, urokinase, tPA, aspirin and streptokinase. Although results indicate that bivalirudin decreased the clearance of tPA in monkeys, the data show that this interaction was also observed with heparin, and there were no unexpected toxic effects observed as a result of this interaction. It is unknown whether there is a pharmacokinetic interaction with tPA in humans.

Toxicology
Single and repeated dose toxicity studies were performed in the cynomolgus monkey and in the rat. Also, some single dose toxicity studies were carried out in the mouse.

In the absence of any blood sampling procedures, rats tolerated continuous infusion of doses up to 1,000 $mg \cdot kg^{-1} \cdot day^{-1}$ for 28 days and up to 2,000 $mg \cdot kg^{-1} \cdot day^{-1}$ for 14 days without evidence of target organ toxicity. At this dose, plasma levels of bivalirudin were approximately 10 times those seen in clinical use.

The cynomolgus monkey appeared to be more sensitive to the pharmacological effect of bivalirudin than the rat. The maximum tolerated dose was only 150 $mg \cdot kg^{-1} \cdot day^{-1}$. Although the plasma drug levels achieved at this dose did not exceed the expected human therapeutic level, the prolonged duration of exposure at a constant level at least similar to that seen in clinical use should have revealed any potential target organ toxicity.

Bivalirudin is intended for short-term administration and therefore no data on the long-term carcinogenic potential of bivalirudin are available. However, bivalirudin was not mutagenic or clastogenic in standard assays for such effects.

Although a toxic level was not reached in the study covering the period of organogenesis in the rabbit, no teratogenic effects were seen in the range finding study, which included a dose that produced maternal systemic toxicity.

Although reactions were observed at the administration site after prolonged administration, these reactions can be ascribed to excessive pharmacological effect and local tolerance of bivalirudin is acceptable.

No apparent toxicity linked to the presence of the D-Phe amino acid portion of the bivalirudin molecule was observed. Acute and repeated dose studies in rats demonstrated no toxicity associated with the major impurities and degradation products of bivalirudin.

In conclusion, these toxicity studies offer reasonable support for an expected safe use of bivalirudin in the currently proposed clinical conditions (low dose level and short duration of exposure). The results reported indicate that the main toxicity observed after single, repeated or continuous exposure is related to the anticoagulant effect of the product. No other toxicity – unrelated to the haemostatic alteration - was found. Toxicity was reversible after stopping the drug administration and no delayed toxicity was apparent. Undesirable effects that resulted from prolonged physiological stress in response to a non-homeostatic state of coagulation were not seen after short exposure comparable to that in clinical use, even at much higher doses. Based on this, no special hazard is foreseen in humans.

4. Clinical aspects

GCP

All clinical studies were conducted in line with GCP standards.

Pharmacokinetics

Absorption – Bioavailability

C90-010: This was a three-part study in 72 healthy volunteers. Part I was a single blind, placebo-controlled, sequential dose-escalation, part II a single blind, placebo-controlled crossover evaluation with and without aspirin and part III an open-label, sequential dose-escalation evaluation with aspirin.
The results showed dose-dependent increase in aPTT, PT and TT with good correlation with plasma concentrations. The anticoagulant activity was quick in onset and reversed rapidly.
Kinetic parameter following IV administration showed mean elimination half-life of 24 minutes with volume of distribution of 13L. Approximately 10 –22% of drug was excreted renally. Following subcutaneous administration, prolongation of anticoagulant activity was noted with increasing doses with peak anticoagulant effect at 0.25 – 2 h.
No PK/PD interaction was noted with aspirin and there was no effect on platelets. A single subject had a positive post-dose antibody titre.

C92-305: This was an open, randomised, crossover comparison of two formulations of bivalirudin administered intravenously. A total of 20 healthy volunteers were recruited. Each subject received two IV infusions separated by 7 days. Bivalirudin `A' 0.5mg/kg/h *4h bivalirudin `D' 0.5mg/kg/h *4h was administered.
Results showed similar kinetic parameters and anticoagulant activity for both formulations but greater incidence of nausea and vomiting with bivalirudin `D'. A statistically significant difference, however was observed for Tmax and urinary clearance.

C93-310: This was a double blind, placebo-controlled study in 15 healthy male subjects were given 4-hour infusions of either a frozen (bivalirudin "A") or a lyophilised formulation (bivalirudin "E1"), or a placebo. Each treatment was separated by 7 days. The AUC_{0-28} and Cmax values were similar and there was a strong correlation of aPTT values with plasma concentrations of bivalirudin. No antibodies were detected.

	AUC_{0-28} (h.ng/mL)	**$AUC_{0-\infty}$ (h.ng/mL)**	**C_{max} (ng/mL)**	**T_{max} (h)**	**Half-life (h)**	**Clearance (mL/min/kg)**	**Vdss (L/kg)**
Frozen	5638.44	5915.52	1474.13	3.36	0.61	6.52	0.22
Lyophilised	6202.46	6543.19	1667.54	3.14	0.61	6.24	0.21
Lyophilised (Rechallenge)	7568.56	7368.56	1935.08	3.83	0.62	4.63	0.20

C93-316: This double blind, randomised, crossover study compared a pilot chemistry frozen formulation with a commercial chemistry lyophilised formulation of bivalirudin in 20 healthy male subjects. The pharmacodynamic response to the two formulations was similar based on aPTT.

Bioequivalence

Six formulations of bivalirudin have been used in human studies. These are bivalirudin `A' (frozen solution with sodium phosphate and mannitol), bivalirudin `B' (frozen solution with sodium hydroxide), bivalirudin `C' (frozen solution with tromethamine, used only in a comparative study with formulations A & B), bivalirudin `D' (refrigerated solution with sodium hydroxide and trifluoroacetic acid) and bivalirudin formulations `E1' & `E2' (lyophilised formulations, in pilot and commercial scales respectively). The lyophilised formulation is used commercially.
The pharmacokinetic and pharmacodynamic profile of the frozen and lyophilised formulation was bioequivalent (Studies C93-310 and C93-316).
In the proposed indication, studies C90-041 & C92-304/1-2 used frozen formulation `A' and studies TMC-97-01, TMC-BIV-00-01 and TMC-BIV-01-03 and TMC-98-10 were all conducted with the lyphilised formulation.

Distribution

Bivalirudin is rapidly distributed between plasma and extracellular fluid. The steady-state volume of distribution is 0.1 litre/kg. Bivalirudin does not bind to plasma proteins (other than thrombin) or to red blood cells.

Biotransformation

As a peptide, bivalirudin is expected to undergo catabolism to its constituent amino acids, with subsequent recycling of the amino acid in the body pool. Bivalirudin is metabolized by proteases, including thrombin. The primary metabolite resulting from the cleavage of Arg_3-Pro_4 bond of the N-terminal sequence by thrombin is not active because of the loss of affinity to the catalytic active site of thrombin. About 20% of bivalirudin is excreted unchanged in the urine.

