

# DRUG SAFETY

## Evaluation of the immunogenicity of the dabigatran reversal agent idarucizumab during Phase I studies

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### AIMS

Idarucizumab, a humanized monoclonal anti-dabigatran antibody fragment, is effective in emergency reversal of dabigatran anticoagulation. Pre-existing and treatment-emergent anti-idarucizumab antibodies (antidrug antibodies; ADA) may affect the safety and efficacy of idarucizumab. This analysis characterized the pre-existing and treatment-emergent ADA and assessed their impact on the pharmacokinetics and pharmacodynamics (PK/PD) of idarucizumab.

### METHODS

Data were pooled from three Phase I, randomized, double-blind idarucizumab studies in healthy Caucasian subjects; elderly, renally impaired subjects; and healthy Japanese subjects. In plasma sampled before and after idarucizumab dosing, ADA were detected and titrated using a validated electrochemiluminescence method. ADA epitope specificities were examined using idarucizumab and two structurally related molecules. Idarucizumab PK/PD data were compared for subjects with and without pre-existing ADA.

### RESULTS

Pre-existing ADA were found in 33 out of 283 individuals (11.7%), seven of whom had intermittent ADA. Titres of pre-existing and treatment-emergent ADA were low, estimated equivalent to <0.3% of circulating idarucizumab after a 5 g dose. Pre-existing ADA had no impact on dose-normalized idarucizumab maximum plasma levels and exposure and, although data were limited, no impact on the reversal of dabigatran-induced anticoagulation by idarucizumab. Treatment-emergent ADA were detected in 20 individuals (19 out of 224 treated [8.5%]; 1 out of 59 received placebo [1.7%]) and were transient in ten. The majority had specificity primarily toward the C-terminus of idarucizumab. There were no adverse events indicative of immunogenic reactions.

### CONCLUSION

Pre-existing and treatment-emergent ADA were present at extremely low levels relative to the idarucizumab dosage under evaluation. The PK/PD of idarucizumab appeared to be unaffected by the presence of pre-existing ADA.

## WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Idarucizumab is a humanized monoclonal antibody that binds specifically to dabigatran, reversing its anticoagulant activity.
- Hypothetically, antibody formation has the potential to interfere with the pharmacologic action of idarucizumab.
- Hypersensitivity or allergic reactions to idarucizumab have not been identified to date.

## WHAT THIS STUDY ADDS

- Assessment of the presence or formation of anti-idarucizumab antibodies, their specificity and impact on the pharmacokinetics/pharmacodynamics of idarucizumab.
- Pre-existing and treatment emergent anti-idarucizumab antibodies are present at extremely low levels relative to idarucizumab dose.
- The pharmacokinetics/pharmacodynamics of idarucizumab are unaffected by the presence of pre-existing anti-idarucizumab antibodies.

## Tables of Links

TARGETS
<b>Enzymes</b> [2]
Thrombin

LIGANDS
Idarucizumab
Dabigatran etexilate
Abciximab

These Tables list key protein targets and ligands in this article that are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY [1], and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 [2].

## Introduction

Dabigatran etexilate is the oral prodrug of the direct thrombin inhibitor dabigatran. The safety and efficacy of dabigatran have been shown in Phase III trials in patients with non-valvular atrial fibrillation (AF) and venous thromboembolism, and have also been evaluated in real-world studies of patients with AF [3–8]. Despite the overall positive benefit–risk profile of dabigatran, life-threatening bleeding in patients being treated with anticoagulant therapies remains an infrequent but real risk [9]. Hence, reversal agents for dabigatran and the other non-vitamin K oral anticoagulants are in development [10–14].

Idarucizumab is a specific reversal agent for dabigatran, currently approved for use in over 35 countries, including the United States, the countries of the European Union, Japan, Canada, Australia, New Zealand, and many others. Its efficacy and safety have been demonstrated in Phase I studies and in an initial analysis of data from an ongoing Phase III study in patients with serious bleeding or who required urgent major invasive procedures [10, 11, 15]. Idarucizumab is a humanized antigen-binding fragment (Fab) derived from a mouse monoclonal antibody and directed specifically against dabigatran [16]. With no known endogenous targets [16], idarucizumab binds dabigatran in a 1:1 stoichiometric relationship and with an affinity >300 times the affinity of dabigatran for thrombin [16]. It thereby effectively competes with thrombin for thrombin-bound dabigatran and thus neutralizes the thrombin-inhibiting activity of dabigatran. The dabigatran–idarucizumab complexes formed are then predominantly cleared renally [10, 16].

There is an inherent risk that the patient will have an immune response when treated with a biologic agent [17–20]. Such responses may vary considerably in severity, from asymptomatic with production of low titres of antidrug antibodies (ADA), to mild or moderate adverse drug reactions, to anaphylaxis. Hence, safety considerations and regulatory guidance indicate that the immunogenic potential of therapeutic protein products should be evaluated and characterized as part of risk-based mitigation strategies during clinical development [17, 18]. ADA can mediate immune reactions affecting safety or can reduce the efficacy of an agent [21–23]. To explore these effects of ADA, a pooled analysis of data from idarucizumab Phase I studies was conducted to characterize pre-existing ADA and treatment-emergent ADA as well as to evaluate the impact of pre-existing ADA on the pharmacokinetics (PK) and pharmacodynamics (PD) of idarucizumab.

