

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

761025Orig1s000

PHARMACOLOGY REVIEW(S)

MEMORANDUM

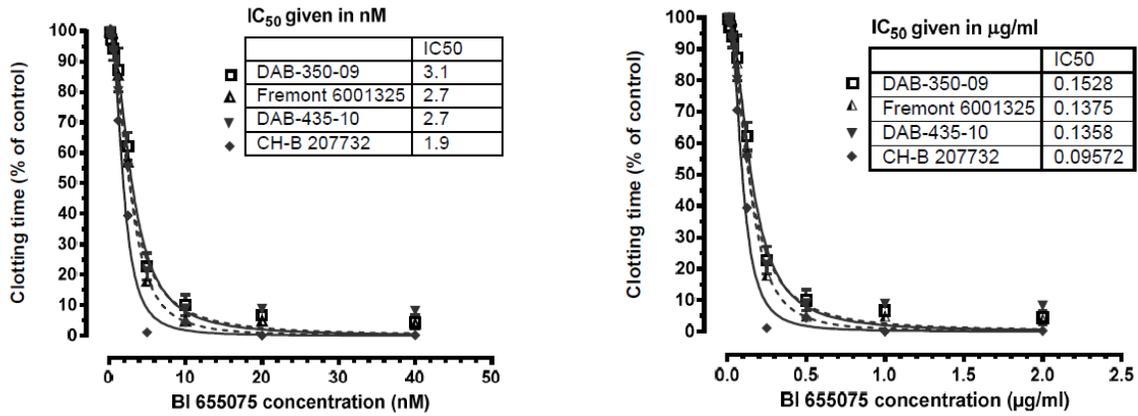
Date: October 13, 2015
From: Emily Place, PhD MPH
Pharmacology/Toxicology Reviewer
Division of Hematology Oncology Toxicology (DHOT)
Office of Hematology and Oncology Products (OHOP)
Re: Approvability for Pharmacology and Toxicology
Addendum to Primary Review
BLA: 761025
Drug: Praxbind, Idarucizumab
Indications: Rapid reversal of anticoagulant effects of dabigatran
Applicant: Boehringer Ingelheim Pharmaceuticals, Inc.

This addendum to the original pharmacology/toxicology BLA and labeling reviews for idarucizumab summarizes the data reviewed in support of the labeling claim that “(b) (4)”. Based on the review of study MD2014/01/PPSS, the proposed labeling text will be updated to state “(b) (4)”. ”.

Primary Pharmacology Study MD2014/01/PPSS:

Dabigatran (BIBR 953 ZW) is a direct inhibitor of thrombin, and idarucizumab (BI 655075) is a humanized Fab being developed as an antidote for dabigatran. The aim of the present study was to examine idarucizumab-mediated neutralization of the anticoagulant activity of dabigatran (Figure 1) as well as to a mix of acylglucuronide metabolites of dabigatran (Figure 2), in vitro, using a modified thrombin time (TT) coagulation assay. The anticoagulant activity of dabigatran or dabigatran-acylglucuronides was considered achieved when an approximately 40 second increase was observed in clotting time between the highest concentrations of dabigatran (or acylglucuronide mix) tested and baseline clotting in the absence of dabigatran when using thrombin as the clotting stimulus. In a pure plasma system, 7 nM of dabigatran was required to achieve an approximately 40 second prolongation. Higher concentrations (10 nM) were required in plasma when testing the acylglucuronidated forms of dabigatran (the ¹⁴C-acylglucuronide dabigatran metabolites used in the assay were isolated from the urine of ¹⁴C-dabigatran exposed monkeys). Plasma without dabigatran clotted in 60.9 ± 1.7 seconds in the presence of 0.4 U/mL thrombin; this was defined as 0% for the plasma-based assay. The plasma containing 7 nM dabigatran clotted in 97.9 ± 2.6 seconds. IC₅₀ values were determined for different idarucizumab production batches (DAB-350-09, Fremont 6001325, DAB-435-10, and CH-B 207732) in dabigatran-supplemented (7 nM) pooled plasma stimulated with thrombin (0.4 U/mL) and ranged from 1.9 to 3.1 nM (Figure 1, left panel). The plasma containing 10 nM of a mix of dabigatran acylglucuronide metabolites clotted in 106.0 ± 0.9 seconds. The IC₅₀ values for idarucizumab associated restoration of in vitro clotting time in the presence of 10 nM acylglucuronidated dabigatran metabolites is 2.4 nM (Figure 2, left panel). Dabigatran and dabigatran metabolites were shown to prolong clotting time in the assay and idarucizumab was shown to affect a concentration-dependent neutralization of prolonged clotting time to both.

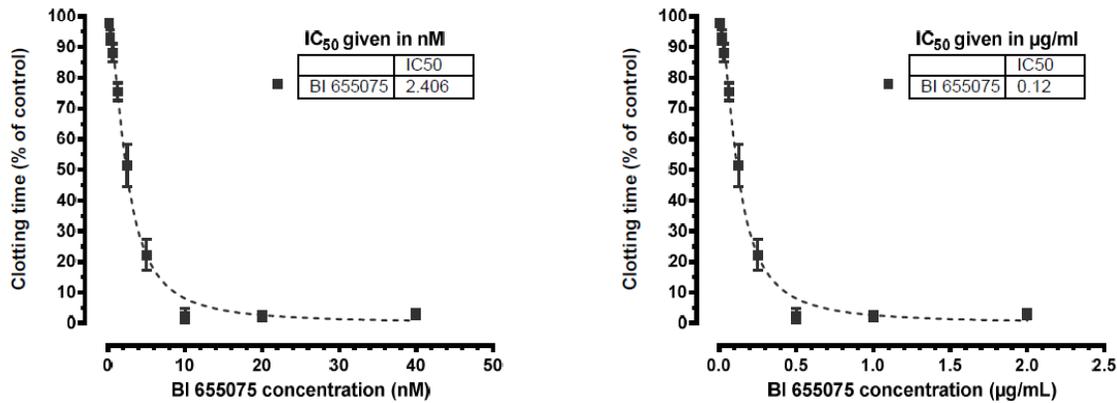
Figure 1. Restoration of Clotting Time in Plasma Supplemented with Dabigatran



(Excerpted from the submission)

Concentration-dependent restoration of in vitro clotting time in a modified TT time assay by different idarucizumab (BI 655075) production batches in dabigatran-supplemented (7 nM) pooled plasma stimulated with thrombin (0.4U/mL). Baseline clotting without dabigatran was defined as 0%. DAB-350-09 data expressed as mean ± SEM, N=3.

Figure 2. Restoration of Clotting Time in Plasma Supplemented with a Mix of Acylglucuronide Metabolites of Dabigatran



Tested against 10nM ¹⁴C-BIBR953-acylglucuronide-mix

Tested against 10nM ¹⁴C-BIBR953-acylglucuronide-mix

(Excerpted from the submission)

Concentration-dependent restoration of in vitro clotting time in a modified TT assay by idarucizumab (BI 655075) in ¹⁴C-labeled dabigatran acylglucuronide-supplemented (10 nM) plasma stimulated with thrombin (0.4U/mL). Baseline clotting without dabigatran was defined as 0%. Data expressed as mean ± SEM, N=3.

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/s/

EMILY J PLACE
10/14/2015

CHRISTOPHER M SHETH
10/14/2015

MEMORANDUM

Praxbind (idarucizumab)

Date: October 1, 2015

To: File for BLA 761025

From: John K. Leighton, PhD, DABT

Director, Division of Hematology Oncology Toxicology
Office of Hematology and Oncology Products

I have examined pharmacology/toxicology supporting and labeling reviews for Praxbind conducted by Dr. Place, and secondary memorandum and labeling provided by Dr. Sheth. Dr. Sheth discussed in his memo that the Applicant proposed Pregnancy Category ^(b)₍₄₎, and that this was considered acceptable by the review team. The labeling will be following the Pregnancy and Lactation Labeling Rule (PLLR) and thus no Pregnancy Category will be included in the label. I concur with Dr. Sheth's conclusion that Praxbind may be approved for the proposed indication.

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/s/

JOHN K LEIGHTON
10/01/2015

MEMORANDUM

Date: September 11, 2015
From: Christopher Sheth, PhD
Division of Hematology Oncology Toxicology (DHOT)
Office of Hematology and Oncology Products (OHOP)
Re: Approvability for Pharmacology and Toxicology
BLA: 761025
Drug: PRAXBIND (idarucizumab) injection; for intravenous use
Indication: PRAXBIND is a humanized monoclonal antibody fragment (Fab) indicated in patients treated with Pradaxa® when rapid reversal of the anticoagulant effects of dabigatran is required
Applicant: Boehringer Ingelheim Pharmaceuticals, Inc.

Idarucizumab is a humanized monoclonal antibody fragment (Fab) that binds to the thrombin inhibitor anticoagulant dabigatran with a higher affinity than the binding of dabigatran to thrombin. There is no endogenous target for idarucizumab. Based on its chemical structure, the pharmacologic class assigned to idarucizumab is “humanized monoclonal antibody fragment (Fab)”. Pharmacology, safety pharmacology, and toxicology studies were conducted in in vitro and in vivo models. The biologic product idarucizumab is only intended to be administered in acute situations where a rapid reversal of the anticoagulant effects of dabigatran are required; as such, in accordance with the ICH S6 and S9 Guidances and conditions of use, genetic toxicology, reproductive and developmental, and carcinogenicity studies were not needed.

Data from in vitro studies demonstrated that idarucizumab forms complexes with dabigatran and with dabigatran metabolites. Several in vivo nonclinical bleeding models were utilized to show that idarucizumab administration results in reversal of the anticoagulant effects of dabigatran. Additional data submitted indicate the effects of idarucizumab are in part mediated by increasing fibrin coverage and increasing fibrin masses around damaged subendothelium.

No major target organs for toxicity were identified in rats or monkeys administered idarucizumab, likely due to the specificity of the molecule for an exogenously administered target (i.e., dabigatran). The Applicant proposed a Pregnancy Category ^(b)₍₄₎ for idarucizumab, which was found to be acceptable. In general, women should not become pregnant while taking anticoagulants, thus it is unlikely idarucizumab will be administered to women of childbearing potential who are not taking measures to prevent pregnancy. The nonclinical studies needed to support product labeling were reviewed by Dr. Emily Place. The nonclinical findings are summarized in the “Executive Summary” of the BLA review and reflected in the product label.

Recommendation: I concur with the pharmacology/toxicology reviewer that from a nonclinical perspective, PRAXBIND may be approved and that no additional nonclinical studies are needed to support approval of PRAXBIND in patients with treated with Pradaxa® when rapid reversal of the anticoagulant effects of dabigatran is required.

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/s/

CHRISTOPHER M SHETH
09/11/2015

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

PHARMACOLOGY/TOXICOLOGY BLA REVIEW AND EVALUATION

Application number: 761025
Supporting document: 2
Applicant's letter date: February 18, 2015
CDER stamp date: February 19, 2015
Product: Praxbind (idarucizumab)
Indication: Rapid reversal of anticoagulant effects of dabigatran
Applicant: Boehringer Ingelheim Pharmaceuticals, Inc.
Review Division: Division of Hematology Oncology Toxicology (DHOT) for Division of Hematology Products (DHP)
Reviewer: Emily Place PhD MPH
Supervisor/Team Leader: Chris Sheth PhD
Division Director: John Leighton PhD, DABT (DHOT)
Ann Farrell MD (DHP)
Project Manager: Alycia Anderson CCRP

Disclaimer

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1 Executive Summary

1.1 Introduction

Idarucizumab is a reversal agent for the thrombin inhibitor dabigatran. It is a humanized murine monoclonal antibody fragment antigen-binding (Fab) molecule directed against dabigatran and its metabolites and forms a stable complex to neutralize the anticoagulant effects dabigatran. The applicant is seeking approval for the intravenous route of administration and the proposed clinical dose is 5.0 g. Nonclinical pharmacology, pharmacokinetic, and chronic toxicology studies in the rat and the monkey have been submitted.

The pharmacology/toxicology studies conducted support approval of idarucizumab for the proposed indication (patients treated with dabigatran when rapid reversal of the anticoagulant effects of dabigatran is required for emergency surgery/urgent procedures or in life-threatening or uncontrolled bleeding).

1.2 Brief Discussion of Nonclinical Findings

Idarucizumab binds dabigatran with higher affinity than thrombin (~300 times higher) and thrombin substrates in vitro. Results from in vitro studies show that idarucizumab forms a stable complex with dabigatran (with 50% of bound complex remaining after 260 hours). Idarucizumab also binds dabigatran metabolites. In vitro data showed that idarucizumab reverses the anticoagulant effect of dabigatran, in part by increasing fibrin coverage and increasing fibrin masses around damaged subendothelium. Three animal models of activity were submitted in support of the application: a mouse intracranial hemorrhage model; a rat tail cut bleeding model, and a pig blunt liver trauma model. All 3 animal pharmacology models showed the effectiveness of the neutralization activity of idarucizumab, and its ability to significantly reduce anticoagulation and blood loss. Based on the nonclinical data submitted in the BLA and its chemical structure, the Established Pharmacological Class (EPC) of “humanized monoclonal antibody fragment (Fab)” was determined to be both clinically meaningful and scientifically valid for idarucizumab.

Safety pharmacology studies showed no adverse respiratory findings. Cardiovascular safety pharmacology studies were not performed independently but ECG measurements assessed during the 2 week repeat dose toxicology study in monkeys were unremarkable at doses up to 500 mg/kg.

In the pharmacokinetic studies in both rats and monkeys, there was a rapid increase in dabigatran plasma concentration following dosing with idarucizumab suggesting redistribution of dabigatran from the tissue to the plasma. Based on the data collected in general toxicology studies, there were no gender differences in exposure, and increased in C_{max} and AUC values were approximately dose proportional. Idarucizumab was rapidly eliminated in the blood following intravenous dosing and exhibited biphasic plasma concentration-time profiles; initial phase half-lives were approximately 0.25 hrs.

(both species) and terminal phase half-lives were approximately 6 hrs in the rat and 5.5 hrs. in the monkey.

The general toxicology studies were conducted in the rat and monkey via I.V., which is the intended route of administration for idarucizumab. The rat studies were performed using only idarucizumab; the monkey studies were performed in the presence and absence of orally administered dabigatran. The 4 week repeat dose toxicity study in rat and 2 week repeat dose toxicity study in the monkey are reviewed. All appropriate studies were conducted in compliance with Good Laboratory Practice (GLP) regulations. There were no major target organs in rat or monkey.

The following types of toxicological assessments for idarucizumab were not deemed essential for marketing due to the specific nature of its intended use, which is as a single administration only when needed to rapidly reverse the anticoagulant effects of dabigatran: in vitro and in vivo genotoxicity, carcinogenicity, reproductive and developmental toxicity, fertility, embryo-fetal development; and pre- and postnatal development toxicity testing.

1.3 Recommendations

1.3.1 Approvability

From the Pharmacology/Toxicology perspective “idarucizumab” may be approved for the proposed indications.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

The content for the labeling of idarucizumab is contained in this review. Based on the nonclinical data submitted in the BLA, the Established Pharmacological Class (EPC) of “humanized monoclonal antibody fragment (Fab)” was determined to be both clinically meaningful and scientifically valid for idarucizumab.

2 Drug Information

2.1 Drug

CAS Registry Number (Optional): 1362509-93-0

Code Name: BI 655075, 655075-01, idarucizumab

Chemical Name: N/A

Molecular Formula/Molecular Weight: $C_{2131}H_{3299}N_{555}O_{671}S_{11}$ / 47,766 Da.

Structure or Biochemical Description:

Figure 1. Structure of idarucizumab



(Excerpted from the submission)

Pharmacologic Class: humanized monoclonal antibody fragment (Fab)

2.2 Relevant INDs, NDAs, BLAs and DMFs

None

2.3 Drug Formulation

Table 1. Idarucizumab drug formulation

Ingredient	Amount per vial [mg] ¹	Concentration [mg / mL]	Function	Reference to standards
Idarucizumab	2500.00	50.00	Active ingredient	Internal standard
Acetic acid glacial	10.05	0.20	(b) (4)	Ph. Eur., USP
Polysorbate 20	10.00	0.20		Ph. Eur., USP/NF
Sodium acetate trihydrate	147.35	2.95		Ph. Eur., USP
Sorbitol	2004.20	40.08		Ph. Eur., USP/NF
Water for Injection	q.s.ad 50.00 mL	n/a		Ph. Eur., USP
Total volume	50 mL	-		-

¹ (b) (4)

(Excerpted from the submission)

2.4 Comments on Novel Excipients

There are no Pharmacology/Toxicology concerns with the excipients or their levels in the drug formulation.

2.5 Comments on Impurities/Degradants of Concern

N/A – Division of Therapeutic Proteins

2.6 Proposed Clinical Population and Dosing Regimen

The proposed indication as it will appear on the label is: PRAXBIND is a humanized monoclonal antibody fragment (Fab) indicated in patients treated with Pradaxa® when rapid reversal of the anticoagulant effects of dabigatran is required:

- For emergency surgery/urgent procedures
- In life-threatening or uncontrolled bleeding

The proposed dose of idarucizumab is 5.0 g. It is currently provided as two separate 50 mL vials each containing 2.5 g idarucizumab, which will be administered intravenously.

2.7 Regulatory Background

NDA 761025 was submitted on February 19, 2015 for the rapid reversal of anticoagulant effects of dabigatran.

3 Studies Submitted

3.1 Studies Reviewed

Study Title	Study No.	Module
Primary Pharmacodynamics		
<i>In vitro Pharmacology</i>		
Affinity of BI 655075 for dabigatran, pH dependence and binding to plasma proteins	MD2013/03/P PSS	4.2.1.1
Neutralization of the anticoagulant activity of dabigatran and its acylglucuronides by BI 655075 in vitro using a modified thrombin time assay in plasma or whole blood	MD2014/01/P PSS	4.2.1.1
Lack of potential prothrombotic activity of BI 655075 investigated in human plasma in vitro and in rats in vivo	MD2014/02/P PSS	4.2.1.1
Effects of dabigatran on platelet interaction and fibrin generation and reversal of its antihemostatic action by procoagulant strategies or a specific antidote (BI655075): Studies in vitro with circulating human blood.	MD2014/11/P PSS	4.2.1.1
Inhibition of dabigatran anticoagulant activity by BI 655075 in the presence of coagulation factor concentrates in vitro	MD2014/14/P PSS	4.2.1.1
<i>In vivo Pharmacology</i>		
Efficacy of hemostatic therapy with BI 655075 in experimental intracerebral hemorrhage associated with the oral anticoagulant Dabigatran Etxilate	MD2014/08/P PSS	4.2.1.1
Neutralization of the anticoagulant activity of dabigatran with BI 655075 after intravenous administration in a rat model of anticoagulation	MD2013/05/P PSS	4.2.1.1
Reversal of dabigatran-induced bleeding with BI 655075 after single oral administration of dabigatran etexilate in an in vivo rat tail bleeding model	MD2013/06/P PSS	4.2.1.1
Reversal of supra-therapeutic dabigatran anticoagulation and subsequent bleeding with BI 655075 in an in vivo rat model	MD2014/05/P PSS	4.2.1.1
Effect of BI 655075 on bleeding induced by dabigatran in combination with the antiplatelet agents ASA, clopidogrel and ticagrelor (rat in vivo)	MD2014/09/P PSS	4.2.1.1
Neutralization of dabigatran by BI 655075, the specific antidote for dabigatran, after haemodilution and administration of various volume expanders in a pig model.	MD2013/07/P PSS/2012- 008 A	4.2.1.1
Effects of BI 655075 on dabigatran-induced bleeding and hemorrhagic shock in a porcine blunt liver trauma model	MD2013/08/P PSS	4.2.1.1
Effects of BI 655075 as a split dose (2 bolus injections 60 min apart) on dabigatran-induced bleeding and hemorrhagic shock in a porcine blunt liver double trauma model	MD2014/12/P PSS	4.2.1.1
Safety Pharmacology		
BI 655075: Evaluation of Respiratory Parameters in the conscious Male Rat using Whole Body Bias Flow Plethysmography (Intravenous Bolus Administration)	12r006	4.2.1.3
Pharmacokinetics		
<i>Absorption</i>		
Pharmacokinetics and urinary excretion of BI 655075 and the effect of BI 655075 on the pharmacokinetics and urinary excretion of dabigatran in the Wistar Han rat	U12-3083-01	4.2.2.2.1
BI 655075: Pharmacokinetic and Pharmacodynamic Study in rhesus Monkeys Pretreated with Dabigatran Etxilate	U13-3539-01	4.2.2.2.1

Study Title	Study No.	Module
Repeat Dose Toxicology <i>(including supportive toxicokinetics)</i>		
BI 655075: Toxicity Study by Intravenous Administration to Han Wistar Rats for 4 Weeks Followed by a 4 Week Recovery Period	DDB0150	4.2.3.2.1
Study title: Report BI 655075 and Dabigatran Etexilate: Toxicity Study by Intravenous and Oral Gavage Administration to rhesus Monkeys for 14 Days Followed by a 4 Week Recovery Period and Dabigatran Etexilate Re-Administration	DDB0331	4.2.3.2.1

3.2 Studies Not Reviewed

Study Title	Study No.	Module
Pharmacokinetics-		
<i>Analytic Methods and Validation Reports</i>		
Validation of a Radioimmuno-precipitation Assay for Detection of Anti-Dabigatran Antibodies in rhesus Monkey Plasma	8295-001	4.2.2.1
Evaluation of dabigatran interference in ELISA assays used to quantify BI 655075 in nonclinical studies	BB-14-1001	4.2.2.1
Validation of an ELISA Method for the Quantitation of BI 655075 in rhesus Monkey Plasma (K3EDTA) (Method Number: BBM-12-1001)	BBM-12-1001	4.2.2.1
Validation of an Electrochemiluminescence Method for the Detection of Anti BI 655075 Antibodies in Wistar Hannover K3EDTA Rat Plasma	dm-12-1036	4.2.2.1
Validation of an ELISA Method for the Quantitation of BI 655075 in Rat Plasma (k3EDTA) (Method Number: BBM-12-1003)	dm-12-1044	4.2.2.1
Validation of an Electrochemiluminescence Method for the Detection of Anti BI 655075 Antibodies in rhesus K3EDTA Monkey Plasma	dm-12-1058	4.2.2.1
Evaluation of Potential Assay Interference for Plasma Samples from rhesus Monkeys Administered Dabigatran Etexilate	dm-13-1011	4.2.2.1
Validation of an Electrochemiluminescence Method for the Detection of Anti BI 655075 Antibodies in rhesus Monkey K3EDTA Plasma (Method Number: BBM-13-1001)	dm-13-1086	4.2.2.1
Method validation of determination of pharmacodynamic action of BIBR 953 on Hemoclot® Thrombin Inhibitors Clotting Time, activated Partial Thromboplastin Clotting Time, Thrombin Clotting Time and Ecarin Clotting Time in rhesus monkey plasma	MEN1011	4.2.2.1
Method validation of a modified TT based assay for detection of neutralising anti-Dabigatran antibodies in rhesus monkey plasma	MEN1313	4.2.2.1
Method validation of Prothrombin fragment F 1+2 in rhesus monkey plasma by specific commercial ELISA kit.	MEN1414	4.2.2.1
Method validation of determination of pharmacodynamic action of BIBR 953 on Hemoclot® Thrombin Inhibitors Clotting Time, activated Partial Thromboplastin Clotting Time and Ecarin Clotting Time in Rat plasma	MEN2212	4.2.2.1
Method validation of D-Dimer and Prothrombin fragment F 1+2 in human and rhesus monkey plasma by specific commercial ELISA kits.	MEN2513	4.2.2.1
Revalidation of a LC-MS/MS method for the determination of BIBR 953 ZW in EDTA-rat plasma in the presence of BI 655075	n-a-bio-11-143	4.2.2.1
Validation of a LC-MS/MS method for the determination of BIBR 953 ZW in rat urine in the presence of BI 655075	n-a-bio-11-144	4.2.2.1
VALIDATION OF A LC-MS/MS METHOD FOR THE DETERMINATION OF BIBR 953 ZW IN EDTA RHESUS MONKEY	n-a-bio-11-145	4.2.2.1

Study Title	Study No.	Module
PLASMA IN PRESENCE OF BI 655075		
VALIDATION OF A LC-MS/MS METHOD FOR THE DETERMINATION OF SUM BIBR 953 ZW IN EDTA RHESUS MONKEY PLASMA IN PRESENCE OF BI 655075	n-a-bio-11-146	4.2.2.1
Validation of a LC-MS/MS method for the determination of BIBR 953 ZW in monkey urine in the presence of BI 655075	n-a-bio-11-147	4.2.2.1
Validation of a LC-MS/MS method for the determination of SUM-BIBR 953 ZW in monkey urine in the presence of BI 655075	n-a-bio-11-148	4.2.2.1
Partial validation of a LC-MS/MS method for the determination of SUM-BIBR 953 ZW in EDTA-pig plasma in the presence of BI 655075 -Additional long term stability data	n-a-bio-12-224-am1	4.2.2.1
Evaluation of interference in an anti-BI 655075 antibody assay in plasma from dabigatran-treated rhesus monkeys	PK-12-1024 and PK-13-1036	4.2.2.1
<i>Absorption</i>		
Metabolism of BIBR 1048 MS and BIBR 953 ZW in rats	A289_01BC A344_02BC	4.2.2.2
BI 655075: Pharmacokinetic and Pharmacodynamic Study in rhesus Monkeys Pretreated with Dabigatran Etxilate	U12-3849-01	4.2.2.2
Prediction of human pharmacokinetic parameters of BI 655075 based on rhesus monkey pharmacokinetic data	U12-3374-01	4.2.2.2
The disposition of 14C BIBR953 ZW in rhesus monkey following IV administration.	IRI164291	4.2.2.2
Development of a preliminary semi-mechanistic model to describe the interaction between dabigatran and its antidote, idarucizumab, in animals and humans	pk-14-1019	4.2.2.2
<i>Other pharmacokinetic studies</i>		
Effect of renal impairment on the pharmacokinetics and urinary excretion of dabigatran or its antidote BI 655075 in the rat	U13-3340-01	4.2.2.7
Impact of renal impairment on pharmacokinetics and pharmacodynamics of BI 655075 in rats treated with dabigatran	dm-13-1030	4.2.2.7
Single Dose Toxicology		
BI 655075: Single Dose Study by Intravenous Bolus	U12-3325-01	4.2.3.1
Repeat Dose Toxicology <i>(including supportive toxicokinetics)</i>		
BI 655075: Exploratory Study in rhesus Monkeys	U12-3326-01	4.2.3.2.1
BI 655075: Toxicity Study by Intravenous Administration to rhesus Monkeys for 2 Days Followed by a 2 Week Recovery Period	11r149	4.2.3.2.1
BI 655075: Renal Investigation Study by Intravenous Administration to rhesus Monkeys for 2 Days Followed by a 2 Week Recovery Period	12r169	4.2.3.2.1
Local Tolerance		
Single Dose Perivascular Tolerance Study in Male Rabbits	13r064	4.2.3.6
Other Toxicity Studies		
A Tissue Cross-Reactivity Study of BI 655075 in Normal Human, rhesus Monkey and Wistar-Han Rat Tissues	11r152	4.2.3.7.7
BI 655075: Determination of Hemocompatibility in Human Whole Blood	12r021	4.2.3.7.7

Study Title	Study No.	Module
Nonclinical Safety Assessment: Kappa Select Protein	n00236993-01	4.2.3.7.7
Nonclinical Safety Assessment: Leachables in idarucizumab Process Equipment	n00237442-01	4.2.3.7.7
BIBR 1048 MS: Acyl glucuronides of dabigatran etexilate	U07-1334	4.2.3.7.7

3.3 Previous Reviews Referenced

None

4 Pharmacology

4.1 Primary Pharmacology

Mechanism of Action

Idarucizumab is a reversal agent for dabigatran. It is a humanized monoclonal antibody fragment (Fab) directed against the thrombin inhibitor dabigatran. Binding of idarucizumab to dabigatran results in formation of a stable complex. Idarucizumab can also bind to dabigatran metabolites. Idarucizumab neutralizes the anticoagulant effect of dabigatran.

Idarucizumab binding to dabigatran in vitro

Methods

Affinity determinations and binding kinetics were determined using Kinexa technologies. Dissociation constants were calculated and K_D determinations were performed at different pH values.

Results

Binding of idarucizumab to dabigatran was assessed using Biacore technology. The calculated K_D value for idarucizumab was 2.1 pM. The K_D for binding of dabigatran to thrombin was 0.7nM (~300 times lower affinity).

Table 2. Antibody affinity for BI 655075 binding to dabigatran at pH 7.4

Fab	K_D (pM)	$k_a \times 10^5 M^{-1}s^{-1}$	$k_a \times 10^{-6} s^{-1}$ (calculated)	estimated $T_{1/2}$ of complex
Idarucizumab	2.1 ± 0.6	3.4 ± 0.4	0.7 ± 0.2	~260 h

(Excerpted from the submission)

Inhibition of dabigatran by idarucizumab in Human Plasma and Whole Blood

Methods

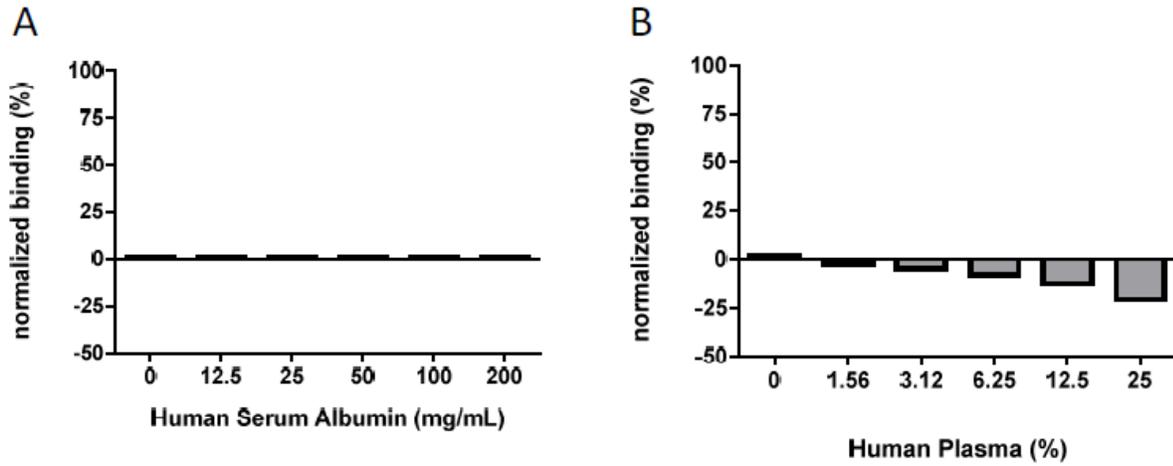
Binding to plasma proteins and thrombin substrates was determined.

Idarucizumab was coupled to the matrix of a CM-5 sensor chip. Surface plasmon resonance (Biacore) experiments were performed using idarucizumab and dabigatran with aliquot of human plasma pooled from 10 volunteers. Idarucizumab binding was tested using a modified thrombin time (TT) assay. IC₅₀ values were determined using concentrations of idarucizumab added to plasma containing dabigatran and the clotting time was measured in whole blood and plasma.

