The IgG-degrading enzyme, Imlifidase, restores the therapeutic activity of FVIII in inhibitor-positive hemophilia A mice

Melissa Bou-Jaoudeh, Sandrine Delignat, Victoria Daventure, Jan Astermark, Hervé Lévesque, Jordan D Dimitrov, Claire Deligne, Valérie Proulle, Sébastien Lacroix-Desmazes

To cite this version:

Melissa Bou-Jaoudeh, Sandrine Delignat, Victoria Daventure, Jan Astermark, Hervé Lévesque, et al.. The IgG-degrading enzyme, Imlifidase, restores the therapeutic activity of FVIII in inhibitor-positive hemophilia A mice. Haematologica, 2023, 108, pp.1322 - 1334. 10.3324/haematol.2022.281895 . hal-04117801

HAL Id: hal-04117801
https://hal.sorbonne-universite.fr/hal-04117801

ARTICLE -

The IgG-degrading enzyme, Imlifidase, restores the therapeutic activity of FVIII in inhibitor-positive hemophilia A mice.

Melissa Bou-Jaoudeh,[1] Sandrine Delignat,[1] Victoria Daventure,[1] Jan Astermark,[2] Hervé Lévesque,[3] Jordan D Dimitrov,[1] Claire Deligne,[1] Valérie Proulle[1,4] and Sébastien Lacroix-Desmazes[1]

[1]Institut National de la Santé et de la Recherche Médicale, Centre de Recherche des Cordeliers, CNRS, Sorbonne Université, Université Paris Cité, Paris, France; [2]Department for Translational Medicine, Skane University Hospital, Lund University, Malmö, Sweden; [3]Service de Médecine Interne, Normandie Université, UNIROUEN, Rouen, France and [4]Service d'Hématologie Biologique et Unité Fonctionnelle d'Hémostase, Hôpital Cochin, AP-HP Centre, Université Paris Cité, Paris, France

Correspondence: S. Lacroix-Desmazes
sebastien.lacroix-desmazes@inserm.fr
V. Proulle
Valerie.proulle@aphp.fr

Received: August 5, 2022.
Accepted: January 11, 2023.
Early view: January 19, 2023.

https://doi.org/10.3324/haematol.2022.281895

Abstract

Neutralizing anti-factor VIII (FVIII) antibodies, known as FVIII inhibitors, represent a major drawback of replacement therapy in persons with congenital hemophilia A (PwHA), rendering further infusions of FVIII ineffective. FVIII inhibitors can also appear in non-hemophilic individuals causing acquired hemophilia A (AHA). The use of non-FVIII bypassing agents in cases of bleeds or surgery in inhibitor-positive patients is complicated by the lack of reliable biological monitoring and increased thrombotic risk. Imlifidase (IdeS) is an endopeptidase that degrades human immunoglobulin G (IgG); it was recently approved for hyperimmune patients undergoing renal transplants. Here we investigated the ability of IdeS to eliminate FVIII inhibitors *in vitro* and in a model of inhibitor-positive HA mice. IdeS cleaved anti-FVIII plasma IgG from PwHA and AHA patients, and hydrolyzed recombinant human anti-FVIII IgG independently from their subclass or specificity for the A2, A3, C1 or C2 domains of FVIII. In HA mice passively immunized with recombinant human anti-FVIII IgG, IdeS restored the hemostatic efficacy of FVIII, as evidenced by the correction of the bleeding tendency. Our results provide the proof of concept for the transient removal of FVIII inhibitors by IdeS, thereby opening a therapeutic window for efficient FVIII replacement therapy in inhibitor-positive patients.

Introduction

Up to 30% of the persons with hemophilia A (PwHA) may develop neutralizing anti-factor VIII (FVIII) allo-antibodies (FVIII inhibitors) after replacement therapy,[1] with approximately 60% exhibiting high inhibitory titers. The onset of FVIII inhibitors is favored by genetic (ethnicity, mutations in the *F8* gene) and environmental (exposure) factors.[2] Neutralizing auto-antibodies against FVIII can also appear in individuals with no previous history of bleeding, typically in elderly individuals or in the postpartum period,[3] causing acquired hemophilia A (AHA).

The management of clinically relevant acute bleeds and/or surgeries in patients with high FVIII inhibitor titers is particularly challenging. Bypassing agents (BPA), such as recombinant activated FVII (rFVIIa) and activated prothrombin complex concentrates (aPCC), or recombinant porcine FVIII, are recommended as first-line treatments. Apart from their proven efficacy, BPA have major drawbacks, including the need for frequent dosing, the lack of reliable biomarkers for hemostatic efficacy other than clinical improvement, and the increased thrombotic risk.[4–7] The development of emicizumab, a humanized bispecific antibody that mimics the cofactor function of FVIII, has revolutionized prophylaxis for PwHA and inhibitors.[8,9] Emicizumab dramatically reduces annualized bleeding rates with once-weekly or fewer subcutaneous injections.[10] However, emicizumab does not completely restore hemostasis, and standard hemostatic treatments are still required for patients undergoing breakthrough bleeds or surgery.[11,12] Further, the concomitant use of emicizumab and BPA, particularly aPCC, carries an increased risk of thrombotic microangiopathies and thromboembolic events.[13] Elderly hospitalized patients with acquired HA (PwAHA) with multiple comorbidities are also at increased risks of arterial and venous thrombotic events while receiving high

BPA doses.[3,7] As a result, on-demand replacement therapy with exogenous FVIII remains the best option for managing acute bleeds or surgery in PwHA and PwAHA. Eliminating neutralizing anti-FVIII antibodies to temporarily restore the hemostatic efficacy of FVIII while avoiding the use of BPA is an appealing new therapeutic option in patients with FVIII inhibitors.

Streptococcus pyogenes, an important human pathogen, produces IdeS (immunoglobulin G [IgG]-degrading enzyme of *Streptococcus pyogenes)* as a defense mechanism against antibody attack and complement activation.[14] IdeS is a cysteine proteinase that can cleave all four human IgG subclasses with a unique degree of specificity below the disulfide bridge in the hinge region.[15] However, IdeS only partially hydrolyzes mouse IgG.[16] IdeS sequentially cleaves the two heavy chains of IgG with different kinetics, thus releasing the $F(ab')_2$ fragment from the Fc fragment. A recombinant IdeS is commercially available (Imlifidase, Ideferix®) and is the only desensitization treatment European Medicines Agency-approved for kidney transplant patients with donor-specific antibodies.[17] IdeS is also being studied for its therapeutic potential in several autoimmune diseases[18,19,20] as well as in oncology and gene therapy.[21,22]

Here, we hypothesized that the cleavage of circulating IgG by IdeS, leading to the fast, though temporary, clearance of IgG, may provide a new therapeutic opportunity for patients with FVIII inhibitors. We demonstrate that IdeS efficiently hydrolyzes polyclonal anti-FVIII IgG in patients' plasma and monoclonal recombinant human anti-FVIII IgG (anti-FVIII rhIgG) *in vitro*. We developed a mouse model of inhibitor-positive severe HA by passively immunizing HA mice with anti-FVIII rhIgG. IdeS restored the hemostatic efficacy of FVIII infusions in inhibitor-positive HA mice. Our results provide the proof of concept for temporarily removing FVIII inhibitors by IdeS and opening a therapeutic window for efficient FVIII replacement therapy and better management of patients with FVIII inhibitors.

Methods

Plasma samples from patients with congenital or acquired hemophilia A

Plasma from 102 PwHA was obtained from the MIBS registry (Malmö International Brother Study) that includes siblings with and without a history of inhibitors.[23] Plasma from 43 PwAHA was obtained from the SACHA (Surveillance des Auto antiCorps au cours de l'Hémophilie Acquise) French registry at the time of inclusion with titers ≥1 Bethesda units (BU)/mL.[7] Procedures were in accordance with the ethical standards of the responsible committees on human experimentation for both cohorts and with the Declaration of Helsinki. MIBS and SACHA are registered (*clinicaltrails.gov. Identifier: NCT00231751* and *NCT00213473*, respectively).[7,23]

Generation of recombinant human anti-FVIII immunoglobulin G

Four anti-FVIII $rhIgG_k$ were produced: BOIIB2 (patent US20070065425A1), KM41,[24] LE2E9[25] and BO2C11[26] that are specific for the A2, A3, C1 and C2 domains of FVIII, respectively. The genes encoding the VH regions of the IgG and the VL regions of the Ig_k were cloned in eukaryotic expression vectors (kindly provided by Dr. Hugo Mouquet, INSERM, Paris). The corresponding $IgG1_k$ and $IgG4_k$ were produced in HEK293 cells using the Expi293 protocol (Thermo Scientific) and purified from the culture supernatant by affinity chromatography on protein G-agarose beads (GE Healthcare). Monoclonal IgG were validated by SDS-PAGE, enzyme-linked immunosorbant assay (ELISA) and modified Nijmegen-Bethesda assay.

Determination of anti-FVIII antibody inhibitory titers

The inhibitory activity of the anti-FVIII rhIgG was measured using the modified Nijmegen-Bethesda assay (MNBA).[27] Monoclonal IgG in phosphate-buffered saline (PBS, pH 7.4, Life Technologies) or in mouse plasma were serially diluted in veronal buffer and incubated vol/vol with a standard pool of human plasma (Siemens Healthcare), used as a source of FVIII, for 2 hours (h) at 37°C. The residual pro-coagulant FVIII activity (FVIII:C) was measured using a chromogenic assay following the manufacturer's instructions (Siemens Healthcare). In the case of purified IgG, the inhibitory activity of the IgG was expressed in BU/μg IgG, defined as the inverse of the concentration of IgG needed to inhibit 50% of FVIII:C. In the case of IgG in mouse plasma, the inhibitory titers were expressed in BU/mL, defined as the plasma dilution that neutralizes 50% of normal plasma FVIII:C. Titers ≥0.6 BU/mL were considered as positive.

