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Endopeptidase Cleavage of Anti-Glomerular Basement Membrane Antibodies *in vivo* in Severe Kidney Disease: An Open-Label Phase 2a Study

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ABSTRACT

Background The prognosis for kidney survival is poor in patients presenting with circulating anti–glomerular basement membrane (GBM) antibodies and severe kidney injury. It is unknown if treatment with an endopeptidase that cleaves circulating and kidney bound IgG can alter the prognosis.

Methods An investigator-driven phase 2a one-arm study (EudraCT 2016–004082–39) was performed in 17 hospitals in five European countries. A single dose of 0.25 mg/kg of imlifidase was given to 15 adults with circulating anti-GBM antibodies and an eGFR <15 ml/min per 1.73m^2. All patients received standard treatment with cyclophosphamide and corticosteroids, but plasma exchange only if autoantibodies rebounded. The primary outcomes were safety and dialysis independency at 6 months.

Results At inclusion, ten patients were dialysis dependent and the other five had eGFR levels between 7 and 14 ml/min per 1.73m^2. The median age was 61 years (range 19–77), six were women, and six were also positive for anti–neutrophil cytoplasmic antibodies. Then 6 hours after imlifidase infusion, all patients had anti-GBM antibodies levels below the reference range of a prespecified assay. At 6 months 67% (ten out of 15) were dialysis independent. This is significantly higher compared with 18% (nine out of 50) in a historical control cohort (P<0.001, Fisher's exact test). Eight serious adverse events (including one death) were reported, none assessed as probably or possibly related to the study drug.

Conclusions In this pilot study, the use of imlifidase was associated with a better outcome compared with earlier publications, without major safety issues, but the findings need to be confirmed in a randomized controlled trial.

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Kidney survival is poor in patients presenting with circulating anti–glomerular basement membrane (anti-GBM) antibodies and advanced kidney failure.[1,2] Most patients become dialysis dependent, despite aggressive therapy combining daily plasma exchange (PLEX), pulse doses of intravenous corticosteroids, and cytotoxic drugs. The antibodies, which are directed against non-collagenous domain of type IV collagen,[3] have in

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animal transfer experiments been shown to be able to inflict injury.[4] The current guidelines state the cornerstone of the treatment is rapid removal of pathogenic autoantibodies.[5] Imlifidase, which is the international nonproprietary name for the IgG degrading enzyme of *Streptococcus pyogenes*, causes rapid cleavage of all circulating IgG in healthy subjects,[6] and is now licensed in Europe for desensitization treatment of highly sensitized adult patients who have undergone a kidney transplant.[7] Imlifidase has also been shown to cleave IgG bound to the GBM *in vivo*, both in an animal model and in patients,[8,9] leaving the $F(ab')_2$-fragment in the membrane without the ability to recruit effector mechanisms. We hypothesized that imlifidase on top of standard of care would be safe, and increase the chances of kidney survival compared with historical controls.

Significance Statement

In vivo cleavage of IgG by an endopeptidase is a novel therapeutic strategy for anti-GBM disease. Despite plasma exchange, most patients become dependent on dialysis, especially those with AKI at diagnosis. In an encouraging pilot study, two thirds of 15 patients selected because of poor prognosis exhibited kidney survival at 6 months without major safety issues after receiving a single infusion of imlifidase. The drug has been used in patients who have undergone a transplant with multiple HLA antibodies. Our study supports further use of the drug in clinical situations in which IgG autoantibodies threaten vital organ function. However, randomized trials are necessary to confirm the findings.

METHODS

Trial Registration and Inclusion/exclusion Criteria

In the GOOD-IDES-01 trial (treating GOODpasture's disease with Imunoglobulin G Degrading Enzyme of Streptococcus pyogenous) (EUDRACT 2016–004082–39 first posted January 13, 2017, ClinicalTrials.gov: NCT03157037, first posted May 17, 2017), the main inclusion criteria were the presence of circulating anti-GBM antibodies and an eGFR <15 ml/min per $1.73m^2$ (complete list in Supplemental Table 1). The main exclusion criteria were anuria for >48 hours or dialysis dependency for >5 days. In addition, patients could not be included if they had moderate to severe lung hemorrhage, defined as hemorrhage requiring medical treatment (oxygen, ventilator, blood transfusion, etc.). To avoid delay in the start of treatment, kidney biopsy before inclusion was not a requirement. No other demographic information than age and sex was collected.