Results

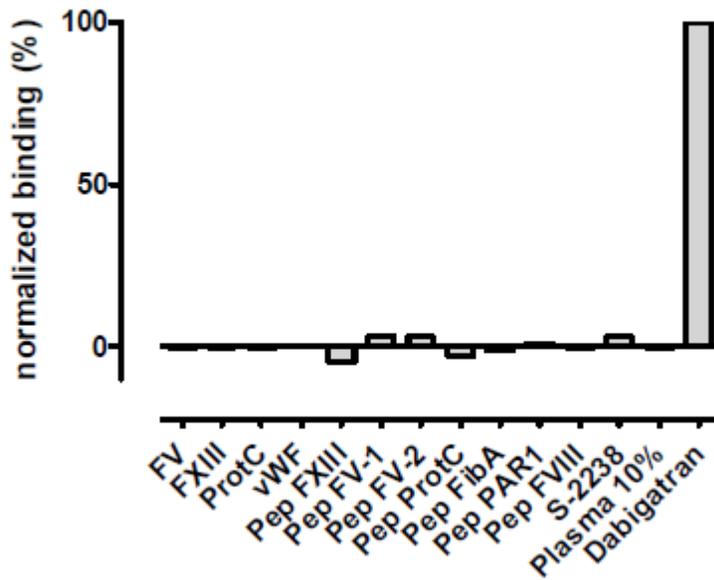
There was no specific binding of human plasma, serum, albumin or fibrinogen by idarucizumab- dabigatran complex. There was no specific binding to the thrombin substrates displayed by idarucizumab including the following substrates: S-2238, factors V, VIII, XIII, fibrinogen, von Willebrand factor, protease-activated receptor-1 (PAR-1) peptide or protein C. IC₅₀ values for the reversal of anticoagulant activity of dabigatran by idarucizumab in human plasma and whole blood are shown below for 7 nm (A) and 30nm (B) (n=4). Reversal of the anticoagulant effect of dabigatran was similar in whole blood and plasma (IC₅₀ 11.3 and 10.9 nM, respectively); IC₅₀ is approximately half the total dabigatran in each respective assay. The Applicant points to evidence of a 1:1 stoichiometric relationship between dabigatran and idarucizumab.

Figure 2. Idarucizumab binding to human serum albumin and plasma proteins



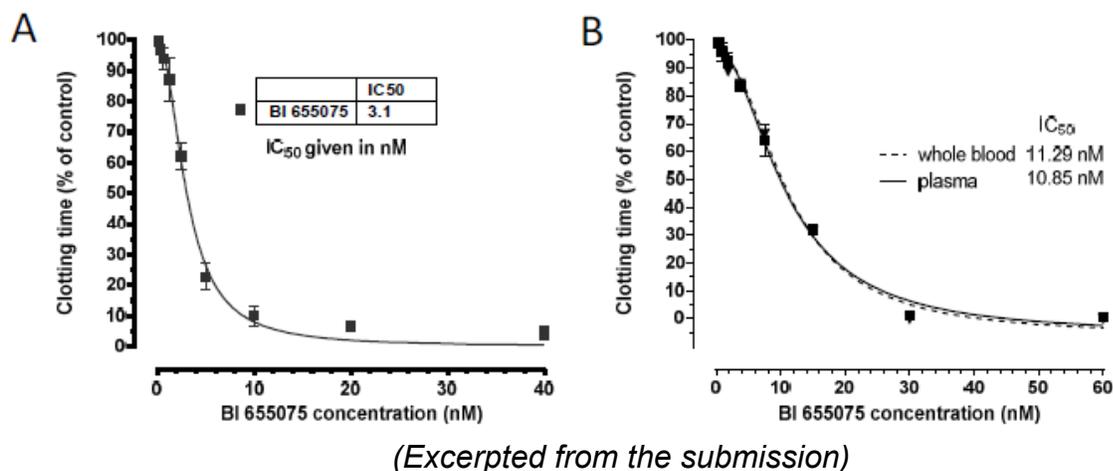
(Excerpted from the submission)

Figure 3. Idarucizumab binding to thrombin substrates



(Excerpted from the submission)

Figure 4. IC₅₀ values for the reversal of anticoagulant activity of dabigatran by idarucizumab in human plasma and whole blood



Idarucizumab displays thrombin-like activity in vitro and in vivo

Methods

Based on structural similarities to thrombin and its interactions with dabigatran, experiments were performed to determine if idarucizumab had any thrombin-like enzymatic activity. Activity was measured in human test systems in vitro and in rat plasma ex vivo using a thrombin-based clotting assay and platelet stimulation assays.

Results

Thrombin generation was not significantly increased relative to controls in a number of parameters tested. In prothrombin depletion coagulation assays, idarucizumab displayed a lack of procoagulant activity with long mean clotting times. Results also indicated that idarucizumab does not convert fibrinogen into fibrin in human plasma in vitro or in rats in vivo. There was also a lack of interaction by idarucizumab with the PAR-1 receptor on platelets in platelet rich plasma. The data indicated idarucizumab lacks thrombin-like activity and lacks the ability to stimulate aggregation of platelets.

Effects of idarucizumab and dabigatran on platelet interaction and fibrin generation in vitro

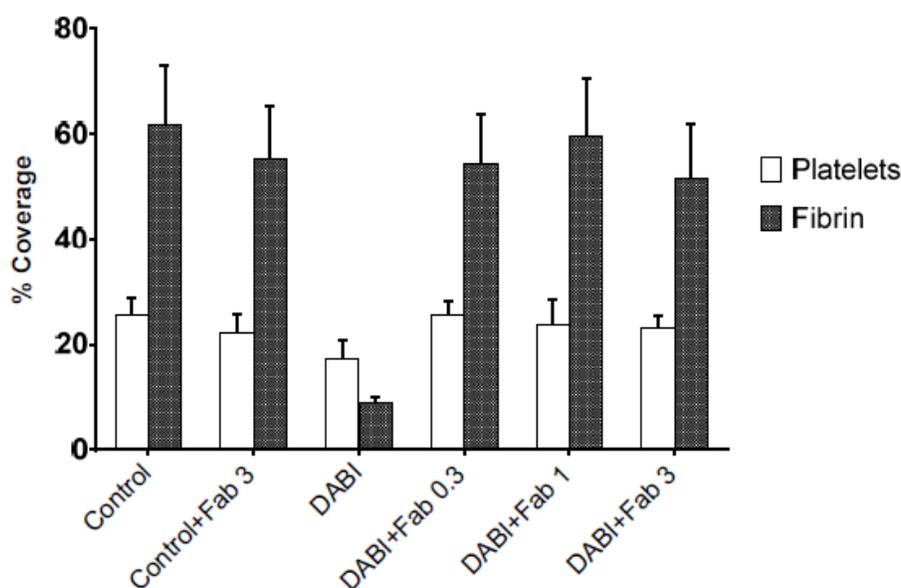
Methods

Experiments were performed to evaluate thrombus formation on damaged vascular surfaces using idarucizumab and dabigatran with an aliquot of human plasma pooled from 11 volunteers. The blood was then perfused through annular chambers exposing the thrombogenic substrate (a damaged vessel segment, rabbit aorta). Perfusion studies were performed using light microscopy to quantify the amount of fibrin and platelet deposition onto the damaged vessel. Routine hematology parameters including platelet count, hematocrit, and white blood cell differential, PT, aPTT and fibrinogen levels were collected using the BCS TM XP system.

Results

Dabigatran (DABI, 390 nM) supplemented blood significantly decreased fibrin coverage on the perfused damaged subendothelium and reduced the percentage of the vessel covered by platelets. Idarucizumab in the absence of dabigatran did not alter fibrin or platelet deposition. Idarucizumab added to blood previously treated with dabigatran at concentrations at 0.3, 1 or 3 mg/mL (Fab 0.3, 1, 3) restored the amount of fibrin coverage and size of fibrin masses to those originally observed in control samples (idarucizumab only).

Figure 5. Effects of idarucizumab and dabigatran on platelet interaction and fibrin generation



(Excerpted from the submission)

Effects of coagulation factors on idarucizumab and dabigatran in vitro

Methods

Prothrombin complex concentrates (PCCs), activated prothrombin complex concentrates (aPCC), and recombinant activated factor VIIa (rFVIIa) were measured using diluted thrombin time (dTT, clotting time) and tested in human plasma with increasing concentrations of both idarucizumab and dabigatran.

Results

Coagulation factor concentrates (PCCs, aPCC, rFVIIa) in human plasma did not influence the anticoagulant effect of dabigatran as measured using dTT (clotting time), and they did not interfere with inhibition of dabigatran by idarucizumab.

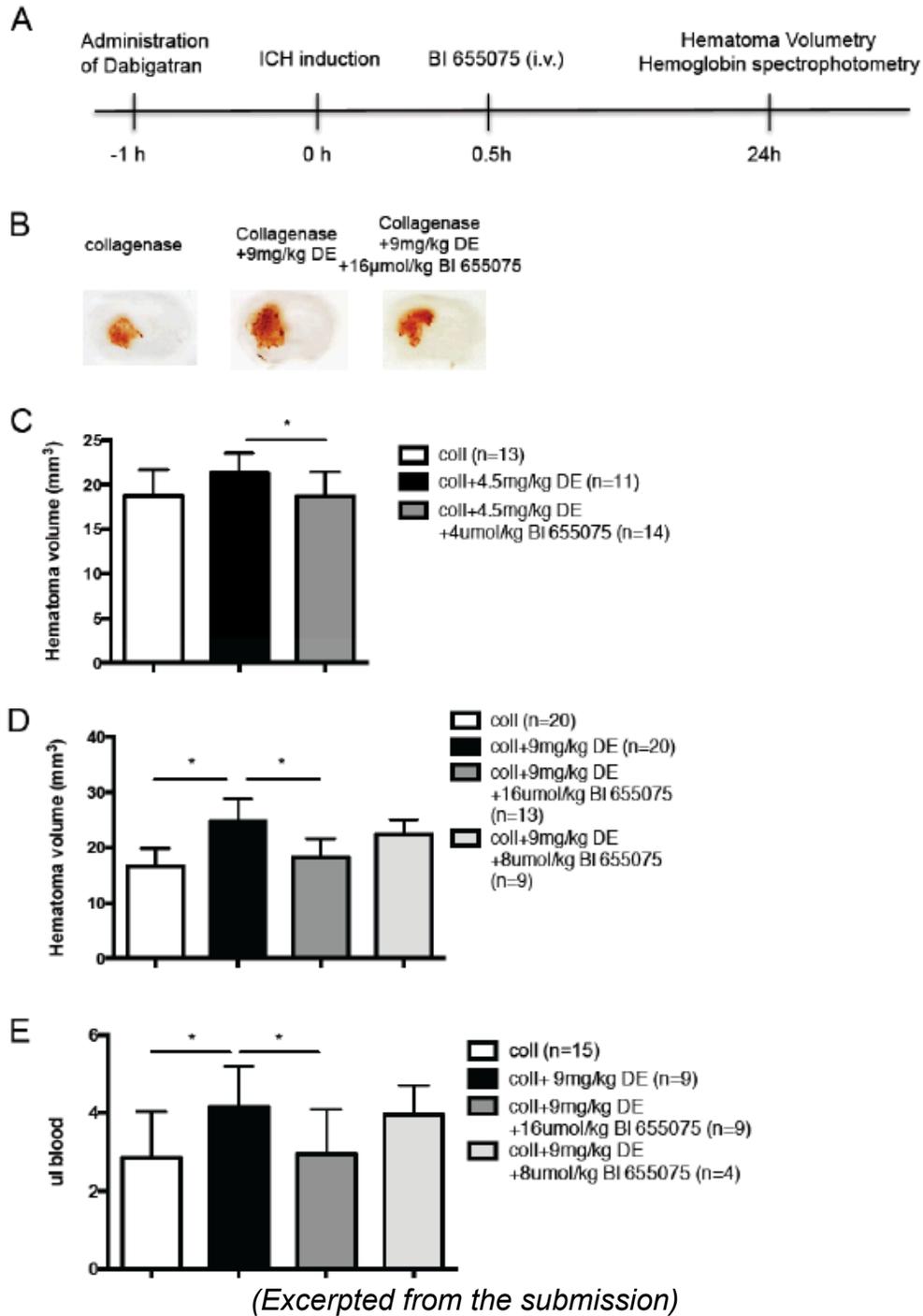
**Animal models of activity:
Intracranial hemorrhage mouse model**Methods

Experiments were performed to determine if idarucizumab in addition to dabigatran etexilate (DE) influences hematoma volume in a mouse model of intracranial hemorrhage (ICH). DE was administered intraperitoneally and ICH was induced by intrastriatal collagenase-injection. Idarucizumab or saline was then administered 30 minutes following induction of ICH by tail vein injection. ICH volume and intracerebral blood content were quantified using hemoglobin spectrophotometry and by examination of brain cryosections.

Results

Hematoma enlargement occurs rapidly after ICH induction and DE is administered within 1 hour. Administration of idarucizumab at equimolar doses of DE is effective at reducing hematoma volume (8-16uM idarucizumab, 4.5-9.0 mg/kg DE). Hematoma volumes returned to levels observed in non-anticoagulated animals.

Figure 6. Intracranial hemorrhage model in mouse- hematoma volumes



Rat tail cut bleeding model

Neutralization of dabigatran induced bleeding after IV administration in rat

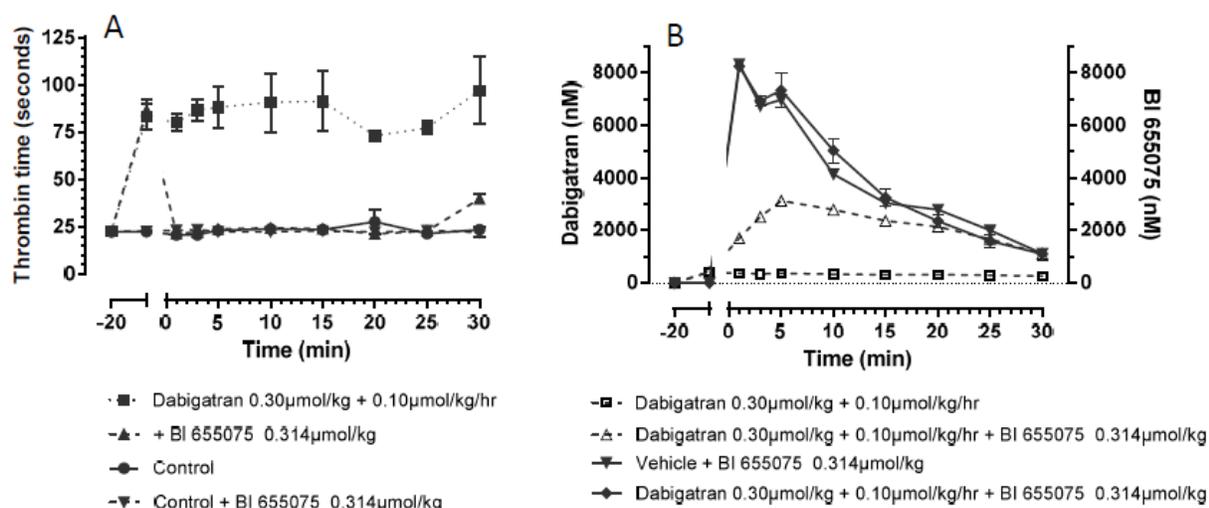
Methods

Neutralization activity of dabigatran by idarucizumab was measured using an assay for thrombin time (seconds).

Results

Dabigatran prolonged thrombin time ~4fold (25sec to 100sec). Addition of idarucizumab at a single bolus dose of equimolar concentration completely reversed the activity within one minute (0.3uM/kg). Plasma levels of the drug are shown in Figure B below.

Figure 7. Neutralization of dabigatran by idarucizumab in rat tail cut bleeding model



(Excerpted from the submission)

Reversal of dabigatran induced bleeding after single oral administration

Methods

Dabigatran etexilate (DE) was administered orally to rats at a dose of 30 mg/kg followed by a single bolus of idarucizumab (33 mg/kg, 0.69 μmol/kg) administered intravenously (at t=0 below; 40 minutes post DE). Following idarucizumab administration a bleeding time assay was performed by making a standardized incision in the rat tail and measuring the length of time required for bleeding to stop. Anticoagulant activity was measured by various clotting time assays (TT, aPTT, and ECT; thrombin time, activated prothrombin time and Ecarin clotting time).

Results

There was a significant reduction in bleeding time prolongation sustained following treatment with idarucizumab after dosing dabigatran (120 minutes post dose). There was also a significant reduction in anticoagulant activity (TT, aPTT, and ECT). dTT is shown in Figure 9A below. Plasma bioanalysis of total dabigatran (shown as solid black lines) and idarucizumab (shown as solid grey lines) are shown in ng/mL in Figure 9B below.

Figure 8. Rat tail bleeding time in the rat tail cut model, single dose

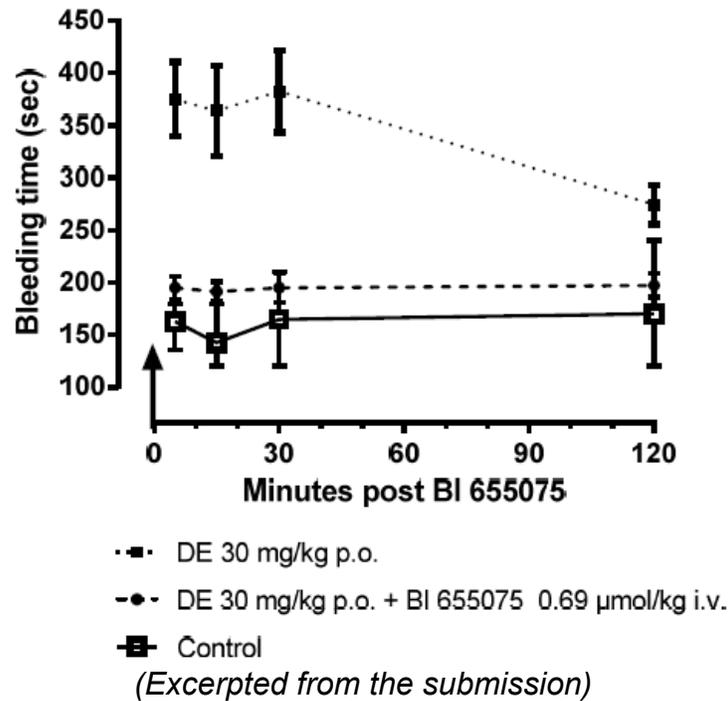
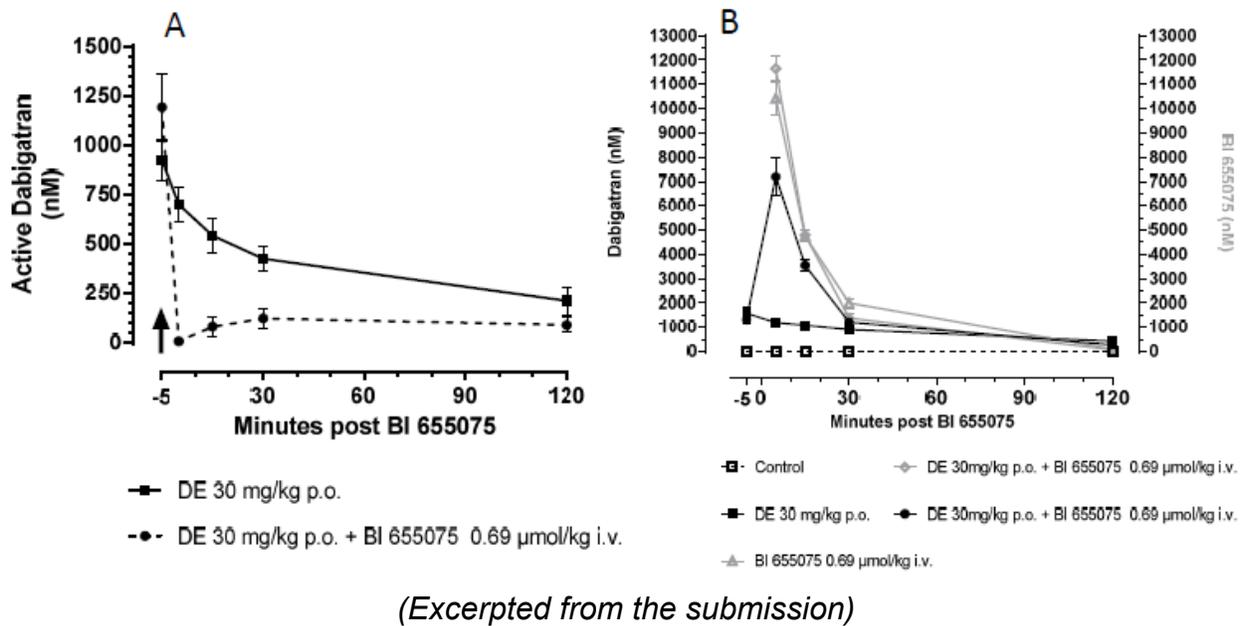


Figure 9. Anticoagulant effects of idarucizumab in rat tail cut model



Reversal of dabigatran induced bleeding after supra-therapeutic dosing (split dose)

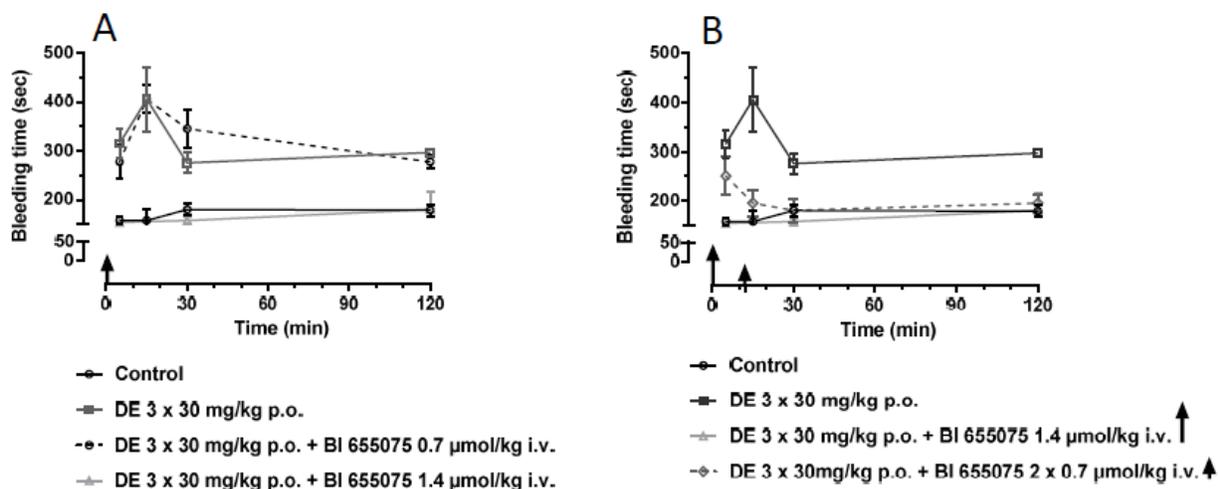
Methods

Binding and clearance of dabigatran by idarucizumab in vivo occurs rapidly so split dosing was tested to determine effectiveness over one large single dose. DE (30 mg/kg) was administered orally 3 times every 8 hours. Idarucizumab was given in increasing split doses, 0.7 and 1.4 $\mu\text{mol/kg}$ or as two 0.7 $\mu\text{mol/kg}$ bolus doses 12 min apart. One hour following the last dose of DE, reversal of dabigatran-prolonged bleeding time and anticoagulation activity by idarucizumab was tested in an in vivo rat tail bleeding model.

Results

Low dose idarucizumab (33 mg/kg) did not have a significant effect on reversal of dabigatran induced bleeding time, while the high dose idarucizumab (66 mg/kg) completely reversed dabigatran induced bleeding time to baseline. When comparing the large bolus dose vs. split dosing, reversal of bleeding time was effective with both methods of dosing and significantly reduced anticoagulation as measured by rat tail bleeding time.

Figure 10. Rat tail bleeding time in rat tail cut model



(Excerpted from the submission)

Specificity of idarucizumab- Effect of idarucizumab on bleeding induced by dabigatran in combination with antiplatelet agents

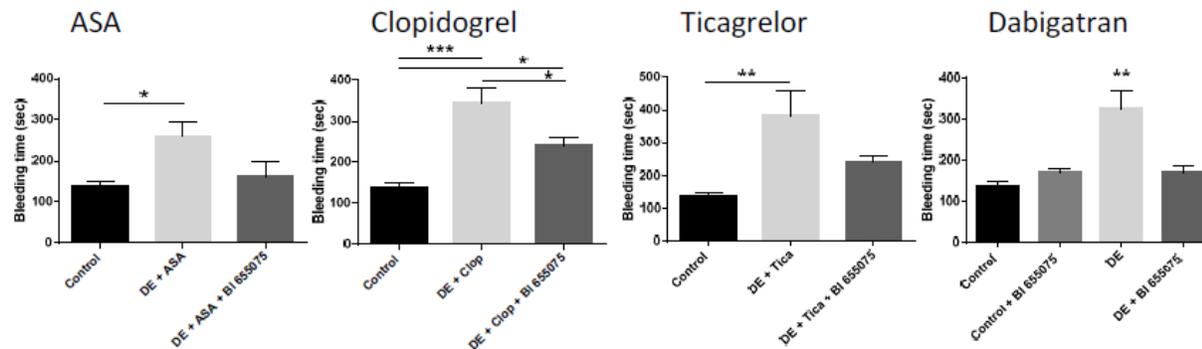
Methods

Antiplatelet agents, acetylsalicylic acid (100 mg/kg, Aspirin®, ASA), clopidogrel (4 mg/kg, Plavix®) or ticagrelor (3 mg/kg, Brilique®) were administered orally to rats and measured ex vivo using aggregometry as a confirmatory assay. Dabigatran etexilate (DE) was also administered orally at a dose of 30 mg/kg following administration of the antiplatelet agents. Idarucizumab (0.7 $\mu\text{mol/kg}$) or vehicle was administered intravenously 45 minutes following DE. Bleeding time was assessed by standardized tail cuts and measuring the time required for bleeding to stop (hemostasis).

Results

The combination of antiplatelet agents together with dabigatran reduced the inhibition of dabigatran induced prolonged bleeding time by idarucizumab compared to dabigatran alone. Antiplatelet agents in the presence of dabigatran may not result in the complete reversal of bleeding/anticoagulation.

Figure 11. Bleeding time following administration of antiplatelet agents with idarucizumab and dabigatran in rat tail cut model



(Excerpted from the submission)

Pig liver trauma models (single and double trauma models below)

Reversal of supratherapeutic levels of dabigatran induced bleeding after hemorrhagic shock, single dose idarucizumab

Methods

Experiments were designed to test doses of idarucizumab in reversing bleeding in a more severe model of trauma of trauma in pigs in exposure to supratherapeutic doses of dabigatran. Briefly, dabigatran etexilate (DE) was administered orally to 18 male Landrace swine (n=18) for three days twice daily at 30 mg/kg. Animals were anesthetized on Day 4 and DE was infused over 90 minutes to achieve supratherapeutic plasma levels. Standardized blunt liver injury was made and the injury bled for 12 minutes to induce hemorrhagic shock. The only intervention made was resuscitation after five minutes with Ringer's solution (to maintain blood pressure). Blood loss was recorded and animals were randomized to different doses of idarucizumab (30, 60, or 120 mg/kg) or vehicle control groups. Serial blood sampling and blood loss was measured over time (4 hours).

Results

Diluted thrombin time was used to measure the anticoagulant activity of dabigatran. Supratherapeutic levels of dabigatran (resulting in systemic levels reaching 2310 nM to 2740 nM, or 1090 ng/mL to 1290 ng/mL) were administered prior to injury and idarucizumab dosing. Blood loss doubled in pigs treated with dabigatran prior to blunt liver trauma; pigs that received vehicle and not idarucizumab, and due to ongoing bleeding there was 100% mortality in this group. Continuous bleeding was associated

with severe shock as characterized by increased lactate levels, decreases in blood bicarbonate levels, decreased mean arterial pressure, and a decrease in hemoglobin, and thrombocytopenia. There was a 47%, 64%, and 62% reduction in blood loss with 30, 60 and 120 mg/kg idarucizumab, respectively, vs the dabigatran-treated group. Survival significantly improved in all dose groups of idarucizumab and dabigatran compared to dabigatran alone, with all of the animals surviving the 4 hours in the 60 and 120 mg/kg dose groups and 5/6 animals surviving in the 30 mg/kg dose group. The 30 and 60 mg/kg dose of idarucizumab were not sufficient to eliminate anticoagulant activity of the supratherapeutic dosing of active dabigatran but the 120 mg/kg dosing almost completely inhibited anticoagulant activity over the 4 hour period tested as measured by dTT. The 120 mg/kg dose of idarucizumab was approximately equimolar to the total dabigatran infusion (0.905 mg/kg dabigatran was infused over 90 minutes).