Generation of Imlifidase

The DNA sequence encoding IdeS from *S. pyogenes* was obtained from Geneart (Thermo Scientific). It was cloned into a pEX-N-His-tagged expression vector for expression in *E. coli* strain BL21. Protein expression was induced by 0.5 mM IPTG for 4 h at 37°C. Proteins were purified by immobilized metal affinity chromatography (HisTrap FF column, GE Healthcare). Buffer was exchanged with PBS using a PD-10 desalting column (GE Healthcare) and endotoxins were removed using the Pierce endotoxin removal kit (Thermo Scientific). Integrity of IdeS was confirmed by SDS-PAGE and concentration was determined using NanoDrop™ with a 50880 $M^{-1}cm^{-1}$ extinction coefficient.

Hydrolysis of immunglobulin G by Imlifidase

For IgG in patients' plasma, 10-fold diluted plasma was incubated in PBS alone or with 0.54 μM IdeS (yielding an approximate 12:1 molar ratio of IgG:IdeS) for 24 h at 37°C. For anti-FVIII rhIgG1$_k$ and rhIgG4$_k$, IgG (1.66 μM) were incubated alone or with IdeS (0.14 μM) at a 12:1 IgG:IdeS molar ratio for 24 h at 37°C.

Mouse model of inhibitor-positive severe hemophilia A

Eight- to 12-week-old male and female exon 16 FVIII-deficient mice[28] on a C57BL/6 background (HA mice) were housed and handled in accordance with French regulations and the experimental guidelines of the European Community (Comité d'éthique en expérimentation animale no.005, protocol APAFIS#24748-2020032014465347). Naive HA mice were passively immunized by intravenous injection of the human recombinant BO2C11 IgG1$_k$ (600 BU/kg). For determination of IgG half-life, blood was collected at 5 minutes (min), 4 h, 1, 2, 5, and 7 days post-injection. Inhibitory titers were measured in plasma using MNBA.

In vivo efficacy of Imlifidase in inhibitor-positive hemophilia A mice

HA mice were passively immunized by intravenous injection of BO2C11 IgG1$_k$ alone at 1,200 BU/kg or 24,000 BU/kg, or of equimolar amounts of BOIIB2, KM41, LE2E9 and BO2C11 in IgG1$_k$ format (2,800 BU/kg). Mice were treated 1 day later by intravenous injection of IdeS (0.6 mg/kg or 0.29 μM) or PBS as control. When indicated, mice received a second injection of IdeS 24 h later. Residual levels of intact anti-FVIII rhIgG, partially hydrolyzed single-~~chain~~ (sc) IgG, F(ab')$_2$ fragments, and inhibitory activities levels were determined by ELISA and MNBA in plasma collected up to 6 days after IdeS or PBS injection.

Evaluation of bleeding tendency and hemostasis

Inhibitor-positive HA mice treated with PBS or IdeS were injected with therapeutic recombinant human FVIII (Helixate®, 200 U/kg) via the retro-orbital route 3 days after IdeS or PBS injection. The bleeding tendency and hemostatic parameters were analyzed 2 h later. The bleeding tendency was evaluated using a standardized tail clipping assay in isoflurane-anesthetized mice (2% isoflurane in 30% O_2 and 70% N_2O; flow: 1 L/min) maintained at 37°C on a heating pad. Three mm of the distal tail was amputated and blood was collected over 10 min. Blood loss in each sample was calculated from a standard curve, as already described.[29] The FVIII:C was measured in plasma using a chromogenic test (Siemens Healthcare). Thrombin generation in platelet-poor plasma (PPP) was measured using the Calibrated Automated Thrombrogram and PPP Reagent Low (Stago) as already described,[30] except that PPP was diluted 1/6 in HEPES-buffered saline containing 0.5% bovine serum albumin (BSA).

SDS-PAGE and western blot

Purified IgG or IgG in human plasma (5 μg), incubated alone or with IdeS, were separated by SDS-PAGE in NuPAGE 4-12% gradient Bis-Tris protein gels (Thermo Scientific) under non-reducing conditions, and transferred to nitrocellulose membranes using a semi-dry iBlot system (Invitrogen). Membranes were blocked and incubated with a polyclonal goat anti-human F(ab')$_2$ fragment-specific antibody (Invitrogen) or a polyclonal rabbit anti-human Fc©-specific antibody (Sigma-Aldrich). Bound antibodies were revealed using appropriate secondary antibodies: an HRP-coupled rabbit anti-goat IgG (R&D System) or an HRP-coupled goat anti-rabbit IgG (Cell signaling), and the Pierce™ ECL Western Blotting Substrate and iBright™ FL1000 Imaging System (Thermo Scientific).

Human anti-FVIII immunoglobulin G enzyme-linked immunosorbant assay

ELISA plates (Maxisorp, Nunc) were coated with rhFVIII (Advate®, 2.5 μg/mL). Patients' plasma or purified anti-FVIII rhIgG were added to the wells. Bound anti-FVIII IgG or F(ab')$_2$ were revealed using an HRP-labeled mouse monoclonal antibody specific for human Fcγ (Southern Biotech) or an HRP-labeled goat anti-human IgG F(ab')$_2$ fragment secondary antibody (Thermo Scientific), respectively, and the o-phenylenediamine dihydrochloride (OPD, Sigma-Aldrich) substrate. Absorbances were read at 492 nm. The titers of anti-FVIII IgG in patients' plasma were defined as the highest dilution of plasma yielding an optical density (OD) ≥cutoff. The cutoff was computed as the mean OD calculated for the plasma from 22 healthy individuals + 95% percentile*standard deviation.[31]

Human immunoglobulin G and F(ab')$_2$ fragments enzyme-linked immunosorbant assay

ELISA plates were coated with a goat anti-human Ig κ antibody (2.5 μg/mL; Southern Biotech). Purified anti-FVIII rhIgG or mouse plasma containing anti-FVIII rhIgG were added to the wells. Bound IgG were revealed using an horseradish peroxidase (HRP)-labeled mouse monoclonal antibody specific for human Fcγ (Southern Biotech). Bound F(ab')$_2$ fragments were detected using an HRP-labeled goat anti-human IgG F(ab')$_2$ secondary antibody (Thermo Scientific). Absorbance was read at 492 nm after addition of the OPD substrate. Concentrations were calculated in μg/mL using BO2C11 as a standard.

Results

Imlifidase hydrolyzes anti-FVIII immunglobulin G in plasma from persons with hemophilia A and patients with acquired hemophilia A

We investigated whether IdeS hydrolyzes IgG in the plasma from 43 PwAHA and 102 PwHA. Twenty-two of the 102 PwHA plasma tested positive for FVIII inhibitors (mean ± standard deviation [SD]: 9.8±15.6 BU/mL, ranging from 0.6

to 63 BU/mL; Figure 1). Inhibitor-negative PwHA had titers below 0.6 BU/mL. Ten-fold diluted plasma was incubated alone or with IdeS (0.54 μM) for 24 h at 37°C. Samples from five randomly selected PwHA were analyzed by western blot to detect F(ab')$_2$ and Fc fragments before and after IdeS treatment. As expected,[32] incubation in the presence of IdeS led to a close to complete degradation of total IgG and the detection of traces of scIgG, together with the accumulation of F(ab')$_2$ and Fc fragments at 100 and 25 kDa, respectively (Figure 1A).

We confirmed the cleavage of anti-FVIII IgG in plasma from PwHA and PwAHA using an anti-FVIII IgG ELISA. As reported,[33] some inhibitor-negative PwHA had detectable levels of FVIII-binding IgG, but at significantly lower levels than inhibitor-positive PwHA (Figure 1B; *P*<0.0001). Treatment with IdeS resulted in undetectable anti-FVIII IgG titers in the plasma from inhibitor-positive and inhibitor-negative PwHA, and PwAHA (*P*<0.0001 in all cases). This is consistent with the release of the Fc fragments from the F(ab')$_2$ fragments of the IgG upon IdeS-mediated cleavage and the associated loss of detection of the bound anti-FVIII F(ab')$_2$ fragments by the anti-human Fc antibody in ELISA.

Imlifidase hydrolyzes anti-FVIII immunoglobulin G irrespective of their subclass and epitope specificity

Anti-FVIII IgG in PwHA and PwAHA belong in the large majority to the IgG1 and IgG4 subclasses.[33] In order to further decipher the action of IdeS on anti-FVIII IgG, we generated four monoclonal anti-FVIII rhIgG expressed in both the IgG1$_k$ and IgG4$_k$ formats, specific for the A2, A3, C1 or C2 domain of human FVIII.