Study Drug, Treatment Regimen, and Follow-up

Imlifidase was provided as a frozen concentrate for the first seven patients and as a lyophilized powder for the remaining eight. A single infusion of 0.25 mg/kg bodyweight was given within 24 hours after signing informed consent. Imlifidase was added to standard of care, which at all sites included pulse methylprednisolone, oral corticosteroids, cyclophosphamide (CYC), and PLEX. PLEX should be tailored for each patient on the basis of repeat measurements using local assays. The frequency of PLEX was adjusted to keep anti-GBM antibodies below the level of detection or a threshold considered as toxic by the principal investigator. PLEX was not allowed within the first 36 hours after imlifidase dosage, and could be restarted only if anti-GBM antibodies had rebounded. CYC could be given either as daily oral doses or intermittent pulses. Doses of corticosteroids and CYC were in accordance with recent trials in systemic small-vessel vasculitis, such as the PEXIVAS[10] (**P**lasma **EX**change and glucocorticoid dosing In the treatment of ANCAassociated **VAS**culitis: a multicentre randomized controlled trial) and CYCLOPS[11] (Randomised trial of daily oral versus pulse **CYCLO**phosphamide as therapy for ANCA-associated **S**ystemic Vasculitis (CYCLOPS) (Supplemental Table 2). Prophylaxis with phenoxymethylpenicillin was strongly recommended during the first 10 days of the study. Prophylaxis against *Pneumocystis jirovecii*, *Candida albicans*, osteoporosis, and gastric/duodenal ulcer was to be given according to local practice. Patients were followed for 6 months, with ten clinical visits and collection of samples for central laboratory analysis.

Laboratory Analysis

Clinical chemistry and immunology analyses were performed for safety and clinical monitoring at the local hospitals during the trial. Central analyses of selected electrolytes and proteins were performed after the trial was finished at the Department of Clinical Chemistry, University and Regional Laboratories, Region Skåne, Lund, Sweden. Central analysis of anti-GBM antibodies was performed at SVAR AB, Malmö, Sweden, using the Wieslab anti-GBM ELISA. Measurements of other autoantibodies were conducted at the Department of Clinical Immunology and Transfusion Medicine, University Hospital, Linköping, Sweden where the Thermo Fisher EliA system was used to analyze proteinase 3–ANCA and myeloperoxidase (MPO)-ANCA.

Pathology

Light microscopy slides or scanned images were re-examined by central evaluation and scored according to a predefined protocol by two independent pathologists in Leiden, the Netherlands, and Gothenburg, Sweden. Discrepancies were solved with consensus discussions. Glomerular, interstitial, and vascular lesions were scored in detail, and the biopsies were classified, according to the Berden classification developed for ANCA-associated vasculitis, as crescentic ($>50\%$ of glomeruli with cellular crescents), sclerotic ($>50\%$ of glomeruli with global sclerosis),

focal (>50% normal glomeruli), or mixed (no majority of glomeruli with either cellular crescents, glomerulosclerosis or absence of lesions).[2,12]

IgG Cleavage, Pharmacokinetics, and Antidrug Antibodies

To study pharmacodynamics, intact IgG and single cleaved IgG were determined using a sandwich electrochemiluminescence immunoassay and anti-IgG–degrading enzyme of *Streptococcus pyogenes* antibodies were determined using ImmunoCAP (Thermo Fisher Scientific), as previously described.[13] Each subject underwent serial sampling for pharmacokinetic evaluation; the serum concentration of imlifidase was determined using an electrochemiluminescence immunoassay, and the results were evaluated using WinNonlinProfessional (Pharsight Corporation, St Louis, MO).[14]

Primary and Secondary Outcomes

The primary outcome was safety and dialysis independency at 6 months. Safety was measured as number of adverse events (AEs) and serious AEs (SAEs), and their relationship to the study drug. Secondary outcomes included dialysis dependency at 3 months, changes in eGFR from baseline to 6 months, changes in albuminuria, changes in hematuria, anti-GBM cleavage and rebound, use of PLEX, imlifidase pharmacokinetics, and the presence of antidrug antibodies (ADA).

Historical Controls

An observational study published in 2017 by McAdoo *et al.* was used as a source for historical controls.[15] This study contains observational data from four centers, of which three participated in this study, and all four centers adhered to same principles for standard therapy as in this study. To be included as a control in this study, the patients in McAdoo's study had to fulfill the following criteria: (*1*) outcome at 6 months available, (*2*) had received standard treatment including PLEX, and (*3*) had an eGFR of <15 ml/min per 1.73 m^2 at the start of such therapy. This analysis was *post hoc*, prespecified, but not detailed in the protocol and statistical analysis plan.

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics, version 27.0.0. Proportions were compared using Fisher's exact test and parametric variables were compared using Mann–Whitney *U* test. The sample size was determined on the basis of power calculations assuming imlifidase treatment would lead to a kidney survival of 30%–50%, compared with 7% in our previous experience.[16] With 40% survival, a sample size of 15 would have a power of 0.93 to detect a difference with an alpha of 0.05; if only ten patients could be evaluated, the power would drop to 0.83.