Figure 12. Blood loss and survival in pig liver trauma model following supratherapeutic doses of dabigatran and single dose idarucizumab

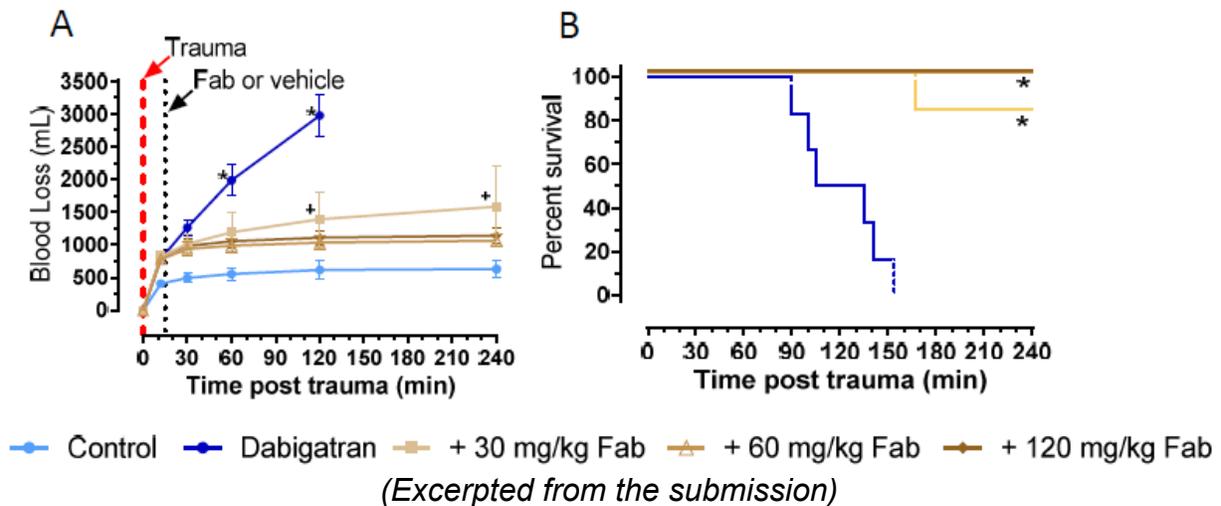


Figure 13. Shock parameters in pig liver trauma model following supratherapeutic doses of dabigatran and single dose idarucizumab

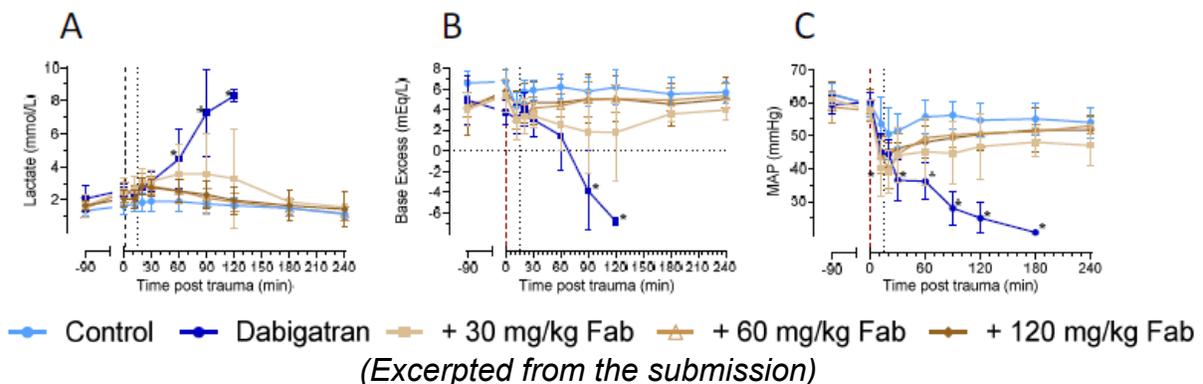
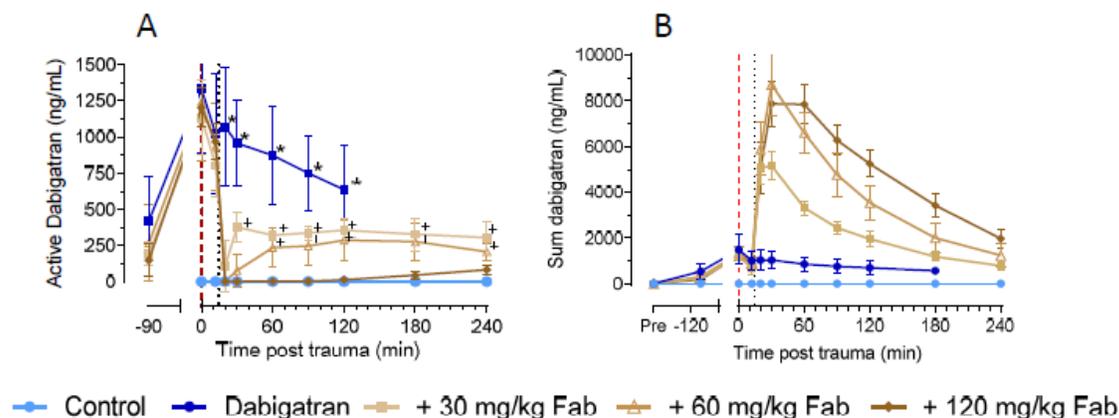


Figure 14. Dabigatran levels in plasma in pig liver trauma model following supratherapeutic doses of dabigatran and single dose idarucizumab



(Excerpted from the submission)

Reversal of dabigatran induced bleeding after hemorrhagic shock, split dose, pig liver double trauma model

Methods

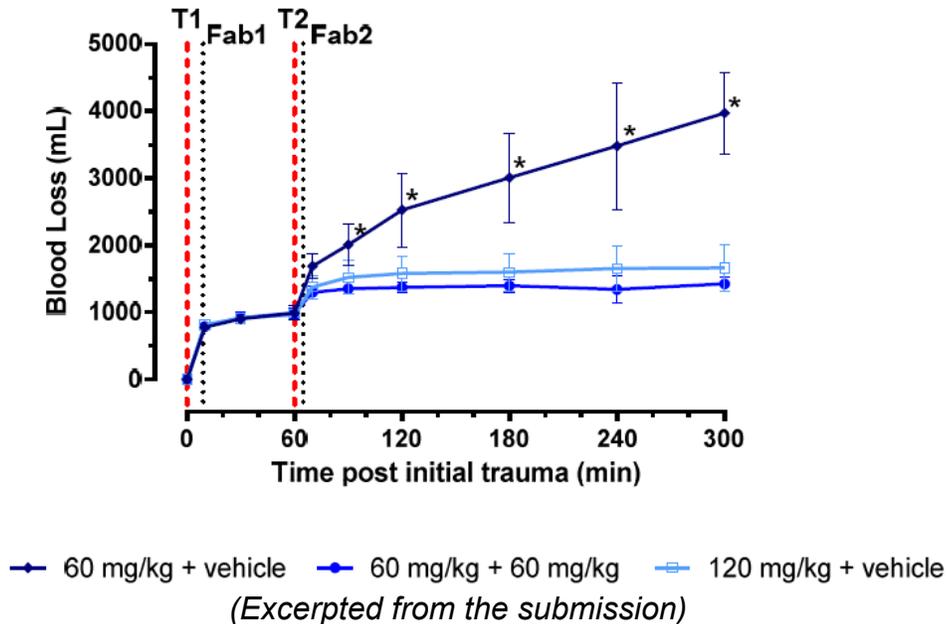
Experiments were performed to determine activity of split dosing over single bolus administration of idarucizumab following dabigatran dosing. Dabigatran etexilate (DE) was administered orally to 18 (6 per dose group) male Landrace swine for three days twice daily at 30 mg/kg. Animals were anesthetized on Day 4 and DE was infused over 90 minutes to achieve ideal plasma levels (1130 +/- 160 ng/mL). Standardized blunt liver injury was made and the injury bled for 12 minutes to induce hemorrhagic shock. The only intervention made was resuscitation after five minutes with Ringer's solution (to maintain blood pressure). Blood loss was recorded and animals were randomized to different doses of idarucizumab (60 mg/kg, 120 mg/kg) or vehicle control groups 15 minutes post injury. A second trauma was made 60 minutes post injury, on a second liver lobe. Fifteen minutes following the second trauma, idarucizumab was administered at 60 mg/kg or vehicle. Serial blood sampling and blood loss was measured over time (4 hours).

Results

Both the 60 mg/kg and 120 mg/kg doses of idarucizumab reduced dabigatran induced blood loss following the first blunt liver trauma (first 60 minutes). Following the second injury, animals started to bleed again, with 83% mortality but with no further bleeding from the first wound site if a clot had formed in the previous time span. Upon administration of the second bolus dose of idarucizumab, 60 mg/kg, there was a rapid reversal of bleeding from the second injury. This response was also observed in the single high dose 120 mg/kg group (n=6/group). In the figure below, the dashed line at t=0, T1 represents the first trauma and T2 the second trauma. The dotted line, Fab1, represents the first dose of idarucizumab, and the second dose Fab 2, or vehicle. Both

the single 120 mg/kg dose (~5 g total dose) or 2x60 mg/kg (2 x 2.5 g total dose) were effective in reversing bleeding, even after two injuries sustained one hour apart.

Figure 15. Blood loss in pig liver double trauma model, split dosing



Specificity of idarucizumab - Hemodilution using colloid and crystalloid volume replacement- Neutralization of dabigatran induced bleeding after hemodilution

Methods

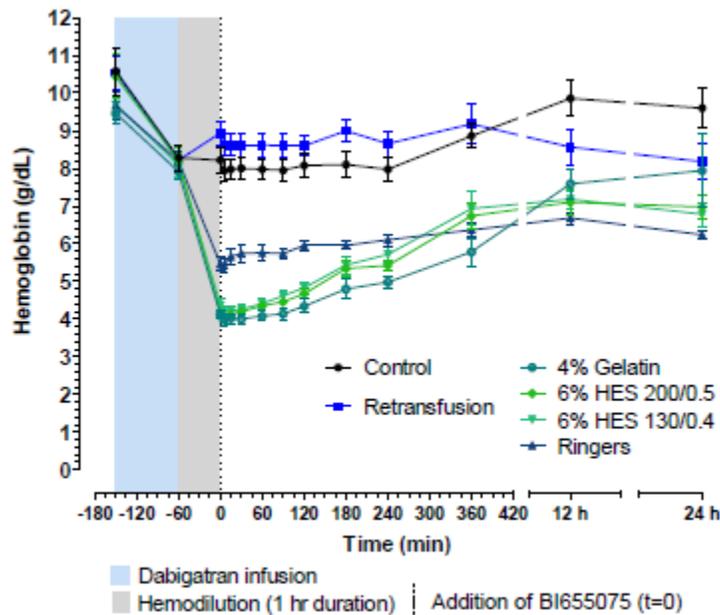
In trauma, volume expanders are used for resuscitation to compensate for blood loss and hemorrhagic shock. Experiments were performed to determine if volume expanders can influence binding of dabigatran to idarucizumab in an in vivo pig liver trauma model (under severe blood loss or hemorrhagic shock). Dabigatran etexilate (DE) was administered to male Landrace pigs (30 mg/kg) twice daily for three days. Animals were anesthetized on Day 4 and DE was infused to achieve an ideal mean plasma level (1130 +/- 160 ng/mL). Following DE infusion, ~50% of total blood volume was withdrawn to mimic hemorrhagic shock. Animals were randomized by the follow groups (n=5): balanced Ringer's solution, 6% hydroxyethyl starch (HES) 130/0.4, 6% HES 200/0.5, 4% gelatin, retransfusion of washed red blood cells (RBCs) or control. Resuscitation was comprised of: 1:1 to blood loss for crystalloids, 25 mL/kg for colloids, and 12 mL/kg for retransfusion. Idarucizumab (30 mg/kg i.v.) was administered immediately following hemodilution (dotted line, t=0) and dabigatran levels (binding to idarucizumab) were followed over time by taking serial blood samples over 24 hours (as measured by dTT assay).

Results

Control and retransfusion samples are the only samples that show normal levels of hemoglobin after hemodilution. Animal weights, blood cell counts, and coagulation

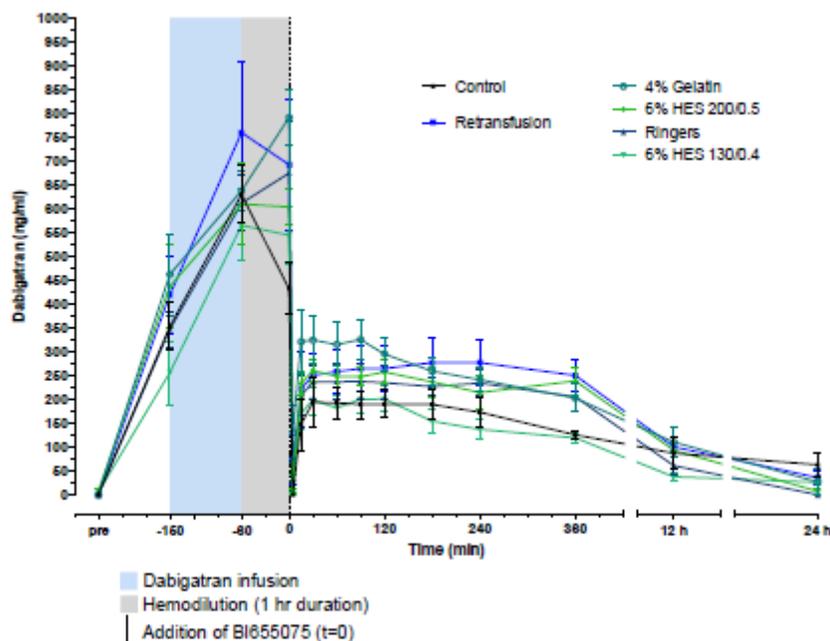
parameters were comparable between all groups, there were no significant differences. Dabigatran values decreased immediately following administration of idarucizumab. In summary, there was no significant difference in the binding of idarucizumab to dabigatran following retransfusion or the volume expanders tested as measured by plasma or active dabigatran. There were only minimal differences in anticoagulation as measured by different assays which can be explained at least in part by the response to hemodilution on coagulation.

Figure 16. Hemoglobin levels over time during hemodilution experiments in pig liver double trauma model



(Excerpted from the submission)

Figure 17. Plasma dabigatran levels in the presence of volume expanders in pig liver double trauma model.



(Excerpted from the submission)

4.2 Secondary Pharmacology

Studies not conducted.

4.3 Safety Pharmacology

Respiratory (GLP):

The pharmacological effects of idarucizumab on the respiratory system were determined in study 12r006 by examining Wistar rats treated i.v. with a single dose of 150 mg/kg or 500 mg/kg, drug or vehicle (10 mL/kg). The vehicle was 25 mM acetate, 240 mM sorbitol, 0.02% Polysorbate 20, pH5.5. Measures of respiratory function (tidal volume, respiratory rate and derived minute volume) were captured using plethysmographs over 30 minute periods at 0, 30, 60, 90, 120, 150, 180, 210, and 240 minutes following dosing.

Results:

- No adverse effects in treatment groups or control; all respiratory measures were within limits for healthy animals at doses up to 500 mg/kg, i.v.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Absorption

Pharmacokinetics and urinary excretion of BI 655075 and the effect of BI 655075 on the pharmacokinetics and urinary excretion of dabigatran in the Wistar Han rat

Key study findings:

- Idarucizumab was rapidly eliminated in the blood following intravenous dosing (initial phase half-life = 0.24hr; terminal phase half-life 5.81 hrs); half-life with dabigatran was 0.2 hrs.
- Dabigatran clearance (CL) was lower in rats treated with idarucizumab (4.36 mL/min/kg) than without (9.16 mL/min/kg).
- Idarucizumab excretion is 21% in the urine 24 hour after dosing and pretreatment with dabigatran.

Study no.:	U12-3083-01
Study report location:	eCTD 4.2.2.2
Conducting laboratory and location:	Boehringer Ingelheim Ridgefield, CT, USA
Date of study initiation:	13 February 2012
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	Idarucizumab, lot # 1257346, purity >95%

Methods: Blood collection by femoral vein; urine into polypropylene containers

Species/strain: HanWistar rats

N: 3 males/group

Dose: 20 mg/kg idarucizumab; 0.2 mg/kg dose dabigatran

Frequency: single dose

Route: intravenous bolus, femoral vein cannula

Volume: (1.39 (idarucizumab or 0.5mL/kg (dabigatran)

Observations and times: 400 µl of blood was collected at various time points. Blood was collected at approximately 0.0333, 0.25, 1, 2, 3, 4, 6, 8, and 24 hrs. after dosing from group 1 (20 mg/kg idarucizumab) rats (See study design table following page). Urine samples were collected in a polypropylene container at intervals of 0-8, 8-24, and 24-48 hours after dosing and volume was determined by weight. Idarucizumab was detected in urine using a qualified ELISA method specifically for Wistar rat urine, similar to the assay for plasma. The ELISA assay was qualified to detect idarucizumab over the range 0.0156 to 0.250 µg/mL with 0.0156 µg/mL as the lower limit of quantitation (LLOQ). Rats dosed with dabigatran only, 0.2 mg/kg, blood was collected at 0.0333, 0.2, 0.283, 1, 2, 3, 4, 6, and 8 hrs. after dosing from (Group 5 rats). For group 6 rats (dabigatran and idarucizumab) 600 µl of blood was collected at 0.0333, 0.2, 1, 3, 6, and 24 hrs. after

dosing from the first 3 rats and at 0.283, 0.5, 2, 4, and 8 hrs. after dosing from the last 3 rats.

Table 3. Study design for pharmacokinetic and excretion study in Wistar rats

Group	End point	Number	Rat ID	Test article	Dose (IV)	Samples	Assay
1	PK	3	1-3	BI 655075	20 mg/kg	Blood	BI 655075
2	Urinary excretion	3	4-6	BI 655075	20 mg/kg	Urine	BI 655075
3	Urinary excretion	3	7-9	Dabigatran	0.2 mg/kg	Urine	Dabigatran
4	Urinary excretion	3	10-12	Dabigatran + BI 655075	0.2 mg/kg + 20 mg/kg	Urine	BI 655075, Dabigatran
5	PK	3	13-15	Dabigatran	0.2 mg/kg	Blood	Dabigatran
6	PK	6	16-21	Dabigatran + BI 655075	0.2 mg/kg + 20 mg/kg	Blood	BI 655075, Dabigatran

(Excerpted from the submission)

A summary of the pharmacokinetic parameters of idarucizumab and dabigatran are shown below. Three groups of male Wistar Han rats (n=3 per group) received an intravenous bolus dose of 0.2 mg/kg of dabigatran, 20 mg/kg of idarucizumab (BI655075), or 0.2 mg/kg dabigatran followed with 20 mg/kg of idarucizumab (BI 655075). In columns three and five below, idarucizumab and dabigatran parameters are shown respectively for the drugs as they are given in combination.

Table 4. Summary pharmacokinetic parameters of idarucizumab and dabigatran in rats

Summary of PK parameters (n=3) of BI 655075 and dabigatran administered alone or in combination				
PK Parameter	BI 655075 ^a	BI 655075 ^b (with dabigatran)	Dabigatran ^a	Dabigatran ^b (with BI 655075)
Dose (mg/kg)	20	20	0.2	0.2
AUC _{0-∞} (nM)	3,730±875	2,990	787±128	1,620
CL (mL/min/kg)	1.95±0.494	2.33	9.16±1.59	4.36
CL _r (mL/min/kg)	0.263±0.068	0.484	5.83±3.42	2.59
V _{ss} (L/kg)	0.0688±0.0291	0.0771	0.561±0.0780	0.180
Terminal t _{1/2} (h)	6.68±0.493	6.34	1.40±0.447	1.36
MRT (h)	0.570±0.0943	0.550	1.03±0.191	0.688
t _{1/2,α} (h) ^c	0.241±0.0202	0.198	Not determined	Not determined
t _{1/2,β} (h) ^c	5.81±0.481	5.15	Not determined	Not determined

^a Mean±SD.^b Parameters were calculated based on composite mean concentration data.^c Compartmental analysis was also conducted to determine the half-lives of BI 655075 in initial and later phases (b) (4)*(Excerpted from the submission)***Table 5. Mean percentage of idarucizumab and dabigatran excretion in urine following intravenous dosing in rats**

Summary of urinary excretion (% of dose) of BI 655075 and dabigatran administered alone or in combination				
Interval (h)	BI 655075 urinary excretion		Dabigatran urinary excretion	
	Alone	With dabigatran	Alone	With BI 655075
0-8	13.2±6.08	16.0±5.04	50.9±29.2	42.5±11.2
8-24	1.12±0.76	4.18±3.17	5.99±1.39	15.1±8.87
24-48	0.25±0.38	0.57±0.85	0.39±0.37	1.70±1.22
0-48 (total)	14.5±6.91	20.8±6.68	57.2±30.6	59.3±17.1

*(Excerpted from the submission)***BI 655075: Pharmacokinetic and Pharmacodynamic Study in Rhesus Monkeys Pretreated with Dabigatran Etxilate**

Key study findings:

- Idarucizumab was rapidly eliminated in the blood following intravenous dosing (terminal phase half-life = 5.64h).
- The mean residence time (MRT) was short (1.38 hrs.).
- Idarucizumab clearance (CL) averaged 0.902 mL/min/kg.
- In excretion studies, 10% of the idarucizumab was recovered in the urine of the monkey; and 2% or less of dabigatran with or without idarucizumab was recovered in the urine of the monkey.
- Idarucizumab exposure was not affected by the presence of anti-drug antibodies.

Study no.: U13-3539-01
 Study report location: eCTD 4.2.2.2
 Conducting laboratory and location: Boehringer Ingelheim Ridgefield, CT, USA
 Date of study initiation: 27 June 2013
 GLP compliance: No
 QA statement: No
 Drug, lot #, and % purity: Idarucizumab, lot # E2739F01, purity >95%

Methods: Blood collection by femoral vein (see Table 6);
 Urine was collected using metabolism cages, immediately after the dabigatran etexilate dose and until 48 hrs (24 hrs for Day 3) after dabigatran etexilate dose. Urine was collected on ice on Days 3, 4, 11, and 33 at the following intervals: 0-8, 8- 24, 24-32, and 32-48 hrs.

Species/strain: male naïve rhesus monkeys
 N: 4 males/group
 Dose: 30 or 60 mg/kg idarucizumab; 12 mg/kg dose dabigatran
 Frequency: single dose dabigatran; single and double dosing idarucizumab
 Route: intravenous infusion idarucizumab; oral dabigatran
 Volume: 10 mL/kg for both (I.V. and oral gavage)

Observations and times: Plasma and urine samples were analyzed for idarucizumab using ELISA. For plasma, the detection range for the ELISA was 0.0500 to 0.500 µg/mL with 0.0500 µg/mL as the LLOQ. For urine, the detection range for the ELISA was 0.0250 to 0.250 µg/mL with 0.0250 µg/mL as the LLOQ.

Table 6. Blood sampling and collection times for pharmacokinetic analysis in rhesus monkeys

Occasion	Animals	Sample times
Pre-treatment	All	During pre-test (before dabigatran etexilate was dosed)
Day 3	All	1.5, 3, 6 and 9 hours post-dose
Day 4	All	Pre-dose, 1.5, 2.5 (immediately after IV infusion), 3, 6, 9 and 24 hours post-dose
Day 11	All	Pre-dose, 1.5 (prior to 1 st IV infusion), 1.67 (Immediately after 1 st IV infusion), 3 (prior to 2 nd IV infusion), 3.17 (Immediately after 2 nd IV infusion), 6, 9 and 24 hours post-dose
Day 33	All	Pre-dose, 1.5 (prior to IV infusion), 1.67 (immediately after IV infusion), 3, 6, 9 and 24 hours post-dose

(Excerpted from the submission)

Table 7. Study design for pharmacokinetic and excretion study in rhesus monkeys

Day	Group	Number/ Sex	Dose route	Test Substance	Dose level (mg/kg)	Monkey ID no.
1 – 4	1	4M	Oral Gavage	Dabigatran etexilate	12	205, 207, 209, 211
1 – 4	2	4M	Oral Gavage	Dabigatran etexilate	12	213, 215, 217, 219
4	1	4M	Intravenous Infusion ^a	BI 655075	30	205, 207, 209, 211
4	2	4M	Intravenous Infusion ^a	BI 655075	60	213, 215, 217, 219
8 – 11	1	4M	Oral Gavage	Dabigatran etexilate	12	205, 207, 209, 211
8 – 11	2	4M	Oral Gavage	Dabigatran etexilate	12	213, 215, 217, 219
11	1	4M	Intravenous Infusion ^b	BI 655075	30 + 30	205, 207, 209, 211
11	2	4M	Intravenous Infusion ^b	BI 655075	60 + 60	213, 215, 217, 219
30 – 33	1	4M	Oral Gavage	Dabigatran etexilate	12	205, 207, 209, 211
30 – 33	2	4M	Oral Gavage	Dabigatran etexilate	12	213, 215, 217, 219
33	1	4M	Intravenous Infusion ^c	BI 655075	30	205, 207, 209, 211
33	2	4M	Intravenous Infusion ^c	BI 655075	60	213, 215, 217, 219

^a A 10-min IV infusion was scheduled to be administered at 1.5 hours, but was actually given at 2.33 h after receiving the oral administration of dabigatran etexilate due to an error of timing in dose preparation. Therefore, a third phase of study on Days 30-33 was added to conduct the study with the originally designed timing.

^b Two 10-min IV infusions were administered at 1.5 and 3 h after receiving the oral administration of dabigatran etexilate.

^c A 10-min IV infusion was administered at 1.5 h after receiving the oral administration of dabigatran etexilate.

(Excerpted from the submission)

Table 8. Summary pharmacokinetics of idarucizumab (I.V.) pretreated with dabigatran (oral gavage) in rhesus monkeys

Summary PK parameters of BI 655075 after IV dosing in monkeys (n=4/group) pretreated with PO dabigatran etexilate MS (Study no. DDB0210)								
PK Parameter	Group	Dose (mg/kg)	Descriptive statistics	Day 4 single dose at 2.33 h	Day 11 two doses at 1.5 and 3 h	Day 33 ^b single dose at 1.5 h	Overall mean, SD, %CV	
C_{max} (nM)	1	30	Mean	10,100	11,300	10,100	Not applicable	
			SD	746	520	395		
			%CV	7.38	4.58	3.93		
	2	60	Mean	15,000	21,200	19,700		
SD			2,560	5,390	705			
%CV			17.0	25.5	3.59			
$AUC_{0-\infty}$ (nM•h)	1	30	Mean	11,400	27,100	11,600		
			SD	820	3,610	305		
			%CV	7.17	13.3	2.63		
	2	60	Mean	26,100	54,200	23,700		
SD			5,320	13,600	3,360			
%CV			20.4	25.1	14.2			
t_{max} (h) ^a	1	30	Median	2.5	3.17	1.67		
			Range	2.5-2.5	1.67-3.17	1.67-1.67		
	2	60	Median	2.5	3.17	1.67		
			Range	2.5-2.5	1.67-3.17	1.67-1.67		
CL (mL/min/kg)	1	30	Mean	0.919	0.784	0.902	0.902 0.177 19.6	
			SD	0.0670	0.118	0.0241		
			%CV	7.29	15.1	2.67		
	2	60	Mean	1.09	0.822	0.895		
SD			0.231	0.266	0.112			
%CV			21.1	32.4	12.5			
V_{ss} (L/kg)	1	30	Mean	0.0739	0.0592	0.0719		0.0763 0.0265 34.8
			SD	0.0167	0.0181	0.0103		
			%CV	22.6	30.5	14.3		
	2	60	Mean	0.107	0.0712	0.0740		
SD			0.0323	0.0411	0.00866			
%CV			30.2	57.7	11.7			
$t_{1/2}$ (h)	1	30	Mean	5.59	5.62	6.15	5.64 0.585 10.4	
			SD	0.717	0.457	0.890		
			%CV	12.8	8.13	14.5		
	2	60	Mean	5.29	5.32	6.01		
SD			0.348	0.552	0.252			
%CV			6.58	10.4	4.19			
MRT (h)	1	30	Mean	1.34	1.24	1.33		1.38 0.227 16.4
			SD	0.286	0.186	0.187		
			%CV	21.4	15.0	14.1		
	2	60	Mean	1.61	1.37	1.38		
SD			0.195	0.298	0.0899			
%CV			12.1	21.7	6.51			

^a The time was based on the PO dosing of dabigatran etexilate. BI 655075 was administered via a 10-min IV infusion.

^b n=3 for Group 1 on Day 33, due to early termination of one monkey.

(Excerpted from the submission)

Table 9. Mean percentage of idarucizumab excretion in urine in dabigatran pretreated rhesus monkey

Summary urinary excretion of BI 655075 (% of dose) after IV dosing in monkeys (n=4/group) pretreated with PO dabigatran etexilate MS (study no. DDB0210)			
Group	Dose	Mean ± SD (%CV)	Overall Mean ± SD (%CV)
1	30 mg/kg	10.3 ± 6.35 (61.4%)	9.98 ± 6.32 (63.3%)
2	60 mg/kg	9.62 ± 4.53 (47.0%)	

(Excerpted from the submission)

Table 10. Summary pharmacokinetics of dabigatran (oral gavage) with and without idarucizumab (I.V.)

Summary PK parameters of dabigatran in monkeys (n=4/group) receiving oral dabigatran etexilate with and without IV BI 655075 treatment (study no. DDB0210)							
PK Parameter	Group	BI 655075 Dose (mg/kg)	Descriptive statistics	Day 3	Day 4	Day 11	Day 33 ^a
				No BI 655075	one BI 655075 dose at 2.33h	two BI 655075 doses at 1.5 and 3 h	one BI 655075 dose at 1.5 h
C_{max} (nM)	1	30	Mean	120	476	701	513
			SD	122	275	637	478
			%CV	102	57.8	90.9	93.2
	2	60	Mean	245	1,320	1,150	2,500
			SD	294	709	1,030	2,490
			%CV	120	53.6	89.6	99.8
AUC_{0-24} (nM·h)	1	30	Mean	535	1,740	2,100	1,710
			SD	478	777	1,280	1,410
			%CV	89.3	44.7	61.1	82.5
	2	60	Mean	1,030	4,650	4,430	6,200
			SD	1,060	2,260	4,190	6,410
			%CV	102	48.6	94.4	103
t_{max} (h)	1	30	Median	3.75	2.5	2.34	1.67
			Range	1.5-6	2.5-3	1.67-6	1.67-6
	2	60	Median	2.25	3	1.67	1.67
			Range	1.5-6	2.5-3	1.67-3	1.67-1.67

(Excerpted from the submission)

Table 11. Summary of pharmacokinetics of dabigatran (with glucuronides) with and without idarucizumab in monkeys

Summary PK parameters of sum dabigatran (dabigatran + glucuronides) in monkeys (n=4/group) receiving oral dabigatran etexilate with and without IV BI 655075 treatment (Study no. DDB0210)							
PK Parameter	Group	BI 655075 Dose (mg/kg)	Descriptive statistics	Day 3	Day 4	Day 11	Day 33 ^a
				No BI 655075	one BI 655075 dose at 2.33h	two BI 655075 doses at 1.5 and 3 h	one BI 655075 dose at 1.5 h
C_{max} (nM)	1	30	Mean	672	2,120	2,570	1,690
			SD	693	1,360	2,470	1,640
			%CV	103	64.1	96.2	96.9
	2	60	Mean	934	4,730	4,010	6,700
			SD	963	2,540	2,920	5,390
			%CV	103	53.8	72.9	80.5
AUC_{0-24} (nM·h)	1	30	Mean	4,220	6,860	5,810	4,880
			SD	3,540	3,790	4,150	4,250
			%CV	83.9	55.2	71.4	87.1
	2	60	Mean	6,510	15,900	11,400	17,000
			SD	6,010	8,000	9,520	15,600
			%CV	92.4	50.3	83.2	91.8
t_{max} (h)	1	30	Median	3.75	2.5	1.67	1.67
			Range	1.5-6	2.5-2.5	1.67-3	1.67-1.67
	2	60	Median	2.25	2.5	1.67	1.67
			Range	1.5-6	2.5-3	1.67-1.67	1.67-1.67

^a n=3 for Group 1 on Day 33, due to early termination of one monkey.

(Excerpted from the submission)

Table 12. Urinary excretion of dabigatran with and without idarucizumab

Summary urinary excretion of sum dabigatran (% of dose) in monkeys (n=4/group) with and without BI 655075 treatment (Study no. DDB0210)					
Group, Dabigatran dose	Monkey ID	Day 3	Day 4	Day 11	Day 33
		No BI 655075	one BI 655075 dose at 2.33h	two BI 655075 doses at 1.5 and 3 h	one BI 655075 dose at 1.5 h
1, 12 mg/kg ^a	Mean	1.38	1.40	1.03	1.07
	SD	1.29	1.06	0.91	0.94
	%CV	93.2	75.9	88.0	87.8
2, 12 mg/kg ^a	Mean	1.47	1.83	1.83	2.41
	SD	1.18	0.72	1.34	2.46
	%CV	80.4	39.3	73.1	102
Overall Mean		1.43	1.59		
Overall SD		1.15	1.31		
Overall %CV		80.3	81.9		

^a The dose was based on the dabigatran etexilate free base. It was equivalent to 9.0 mg/kg of active component dabigatran, which was used for calculation. The BI 655075 dose was 30 mg/kg for Group 1 and 60 mg/kg for Group 2.

(Excerpted from the submission)

Table 13. Immunogenicity to idarucizumab following I.V. dosing in rhesus monkeys

Summary immunogenicity (ADA) to BI 655075 after IV dosing in monkeys (Study no. DDB0210)			
Group (BI 655075 dose)	Money ID	Day 1 (pretreatment)	Day 30 (predose)
Group 1 (30 mg/kg)	205	Negative	Putative Positive
	207	Negative	Putative Positive
	209	Negative	Putative Positive
	211	No Sample ^a	Negative
Group 2 (60 mg/kg)	213	Negative	Putative Positive
	215	Negative	Putative Positive
	217	Negative	Negative
	219	No sample ^a	Putative Positive

^a No sample due to the limitation of blood volume for sampling.