The recombinant IgG1$_k$ and IgG4$_k$ versions of each monoclonal IgG exhibited identical dose-dependent binding to FVIII in ELISA (Figure 2A) and neutralized FVIII:C within identical orders of magnitude (Table 1). The four IgG were cleaved equally by IdeS, irrespective of their epitope specificity or IgG subclass. Indeed, incubation of each IgG with IdeS at a 12:1 molar excess for 24 h at 37°C resulted in the complete disappearance of the intact IgG and the generation of the F(ab')$_2$ and Fc fragments (Figure 2B). Time-dependent analyses of IgG cleavage by IdeS, performed using BO2C11, demonstrated that IgG1$_k$ and IgG4$_k$ are cleaved with similar kinetics. More than 90% of the IgG were hydrolyzed as scIgG within the first 5 min of *in vitro* incubation and fully hydrolyzed F(ab')$_2$ fragments were detected from 20 min onwards (Figure 2C). The physical dissociation between F(ab')$_2$ and Fc fragments upon IdeS cleavage was confirmed by ELISA (Figures 3A; *Online Supplementary Figure S1*). Under static conditions (i.e., in a test tube), the F(ab')$_2$ fragments of neutralizing anti-FVIII IgG, generated upon IdeS cleavage, are not eliminated and are presumably still able to neutralize the procoagulant activity of FVIII. Indeed,

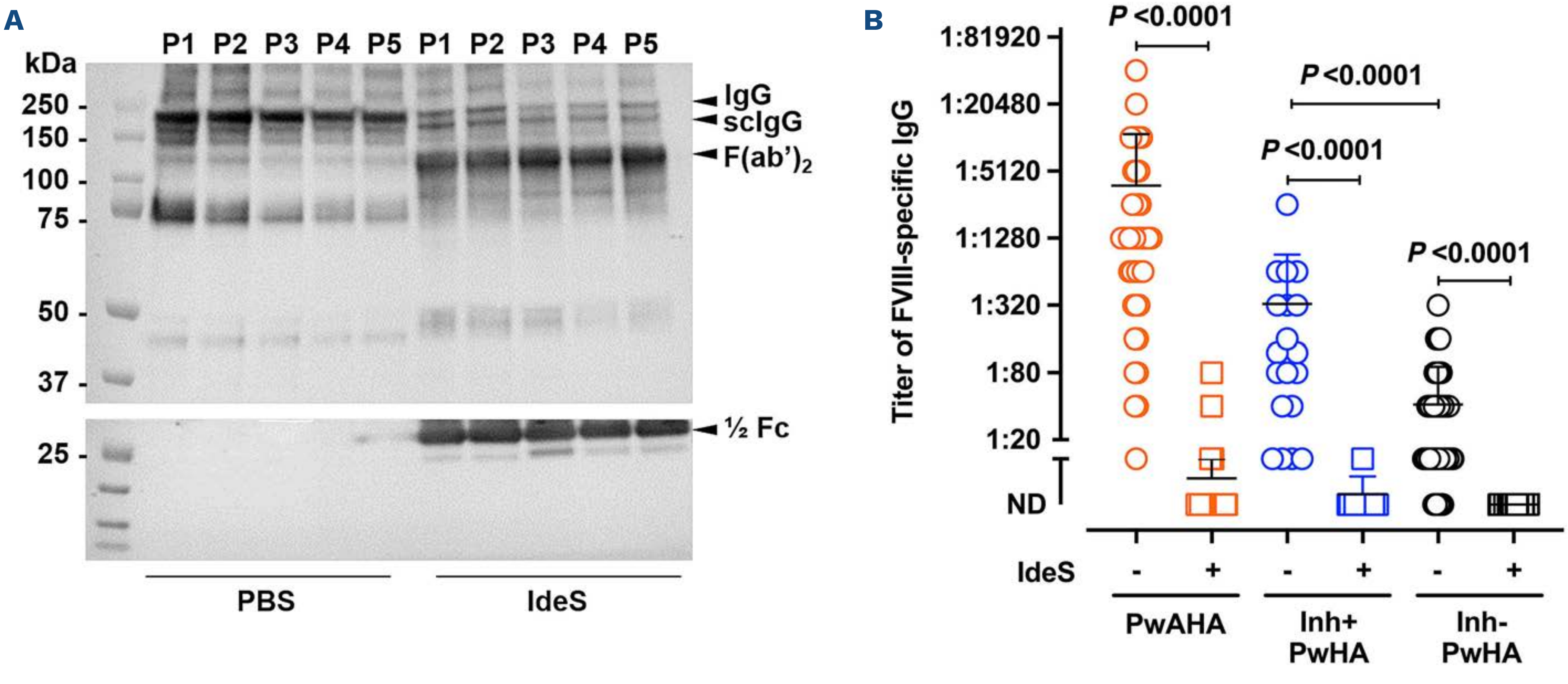

Figure 1. Hydrolysis of immunglobulin G in the plasma from patients with congenital and acquired hemophilia A. (A) Plasma samples obtained from 5 congenital hemophilia A (HA) patients (P1-P5, MIBS cohort) were pre-incubated for 24 hours (h) at 37°C with Imlifidase (IdeS) (0.54 μM) or phosphate-buffered saline (PBS), and subjected to western blot. Immunoglobulin G (IgG) were recognized with a F(ab')$_2$-specific antibody (top) or a Fc-specific antibody (bottom). Molecular weight markers are shown at the left of the blot. The predicted molecular weights of intact IgG, scIgG, F(ab')$_2$ and Fc fragments are shown. (B) Ten-fold diluted plasma from 43 patients with acquired HA (PwAHA) and from 102 patients with mild, moderate or severe HA, with (Inh+ PwHA, n=22) or without (Inh- PwHA, n=80) FVIII inhibitors, were incubated for 24 h at 37°C with IdeS (0.54 μM) or PBS. Inhibitor-positive patients were defined by inhibitory titers ≥ 0.6 BU/mL. The graph depicts the titers of FVIII-specific IgG. Plasma samples were diluted at least 1:20. Samples that did not give a positive signal at this minimum dilution were considered as negative (ND: not detectable). Statistical differences were assessed using the two-sided Mann-Whitney test.

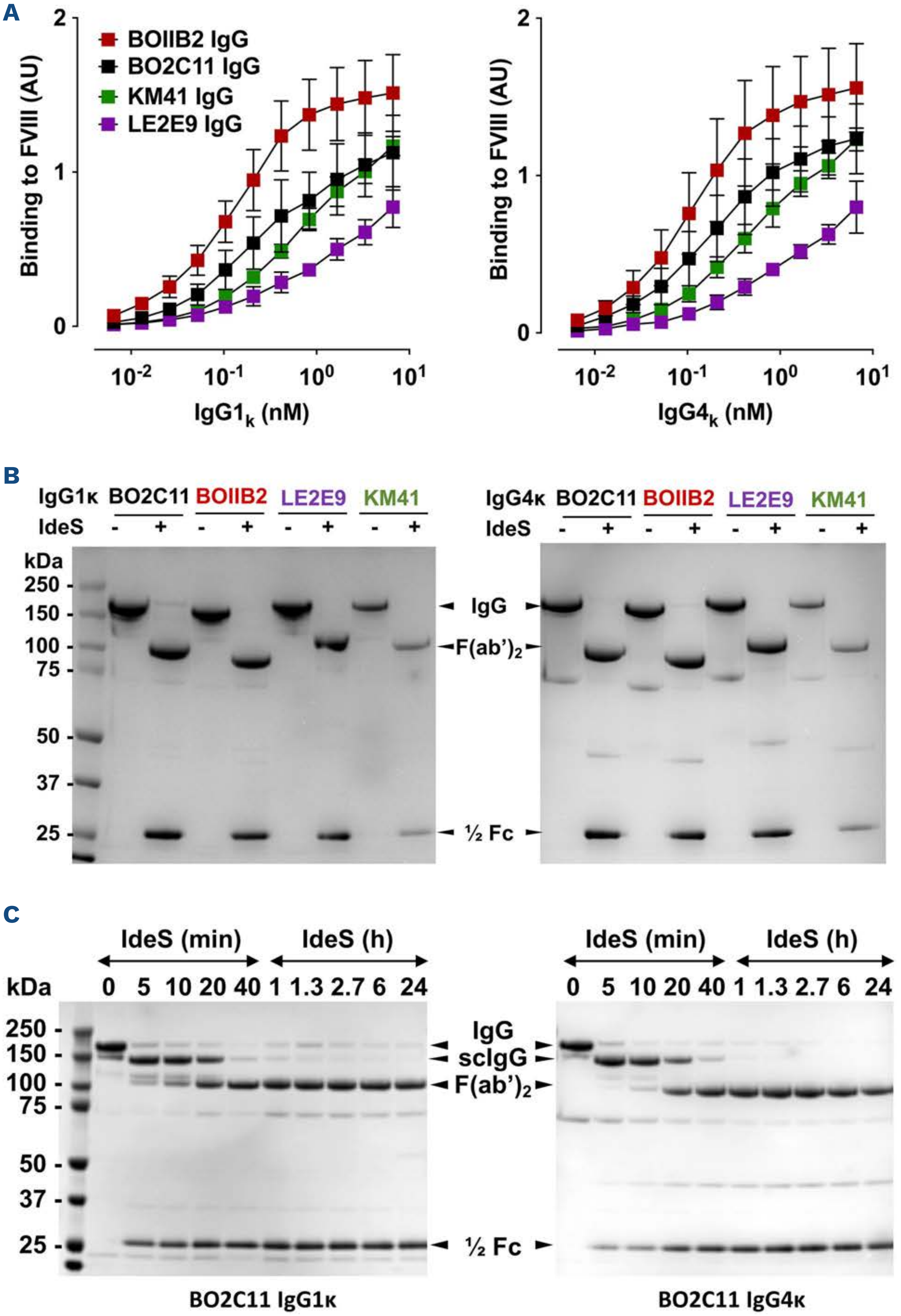

Figure 2. Cleavage of human monoclonal anti-FVIII immunglobulin G by Imlifidase. (A) Validation of human recombinant monoclonal anti-FVIII immunoglobulin G (IgG). Four human monoclonal neutralizing anti-FVIII IgG were cloned and expressed as IgG1_k (left panel) or IgG4_k (right panel). Their binding to FVIII was validated in a human anti-FVIII IgG enzyme-linked immunosorbant assay. Results are expressed in arbitrary units (AU, mean ± standard deviation from 3 independent experiments) using the optical densities measured at 492 nm. (B, C) IdeS-mediated hydrolysis of human monoclonal anti-FVIII IgG. The monoclonal anti-FVIII IgG1_k (left panel) or IgG4_k (right panel) were incubated with Imlifidase (IdeS) at a 12 IgG:1 IdeS molar ratio (1.66 μM IgG *vs.* 0.14 μM IdeS) for 24 hours (panels B) or for different periods of time (C) at 37°C. Samples were separated by SDS-PAGE under non-reducing conditions. Molecular weight markers and the predicted molecular weights of intact IgG, scIgG, F(ab')_2 and Fc fragments are shown on the left and right of each gel, respectively.