## Methods

### *Study participants and dosing regimens*

Data were obtained from three Phase I studies of idarucizumab in healthy, predominantly Caucasian (with the exception of one Asian, one black/African American and one American Indian/Alaskan) volunteers (aged 18–45 years,  $n = 157$ ) (NCT01688830) [10, 15]; in healthy elderly subjects (aged 65–80 years,  $n = 16$ ), healthy middle-aged subjects (aged 45–64 years,  $n = 12$ ), and renally impaired subjects (aged 45–80 years,  $n = 18$ , with

mild or moderate renal impairment,  $CL_{CR}$  60 to <90 or 30 to <60 [ $ml\ min^{-1}$ ], respectively) (NCT01955720) [24]; and in healthy Japanese volunteers (aged 20–45 years,  $n = 80$ ) (NCT02028780) [25]. Details of the study designs and dosing regimens are outlined in Table 1. Of a total of 283 subjects studied, 59 received placebo (i.e., neither dabigatran nor idarucizumab), 107 received only idarucizumab, and 117 received dabigatran (dosed to steady state) followed by idarucizumab. The studies were approved by all appropriate regulatory authorities and ethics committees, and all participants provided written, informed consent.

### Study outcomes

The outcomes of this study were to obtain detailed characterizations of any pre-existing or treatment-emergent ADA in terms of titres and idarucizumab epitopes and to evaluate the impact of pre-existing ADA on the PK and PD of idarucizumab and the extent of treatment-emergent ADA.

### Detection and characterization of ADA

Plasma for determination of idarucizumab ADA was taken from participants in the three Phase I studies at the predose visit, at the end of each study, and then 4 weeks and 3 months

later (Table 1). Blood samples collected from forearm veins were drawn into  $K_3$ -EDTA anticoagulant blood-drawing vials and plasma isolated by centrifugation at 2000–4000  $g$  at 4–8°C for ~10 min. ADA were detected in these plasma samples by the use of a validated electrochemiluminescence (ECL) method at Covance Laboratories, Inc. (Chantilly, VA). In brief, for this procedure, acid-dissociated samples were added to a mix of Tris base, biotin-labelled idarucizumab, and sulfo-TAG-labelled idarucizumab so that complexes could form with any ADA present. Labelled ADA complexes were captured on streptavidin plates, and after washing, plates were read on an MSD Sector Imager 6000 (Meso Scale Diagnostics LLC, Rockville, MD, USA). A monoclonal anti-idiotypic anti-idarucizumab antibody was used as a positive reference control. Validation of the ADA assay utilized commercial human plasma samples (with  $K_3$ -EDTA anticoagulant) obtained from BioreclamationIVT, Chestertown, MD, USA.

Validation of the method followed recommendations found in the literature [26] and in regulatory guidance [27] and included assessments of sensitivity, precision, specificity, selectivity, drug tolerance and stability. The screening assay cut point was determined using the 95th percentile (5% false-positive rate), with exclusion of outliers. Using an

**Table 1**

Design, dosing regimens, and analysis time points in the Phase 1 trials analysed

Trial and design	Dosing regimen		Dabigatran status	Number per cohort	ADA evaluation time points
	Infusion length (min)	Treatment			
<b>Healthy Caucasian volunteers (NCT01688830) [10, 15]</b>	5 or 60	Placebo	None	39	Predose, end of study, <sup>a</sup> 4 weeks and 3 months after idarucizumab
	60	20, 60, 200 or 600 mg, or 1.2, 3, 4, 6 or 8 g	None	6 (5 for 600 mg)	
	60	2 g	None	12	
	5	1, 2 or 4 g	None	6	
	5	1, 2, 4 or 7.5 g	220 mg b.i.d.	9 (8 for 4 g)	
<b>Elderly, middle-aged and renally impaired subjects (NCT01955720) [24]</b>	5	1 g	150/220 mg b.i.d. <sup>b</sup>	14 <sup>c</sup>	Predose, end of study, follow-up 1, follow-up 2 <sup>f</sup>
	5	2.5 g followed by 2.5 g 2 months later	220 mg b.i.d.	6 <sup>d</sup>	
	5	5 g	150/220 mg b.i.d. <sup>b</sup>	26 <sup>e</sup>	
<b>Healthy Japanese volunteers (NCT02028780) [25]</b>	5 or 60	Placebo	None	20	Predose, end of study, 4 weeks and 3 months after idarucizumab <sup>g</sup>
	5	1, 2 or 4 g	None	6	
	60	8 g	None	6	
	5	1, 2, 4 or 5 g	220 mg b.i.d.	9	

b.i.d., twice daily.

<sup>a</sup>End of study sample time point ranged from 7–20 days after idarucizumab administration.

<sup>b</sup>220 mg b.i.d. healthy subjects and 150 mg b.i.d. in subjects with renal impairment.

<sup>c</sup>Eight healthy elderly subjects aged 65–80 years and six subjects aged 45–80 years with mild renal impairment ( $CrCl$  60–90  $ml\ min^{-1}$ ).

<sup>d</sup>Healthy subjects aged 45–64 years.

<sup>e</sup>Six healthy subjects aged 45–64 years, eight healthy elderly subjects aged 65–80 years, six subjects aged 45–80 years with mild renal impairment ( $CrCl$  60–90  $ml\ min^{-1}$ ) and six subjects aged 45–80 years with moderate renal impairment ( $CrCl$  30–60  $ml\ min^{-1}$ ).

<sup>f</sup>End-of-study sample time point ranged from 5–24 days after idarucizumab administration for subjects who received a single idarucizumab treatment and from 73–88 days after first treatment for subjects who received two treatments; follow-up 1 and follow-up 2 were from 31–49 days and 87–105 days, respectively, after 1 idarucizumab treatment and 93–113 days and 157–173 days, respectively, after the first treatment in subjects who received two treatments in this study.