In the absence of idarucizumab, the assay sensitivity was 0.29 ng/mL in monkey plasma. The drug tolerance of the assay in monkey plasma was such that 100 ng/mL ADA positive control could be detected in the presence of up to 125 µg/mL idarucizumab. False positive anti-idarucizumab antibody signals were detected using a sensitive ECL method (method # BBM-12-1002) in plasma samples that were taken from monkeys treated only with dabigatran etexilate.

(Excerpted from the submission)

5.2 Toxicokinetics

Toxicokinetic parameters were reviewed in the context of the general toxicology studies.

6 General Toxicology

6.1 Single-Dose Toxicity

Studies not reviewed.

6.2 Repeat-Dose Toxicity

Study title: BI 655075: Toxicity Study by Intravenous Administration to Han Wistar Rats for 4 Weeks Followed by a 4 Week Recovery Period

Study no.:	DDB0150/BI no. 11R141
Study report location:	eCTD 4.2.3.2
Conducting laboratory and location:	 (b) (4)
Date of study initiation:	January 31, 2012
GLP compliance:	Signed and included
QA statement:	Signed and included
Drug, lot #, and % purity:	BI-655075-01, DAB-FTOX-01, assumed 100% purity

Key Study Findings

- There were no mortalities during the study.
- Idarucizumab related changes in hematology findings included increased white blood cells and lymphocytes at doses of 150 and 500 mg/kg at terminal sacrifice, which were present in high dose animals at the end of the recovery period.
- Toxicologically significant idarucizumab related changes in clinical chemistry findings included decreased blood urea and creatinine levels both of which were present in high dose male animals only at the recovery period (Day 58, terminal sacrifice). Values for female animals returned to levels similar to controls.
- Female rats dosed at both 150 and 500 mg/kg had elevated absolute thymus weights, thymus weight relative to body weight and thymus weight relative to brain weight.
- Histopathology findings included atrophy in the pancreas at high doses (500 mg/kg).

Methods

Doses: 0, 150, 500 mg/kg/day
 Frequency of dosing: Once daily
 Route of administration: Intravenous injection (bolus- right caudal vein)
 Dose volume: 10mL/kg
 Formulation/Vehicle: 25 mM acetate, 240 mM sorbitol, and 0.02% polysorbate 20,pH 5.5
 Species/Strain: RccHan™:WIST strain Rat
 Number/Sex/Group: 15 sex/group; 10/sex/group recovery
 Age: 36-42days
 Weight: 103-135g
 Satellite groups: 3/sex/control; 9/sex/treatment
 Unique study design: N/A
 Deviation from study protocol: N/A

Observations and Results**Table 14. Study design for 4-week repeat dose toxicology study in rats**

The study consisted of one Control and two treated groups of rats, identified as follows:

Group	Treatment	Dose# (mg/kg/day)	No. of animals		Main study (4 weeks) Animal numbers		Cage numbers	
			Male	Female	Male	Female	Male	Female
1	Control	0	15	15	1-15	87-101	1-3	21-23
2	BI 655075	150	15	15	29-43	115-129	7-9	27-29
3	BI 655075	500	15	15	53-67	139-153	13-15	33-35

Expressed in terms of test material as supplied.

(Excerpted from the submission)

Mortality

There were no mortalities during the study.

Clinical Signs

Unremarkable

Body Weights

Unremarkable

Feed Consumption

Unremarkable

Ophthalmoscopy

Unremarkable

ECG

Unremarkable

Hematology

Table 15. Erythroid parameters in 4 week rat toxicology study

Hematology parameter	% change from control at sacrifice			
	males		females	
dose (mg/kg/day)	150	500	150	500
RBC (x10¹²/L)				
Day 14	-2.0	-4.7	-1.8	0.4
Day 29	3.1	4.4	-1.6	0.3
Day 58 (Recovery)		7.8		2.2
HCT (L/L)				
Day 14	-1.7	-4.8	-1.6	1.6
Day 29	3.8	4.7	0.2	2.5
Day 58 (Recovery)		6.1		3.8
MCH (pg)				
Day 14	2.1	4.8	-1.1	-1.1
Day 29	0.5	-1.1	2.1	1.1
Day 58 (Recovery)		-3.8		-2.5
MCHC (g/dL)				
Day 14	1.8	4.5	-1.2	-1.2
Day 29	0.0	-0.9	0.0	-1.2
Day 58 (Recovery)		-2.6		-3.2
Hemoglobin (g/dL)				
Day 14	0.0	0.0	-2.7	0.7
Day 29	4.1	4.1	0.7	1.4
Day 58 (Recovery)		3.3		0.6
Reticulocytes (%)				
Day 14	-2.4	2.8	17.7	6.8
Day 29	-1.9	6.8	28.3	23.3
Day 58 (Recovery)		-14.0		7.0

P<0.05

P<0.01

Day 14 and Day 29 were during dosing phase (main study). Day 29 was last day of dosing. Day 58 was last day of recovery.

Table 16. White blood parameters and coagulation findings in 4 week rat toxicology study

Hematology parameter	% change from control at sacrifice			
	males		females	
sex	150	500	150	500
dose (mg/kg/day)				
WBC (x10⁹/L)				
Day 14	-5.4	-6.0	7.4	20.1
Day 29	23.3	20.3	37.7	38.4
Day 58 (Recovery)		31.7		23.0
Lymphocytes (x10⁹/L)				
Day 14	-8.2	-7.7	7.4	22.3
Day 29	26.0	25.8	43.4	47.8
Day 58 (Recovery)		42.4		24.1
Platelets (x10⁹/L)				
Day 14	-1.4	4.7	4.0	8.8
Day 29	1.2	11.8	9.5	7.9
Day 58 (Recovery)		-7.3		-1.2
PT (sec)				
Day 29	-1.0	5.0	1.4	2.3
Day 58 (Recovery)		-1.5		-1.4

P<0.05

P<0.01

Day 14 and Day 29 were during dosing phase (main study). Day 29 was last day of dosing. Day 58 was last day of recovery.

Clinical Chemistry

Table 17. Clinical chemistry findings in 4 week rat toxicology study

% change from control at sacrifice				
sex	males		females	
	dose (mg/kg/day)	150	500	150
Clinical chemistry parameter				
Urea (mmol/L)				
Day 14	2.5	-1.8	-2.7	-6.9
Day 29	-10.4	-21.6	-8.6	-27.9
Day 58		-23.1		-3.9
Creatinine (µmol/L)				
Day 14	3.3	3.3	-7.7	-7.7
Day 29	-8.8	-17.6	-16.7	-26.2
Day 58		-14.3		0.0
Cholesterol (mmol/L)				
Day 14	-4.2	2.5	6.5	7.0
Day 29	11.5	15.8	11.3	11.9
Day 58		0.5		4.8
Total Protein (g/L)				
Day 14	-1.6	0.0	1.6	6.3
Day 29	4.9	4.9	0.0	4.5
Day 58		0.0		-1.4
Albumin (g/L)				
Day 14	0.0	3.2	0.0	35.3
Day 29	6.7	6.7	0.0	2.9
Day 58		-3.2		0.0
A/G (ratio)				
Day 14	6.3	7.4	0.0	2.7
Day 29	4.3	5.3	-0.9	-1.8
Day 58		-6.3		0.0
Phosphorus (mmol/L)				
Day 14	-5.6	-1.4	11.9	10.5
Day 29	4.1	5.8	21.3	7.7
Day 58		13.5		-13.7

P<0.05

P<0.01

Day 14 and Day 29 were during dosing phase (main study). Day 29 was last day of dosing. Day 58 was last day of recovery.

Urinalysis

Unremarkable

Gross Pathology

Unremarkable

Organ Weights**Table 18. Organ weight changes in the thymus in the 4 week rat toxicology study**

Organ	% change from control at sacrifice (g/kg body weight)			
	males		females	
sex				
dose (mg/kg/day)	150	500	150	500
Thymus (absolute)				
Day 29	-0.1	-0.8	19.3	9.8
Day 58		-6.3		-4.4
Thymus (% BdWgt)				
Day 29	-2.5	-1.2	16.9	10.7
Day 58		-8.7		-4.2
Thymus (% BrainWgt)				
Day 29	0.0	-2.1	20.1	11.6
Day 58		-5.9		-8.0

P<0.01

Day 29 was the day of terminal necropsy during the main study. Day 58 was terminal necropsy for the recovery period.

%BdWgt is the change in organ weight based on change relative to body weight.

% BrainWgt is the change in organ weight based on change relative to brain weight.

Histopathology

Adequate Battery: Yes

Peer Review: Yes

Histological Findings

Table 19. Histopathology findings in 4 week rat toxicology study- main study

Treatment related microscopic findings		males			females		
idarucizumab group (mg/kg/day)		0	150	500	0	150	500
Number of animals examined		15	15	15	15	15	15
grade							
Thyroids	ectopic thymic tissue	1	1		3	1	3
Pancreas	acinar atrophy, focal	1			1		1
Kidneys	pelvic dilatation	1		2			

Grade key: 1 minimal, 2 mild, 3 moderate, 4 severe

Table 20. Histopathology findings in 4 week rat toxicology study- recovery

Treatment related microscopic findings- recovery		males			females		
idarucizumab group (mg/kg/day)		0	150	500	0	150	500
Number of animals examined		15	15	15	15	15	15
grade							
Thyroids	ectopic thymic tissue	1					1

Grade key: 1 minimal, 2 mild, 3 moderate, 4 severe

Special Evaluation- Irwin Screen

The Irwin screen for neurobehavior and body temperature was unremarkable.

Special Evaluation-Immunogenicity

Anti-drug antibodies (ADA) were positively detected in 26%, 38%, and 56% of rats in the control, 150 mg/kg, and 500 mg/kg dose groups, respectively.

Following recovery (Day 57) anti-drug antibodies were positively detected in 5% and 60% in the control and 500 mg/kg dose groups of rats, respectively. There were no gender differences in detection of anti-drug antibodies in rats. In toxicokinetic rats (TK), 15 out of 35 idarucizumab treated rats were positive for ADA, however this did not result in differences in idarucizumab exposure during the dosing period (between Day 1 and Day 28).

Toxicokinetics

- Increases in idarucizumab exposure were proportional to increases in dose from 150 mg/kg to 500 mg/kg (C_{max} and AUC) on Days 1 and 28 (with the exception of C_{max} in male rats on Day 28).
- Idarucizumab exposure was similar between male and female rats with the exception a lower C_{max} on Day 28 in the 150 mg/kg/day dosing group in females compared to males.
- There were no apparent differences in exposure when comparing Days 1 and 28 in both C_{max} and AUC (>2 fold).

Table 21. Toxicokinetic parameters of idarucizumab in rats following I.V. injection (ug/mL)

TK Parameter ^a	Day	Sex	BI 655075 dose (mg/kg/day)	
			150	500
C _{max} (ug/mL)	1	Male	2,530	7,520
		Female	2,560	8,510
	28	Male	1,590 ^b	9,420
		Female	2,460	7,920
AUC ₀₋₂₄ (ug·h/mL)	1	Male	1,300	3,950
		Female	1,260	4,010
	28	Male	1,180	5,070
		Female	1,330	5,220
t _{max} (h)	1	Male	0.05	0.05
		Female	0.05	0.05
	28	Male	0.05	0.05
		Female	0.05	0.05

^a TK parameters were calculated from composite concentrations data with up to 3 rats/sex/timepoint/group. n<3 at a few time points or occasions due to death or dosing difficulty in TK rats.

^b C_{max} was underestimated for male rats at 150 mg/kg on Day 28 due to delayed sampling (approximately 5-10 min delay).

(Excerpted from the submission)

Prior to dosing, 10 of 169 rats (5.9%) screened ADA positive which is in line with the expected 5% false positive rate for this assay. At the end of the dosing or experimental phase, (Days 28-30), ADA positive rats in the control, 150 mg/kg, and 500 mg/kg dose groups were reported at 26%, 38%, and 56%, respectively. At the end of the recovery phase (Day 58), the reported percentage for ADA positive rats in the control and 500 mg/kg dose groups were 5% and 60%, respectively.

Table 22. Summary of screening assay for anti-drug antibodies in 4 week rat toxicology study (main study, recovery, and TK satellite)

Group	Dose (mg/kg)	Sex	No. of rat screened ADA positive / No. of rats sampled					
			Pre-treatment	Day 15	End of dosing			Recovery
			Day 0		Day 28	Day 29	Day 30	Day 57
1	0	Male	3/28	1/3	-- ^a	5/18	--	0/10
		Female	1/28	0/3	0/3	--	4/14	1/10
		Combined	4/56	1/6	9/35			1/20
2	150	Male	3/24	3/8	--	9/23	--	--
		Female	0/24	1/9	1/9	--	8/15	--
		Combined	3/48	4/17	18/47			--
3	500	Male	2/34	4/9	--	11/24	--	6/10
		Female	1/31	4/9	4/9	--	12/15	6/10
		Combined	3/65	8/18	27/48			12/20

^a No sample on this day.

(Excerpted from the submission)

Table 23. Summary of screening assay for anti-drug antibodies in 4 week rat toxicology study in TK satellite rats

Group	Dose (mg/kg)	Sex	No. of rat screened ADA positive / No. of TK rats sampled			
			Pre-treatment		End of dosing	
			Day 0		Day 28	Day 29
1	0	Male	0/3		-- ^a	0/3
		Female	0/3		0/3	--
		Combined	0/6		0/6	
2	150	Male	1/9		--	5/8
		Female	0/9		1/9	--
		Combined	1/18		6/17	
3	500	Male	0/9		--	5/9
		Female	1/9		4/9	--
		Combined	1/18		9/18	

^a No sample on this day.

(Excerpted from the submission)

Dosing Solution Analysis

Concentration and Stability

Stability was confirmed in standard storage buffer for idarucizumab in 25 mM acetate, 220 mM sorbitol and 0.02% polysorbate 20, pH 5.5 formulations at nominal concentrations of 7.5 mg/mL, 15 mg/mL and 50 mg/mL in (b) (4) storage for (b) (4) and (b) (4) storage for up to (b) (4). The mean concentrations of idarucizumab in solution analyzed during the study were within acceptability criteria of (b) (4) % of nominal concentrations, indicating accurate formulation.

Table 24. Dosing solution analysis for concentration in 4 week rat toxicology study

Occasion	Group	Nominal inclusion (mg/mL)	Analysed concentration (mg/mL)			RME (%)	CV (%)
			Analysis 1	Analysis 2	Mean		
Day 1	1	0	(b) (4)				
	2	15					
	3	50					
Day 28	1	0					
	2	15					
	3	50					

RME Relative mean error, representing the deviation from nominal
 CV Coefficient of variation
 ND Not detected

(Excerpted from the submission)

Table 25. Dosing solution analysis for stability in 4 week rat toxicology study

Nominal inclusion (mg/mL)	Bottle No.	Storage conditions			Analysed concentration (mg/mL)			CV (%)	Relative mean error (%)	
		Day	Hour	°C	Sample 1	Sample 2	Mean		A	B
7.5	1	(b) (4)								
	1									
	2									
	2									
	2									
	2									
15	1									
	1									
	2									
	2									
	2									
	2									
50	1									
	1									
	2									
	2									
	2									
	2									

CV Coefficient of variation
A Relative mean error, representing the deviation from nominal
B Relative mean error, representing the deviation from time zero

(Excerpted from the submission)

Study title: Report BI 655075 and Dabigatran Etxilate: Toxicity Study by Intravenous and Oral Gavage Administration to Rhesus Monkeys for 14 Days Followed by a 4 Week Recovery Period and Dabigatran Etxilate Re-Administration

Study no.:	DDB0331/n00230533
Study report location:	eCTD 4.2.3.2
Conducting laboratory and location:	 (b) (4)
Date of study initiation:	December 5, 2013
GLP compliance:	Signed and included
QA statement:	Signed and included
Drug, lot #, and % purity:	BI 655075-01; Lot # 207733; assumed 100% purity

Key Study Findings

- There were no mortalities during the study
- There were no major target organs of toxicity.
- There was no evidence of a prothrombic effect of idarucizumab in monkeys based on the comparison of measured concentrations of respective markers D-dimer and F1+2 between control and treatment groups.
- There was no evidence in any of the three complement assays analyzed: Bb, C3a and C4a. All values were within normal ranges for all dose groups.
- Antidrug antibodies were detected in 9/10 monkeys dosed at 150 mg/kg idarucizumab (with dabigatran) and 3/10 of monkeys dosed at 150 mg/kg idarucizumab (with and without dabigatran; groups combined). The presence of antidrug antibodies did not affect exposure to idarucizumab.

Methods

Doses:	150 or 500 mg/kg
Frequency of dosing:	Once daily
Route of administration:	Intravenous injection, slow bolus; left and right cephalic veins, left and right saphenous veins
Dose volume:	10mL/kg
Formulation/Vehicle:	Formulation: 50 mg/mL solution in 25 mM acetate, 220 mM sorbitol and 0.02% polysorbate 20 (Tween 20), pH 5.5; oral vehicle: 0.5% hydroxyethylcellulose; intravenous vehicle: Placebo buffer (25 mM acetate, 240 mM sorbitol, 0.02% polysorbate 20, pH 5.5), Lot number DAB-488
Species/Strain:	rhesus monkeys
Number/Sex/Group:	5/sex/group
Age:	40 to 47 months
Weight:	Males: 3.41 kg to 6.36 kg; Females: 3.25 kg to 5.01 kg
Satellite groups:	N/A
Unique study design:	N/A
Deviation from study protocol:	None

Table 26. Study design for 2 week toxicity study in rhesus monkeys

Group	Treatment	Dose (mg/kg/day)	Nominal concentration (mg/mL)	Formulated concentration (mg/mL)	Volume dose (mL/kg)
1	Oral vehicle or placebo buffer	0	0	0	10
2	Oral vehicle or BI 655075	0	0	0	10
3	dabigatran etexilate and BI 655075	12	1.2	1.38	10
4	dabigatran etexilate and BI 655075	150	15	15	10
		12	1.2	1.38	10
		500	50	50	10

(Excerpted from the submission)

Observations and Results**Mortality**

There were no mortalities during the study.

Clinical Signs

There were no clinical signs attributable to idarucizumab or dabigatran. Non-drug related clinical signs were related to dosing and administration of the drug (vomiting, injection site findings).

Body Weights

Unremarkable

Feed Consumption

Unremarkable

Ophthalmoscopy

Unremarkable

ECG

Unremarkable

Hematology

Unremarkable

Clinical Chemistry

Unremarkable

Urinalysis

Unremarkable

Gross Pathology

Unremarkable

Organ Weights**Table 27. Organ weight changes in 2 week toxicity study in rhesus monkey**

	males			females		
dabigatran group (mg/kg/day)	0	12	12	0	12	12
idarucizumab group (mg/kg/day)	500	150	500	500	150	500
Spleen						
Day 14	-2.228	-22.65	-26.48	22.968	-0.407	8.894

P<0.01

Histopathology

Adequate Battery: Yes

Peer Review: Signed and Included

Histological Findings

Although there were a number of histological findings in the monkey study at both 150 mg/kg and 500 mg/kg they were not present in the recovery group animals and these findings had no correlation to other pathological changes in blood chemistry, clinical chemistry, or gross pathology. They were incidental.

Table 28. Histopathology findings in 2 week toxicity study in rhesus monkey (main study)

Treatment related microscopic findings										
			males				females			
dabigatran group (mg/kg/day)			0	0	12	12	0	0	12	12
idarucizumab group (mg/kg/day)			0	500	150	500	0	500	150	500
Number of animals examined			3	3	3	3	3	3	3	3
Organ	Finding	Grade								
Heart	Inflammatory cells, myocardial	1	1		1	2				1
	mineralization, myocardium	1							1	
Kidneys	cysts cortical	1							1	
	cysts papilla	1							1	
	deposits hyaline, tubular	1				1				
Liver	hydropic degeneration	1				1				
Lymph node, axillary	Erythrocytosis/ Erythrophagocytosis, Sinuses	1				1	1		1	
Lymph node, mesenteric	Erythrocytosis/ Erythrophagocytosis, Sinuses	1			1					
Pancrease	infiltration, inflammatory cells	1			1				1	1
Spleen	germinal center development decreased	1				1				
thymus	cysts	1		2	2	1	1		2	
thyroid	cysts	1				1				1
		2		1					1	

Grade key: 1 minimal, 2 mild, 3 moderate, 4 severe

No toxicologically significant findings were present at recovery.

Additional findings present in all dose groups, including control were subcutaneous (dermal) inflammatory cell infiltration; vascular inflammatory cell infiltration and

subcutaneous hemorrhage were observed at the injection sites specifically at the left and right cephalic and saphenous veins.

Special Evaluation- Immunogenicity

Nine of 10 monkeys dosed at 150 mg/kg/day idarucizumab (with dabigatran) were reported positive for anti-idarucizumab antibodies. At the 500 mg/kg dose 3 of 10 monkeys were reported positive for anti-idarucizumab antibodies in the combined two groups of monkeys (with and without dabigatran).

Table 29. Summary of results for anti-idarucizumab antibodies in 2 week toxicity study in rhesus monkey

Group	Treatment (mg/kg/day)		Sex	# of monkeys screened positive / total # of monkeys ^a		
	Dabigatran etexilate	BI 655075		Pretreatment	Day 15	Recovery Day 28
1	0	0	Male	1/5	1/5	0/2
			Female	0/5	0/5	0/2
			Combined	1/10	1/10	0/4
2	0	500	Male	0/5	1/5	2/2
			Female	1/5	2/5	2/2
			Combined	1/10	3/10	4/4
3	12	150	Male	2/5	5/5	2/2
			Female	0/5	4/5	2/2
			Combined	2/10	9/10	4/4
4	12	500	Male	0/5	1/5	2/2
			Female	0/5	2/5	2/2
			Combined	0/10	3/10	4/4

(Excerpted from the submission)

Table 30. Summary of results for anti-dabigatran antibodies in 2 week toxicity study in rhesus monkey

Group	Treatment (mg/kg/day)		Sex	# of monkeys screened putative positive / total # of monkeys *		
	Dabigatran etexilate	BI 655075		Pretreatment	Recovery Day 15	Recovery Day 28
1	0	0	Male	0/5	0/2	0/2
			Female	0/5	0/2	0/2
			Combined	0/10	0/4	0/4
2	0	500	Male	0/5	2/2	0/2
			Female	0/5	1/2	0/2
			Combined	0/10	3/4	0/4
3	12	150	Male	0/5	1/2	0/2
			Female	0/5	2/2	0/2
			Combined	0/10	3/4	0/4
4	12	500	Male	0/5	2/2	2/2
			Female	0/5	2/2	0/2
			Combined	0/10	4/4	2/4

(Excerpted from the submission)

Special Evaluation- Indices of Thrombosis

Unremarkable. There was no evidence to suggest a prothrombic effect of idarucizumab in monkeys based on the comparison of measured concentrations of respective markers D-dimer and F1+2 between control and treatment groups.

Special Evaluation- Complement and Immune Complex Analysis

Unremarkable. There was no evidence to suggest changes in any of the three complement assays analyzed: Bb, C3a and C4a. All values were within normal ranges for all dose groups.

Toxicokinetics

On Days 1 and 14, blood samples were collected at 1.5 hours following dosing of dabigatran vehicle (prior to IV dosing of idarucizumab or vehicle), and 1 hour 40 minutes (immediately following IV infusion), 3 hrs., 6 hrs., 9 hrs., and 24 hrs. following dabigatran dosing. On Day 6, additionally samples were collected at 1.5 hrs. following dabigatran dosing and immediately following idarucizumab dosing (1 hr. 40 minutes following dabigatran dosing). Samples to analyze dabigatran and sum dabigatran (with gluconurides) were collected on recovery Day 29 (Day 58) pre-dose and 1.5 hrs. following re-administration of dabigatran to recovery animals. These samples were also analyzing for their idarucizumab concentrations.

Table 31. Summary toxicokinetics of idarucizumab in 2 week toxicity study in rhesus monkey

Summary TK parameters of BI 655075 in monkeys							
TK Parameter	Day	Sex	Descriptive statistics (n=5)	BI 655075 dose (mg/kg/day)			
				500 (no dabigatran)	150 (+ dabigatran)	500 (+ dabigatran)	
C_{max} ($\mu\text{g/mL}$)	1	Male	Mean SD	8,100 628	2,520 303	7,670 364	
		Female	Mean SD	8,380 526	2,520 76.0	8,040 789	
	14	Male	Mean SD	7,780 1,120	2,850 259	8,670 1,350	
		Female	Mean SD	8,880 934	2,340 293	7,860 471	
	AUC_{0-24} ($\mu\text{g}\cdot\text{h/mL}$)	1	Male	Mean SD	9,080 823	2,610 366	8,690 986
			Female	Mean SD	9,500 1,070	2,560 202	8,700 782
14		Male	Mean SD	8,960 396	2,950 411	9,960 1,830	
		Female	Mean SD	10,300 883	2,470 321	9,360 874	
t_{max} (h) ^a		1	Male	Median Range	1.67 1.67 – 1.67	1.67 1.67 – 1.67	1.67 1.67 – 1.67
			Female	Median Range	1.67 1.67 – 1.67	1.67 1.67 – 1.67	1.67 1.67 – 1.67
	14	Male	Median Range	1.67 1.67 – 1.67	1.67 1.67 – 1.67	1.67 1.67 – 1.67	
		Female	Median Range	1.67 1.67 – 1.67	1.67 1.67 – 1.67	1.67 1.67 – 1.67	

^a The time was based on the oral dosing of dabigatran etexilate. BI 655075 was administered intravenously between 1.5 and 1.67 h after oral dosing of dabigatran etexilate.

(Excerpted from the submission)

Key findings

- Increases in idarucizumab exposure were proportional to increases in dose from 150 mg/kg to 500 mg/kg (C_{max} and AUC) on Days 1 and 14.
- Idarucizumab exposure was similar between male and female monkeys.
- Dabigatran treatment did not affect exposure to idarucizumab.
- The presence of antidrug antibodies did not affect exposure to idarucizumab.
- T_{max} occurs at approximately 1.67 hours following idarucizumab administration.

Table 32. Summary toxicokinetics of dabigatran in 2 week toxicity study in rhesus monkey

Summary TK parameters of dabigatran and sum dabigatran in monkeys							
TK Parameter	Day	Sex	Descriptive statistics (n=5)	Dabigatran TK		Sum dabigatran TK	
				BI 655075 dose (mg/kg/day)		BI 655075 dose (mg/kg/day)	
				150	500	150	500
C _{max} (ng/mL)	1	Male	Mean SD	201 130	175 82.6	1,060 688	611 261
		Female	Mean SD	156 66.1	156 73.5	803 464	755 606
	14	Male	Mean SD	152 147	199 270	948 1,170	560 595
		Female	Mean SD	127 92.2	107 34.0	710 615	576 277
AUC ₀₋₂₄ (ng•h/mL)	1	Male	Mean SD	1,230 719	1,810 480	3,430 1,780	3,480 971
		Female	Mean SD	1,030 155	1,560 450	2,500 831	3,850 1,220
	14	Male	Mean SD	1,130 751	1,600 1,000	3,310 2,740	3,800 2,220
		Female	Mean SD	877 225	1,180 124	2,450 992	3,560 478
t _{max} (h) ^a	1	Male	Median Range	1.67 1.67 – 6.00	1.67 1.67 – 9.00	1.67 1.67 – 6.00	1.67 1.67 – 1.67
		Female	Median Range	1.67 1.67 – 3.00	3.00 1.67 – 6.00	1.67 1.67 – 1.67	1.67 1.67 – 6.00
	14	Male	Median Range	1.67 1.67 – 6.00	1.67 1.67 – 24.0	1.67 1.67 – 1.67	1.67 1.67 – 3.00
		Female	Median Range	1.67 1.67 – 3.00	1.67 1.67 – 9.00	1.67 1.67 – 1.67	1.67 1.67 – 1.67

^a The time was based on the oral dosing of dabigatran etexilate. BI 655075 was administered intravenously between 1.5 and 1.67 h after oral dosing of dabigatran etexilate.

(Excerpted from the submission)

Key findings

- Dabigatran exposure was similar between male and female monkeys.
- Idarucizumab treatment did not consistently cause a trend in exposure differences to dabigatran.

Dosing Solution Analysis

Concentration

The mean concentrations of idarucizumab in the solutions that were analyzed were within acceptability criteria of ^{(b) (4)}% of nominal concentrations, indicating accurate formulation; actual values were between ^{(b) (4)}%.

The mean concentrations of dabigatran in the solutions that were analyzed were within acceptability criteria of \pm ^{(b) (4)}% of nominal concentrations, indicating accurate formulation; actual values were between ^{(b) (4)}%.

Table 33. Dosing solution analysis for idarucizumab concentration in 4 week toxicology study in rhesus monkey

Occasion	Group	Nominal inclusion (mg/mL)	Analysed concentration (mg/mL)			RME (%)
			Analysis 1	Analysis 2	Mean	
Day 1M	1M	0	(b) (4)			
	3M	15				
	2+4M	50				
Day 14M	1M	0				
	3M	15				
	2+4M	50				
Day 1F	1F	0				
	3F	15				
	2+4F	50				
Day 14F	1F	0				
	3F	15				
	2+4F	50				

RME Relative mean error, representing the deviation from nominal
 ND Not detected

Table 34. Dosing solution analysis for dabigatran (0.5% hydroxyethylcellulose) concentration in 4 week toxicology study in rhesus monkey

Occasion	Group	Nominal inclusion (mg/mL)	Analysed concentration (mg/mL)				RME (%)
			Top	Middle	Bottom	Mean	
Day 1 (M)	1+2M	0	(b) (4)				
	3+4M	1.2					
Day 14 (M)	1+2M	0					
	3+4M	1.2					
Day 1 (F)	1+2F	0					
	3+4F	1.2					
Day 14 (F)	1+2F	0					
	3+4F	1.2					
Recovery Day 29(M)	1+2M	0					
	3+4M	1.2					
Recovery Day 29(F)	1+2F	0					
	3+4F	1.2					

RME Relative mean error, representing the deviation from nominal
ND Not detected

7 Genetic Toxicology

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Studies not conducted.

7.2 *In Vitro* Assays in Mammalian Cells

Studies not conducted.

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Studies not conducted.

7.4 Other Genetic Toxicity Studies

Studies not conducted.

8 Carcinogenicity

Studies not conducted.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Studies not conducted.

9.2 Embryonic Fetal Development

Studies not conducted.

9.3 Prenatal and Postnatal Development

Studies not conducted.

10 Special Toxicology Studies

Studies not conducted.

11 Integrated Summary and Safety Evaluation

The nonclinical studies submitted to this NDA provide sufficient information to support the use of idarucizumab (PRAXBIND) for the treatment of patients when rapid reversal of the anticoagulant effects of dabigatran is required: for emergency situations or in life threatening or uncontrolled bleeding.

Idarucizumab is a reversal agent for dabigatran. It is a humanized murine monoclonal antibody fragment antigen-binding (Fab) molecule that binds to dabigatran and forms a stable complex. Idarucizumab can also bind to dabigatran metabolites.