Table 1. Inhibitory activity of monoclonal anti-FVIII IgG1k and IgG4k.

mAb	Domain	Inhibitory activity (BU/μg)		Reference
		IgG1k	IgG4k	
BOIIB2	A2	6.3 ± 3	7.7 ± 0.5	Patent US20070065425
BO2C11	C2	2.6 ± 1.6	1.6 ± 0.8	Jacquemin *et al.* Blood 1998[26]
KM41	A3	0.023 ± 0.001	0.054 ± 0.011	van den Brink *et al.* Blood 2001[24]
LE2E9	C1	0.36 ± 0.04	0.77 ± 0.25	Jacquemin *et al.* Blood 2000[25]

The inhibitory activity of the 4 monoclonal IgG1 and IgG4 antibodies was measured in a modified Nijmegen Bethesda assay. The table depicts, for each immunglobulin G (IgG), the domain specificity, the inhibitory activity in the IgG1 and IgG4 formats and the original reference. mAb: monoclonal antibody.

samples of native or IdeS-cleaved BO2C11 IgG neutralized FVIII:C to a similar extent *in vitro* in a MNBA, irrespective of the IgG subclass (Figure 3B). Accordingly, plasma from an inhibitor-positive PwHA neutralized FVIII:C to similar extent *in vitro* following incubation alone or with IdeS (Figure 3C).

Validation of a mouse model of inhibitor-positive hemophilia A

In order to develop a mouse model of inhibitor-positive HA, we first determined the half-life of BO2C11 IgG1$_k$, used as model IgG, in FVIII-deficient mice. The intravenous injection of 600 BU/kg of BO2C11 IgG1$_k$ was followed by a two-phase elimination pattern. Fitting the experimental data to a double exponential decay curve yielded fast and slow elimination half-lives of 0.2 and 9 days, respectively (Figure 4A). Inhibitory titers measured in mice plasma were 12.8±1.2 BU/mL at 5 min and 5.7±1.1 BU/mL at 24 h, representing a 45% reduction. The inhibitory titers remained relatively stable for the next 6 days (i.e., 3.3±1.9 BU/mL at day 7).

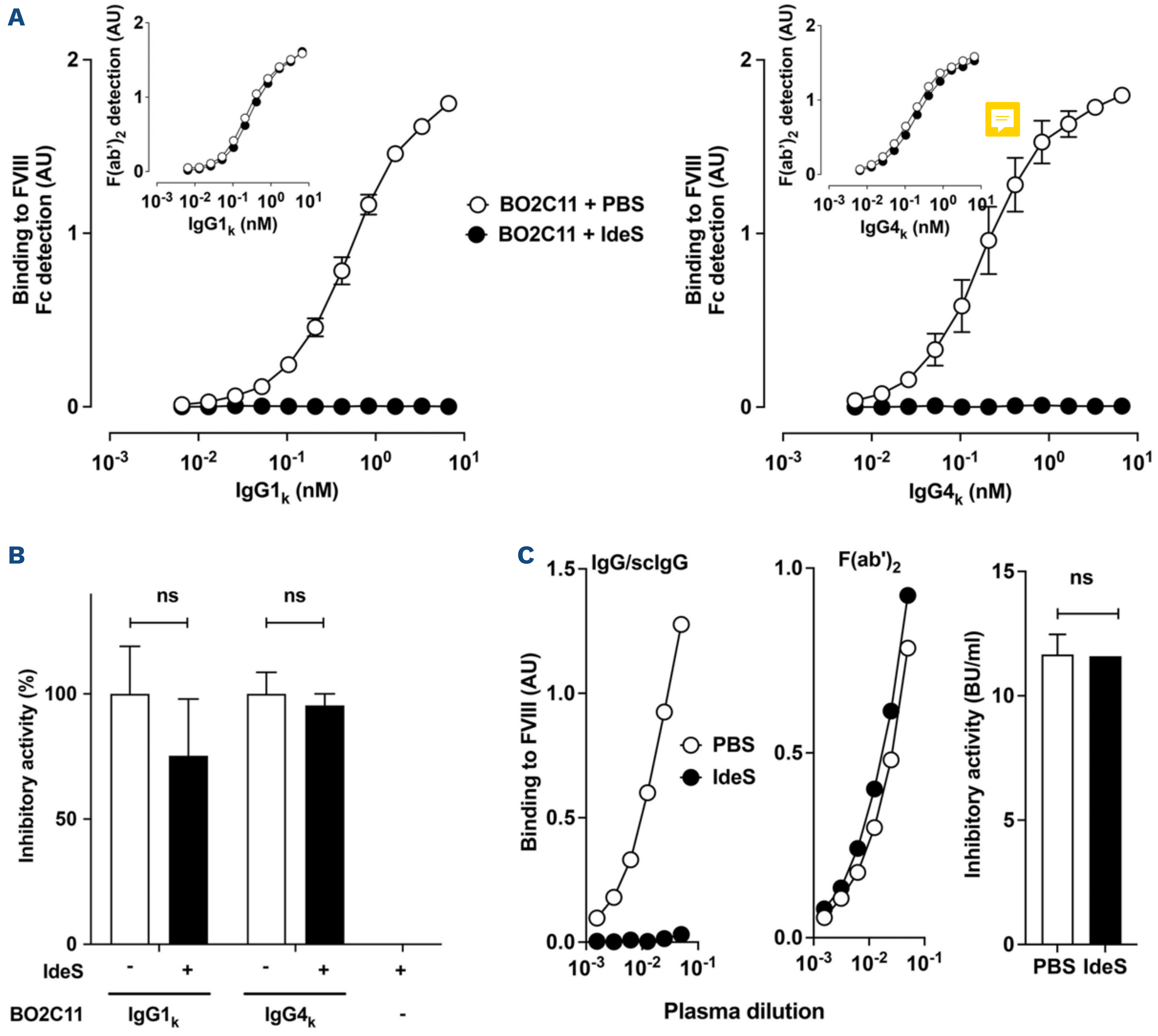

Figure 3. Inhibitory activity of F(ab')$_2$ fragments generated upon Imlifidase cleavage. (A) Binding of BO2C11 immunoglobulin G (IgG) to FVIII following cleavage by Imlifidase (IdeS). BO2C11 IgG1$_k$ (left panel) and IgG4$_k$ (right panel) at 1.66 μM were incubated alone or with IdeS (0.14 μM) for 24 hours (h) at 37°C (12 IgG:1 IdeS molar ratio). The binding of the intact IgG and/or scIgG to FVIII and that of F(ab')$_2$ fragments (showed as insets) was validated by enzyme-linked immunsorbant assay. Results are expressed in arbitrary units (AU, representative of 2 experiments) from optical density measured at 492 nm. (B) Inhibitory activity of IdeS-cleaved BO2C11 IgG. The inhibitory activity of BO2C11 IgG1$_k$ and IgG4$_k$ incubated in phosphate-buffered saline (PBS) alone (-) or with IdeS (+) was measured in a Bethesda assay. As a control, IdeS was introduced alone in the assay. Values depict the respective % of residual inhibitory activities as compared to the activity measured in the absence of IdeS for IgG1$_k$ and for IgG4$_k$ (means ± standard deviation of 3 independent experiments). (C) Inhibitory activity of IdeS-cleaved polyclonal anti-FVIII IgG. Plasma (1/10) from an inhibitor-positive patient with hemophilia A (PwHA) was incubated for 24 h at 37°C with IdeS (0.54 μM) or PBS. The binding to FVIII of intact IgG/scIgG and F(ab')$_2$ fragments was measured by enzyme-linked immunsorbant assay. Results are expressed in AU using the optical densities measured at 492 nm. The inhibitory titer was measured in the plasma treated with PBS or IdeS using a modified Nijmegen-Bethesda assay (n=2, mean ± standard deviation).

In vivo Imlifidase efficacy and pharmacokinetics

In a first series of experiments, HA mice were passively immunized with 1,200 BU/kg of BO2C11 IgG1$_k$. This amount of IgG1$_k$ achieved reproducible inhibitory titers of 9.4±2.3 BU/mL and 5.2±2.7 BU/mL 24 and 96 h later, respectively (Figure 4D), titers for which administration of therapeutic FVIII is inefficient in patients. Inhibitor-positive HA mice were treated with 0.6 mg/kg IdeS 24 h after the injection of BO2C11 IgG1$_k$ (Figure 4B). As compared to PBS-treated control mice, IdeS-treated mice experienced a drastic 94% drop in IgG levels (either intact IgG or scIgG that are both detected in the human IgG ELISA) 6 h after IdeS injection (Figure 4C). The rapid loss of detection of IgG in mouse plasma was associated with a slower disappearance of the inhibitory activity towards FVIII that was still detectable at least 24 h following IdeS injection (Figure 4D). Interestingly, the progressive decrease in inhibitory activity in plasma demonstrated a statistically significant linear correlation with the disappearance of the F(ab')$_2$ fragments of BO2C11 from the circulation (Figures 4E, F; $r^2=0.93$; $P<0.0001$). The inhibitory activity was below the detection threshold of the assay 2-3 days after IdeS injection. Similar results were obtained when HA mice were passively immunized with a pool of BOIIB2, KM41, LE2E9 and BO2C11 IgG1$_k$ (*Online Supplementary Figure S2*).

Fitting the experimental data of F(ab')$_2$ catabolism (Figure 4E), from 6 h following IdeS injection onwards, to a one-phase decay curve yielded a 11.7 h half-life of human F(ab')$_2$ fragments in mice (range, 10.4 to 13.1 h).