Elimination

The concentration-time profile following intravenous administration is well described by a two-compartment model. Elimination follows a first order process with a terminal half-life of 25 ± 12 minutes in patients with normal renal function. The corresponding clearance is about 3.4 ± 0.5 ml/min/kg

Dose proportionality and time dependencies

Data regarding dose-linearity was derived from four studies (C90-10, C90-041, C92-301 and TMC98-09). Study C90-10 in 72 healthy volunteers demonstrated doubling of AUC when the dose was increased from 0.3 to 0.6 mg/kg. The Cl and t1/2 did not show any significant difference. Study C90-041 in patients with PTCA showed a significant linear dose-response relationship with increase in plasma concentration of bivalirudin noted with increase in dose.
Study C92-301, a double-blind study in 410 patients with unstable angina supported a linear dose relationship by steady state plasma concentration. Study TMC98-09 was PK study in 26 PTCA patients with normal renal function or mild or moderate renal impairment.
In study C93-310, a lower total clearance was noted after 3 months (4.63 ± 0.8 vs. 6.24 ± 3.8).

Pharmacokinetics in target population

In patients undergoing PTCA, the percentage of patients for whom ACT (activated clotting time) >300 sec (target ACT) was achieved increased from 12% in the lowest-dose group (0.15 mg/kg/ bolus + 0.6 mg/kg/hr infusion) to 100% in the highest-dose group (1.0 mg/kg bolus + 2.5 mg/kg/hr infusion) in a dose-dependent manner (Study C90-041).

In study C92-301, a double blind, parallel-group comparison of 4 doses of bivalirudin (0.02, 0.25, 0.5 and 1.0 mg/kg/hr) administered as IV infusions over 72 hours in patients with unstable angina, a correlation between aPTT (activated partial thromboplastin time) and plasma concentrations was observed at 12-24 and 36-48 hours after the start of the infusion.

Special populations

Impaired renal function - Four studies looked at the pharmacology in patients with renal impairment (C93-313, C94-321, TMC-98-09 and TMC-BIV-00-02). The systemic clearance of bivalirudin decreases with glomerular filtration rate (GFR). The clearance of bivalirudin is similar in patients with normal renal function and those with mild renal impairment. Clearance is reduced by approximately 20% in patients with moderate or severe renal impairment, and 80% in dialysis-dependent patients.

Pharmacokinetic parameters for bivalirudin in patients with normal and impaired renal function.

Renal function (GFR)	Clearance (ml/min/kg)	Half-life (minutes)
Normal renal function (≥ 90ml/min)	3.4	25
Mild renal impairment (60-89 ml/min)	3.4	22
Moderate renal impairment (30-59 ml/min)	2.7	34
Severe renal impairment (10-29 ml/min)	2.8	57
Dialysis dependent patients (off-dialysis)	1.0	3.5 hours

Impaired liver function - The pharmacokinetics of bivalirudin have not been studied in patients with hepatic impairment but are not expected to be altered because bivalirudin is not metabolized by liver enzymes such as cytochrome P-450 isozymes.

Gender/Race/Weight - The pharmacodynamics does not differ between males and females. In study C90-041 there was lack of difference in activity and safety of bivalirudin based on age, gender and weight.

Elderly - An increase in bleeding event has been noted in patients ≥ 65 years of age compared to younger patients in heparin treated patients as well as bivalirudin treated patients. Pharmacokinetics have been evaluated in elderly patients as part of a renal pharmacokinetic study. Dose adjustments for this age group should be on the basis of renal function.

Children - The product is not recommended for use in children as safety and effectiveness has not been established in this age group.

Interaction studies

In CACHET study (parts B & C), use of provisional abciximab in 34 patients randomised to bivalirudin did not markedly affect the ACT levels achieved at 5 min or thereafter. The mean ACT was 301, 307 and 348 seconds at 5 min, 30 min, and the end of the procedure respectively. Thrombin inhibition was more effective with bivalirudin + abciximab at 4 hours than with heparin + abciximab.
In another study (TMC-BIV-00-03), the combination of bivalirudin and eptifibatide produced a mean steady state activated clotting time (ACTs) of around 339 seconds for the lower dose bivalirudin and 349.5 seconds for the higher dose bivalirudin. The mean peak ACT for heparin and eptifibatide was 234.9 seconds.
The combination of bivalirudin and tirofiban produced a mean steady state ACT of around 350 seconds while heparin and tirofiban produced a mean peak ACT of 225 seconds 5 minutes after injection.
The combination of bivalirudin and ticlopidin produced steady state ACT values of approximately 350 seconds. These were significantly higher than the mean peak ACT of 282 seconds for heparin.
Bivalirudin can be administered to patients undergoing PCI following discontinuation of LMWH for at least 8 hours based on ACT data in conjunction with the antifactor Xa (TMC-99-05).

Pharmacodynamics

Mechanism of action

Bivalirudin is a short acting thrombin inhibitor that binds specifically to both the anion-binding (recognition) and catalytic (active) sites of thrombin. While heparin and antithrombin III only inhibit soluble thrombin, bivalirudin inhibits both soluble and clot-bound thrombin.

Primary and Secondary pharmacology

In healthy volunteers and in patients with unstable angina and PCI, bivalirudin exhibits linear dose-dependent anticoagulant activity.
Following intravenous administration, bivalirudin has a rapid anticoagulation effect, which reversed quickly upon cessation. ACT monitors this in the PCI setting. ACT is the preferred parameter in interventional cardiology as higher levels of anticoagulation is required and aPTT is less useful for this.

The initial dose ranging study (C90-041) showed ACT of >300 seconds with ascending doses in increasing proportion of patients. More than 80% of patients achieved ACT values of >300 seconds with bivalirudin doses of 0.45 mg/kg bolus and 1.6 mg/kg/h infusion.
In a subsequent study with GPIIb/IIIa blockers (TMC-97-01-A/B/C), the goal was to find a dose at which >80% of patients achieved ACT of >280 seconds at the time of PCI device activation. The median was 305 seconds for bolus doses of 0.5 mg/kg/ and 321 seconds for 0.75 mg/kg but the porportion of patients >280 seconds at 5 minutes reached 80% only with 0.75mg /kg dose level.

Bivalirudin inhibits thrombin induced platelet aggregation.

<u>Discussion on Clinical Pharmacology</u>

Pharmacokinetics

Bivalirudin is a twenty-residue polypeptide with a small molecular weight. Bivalirudin is currently administered only by the iv route. However, its absorption was also explored after subcutaneous injection. Pharmacokinetic parameters were not measured following subcutaneous administration because most values fell below the limit of quantification and the only sensitive markers for drug absorption were coagulation parameters.

Bivalirudin pharmacokinetics has been evaluated in healthy volunteers in different doses that included iv injections and infusions. Doses in the therapeutic range have only been investigated in patients

undergoing PTCA. Although complete profiles were not characterised, the measurements did provide an approximate measure of exposure.

There was no evidence for binding bivalirudin to plasma proteins but binding to red blood cells may occur to a minor extent. The kinetic of bivalirudin was dose related in the dose range tested. The volume of distribution is approximately equal to the sum of plasma and interstitial fluid i.e. approximately 0.24 L/kg. In humans, bivalirudin has a mean half-life around 25 minutes.

The onset of anticoagulant activity was rapid and reversible within 30 to 60 minutes upon cessation of drug administration. Renal excretion was approximately 10-22% of the total elimination with remainder of the drug being eliminated via reabsorption in the kidney and proteolysis by intracellular lysosomes. The kinetics was not affected by co-administration with aspirin.