<sup>g</sup>End-of-study sample time point ranged from 5–7 days after idarucizumab administration.

additive normalization factor of 80.2 relative luminescence units (RLU), the screening cut points were quite similar across all three clinical studies, ranging from (mean  $\pm$  SD) 171.0  $\pm$  6.5 to 176.6  $\pm$  23.3 RLU. Putative ADA-positive samples underwent a confirmatory assay (confirmatory cut point set at a 1% false-positive rate: 84.7% inhibition), titres were determined, and epitope specificity characterized. At a titre of 1 (i.e., a sample was positive without any dilution beyond the minimum required), the concentration of antibody was considered to be approximately equivalent to the sensitivity of the assay as determined with three different positive controls: the assay sensitivity ranged from 11.5 ng ml<sup>-1</sup> with a monoclonal anti-idarucizumab antibody to 31.3 ng ml<sup>-1</sup> and 81.3 ng ml<sup>-1</sup>, respectively, with two different preparations of rabbit polyclonal antibodies. Regarding drug tolerance of the screening assay, in the presence of 7.5  $\mu$ g ml<sup>-1</sup> idarucizumab, 250 ng ml<sup>-1</sup> of the monoclonal positive control antibody could be detected.

The specificities of the ADA for different idarucizumab epitopes were assessed in a competitive-format ECL assay. This was analogous to the method above, except that molecules structurally related to idarucizumab were evaluated for their potential to block the signal in the ECL assay that was due to the ADA. Two molecules were utilized: a full-length IgG1 molecule containing two idarucizumab Fab fragments (molecule 1) and a Fab with constant regions C<sub>H</sub>1 and C<sub>L</sub> identical to those in idarucizumab, but with different variable regions (molecule 2). Blocking by only the first would suggest that an ADA was binding to variable regions of idarucizumab; blocking by both of these test molecules would be indicative of an ADA binding to the constant regions or binding with a mixed specificity; and blocking by the second alone (molecule 2) would suggest that an ADA was binding to an epitope near the C-terminus of idarucizumab, but one that is disrupted by the presence of C<sub>H</sub>2 and C<sub>H</sub>3 in the test molecule 1. The presence of pre-existing ADA in some individuals complicates the usual approach of utilizing a screening cut point to identify either boosted ADA or idarucizumab-specific, treatment-induced ADA. In those individuals who have pre-existing ADA, changes in ADA following idarucizumab were determined based on an increase in titre over baseline.

### ADA terminology

A pre-existing ADA was defined by a positive response to the biologic drug before treatment and was described as intermittent if the response was not consistently positive at all time points.

The descriptions of treatment-emergent (boosted or induced) ADA responses used in the present analysis were as defined previously [28, 29]. A treatment-boosted ADA response was a response where at least one postdose sample was ADA-positive and had a titre greater than any positive predose ADA titre. A treatment-induced ADA response was a response where at least one postdose sample was ADA-positive, and the predose sample was ADA-negative. A transient, treatment-emergent ADA response was a response occurring at an intermediate postdose time point, but then followed by an ADA-negative response in the sample collected at the final time point. Where a treatment-emergent

response occurred and was still present at the last sample time point, it was considered to be a possibly persistent ADA response. The term 'possibly persistent' was selected rather than 'persistent' because the design of the studies, with samples collected only up to roughly 3 months, did not allow a definitive conclusion that a response was persistent. A false-positive response was defined as a result that was ADA-positive in the screening assay and ADA-negative in the confirmatory assay.

### PK and PD analyses

The methods employed for the evaluation of idarucizumab PK, as well as its reversal of dabigatran-mediated anticoagulation (PD), have been consistent across studies and described in detail in their original reports [10, 15]. PK data for idarucizumab and PD results were compared descriptively for subjects with and without pre-existing ADA.