Idarucizumab binds dabigatran with higher affinity than thrombin (~300 times higher) and thrombin substrates in vitro. As a complex with dabigatran, idarucizumab is very stable with a long half-life in vitro (260hr). Idarucizumab also binds dabigatran metabolites. In vitro data showed that idarucizumab reverses the anticoagulant effect of dabigatran, in part by increasing fibrin coverage and increasing fibrin masses around damaged subendothelium. Three animal models of activity were conducted: a mouse intracranial hemorrhage model; a rat tail cut bleeding model and a pig blunt liver trauma model. In the mouse intracranial hemorrhage model, idarucizumab reduced hematoma volume following administration returning levels to those observed in non-anticoagulated animals. In the rat tail cut model showed neutralization of dabigatran by idarucizumab, effective single and split dosing regimens with activity measured by significantly reduced anticoagulation and bleeding time. The pig blunt liver trauma model also tested single and split dosing measuring activity of idarucizumab through reduced anticoagulation, blood loss and survival of pigs after a given time period.

Safety pharmacology studies showed no adverse respiratory findings. Cardiovascular safety pharmacology studies were not performed independently but ECG measurements assessed during the 2 week repeat dose toxicology study in monkeys were unremarkable at doses up to 500 mg/kg.

In the pharmacokinetic studies in both rats and monkeys, there was a rapid increase in dabigatran plasma concentration following dosing with idarucizumab suggesting redistribution of dabigatran from the tissue to the plasma. This was evidenced by the high average dabigatran C_{max} and AUC_{0-24} values in animals treated with idarucizumab vs. without idarucizumab (dabigatran only). Dabigatran and average sum dabigatran values were many fold values higher in the idarucizumab treated animals (C_{max} and AUC_{0-24} values). Idarucizumab was rapidly eliminated in the blood following intravenous dosing and exhibited biphasic plasma concentration-time profiles; initial phase half-lives were approximately 0.25 hrs. (both species) and terminal phase half-lives were approximately 6 hrs in the rat and 5.5 hrs. in the monkey. Excretion in the urine was only 10% in the monkeys and 21% in the rats. Based on the data collected in general toxicology studies, there were relatively no gender differences in exposure, and increased in C_{max} and AUC values were approximately dose proportional.

The general toxicology studies were conducted in the rat and monkey via I.V., which is the intended route of administration. The rat studies were performed using only idarucizumab; the monkey studies were performed in the presence and absence of dabigatran. The 4 week repeat dose toxicity study in rat and 2 week repeat dose toxicity study in the monkey are reviewed. All appropriate studies were conducted in compliance with Good Laboratory Practice (GLP) regulations. There were no major target organs in rat or monkey. There were no mortalities in the rat study or the monkey study. There were some hematology findings in rats including increased white blood cells and lymphocytes at 150 and 500 mg/kg dose levels; and clinical chemistry findings of decreased blood urea and creatinine levels at 500 mg/kg. Additionally, female rats dosed at both 150 and 500 mg/kg had elevated thymus weights (absolute, relative to body weight and relative to brain weight). Histopathology findings in the rat included atrophy in the pancreas at high doses (500 mg/kg) in two animals. Although there were a number of histological findings in the monkey study at both 150 mg/kg and 500 mg/kg they were not present in the recovery group animals and these findings had no correlation to other pathological changes in blood chemistry, clinical chemistry, or gross pathology. They were incidental. Markers of thrombosis were examined (D-dimer and F1+2) between control and treatment groups. There was no evidence of a prothrombic effect of idarucizumab in monkeys based on the analysis of these markers. The effect of idarucizumab on complement activation was also assessed using assays directed at Bb, C3a and C4a and these were unremarkable for all dose groups. Anti-drug antibodies were detected in monkeys at both doses tested and detected in more monkeys at lower dose groups (in 9/10 monkeys dosed at 150 mg/kg idarucizumab (with dabigatran) and 3/10 of monkeys dosed at 150 mg/kg idarucizumab (with and without dabigatran; groups combined). The presence of antidrug antibodies did not affect exposure to idarucizumab.

Studies specifically addressing in vitro and in vivo genotoxicity and carcinogenicity have not been conducted. Studies addressing reproductive and developmental toxicity; fertility and early embryonic development to implantation; effects on embryo-fetal development; pre- and postnatal development, including maternal function and toxicity in juvenile animals have not been conducted.

12 Appendix/Attachments

None

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

/s/

EMILY J PLACE
09/11/2015

CHRISTOPHER M SHETH
09/11/2015

MEMORANDUM

Date: July 28, 2015
From: Christopher Sheth, PhD
Division of Hematology Oncology Toxicology (DHOT)
Office of Hematology and Oncology Products (OHOP)
Re: Approvability for Pharmacology and Toxicology
BLA: 761025
Drug: PRAXBIND (idarucizumab) injection; for intravenous use
Indication: PRAXBIND is a reversal agent for dabigatran
Applicant: Boehringer Ingelheim Pharmaceuticals, Inc.

Idarucizumab is a humanized monoclonal antibody fragment (Fab) that binds to the thrombin inhibitor anticoagulant dabigatran with a higher affinity than the binding of dabigatran to thrombin. There is no endogenous target for idarucizumab. Based on its mechanism of action, the pharmacologic class assigned to idarucizumab is “reversal agent for dabigatran”.

Pharmacology, safety pharmacology, and toxicology studies were conducted in in vitro and in vivo models. The biologic product idarucizumab is only intended to be administered in acute situations where a rapid reversal of the anticoagulant effects of dabigatran are required; as such, in accordance with the ICH S6 and S9 Guidances and conditions of use, genetic toxicology, reproductive and developmental, and carcinogenicity studies were not needed.

Data from in vitro studies demonstrated that idarucizumab forms complexes with dabigatran and with dabigatran metabolites. Several in vivo nonclinical bleeding models were utilized to show that idarucizumab administration results in reversal of the anticoagulant effects of dabigatran. Additional data submitted indicate the effects of idarucizumab are in part mediated by increasing fibrin coverage and increasing fibrin masses around damaged subendothelium.

No major target organs for toxicity were identified in rats or monkeys administered idarucizumab, likely due to the specificity of the molecule for an exogenously administered target (i.e., dabigatran). The Applicant proposed a Pregnancy Category ^(b)₍₄₎ for idarucizumab, which was found to be acceptable. In general, women should not become pregnant while taking anticoagulants, thus it is unlikely idarucizumab will be administered to women of childbearing potential who are not taking measures to prevent pregnancy. The nonclinical studies needed to support product labeling were reviewed by Dr. Emily Place. The nonclinical findings are summarized in the “Executive Summary” of the BLA review and reflected in the product label.

Recommendation: I concur with the pharmacology/toxicology reviewer that from a nonclinical perspective, PRAXBIND may be approved and that no additional nonclinical studies are needed to support approval of PRAXBIND in patients with treated with Pradaxa® when rapid reversal of the anticoagulant effects of dabigatran is required.

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/s/

CHRISTOPHER M SHETH
07/28/2015

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

PHARMACOLOGY/TOXICOLOGY BLA REVIEW AND EVALUATION

Application number: 761025
Supporting document: 2
Applicant's letter date: February 18, 2015
CDER stamp date: February 19, 2015
Product: Praxbind (idarucizumab)
Indication: Rapid reversal of anticoagulant effects of dabigatran
Applicant: Boehringer Ingelheim Pharmaceuticals, Inc.
Review Division: Division of Hematology Oncology Toxicology (DHOT) for Division of Hematology Products (DHP)
Reviewer: Emily Place PhD MPH
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1 Executive Summary

1.1 Introduction

Idarucizumab is a reversal agent for the thrombin inhibitor dabigatran. It is a humanized murine Fab fragment directed against dabigatran and its metabolites and forms a stable complex to neutralize the anticoagulant effects dabigatran. The applicant is seeking approval for the intravenous route of administration and the proposed clinical dose is 5.0 g. Nonclinical pharmacology, pharmacokinetic, and chronic toxicology studies in the rat and the monkey have been submitted.

The pharmacology/toxicology studies conducted support approval of idarucizumab for the proposed indication (patients treated with dabigatran when rapid reversal of the anticoagulant effects of dabigatran is required for emergency surgery/urgent procedures or in life-threatening or uncontrolled bleeding).

1.2 Brief Discussion of Nonclinical Findings

Idarucizumab binds dabigatran with higher affinity than thrombin (~300 times higher) and thrombin substrates in vitro. Results from in vitro studies show that idarucizumab forms a stable complex with dabigatran (with 50% of bound complex remaining after 260 hours). Idarucizumab also binds dabigatran metabolites. In vitro data showed that idarucizumab reverses the anticoagulant effect of dabigatran, in part by increasing fibrin coverage and increasing fibrin masses around damaged subendothelium. Three animal models of activity were submitted in support of the application: a mouse intracranial hemorrhage model; a rat tail cut bleeding model, and a pig blunt liver trauma model. All 3 animal pharmacology models showed the effectiveness of the neutralization activity of idarucizumab, and its ability to significantly reduce anticoagulation and blood loss. Based on the pharmacology data submitted in the BLA, the Established Pharmacological Class (EPC) of “reversal agent for dabigatran” was determined to be both clinically meaningful and scientifically valid for idarucizumab.

Safety pharmacology studies showed no adverse respiratory findings. Cardiovascular safety pharmacology studies were not performed independently but ECG measurements assessed during the 2 week repeat dose toxicology study in monkeys were unremarkable at doses up to 500 mg/kg.

In the pharmacokinetic studies in both rats and monkeys, there was a rapid increase in dabigatran plasma concentration following dosing with idarucizumab suggesting redistribution of dabigatran from the tissue to the plasma. Based on the data collected in general toxicology studies, there were no gender differences in exposure, and increased in C_{max} and AUC values were approximately dose proportional. Idarucizumab was rapidly eliminated in the blood following intravenous dosing and exhibited biphasic plasma concentration-time profiles; initial phase half-lives were approximately 0.25 hrs. (both species) and terminal phase half-lives were approximately 6 hrs in the rat and 5.5 hrs. in the monkey.

The general toxicology studies were conducted in the rat and monkey via I.V., which is the intended route of administration for idarucizumab. The rat studies were performed using only idarucizumab; the monkey studies were performed in the presence and absence of orally administered dabigatran. The 4 week repeat dose toxicity study in rat and 2 week repeat dose toxicity study in the monkey are reviewed. All appropriate studies were conducted in compliance with Good Laboratory Practice (GLP) regulations. There were no major target organs in rat or monkey.

The following types of toxicological assessments for idarucizumab were not deemed essential for marketing due to the specific nature of its intended use, which is as a single administration only when needed to rapidly reverse the anticoagulant effects of dabigatran: in vitro and in vivo genotoxicity, carcinogenicity, reproductive and developmental toxicity, fertility, embryo-fetal development; and pre- and postnatal development toxicity testing.

1.3 Recommendations

1.3.1 Approvability

From the Pharmacology/Toxicology perspective “idarucizumab” may be approved for the proposed indications.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

The content for the labeling of idarucizumab is contained in this review. Based on the pharmacology data submitted in the BLA, the Established Pharmacological Class (EPC) of “reversal agent for dabigatran” was determined to be both clinically meaningful and scientifically valid for idarucizumab.

2 Drug Information

2.1 Drug

CAS Registry Number (Optional): 1362509-93-0

Code Name: BI 655075, 655075-01, idarucizumab

Chemical Name: N/A

Molecular Formula/Molecular Weight: $C_{2131}H_{3299}N_{555}O_{671}S_{11}$ / 47,766 Da.

Structure or Biochemical Description:

Figure 1. Structure of idarucizumab



(Excerpted from the submission)

Pharmacologic Class: reversal agent for dabigatran

2.2 Relevant INDs, NDAs, BLAs and DMFs

None

2.3 Drug Formulation

Table 1. Idarucizumab drug formulation

Ingredient	Amount per vial [mg] ¹	Concentration [mg / mL]	Function	Reference to standards
Idarucizumab	2500.00	50.00	Active ingredient	Internal standard
Acetic acid glacial	10.05	0.20	(b) (4)	Ph. Eur., USP
Polysorbate 20	10.00	0.20		Ph. Eur., USP/NF
Sodium acetate trihydrate	147.35	2.95		Ph. Eur., USP
Sorbitol	2004.20	40.08		Ph. Eur., USP/NF
Water for Injection	q.s.ad 50.00 mL	n/a		Ph. Eur., USP
Total volume	50 mL	-		-

¹ (b) (4)

(Excerpted from the submission)

2.4 Comments on Novel Excipients

There are no Pharmacology/Toxicology concerns with the excipients or their levels in the drug formulation.

2.5 Comments on Impurities/Degradants of Concern

N/A – Division of Therapeutic Proteins

2.6 Proposed Clinical Population and Dosing Regimen

The proposed indication for idarucizumab is patients treated with dabigatran when rapid reversal of the anticoagulant effects of dabigatran is required for emergency surgery/urgent procedures or in life-threatening or uncontrolled bleeding.

The proposed dose of idarucizumab is 5.0 g. It is currently provided as two separate 50 mL vials each containing 2.5 g idarucizumab, which will be administered intravenously.

2.7 Regulatory Background

NDA 761025 was submitted on February 19, 2015 for the rapid reversal of anticoagulant effects of dabigatran.

3 Studies Submitted

3.1 Studies Reviewed

Study Title	Study No.	Module
Primary Pharmacodynamics		
<i>In vitro Pharmacology</i>		
Affinity of BI 655075 for dabigatran, pH dependence and binding to plasma proteins	MD2013/03/P PSS	4.2.1.1
Neutralization of the anticoagulant activity of dabigatran and its acylglucuronides by BI 655075 in vitro using a modified thrombin time assay in plasma or whole blood	MD2014/01/P PSS	4.2.1.1
Lack of potential prothrombotic activity of BI 655075 investigated in human plasma in vitro and in rats in vivo	MD2014/02/P PSS	4.2.1.1
Effects of dabigatran on platelet interaction and fibrin generation and reversal of its antihemostatic action by procoagulant strategies or a specific antidote (BI655075): Studies in vitro with circulating human blood.	MD2014/11/P PSS	4.2.1.1
Inhibition of dabigatran anticoagulant activity by BI 655075 in the presence of coagulation factor concentrates in vitro	MD2014/14/P PSS	4.2.1.1
<i>In vivo Pharmacology</i>		
Efficacy of hemostatic therapy with BI 655075 in experimental intracerebral hemorrhage associated with the oral anticoagulant Dabigatran Etexilate	MD2014/08/P PSS	4.2.1.1
Neutralization of the anticoagulant activity of dabigatran with BI 655075 after intravenous administration in a rat model of anticoagulation	MD2013/05/P PSS	4.2.1.1
Reversal of dabigatran-induced bleeding with BI 655075 after single oral administration of dabigatran etexilate in an in vivo rat tail bleeding model	MD2013/06/P PSS	4.2.1.1
Reversal of supra-therapeutic dabigatran anticoagulation and subsequent bleeding with BI 655075 in an in vivo rat model	MD2014/05/P PSS	4.2.1.1
Effect of BI 655075 on bleeding induced by dabigatran in combination with the antiplatelet agents ASA, clopidogrel and ticagrelor (rat in vivo)	MD2014/09/P PSS	4.2.1.1
Neutralization of dabigatran by BI 655075, the specific antidote for dabigatran, after haemodilution and administration of various volume expanders in a pig model.	MD2013/07/P PSS/2012- 008 A	4.2.1.1
Effects of BI 655075 on dabigatran-induced bleeding and hemorrhagic shock in a porcine blunt liver trauma model	MD2013/08/P PSS	4.2.1.1
Effects of BI 655075 as a split dose (2 bolus injections 60 min apart) on dabigatran-induced bleeding and hemorrhagic shock in a porcine blunt liver double trauma model	MD2014/12/P PSS	4.2.1.1
Safety Pharmacology		
BI 655075: Evaluation of Respiratory Parameters in the conscious Male Rat using Whole Body Bias Flow Plethysmography (Intravenous Bolus Administration)	12r006	4.2.1.3
Pharmacokinetics		
<i>Absorption</i>		
Pharmacokinetics and urinary excretion of BI 655075 and the effect of BI 655075 on the pharmacokinetics and urinary excretion of dabigatran in the Wistar Han rat	U12-3083-01	4.2.2.2.1
BI 655075: Pharmacokinetic and Pharmacodynamic Study in rhesus Monkeys Pretreated with Dabigatran Etexilate	U13-3539-01	4.2.2.2.1

Study Title	Study No.	Module
Repeat Dose Toxicology <i>(including supportive toxicokinetics)</i>		
BI 655075: Toxicity Study by Intravenous Administration to Han Wistar Rats for 4 Weeks Followed by a 4 Week Recovery Period	DDB0150	4.2.3.2.1
Study title: Report BI 655075 and Dabigatran Etexilate: Toxicity Study by Intravenous and Oral Gavage Administration to rhesus Monkeys for 14 Days Followed by a 4 Week Recovery Period and Dabigatran Etexilate Re-Administration	DDB0331	4.2.3.2.1

3.2 Studies Not Reviewed

Study Title	Study No.	Module
Pharmacokinetics-		
<i>Analytic Methods and Validation Reports</i>		
Validation of a Radioimmunoassay for Detection of Anti-Dabigatran Antibodies in rhesus Monkey Plasma	8295-001	4.2.2.1
Evaluation of dabigatran interference in ELISA assays used to quantify BI 655075 in nonclinical studies	BB-14-1001	4.2.2.1
Validation of an ELISA Method for the Quantitation of BI 655075 in rhesus Monkey Plasma (K3EDTA) (Method Number: BBM-12-1001)	BBM-12-1001	4.2.2.1
Validation of an Electrochemiluminescence Method for the Detection of Anti BI 655075 Antibodies in Wistar Hannover K3EDTA Rat Plasma	dm-12-1036	4.2.2.1
Validation of an ELISA Method for the Quantitation of BI 655075 in Rat Plasma (k3EDTA) (Method Number: BBM-12-1003)	dm-12-1044	4.2.2.1
Validation of an Electrochemiluminescence Method for the Detection of Anti BI 655075 Antibodies in rhesus K3EDTA Monkey Plasma	dm-12-1058	4.2.2.1
Evaluation of Potential Assay Interference for Plasma Samples from rhesus Monkeys Administered Dabigatran Etexilate	dm-13-1011	4.2.2.1
Validation of an Electrochemiluminescence Method for the Detection of Anti BI 655075 Antibodies in rhesus Monkey K3EDTA Plasma (Method Number: BBM-13-1001)	dm-13-1086	4.2.2.1
Method validation of determination of pharmacodynamic action of BIBR 953 on Hemoclot® Thrombin Inhibitors Clotting Time, activated Partial Thromboplastin Clotting Time, Thrombin Clotting Time and Ecarin Clotting Time in rhesus monkey plasma	MEN1011	4.2.2.1
Method validation of a modified TT based assay for detection of neutralising anti-Dabigatran antibodies in rhesus monkey plasma	MEN1313	4.2.2.1
Method validation of Prothrombin fragment F 1+2 in rhesus monkey plasma by specific commercial ELISA kit.	MEN1414	4.2.2.1
Method validation of determination of pharmacodynamic action of BIBR 953 on Hemoclot® Thrombin Inhibitors Clotting Time, activated Partial Thromboplastin Clotting Time and Ecarin Clotting Time in Rat plasma	MEN2212	4.2.2.1
Method validation of D-Dimer and Prothrombin fragment F 1+2 in human and rhesus monkey plasma by specific commercial ELISA kits.	MEN2513	4.2.2.1
Revalidation of a LC-MS/MS method for the determination of BIBR 953 ZW in EDTA-rat plasma in the presence of BI 655075	n-a-bio-11-143	4.2.2.1
Validation of a LC-MS/MS method for the determination of BIBR 953 ZW in rat urine in the presence of BI 655075	n-a-bio-11-144	4.2.2.1
VALIDATION OF A LC-MS/MS METHOD FOR THE DETERMINATION OF BIBR 953 ZW IN EDTA RHESUS MONKEY	n-a-bio-11-145	4.2.2.1

Study Title	Study No.	Module
PLASMA IN PRESENCE OF BI 655075		
VALIDATION OF A LC-MS/MS METHOD FOR THE DETERMINATION OF SUM BIBR 953 ZW IN EDTA RHESUS MONKEY PLASMA IN PRESENCE OF BI 655075	n-a-bio-11-146	4.2.2.1
Validation of a LC-MS/MS method for the determination of BIBR 953 ZW in monkey urine in the presence of BI 655075	n-a-bio-11-147	4.2.2.1
Validation of a LC-MS/MS method for the determination of SUM-BIBR 953 ZW in monkey urine in the presence of BI 655075	n-a-bio-11-148	4.2.2.1
Partial validation of a LC-MS/MS method for the determination of SUM-BIBR 953 ZW in EDTA-pig plasma in the presence of BI 655075 -Additional long term stability data	n-a-bio-12-224-am1	4.2.2.1
Evaluation of interference in an anti-BI 655075 antibody assay in plasma from dabigatran-treated rhesus monkeys	PK-12-1024 and PK-13-1036	4.2.2.1
<i>Absorption</i>		
Metabolism of BIBR 1048 MS and BIBR 953 ZW in rats	A289_01BC A344_02BC	4.2.2.2
BI 655075: Pharmacokinetic and Pharmacodynamic Study in rhesus Monkeys Pretreated with Dabigatran Etxilate	U12-3849-01	4.2.2.2
Prediction of human pharmacokinetic parameters of BI 655075 based on rhesus monkey pharmacokinetic data	U12-3374-01	4.2.2.2
The disposition of 14C BIBR953 ZW in rhesus monkey following IV administration.	IRI164291	4.2.2.2
Development of a preliminary semi-mechanistic model to describe the interaction between dabigatran and its antidote, idarucizumab, in animals and humans	pk-14-1019	4.2.2.2
<i>Other pharmacokinetic studies</i>		
Effect of renal impairment on the pharmacokinetics and urinary excretion of dabigatran or its antidote BI 655075 in the rat	U13-3340-01	4.2.2.7
Impact of renal impairment on pharmacokinetics and pharmacodynamics of BI 655075 in rats treated with dabigatran	dm-13-1030	4.2.2.7
Single Dose Toxicology		
BI 655075: Single Dose Study by Intravenous Bolus	U12-3325-01	4.2.3.1
Repeat Dose Toxicology <i>(including supportive toxicokinetics)</i>		
BI 655075: Exploratory Study in rhesus Monkeys	U12-3326-01	4.2.3.2.1
BI 655075: Toxicity Study by Intravenous Administration to rhesus Monkeys for 2 Days Followed by A 2 Week Recovery Period	11r149	4.2.3.2.1
BI 655075: Renal Investigation Study by Intravenous Administration to rhesus Monkeys for 2 Days Followed by a 2 Week Recovery Period	12r169	4.2.3.2.1
Local Tolerance		
Single Dose Perivascular Tolerance Study in Male Rabbits	13r064	4.2.3.6
Other Toxicity Studies		
A Tissue Cross-Reactivity Study of BI 655075 in Normal Human, rhesus Monkey and Wistar-Han Rat Tissues	11r152	4.2.3.7.7
BI 655075: Determination of Hemocompatibility in Human Whole Blood	12r021	4.2.3.7.7

Study Title	Study No.	Module
Nonclinical Safety Assessment: Kappa Select Protein	n00236993-01	4.2.3.7.7
Nonclinical Safety Assessment: Leachables in idarucizumab Process Equipment	n00237442-01	4.2.3.7.7
BIBR 1048 MS: Acyl glucuronides of dabigatran etexilate	U07-1334	4.2.3.7.7

3.3 Previous Reviews Referenced

None

4 Pharmacology

4.1 Primary Pharmacology

Mechanism of Action

Idarucizumab is a reversal agent for dabigatran. It is a humanized murine Fab fragment directed against the thrombin inhibitor dabigatran. Binding of idarucizumab to dabigatran results in formation of a stable complex. Idarucizumab can also bind to dabigatran metabolites. Idarucizumab neutralizes the anticoagulant effect of dabigatran.

Idarucizumab binding to dabigatran in vitro

Methods

Affinity determinations and binding kinetics were determined using Kinexa technologies. Dissociation constants were calculated and K_D determinations were performed at different pH values.

Results

Binding of idarucizumab to dabigatran was assessed using Biacore technology. The calculated K_D value for idarucizumab was 2.1 pM. The K_D for binding of dabigatran to thrombin was 0.7nM (~300 times lower affinity).

Table 2. Antibody affinity for BI 655075 binding to dabigatran at pH 7.4

Fab	K_D (pM)	$k_a \times 10^5 M^{-1}s^{-1}$	$k_a \times 10^{-6} s^{-1}$ (calculated)	estimated $T_{1/2}$ of complex
Idarucizumab	2.1 ± 0.6	3.4 ± 0.4	0.7 ± 0.2	~260 h

(Excerpted from the submission)

Inhibition of dabigatran by idarucizumab in Human Plasma and Whole Blood

Methods

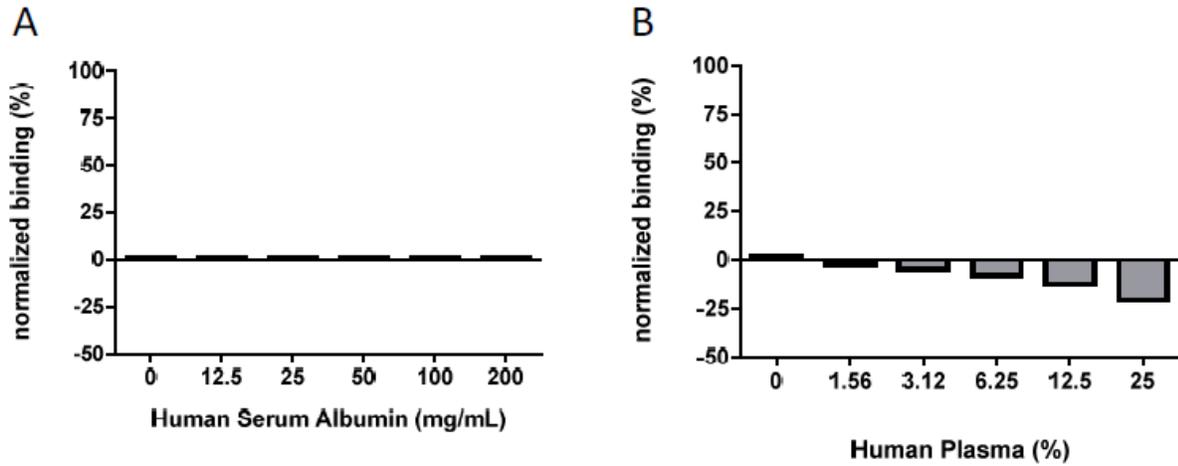
Binding to plasma proteins and thrombin substrates was determined.

Idarucizumab was coupled to the matrix of a CM-5 sensor chip. Surface plasmon resonance (Biacore) experiments were performed using idarucizumab and dabigatran with aliquot of human plasma pooled from 10 volunteers. Idarucizumab binding was tested using a modified thrombin time (TT) assay. IC50 values were determined using concentrations of idarucizumab added to plasma containing dabigatran and the clotting time was measured in whole blood and plasma.

Results

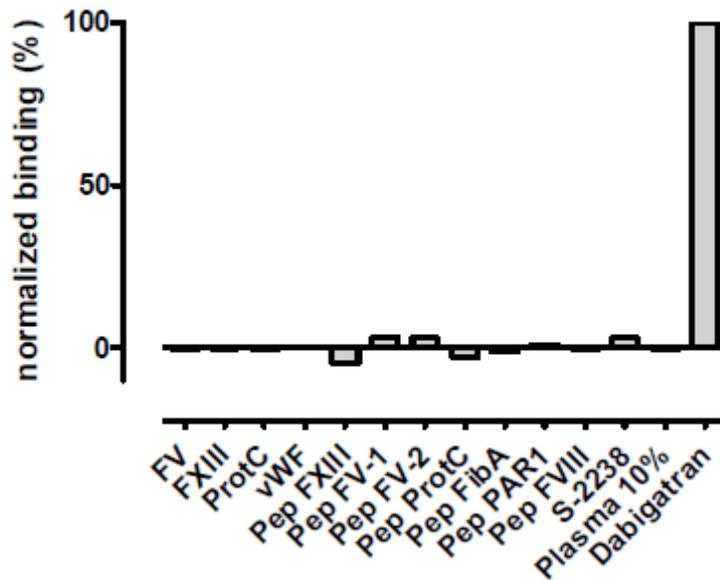
There was no specific binding of human plasma, serum, albumin or fibrinogen by idarucizumab- dabigatran complex. There was no specific binding to the thrombin substrates displayed by idarucizumab including the following substrates: S-2238, factors V, VIII, XIII, fibrinogen, von Willebrand factor, protease-activated receptor-1 (PAR-1) peptide or protein C. IC50 values for the reversal of anticoagulant activity of dabigatran by idarucizumab in human plasma and whole blood are shown below for 7 nm (A) and 30nm (B) (n=4). Reversal of the anticoagulant effect of dabigatran was similar in whole blood and plasma (IC50 11.3 and 10.9 nM, respectively); IC50 is approximately half the total dabigatran in each respective assay. The Applicant points to evidence of a 1:1 stoichiometric relationship between dabigatran and idarucizumab.

Figure 2. Idarucizumab binding to human serum albumin and plasma proteins



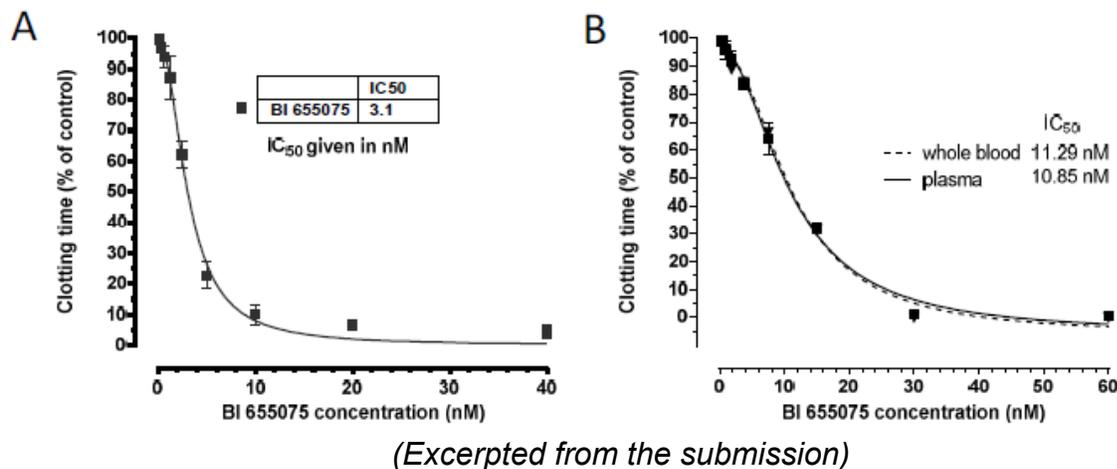
(Excerpted from the submission)

Figure 3. Idarucizumab binding to thrombin substrates



(Excerpted from the submission)

Figure 4. IC₅₀ values for the reversal of anticoagulant activity of dabigatran by idarucizumab in human plasma and whole blood



Idarucizumab displays thrombin-like activity in vitro and in vivo

Methods

Based on structural similarities to thrombin and its interactions with dabigatran, experiments were performed to determine if idarucizumab had any thrombin-like enzymatic activity. Activity was measured in human test systems in vitro and in rat plasma ex vivo using a thrombin-based clotting assay and platelet stimulation assays.