Pharmacokinetics studies were performed in patients with renal disease. Bivalirudin was well tolerated in these patients. Pharmacokinetics was proportional to the degree of renal insufficiency.
In patients with renal insufficiency, monitoring of clotting time, such as the ACT is recommended. Angiox is contraindicated in patients with severe renal insufficiency (GFR <30 ml/min) and also in dialysis-dependent patients and the dose should be reduced in case of moderate renal impairment.

No dose adjustment is necessary in the elderly, provided renal functions are normal. However, caution should be exercised in the elderly due to age-related decrease in renal function. In the presence of renal insufficiency the infusion dose/rate should be adjusted.

Pharmacodynamics
Bivalirudin is a direct thrombin inhibitor, which inhibits free and clot bound thrombin. The pharmacodynamic profile of bivalirudin has been sufficiently explored *in vitro* and *in vivo*.

In vitro, bivalirudin has prolonged coagulation parameters (aPTT, PT, TT). *In vivo* studies have been performed in healthy subjects, patients and patients with renal impairment. In healthy subjects and in patients bivalirudin also prolonged the coagulation parameters (aPTT, PT, TT, ACT). In patients with moderate and severe renal impairment and in dialysis dependent patients, these values were prolonged but this prolongation was higher than in normal patients or mild renal impairment.

Specific drug-drug interaction studies have been performed with drugs usually used in the proposed indication (aspirin, ticlopidine, inhibitor GPIIb/IIIa, streptokinase, unfractionated heparin and low molecular heparin). The results of these studies have shown that the coagulation parameters were not modified by the combination of bivalirudin with these drugs.

The antigenicity of bivalirudin was studied *in vivo* in healthy subjects and *in vitro* in patients with lepirudin antibodies. In the *in vivo* studies undertaken, all pre- and post-dosing assays for anti-bivalirudin antibodies were negative. The *in vitro* studies showed that it is probable that not all lepirudin antibodies can actually bind bivalirudin.

Clinical efficacy

Summary of Bivalirudin Efficacy Trials in PCI

The initial development programme was for PCI in patients with unstable angina and acute coronary syndromes (ACS). The studies conducted in 1993 for that indication reflected the standard care at the time and therefore uses high dose of heparin as comparator. The role of GPIIb/IIIa inhibitor and antiplatelet therapy apart from aspirin was not established. The use of stent was not so common and in those trials only rescue stenting was allowed.

Having evaluated the earlier pooled data from PCI studies with heparin and GPIIb/IIIa inhibitors, a series of studies were undertaken to test the hypothesis that bivalirudin may replace heparin and may possibly limit the need for GPIIb/IIIa inhibition.

The clinical trial programme related to PCI indication comprised five studies (C90-041, C92-304-1/-2, TMC-97-01A/B/C, TMC-BIV-00-01 and TMC-BIV-01-03) with a total of 6113 patients on bivalirudin and 5757 on heparin.

At present, the pivotal data supporting this application come mainly from study TMC-BIV-01-03 (REPLACE-2). Data on studies TMC-97-01A/B/C (CACHET A/B/C) and TMC-BIV-00-01 (REPLACE-1) allowed to optimised the dose to be used in study REPLACE-2 and to obtain additional information on efficacy and safety. Data on studies previously submitted by the Applicant can now be considered as supportive evidence.

Study	**Indication**	**Design**	**Patients**	
			Bivalirudin	Heparin
Studies performed 1990-1995				
C90-041	PTCA	Sequential dose groups	291	
C92-304-1/-2	PTCA	Randomised double- blind	2151	2161
Studies performed 1997-2003				
TMC-97-01A/B/C *(CACHET)*	PTCA ± Stent	Randomised open-label	145	64
TMC-BIV-00-01 *(REPLACE-1)*	PTCA ± Stent	Randomised open-label	532	524
TMC-BIV-01-03 *(REPLACE-2)*	PTCA ± Stent	Randomised double-blind	2994	3008
Total			6113	5757

Dose-response studies and main clinical studies

Study TMC-BIV-00-01 (REPLACE – 1)

Description
This pilot study evaluated any link between the use of bivalirudin and reduced clinical events in PCI. The trial was a phase IIIb study, randomised and open label. Patients were enrolled in 1:1 ratio to either heparin or bivalirudin with use of GPIIbIIIa inhibitors in both groups according to the investigator's choice.

Following randomisation, bivalirudin was given as 0.75mg/kg i.v bolus prior to device activation, immediately followed by a 1.75mg/kg/hr i.v infusion. Heparin was given as 60 – 70mg/kg i.v bolus, to target an activated clotting time (ACT) of 200 – 300 seconds (not to exceed 7000 U), followed by additional weight-adjusted boluses as per institutional practice to maintain the target ACT. Both treatments were given for the duration of PCI and up to 4 hours post-bolus dose at the discretion of the investigator. Recommended adjunctive medications were aspirin and clopidogrel while ticlopidine was not recommended.

The primary criterion for evaluation was a composite quadruple endpoint evaluated at 48 hours or at hospital discharge, whichever occurred first. The secondary endpoints were composite of individual components of death/MI/revascularisation, major and minor bleeding, adverse events and health economic parameters.

Results
In this study stents were used in approximately 85% of patients, thienopyridines in 56% and GPIIbIIIa inhibitors in 72%. No formal hypotheses were tested in this trial.

While baseline ACT were comparable in both groups, the ACT values in the bivalirudin group were significantly higher prior to device activation, end of procedure and sheath removal. Fewer patients in the bivalirudin group (0.6%) failed to meet the minimum ACT of 200 seconds compared to heparin group (3.7%).

ACT coagulation measurements – Safety population

Assessment Time Point	**Number Assessed**		**Bivalirudin Mean ± SD [Median] (sec)**	**Heparin Mean ± SD [Median] (sec)**	**P-value (NP)**
	Bivalirudin	**Heparin**			
Baseline	491	463	136.3 ± 27.88 [135.0]	138.5 ± 32.47 [136.0]	0.465
Prior to device activation	475	488	370.5 ± 101.80 [359.0]	303.8 ± 88.80 [292.5]	<0.001
End of procedure	490	498	334.3 ± 62.98 [329.0]	267.0 ± 57.62 [261.5]	<0.001
Sheath removal	351	337	186.1 ± 81. 71 [159.0]	168.4 ± 54.28 [154.0]	0.014

ACT = activated clotting time: NP = nonparametric test: SD = standard deviation

As regards the composite quadruple endpoint and triple endpoint, there was a trend in favour of bivalirudinalthough no statistical significance was shown.