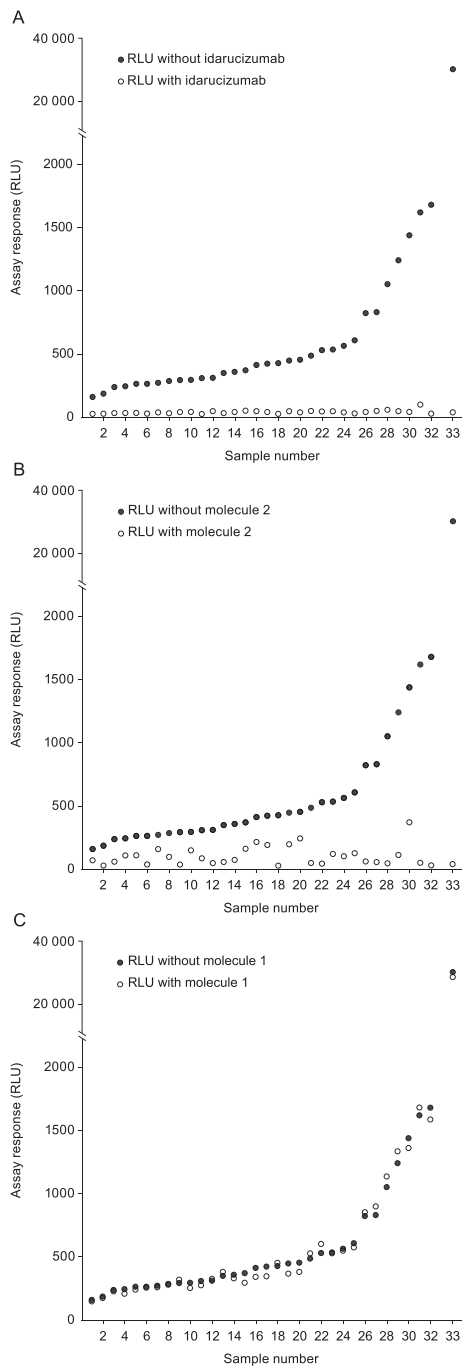
## Results

### Detection and characterization of pre-existing ADA

Pre-existing ADA were detected in 33 out of 283 individuals (11.7%) from the three studies (Figure 1). In the healthy Caucasian volunteer study, 19 out of 157 participants (12.1%) had pre-existing ADA (7 out of 39 in the placebo group [17.9%] and 12 out of 118 in the treated group [10.2%]). In all cases, these pre-existing ADA were anti-C terminus antibodies (Figure 1). The observed pre-existing ADA were intermittent in two subjects in the placebo group and three in the treated group. In the study in elderly, middle-aged, and renally impaired subjects, none of the 46 participants had pre-existing ADA. In the healthy Japanese volunteer study, 14 out of 80 participants (17.5%) had pre-existing ADA (4 out of 20 in the placebo group [20%] and 10 out of 60 from the treated group [16.7%]). Again, the pre-existing ADA were C-terminus-specific or primarily C-terminus-specific (Figure 1). Intermittent pre-existing ADA were found in two participants in the treated group only. In all three of the Phase I studies, titres of pre-existing ADA ranged from 1 to 160. Screening assay RLU values corresponding to these titres ranged from 234 to 30 400. Peak titre in these 33 subjects were distributed as follows: titre (number of subjects) = 1 (7), 2 (12), 4 (4), 8 (2), 10 (2), 20 (4), 40 (1) and 160 (1). Other than in the intermittent cases, titres of pre-existing ADA tended to remain constant over the course of ADA sampling time points (Figure S1 in the supporting information).

### Treatment-emergent ADA

*Assay evaluation.* The tested drug tolerance level during the ADA assay validation (utilizing positive controls spiked with concentrations of idarucizumab within the expected range in circulation) demonstrated that the assay had sufficient sensitivity for the detection of anti-idarucizumab antibodies for each of the three populations examined (healthy Caucasian, middle-aged, elderly and renally impaired, and healthy Japanese). The detection method utilized was precise enough to distinguish readily between samples with



**Figure 1**

Pre-existing anti-idarucizumab responses in plasma from treatment-naïve individuals from the three Phase I studies evaluated. (A) blocking of ADA assay responses by idarucizumab, (B) blocking of ADA assay responses with molecule 2 (a Fab with constant regions C<sub>H</sub>1 and C<sub>L</sub> identical to those in idarucizumab, but with different variable regions), and (C) lack of blocking of ADA assay response by molecule 1 (a full-length IgG1 molecule containing two idarucizumab Fab fragments). ADA, antidrug antibody; RLU, relative luminescence units

37.5 and 75 ng ml<sup>-1</sup> ADA (mean RLUs of 436 and 779, respectively). There was a difference in RLU of 343 for

these positive control concentrations (Figure S2 in the supporting information), which was judged to be sufficiently sensitive to detect treatment-boosted ADA on top of any moderate pre-existing ADA signal, which was typically <1000 RLU.

Rates of false-positive ADA screening results were 6.4% (36 false-positive samples, 561 negative samples) in the healthy Caucasian study; 9.4% (17 false-positive, 181 negative) in the elderly, middle-aged and renally impaired study; and 5.6% (15 false-positive, 266 negative) in the healthy Japanese volunteer study.

**Pooled analysis findings.** The findings for treatment-emergent ADA are summarized in Table 2. In the healthy Caucasian volunteer study, 8 out of 157 subjects (5.1%) developed treatment-induced ADA (i.e., these subjects did not have pre-existing ADA). One subject in the placebo arm and six in the treated arm developed transient ADA, and one in the treated arm developed a possibly persistent response. In all cases, these were anti-C terminus ADA. In the elderly, middle-aged and renally impaired study (in which all subjects received idarucizumab), 6 out of 46 subjects (13.0%) developed treatment-induced ADA. One participant developed transient anti-C-terminus ADA. Five participants developed possibly persistent ADA (four were anti-C terminus ADA, one was anti-variable region, and specificity was not determined for one). In the healthy Japanese volunteer study, 6 out of 80 subjects (7.5%) developed treatment-emergent ADA, and all of these individuals had received idarucizumab. Four of these treatment-emergent responses were treatment-induced, and two were treatment-boosted. Interestingly, the specificity profile of the ADA in one of the subjects with a treatment-boosted response changed from primarily anti-C terminus in the predose and 4-week samples to primarily anti-variable region in the 3-month sample. Two participants had transient ADA (one was anti-C-terminus and one had anti-constant region/mixed specificities), and four had possibly persistent ADA (two were anti-C-terminus specific, one was anti-C-terminus switching to primarily anti-variable region-specific and one had anti-constant region/mixed specificity).

Of the 20 out of 283 (7.1%) subjects in these studies who developed ADA during these studies, 12 out of 117 (10.3%) had been exposed to both dabigatran and idarucizumab, 7 out of 107 (6.5%) only to idarucizumab and one to placebo (i.e., 19 out of 224 treated with idarucizumab [8.5%] and 1 of 59 treated with placebo [1.7%]). ADA were transient in 9 out of 224 (4.0%) and possibly persistent in 10 out of 224 (4.5%) idarucizumab-treated individuals. The titres of treatment-emergent ADA were mainly ≤4, with a maximum of 40 reported in one subject (Table 2).

**Pre-existing ADA and idarucizumab PK and PD.** Pooled PK data were analysed by pre-existing ADA-positive or -negative status for subjects with normal renal function; no subjects with renal impairment had pre-existing ADA. Presence vs. absence of ADA had no impact on dose-normalized maximum plasma concentrations of idarucizumab or on the dose-normalized area under the plasma idarucizumab concentration–time curve (Figure 2).