Ethics

The study was conducted abiding to the Declaration of Helsinki and all patients provided written informed consent. Independent ethics committees and regulatory authorities in all participating countries approved the study.

Data Sharing

After de-identification, individual participant data that underlie the results reported in this article (text, tables, figures, and appendices) will be made available on request, beginning 6 months and ending 5 years after publication, for meta-analysis or similar purposes. A request accompanied by ethical permit and study protocol should be addressed to the corresponding author.

RESULTS

Patients

From March 2017 to January 2020, 17 sites at tertiary referral hospitals in five European countries (Sweden, Denmark, France, Austria, and Czech Republic) opened for inclusion in the GOOD-IDES-01 study. A total of 26 patients with anti-GBM antibodies were prescreened, among whom five had an eGFR >15 ml/min per 1.73m^2 and six failed other inclusion and/or exclusion criteria (Figure 1). Informed consent was provided from 15 subjects (six women), with a median age of 61 years (range, 19–77, Table 1).

Clinical Characteristics at Baseline

At inclusion, ten patients needed dialysis, five of them were anuric/oliguric. One patient was included when experiencing a rebound of anti-GBM antibodies combined with a deterioration of kidney function. This patient was dialysis dependent at diagnosis, but not at inclusion in the study. Finally, four patients were included with a predose eGFR of 7, 7, 9, and 14 ml/min per 1.73 m^2.

Two patients had hemoptysis before inclusion, and four more had unspecific pulmonary symptoms in combination with radiographic findings, suggesting lung involvement of the disease. Previous autoimmune diseases were recorded in five (33%) patients, including diagnoses such as ulcerative colitis, rheumatoid arthritis, Sjögren's syndrome, thyroid disease, multiple sclerosis, and type 1 diabetes mellitus. Hypertension had been diagnosed in four patients and diabetes in three. All patients were anemic at inclusion, the median hemoglobin concentration according to local laboratories was 8.8 g/dl (range, 6.9–11.1).

Histology

Kidney biopsies were available from 14 patients, ten before (median, 4.5 days; range, 0–33) and four after administration of the study drug (median, 3.5 days; range, 3–6). According to the Berden classification, crescentic class was seen in nine

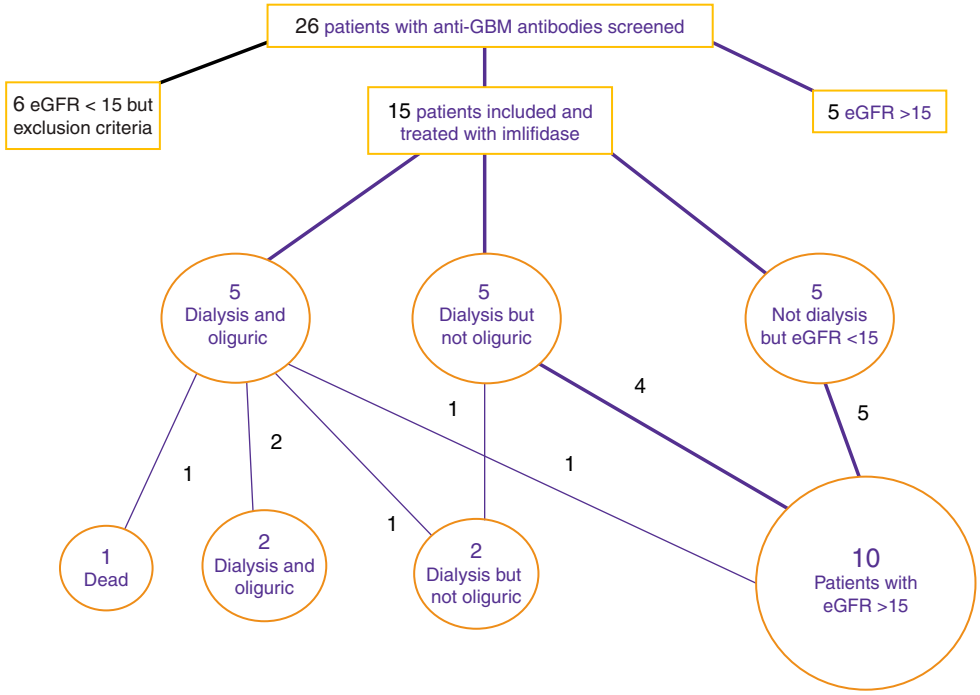

Figure 1. Flow chart. Upper panel: All patients with anti-GBM antibodies treated or reported to the investigators were prescreened for participation during the study period March 2017 until January 2020. Middle panel: Groups on the basis of kidney function at inclusion. Lower panel: Groups on the basis of kidney function at 3 and 6 months.