Primary composite efficacy outcomes and components at discharge/48 Hours - ITT population

	Bivalirudin (N = 532)	**Heparin (N = 524)**	**% Diff [a]**	**% Relative Diff [a]**	**P-value**
	n (%)	n (%)			
Quadruple endpoint	36 (6.8)	41[b] (7.8)	1.07	-13.5	0.503
Triple endpoint	27 (5.1)	31[b] (5.9)	0.85	-13.6	0.544
Death	0 (0)	3[c] (0.6)	0.57	-95.0	0.122EX
MI[d]	26 (4.9)	27[c] (5.2)	0.27	-5.2	0.843
Urgent revascularisation	4 (0.8)	7[b] (1.3)	0.59	-39.8	0.348
Major haemorrhage[d]	11 (2.1)	14[c] (2.7)	0.60	-22.6	0.519

% Diff is the difference in the event rates between the bivalirudin and heparin treatment arms.
% relative difference is % difference/% heparin
Number assessed = 523
Number assessed = 524
CRF declared MI (74%) or not declared but met MI enzyme criteria (26%). MI enzyme criteria defined as atleast two post baseline CK-MB elevations >3*ULN with atleast one of the elevations ≥50% over previous nadir (CK used if CK-MB was missing)
Diff = difference: EX = Fisher's exact test: MI = myocardial infarction

Study TMC-9701A (CACHET Pilot study A)

Description

This phase III, open label, randomised study investigated the effect of bivalirudin in combination with ReoPro on laboratory coagulation parameters and the incidence of clinically significant bleeding in patients undergoing PTCA.

The primary objective was to compare the ACT levels, thrombin levels and platelet inhibition, incidence of death and/or haemorrhagic stroke, incidence of major bleeding and/or red blood cell transfusion and incidence of adverse experiences following administration of bivalirudin + ReoPro or ReoPro + low-dose heparin in patients with PCI

Bivalirudin was administered i.v as 1.0mg/kg bolus followed by a 2.5 mg/kg/hour infusion for 4 hours. ReoPro was administered i.v as a 0.25 mg/kg/bolus followed by a 0.125 microgramme/kg/minute infusion (to a maximum of 10 microgramme/minute) for 12 hours. In the ReoPro + heparin arm, additional boluses of heparin were administered every 30 minutes, as needed during the procedure to maintain the ACT between 200 – 300 seconds or a 7 units/kg/hour infusion of heparin was begun.

A total of 60 patients were randomised (30 in each group). Approximately a similar number of patients received stent in each group (25/30 vs. 27/30).

Results

Results showed that the median ACT of 350 seconds was reached rapidly and was maintained throughout the procedure in the bivalirudin + Reopro group and was significantly higher than Reopro + heparin group. Thrombin inhibition was also more effective and less prolonged in the bivalirudin + ReoPro group. There were no deaths, haemorrhagic strokes, or revascularisation in either group but a single patient in bivalirudin + ReoPro group had a major haemorrhage while a single patient in Reopro + heparin group had myocardial infarction following intervention.

CACHET B/C

Description

This study evaluated bivalirudin (with provisional abciximab) versus low-dose, weight-adjusted heparin (with up-front abciximab). Assessments included ACT levels, thrombin activity, anticoagulation and haemostasis and clinical safety outcomes.

A total of 209 patients were enrolled (144 randomised to bivalirudin plus provisional abciximab and 64 were randomised to receive heparin plus abciximab). All patients received aspirin. Approximately 68% in the bivalirudin group and 56% in the heparin plus abciximab group received pretreatment with clopidogrel and ticlopidine.

Bivalirudin 0.5 mg/kg IV bolus (CACHET B) or 0.75 mg/kg IV bolus (CACHET C), followed by a 1.75 mg/kg/h IV infusion for the duration of procedure and upto 4 hours. Heparin 70 U/kg by IV bolus administered prior to the start of the procedure. Additional weight adjusted heparin boluses were administered every 30 minutes, as needed to maintain an ACT between 200 and 300 seconds.

Results

The results showed a statistically significant benefit with reference to the quadruple endpoint for bivalirudin. The incidence of triple endpoint and major bleeding was numerically low in the bivalirudin group but the incidence of MI was higher.

Study TMC-BIV-01-03 (REPLACE-2)

Description

This multicentre (233 centres) study was the main therapeutic study and compared the use of bivalirudin + provisional GPIIbIIIa inhibitor with heparin + planned GPIIbIIIa administration in patients undergoing PCI. The study was double blind, randomised and multinational. The objectives were to validate the superiority compared to imputed heparin, simulating the comparison by which the efficacy of GPIIbIIIa inhibitors has been defined (ESPRIT and EPISTENT) and to demonstrate the non-inferiority of bivalirudin as foundation anticoagulant with provisional use of GPIIbIIIa inhibitors compared to heparin and routine use of GPIIbIIIa inhibitors in patients undergoing PCI.

The primary efficacy criteria were a composite of quadruple endpoints (Death, MI, Urgent Revascularisation and Major haemorrhage). The secondary endpoints were the composite triple endpoint (Death, MI, Urgent Revascularisation), major haemorrhage, the individual components of the primary endpoint and safety endpoints.

Results

Results for the primary endpoint and the triple composite endpoint

Endpoint	Statistic	Bivalirudin (N=2994)	Heparin + GPIIb/IIIa (N=3008)	P-value
Quadruple Endpoint		**n (%)**	**n (%)**	
	N (%)	275/2975 (9.2)	299/2991 (10.0)	0.32
	OR (LL,UL)[b]	0.92 (0.77, 1.09)		
	OR (LL,UL)[c]	0.62 (0.47,0.82)		
Triple Endpoint	**N (%)**	227/2975 (7.6)	211/2990 (7.1)	0.40
	OR (LL,UL)[b]	1.09 (0.90, 1.32)		
	OR (LL,UL)[d]	0.61 (0.44,0.83)		
Major haemorrhage Endpoint	**N (%)**	71/2993 (2.4)	123/3008 (4.1)	<0.001

b Odds ratio and 95% CI for bivalirudin vs heparin + GPIIb/IIIa inhibitor
c Odds ratio and 95% CI for bivalirudin vs imputed heparin for death/MI/Urgent revascularisation/bleeding endpoint
d Odds ratio and 95% CI for bivalirudin vs imputed heparin for death/MI/Urgent revascularisation endpoint

Discussion on clinical efficacy

The proposed dose has been well justified. In the present submission, the dosing schedule recommended was mainly based on the results of studies CACHET A/B/C and REPLACE-1. In these studies, bivalirudin at a bolus dose of 0.75 mg/kg achieved an immediate ACT of approximately 280 to 320 seconds that was maintained for the duration of the PCI procedure by a subsequent infusion of 1.75 mg/kg/hr. Additionally, bivalirudin directly inhibits thrombin, but has no direct effect on platelets. Bivalirudin shows no apparent pharmacological interaction with abciximab, and their actions on thrombin and platelets are independent when used together.
The clinical efficacy of bivalirudin as an anticoagulant in the setting of PCI has been shown based on the 30-day results based on quadruple and triple endpoints from the randomised, double blind trial of over 6,000 patients undergoing PCI (REPLACE-2).

REPLACE-2 Study results: 30-day endpoints (intent-to-treat and per-protocol populations)

Endpoint	Intent-to-treat		Per-protocol	
	bivalirudin (N=2994) %	**Heparin + GPIIb/IIIa inhibitor (N=3008) %**	**bivalirudin (N=2902) %**	**heparin + GPIIb/IIIa inhibitor (N=2882) %**
Quadruple endpoint	9.2	10.0	9.2	10.0
Triple endpoint*	7.6	7.1	7.8	7.1
Components:				
Death	0.2	0.4	0.2	0.4
Myocardial Infarction	7.0	6.2	7.1	6.4
Major bleeding** (based on non-TIMI criteria - see section 4.8)	2.4	4.1	2.2	4.0
Urgent revascularization	1.2	1.4	1.2	1.3

* excludes major bleeding component. **p<0.001

Based on the main therapeutic study, bivalirudin with provisional GPIIb/IIIa has been shown non-inferior to heparin with planned GPIIb/IIIa as regards the quadruple endpoint. The statistically significant difference is largely due to a beneficial effect on major bleeding which has been included as one of the components of the quadruple endpoint. This is unusual but was defined in the protocol a priory.