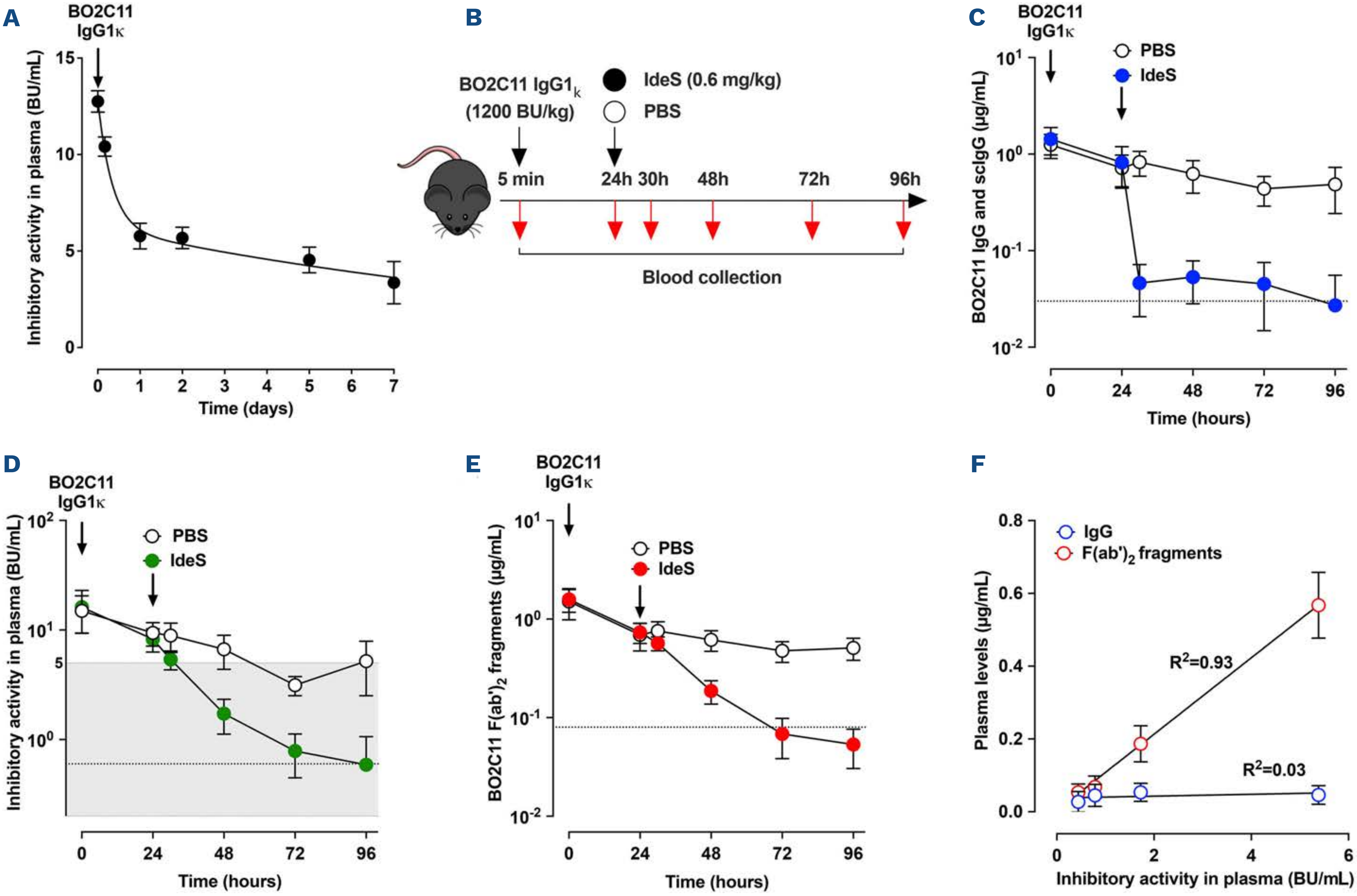

Figure 4. Imlifidase-mediated elimination of a FVIII inhibitor in inhibitor-positive hemophila A mice. (A) Half-life of BO2C11 immunoglobulin G (IgG1$_k$) in hemophila A (HA) mice. C57BL/6 HA mice (n=5) were passively immunized with BO2C11 IgG1$_k$ (600 Bethesda units [BU]/kg). The graph depicts the inhibitory activity towards FVIII measured in plasma over time. (B) HA mice (n=6 per group) were passively immunized with 1,200 BU/kg of BO2C11 IgG1$_k$ to reach 10 BU/mL after 24 hours (h), and injected with Imlifidase (IdeS) (0.6 mg/kg, 0.29 µM) or phosphate-buffered saline (PBS) 24 h later. (C-E). The levels of intact IgG and/or scIgG (panel C, IgG concentration at 24 h: 5.1±0.3 nM), the inhibitory titers (D) and the levels of F(ab')$_2$ fragments (E) were determined over time by enzyme-linked immunsorbant assay and Bethesda assay (n=4, mean ± standard deviation). The dotted lines represent the respective detection thresholds: 0.03 µg/mL, 0.6 BU/mL and 0.08 µg/mL. (F) The graph shows the plasma levels of IgG (C) and F(ab')$_2$ fragments (E) as a function of the inhibitory activity in plasma (D) for the condition where mice were treated with IdeS at 30, 48, 72 and 96 h following BO2C11 injection. The experimental data were interpolated using a linear curve (R^2: goodness of fit). The grey zone in panel (D) depicts inhibitory titers below 5 BU/mL, a titer that is compatible with the hemostatic efficacy of exogenous FVIII.

Imlifidase corrects the bleeding tendency and restores FVIII hemostatic efficacy

In order to provide proof of concept towards the transient removal of FVIII inhibitors by IdeS, thereby opening a therapeutic window for efficient FVIII replacement therapy, inhibitor-positive HA mice were given 200 IU/kg of FVIII 96 h (3 days) after IdeS or PBS treatment (Figure 5A). Two hours later, the bleeding tendency and hemostatic efficacy of therapeutic FVIII were evaluated. The blood loss that followed tail tip amputation of IdeS-treated mice was significantly lower than that measured in PBS-treated mice (Figure 5B; 13±26 μL *vs.* 74±65 μL; *P*=0.0047), but was not different from that measured in naive inhibitor-negative HA mice that had received FVIII alone (21±16 μL). The reduction in blood loss was explained by a restoration of the hemostatic efficacy of therapeutic FVIII. FVIII:C recovery in IdeS-treated mice was significantly higher than that in PBS-treated mice (Figure 5C; 84.2±29.7% *vs.* 2.0±1.5%; *P*=0.0015) and did not differ from that in naive inhibitor-negative mice injected with FVIII alone (112.4±58.7%). Accordingly, thrombin generation was significantly increased in IdeS-treated mice as compared to PBS-treated mice (Figures 5D, E; thrombin peak: 52±8 nM *vs.* 25±19 nM; *P*=0.0386).

Imlifidase efficacy in the context of very high inhibitory titers

In order to mimic the situation of patients with very high inhibitory titers, we passively immunized HA mice with 24,000 BU/kg of BO2C11 $IgG1_k$ to reach inhibitory titers of 171±48 BU/mL and 97±7 BU/mL 24 and 168 h later, respectively. Mice then received either one or two injections of IdeS (0.6 mg/kg) with a 24-h interval. The circulating levels of IgG/scIgG and $F(ab')_2$ fragments and the inhibitory titers were followed over time. The loss of detection of IgG/scIgG was faster than the decrease in detection of circulating $F(ab')_2$ fragments and inhibitory activity (Figure 6). As compared to PBS-treated mice, the decrease in inhibitory activity was 27-fold and 68-fold 3 and 6 days after a single IdeS injection, respectively. Redosing of IdeS yielded a further reduction in inhibitory activity below 5 BU/mL (*P*<0.05 at 96 and 168 h).

Discussion

The promising therapeutic effect of IdeS has already been suggested in several preclinical models of human autoimmune diseases,[34–37] and in the context of gene therapy.[21] In humans, IdeS potency has been explored in patients with anti-HLA allo-antibodies undergoing kidney transplant[38,39] and in patients with Goodpasture syndrome and auto-antibodies directed against the non-collagenous domain of the α3 chain of type IV collagen.[19] Our work further substantiates the efficacy of IdeS treatment in both allo- and auto-immune settings. There are alternatives to IdeS for removing pathogenic antibodies, such as plasmapheresis,[40] molecules that block the neonatal Fc receptor (FcRn),[41,42] immunosuppressive drugs,[43,44] or therapeutic antibodies that deplete B cells.[45] However, IdeS offers several benefits in terms of specificity and efficacy, fast elimination rate, and long-lasting effects. Importantly, the presence of pre-existing anti-IdeS IgG or the onset of an anti-IdeS immune response, which peaks around 2 weeks after IdeS administration, do not preclude repeated dosing of IdeS for several consecutive days, or at a 6-month distance from the first treatment.[46] Furthermore, IdeS can cleave anti-IdeS antibodies with an IgG isotype,[21] and neutralization of IdeS by anti-IdeS antibodies has never been proven convincingly.

All pathogenic IgG hydrolyzed by IdeS in the disorders and disease models listed above are specific for antigens exposed at the surface of cells, platelets or viruses. In contrast, FVIII circulates in the blood. The soluble/membrane location of the antigen targeted by the pathogenic IgG determines the functional outcome of IdeS-mediated cleavage. Indeed, IdeS hydrolyzes IgG in two steps, starting with a rapid cleavage of one of the two heavy chains to generate a scIgG, followed by a slow cleavage of the second heavy chain that releases the $F(ab')_2$ fragment from the Fc fragment.[14,15] While scIgG lose their capacity to bind and activate complement, as well as to mediate antibody-dependent cell cytotoxicity (ADCC), they retain their capacity to bind their target antigen and have a normal half-life owing to the preserved binding to the FcRn.[14] In contrast, the $F(ab')_2$ fragments of completely digested IgG lose all Fc fragment-mediated functions but maintain antigen-binding (and possibly neutralization) capacity during their life span in the circulation. As a result, IdeS-mediated IgG cleavage has an immediate functional repercussion when the pathogenic IgG are directed against membrane antigens and exert their pathogenic effects by complement activation, phagocytosis or ADCC. In contrast, when the pathogenic IgG neutralize soluble antigens, as is the case of neutralizing anti-FVIII IgG, the functional consequence of IdeS-mediated cleavage is delayed until elimination of the $F(ab')_2$ fragments from the circulation. Hence, in test tubes, the mere *in vitro* cleavage of monoclonal and polyclonal anti-FVIII IgG failed to abrogate the neutralizing activity of the residual $F(ab')_2$ fragments towards FVIII:C. *In vivo*, the disappearance of the FVIII inhibitory titers from the plasma of passively immunized inhibitor-positive HA mice required 48 h after dosing with IdeS, which correlated with changes in plasma levels of $F(ab')_2$ fragments and is consistent with the 12-h half-life of $F(ab')_2$ fragments that we determined in HA mice.