Table 1. Baseline clinical characteristics

Characteristics	Men	Women	All	Historical Controls
Number, *n* (%)	9 (60)	6 (40)	15 (100)	50 (100)
Country: France/Sweden/Denmark Austria/Czech Republic, *n*	3/1/2/1/1	2/3/1/0/0	5/4/3/2/1	NA
Age years median (range)	60 (19–77)	66 (32–71)	61 (19–77)	63 (16–88)
ANCA MPO/PR3/none, *n* (%)	3/2/5 (33/22/56)	1/0/4 (17/0/67)	4/2/9 (27/13/60)	15[a]/7[a]/29 (30/14/58)
ANA positive, *n* (%)	3 (33%)	0	3 (20%)	NA
Renal function oliguria/dialysis/no dialysis, *n* (%)	4/4/1 (44/44/11)	1/1/4 (17/17/67)	5/5/5 (33/33/33)	41[7]/9 (82/18)
Pulmonary disease AH/other/none, *n* (%)[b]	1/2/6 (11/22/67)	1/2/3 (17/33/50)	2/4/9 (13/27/60)	21/NA/NA (42/NA/NA)
Smoking current/previous/never, *n* (%)	2/7/0 (22/78/0)	0/3/3 (0/50/50)	2/10/3 (13/67/20)	NA
Urinary albumin-creatinine, mg/g median (range) Reference range <27 mg/g[c]	2230 (434–30,531)	1932 (195–3540)	1982 (195–30,531)	NA
CRP mg/L median (range) Reference range <0.3 mg/dL[c]	3.4 (<0.07–5.2)	1.2 (<0.07–8.9)	3.2 (<0.07–8.9)	NA
Hb g/dL median (range)[c]	8.4 (6.9–11.1)	9.3 (7.3–11.5)	8.8 (6.9–11.5)	NA
Platelets 10^9/L median (range)[c]	318 (131–505)	320 (201–384)	320 (131–505)	NA
Berden class crescentic/mixed/sclerotic/focal, *n* (%)	6/2/0/0 (75/25/0/0)	3/2/1/0 (50/33/17/0)	9/4/1/0 (65/29/7/0)	25/3/1/0 (86/10/3/0)
Normal glomeruli, % median (range)	11% (0–29)	9% (0–35)	9.5% (0–35)	6.5% (0–43)
Linear staining for IgG, *n/n* (%)	5/8 (62)	6/6 (100)	11/14 (79)	27/29 (93)

NA, not available; ANCA, anti-neutrophil cytoplasm antibodies; PR3, proteinase 3; CRP, C reactive protein; Hb, hemoglobulin.
[a]One double positive.
[b]Other, other pulmonary symptoms and signs not assessed as water overload.
[c]Separation between oliguric and nonoliguric not available. Reference range is provided for analysis performed at central laboratory, but not for assays performed at local laboratories.

patients (64%), mixed in four (29%), and sclerotic in one (7%) (Table 2). The median percentage of normal glomeruli was 9.5% (range, 0–35). Immunofluorescence performed at the local hospitals revealed linear IgG deposits in 11 of the 14 biopsies.

Standard Treatment

PLEX had been given before the study drug with a median of one session (range, 1–13) in 14 patients (Supplemental Table 3). PLEX was (re)started after imlifidase due to rebound of anti-GBM antibodies in ten patients, within a

Table 2. Histologic findings

Patient	Class	Normaln (%)	Cell Crescentsn (%)	Fibrous Crescentsn (%)	Global Sclerosisn (%)	IFTA%	ANCA	Linear IgG[a]
1	Crescentic	0/11 (0%)	10 (91%)	0	1/ 9%	10%	neg	Yes
2	Crescentic	3/25 (12%)	17 (68%)	0	5/ 20%	15%	neg	Yes
3	Crescentic	5/42 (12%)	15 (36%)	2/ 4%	20/48%	30%	MPO	No
4	Crescentic	2/14 (14%)	10 (72%)	0	2/ 14%	15%	neg	Yes
5	Crescentic	2/47 (4%)	32 (68%)	4/ 9%	9/ 19%	25%	MPO	Yes
6	Crescentic	0/20 (0%)	18 (90%)	0	2/ 10%	<10%	PR3	No
7	Crescentic	5/17 (29%)	10 (59%)	1/ 6%	1/ 6%	<10%	MPO	Yes
8	Mixed	7/20 (35%)	8 (40%)	2/ 10%	3/ 15%	30%	neg	Yes
9	Mixed	2/7 (29%)	3 (43%)	1/ 14%	1/ 14%	60%	PR3	Yes
10	Crescentic	2/34 (6%)	22 (65%)	2/ 6%	4/ 12%	25%	neg	Yes
11	Mixed	1/7 (14%)	4 (57%)	1/ 14%	1/ 14%	20%	neg	Yes
12	Sclerotic	0/12 (0%)	2 (17%)	1/ 8%	7/ 58%	45%	neg	Yes
13	Mixed	2/21 (10%)	2 (10%)	0	2/ 10%	30%	neg	No
14	Crescentic	0/15 (0%)	10 (67%)	0	5/ 33%	55%	MPO	Yes