It is true that earlier trials in patients with unstable angina and PCI have used a triple endpoint with major bleeding evaluated as a safety criterion. Although this was not the primary efficacy criterion in the REPLACE-2 study, the Applicant had included this as one of the secondary efficacy criteria. Additional analyses provided are considered sufficient to make an informed judgement.

Analysis of individual components shows that there is a higher incidence of myocardial infarction in the bivalirudin group but that this is balanced by a lower death rate overall and an advantage regarding major bleeding. The 6-month follow-up evaluated the triple composite endpoint of death/MI/revascularisation (target and any) and its individual components while evaluation at 1-year was for mortality only. The mortality benefit observed at 30 days is sustained at 1 year. At 6 months, the observed mortality benefit was (28/2994 (0.97%) in the bivalirudin group vs 40/3008 (1.37%) in the heparin + GPIIb/IIIa group. At 30 days the advantageous effect of bivaluridin is noted for the quadruple endpoint but the effect on the triple endpoint is marginally verse, although within the criteria of non-inferiority. Benefit has therefore also been demonstrated for the triple endpoint, which has been used in earlier trials for acute PCI and stent. This is based on the protocol-defined criteria. If more stringent statistical criteria are used the non-inferiority on the basis of quadruple endpoint will still be met but will just fail for the triple endpoint. This should be interpreted in the light that the protocol defined quadruple endpoint *a priori*.

The majority of mortality observed was due to cardiovascular causes. The increased incidence of MI were biochemical in nature as most increases were in the group with CK-MB of >5-<10.
At one year there was 22% relative risk reduction of death although the difference between the groups was not statistically significant. However, it should be noted that the Absolute Risk Reduction (ARR) at 1 year was only 0.5%.

Superiority over heparin has also been acceptably demonstrated but the clinical relevance of this is not clear, since the use of heparin alone in the PCI setting in not justified in current practice.

The incidence of thrombocytopaenia was lower in the bivalirudin group compared to the heparin group (0.7% vs 1.7%). This was true for severe thrombocytopaenia (0.3% vs 0.6%) and profound thrombocytopaenia (0.1% vs 0.3%).

A sub-group analysis by age shows no significant difference between patient <75 years and >75 years of age for the quadruple and triple endpoints at day 30. However 12-month mortality data showed that patients >75 years of age faired better than those <75 years (3.6% vs 6.9%, p=0.039).
Based on baseline creatinine clearance, 30-day quadruple and triple endpoints were higher in patients with severe renal impairment receiving Angiox than those receiving heparin + GPIIb. The mortality at 12 months was however less in these patients. All this was based on a small number of patients.
Old age itself is not a barrier to the use of bivalirudin provided recommendations proposed in the SPC regarding use in renal impairment is taken into account.

Heparin-induced thrombocytopenia (HIT) and heparin-induced thrombocytopenia-thrombosis syndrome (HIT/HITTS): Clinical trials in a small number of patients have provided limited information about the use of Angiox in patients with HIT/HITTS.

Clinical safety

Patient exposure

The safety database comprised 30,402 patients including 6010 patients from the REPLACE-2 trial, pooled data from 6,191 patients in 11 PCI trials and pooled data from 15,817 patients from all clinical trials. There is additional information from post-marketing experience. It is estimated that approximately 164,300 patients have been exposed to bivalirudin in the past two years.

The demographic characteristics of patients in all PCI studies were similar to all patient studies and to patients in main therapeutic study REPLACE-2 (TMC-BIV-01-03).

Adverse events

Common adverse events
Back pain was the most common adverse events in both groups and in all studies, in all PCI studies and in the main therapeutic study.

Incidence of common adverse events regardless of causal relationship (through 30 days

COSTART Preferred Term for AE	**All Studies**[1]		**Pooled PCI Studies**[2]		**TMC-BIV-01-03**	
	Bivalirudin [n (%)] (n=7378)	**Heparin [n (%)] (n=6091)**	**Bivalirudin [n (%)] (n=6191)**	**Heparin [n (%)] (n=5844)**	**Bivalirudin [n (%)] (n=2914)**	**Heparin**[3] **[n (%)] (n=2987)**
Pain back	1477 (20)	1335 (22)	1345 (22)	1290 (22)	268 (9)	263 (9)
Hypotension	466 (6)	545 (9)	393 (6)	517 (9)	91 (3)	120 (4)
Headache	581 (8)	359 (6)	386 (6)	339 (6)	75 (3)	83 (3)
Pain	592 (8)	482 (8)	476 (8)	455 (8)	98 (3)	72 (2)
Injection Site Pain	315 (4)	369 (6)	267 (4)	364 (6)	80 (3)	80 (3)
Fever	305 (4)	167 (3)	157 (3)	131 (2)	30 (1)	16 (<1)
Pain Chest	256 (3)	188 (3)	210 (3)	182 (3)	68 (2)	69 (2)
Pain Abdominal	197 (3)	148 (2)	154 (2)	138 (2)	30 (1)	24 (<1)
Pain Pelvic	186 (3)	197 (3)	150 (2)	183 (3)	2 (<1)	4 (<1)
Angina Pectoris	320 (4)	183 (3)	286 (5)	174 (3)	155 (5)	156 (5)
Bradycardia	223 (3)	232 (4)	185 (3)	218 (4)	35 (1)	37 (1)
Hypertension	208 (3)	161 (3)	178 (3)	158 (3)	34 (1)	30 (1)
Syncope	80 (1)	89 (1)	70 (1)	84 (1)	26 (<1)	22 (<1)

1Does not include data from study TMC-97-03
2Except for study TMC-98-10
3Plus GPIIb/IIIa inhibitor in all patients

Bleeding events
The overall bleeding events were less in bivalirudin group than the heparin group: 23% Vs 27% in all studies, 33% Vs 49% in all PCI studies and 16% Vs 30% in the main therapeutic study REPLACE-2 (TMC-BIV-01-03).
The major bleeding in the main therapeutic study was defined as transfusion of whole blood or packed cell ≥2 units, intracranial haemorrhage, retroperitoneal haemorrhage, drop in haemoglobin >4 g/dL with no bleeding site identified and a spontaneous or non-spontaneous blood loss associated with drop in haemoglobin of >3 g/dL.
The incidence of major bleeding was clearly less in bivalirudin group than in the heparin group with GPIIbIIIa inhibitor (2.3% Vs 4.0%, p<0.001). The advantage in bivalirudin group was also seen for non-CABG bleed but the incidences were similar for CABG bleed. Minor bleeding was also significantly less in the bivalirudin group.