**Table 2**

Characterization of individual treatment-emergent ADA observed in 20 of 283 subjects from Phase 1 studies, showing anti-idarucizumab ADA titres and percentage blocking of ADA by alternative molecules

Idarucizumab dose <sup>a</sup>	Assay result (titre and % signal inhibition)					Comments (type and specificity of response)
	Assay <sup>b</sup>	Predose <sup>c</sup>	EOS <sup>d</sup>	4 weeks <sup>e</sup>	3 months <sup>f</sup>	
<b>Healthy, mostly Caucasian volunteer study (8/157 subjects)</b>						
<b>Placebo (– dabigatran)</b>	Titre	0	0	2	0	Transient induced
	Mol 1, 2			–4.4, 89.5		Anti-C terminus
<b>60 mg (– dabigatran)</b>	Titre	0	1	0	0	Transient induced
	Mol 1, 2		3.5, 89.4			Anti-C terminus
<b>60 mg (– dabigatran)</b>	Titre	0	4	0	0	Transient induced
	Mol 1, 2		–17.3, 92.4			Anti-C terminus
<b>4000 mg (– dabigatran)</b>	Titre	0	2	0	0	Transient induced
	Mol 1, 2		–4.8, 88.5			Anti-C terminus
<b>1 g (+ dabigatran)</b>	Titre	0	1	0	0	Transient induced
	Mol 1, 2		16.9, 52.4			Anti-C terminus
<b>7.5 g (+ dabigatran)</b>	Titre	0	8	0	0	Transient induced
	Mol 1, 2		2.9, 94.8			Anti-C terminus
<b>7.5 g (+ dabigatran)</b>	Titre	0	0	8	16	Possibly persistent induced
	Mol 1, 2			–8.6, 76.4	–3.9, 93.4	Anti-C terminus
<b>7.5 g (+ dabigatran)</b>	Titre	0	4	0	0	Transient induced
	Mol 1, 2		4.8, 78.3			Anti-C terminus
<b>Elderly, middle-aged, renally impaired volunteer study (6/46 subjects)</b>						
<b>5 g (+ dabigatran)</b>	Titre	0	0	4	4	Possibly persistent induced
	Mol 1, 2			–0.9, 57.1	0.4, 65.5	Anti-C terminus
<b>2 × 2.5 g (+ dabigatran)</b>	Titre	0	0	0	4	Possibly persistent induced
	Mol 1, 2				86.5, 4.2	Anti-variable region
<b>5 g (+ dabigatran)</b>	Titre	0	0	1	0	Transient induced
	Mol 1, 2			3.1, 34.0		Anti-C terminus
<b>1 g (+ dabigatran)</b>	Titre	0	0	0	2	Possibly persistent induced
	Mol 1, 2				–5.8, –4.5	Specificity not determined
<b>5 g (+ dabigatran)</b>	Titre	0	0	8	4	Possibly persistent induced
	Mol 1, 2			–1.6, 26.4	–5.9, 28.4	Anti-C terminus
<b>5 g (+ dabigatran)</b>	Titre	0	0	2	1	Possibly persistent induced
	Mol 1, 2			6.8, 68.1	1.2, 66.5	Anti-C terminus
<b>Healthy Japanese volunteer study (6/80 subjects)</b>						
<b>1 g (– dabigatran)</b>	Titre	2	1	2	40	Possibly persistent boosted
	Mol 1, 2	8.1, 80.2	8.6, 73.9	3.9, 76.2	0.8, 86.5	Anti-C terminus
<b>4 g (– dabigatran)</b>	Titre	0	2	1	0	Transient induced
	Mol 1, 2		–1.9, 71.0	10.0, 63.6		Anti-C terminus
<b>8 g (– dabigatran)</b>	Titre	2	0	2	10	Possibly persistent boosted
	Mol 1, 2	11.1, 73.0		7.5, 69.6	73.9, 19.7	Anti-C term switch to anti-var
<b>8 g (– dabigatran)</b>	Titre	0	0	0	1	Possibly persistent induced
	Mol 1, 2				45.4, 28.2	Anti-constant region or mixed
<b>1 g (+ dabigatran)</b>	Titre	0	0	2	2	Possibly persistent induced
	Mol 1, 2			–0.4, 25.0	13.8, 33.0	Anti-C terminus
<b>4 g (+ dabigatran)</b>	Titre	0	4	0	0	Transient induced
	Mol 1, 2		27.6, 41.5			Anti-constant region or mixed

Mol, molecule.

<sup>a</sup>The dose of idarucizumab is indicated in this column along with an indication of whether dabigatran was also dosed (+ dabigatran) or not (– dabigatran).