IFTA, interstitial fibrosis and tubular atrophy; PR3, proteinase 3.
[a]Direct immunofluorescence performed at local pathology laboratories showing linear deposits of IgG.

median of 6.5 days (range, 3–22) after imlifidase infusion; six needed 2–5 sessions and four 10–16 sessions; one patient received a session without a rebound (protocol deviation), whereas four patients did not receive any PLEX after imlifidase. All subjects received CYC, five as daily oral therapy (median, 7200 mg; interquartile range, 6750–8700) and ten as intermittent pulses (3750 mg; 2450–5540). In one patient, CYC was stopped after two pulses due to concerns of infertility, and replaced by rituximab, CYC was stopped early in two other patients due to infection/death and perceived futility. Pulse corticosteroids were administered to 14 of the 15 patients, in most cases 1500 mg (range, 500–4000 mg). The oral corticosteroid dosing is presented in Supplemental Table 2.

Primary Outcome: Kidney Function at the End of the Study

At the last study visit, 6 months after imlifidase treatment, 14 patients were alive, ten with independent kidney function, and four patients received maintenance dialysis treatment. Among the five patients who were anuric/oliguric at the start of the trial, one died 2 months after inclusion. Two could stop dialysis after 17 and 26 days; however, one of them had to restart dialysis at day 91, whereas the other had an eGFR of 16 ml/min per 1.73m^2 at 6 months. Among the five patients who were dialysis dependent, but not oliguric, when receiving the study drug, none developed oliguria and four could stop dialysis (on days 7, 22, 26, and 90, respectively). At 6 months, their eGFR were between 21 and 34 ml/min per 1.73 m^2. All patients who were not dialysis dependent remained so during the trial, with an eGFR at 6 months between 19 and 59 ml/min per 1.73 m^2. Possible prognostic factors associated with the primary outcome included oliguria at the start of treatment, sex, initial levels of anti-GBM antibodies, and ADA (Supplemental Table 3).

Historical Controls

Patients in the study by McAdoo *et al.*[15] that fulfilled the inclusion criteria described the *Methods*, resembling those of this study, were used as a historical control cohort (n=50). At start of treatment for anti-GBM disease, all 50 had an eGFR <15 ml/min per 1.73 m^2, and 41 were dialysis dependent (Table 1). After 6 months, eight (16%) had died, 33 (67%) were dialysis dependent, and nine (18%) were alive, with functioning native kidneys (Table 3). The proportion of kidney survival of ten out of 15 (67%) patients in this study is significantly higher compared with the nine out of 50 (18%) in the historical control cohort (P<0.001, Fisher's exact test). The difference remains

Table 3. Comparison with historical controls

COLUMN ONE	This Study			McAdoo *et al.*[a]		
	Dialysis at Study Drug	No Dialysis at Study Drug	All	Dialysis at PLEX	No Dialysis at PLEX	All
Total	10 (67)	5 (33)	15 (100)	41 (82)	9 (18)	50 (100)
Dead at 6 months, *n* (%)	1 (10)	0	1 (6.7)	5 (12)	3 (33)	8 (16)
Dialysis at 6 months, *n* (%)	4 (40)	0	4 (27)	29 (77)	4 (43)	33 (67)
Dialysis independent at 6 months, *n* (%)	5 (50)	5 (100)	10 (67)	7 (17)	2 (22)	9 (18)

[a]Patients selected from McAdoo *et al.*[12] to meet inclusion criteria of this study.

significant also when removing 21 patients with alveolar hemorrhage from the control group (ten out of 15 versus six out of 29; $P=0.007$).

Secondary Outcomes

ELISA for anti-GBM antibodies fell below the reference level for positive results in all samples 6 hours after start of imlifidase infusion (Figure 2A and Supplemental Table 3). The highest tendency for rebound was seen at the day 10 visit, when three patients had results above the reference level for negative results, but at that timepoint six patients had already restarted PLEX on the basis of results from local laboratories. At 3 and 6 months, all anti-GBM ELISA results were in the negative range for all patients.