Results

Thrombin generation was not significantly increased relative to controls in a number of parameters tested. In prothrombin depletion coagulation assays, idarucizumab displayed a lack of procoagulant activity with long mean clotting times. Results also indicated that idarucizumab does not convert fibrinogen into fibrin in human plasma in vitro or in rats in vivo. There was also a lack of interaction by idarucizumab with the PAR-1 receptor on platelets in platelet rich plasma. The data indicated idarucizumab lacks thrombin-like activity and lacks the ability to stimulate aggregation of platelets.

Effects of idarucizumab and dabigatran on platelet interaction and fibrin generation in vitro

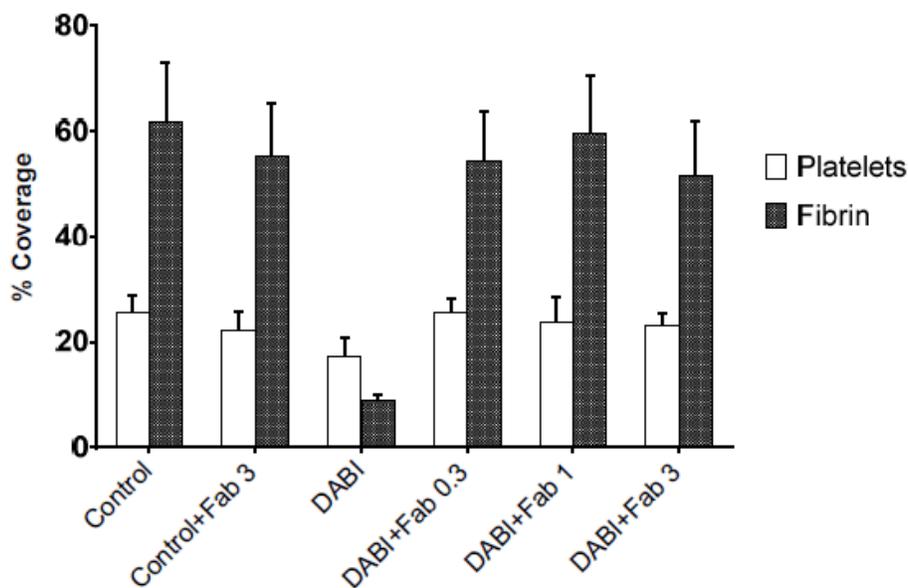
Methods

Experiments were performed to evaluate thrombus formation on damaged vascular surfaces using idarucizumab and dabigatran with an aliquot of human plasma pooled from 11 volunteers. The blood was then perfused through annular chambers exposing the thrombogenic substrate (a damaged vessel segment, rabbit aorta). Perfusion studies were performed using light microscopy to quantify the amount of fibrin and platelet deposition onto the damaged vessel. Routine hematology parameters including platelet count, hematocrit, and white blood cell differential, PT, aPTT and fibrinogen levels were collected using the BCS TM XP system.

Results

Dabigatran (DABI, 390 nM) supplemented blood significantly decreased fibrin coverage on the perfused damaged subendothelium and reduced the percentage of the vessel covered by platelets. Idarucizumab in the absence of dabigatran did not alter fibrin or platelet deposition. Idarucizumab added to blood previously treated with dabigatran at concentrations at 0.3, 1 or 3 mg/mL (Fab 0.3, 1, 3) restored the amount of fibrin coverage and size of fibrin masses to those originally observed in control samples (idarucizumab only).

Figure 5. Effects of idarucizumab and dabigatran on platelet interaction and fibrin generation



(Excerpted from the submission)

Effects of coagulation factors on idarucizumab and dabigatran in vitro

Methods

Prothrombin complex concentrates (PCCs), activated prothrombin complex concentrates (aPCC), and recombinant activated factor VIIa (rFVIIa) were measured using diluted thrombin time (dTT, clotting time) and tested in human plasma with increasing concentrations of both idarucizumab and dabigatran.

Results

Coagulation factor concentrates (PCCs, aPCC, rFVIIa) in human plasma did not influence the anticoagulant effect of dabigatran as measured using dTT (clotting time), and they did not interfere with inhibition of dabigatran by idarucizumab.

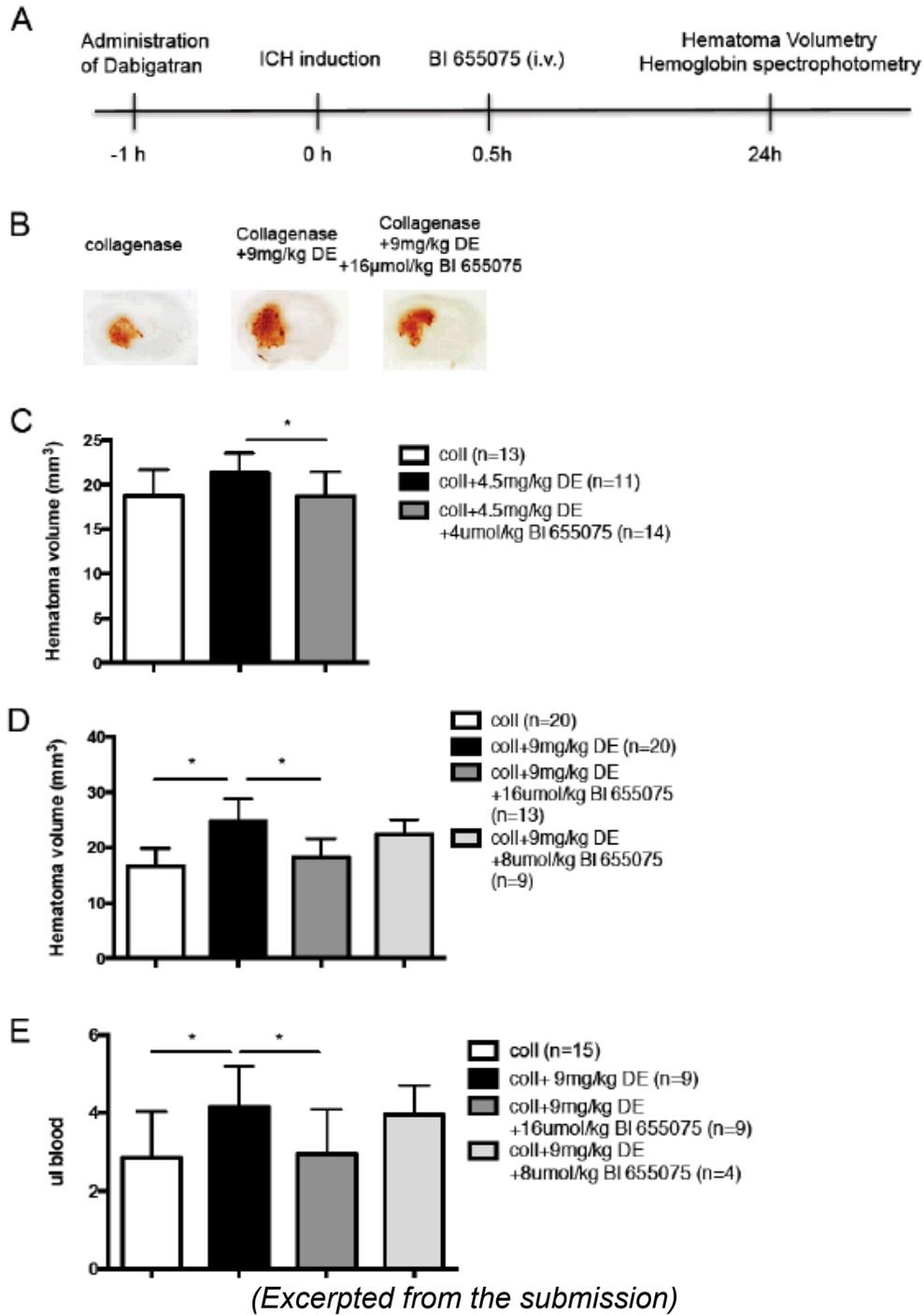
**Animal models of activity:
Intracranial hemorrhage mouse model****Methods**

Experiments were performed to determine if idarucizumab in addition to dabigatran etexilate (DE) influences hematoma volume in a mouse model of intracranial hemorrhage (ICH). DE was administered intraperitoneally and ICH was induced by intrastriatal collagenase-injection. Idarucizumab or saline was then administered 30 minutes following induction of ICH by tail vein injection. ICH volume and intracerebral blood content were quantified using hemoglobin spectrophotometry and by examination of brain cryosections.

Results

Hematoma enlargement occurs rapidly after ICH induction and DE is administered within 1 hour. Administration of idarucizumab at equimolar doses of DE is effective at reducing hematoma volume (8-16uM idarucizumab, 4.5-9.0 mg/kg DE). Hematoma volumes returned to levels observed in non-anticoagulated animals.

Figure 6. Intracranial hemorrhage model in mouse- hematoma volumes



Rat tail cut bleeding model

Neutralization of dabigatran induced bleeding after IV administration in rat

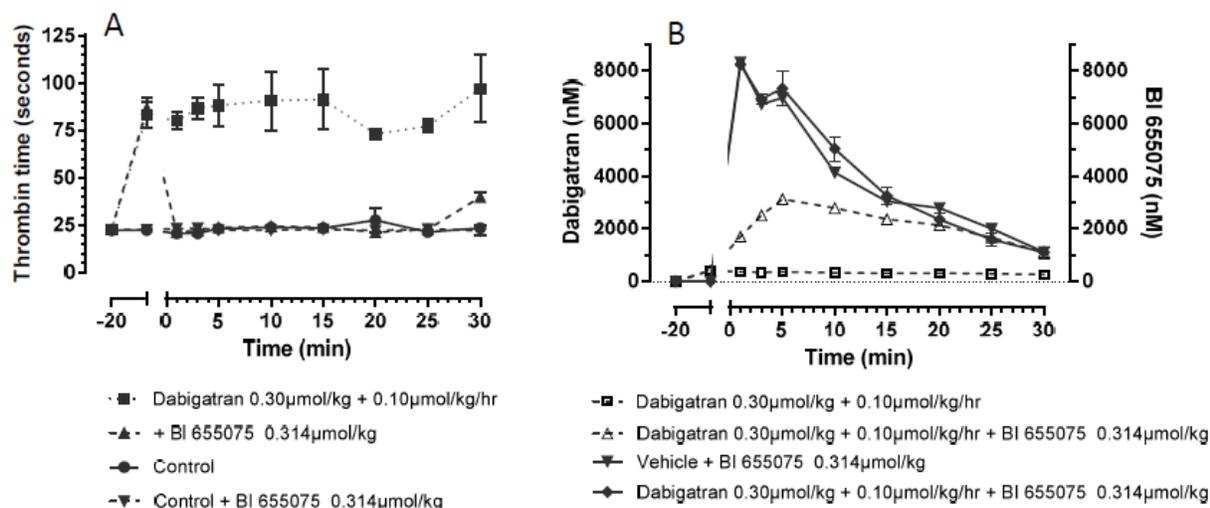
Methods

Neutralization activity of dabigatran by idarucizumab was measured using an assay for thrombin time (seconds).

Results

Dabigatran prolonged thrombin time ~4fold (25sec to 100sec). Addition of idarucizumab at a single bolus dose of equimolar concentration completely reversed the activity within one minute (0.3uM/kg). Plasma levels of the drug are shown in Figure B below.

Figure 7. Neutralization of dabigatran by idarucizumab in rat tail cut bleeding model



(Excerpted from the submission)

Reversal of dabigatran induced bleeding after single oral administration

Methods

Dabigatran etexilate (DE) was administered orally to rats at a dose of 30 mg/kg followed by a single bolus of idarucizumab (33 mg/kg, 0.69 μmol/kg) administered intravenously (at t=0 below; 40 minutes post DE). Following idarucizumab administration a bleeding time assay was performed by making a standardized incision in the rat tail and measuring the length of time required for bleeding to stop. Anticoagulant activity was measured by various clotting time assays (TT, aPTT, and ECT; thrombin time, activated prothrombin time and Ecarin clotting time).

Results

There was a significant reduction in bleeding time prolongation sustained following treatment with idarucizumab after dosing dabigatran (120 minutes post dose). There was also a significant reduction in anticoagulant activity (TT, aPTT, and ECT). dTT is shown in Figure 9A below. Plasma bioanalysis of total dabigatran (shown as solid black lines) and idarucizumab (shown as solid grey lines) are shown in ng/mL in Figure 9B below.

Figure 8. Rat tail bleeding time in the rat tail cut model, single dose

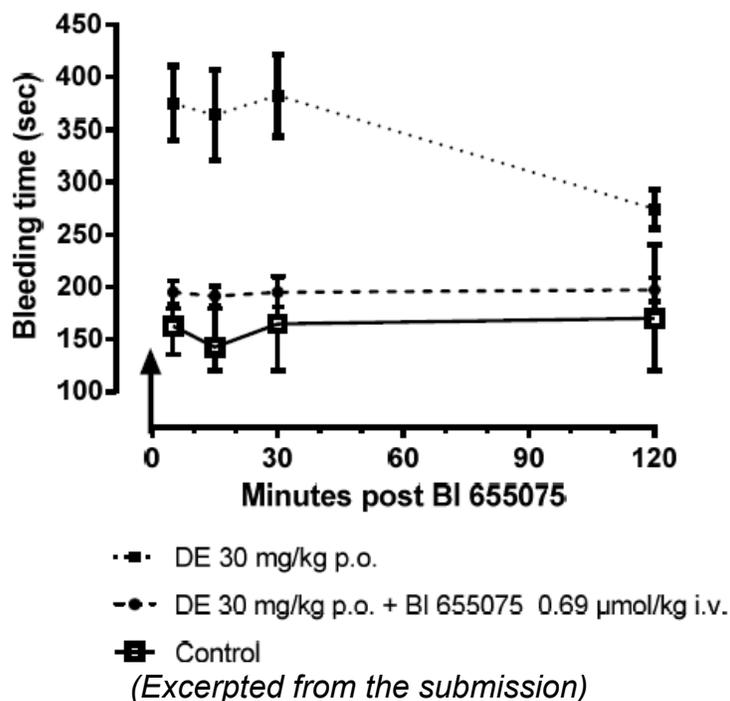
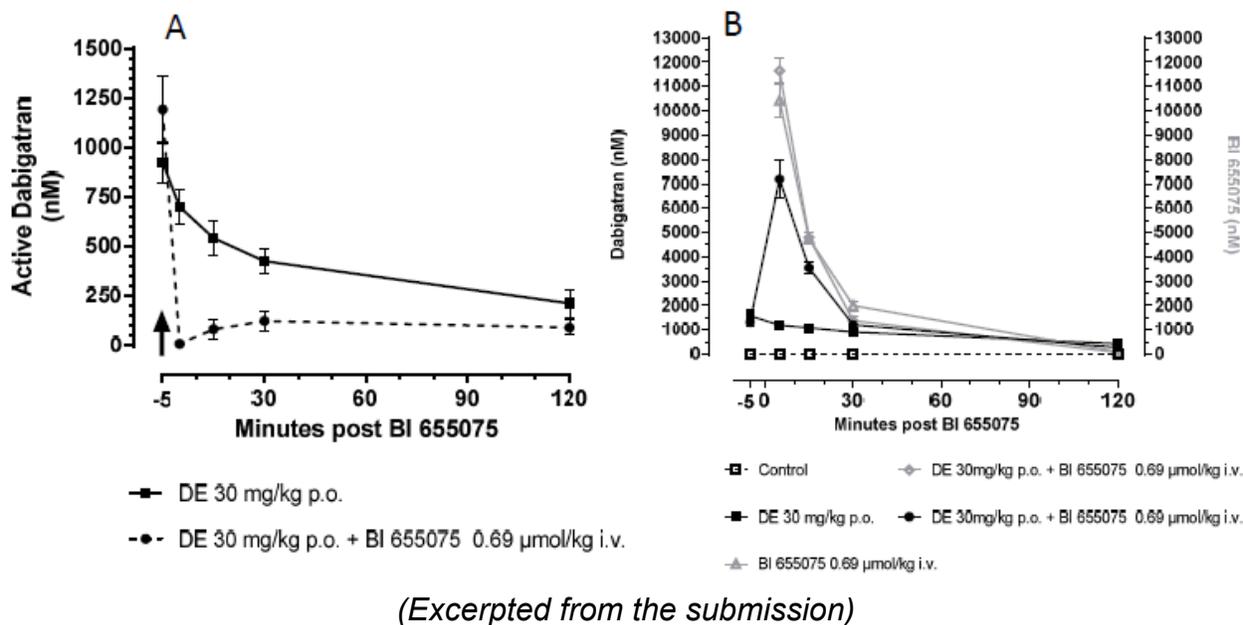


Figure 9. Anticoagulant effects of idarucizumab in rat tail cut model



Reversal of dabigatran induced bleeding after supra-therapeutic dosing (split dose)

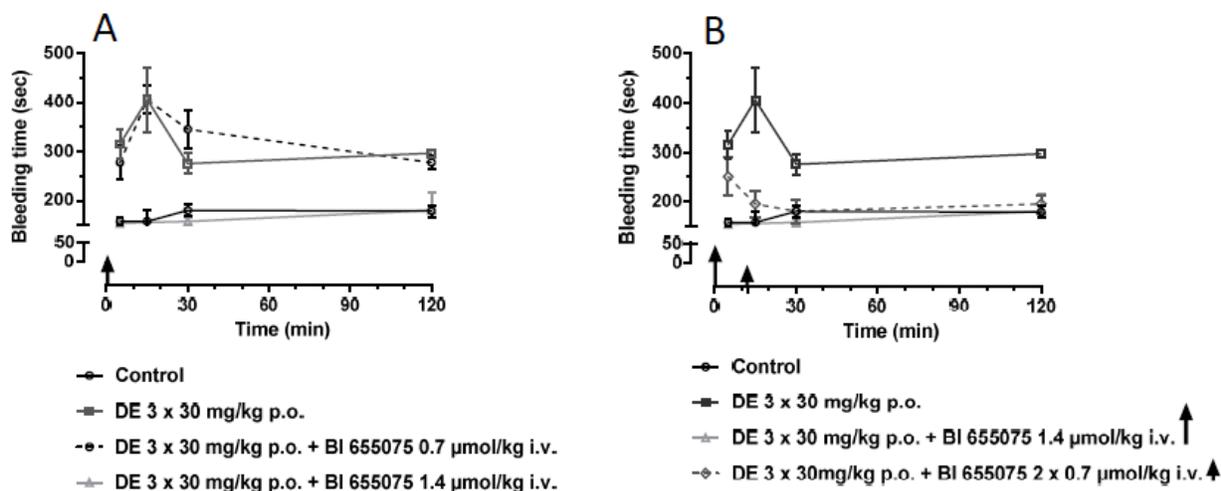
Methods

Binding and clearance of dabigatran by idarucizumab in vivo occurs rapidly so split dosing was tested to determine effectiveness over one large single dose. DE (30 mg/kg) was administered orally 3 times every 8 hours. Idarucizumab was given in increasing split doses, 0.7 and 1.4 $\mu\text{mol/kg}$ or as two 0.7 $\mu\text{mol/kg}$ bolus doses 12 min apart. One hour following the last dose of DE, reversal of dabigatran-prolonged bleeding time and anticoagulation activity by idarucizumab was tested in an in vivo rat tail bleeding model.

Results

Low dose idarucizumab (33 mg/kg) did not have a significant effect on reversal of dabigatran induced bleeding time, while the high dose idarucizumab (66 mg/kg) completely reversed dabigatran induced bleeding time to baseline. When comparing the large bolus dose vs. split dosing, reversal of bleeding time was effective with both methods of dosing and significantly reduced anticoagulation as measured by rat tail bleeding time.

Figure 10. Rat tail bleeding time in rat tail cut model



(Excerpted from the submission)

Specificity of idarucizumab- Effect of idarucizumab on bleeding induced by dabigatran in combination with antiplatelet agents

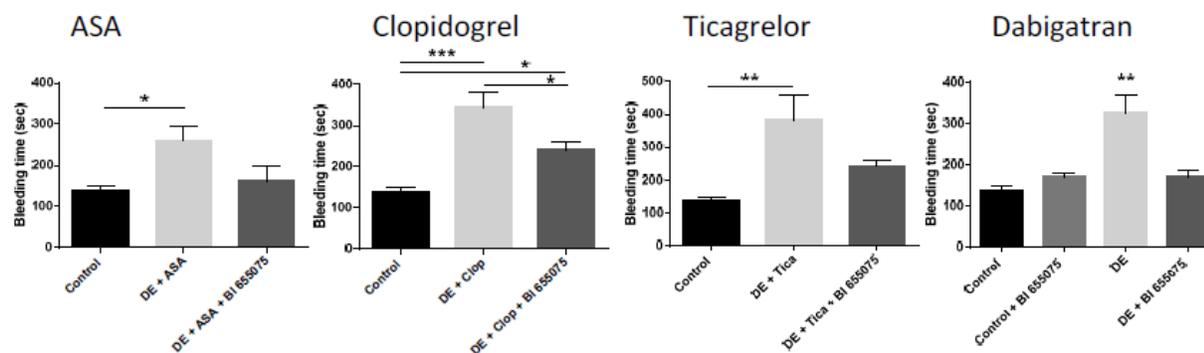
Methods

Antiplatelet agents, acetylsalicylic acid (100 mg/kg, Aspirin®, ASA), clopidogrel (4 mg/kg, Plavix®) or ticagrelor (3 mg/kg, Brilique®) were administered orally to rats and measured ex vivo using aggregometry as a confirmatory assay. Dabigatran etexilate (DE) was also administered orally at a dose of 30 mg/kg following administration of the antiplatelet agents. Idarucizumab (0.7 $\mu\text{mol/kg}$) or vehicle was administered intravenously 45 minutes following DE. Bleeding time was assessed by standardized tail cuts and measuring the time required for bleeding to stop (hemostasis).

Results

The combination of antiplatelet agents together with dabigatran reduced the inhibition of dabigatran induced prolonged bleeding time by idarucizumab compared to dabigatran alone. Antiplatelet agents in the presence of dabigatran may not result in the complete reversal of bleeding/anticoagulation.

Figure 11. Bleeding time following administration of antiplatelet agents with idarucizumab and dabigatran in rat tail cut model



(Excerpted from the submission)

Pig liver trauma models (single and double trauma models below)

Reversal of supratherapeutic levels of dabigatran induced bleeding after hemorrhagic shock, single dose idarucizumab

Methods

Experiments were designed to test doses of idarucizumab in reversing bleeding in a more severe model of trauma of trauma in pigs in exposure to supratherapeutic doses of dabigatran. Briefly, dabigatran etexilate (DE) was administered orally to 18 male Landrace swine (n=18) for three days twice daily at 30 mg/kg. Animals were anesthetized on Day 4 and DE was infused over 90 minutes to achieve supratherapeutic plasma levels. Standardized blunt liver injury was made and the injury bled for 12 minutes to induce hemorrhagic shock. The only intervention made was resuscitation after five minutes with Ringer's solution (to maintain blood pressure). Blood loss was recorded and animals were randomized to different doses of idarucizumab (30, 60, or 120 mg/kg) or vehicle control groups. Serial blood sampling and blood loss was measured over time (4 hours).

Results

Diluted thrombin time was used to measure the anticoagulant activity of dabigatran. Supratherapeutic levels of dabigatran (resulting in systemic levels reaching 2310 nM to 2740 nM, or 1090 ng/mL to 1290 ng/mL) were administered prior to injury and idarucizumab dosing. Blood loss doubled in pigs treated with dabigatran prior to blunt liver trauma; pigs that received vehicle and not idarucizumab, and due to ongoing bleeding there was 100% mortality in this group. Continuous bleeding was associated

with severe shock as characterized by increased lactate levels, decreases in blood bicarbonate levels, decreased mean arterial pressure, and a decrease in hemoglobin, and thrombocytopenia. There was a 47%, 64%, and 62% reduction in blood loss with 30, 60 and 120 mg/kg idarucizumab, respectively, vs the dabigatran-treated group. Survival significantly improved in all dose groups of idarucizumab and dabigatran compared to dabigatran alone, with all of the animals surviving the 4 hours in the 60 and 120 mg/kg dose groups and 5/6 animals surviving in the 30 mg/kg dose group. The 30 and 60 mg/kg dose of idarucizumab were not sufficient to eliminate anticoagulant activity of the suprathereapeutic dosing of active dabigatran but the 120 mg/kg dosing almost completely inhibited anticoagulant activity over the 4 hour period tested as measured by dTT. The 120 mg/kg dose of idarucizumab was approximately equimolar to the total dabigatran infusion (0.905 mg/kg dabigatran was infused over 90 minutes).

Figure 12. Blood loss and survival in pig liver trauma model following suprathereapeutic doses of dabigatran and single dose idarucizumab

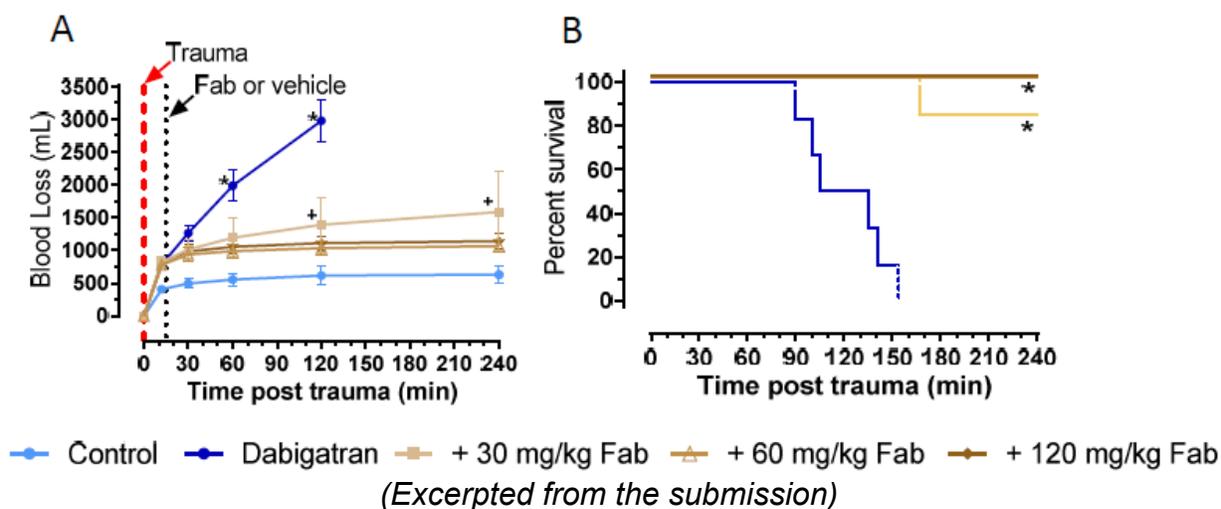


Figure 13. Shock parameters in pig liver trauma model following suprathereapeutic doses of dabigatran and single dose idarucizumab

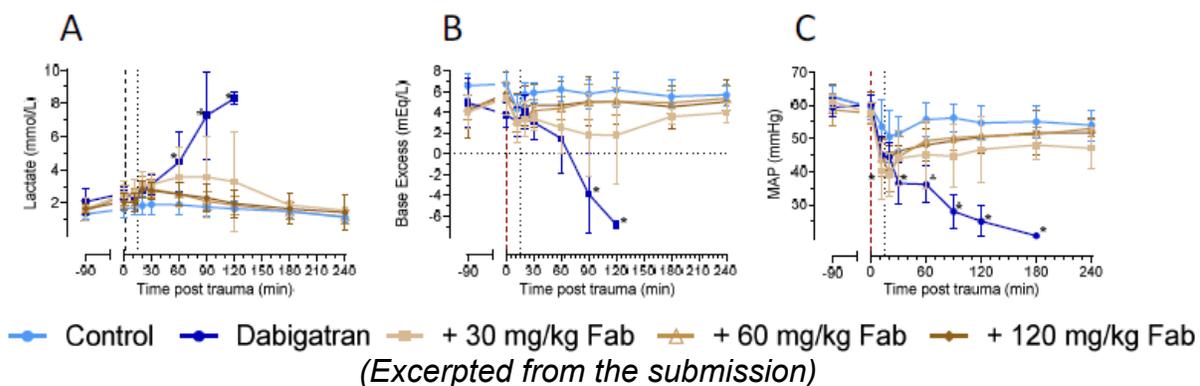
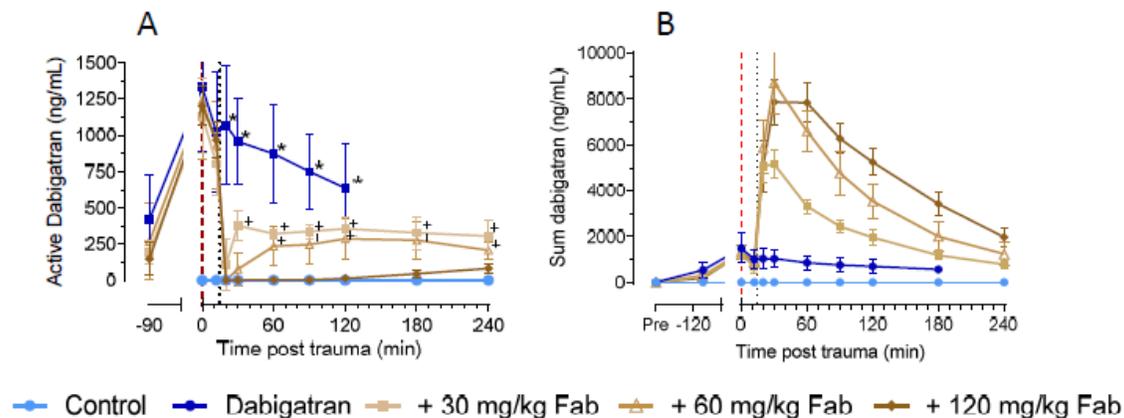


Figure 14. Dabigatran levels in plasma in pig liver trauma model following suprathreshold doses of dabigatran and single dose idarucizumab



(Excerpted from the submission)

Reversal of dabigatran induced bleeding after hemorrhagic shock, split dose, pig liver double trauma model

Methods

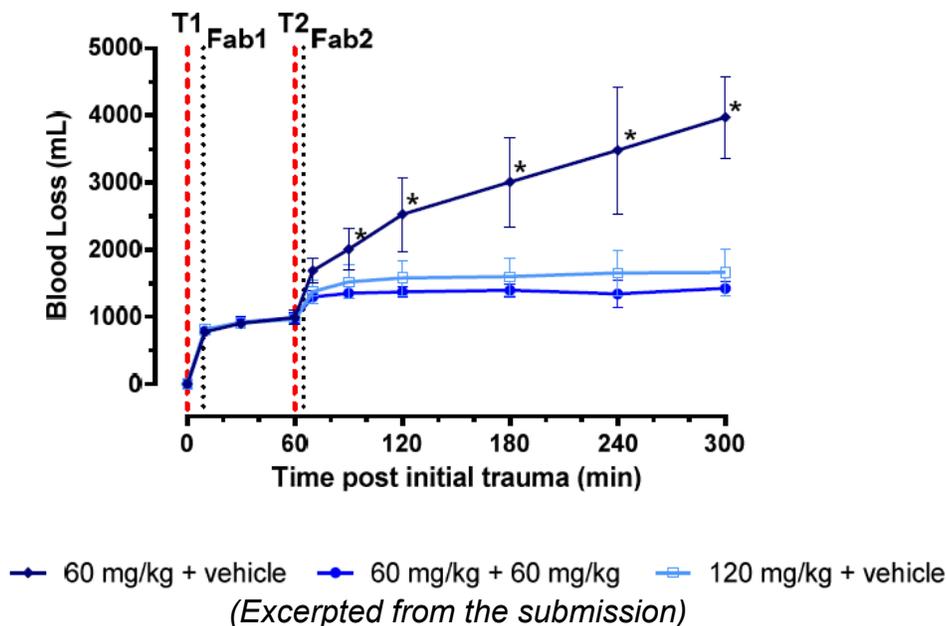
Experiments were performed to determine activity of split dosing over single bolus administration of idarucizumab following dabigatran dosing. Dabigatran etexilate (DE) was administered orally to 18 (6 per dose group) male Landrace swine for three days twice daily at 30 mg/kg. Animals were anesthetized on Day 4 and DE was infused over 90 minutes to achieve ideal plasma levels (1130 +/- 160 ng/mL). Standardized blunt liver injury was made and the injury bled for 12 minutes to induce hemorrhagic shock. The only intervention made was resuscitation after five minutes with Ringer's solution (to maintain blood pressure). Blood loss was recorded and animals were randomized to different doses of idarucizumab (60 mg/kg, 120 mg/kg) or vehicle control groups 15 minutes post injury. A second trauma was made 60 minutes post injury, on a second liver lobe. Fifteen minutes following the second trauma, idarucizumab was administered at 60 mg/kg or vehicle. Serial blood sampling and blood loss was measured over time (4 hours).