Incidence of Major Bleeding Events[1]

Bleeding Event	All Studies[2]		Pooled PCI studies[3]		Study TMC-BIV-01-03	
	Bivalirudin [n (%)] (n =15,817)	Heparin [n (%)] (n = 14,585)	Bivalirudin [n (%)] (n = 6191)	Heparin [n (%)] (n = 5844)	Bivalirudin [n (%)] (n = 2914)	Heparin[4] [n (%)] n = 2987)
Any Major Bleeding Event	238 (2)	389 (3)	160 (3)	335 (6)	67 (2)	118 (4)
Catheterisation Site Haematoma	56 (<1)	127 (<1)	32 (<1)	122 (2)	12 (<1)	44 (1)
Puncture Site Haemorrhage	54 (<1)	99 (<1)	40 (<1)	83 (1)	7 (<1)	15 (<1)
Venipuncture Site Prolonged Bleeding	12 (<1)	26 (<1)	12 (<1)	26 (<1)	8 (<1)	17 (<1)
Anaemia	25 (<1)	46 (<1)	19 (<1)	39 (<1)	0	0
Catheterisation Site Ecchymosis Without Haematoma	21 (<1)	44 (<1)	21 (<1)	44 (<1)	12 (<1)	39 (1)
Other Bleeding	19 (<1)	27 (<1)	10 (<1)	23 (<1)	9 (<1)	14 (<1)

[1]Patients are included in each event where the patient had at least 1 bleeding episode
[2]All studies, excluding healthy volunteers, safety population (all who received study drug)
[3]All PCI studies except TMC-98-10
[4]Plus GPIIb/IIIa Inhibitor in all patients

Treatment Emergent Adverse Events

The overall treatment–emergent adverse events were similar in the bivalirudin group and the heparin + GP IIb/IIIa inhibitor group in all patient studies, all PCI studies and in TMC-BIV-01-03 study.
In the main therapeutic study, the most frequent treatment-related adverse event (through 30 days) were less, except for nausea, in bivalirudin treated group compared to heparin + GPIIb/IIIa group, as shown below.

Most frequent treatment-related adverse event (through 30 days) – Safety population – TMC-BIV-01-03

COSTART Term	Bivalirudin (n = 2914) [n (%)]	Heparin + GP IIb/IIIa (n = 2987) [n (%)]
Patients with at least one treatment related AE	78 (2.7)*	115 (3.9)*
Thrombocytopenia	9 (11.5)	30 (26.1)
Nausea	15 (19.2)	7 (6.1)
Hypotension	7 (9.0)	11 (9.6)
Angina pectoris	5 (6.4)	12 (10.4)
Headache	6 (7.7)	5 (4.3)
Injection site pain	3 (3.8)	8 (7.0)
Nausea & vomiting	2 (2.6)	6 (5.2)
Vomiting	3 (3.8)	5 (4.3)

A patient could have more than one event in any category. Percentages are of the subpopulation with at least one treatment-related AE
*p-value = 0.0113

Serious adverse events

The incidence of major bleeding has been mentioned above as this was one of the components of quadruple primary endpoint. The incidence of serious adverse events in the main therapeutic study was 0.5% in bivalirudin group compared to 1.2% for the heparin group.

There was no difference between the groups regarding incidence of serious adverse events in all patients' studies, all PCI studies and study TMC-BIV-01-03. The commonest serious adverse events reported in the main therapeutic study were thrombocytopaenia (4 in bivalirudin group and 18 in heparin group), and events related to cardiovascular system (7 vs 11). In this study transfusion was needed for 1.5% patients in bivalirudin group and 2.5% in heparin group (P=0.009).

Deaths
Death rates were similar in bivalirudin and heparin groups in all studies (6% vs 7% respectively) and in all PCI studies (1% each group). In the main therapeutic study TMC-BIV-01-03 it was lower in bivalirudin group (0.2%) compared to heparin group (0.4%) (table 2.7.4.15, safety summary). This included all deaths upto 30 days. There was a trend towards lower death rate, although not statistically significant, in bivalirudin group over six months (0.93% vs 1.34%, P value=0.128).

Laboratory findings
Comparative laboratory data were provided from study TMC-BIV-01-03. The maximum haemoglobin drop was 0.90 g/dL in bivalirudin group and 1.00 g/dL in the heparin group. Confirmed thrombocytopenia was lower in the bivalirudin group compared to heparin + GPIIb/IIIa group (0.7% vs 1.7%, P<0.001).
For all studies taken together there were no significant differences between the groups or between the baseline and the final visit.

Safety in special populations

Renal
As bivalirudin clearance is diminished in patients with moderate to severe renal impairment, the dose needs to be adjusted in these patients based on evidence from four studies, which evaluated bivalirudin in patients with renal impairment. Patients with renal impairment have lower clearance of bivalirudin.
Safety data in renal impairment patients were collected from four studies: C93-313 (39 patients), C94-321 (11 patients), TMC-98-09 (26 PTCA patients with normal, and mild to moderate renal impairment), and study TMC-BIV-00-02 (10 patients). Patients with severe renal impairment had higher adverse events.

HIT/HITTS
Study TMC-98-10 specifically looked at the safety and tolerability of bivalirudin in patients with heparin-induced thrombocytopaenia with/without thrombosis syndrome, requiring PCI. A total of 52 patients were recruited in the study, 27 received bivalirudin 1mg/kg IV bolus followed by 2.5mg/kg/hr infusion and 25 received 0.75mg/kg/bolus and 1.75mg/kg/hr infusion. The primary outcome measures were the composite incidence of bleeding.
Only one episode of major bleeding was reported in a patient receiving higher dose. This patient went on to have CABG and not PCI. The effect on platelet was not significant.

	Dose 0.75mg/kg bolus then 1.75mg/kg/h	**Dose 1.0mg/kg bolus then 2.5mg /kg/h**	**All Subjects**
Nadir platelet count	N= 25	N=27	N=52
≥ 100,000	21 (84%)	20 (74%)	41 (79%)
≥ 50,000 – 99,999	4 (16%)	7 (26%)	11 (21%)
≥ 20,000 – 49,999	0	0	0
< 20,000	0	0	0

In study C93-312 in patients with HIT/HITTS, three cases of major bleeding were reported, two probably related to the drug. Four deaths were reported in these patients in study C93-312 & C94-322, none related to bivalirudin.

Gender/Age
Although bleeding rates are reportedly higher in females and in older patients undergoing PCI, bivalirudin was safer than heparin even in these sub-populations. Safety has not been assessed in pregnant women and in paediatric population. PCI procedures are not commonly used in paediatric patients. Age related withdrawal was similar in both treatment groups.

Death rates were higher in patients >65 years of age in both groups but numerically lower in the bivalirudin group. Death rate was higher in female than male patients in both the treatment groups in all studies and in all PCI studies but in TMC-BIV-01-03 fewer patients died in bivalirudin group than the heparin group (0.4% vs 0.8% in females and 0.1% vs 0.3% in males).

Brachytherapy
In patients receiving gamma brachytherapy (11 bivalirudin, 13 heparin), numerically greater quadruple endpoint, triple endpoint, MI and provisional use of GPIIbIIIa inhibitor were reported in bivalirudin group.