Total IgG and other autoantibodies were also cleaved rapidly (Figure 2B). After 2 hours, the median total IgG was 0.15 g/L (range, 0.03–0.44; reference range, 6.7–14.5 g/L) and no ANCA or ANA could be detected. After 1, 3, and 6 months, the median IgG levels were 3.3, 3.9, and 5.2 g/L, respectively. ANCA rebounded in three patients at day 15, but only one patient who was proteinase 3 positive remained positive in the 3- and 6-month samples. Albuminuria showed a statistically nonsignificant (paired t test) decline during the trial, the median value was reduced from 1982 g/mg (range, 195–30531) to 726 (range, 35–6646). Four patients had negative dipstick tests for hematuria at the final visit.

The imlifidase plasma concentration curves showed a fast distribution phase with a mean $t_{1/2}$ of 2.6 hours and a slow elimination phase with a mean $t_{1/2}$ of 53 hours (range, 26–114 hours) and were well described by a two-compartment model, compatible with initial distribution to the plasma volume and final distribution in the extracellular space (Supplemental Tables 4 and 5). Levels of ADA varied at predose, between 3.5 and 16.7 mg/L. Despite pulse steroids and CYC, there was a sharp rise in ADA in the day 14 sample, which faded slowly during the study (Figure 2C).

AEs

There were eight SAEs and 83 other AEs reported in the study (Table 4). No SAE was assessed as probably or possibly related to the study drug. This includes the death of one patient due to pneumonia at day 58. This was the oldest patient in the study, and he remained anuric during the trial. At the last study visit, 1 week after stopping PLEX and visit 3 weeks before death, the plasma IgG concentration was 4.5 g/L (ref 6.7–14.5). Four of the AEs were considered possibly related to the study drug, including a urinary tract infection on day 25 and shingles on day 41. The most common AEs were infections ($n=18$), most of them occurring after the first month, and were gastrointestinal or urinary tract origin. No AE was reported during the infusion of the study drug. There were two patients with deep vein thrombosis (day 20 and 121) and one patient with superficial thrombophlebitis (day 10).

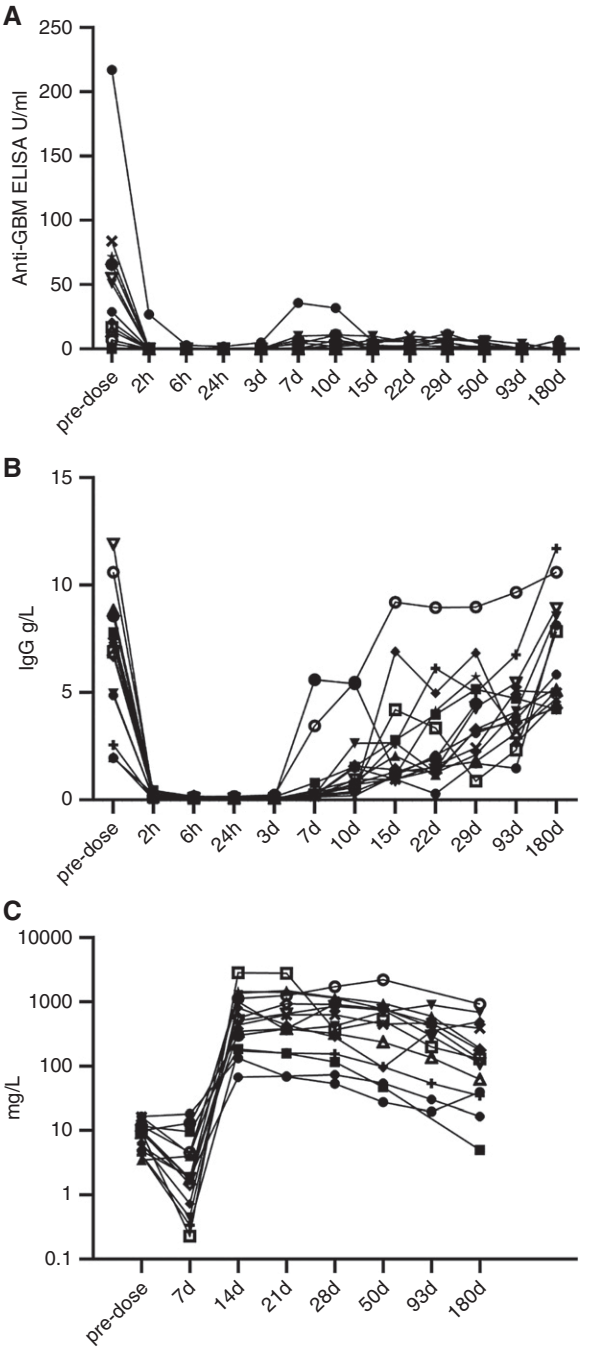

Figure 2. Immunoglubulins before and after imlifidase. ELISA determinations of (A) anti-GBM antibodies, (B) total IgG, and (C) ADA. Samples taken before imlifidase (predose), 2–24 hours after dosing and at study visits (day 3–180). The y axis in (C) is logarithmic.