Results

Both the 60 mg/kg and 120 mg/kg doses of idarucizumab reduced dabigatran induced blood loss following the first blunt liver trauma (first 60 minutes). Following the second injury, animals started to bleed again, with 83% mortality but with no further bleeding from the first wound site if a clot had formed in the previous time span. Upon administration of the second bolus dose of idarucizumab, 60 mg/kg, there was a rapid reversal of bleeding from the second injury. This response was also observed in the single high dose 120 mg/kg group (n=6/group). In the figure below, the dashed line at t=0, T1 represents the first trauma and T2 the second trauma. The dotted line, Fab1, represents the first dose of idarucizumab, and the second dose Fab 2, or vehicle. Both

the single 120 mg/kg dose (~5 g total dose) or 2x60 mg/kg (2 x 2.5 g total dose) were effective in reversing bleeding, even after two injuries sustained one hour apart.

Figure 15. Blood loss in pig liver double trauma model, split dosing



Specificity of idarucizumab - Hemodilution using colloid and crystalloid volume replacement- Neutralization of dabigatran induced bleeding after hemodilution

Methods

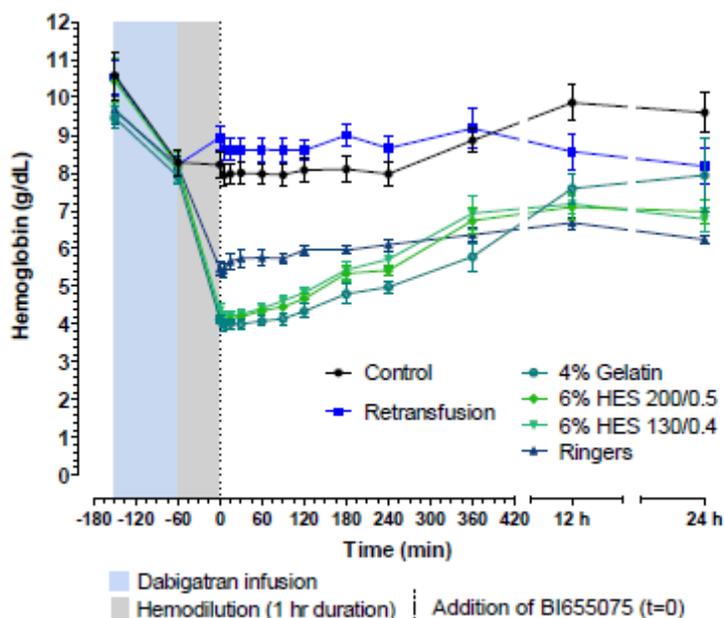
In trauma, volume expanders are used for resuscitation to compensate for blood loss and hemorrhagic shock. Experiments were performed to determine if volume expanders can influence binding of dabigatran to idarucizumab in an in vivo pig liver trauma model (under severe blood loss or hemorrhagic shock). Dabigatran etexilate (DE) was administered to male Landrace pigs (30 mg/kg) twice daily for three days. Animals were anesthetized on Day 4 and DE was infused to achieve an ideal mean plasma level (1130 +/- 160 ng/mL). Following DE infusion, ~50% of total blood volume was withdrawn to mimic hemorrhagic shock. Animals were randomized by the follow groups (n=5): balanced Ringer's solution, 6% hydroxyethyl starch (HES) 130/0.4, 6% HES 200/0.5, 4% gelatin, retransfusion of washed red blood cells (RBCs) or control. Resuscitation was comprised of: 1:1 to blood loss for crystalloids, 25 mL/kg for colloids, and 12 mL/kg for retransfusion. Idarucizumab (30 mg/kg i.v.) was administered immediately following hemodilution (dotted line, t=0) and dabigatran levels (binding to idarucizumab) were followed over time by taking serial blood samples over 24 hours (as measured by dTT assay).

Results

Control and retransfusion samples are the only samples that show normal levels of hemoglobin after hemodilution. Animal weights, blood cell counts, and coagulation

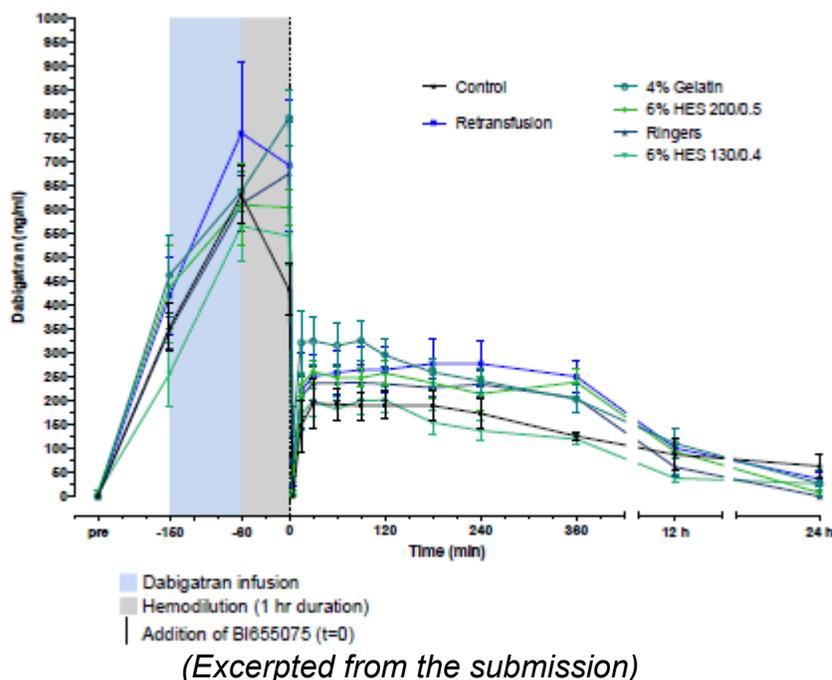
parameters were comparable between all groups, there were no significant differences. Dabigatran values decreased immediately following administration of idarucizumab. In summary, there was no significant difference in the binding of idarucizumab to dabigatran following retransfusion or the volume expanders tested as measured by plasma or active dabigatran. There were only minimal differences in anticoagulation as measured by different assays which can be explained at least in part by the response to hemodilution on coagulation.

Figure 16. Hemoglobin levels over time during hemodilution experiments in pig liver double trauma model



(Excerpted from the submission)

Figure 17. Plasma dabigatran levels in the presence of volume expanders in pig liver double trauma model.



4.2 Secondary Pharmacology

Studies not conducted.

4.3 Safety Pharmacology

Respiratory (GLP):

The pharmacological effects of idarucizumab on the respiratory system were determined in study 12r006 by examining Wistar rats treated i.v. with a single dose of 150 mg/kg or 500 mg/kg, drug or vehicle (10 mL/kg). The vehicle was 25 mM acetate, 240 mM sorbitol, 0.02% Polysorbate 20, pH5.5. Measures of respiratory function (tidal volume, respiratory rate and derived minute volume) were captured using plethysmographs over 30 minute periods at 0, 30, 60, 90, 120, 150, 180, 210, and 240 minutes following dosing.

Results:

- No adverse effects in treatment groups or control; all respiratory measures were within limits for healthy animals at doses up to 500 mg/kg, i.v.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Absorption

Pharmacokinetics and urinary excretion of BI 655075 and the effect of BI 655075 on the pharmacokinetics and urinary excretion of dabigatran in the Wistar Han rat

Key study findings:

- Idarucizumab was rapidly eliminated in the blood following intravenous dosing (initial phase half-life = 0.24hr; terminal phase half-life 5.81 hrs); half-life with dabigatran was 0.2 hrs.
- Dabigatran clearance (CL) was lower in rats treated with idarucizumab (4.36 mL/min/kg) than without (9.16 mL/min/kg).
- Idarucizumab excretion is 21% in the urine 24 hour after dosing and pretreatment with dabigatran.

Study no.:	U12-3083-01
Study report location:	eCTD 4.2.2.2
Conducting laboratory and location:	Boehringer Ingelheim Ridgefield, CT, USA
Date of study initiation:	13 February 2012
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	Idarucizumab, lot # 1257346, purity >95%

Methods: Blood collection by femoral vein; urine into polypropylene containers

Species/strain: HanWistar rats

N: 3 males/group

Dose: 20 mg/kg idarucizumab; 0.2 mg/kg dose dabigatran

Frequency: single dose

Route: intravenous bolus, femoral vein cannula

Volume: (1.39 (idarucizumab or 0.5mL/kg (dabigatran)

Observations and times: 400 µl of blood was collected at various time points. Blood was collected at approximately 0.0333, 0.25, 1, 2, 3, 4, 6, 8, and 24 hrs. after dosing from group 1 (20 mg/kg idarucizumab) rats (See study design table following page). Urine samples were collected in a polypropylene container at intervals of 0-8, 8-24, and 24-48 hours after dosing and volume was determined by weight. Idarucizumab was detected in urine using a qualified ELISA method specifically for Wistar rat urine, similar to the assay for plasma. The ELISA assay was qualified to detect idarucizumab over the range 0.0156 to 0.250 µg/mL with 0.0156 µg/mL as the lower limit of quantitation (LLOQ). Rats dosed with dabigatran only, 0.2 mg/kg, blood was collected at 0.0333, 0.2, 0.283, 1, 2, 3, 4, 6, and 8 hrs. after dosing from (Group 5 rats). For group 6 rats (dabigatran and idarucizumab) 600 µl of blood was collected at 0.0333, 0.2, 1, 3, 6, and 24 hrs. after

dosing from the first 3 rats and at 0.283, 0.5, 2, 4, and 8 hrs. after dosing from the last 3 rats.

Table 3. Study design for pharmacokinetic and excretion study in Wistar rats

Group	End point	Number	Rat ID	Test article	Dose (IV)	Samples	Assay
1	PK	3	1-3	BI 655075	20 mg/kg	Blood	BI 655075
2	Urinary excretion	3	4-6	BI 655075	20 mg/kg	Urine	BI 655075
3	Urinary excretion	3	7-9	Dabigatran	0.2 mg/kg	Urine	Dabigatran
4	Urinary excretion	3	10-12	Dabigatran + BI 655075	0.2 mg/kg + 20 mg/kg	Urine	BI 655075, Dabigatran
5	PK	3	13-15	Dabigatran	0.2 mg/kg	Blood	Dabigatran
6	PK	6	16-21	Dabigatran + BI 655075	0.2 mg/kg + 20 mg/kg	Blood	BI 655075, Dabigatran

(Excerpted from the submission)

A summary of the pharmacokinetic parameters of idarucizumab and dabigatran are shown below. Three groups of male Wistar Han rats (n=3 per group) received an intravenous bolus dose of 0.2 mg/kg of dabigatran, 20 mg/kg of idarucizumab (BI655075), or 0.2 mg/kg dabigatran followed with 20 mg/kg of idarucizumab (BI 655075). In columns three and five below, idarucizumab and dabigatran parameters are shown respectively for the drugs as they are given in combination.

Table 4. Summary pharmacokinetic parameters of idarucizumab and dabigatran in rats

Summary of PK parameters (n=3) of BI 655075 and dabigatran administered alone or in combination				
PK Parameter	BI 655075 ^a	BI 655075 ^b (with dabigatran)	Dabigatran ^a	Dabigatran ^b (with BI 655075)
Dose (mg/kg)	20	20	0.2	0.2
AUC _{0-∞} (nM)	3,730±875	2,990	787±128	1,620
CL (mL/min/kg)	1.95±0.494	2.33	9.16±1.59	4.36
CL _r (mL/min/kg)	0.263±0.068	0.484	5.83±3.42	2.59
V _{ss} (L/kg)	0.0688±0.0291	0.0771	0.561±0.0780	0.180
Terminal t _{1/2} (h)	6.68±0.493	6.34	1.40±0.447	1.36
MRT (h)	0.570±0.0943	0.550	1.03±0.191	0.688
t _{1/2,α} (h) ^c	0.241±0.0202	0.198	Not determined	Not determined
t _{1/2,β} (h) ^c	5.81±0.481	5.15	Not determined	Not determined

^a Mean±SD.^b Parameters were calculated based on composite mean concentration data.^c Compartmental analysis was also conducted to determine the half-lives of BI 655075 in initial and later phases

(b) (4)

*(Excerpted from the submission)***Table 5. Mean percentage of idarucizumab and dabigatran excretion in urine following intravenous dosing in rats**

Summary of urinary excretion (% of dose) of BI 655075 and dabigatran administered alone or in combination				
Interval (h)	BI 655075 urinary excretion		Dabigatran urinary excretion	
	Alone	With dabigatran	Alone	With BI 655075
0-8	13.2±6.08	16.0±5.04	50.9±29.2	42.5±11.2
8-24	1.12±0.76	4.18±3.17	5.99±1.39	15.1±8.87
24-48	0.25±0.38	0.57±0.85	0.39±0.37	1.70±1.22
0-48 (total)	14.5±6.91	20.8±6.68	57.2±30.6	59.3±17.1

*(Excerpted from the submission)***BI 655075: Pharmacokinetic and Pharmacodynamic Study in Rhesus Monkeys Pretreated with Dabigatran Etexilate**

Key study findings:

- Idarucizumab was rapidly eliminated in the blood following intravenous dosing (terminal phase half-life = 5.64h).
- The mean residence time (MRT) was short (1.38 hrs.).
- Idarucizumab clearance (CL) averaged 0.902 mL/min/kg.
- In excretion studies, 10% of the idarucizumab was recovered in the urine of the monkey; and 2% or less of dabigatran with or without idarucizumab was recovered in the urine of the monkey.
- Idarucizumab exposure was not affected by the presence of anti-drug antibodies.

Study no.: U13-3539-01
 Study report location: eCTD 4.2.2.2
 Conducting laboratory and location: Boehringer Ingelheim Ridgefield, CT, USA
 Date of study initiation: 27 June 2013
 GLP compliance: No
 QA statement: No
 Drug, lot #, and % purity: Idarucizumab, lot # E2739F01, purity >95%

Methods: Blood collection by femoral vein (see Table 6);
 Urine was collected using metabolism cages, immediately after the dabigatran etexilate dose and until 48 hrs (24 hrs for Day 3) after dabigatran etexilate dose. Urine was collected on ice on Days 3, 4, 11, and 33 at the following intervals: 0-8, 8- 24, 24-32, and 32-48 hrs.

Species/strain: male naïve rhesus monkeys
 N: 4 males/group
 Dose: 30 or 60 mg/kg idarucizumab; 12 mg/kg dose dabigatran
 Frequency: single dose dabigatran; single and double dosing idarucizumab
 Route: intravenous infusion idarucizumab; oral dabigatran
 Volume: 10 mL/kg for both (I.V. and oral gavage)

Observations and times: Plasma and urine samples were analyzed for idarucizumab using ELISA. For plasma, the detection range for the ELISA was 0.0500 to 0.500 µg/mL with 0.0500 µg/mL as the LLOQ. For urine, the detection range for the ELISA was 0.0250 to 0.250 µg/mL with 0.0250 µg/mL as the LLOQ.

Table 6. Blood sampling and collection times for pharmacokinetic analysis in rhesus monkeys

Occasion	Animals	Sample times
Pre-treatment	All	During pre-test (before dabigatran etexilate was dosed)
Day 3	All	1.5, 3, 6 and 9 hours post-dose
Day 4	All	Pre-dose, 1.5, 2.5 (immediately after IV infusion), 3, 6, 9 and 24 hours post-dose
Day 11	All	Pre-dose, 1.5 (prior to 1 st IV infusion), 1.67 (Immediately after 1 st IV infusion), 3 (prior to 2 nd IV infusion), 3.17 (Immediately after 2 nd IV infusion), 6, 9 and 24 hours post-dose
Day 33	All	Pre-dose, 1.5 (prior to IV infusion), 1.67 (immediately after IV infusion), 3, 6, 9 and 24 hours post-dose

(Excerpted from the submission)

Table 7. Study design for pharmacokinetic and excretion study in rhesus monkeys

Day	Group	Number/ Sex	Dose route	Test Substance	Dose level (mg/kg)	Monkey ID no.
1 – 4	1	4M	Oral Gavage	Dabigatran etexilate	12	205, 207, 209, 211
1 – 4	2	4M	Oral Gavage	Dabigatran etexilate	12	213, 215, 217, 219
4	1	4M	Intravenous Infusion ^a	BI 655075	30	205, 207, 209, 211
4	2	4M	Intravenous Infusion ^a	BI 655075	60	213, 215, 217, 219
8 – 11	1	4M	Oral Gavage	Dabigatran etexilate	12	205, 207, 209, 211
8 – 11	2	4M	Oral Gavage	Dabigatran etexilate	12	213, 215, 217, 219
11	1	4M	Intravenous Infusion ^b	BI 655075	30 + 30	205, 207, 209, 211
11	2	4M	Intravenous Infusion ^b	BI 655075	60 + 60	213, 215, 217, 219
30 – 33	1	4M	Oral Gavage	Dabigatran etexilate	12	205, 207, 209, 211
30 – 33	2	4M	Oral Gavage	Dabigatran etexilate	12	213, 215, 217, 219
33	1	4M	Intravenous Infusion ^c	BI 655075	30	205, 207, 209, 211
33	2	4M	Intravenous Infusion ^c	BI 655075	60	213, 215, 217, 219

^a A 10-min IV infusion was scheduled to be administered at 1.5 hours, but was actually given at 2.33 h after receiving the oral administration of dabigatran etexilate due to an error of timing in dose preparation. Therefore, a third phase of study on Days 30-33 was added to conduct the study with the originally designed timing.

^b Two 10-min IV infusions were administered at 1.5 and 3 h after receiving the oral administration of dabigatran etexilate.

^c A 10-min IV infusion was administered at 1.5 h after receiving the oral administration of dabigatran etexilate.

(Excerpted from the submission)

Table 8. Summary pharmacokinetics of idarucizumab (I.V.) pretreated with dabigatran (oral gavage) in rhesus monkeys

Summary PK parameters of BI 655075 after IV dosing in monkeys (n=4/group) pretreated with PO dabigatran etexilate MS (Study no. DDB0210)								
PK Parameter	Group	Dose (mg/kg)	Descriptive statistics	Day 4 single dose at 2.33 h	Day 11 two doses at 1.5 and 3 h	Day 33 ^b single dose at 1.5 h	Overall mean, SD, %CV	
C_{max} (nM)	1	30	Mean	10,100	11,300	10,100	Not applicable	
			SD	746	520	395		
			%CV	7.38	4.58	3.93		
	2	60	Mean	15,000	21,200	19,700		
SD			2,560	5,390	705			
%CV			17.0	25.5	3.59			
$AUC_{0-\infty}$ (nM•h)	1	30	Mean	11,400	27,100	11,600		
			SD	820	3,610	305		
			%CV	7.17	13.3	2.63		
	2	60	Mean	26,100	54,200	23,700		
SD			5,320	13,600	3,360			
%CV			20.4	25.1	14.2			
t_{max} (h) ^a	1	30	Median	2.5	3.17	1.67		
			Range	2.5-2.5	1.67-3.17	1.67-1.67		
	2	60	Median	2.5	3.17	1.67		
			Range	2.5-2.5	1.67-3.17	1.67-1.67		
CL (mL/min/kg)	1	30	Mean	0.919	0.784	0.902	0.902 0.177 19.6	
			SD	0.0670	0.118	0.0241		
			%CV	7.29	15.1	2.67		
	2	60	Mean	1.09	0.822	0.895		
SD			0.231	0.266	0.112			
%CV			21.1	32.4	12.5			
V_{ss} (L/kg)	1	30	Mean	0.0739	0.0592	0.0719		0.0763 0.0265 34.8
			SD	0.0167	0.0181	0.0103		
			%CV	22.6	30.5	14.3		
	2	60	Mean	0.107	0.0712	0.0740		
SD			0.0323	0.0411	0.00866			
%CV			30.2	57.7	11.7			
$t_{1/2}$ (h)	1	30	Mean	5.59	5.62	6.15	5.64 0.585 10.4	
			SD	0.717	0.457	0.890		
			%CV	12.8	8.13	14.5		
	2	60	Mean	5.29	5.32	6.01		
SD			0.348	0.552	0.252			
%CV			6.58	10.4	4.19			
MRT (h)	1	30	Mean	1.34	1.24	1.33		1.38 0.227 16.4
			SD	0.286	0.186	0.187		
			%CV	21.4	15.0	14.1		
	2	60	Mean	1.61	1.37	1.38		
SD			0.195	0.298	0.0899			
%CV			12.1	21.7	6.51			

^a The time was based on the PO dosing of dabigatran etexilate. BI 655075 was administered via a 10-min IV infusion.

^b n=3 for Group 1 on Day 33, due to early termination of one monkey.

(Excerpted from the submission)

Table 9. Mean percentage of idarucizumab excretion in urine in dabigatran pretreated rhesus monkey

Summary urinary excretion of BI 655075 (% of dose) after IV dosing in monkeys (n=4/group) pretreated with PO dabigatran etexilate MS (study no. DDB0210)			
Group	Dose	Mean ± SD (%CV)	Overall Mean ± SD (%CV)
1	30 mg/kg	10.3 ± 6.35 (61.4%)	9.98 ± 6.32 (63.3%)
2	60 mg/kg	9.62 ± 4.53 (47.0%)	

(Excerpted from the submission)

Table 10. Summary pharmacokinetics of dabigatran (oral gavage) with and without idarucizumab (I.V.)

Summary PK parameters of dabigatran in monkeys (n=4/group) receiving oral dabigatran etexilate with and without IV BI 655075 treatment (study no. DDB0210)							
PK Parameter	Group	BI 655075 Dose (mg/kg)	Descriptive statistics	Day 3	Day 4	Day 11	Day 33 ^a
				No BI 655075	one BI 655075 dose at 2.33h	two BI 655075 doses at 1.5 and 3 h	one BI 655075 dose at 1.5 h
C_{max} (nM)	1	30	Mean	120	476	701	513
			SD	122	275	637	478
			%CV	102	57.8	90.9	93.2
	2	60	Mean	245	1,320	1,150	2,500
			SD	294	709	1,030	2,490
			%CV	120	53.6	89.6	99.8
AUC_{0-24} (nM·h)	1	30	Mean	535	1,740	2,100	1,710
			SD	478	777	1,280	1,410
			%CV	89.3	44.7	61.1	82.5
	2	60	Mean	1,030	4,650	4,430	6,200
			SD	1,060	2,260	4,190	6,410
			%CV	102	48.6	94.4	103
t_{max} (h)	1	30	Median	3.75	2.5	2.34	1.67
			Range	1.5-6	2.5-3	1.67-6	1.67-6
			Median	2.25	3	1.67	1.67
	2	60	Range	1.5-6	2.5-3	1.67-3	1.67-1.67

(Excerpted from the submission)

Table 11. Summary of pharmacokinetics of dabigatran (with glucuronides) with and without idarucizumab in monkeys

Summary PK parameters of sum dabigatran (dabigatran + glucuronides) in monkeys (n=4/group) receiving oral dabigatran etexilate with and without IV BI 655075 treatment (Study no. DDB0210)							
PK Parameter	Group	BI 655075 Dose (mg/kg)	Descriptive statistics	Day 3	Day 4	Day 11	Day 33 ^a
				No BI 655075	one BI 655075 dose at 2.33h	two BI 655075 doses at 1.5 and 3 h	one BI 655075 dose at 1.5 h
C_{max} (nM)	1	30	Mean	672	2,120	2,570	1,690
			SD	693	1,360	2,470	1,640
			%CV	103	64.1	96.2	96.9
	2	60	Mean	934	4,730	4,010	6,700
			SD	963	2,540	2,920	5,390
			%CV	103	53.8	72.9	80.5
AUC_{0-24} (nM·h)	1	30	Mean	4,220	6,860	5,810	4,880
			SD	3,540	3,790	4,150	4,250
			%CV	83.9	55.2	71.4	87.1
	2	60	Mean	6,510	15,900	11,400	17,000
			SD	6,010	8,000	9,520	15,600
			%CV	92.4	50.3	83.2	91.8
t_{max} (h)	1	30	Median	3.75	2.5	1.67	1.67
			Range	1.5-6	2.5-2.5	1.67-3	1.67-1.67
	2	60	Median	2.25	2.5	1.67	1.67
			Range	1.5-6	2.5-3	1.67-1.67	1.67-1.67

^a n=3 for Group 1 on Day 33, due to early termination of one monkey.

(Excerpted from the submission)

Table 12. Urinary excretion of dabigatran with and without idarucizumab

Summary urinary excretion of sum dabigatran (% of dose) in monkeys (n=4/group) with and without BI 655075 treatment (Study no. DDB0210)					
Group, Dabigatran dose	Monkey ID	Day 3	Day 4	Day 11	Day 33
		No BI 655075	one BI 655075 dose at 2.33h	two BI 655075 doses at 1.5 and 3 h	one BI 655075 dose at 1.5 h
1, 12 mg/kg ^a	Mean	1.38	1.40	1.03	1.07
	SD	1.29	1.06	0.91	0.94
	%CV	93.2	75.9	88.0	87.8
2, 12 mg/kg ^a	Mean	1.47	1.83	1.83	2.41
	SD	1.18	0.72	1.34	2.46
	%CV	80.4	39.3	73.1	102
Overall Mean		1.43	1.59		
Overall SD		1.15	1.31		
Overall %CV		80.3	81.9		

^a The dose was based on the dabigatran etexilate free base. It was equivalent to 9.0 mg/kg of active component dabigatran, which was used for calculation. The BI 655075 dose was 30 mg/kg for Group 1 and 60 mg/kg for Group 2.

(Excerpted from the submission)

Table 13. Immunogenicity to idarucizumab following I.V. dosing in rhesus monkeys

Summary immunogenicity (ADA) to BI 655075 after IV dosing in monkeys (Study no. DDB0210)			
Group (BI 655075 dose)	Money ID	Day 1 (pretreatment)	Day 30 (predose)
Group 1 (30 mg/kg)	205	Negative	Putative Positive
	207	Negative	Putative Positive
	209	Negative	Putative Positive
	211	No Sample ^a	Negative
Group 2 (60 mg/kg)	213	Negative	Putative Positive
	215	Negative	Putative Positive
	217	Negative	Negative
	219	No sample ^a	Putative Positive

^a No sample due to the limitation of blood volume for sampling.

In the absence of idarucizumab, the assay sensitivity was 0.29 ng/mL in monkey plasma. The drug tolerance of the assay in monkey plasma was such that 100 ng/mL ADA positive control could be detected in the presence of up to 125 µg/mL idarucizumab. False positive anti-idarucizumab antibody signals were detected using a sensitive ECL method (method # BBM-12-1002) in plasma samples that were taken from monkeys treated only with dabigatran etexilate.

(Excerpted from the submission)

5.2 Toxicokinetics

Toxicokinetic parameters were reviewed in the context of the general toxicology studies.

6 General Toxicology

6.1 Single-Dose Toxicity

Studies not reviewed.

6.2 Repeat-Dose Toxicity

Study title: BI 655075: Toxicity Study by Intravenous Administration to Han Wistar Rats for 4 Weeks Followed by a 4 Week Recovery Period

Study no.:	DDB0150/BI no. 11R141
Study report location:	eCTD 4.2.3.2
Conducting laboratory and location:	 (b) (4)
Date of study initiation:	January 31, 2012
GLP compliance:	Signed and included
QA statement:	Signed and included
Drug, lot #, and % purity:	BI-655075-01, DAB-FTOX-01, assumed 100% purity

Key Study Findings

- There were no mortalities during the study.
- Idarucizumab related changes in hematology findings included increased white blood cells and lymphocytes at doses of 150 and 500 mg/kg at terminal sacrifice, which were present in high dose animals at the end of the recovery period.
- Toxicologically significant idarucizumab related changes in clinical chemistry findings included decreased blood urea and creatinine levels both of which were present in high dose male animals only at the recovery period (Day 58, terminal sacrifice). Values for female animals returned to levels similar to controls.
- Female rats dosed at both 150 and 500 mg/kg had elevated absolute thymus weights, thymus weight relative to body weight and thymus weight relative to brain weight.
- Histopathology findings included atrophy in the pancreas at high doses (500 mg/kg).

Methods

Doses: 0, 150, 500 mg/kg/day
 Frequency of dosing: Once daily
 Route of administration: Intravenous injection (bolus- right caudal vein)
 Dose volume: 10mL/kg
 Formulation/Vehicle: 25 mM acetate, 240 mM sorbitol, and 0.02% polysorbate 20,pH 5.5
 Species/Strain: RccHan™:WIST strain Rat
 Number/Sex/Group: 15 sex/group; 10/sex/group recovery
 Age: 36-42days
 Weight: 103-135g
 Satellite groups: 3/sex/control; 9/sex/treatment
 Unique study design: N/A
 Deviation from study protocol: N/A

Observations and Results

Table 14. Study design for 4-week repeat dose toxicology study in rats

The study consisted of one Control and two treated groups of rats, identified as follows:

Group	Treatment	Dose# (mg/kg/day)	No. of animals		Main study (4 weeks) Animal numbers		Cage numbers	
			Male	Female	Male	Female	Male	Female
1	Control	0	15	15	1-15	87-101	1-3	21-23
2	BI 655075	150	15	15	29-43	115-129	7-9	27-29
3	BI 655075	500	15	15	53-67	139-153	13-15	33-35

Expressed in terms of test material as supplied.

(Excerpted from the submission)

Mortality

There were no mortalities during the study.

Clinical Signs

Unremarkable

Body Weights

Unremarkable

Feed Consumption

Unremarkable

Ophthalmoscopy

Unremarkable

ECG

Unremarkable

Hematology**Table 15. Erythroid parameters in 4 week rat toxicology study**

Hematology parameter	% change from control at sacrifice			
	males		females	
dose (mg/kg/day)	150	500	150	500
RBC (x10¹²/L)				
Day 14	-2.0	-4.7	-1.8	0.4
Day 29	3.1	4.4	-1.6	0.3
Day 58 (Recovery)		7.8		2.2
HCT (L/L)				
Day 14	-1.7	-4.8	-1.6	1.6
Day 29	3.8	4.7	0.2	2.5
Day 58 (Recovery)		6.1		3.8
MCH (pg)				
Day 14	2.1	4.8	-1.1	-1.1
Day 29	0.5	-1.1	2.1	1.1
Day 58 (Recovery)		-3.8		-2.5
MCHC (g/dL)				
Day 14	1.8	4.5	-1.2	-1.2
Day 29	0.0	-0.9	0.0	-1.2
Day 58 (Recovery)		-2.6		-3.2
Hemoglobin (g/dL)				
Day 14	0.0	0.0	-2.7	0.7
Day 29	4.1	4.1	0.7	1.4
Day 58 (Recovery)		3.3		0.6
Reticulocytes (%)				
Day 14	-2.4	2.8	17.7	6.8
Day 29	-1.9	6.8	28.3	23.3
Day 58 (Recovery)		-14.0		7.0

P<0.05

P<0.01

Day 14 and Day 29 were during dosing phase (main study). Day 29 was last day of dosing. Day 58 was last day of recovery.