Antigenicity
Antibodies to bivalirudin were identified by ELISA (enzyme linked immunosorbent assay). One patient each with antibodies was identified in studies C90-041 and C93-313. A total of 8 patients were reported to have antibodies in study C90-039 (2 patients had antibodies pre- and post-dose, 1 had antibodies pre-dose only and 5 had antibodies post-dose only.)
The incidence of allergic reaction was similar in the bivalirudin and heparin groups in all studies, in pooled PCI studies and in study TMC-BIV-01-03. Anaphylactoid reaction has been reported in 2 patients in bivalirudin as opposed to none in heparin group but angioedema was reported in a single patient in heparin group and none in bivalirudin group.

Safety related to drug-drug interactions and other interactions
There was no difference between the bivalirudin group and heparin + GPIIbIIIa group as regards adverse events with concomitant use of ticlodipine or low molecular weight heparin.
In the main therapeutic study, the administration of concomitant drugs (beta blockers, statins, other lipid lowering drugs, ACE inhibitors, oral hypoglycaemics and insulin was similar in both groups. Adverse events in both groups were higher when GPIIbIIIa inhibitor was used concomitantly. In study TMC-BIV–00-01 the incidence of major bleeding in bivalirudin group and heparin group administered with GPIIbIIIa inhibitor was similar.

Discontinuation due to Adverse Events
The overall withdrawal rate in the safety population was similar in both groups (13% in each arm for all studies, 10% vs 13% respectively in bivalirudin and heparin group in all PCI studies and 2% in each arm in the main therapeutic study). Treatment failure as a reason for withdrawal was also similar in both groups (16% vs 14% and 37% vs 29% respectively in all studies and in all PCI studies). There was no withdrawal due to treatment failure in study TMC-BIV-01-03.

The withdrawal due to adverse event in the main therapeutic study was 1.1% in the bivalirudin group and 2.0% in the heparin + GP IIa/IIIb group. The most common adverse events leading to withdrawal in this study were thrombocytopenia (0.1% vs 0 %), hypotension (0.1% vs 0.2%), headache (0.1% in each group), abdominal pain (0.1% vs 0%), back pain (0% vs 0.1%), angina pectoris (0.1% vs 0%), bradycardia (0 % 0.1%), coronary artery disorder (0% vs 0.1%) and cardiac arrest (0% vs 0.1%).

Post marketing experience
Bivalirudin received marketing authorisation in the United States on 15 December 2000, in Canada on 9 October 2002 and in New Zealand on 21 October 1999.
The cut off date for the submitted report was 20 April 2003. Most of the adverse event reports were from the United States. A total of 153 events were reported in 97 patients. Twenty-two deaths were reported.
Adverse events of >5% in system organ classes were Cardiac disorders (24.8%), Vascular disorders (15.7%), Nervous system disorders and General disorders and administration site conditions (9.8%

each), Investigations (9.2%), Gastrointestinal disorders (7.2%), Blood and lymphatic system disorders (5.8%), and Respiratory, thoracic and mediastinal disorders (5.2%).

Out of the 22 deaths, 9 were due to cardiovascular causes, one due to haemarrhagic stroke and one each due to ananphylactic reaction and coagulopathy.

In addition, the applicant provided information during the evaluation about 7 serious adverse events and attached the CIOMs forms. Two of these events occurred in clinical trials and the remainder were spontaneous reports. These events were pseudoaneurysm (related) and hyperkalaemia (possibly related), coronary artery thrombosis (2 cases, unexpected and fatal), sepsis (1 case, unexpected and fatal), vascular injury (one case, unexpected) and ICH (one case, unexpected and fatal).

Discussion on clinical safety

The safety database is considered adequate. There are no major safety concerns related to the use of bivalirudin in the context of PCI. However, there is a problem with increased occurrence of thrombosis in patients treated with gamma brachytherapy. Angiox should be used with caution during beta brachytherapy procedures.
It is also worth pointing out that adverse events were higher in females, in patients >65 years of age and in severe renal impairment in addition to those observed with gamma brachytherapy. A single death with anaphylaxis has been reported.

There is a distinct safety advantage with use of bivalirudin in patients undergoing PCI by way of less bleeding and less incidence of thrombocytopenia which has been observed with heparin + GPIIb/IIIa inhibitor in the PCI setting. Amongst the most frequent treatment-related adverse events (through 30 days) thrombocytopaenia was noted in 11.5% of patients in the bivalirudin group and in 26.1% in the heparin group. Given that non-inferiority has been shown against heparin + GPIIb/IIIa as regards efficacy, this adds to a favourable benefit/risk ratio for this medicinal product.

However, there is some concern regarding the fact that the definition of `major bleeding' used in these studies was not in line with the TIMI classification and was changed throughout the clinical development of the product. The classification of bleeding episodes used in REPLACE-2 could have implied an overestimation of the effect of bivalirudin on major bleeding events. Since the criteria of major bleeding in REPLACE-2 was similar to the combination of TIMI major and some minor bleeds, it is necessary to point out that the differences were mainly driven by vascular access puncture and gastrointestinal tract bleeding episodes. There was little difference between the two groups in terms of real TIMI major bleedings. This will lead to significant dilution of the effect noted with the quadruple endpoint. Although there is no statistically significant difference between the bivalirudin group and the heparin + GPIIb/IIIa group based on TIMI definition of major bleed, there is still a numerical advantage with bivalirudin. All other bleeding criteria show significant difference in favour of bivalirudin

With respect the site of bleeding, there was a lower incidence of major bleeding (defined by protocol) among patients in the bivalirudin group compared with the heparin + GP IIb/IIIa inhibitor group. The treatment difference was significant for bleeding at the sheath puncture site, cardiac/pulmonary bleeds, GI bleeding and non-CABG other bleeding. No difference was found in intracranial bleeding.

The number of patients who received any transfusion was significantly lower in the bivalirudin group than in the heparin group. This difference was also significantly different for patients receiving transfusion ≥ 2 units. Both data would be consistent with the incidence of bleeding reported for both groups of treatment.

In the clinical setting patients must be observed carefully for symptoms and signs of bleeding during treatment. Although most bleeding associated with bivalirudin occurs at the site of arterial puncture in patients undergoing PCI, haemorrhage can occur at any site during therapy. Unexplained decreases in haematocrit, haemoglobin or blood pressure may indicate haemorrhage. Treatment should be stopped if bleeding is observed or suspected.

The applicant has adequately addressed the issue of special populations considering that bivalirudin is intended for use in a particular patient population in acute situation.

The incidence of AEs was similar in the two groups of treatment regardless of age and gender, although elderly and females had a higher rate of death and withdrawals from studies in both groups of treatment. Treatment emergent serious adverse events were higher in patients >65 years of age compared to younger patients (<65 years) in both treatment groups. Bivalirudin has a numerical advantage in age related death.

No dose adjustment is necessary in the elderly, provided renal functions are normal. However, caution should be exercised in the elderly due to age-related decrease in renal function. In the presence of renal insufficiency the infusion dose/rate should be adjusted.

The adverse events rate was higher in patients with severe renal impairment is study C93-313 although this was based on very few patients in this category (8). In the REPLACE-2 study the incidence of major bleeding was higher in patients with severe renal impairment. The incidence of major haemorrhage was significantly higher in the bivalirudin group among patients with a baseline creatinine clearance > 30 ml/min. In patients with renal insufficiency, monitoring of clotting time, such as the ACT is recommended. Angiox is contraindicated in patients with severe renal insufficiency (GFR <30 ml/min) and also in dialysis-dependent patients and the dose should be reduced in case of moderate renal impairment.