DISCUSSION

This is the first study in humans suggesting that *in vivo* degradation of autoantibodies can have the ability to alter the course of an autoimmune disease. In this trial, 67% of the patients

Table 4. AEs and SAEs

MedDRA System Organ Class	AE day 1–28 *n*	AE day 29–183 *n*	SAE day 1–28 *n*	SAE day 29–183 *n*	All AE+SAE *n* (%)
Blood and lymphatic system disorders	6	4	..	..	10 (11)
Cardiac disorders	..	..		1	1 (1.1)
Ear and labyrinth disorders	2	1	..	..	3 (3.3)
Eye disorders	1	1	..	..	2 (2.2)
Gastrointestinal disorders	7	2		1	10 (11)
Immune system disorders	2	1	..	..	3 (3.3)
Infections and infestations	4	10		4[a]	18[a] (20)
Metabolism and nutrition disorders	5	1		1	7 (7.7)
Musculoskeletal and connective tissue disorders	2	3		1	6 (6.6)
Nervous system disorders	3	2	..	..	5 (5.5)
Renal and urinary disorders	1	1	..	..	2 (2.2)
Respiratory, thoracic, and mediastinal disorders	3	3	..	..	6 (6.6)
Skin and subcutaneous tissue disorders	4	1	..	..	5 (5.5)
Vascular disorders	4	3	..	..	7 (7.7)
Miscellaneous	3	3	..	..	6 (6.6)
Total *n* (% of all AE+SAE)	47 (52)	36 (40)	0	8 (8.7)	91 (100)

MedDRA, Medical Dictionary for Regulatory Activities.
[a]One patient died due to bilateral pneumonia.

achieved kidney survival at 6 months, which is considerably better than most published cohorts reporting on patients with anti-GBM disease.[2] When we recently reviewed 12 observational studies published after the year 2000, the median kidney survival was 31%,[17] with kidney survival seen mostly in those with good prognostic signs, and such patients were excluded from this study. Among these 12 studies was McAdoo *et al.*[15] from which historical controls were selected for comparison. A weakness in the comparison with McAdoo study is that it did not include data on urinary output. Another study reported the French Apheresis Society's experience with PLEX in anti-GBM disease.[18] Among 83 patients who required dialysis during the first month, only two recovered renal function by month 6, compared with five out of ten in this study. A somewhat better result was recently reported by Henriksson *et al.* using data from an international apheresis registry; in this study, four out of 20 patients needing dialysis during the initial hospital stay were no longer on dialysis at 6 months.[19] Renal survival in the same range is also found in a study by Margues *et al.*,[20] but exact percentages cannot be extracted due to a large number of patients were lost to follow-up. When van Dahlen *et al.*[2] analyzed the effect of histologic findings on prognosis in 123 patients from three continents, only six of 69 patients who were dialysis dependent at presentation recovered renal function. The histologic appearance using the Berden classification was, however, similar in this and the present study.

There are very few prospective clinical trials aiming at evaluating treatment of patients with anti-GBM disease. In 1985, Johnson *et al.* published a trial where 17 patients were randomized to PLEX or no PLEX on top of CYC and high oral doses of corticosteroids.[21] Kidney survival was only seen in patients who at inclusion displayed a creatinine clearance $\geq$27 ml/min per 1.73m^2. In another study comparing PLEX with immunoadsorption therapy, Stegmayr *et al.* included six patients with anti-GBM disease, none of whom ended up dialysis independent.[22] In the present study, imlifidase treatment led to a rapid decline of anti-GBM antibodies in all patients. PLEX is less effective in removing IgG, but without a control group, we cannot ascertain that the favorable outcome in this study was a direct result of the rapid decline of the causative agent. A head-to-head randomized controlled study is warranted.

The dose, 0.25 mg/kg, was chosen because it had been shown to be safe and efficacious in removing IgG in all healthy subjects and in transplantation studies.[7,13] Although animal experiments have also indicated cleavage of IgG bound to GBM,[8] this was not evaluated in this study, so we do not know if antibodies in all capillaries were cleaved and deactivated. We cannot rule out that a higher initial dose or repeated dosing could have led to an even better outcome.