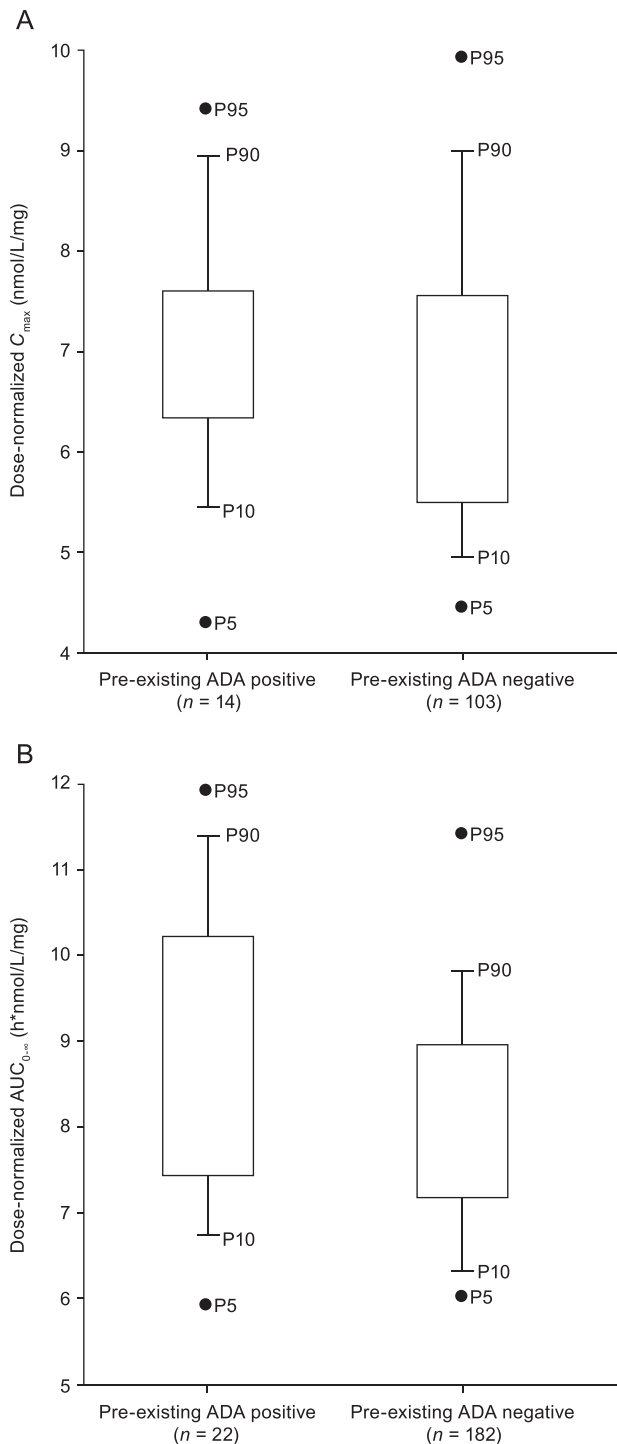
<sup>b</sup>Titre, a titre in ADA assay (0 indicates an ADA-negative sample); Mol 1, 2 refers to the epitope specificity assays using the full length IgG version of idarucizumab (Mol 1, % signal inhibition) or a Fab with constant regions identical to idarucizumab but with different variable regions (Mol 2, % signal inhibition).

<sup>c</sup>Predose, sample collected prior to idarucizumab administration.

<sup>d</sup>EOS, end-of-study sample time point; for details of time points in the three studies, see Table 1.

<sup>e</sup>For the elderly, middle-aged, renally impaired study, this sample is the follow-up 1 sample; for details of time points, see Table 1.

<sup>f</sup>For the elderly, middle-aged, renally impaired study, this sample is the follow-up 2 sample; for details of time points, see Table 1.



**Figure 2**

Comparison of (A) dose-normalized maximum plasma concentrations ( $C_{max}$ ) of idarucizumab for all subjects with normal renal function who received a single 5-min idarucizumab infusion and (B) dose-normalized area under the plasma idarucizumab concentration–time curve ( $AUC_{0-\infty}$ ) values for all subjects with normal renal function with and without pre-existing anti-idarucizumab antibodies; pooled data from the three Phase I studies. ADA, antidrug antibodies;  $AUC_{0-\infty}$ , area under the curve;  $C_{max}$ , maximum plasma concentration; P5, P10, P90 and P95, 5th, 10th, 90th and 95th centiles

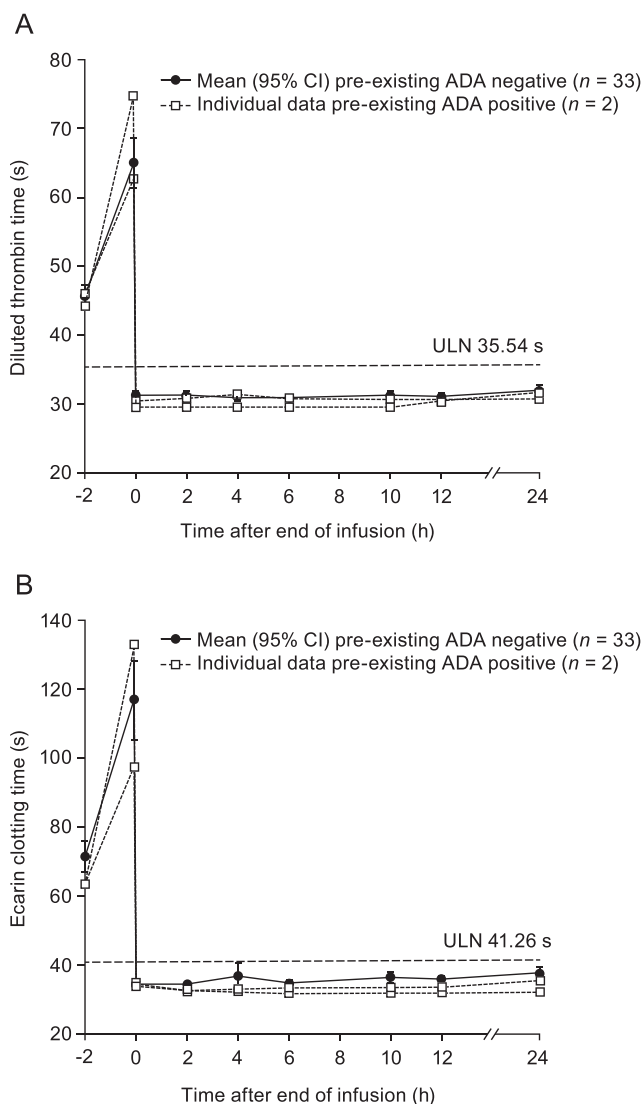
Effect–time profiles of diluted thrombin time and ecarin clotting time assays following anticoagulation with dabigatran and subsequent infusion of a total dose of 5 g idarucizumab were compared in volunteers with ( $n = 2$ ) and without ( $n = 33$ ) pre-existing ADA. The presence of pre-existing ADA in this limited number of subjects was found to have no impact on the inhibition of dabigatran-induced anticoagulation by idarucizumab (Figure 3).