No dose adjustment is needed in patients with hepatic impairment. Pharmacokinetic studies indicate that hepatic metabolism of bivalirudin is limited; therefore the safety and efficacy of bivalirudin have not been specifically studied in patients with hepatic impairment.

The use of bivalirudin in patients with HIT/HITT appears to be safer and beneficial. The major bleeding reported in some patients and unrelated deaths should be seen in light of no alternative to heparin in these groups of patients should they need PCI.

There is a problem with increased occurrence of thrombosis in patients treated with gamma brachytherapy. These patients should be given heparin rather than bivalirudin. Angiox should be used with caution during beta brachytherapy procedures.

Occasional cases of allergy to bivalirudin have been reported. Although anaphylactoid reaction in bivalirudin group is of some concern, its occurrence is rare considering the number of patients who received the drug. Nevertheless, necessary preparations should be made in the clinical setting to deal with this. Patients should be informed of the early signs of hypersensitivity reactions including hives, generalised urticaria, and tightness of chest, wheezing, hypotension and anaphylaxis. In the case of shock, current medical standards for shock treatment should be applied. Anaphylaxis, including anaphylactic shock with fatal outcome has been reported very rarely in post-marketing experience.

Regarding immunological events, pre-dose and post-dose antibody results were available for 494 subjects. Treatment-emergent positive bivalirudin antibodies are rare and have not been associated with clinical evidence of allergic or anaphylactic reactions. Caution should be exercised in patients previously treated with lepirudin who had developed lepirudin antibodies.

There are no safety concerns regarding drug-drug interactions but there was no safety advantage with bivalirudin as regards bleeding when given together with GPIIbIIIa inhibitor as observed in study TMC-BIV-00-01. Drug-drug interaction studies have been conducted with platelet inhibitors, including acetylsalicylic acid, ticlopidine, clopidogrel, abciximab, eptifibatide, or tirofiban. The results do not suggest pharmacodynamic interactions with these medicinal products.
From the knowledge of their mechanism of action, combined use of anti-coagulant drugs (heparin, warfarin, thrombolytics or antiplatelet agents) can be expected to increase the risk of bleeding.
In any case, when bivalirudin is combined with a platelet inhibitor or an anticoagulant drug, clinical and biological parameters of haemostasis should be regularly monitored.

Post-marketing experience, like experience in clinical trials, indicated an association between gamma brachytherapy and an increased incidence of intraprocedural thrombus. In the REPLACE-2 study, event rates for MI, Death/MI/Urgent Revas and bailout stent were much higher among brachytherapy patients.

The safety and effectiveness of bivalirudin in patients under 18 years have not been studied.

The potential safety concerns, which require post-authorisation surveillance are anaphylactic deaths and immune reaction, use during gamma brachytherapy and major bleeding episodes. The Applicant has committed to monitor for immunological reactions, anaphylaxis and major bleeds post-marketing and put particular emphasis on bleeding events in the PSURs. Adverse events during gamma brachytherapy will also be monitored. Additionally, the Applicant will expedite serious bleeding events for a period of one year.

5. Overall conclusions, benefit/risk assessment and recommendation

Quality

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way.

Non-clinical pharmacology and toxicology

The pharmacodynamic and pharmacokinetic profile of bivalirudin has been extensively characterised.

Toxicity in animals upon repeated or continuous exposure (1 day to 4 weeks at exposure levels of up to 10 times the clinical steady state plasma concentration) was limited to exaggerated pharmacological effects. Comparison of the single and repeated dose studies revealed that toxicity was related primarily to duration of exposure. All the undesirable effects, primary and secondary, resulting from excessive pharmacological activity were reversible. Undesirable effects that resulted from prolonged physiological stress in response to a non-homeostatic state of coagulation were not seen after short exposure comparable to that in clinical use, even at much higher doses. Based on this, no special hazard is foreseen in humans.

Bivalirudin is intended for short-term administration and therefore no data on the long-term carcinogenic potential of bivalirudin are available. However, bivalirudin was not mutagenic or clastogenic in standard assays for such effects.

All non-clinical data considered relevant to clinical safety have been included in the Summary of Product Characteristics (SPC) for Angiox.

Efficacy

Bivalirudin is a direct and specific thrombin inhibitor that binds both to the catalytic site and the anion-binding exosite of fluid-phase and clot-bound thrombin. In healthy volunteers and patients, bivalirudin exhibits dose- and concentration dependent anticoagulant activity as evidenced as prolongation of the ACT, aPTT, PT, INR and TT. Intravenous administration of bivalirudin produces measurable anticoagulation within minutes.

In clinical studies bivalirudin has been shown to provide adequate anticoagulation during PCI procedures. The clinical efficacy of bivalirudin as an anticoagulant in the setting of PCI has been shown based on the 30-day results based on quadruple and triple endpoints from the randomised, double blind trial of over 6,000 patients undergoing PCI (REPLACE-2).

Safety

Angiox has demonstrated an acceptable safety profile. There are no major safety concerns related to the use of bivalirudin in the context of PCI, however, there is a problem with increased occurrence of thrombosis in patients treated with gamma brachytherapy. Angiox should be used with caution during beta brachytherapy procedures. It is also worth pointing out that adverse events were higher in females, in patients >65 years of age and in severe renal impairment in addition to those observed with gamma brachytherapy. A single death with anaphylaxis has been reported. Angiox is contraindicated in patients with severe renal insufficiency (GFR <30ml/min) and also in dialysis-dependent patients.

The potential safety concerns, which require post-authorisation surveillance are anaphylactic deaths and immune reaction, use during gamma brachytherapy and major bleeding episodes. The Applicant has committed to monitor for immunological reactions, anaphylaxis and major bleeds post-marketing and put particular emphasis on bleeding events in the PSURs. Adverse events during gamma brachytherapy will also be monitored. Additionally, the Applicant will expedite serious bleeding events for a period of one year.

Benefit/risk assessment

Bivalirudin is an anticoagulant and direct thrombin inhibitor. The pharmacodynamics and pharmacokinetics of the product has been well defined.

The efficacy of the product has been demonstrated in a well-designed large clinical trial. Bivalirudin is non-inferior to a combination of heparin with GPIIb/IIIa inhibitor. The product can be used in patients with HITT and causes less major bleed.

Following the review of the submitted documentation, and the final SPC and Letter of Undertaking, the CHMP agreed that efficacy has been shown that is clinically relevant and that an acceptable safety profile has been demonstrated, which allow a conclusion on an acceptable benefit/risk ratio for the use of Angiox (bivalirudin) as an anticoagulant in patients undergoing percutaneous coronary intervention (PCI).

The Applicant has committed to monitor for immunological reactions, anaphylaxis and major bleeds post-marketing and put particular emphasis on bleeding events in the PSURs. Adverse events during gamma brachytherapy will also be monitored.

Recommendation

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considered by consensus that the benefit/risk ratio of Angiox for the use as an anticoagulant in patients undergoing percutaneous coronary intervention (PCI) was favourable and therefore recommended the granting of the marketing authorisation.