Different preclinical models of HA have been developed, including dogs, rats and minipigs. FVIII-deficient mice, how-

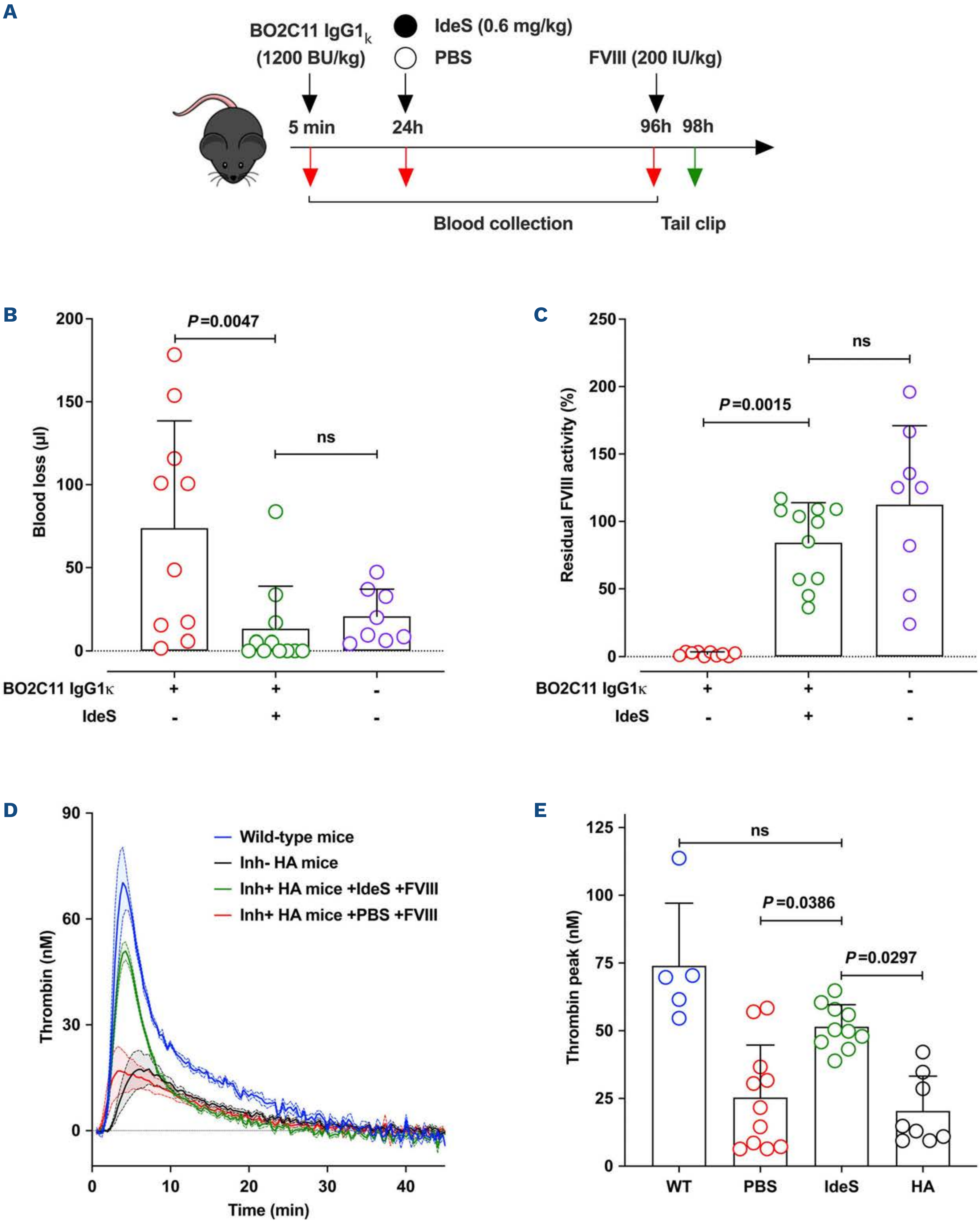

Figure 5. Efficacy of therapeutic FVIII in inhibitor-positive hemophilia A mice treated with Imlifidase. (A) Hemophilia A (HA) mice were passively immunized with BO2C11 immunoglobulin (Ig) G1$_k$ (1,200 Bethesda units [BU]/kg) and treated with Imlifidase (IdeS) (0.6 mg/kg) or phosphate-buffered saline (PBS) 24 hours (h) later. The mice were then administered intravenously with therapeutic FVIII (Helixate®, 200 IU/kg) at day 4. Control mice (wild-type [WT]) were injected with FVIII in the absence of passive immunization with BO2C11 IgG1$_k$ and treatment with IdeS. (B-E) Two h after FVIII injection, the mice tails were amputated at the terminal 3 mm and the blood loss was evaluated over 10 minutes (min). (B) In parallel, plasma was collected to determine the restoration of hemostatic efficacy of therapeutic FVIII by measuring the FVIII:C in a chromogenic assay (C), and the levels of thrombin generation (nM) over time (min) and thrombin peak (nM) using a thrombin generation test (D, E). In the graphs, the horizontal bars represent means ± standard deviation (SD) and each symbol depicts an individual animal. Statistical differences were assessed using the non-parametric Kruskal-Wallis test corrected for multiple comparisons using the Dunn's test (ns: non-significant). In panel (D), the means ± standard error of the means are depicted as plain line and dotted line curves, respectively (n=5-8 mice per group).

ever represent the most widely used model owing to the convenience of breeding and availability of tools for studying the immune system and hemostasis. Most importantly, the immune response to human FVIII in mice resembles that seen in allo-immunized PwHA.[47] IdeS hydrolyzes IgG from a variety of species, including rabbits, pigs, humans and nonhuman primates, but not mouse IgG1 and IgG2b.[16] As a result, the use of mouse models to study the effect of IdeS on induced endogenous IgG-mediated immune responses is not feasible. In order to tackle this limitation, we validated a model of passive transfer to FVIII-deficient HA mice of a neutralizing anti-FVIII rhIgG. In our study, we validated similar hydrolysis profiles *in vitro* for four different monoclonal anti-FVIII rhIgG, irrespective of their specificity for different FVIII domains and of their IgG1/4 subclass.

The administration of anti-FVIII rhIgG to HA mice has already been performed to confirm their inhibitory activity towards FVIII *in vivo*,[48] or to study the effect of antibodies on the pharmacokinetics[48] or immunogenicity of human therapeutic FVIII.[49] Here, we followed the kinetics of one of the anti-FVIII rhIgG (BO2C11 IgG1$_k$) in mice and determined that circulating IgG levels are rather stable 24 h following injection and for up to 5 to 6 days. We also showed that this model allows the precise adjustment and monitoring of the circulating FVIII inhibitory titers. The lack of endogenous production of human IgG is an obvious major limitation of the model, which renders it artificially favorable to IdeS treatment. However, in humans, IdeS administration results in the rapid elimination of IgG from the circulation within 2 to 6 h and *de novo* production of endogenous IgG is detected only after 1 to 2 weeks.[32,38] Taken together, the data suggest that IdeS is expected to achieve a FVIII inhibitor-free time window in PwHA and PwAHA that is wide enough to ensure hemostatic efficacy of FVIII replacement therapy in cases of breakthrough bleeds or major surgeries. In our experiments, mice with FVIII inhibitory titers of 8.3±2.0 BU/mL were successfully treated with IdeS, and therapeutic FVIII hemostatic efficacy was restored within 72 h. Interestingly, despite the persistence of the neutralizing F(ab')$_2$ fragments during the first 48 h after IdeS dosing, inhibitory titers were reduced by 37±13% and 84±8%, respectively, 6 and 24 h after IdeS injection. The inhibitory titers measured at the latter time points, i.e., 5.4±1.1 BU/mL and 1.7±0.6 BU/mL, correspond to the situation of patients with low inhibitory titers who may benefit from high-dose FVIII replacement therapy. Similar observations were made when mice with very high inhibitory titers (i.e., 200 BU/mL) were treated with two doses of IdeS, albeit with a further delay to reach an inhibitory titer <5 BU/mL.