## Discussion

This analysis investigated the incidence of pre-existing ADA and the development of treatment-emergent (boosted or induced) ADA using pooled data of 283 volunteers from three Phase I studies of idarucizumab. Using a validated electrochemiluminescence method, pre-existing ADA were detected in 11.7% of all volunteers, and treatment-emergent ADA were detected in 8.5% of idarucizumab-treated volunteers. No impact of pre-existing ADA on the PK or PD of idarucizumab was observed. The levels of treatment-emergent ADA were low relative to the idarucizumab dose and were primarily C-terminus specific, i.e., bound mostly to epitopes away from the dabigatran-binding site.

Idarucizumab is a recombinant humanized Fab directed against dabigatran that has been shown to be effective for rapid and complete reversal of the anticoagulant effects of dabigatran [10, 11, 15, 30]. There is no evidence of any impact of anti-idarucizumab antibodies on efficacy or safety outcomes [10, 15, 24].

In the present analysis, false-positive rates for the ADA screening assay ranged from 5.6 to 9.4%, suggesting that the assay cut point chosen for the analysis was within acceptable criteria for detecting ADA [27]. Pre-existing ADA in most subjects were found to be completely or predominantly specific to the C-terminus of the Fab, indicating that they bind to the end of the Fab away from the dabigatran-binding site and are therefore unlikely to interfere with idarucizumab binding to dabigatran. The titres of the pre-existing antibodies – which tended to remain constant over the course of ADA sampling – were low, suggesting the potential to react with only an extremely low percentage of circulating idarucizumab when it is administered at the 5 g (i.e., 105  $\mu$ mol of idarucizumab) dosage being evaluated in clinical practice. To evaluate the potential neutralizing activity of ADA, a computation of maximum body load can be made in the sense of a worst-case scenario based on observed titre. An estimate of the highest equivalent concentration of anti-idarucizumab antibody in any of the positive subjects can be obtained by multiplying the peak titre by 81.3  $ng\ ml^{-1}$ , which was the highest value for sensitivity measured with a positive control for the antibody assay. For a titre of 40, this works out to  $\sim 3.3\ \mu g\ ml^{-1}$ . Given an approximate 3000 ml plasma volume of a 70 kg person, the amount of anti-idarucizumab antibody in the circulation is therefore only about 10 mg (or approximately 0.067  $\mu$ mol). This equates to  $<0.1\%$  of the idarucizumab 5 g dose (0.067/105  $\mu$ mol  $\times$  100). For the highest observed pre-existing ADA titre of 160, the amount of ADA is calculated to be  $<0.3\%$  of the idarucizumab dose. The lack of potential



### Figure 3

Comparison of (A) mean diluted thrombin time assay and (B) ecarin clotting time assay results after anticoagulation with dabigatran followed by infusion of 5 g idarucizumab with and without pre-existing anti-idarucizumab antibodies in volunteers.<sup>a</sup> <sup>a</sup>The figure includes all subjects who received a total dose of 5 g idarucizumab:  $n = 6$ , middle-aged;  $n = 8$ , elderly;  $n = 6$ , mild renal impairment;  $n = 6$ , moderate renal impairment;  $n = 9$ , healthy Japanese volunteers. ADA, antidrug antibodies; ULN, upper limit of normal

for such low amounts to relevantly affect idarucizumab efficacy was supported by observations that presence vs. absence of pre-existing ADA had no effect on the PK and PD of idarucizumab. Note that the effect of treatment-emergent ADA on the PK and PD of idarucizumab was not evaluated during these studies due to the study design. Due to its very short half-life, idarucizumab was cleared well before post-dose ADA samples were collected.

Pre-existing ADA are thought to occur because of coincidental cross-reactivity of autoantibodies to unrelated proteins or to epitope molecular mimicry [20, 31, 32]. Perhaps more relevant to the pre-existing ADA described here

is the suggestion that pre-existing ADA result from an autoimmune response to proteolytic cleavage products of IgG [33]. Numerous instances of pre-existing ADA – including anti-Fab and anti-Fc antibodies – that cross-react with therapeutic agents with varying impact on efficacy outcomes have been described [22, 32, 34–36]. In an analysis of 32 trials of 12 biotherapeutic agents, 58% of the biotherapeutics studied were associated with some level of pre-existing antibodies. In the studies where they were present, the prevalence varied from ~4 to 15%, depending on the type of agent [31]. In a study of the immunogenicity of the Fab abciximab (a fibrinogen receptor antagonist), anti-abciximab antibodies were detected in patients, but additionally, pre-existing antibodies specific for the C-terminus of Fab were detected in 77 serum samples from 104 randomly selected healthy subjects [36]. These findings support the hypothesis that pre-existing Fab-specific antibodies are ubiquitous in healthy subjects [36]. Generally, pre-existing ADA have been considered to have little impact on treatment and do not appear to be typically associated with a high risk of developing treatment-emergent ADA [31].