Table 16. White blood parameters and coagulation findings in 4 week rat toxicology study

Hematology parameter	% change from control at sacrifice			
	males		females	
sex				
dose (mg/kg/day)	150	500	150	500
WBC (x10⁹/L)				
Day 14	-5.4	-6.0	7.4	20.1
Day 29	23.3	20.3	37.7	38.4
Day 58 (Recovery)		31.7		23.0
Lymphocytes (x10⁹/L)				
Day 14	-8.2	-7.7	7.4	22.3
Day 29	26.0	25.8	43.4	47.8
Day 58 (Recovery)		42.4		24.1
Platelets (x10⁹/L)				
Day 14	-1.4	4.7	4.0	8.8
Day 29	1.2	11.8	9.5	7.9
Day 58 (Recovery)		-7.3		-1.2
PT (sec)				
Day 29	-1.0	5.0	1.4	2.3
Day 58 (Recovery)		-1.5		-1.4

P<0.05

P<0.01

Day 14 and Day 29 were during dosing phase (main study). Day 29 was last day of dosing. Day 58 was last day of recovery.

Clinical Chemistry

Table 17. Clinical chemistry findings in 4 week rat toxicology study

% change from control at sacrifice				
sex	males		females	
dose (mg/kg/day)	150	500	150	500
Clinical chemistry parameter				
Urea (mmol/L)				
Day 14	2.5	-1.8	-2.7	-6.9
Day 29	-10.4	-21.6	-8.6	-27.9
Day 58		-23.1		-3.9
Creatinine (µmol/L)				
Day 14	3.3	3.3	-7.7	-7.7
Day 29	-8.8	-17.6	-16.7	-26.2
Day 58		-14.3		0.0
Cholesterol (mmol/L)				
Day 14	-4.2	2.5	6.5	7.0
Day 29	11.5	15.8	11.3	11.9
Day 58		0.5		4.8
Total Protein (g/L)				
Day 14	-1.6	0.0	1.6	6.3
Day 29	4.9	4.9	0.0	4.5
Day 58		0.0		-1.4
Albumin (g/L)				
Day 14	0.0	3.2	0.0	35.3
Day 29	6.7	6.7	0.0	2.9
Day 58		-3.2		0.0
A/G (ratio)				
Day 14	6.3	7.4	0.0	2.7
Day 29	4.3	5.3	-0.9	-1.8
Day 58		-6.3		0.0
Phosphorus (mmol/L)				
Day 14	-5.6	-1.4	11.9	10.5
Day 29	4.1	5.8	21.3	7.7
Day 58		13.5		-13.7

P<0.05

P<0.01

Day 14 and Day 29 were during dosing phase (main study). Day 29 was last day of dosing. Day 58 was last day of recovery.

Urinalysis

Unremarkable

Gross Pathology

Unremarkable

Organ Weights**Table 18. Organ weight changes in the thymus in the 4 week rat toxicology study**

Organ	% change from control at sacrifice (g/kg body weight)			
	males		females	
dose (mg/kg/day)	150	500	150	500
Thymus (absolute)				
Day 29	-0.1	-0.8	19.3	9.8
Day 58		-6.3		-4.4
Thymus (%BdWgt)				
Day 29	-2.5	-1.2	16.9	10.7
Day 58		-8.7		-4.2
Thymus (% BrainWgt)				
Day 29	0.0	-2.1	20.1	11.6
Day 58		-5.9		-8.0

P<0.01

Day 29 was the day of terminal necropsy during the main study. Day 58 was terminal necropsy for the recovery period.

%BdWgt is the change in organ weight based on change relative to body weight.

% BrainWgt is the change in organ weight based on change relative to brain weight.

Histopathology

Adequate Battery: Yes
 Peer Review: Yes
 Histological Findings

Table 19. Histopathology findings in 4 week rat toxicology study- main study

Treatment related microscopic findings							
		males			females		
		0	150	500	0	150	500
idarucizumab group (mg/kg/day)		0	150	500	0	150	500
Number of animals examined		15	15	15	15	15	15
grade							
Thyroids	ectopic thymic tissue	1	1	3	1		3
Pancreas	acinar atrophy, focal	1		1			1
Kidneys	pelvic dilatation	1	2				

Grade key: 1 minimal, 2 mild, 3 moderate, 4 severe

Table 20. Histopathology findings in 4 week rat toxicology study- recovery

Treatment related microscopic findings- recovery							
		males			females		
		0	150	500	0	150	500
idarucizumab group (mg/kg/day)		0	150	500	0	150	500
Number of animals examined		15	15	15	15	15	15
grade							
Thyroids	ectopic thymic tissue	1					1

Grade key: 1 minimal, 2 mild, 3 moderate, 4 severe

Special Evaluation- Irwin Screen

The Irwin screen for neurobehavior and body temperature was unremarkable.

Special Evaluation-Immunogenicity

Anti-drug antibodies (ADA) were positively detected in 26%, 38%, and 56% of rats in the control, 150 mg/kg, and 500 mg/kg dose groups, respectively.

Following recovery (Day 57) anti-drug antibodies were positively detected in 5% and 60% in the control and 500 mg/kg dose groups of rats, respectively. There were no gender differences in detection of anti-drug antibodies in rats. In toxicokinetic rats (TK), 15 out of 35 idarucizumab treated rats were positive for ADA, however this did not result in differences in idarucizumab exposure during the dosing period (between Day 1 and Day 28).

Toxicokinetics

- Increases in idarucizumab exposure were proportional to increases in dose from 150 mg/kg to 500 mg/kg (C_{max} and AUC) on Days 1 and 28 (with the exception of C_{max} in male rats on Day 28).
- Idarucizumab exposure was similar between male and female rats with the exception a lower C_{max} on Day 28 in the 150 mg/kg/day dosing group in females compared to males.
- There were no apparent differences in exposure when comparing Days 1 and 28 in both C_{max} and AUC (>2 fold).

Table 21. Toxicokinetic parameters of idarucizumab in rats following I.V. injection (ug/mL)

TK Parameter ^a	Day	Sex	BI 655075 dose (mg/kg/day)	
			150	500
C _{max} (ug/mL)	1	Male	2,530	7,520
		Female	2,560	8,510
	28	Male	1,590 ^b	9,420
		Female	2,460	7,920
AUC ₀₋₂₄ (ug·h/mL)	1	Male	1,300	3,950
		Female	1,260	4,010
	28	Male	1,180	5,070
		Female	1,330	5,220
t _{max} (h)	1	Male	0.05	0.05
		Female	0.05	0.05
	28	Male	0.05	0.05
		Female	0.05	0.05

^a TK parameters were calculated from composite concentrations data with up to 3 rats/sex/timepoint/group. n<3 at a few time points or occasions due to death or dosing difficulty in TK rats.

^b C_{max} was underestimated for male rats at 150 mg/kg on Day 28 due to delayed sampling (approximately 5-10 min delay).

(Excerpted from the submission)

Prior to dosing, 10 of 169 rats (5.9%) screened ADA positive which is in line with the expected 5% false positive rate for this assay. At the end of the dosing or experimental phase, (Days 28-30), ADA positive rats in the control, 150 mg/kg, and 500 mg/kg dose groups were reported at 26%, 38%, and 56%, respectively. At the end of the recovery phase (Day 58), the reported percentage for ADA positive rats in the control and 500 mg/kg dose groups were 5% and 60%, respectively.

Table 22. Summary of screening assay for anti-drug antibodies in 4 week rat toxicology study (main study, recovery, and TK satellite)

Group	Dose (mg/kg)	Sex	No. of rat screened ADA positive / No. of rats sampled					
			Pre-treatment	Day 15	End of dosing			Recovery
			Day 0		Day 28	Day 29	Day 30	Day 57
1	0	Male	3/28	1/3	-- ^a	5/18	--	0/10
		Female	1/28	0/3	0/3	--	4/14	1/10
		Combined	4/56	1/6	9/35			1/20
2	150	Male	3/24	3/8	--	9/23	--	--
		Female	0/24	1/9	1/9	--	8/15	--
		Combined	3/48	4/17	18/47			--
3	500	Male	2/34	4/9	--	11/24	--	6/10
		Female	1/31	4/9	4/9	--	12/15	6/10
		Combined	3/65	8/18	27/48			12/20

^a No sample on this day.

(Excerpted from the submission)

Table 23. Summary of screening assay for anti-drug antibodies in 4 week rat toxicology study in TK satellite rats

Group	Dose (mg/kg)	Sex	No. of rat screened ADA positive / No. of TK rats sampled			
			Pre-treatment		End of dosing	
			Day 0		Day 28	Day 29
1	0	Male	0/3		-- ^a	0/3
		Female	0/3		0/3	--
		Combined	0/6		0/6	
2	150	Male	1/9		--	5/8
		Female	0/9		1/9	--
		Combined	1/18		6/17	
3	500	Male	0/9		--	5/9
		Female	1/9		4/9	--
		Combined	1/18		9/18	

^a No sample on this day.

(Excerpted from the submission)

Dosing Solution Analysis

Concentration and Stability

Stability was confirmed in standard storage buffer for idarucizumab in 25 mM acetate, 220 mM sorbitol and 0.02% polysorbate 20, pH 5.5 formulations at nominal concentrations of 7.5 mg/mL, 15 mg/mL and 50 mg/mL in (b) (4) storage for (b) (4) and (b) (4) storage for up to (b) (4). The mean concentrations of idarucizumab in solution analyzed during the study were within acceptability criteria of (b) (4) % of nominal concentrations, indicating accurate formulation.

Table 24. Dosing solution analysis for concentration in 4 week rat toxicology study

Occasion	Group	Nominal inclusion (mg/mL)	Analysed concentration (mg/mL)			RME (%)	CV (%)
			Analysis 1	Analysis 2	Mean		
Day 1	1	0	(b) (4)				
	2	15					
	3	50					
Day 28	1	0					
	2	15					
	3	50					

RME Relative mean error, representing the deviation from nominal
 CV Coefficient of variation
 ND Not detected

(Excerpted from the submission)

Table 25. Dosing solution analysis for stability in 4 week rat toxicology study

Nominal inclusion (mg/mL)	Bottle No.	Storage conditions			Analysed concentration (mg/mL)			CV (%)	Relative mean error (%)	
		Day	Hour	°C	Sample 1	Sample 2	Mean		A	B
7.5	1	(b) (4)								
	1									
	2									
	2									
	2									
	2									
15	1									
	1									
	2									
	2									
	2									
	2									
50	1									
	1									
	2									
	2									
	2									
	2									

CV Coefficient of variation
A Relative mean error, representing the deviation from nominal
B Relative mean error, representing the deviation from time zero

(Excerpted from the submission)

Study title: Report BI 655075 and Dabigatran Etxilate: Toxicity Study by Intravenous and Oral Gavage Administration to Rhesus Monkeys for 14 Days Followed by a 4 Week Recovery Period and Dabigatran Etxilate Re-Administration

Study no.: DDB0331/n00230533
Study report location: eCTD 4.2.3.2
Conducting laboratory and location:  (b) (4)
Date of study initiation: December 5, 2013
GLP compliance: Signed and included
QA statement: Signed and included
Drug, lot #, and % purity: BI 655075-01; Lot # 207733; assumed 100% purity

Key Study Findings

- There were no mortalities during the study
- There were no major target organs of toxicity.
- There was no evidence of a prothrombic effect of idarucizumab in monkeys based on the comparison of measured concentrations of respective markers D-dimer and F1+2 between control and treatment groups.
- There was no evidence in any of the three complement assays analyzed: Bb, C3a and C4a. All values were within normal ranges for all dose groups.
- Antidrug antibodies were detected in 9/10 monkeys dosed at 150 mg/kg idarucizumab (with dabigatran) and 3/10 of monkeys dosed at 150 mg/kg idarucizumab (with and without dabigatran; groups combined). The presence of antidrug antibodies did not affect exposure to idarucizumab.

Methods

Doses:	150 or 500 mg/kg
Frequency of dosing:	Once daily
Route of administration:	Intravenous injection, slow bolus; left and right cephalic veins, left and right saphenous veins
Dose volume:	10mL/kg
Formulation/Vehicle:	Formulation: 50 mg/mL solution in 25 mM acetate, 220 mM sorbitol and 0.02% polysorbate 20 (Tween 20), pH 5.5; oral vehicle: 0.5% hydroxyethylcellulose; intravenous vehicle: Placebo buffer (25 mM acetate, 240 mM sorbitol, 0.02% polysorbate 20, pH 5.5), Lot number DAB-488
Species/Strain:	rhesus monkeys
Number/Sex/Group:	5/sex/group
Age:	40 to 47 months
Weight:	Males: 3.41 kg to 6.36 kg; Females: 3.25 kg to 5.01 kg
Satellite groups:	N/A
Unique study design:	N/A
Deviation from study protocol:	None

Table 26. Study design for 2 week toxicity study in rhesus monkeys

Group	Treatment	Dose (mg/kg/day)	Nominal concentration (mg/mL)	Formulated concentration (mg/mL)	Volume dose (mL/kg)
1	Oral vehicle or placebo buffer	0	0	0	10
2	Oral vehicle or BI 655075	0	0	0	10
3	dabigatran etexilate and BI 655075	500	50	50	10
4	dabigatran etexilate and BI 655075	12	1.2	1.38	10
		150	15	15	10
		12	1.2	1.38	10
		500	50	50	10

(Excerpted from the submission)

Observations and Results**Mortality**

There were no mortalities during the study.

Clinical Signs

There were no clinical signs attributable to idarucizumab or dabigatran. Non-drug related clinical signs were related to dosing and administration of the drug (vomiting, injection site findings).

Body Weights

Unremarkable

Feed Consumption

Unremarkable

Ophthalmoscopy

Unremarkable

ECG

Unremarkable

Hematology

Unremarkable

Clinical Chemistry

Unremarkable

Urinalysis

Unremarkable

Gross Pathology

Unremarkable

Organ Weights**Table 27. Organ weight changes in 2 week toxicity study in rhesus monkey**

	males			females		
dabigatran group (mg/kg/day)	0	12	12	0	12	12
idarucizumab group (mg/kg/day)	500	150	500	500	150	500
Spleen						
Day 14	-2.228	-22.65	-26.48	22.968	-0.407	8.894
P<0.01						

Histopathology

Adequate Battery: Yes

Peer Review: Signed and Included

Histological Findings

Although there were a number of histological findings in the monkey study at both 150 mg/kg and 500 mg/kg they were not present in the recovery group animals and these findings had no correlation to other pathological changes in blood chemistry, clinical chemistry, or gross pathology. They were incidental.

Table 28. Histopathology findings in 2 week toxicity study in rhesus monkey (main study)

Treatment related microscopic findings										
			males				females			
dabigatran group (mg/kg/day)			0	0	12	12	0	0	12	12
idarucizumab group (mg/kg/day)			0	500	150	500	0	500	150	500
Number of animals examined			3	3	3	3	3	3	3	3
Organ	Finding	Grade								
Heart	Inflammatory cells, myocardial	1	1		1	2				1
	mineralization, myocardium	1							1	
Kidneys	cysts cortical	1							1	
	cysts papilla	1							1	
	deposits hyaline, tubular	1				1				
Liver	hydropic degeneration	1				1				
Lymph node, axillary	Erythrocytosis/ Erythrophagocytosis, Sinuses	1				1	1		1	
Lymph node, mesenteric	Erythrocytosis/ Erythrophagocytosis, Sinuses	1			1					
Pancrease	infiltration, inflammatory cells	1			1				1	1
Spleen	germinal center development decreased	1				1				
thymus	cysts	1		2	2	1	1		2	
thyroid	cysts	1				1				1
		2		1					1	

Grade key: 1 minimal, 2 mild, 3 moderate, 4 severe

No toxicologically significant findings were present at recovery.

Additional findings present in all dose groups, including control were subcutaneous (dermal) inflammatory cell infiltration; vascular inflammatory cell infiltration and

subcutaneous hemorrhage were observed at the injection sites specifically at the left and right cephalic and saphenous veins.

Special Evaluation- Immunogenicity

Nine of 10 monkeys dosed at 150 mg/kg/day idarucizumab (with dabigatran) were reported positive for anti-idarucizumab antibodies. At the 500 mg/kg dose 3 of 10 monkeys were reported positive for anti-idarucizumab antibodies in the combined two groups of monkeys (with and without dabigatran).

Table 29. Summary of results for anti-idarucizumab antibodies in 2 week toxicity study in rhesus monkey

Group	Treatment (mg/kg/day)		Sex	# of monkeys screened positive / total # of monkeys ^a		
	Dabigatran etexilate	BI 655075		Pretreatment	Day 15	Recovery Day 28
1	0	0	Male	1/5	1/5	0/2
			Female	0/5	0/5	0/2
			Combined	1/10	1/10	0/4
2	0	500	Male	0/5	1/5	2/2
			Female	1/5	2/5	2/2
			Combined	1/10	3/10	4/4
3	12	150	Male	2/5	5/5	2/2
			Female	0/5	4/5	2/2
			Combined	2/10	9/10	4/4
4	12	500	Male	0/5	1/5	2/2
			Female	0/5	2/5	2/2
			Combined	0/10	3/10	4/4

(Excerpted from the submission)

Table 30. Summary of results for anti-dabigatran antibodies in 2 week toxicity study in rhesus monkey

Group	Treatment (mg/kg/day)		Sex	# of monkeys screened putative positive / total # of monkeys *		
	Dabigatran etexilate	BI 655075		Pretreatment	Recovery Day 15	Recovery Day 28
1	0	0	Male	0/5	0/2	0/2
			Female	0/5	0/2	0/2
			Combined	0/10	0/4	0/4
2	0	500	Male	0/5	2/2	0/2
			Female	0/5	1/2	0/2
			Combined	0/10	3/4	0/4
3	12	150	Male	0/5	1/2	0/2
			Female	0/5	2/2	0/2
			Combined	0/10	3/4	0/4
4	12	500	Male	0/5	2/2	2/2
			Female	0/5	2/2	0/2
			Combined	0/10	4/4	2/4

(Excerpted from the submission)

Special Evaluation- Indices of Thrombosis

Unremarkable. There was no evidence to suggest a prothrombic effect of idarucizumab in monkeys based on the comparison of measured concentrations of respective markers D-dimer and F1+2 between control and treatment groups.

Special Evaluation- Complement and Immune Complex Analysis

Unremarkable. There was no evidence to suggest changes in any of the three complement assays analyzed: Bb, C3a and C4a. All values were within normal ranges for all dose groups.

Toxicokinetics

On Days 1 and 14, blood samples were collected at 1.5 hours following dosing of dabigatran vehicle (prior to IV dosing of idarucizumab or vehicle), and 1 hour 40 minutes (immediately following IV infusion), 3 hrs., 6 hrs., 9 hrs., and 24 hrs. following dabigatran dosing. On Day 6, additionally samples were collected at 1.5 hrs. following dabigatran dosing and immediately following idarucizumab dosing (1 hr. 40 minutes following dabigatran dosing). Samples to analyze dabigatran and sum dabigatran (with gluconurides) were collected on recovery Day 29 (Day 58) pre-dose and 1.5 hrs. following re-administration of dabigatran to recovery animals. These samples were also analyzing for their idarucizumab concentrations.

Table 31. Summary toxicokinetics of idarucizumab in 2 week toxicity study in rhesus monkey

Summary TK parameters of BI 655075 in monkeys							
TK Parameter	Day	Sex	Descriptive statistics (n=5)	BI 655075 dose (mg/kg/day)			
				500 (no dabigatran)	150 (+ dabigatran)	500 (+ dabigatran)	
C_{max} ($\mu\text{g/mL}$)	1	Male	Mean SD	8,100 628	2,520 303	7,670 364	
		Female	Mean SD	8,380 526	2,520 76.0	8,040 789	
	14	Male	Mean SD	7,780 1,120	2,850 259	8,670 1,350	
		Female	Mean SD	8,880 934	2,340 293	7,860 471	
	AUC_{0-24} ($\mu\text{g}\cdot\text{h/mL}$)	1	Male	Mean SD	9,080 823	2,610 366	8,690 986
			Female	Mean SD	9,500 1,070	2,560 202	8,700 782
14		Male	Mean SD	8,960 396	2,950 411	9,960 1,830	
		Female	Mean SD	10,300 883	2,470 321	9,360 874	
t_{max} (h) ^a		1	Male	Median Range	1.67 1.67 – 1.67	1.67 1.67 – 1.67	1.67 1.67 – 1.67
			Female	Median Range	1.67 1.67 – 1.67	1.67 1.67 – 1.67	1.67 1.67 – 1.67
	14	Male	Median Range	1.67 1.67 – 1.67	1.67 1.67 – 1.67	1.67 1.67 – 1.67	
		Female	Median Range	1.67 1.67 – 1.67	1.67 1.67 – 1.67	1.67 1.67 – 1.67	

^a The time was based on the oral dosing of dabigatran etexilate. BI 655075 was administered intravenously between 1.5 and 1.67 h after oral dosing of dabigatran etexilate.

(Excerpted from the submission)

Key findings

- Increases in idarucizumab exposure were proportional to increases in dose from 150 mg/kg to 500 mg/kg (C_{max} and AUC) on Days 1 and 14.
- Idarucizumab exposure was similar between male and female monkeys.
- Dabigatran treatment did not affect exposure to idarucizumab.
- The presence of antidrug antibodies did not affect exposure to idarucizumab.
- T_{max} occurs at approximately 1.67 hours following idarucizumab administration.

Table 32. Summary toxicokinetics of dabigatran in 2 week toxicity study in rhesus monkey

Summary TK parameters of dabigatran and sum dabigatran in monkeys							
TK Parameter	Day	Sex	Descriptive statistics (n=5)	Dabigatran TK		Sum dabigatran TK	
				BI 655075 dose (mg/kg/day)		BI 655075 dose (mg/kg/day)	
				150	500	150	500
C _{max} (ng/mL)	1	Male	Mean SD	201 130	175 82.6	1,060 688	611 261
		Female	Mean SD	156 66.1	156 73.5	803 464	755 606
	14	Male	Mean SD	152 147	199 270	948 1,170	560 595
		Female	Mean SD	127 92.2	107 34.0	710 615	576 277
AUC ₀₋₂₄ (ng•h/mL)	1	Male	Mean SD	1,230 719	1,810 480	3,430 1,780	3,480 971
		Female	Mean SD	1,030 155	1,560 450	2,500 831	3,850 1,220
	14	Male	Mean SD	1,130 751	1,600 1,000	3,310 2,740	3,800 2,220
		Female	Mean SD	877 225	1,180 124	2,450 992	3,560 478
t _{max} (h) ^a	1	Male	Median Range	1.67 1.67 – 6.00	1.67 1.67 – 9.00	1.67 1.67 – 6.00	1.67 1.67 – 1.67
		Female	Median Range	1.67 1.67 – 3.00	3.00 1.67 – 6.00	1.67 1.67 – 1.67	1.67 1.67 – 6.00
	14	Male	Median Range	1.67 1.67 – 6.00	1.67 1.67 – 24.0	1.67 1.67 – 1.67	1.67 1.67 – 3.00
		Female	Median Range	1.67 1.67 – 3.00	1.67 1.67 – 9.00	1.67 1.67 – 1.67	1.67 1.67 – 1.67

^a The time was based on the oral dosing of dabigatran etexilate. BI 655075 was administered intravenously between 1.5 and 1.67 h after oral dosing of dabigatran etexilate.

(Excerpted from the submission)

Key findings

- Dabigatran exposure was similar between male and female monkeys.
- Idarucizumab treatment did not consistently cause a trend in exposure differences to dabigatran.

Dosing Solution Analysis

Concentration

The mean concentrations of idarucizumab in the solutions that were analyzed were within acceptability criteria of \pm (b) (4) % of nominal concentrations, indicating accurate formulation; actual values were between (b) (4) %.

The mean concentrations of dabigatran in the solutions that were analyzed were within acceptability criteria of (b) (4) % of nominal concentrations, indicating accurate formulation; actual values were between (b) (4) %.

Table 33. Dosing solution analysis for idarucizumab concentration in 4 week toxicology study in rhesus monkey

Occasion	Group	Nominal inclusion (mg/mL)	Analysed concentration (mg/mL)			RME (%)
			Analysis 1	Analysis 2	Mean	
Day 1M	1M	0	(b) (4)			(b) (4)
	3M	15				
	2+4M	50				
Day 14M	1M	0				
	3M	15				
	2+4M	50				
Day 1F	1F	0				
	3F	15				
	2+4F	50				
Day 14F	1F	0				
	3F	15				
	2+4F	50				

RME Relative mean error, representing the deviation from nominal
 ND Not detected

Table 34. Dosing solution analysis for dabigatran (0.5% hydroxyethylcellulose) concentration in 4 week toxicology study in rhesus monkey

Occasion	Group	Nominal inclusion (mg/mL)	Analysed concentration (mg/mL)				RME (%)
			Top	Middle	Bottom	Mean	
Day 1 (M)	1+2M	0					(b) (4)
	3+4M	1.2					
Day 14 (M)	1+2M	0					
	3+4M	1.2					
Day 1 (F)	1+2F	0					
	3+4F	1.2					
Day 14 (F)	1+2F	0					
	3+4F	1.2					
Recovery Day 29(M)	1+2M	0					
	3+4M	1.2					
Recovery Day 29(F)	1+2F	0					
	3+4F	1.2					

RME Relative mean error, representing the deviation from nominal

ND Not detected

7 Genetic Toxicology

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Studies not conducted.

7.2 *In Vitro* Assays in Mammalian Cells

Studies not conducted.

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Studies not conducted.

7.4 Other Genetic Toxicity Studies

Studies not conducted.

8 Carcinogenicity

Studies not conducted.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Studies not conducted.

9.2 Embryonic Fetal Development

Studies not conducted.

9.3 Prenatal and Postnatal Development

Studies not conducted.

10 Special Toxicology Studies

Studies not conducted.

11 Integrated Summary and Safety Evaluation

The nonclinical studies submitted to this NDA provide sufficient information to support the use of idarucizumab (PRAXBIND) for the treatment of patients when rapid reversal of the anticoagulant effects of dabigatran is required: for emergency situations or in life threatening or uncontrolled bleeding.

Idarucizumab is a reversal agent for dabigatran. It is a humanized monoclonal antibody that binds to dabigatran and forms a stable complex. Idarucizumab can also bind to dabigatran metabolites.

Idarucizumab binds dabigatran with higher affinity than thrombin (~300 times higher) and thrombin substrates in vitro. As a complex with dabigatran, idarucizumab is very stable with a long half-life in vitro (260hr). Idarucizumab also binds dabigatran metabolites. In vitro data showed that idarucizumab reverses the anticoagulant effect of dabigatran, in part by increasing fibrin coverage and increasing fibrin masses around damaged subendothelium. Three animal models of activity were conducted: a mouse intracranial hemorrhage model; a rat tail cut bleeding model and a pig blunt liver trauma model. In the mouse intracranial hemorrhage model, idarucizumab reduced hematoma volume following administration returning levels to those observed in non-anticoagulated animals. In the rat tail cut model showed neutralization of dabigatran by idarucizumab, effective single and split dosing regimens with activity measured by significantly reduced anticoagulation and bleeding time. The pig blunt liver trauma model also tested single and split dosing measuring activity of idarucizumab through reduced anticoagulation, blood loss and survival of pigs after a given time period.

Safety pharmacology studies showed no adverse respiratory findings. Cardiovascular safety pharmacology studies were not performed independently but ECG measurements assessed during the 2 week repeat dose toxicology study in monkeys were unremarkable at doses up to 500 mg/kg.

In the pharmacokinetic studies in both rats and monkeys, there was a rapid increase in dabigatran plasma concentration following dosing with idarucizumab suggesting redistribution of dabigatran from the tissue to the plasma. This was evidenced by the high average dabigatran C_{max} and AUC_{0-24} values in animals treated with idarucizumab vs. without idarucizumab (dabigatran only). Dabigatran and average sum dabigatran values were many fold values higher in the idarucizumab treated animals (C_{max} and AUC_{0-24} values). Idarucizumab was rapidly eliminated in the blood following intravenous dosing and exhibited biphasic plasma concentration-time profiles; initial phase half-lives were approximately 0.25 hrs. (both species) and terminal phase half-lives were approximately 6 hrs in the rat and 5.5 hrs. in the monkey. Excretion in the urine was only 10% in the monkeys and 21% in the rats. Based on the data collected in general toxicology studies, there were relatively no gender differences in exposure, and increased in C_{max} and AUC values were approximately dose proportional.

The general toxicology studies were conducted in the rat and monkey via I.V., which is the intended route of administration. The rat studies were performed using only idarucizumab; the monkey studies were performed in the presence and absence of dabigatran. The 4 week repeat dose toxicity study in rat and 2 week repeat dose toxicity study in the monkey are reviewed. All appropriate studies were conducted in compliance with Good Laboratory Practice (GLP) regulations. There were no major target organs in rat or monkey. There were no mortalities in the rat study or the monkey study. There were some hematology findings in rats including increased white blood cells and lymphocytes at 150 and 500 mg/kg dose levels; and clinical chemistry findings of decreased blood urea and creatinine levels at 500 mg/kg. Additionally, female rats dosed at both 150 and 500 mg/kg had elevated thymus weights (absolute, relative to body weight and relative to brain weight). Histopathology findings in the rat included atrophy in the pancreas at high doses (500 mg/kg) in two animals. Although there were a number of histological findings in the monkey study at both 150 mg/kg and 500 mg/kg they were not present in the recovery group animals and these findings had no correlation to other pathological changes in blood chemistry, clinical chemistry, or gross pathology. They were incidental. Markers of thrombosis were examined (D-dimer and F1+2) between control and treatment groups. There was no evidence of a prothrombic effect of idarucizumab in monkeys based on the analysis of these markers. The effect of idarucizumab on complement activation was also assessed using assays directed at Bb, C3a and C4a and these were unremarkable for all dose groups. Anti-drug antibodies were detected in monkeys at both doses tested and detected in more monkeys at lower dose groups (in 9/10 monkeys dosed at 150 mg/kg idarucizumab (with dabigatran) and 3/10 of monkeys dosed at 150 mg/kg idarucizumab (with and without dabigatran; groups combined). The presence of antidrug antibodies did not affect exposure to idarucizumab.

Studies specifically addressing in vitro and in vivo genotoxicity and carcinogenicity have not been conducted. Studies addressing reproductive and developmental toxicity; fertility and early embryonic development to implantation; effects on embryo-fetal development; pre- and postnatal development, including maternal function and toxicity in juvenile animals have not been conducted.

12 Appendix/Attachments

None

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

/s/

EMILY J PLACE
07/20/2015

CHRISTOPHER M SHETH
07/20/2015