The anti-FVIII antibody responses in PwHA and PwAHA are dominated by IgG antibodies.[33,50] Indeed, anti-FVIII IgG titers of 1:20 or more were found in all plasma from the MIBS and SACHA cohorts. Although the presence of FVIII-binding IgM, IgA and IgE was not investigated in our study, the latter iso-

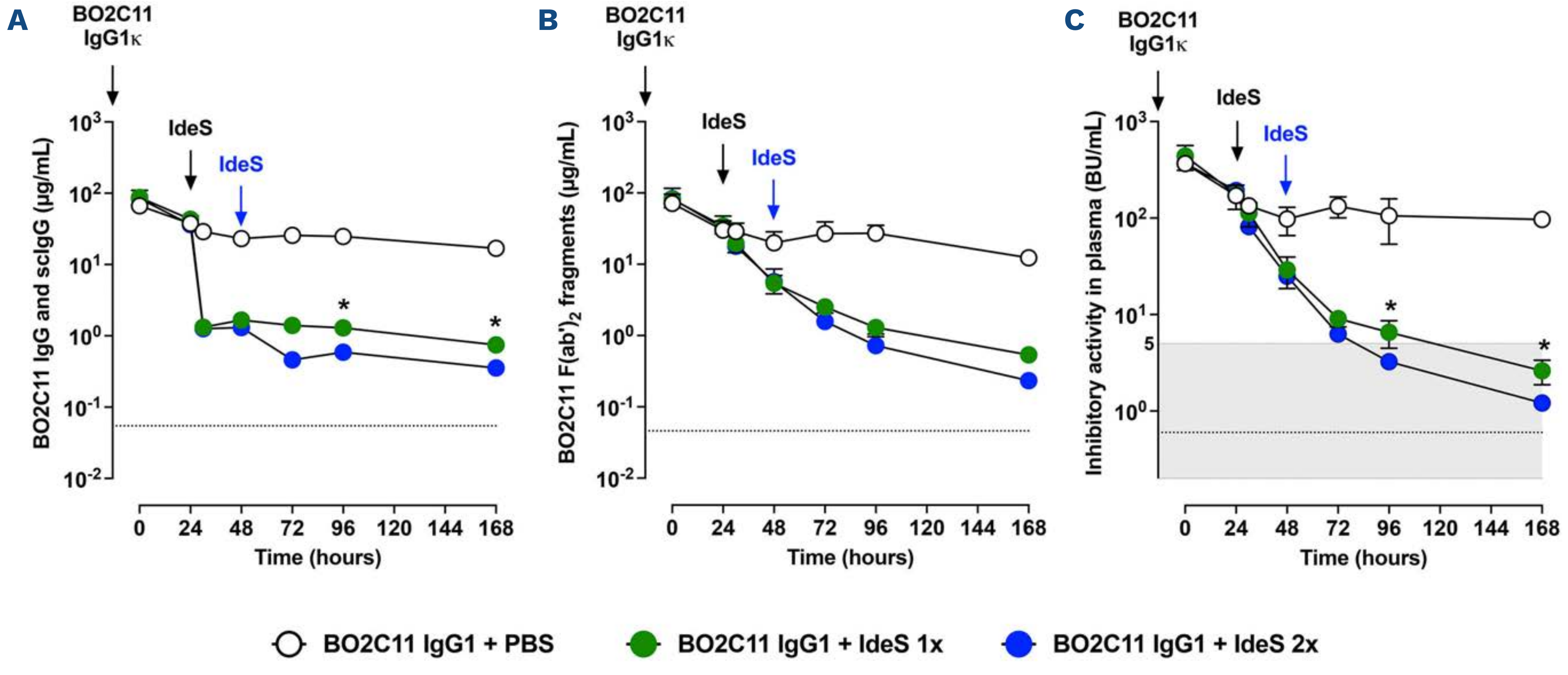

Figure 6. Imlifidase-mediated elimination of very high titer inhibitor. Hemophilia A (HA) mice (n=5 per group) were passively immunized with 24,000 Bethesda units (BU)/kg of BO2C11 immunoglobulin (Ig)G1$_k$ to reach 200 BU/mL after 24 hours (h). Twenty-four hours (h) later, a group of mice was then treated with a single dose of Imlifidase (IdeS) (0.6 mg/kg, 0.29 µM, full green circles) or phosphate-buffered saline (PBS). Another group of mice was treated twice with IdeS (full blue circles) 24 and 48 h after BO2C11 injection. The levels of intact IgG and/or scIgG ((A), IgG concentration at 24 h: 261±8 nM), the levels of F(ab')$_2$ fragments (B) and the inhibitory titers (C) were determined over time by enzyme-linked immunsorbent assay and Bethesda assay (n=2, mean ± standard deviation). The dotted lines represent the respective detection thresholds: 0.03 µg/mL, 0.08 µg/mL and 0.6 BU/mL. The grey zone depicts inhibitory titers below 5 BU/ml. Statistical differences were assessed between mice treated with one or two injections of IdeS (at the 96- and 168-h time points) using the 2-tailed non-parametric Mann-Whitney test (*$P<0.05$; otherwise non-significant).

types may be found in 3-10% of PwHA and 8-36% of PwAHA.[33,50] Although the importance of the latter isotypes in FVIII neutralization *in vivo* is uncertain, their presence may preclude a substantial percentage of patients from receiving IdeS therapy. These observations argue for prescreening patients to determine their eligibility for IdeS treatment.

The injection of IdeS to PwAHA requiring hemostatic treatment would minimize the need for BPA and the associated thrombotic risk while restoring the efficiency of FVIII treatment and monitoring. Based on our *in vivo* results, redosing IdeS 24 h after a first dose, as described in other pathologies,[51] could be indicated for patients with the highest anti-FVIII levels. The use of IdeS as an immediate first-line therapy may be complementary to the use of immunosuppressive agents (i.e., corticosteroids, cyclophosphamide) to remove the inhibitors for a longer time period. On the other hand, the administration of IdeS to PwHA receiving emicizumab should presumably lead to the simultaneous elimination of both neutralizing anti-FVIII IgG and the drug. This would not only restore the clinical hemostatic efficacy of FVIII replacement but would also eliminate emicizumab-related biological interference,[52] ensuring accurate FVIII:C measurement in plasma. Notably, the majority of IdeS will be cleared from circulation within 24 to 48 h, allowing rapid re-administration of emicizumab for prophylaxis.[53] Furthermore, due to limited experience and a lack of guidelines, the management of surgeries in PwHA receiving emicizumab remains an open question. It is further complicated in patients who have inhibitors with variable clinical responses to rFVIIa.[54] In these patients, IdeS would provide a brief but beneficial inhibitor-free therapeutic window for high-risk major surgery or breakthrough bleeds. Finally, our *in vitro* and *in vivo* findings pave the way for a new therapeutic option to improve the management of FVIII inhibitor patients.

Disclosures

SLD and JDD are inventors on patent EP18305971.6 related to the use of IdeS in the context of AAV-mediated gene therapy. All other authors have no conflicts of interest to disclose.

Contributions

MBJ, SD, CD, VP, and SLD designed the research; MBJ, VD, SD, and VP performed experiments; JA and HL contributed essential material; MBJ, SD, CD, VP, and SLD analyzed the results and made the figures; MBJ, VP, and SLD wrote the paper.

Acknowledgments

We wish to thank Dr. Carmen Coxon (National Institute for Biological Standards and Control, Hertfordshire, UK) for sharing the cDNA encoding KM41, as well as the staff from "Centre d'Expérimentation Fonctionnelle" at Centre de Recherche des Cordeliers (Paris) for assistance. Helixate® was a kind gift from CSL-Behring.

Funding

This work was supported by the Institut National de la Santé et de la Recherche Médicale (INSERM), Centre National de la Recherche Scientifique (CNRS), Sorbonne Université, Université de Paris, Assistance Publique des Hôpitaux de Paris and by grants from the Bayer Hemophilia Award Program (BHAP 2019 and BHAP 2021), from Agence National de la Recherche (ANR-18-CE17-0010-02-Exfiltrins and ANR-21-CE17-0043-Persia), from Spark Therapeutics Inc. (Philadelphia, PA), from co-ordination médicale pour l'étude et le traitement des maladies hémorragiques constitutionnelles (CoMETH) and by the Innovative Medicines Initiative 2 Joint Undertaking ARDAT (Accelerating research & development for advanced therapies) project, under grant agreement No 945473. This joint undertaking received support from the European Union's Horizon 2020 research and innovation program and EFPIA. MBJ was the recipient of a fellowship from Ministère de l'enseignement supérieur et de la recherche and of the 2022 Martin Villard Haemostasis Award (Grifols Scientific Awards) in basic research.

Data-sharing statements

Original data and protocols are available upon request to the first and corresponding authors.

References

1. Lusher JM, Arkin S, Abildgaard CF, Schwartz RS. Recombinant factor VIII for the treatment of previously untreated patients with hemophilia A - safety, efficacy, and development of inhibitors. N Engl J Med. 1993;328(7):453-459.
2. Witmer C, Young G. Factor VIII inhibitors in hemophilia A: rationale and latest evidence. Ther Adv Hematol. 2013;4(1):59-72.
3. Tiede A, Collins P, Knoebl P, et al. International recommendations on the diagnosis and treatment of acquired hemophilia A. Haematologica. 2020;105(7):1791-1801.
4. Lloyd Jones M, Wight J, Paisley S, Knight C. Control of bleeding in patients with haemophilia A with inhibitors: a systematic review. Haemophilia. 2003;9(4):464-520.
5. Shapiro AD, Mitchell IS, Nasr S. The future of bypassing agents for hemophilia with inhibitors in the era of novel agents. J Thromb Haemost. 2018;16(12):2362-2374.
6. Baudo F, Collins P, Huth-Kühne A, et al. Management of bleeding in acquired hemophilia A: results from the European Acquired Haemophilia (EACH2) Registry. Blood. 2012;120(1):39-46.