In our analysis, treatment-emergent ADA were mostly directed against the C-terminus of idarucizumab and were almost equally divided into transient and possibly persistent responses. As with the pre-existing ADA, titres of treatment-emergent antibodies, and thus also corresponding total amounts in the body, were low compared with the 5 g idarucizumab dose. This suggests that a relevant impact on idarucizumab efficacy in clinical practice is unlikely. As a reversal agent for use in cases of major bleeding or other serious emergencies, it is expected that only in rare instances will patients be re-exposed to idarucizumab treatment, be it weeks, months or years later. In a subgroup of six subjects, idarucizumab was administered a second time, 2 months after the first administration [37]. No anti-idarucizumab antibodies were detected in these subjects prior to the second administration. Whether there may be an increased immunogenic potential or hypersensitivity reaction following development of treatment-emergent ADA is not known. At this stage in the clinical development of idarucizumab, only the primary response to idarucizumab exposure has been studied and there have been no observations of adverse events indicative of hypersensitivity reactions.

### Study strengths and limitations

Our analysis evaluated a data pool of 283 subjects, of whom 224 had been exposed to idarucizumab in Phase I clinical trials. Because time points up to only ~3 months after idarucizumab dosing were covered in these studies, it is unclear whether the ADA identified as possibly persistent would remain in the longer term. Specific assays to identify neutralizing antibodies were not undertaken; however, the pre-existing and treatment-emergent ADA identified bound mostly to epitopes away from the dabigatran-binding site. Furthermore, given the very low ADA titres vs. the anticipated levels of circulating idarucizumab during treatment, neutralization by ADA is unlikely to be a clinically relevant issue. However, it will be important to confirm the results with observations in the target patient population.



Idarucizumab for dabigatran reversal is currently investigated in the ongoing RE-VERSE AD™ trial in dabigatran-treated patients experiencing life-threatening bleeding or requiring urgent invasive procedures. To date, no clear pattern of immune-associated adverse events has emerged in the clinical study of idarucizumab [38]. Moreover, no relevant information about prior medications was collected during these trials. However, the subjects were mostly healthy volunteers, and it is unlikely that they had received prior treatment with mAbs. Had prior treatment with mAbs occurred, this would complicate interpretation of the pre-existing ADA results.

## Conclusion

Pre-existing idarucizumab ADA were detected in ~12% of subjects, were primarily C-terminus-specific, and were without effects on idarucizumab PK or PD. Treatment-emergent ADA were detected in 8.5% of subjects and most were not specific to the dabigatran-binding regions of idarucizumab; they were possibly persistent in ~4.5% of subjects and transient in the remainder (~4% of subjects). The amounts of circulating pre-existing and treatment-emergent ADA were very low relative to the idarucizumab dose and are therefore unlikely to affect dabigatran reversal by idarucizumab.

## Competing Interests

All authors have completed the Unified Competing Interest form at [http://www.icmje.org/coi\\_disclosure.pdf](http://www.icmje.org/coi_disclosure.pdf) (available on request from the corresponding author). Stephen Norris was a Trial Bioanalyst and is a former employee of Boehringer Ingelheim Pharmaceuticals, Inc. Steven Ramael is a current employee, and Wouter Haazen a former employee, of SGS Life Sciences Clinical Research Services, where the Phase I healthy Caucasian (NCT01688830) and elderly, middle-aged and renally impaired (NCT01955720) studies took place. Ippey Ikushima was the Principal Investigator of the study in Japanese volunteers (NCT02028780). Akiko Harada was a Trial Clinical Pharmacokineticist and is a full-time employee of Nippon Boehringer Ingelheim Co. Ltd. Viktoria Moschetti was a Trial Clinical Monitor and is a full-time employee of Boehringer Ingelheim Pharma GmbH & Co. KG. Susumu Imazu was a Trial Clinical Monitor and is a full-time employee of Nippon Boehringer Ingelheim Co. Ltd, Tokyo, Japan. Paul A. Reilly is a full-time employee of Boehringer Ingelheim Pharmaceuticals, Inc. Benjamin Lang was a Trial Statistician and is a full-time employee of Boehringer Ingelheim GmbH & Co. Joachim Stangier was a Trial Biomarker Analyst and is a full-time employee of Boehringer Ingelheim GmbH & Co. KG. Stephan Glund was a Trial Clinical Pharmacokineticist and is a full-time employee of Boehringer Ingelheim GmbH & Co. KG.

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## Contributors

Substantial contributions to the conception or design of the work: J.S., S.G., S.N., V.M., S.I., P.R., A.H. Analysis and interpretation of data: J.S., S.G., S.N., B.L., P.R., I.I., A.H. Drafting the manuscript or revising it critically for important intellectual content: J.S., S.G., S.H.N., B.L., W.H., V.M., S.I. Final approval of the manuscript: All authors.

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## Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article.

<http://onlinelibrary.wiley.com/doi/10.1111/bcp.13269/supinfo>

**Figure S1** ADA titres over time in 19 subjects<sup>a</sup> from the three Phase I studies who had pre-existing ADA

<sup>a</sup>Excluded from this figure are two subjects who had pre-existing ADA but also had treatment-boosted ADA. Also excluded are 12 subjects with low-titre pre-existing ADA (mostly titres of 1 or 2) for whom one or more of the postdose samples were negative. These could not be illustrated in the format of this figure. Due to overlapping results for several individuals, not all subjects can be seen distinctly at all time

points. ADA, antidrug antibodies; RLU, relative luminescence units

**Figure S2** ADA assay response at different concentrations of ADA-positive control in spiked human plasma  
Results are mean and SD from three separate titration runs on two different days. A comparison between adjacent bars shows the ability to detect changes in assay responses (e.g., a treatment-boosted response compared with a predose response). For example, if a predose response was equivalent to that seen with the 37.5 ng ml<sup>-1</sup> positive control (i.e., RLU = 436), a postdose sample with an RLU equivalent to that seen with the 75 ng ml<sup>-1</sup> positive control (i.e., RLU = 779) would easily be detected as a treatment-boosted response. ADA, antidrug antibodies; RLU, relative luminescence units