7. Borg JY, Guillet B, Le Cam-Duchez V, et al. Outcome of acquired haemophilia in France: the prospective SACHA (Surveillance des Auto antiCorps au cours de l'Hémophilie Acquise) registry. Haemophilia. 2013;19(4):564-570.
8. Shima M, Hanabusa H, Taki M, et al. Factor VIII–mimetic function of humanized bispecific antibody in hemophilia A. N Engl J Med. 2016;374(21):2044-2053.
9. Lenting PJ, Denis CV, Christophe OD. Emicizumab, a bispecific antibody recognizing coagulation factors IX and X: how does it actually compare to factor VIII? Blood. 2017;130(23):2463-2468.
10. Callaghan MU, Negrier C, Paz-Priel I, et al. Long-term outcomes with emicizumab prophylaxis for hemophilia A with or without FVIII inhibitors from the HAVEN 1-4 studies. Blood. 2021;137(16):2231-2242.
11. Collins PW, Liesner R, Makris M, et al. Treatment of bleeding episodes in haemophilia A complicated by a factor VIII inhibitor in patients receiving Emicizumab. Interim guidance from UKHCDO Inhibitor Working Party and Executive Committee. Haemophilia. 2018;24(3):344-347.
12. Lillicrap D, Fijnvandraat K, Young G, Mancuso ME. Patients with hemophilia A and inhibitors: prevention and evolving treatment paradigms. Expert Rev Hematol. 2020;13(4):313-321.
13. Makris M, Iorio A, Lenting PJ. Emicizumab and thrombosis: The story so far. J Thromb Haemost. 2019;17(8):1269-1272.
14. von Pawel-Rammingen U, Johansson BP, Björck L. IdeS, a novel streptococcal cysteine proteinase with unique specificity for immunoglobulin G. EMBO J. 2002;21(7):1607-1615.
15. Vincents B, von Pawel-Rammingen U, Björck L, Abrahamson M. Enzymatic characterization of the Streptococcal endopeptidase, IdeS, reveals that it is a cysteine protease with strict specificity for IgG cleavage due to exosite binding. Biochemistry. 2004;43(49):15540-15549.
16. Agniswamy J, Lei B, Musser JM, Sun PD. Insight of host immune evasion mediated by two variants of group a Streptococcus Mac protein. J Biol Chem. 2004;279(50):52789-52796.
17. Al-Salama ZT. Imlifidase: first approval. Drugs. 2020;80(17):1859-1864.
18. Wang Y, Shi Q, Lv H, et al. IgG-degrading enzyme of Streptococcus pyogenes (IdeS) prevents disease progression and facilitates improvement in a rabbit model of Guillain-Barré syndrome. Exp Neurol. 2017;291:134-140.
19. Soveri I, Mölne J, Uhlin F, et al. The IgG-degrading enzyme of Streptococcus pyogenes causes rapid clearance of anti-glomerular basement membrane antibodies in patients with refractory anti-glomerular basement membrane disease. Kidney Int. 2019;96(5):1234-1238.
20. Montgomery RA, Loupy A, Segev DL. Antibody-mediated rejection: new approaches in prevention and management. Am J Transplant. 2018;18(S3):3-17.
21. Leborgne C, Barbon E, Alexander JM, et al. IgG-cleaving endopeptidase enables in vivo gene therapy in the presence of anti-AAV neutralizing antibodies. Nat Med. 2020;26(7):1096-1101.
22. Järnum S, Runström A, Bockermann R, et al. Enzymatic Inactivation of endogenous IgG by IdeS enhances therapeutic antibody efficacy. Mol Cancer Ther. 2017;16(9):1887-1897.
23. Astermark J, Berntorp E, White GC, Kroner BL. The Malmö International Brother Study (MIBS): further support for genetic predisposition to inhibitor development. Haemophilia. 2001;7(3):267-272.
24. van den Brink EN, Turenhout EAM, Bovenschen N, et al. Multiple VH genes are used to assemble human antibodies directed toward the A3-C1 domains of factor VIII. Blood. 2001;97(4):966-972.
25. Jacquemin M, Benhida A, Peerlinck K, et al. A human antibody directed to the factor VIII C1 domain inhibits factor VIII cofactor activity and binding to von Willebrand factor. Blood. 2000;95(1):156-163.
26. Jacquemin MG, Desqueper BG, Benhida A, et al. Mechanism and kinetics of factor VIII inactivation: study with an IgG4 monoclonal antibody derived from a hemophilia A patient with inhibitor. Blood. 1998;92(2):496-506.
27. Verbruggen B, Novakova I, Wessels H, et al. The Nijmegen modification of the Bethesda assay for factor VIII:C inhibitors: improved specificity and reliability. Thromb Haemost. 1995;73(02):247-251.
28. Bi L, Lawler AM, Antonarakis SE, et al. Targeted disruption of the mouse factor VIII gene produces a model of haemophilia A. Nat Genet. 1995;10(1):119-121.
29. Russick J, Delignat S, Milanov P, et al. Correction of bleeding in experimental severe hemophilia A by systemic delivery of factor VIII-encoding mRNA. Haematologica. 2020;105(4):1129-1137.
30. Hemker HC, Giesen P, Dieri RA, et al. Calibrated automated thrombin generation measurement in clotting plasma. Pathophysiol Haemost Thromb. 2003;33(1):4-15.
31. Jaki T, Lawo J-P, Wolfsegger MJ, et al. A formal comparison of different methods for establishing cut points to distinguish positive and negative samples in immunoassays. J Pharm Biomed Anal. 2011;55(5):1148-1156.
32. Winstedt L, Järnum S, Nordahl EA, et al. Complete removal of extracellular IgG antibodies in a randomized dose-escalation phase I study with the bacterial enzyme IdeS – a novel therapeutic opportunity. PLoS One. 2015;10(7):e0132011.
33. Whelan SFJ, Hofbauer CJ, Horling FM, et al. Distinct characteristics of antibody responses against factor VIII in healthy individuals and in different cohorts of hemophilia A patients. Blood. 2013;121(6):1039-1048.
34. Nandakumar KS, Johansson BP, Björck L, Holmdahl R. Blocking of experimental arthritis by cleavage of IgG antibodies in vivo. Arthritis Rheum. 2007;56(10):3253-3260.
35. Johansson BP, Shannon O, Björck L. IdeS: a bacterial proteolytic enzyme with therapeutic potential. PLoS One. 2008;3(2):e1692.
36. Yang R, Otten MA, Hellmark T, et al. Successful treatment of experimental glomerulonephritis with IdeS and EndoS, IgG-degrading streptococcal enzymes. Nephrol Dial Transplant. 2010;25(8):2479-2486.
37. Kizlik-Masson C, Deveuve Q, Zhou Y, et al. Cleavage of anti-PF4/heparin IgG by a bacterial protease and potential benefit in heparin-induced thrombocytopenia. Blood. 2019;133(22):2427-2435.
38. Jordan SC, Lorant T, Choi J, et al. IgG endopeptidase in highly sensitized patients undergoing transplantation. N Engl J Med. 2017;377(5):442-453.
39. Kjellman C, Maldonado AQ, Sjöholm K, et al. Outcomes at 3 years posttransplant in imlifidase-desensitized kidney transplant patients. Am J Transplant. 2021;21(12):3907-3918.
40. Padmanabhan A, Connelly-Smith L, Aqui N, et al. Guidelines on the use of therapeutic apheresis in clinical practice – evidence-based approach from the writing committee of the American Society for Apheresis: the eighth special issue. J Clin Apher. 2019;34(3):171-354.
41. Wolfe GI, Ward ES, de Haard H, et al. IgG regulation through FcRn blocking: a novel mechanism for the treatment of myasthenia gravis. J Neurol Sci. 2021;430:118074.
42. Blumberg LJ, Humphries JE, Jones SD, et al. Blocking FcRn in humans reduces circulating IgG levels and inhibits IgG immune complex–mediated immune responses. Sci Adv. 2019;5(12):eaax9586.
43. Muntean A, Lucan M. Immunosuppression in kidney

transplantation. Clujul Med. 2013;86(3):177-180.

44. Collins P, Baudo F, Knoebl P, et al. Immunosuppression for acquired hemophilia A: results from the European Acquired Haemophilia Registry (EACH2). Blood. 2012;120(1):47-55.
45. Lee DSW, Rojas OL, Gommerman JL. B cell depletion therapies in autoimmune disease: advances and mechanistic insights. Nat Rev Drug Discov. 2021;20(3):179-199.
46. European Medicines Agency. Idefirix (imlifidase): EU summary of product characteristics. 2020. https://www.ema.europa.eu/en/documents/product information/idefirix epar product information_en.pdf. Accessed 21 Sept 2020.
47. Reipert BM, Ahmad RU, Turecek PL, Schwarz HP. Characterization of antibodies induced by human factor VIII in a murine knockout model of hemophilia A. Thromb Haemost. 2000;84(5):826-832.
48. Batsuli G, Deng W, Healey JF, et al. High-affinity, noninhibitory pathogenic C1 domain antibodies are present in patients with hemophilia A and inhibitors. Blood. 2016;128(16):2055-2067.
49. Gangadharan B, Ing M, Delignat S, et al. The C1 and C2 domains of blood coagulation factor VIII mediate its endocytosis by dendritic cells. Haematologica. 2017;102(2):271-281.
50. Bonnefoy A, Merlen C, Dubé E, et al. Predictive significance of anti-FVIII immunoglobulin patterns on bleeding phenotype and outcomes in acquired hemophilia A: Results from the Quebec Reference Center for Inhibitors. J Thromb Haemost. 2021;19(12):2947-2956.
51. Jordan SC, Legendre C, Desai NM, et al. Imlifidase desensitization in crossmatch-positive, highly sensitized kidney transplant recipients: results of an international phase 2 trial (Highdes). Transplantation. 2021;105(8):1808-1817.
52. Adamkewicz JI, Chen DC, Paz-Priel I. Effects and interferences of emicizumab, a humanised bispecific antibody mimicking activated factor VIII cofactor function, on coagulation assays. Thromb Haemost. 2019;119(07):1084-1093.
53. Huang E, Maldonado AQ, Kjellman C, Jordan SC. Imlifidase for the treatment of anti-HLA antibody-mediated processes in kidney transplantation. Am J Transplant. 2022;22(3):691-697.
54. Jiménez-Yuste V, Rodríguez-Merchán EC, Matsushita T, Holme PA. Concomitant use of bypassing agents with emicizumab for people with haemophilia A and inhibitors undergoing surgery. Haemophilia. 2021;27(4):519-530.