Ten patients exhibited a rebound of anti-GBM antibodies, prompting the reintroduction of PLEX. In a majority, only a few sessions were needed to bring anti-GBM antibodies to levels considered safe by the principal investigator at the site. Only four patients needed more than five PLEX sessions to curb the rebounds.

Three patients in this study did not exhibit linear staining of IgG along the GBM and may thus be contested not to have had anti-GBM disease. This is a result of our study protocol, where we prioritized rapid administration of imlifidase over histologic confirmation. The pathogenicity of circulating anti-GBM antibodies when undetected by immunofluorescence on kidney sections is unknown. It is still possible that antibodies are present below the detection level. It is known that indirect immunofluorescence is less sensitive than solid phase immunoassays for detecting circulating anti-GBM antibodies.[23] Furthermore, it is well

known from animal experiments that subnephritogenic amounts of anti-GBM antibodies can enhance the toxicity of other autoantibodies.[24] For instance, low doses of anti-GBM antibodies given to mice immunized against MPO lead to neutrophil recruitment to the glomerulus resulting in placement of MPO on the endothelium, where a T cell–mediated reaction can ensue.[25]

In this study, moderate to severe lung hemorrhage was an exclusion criterion, which was required by one of the regulatory authorities reviewing the protocol. The concern raised was that imlifidase cleavage could potentially trigger aggravation of pulmonary disease, because aggravation of thrombotic thrombocytopenic purpura has been reported in one patient after the administration of imlifidase.[26] None of the patients in this study had any alveolar hemorrhage or signs of serum sickness after imlifidase.

When giving imlifidase, we saw no infusion reactions, nor did we record any severe AEs assessed as probably or possibly related to the study drug. During the first month of the study, there were no serious infections. One patient died of pneumonia, but this was approximately 2 months after administration of imlifidase. We did, however, see one instance of thrombophlebitis and two instances of deep vein thrombosis. The incidence of thrombosis in anti-GBM disease is to our knowledge unknown, but it is a well-known complication in other inflammatory diseases, such pauci-immune small vessel vasculitis.[27]

In conclusion, when treating 15 patients with circulating anti-GBM antibodies and an eGFR <15 ml/min per $1.73m^2$, we saw a rapid decline of circulating autoantibodies. This seemed to stop the disease process; no more patients became dialysis dependent and half of those who were dialysis dependent regained independent kidney function. Without a control group, however, other explanations for results obtained cannot be ruled out. This novel approach may also prove beneficial in other diseases where IgG autoantibodies cause life-threatening conditions.

DISCLOSURES

A. Bruchfeld reports being a consultant for and receiving honoraria from AstraZeneca, Bayer, Chemocentryx, Fresenius, and Vifor; reports receiving research funding from AstraZeneca; and reports having an advisory or leadership role as Chair of the ERA-EDTA Immunonephrology Working Group, Member of the Eurpean Renal Association scientific advisory board 2018–2024, and Vice-chair of the Swedish Renal Fund. A. Fernstrom reports being a consultant for AstraZeneca and Alnylam; and reports having an ownership interestin Hansa Biopharma, . A. Kronbichler reports being a consultant for Catalyst Biosciences, Delta4, Otsuka, Uri-Salt, Hansa Biopharma and Vifor; and reports receiving research funding from Otsuka (€5500) and Vifor (€2000). A. Lionet reports receiving speakers bureau from Therakos (UK) (Mallinckrodt) and conference fees. C. Elfving reports employment with and an ownership interest in Hansa Biopharma. C. Kjellman reports employment with, an ownership interest in, and patents or royalties with Hansa Biopharma. C. Rafat reports receiving honoraria from M3, Qualworld, and Sermo and having consultancy agrement with Hansa Biopharma. E. Daugas reports being a consultant for Amgen, AstraZeneca, and GlaxoSmithKline (GSK); and reports receiving honoraria from AstraZeneca and GSK. E. Sonesson reports employment with and an ownership interest in Hansa Biopharma. F. Uhlin reports receiving research funding from Hansa Biopharma, Lund, Sweden; and reports being a Project Coordinator for the investigator-driven multicenter study, GOOD-IDES, with a financial support of 50% of salary from Hansa Biopharma during 2017–2020. I. Bajema reports employment with Pathan Laboratories; reports being a consultant for Aurinia, Boehringer Ingelheim, CatBio, GSK, Novartis, and Toleranzia; and reports having other interests or relationships as the Director of Bajema Institute of Pathology, President of Renal Pathology Society, and Vice-President of European Vasculitis Society. I. Soveri reports being a consultant for Vifor; reports receiving research funding from Bayer and Vifor; and reports receiving honoraria from Sandoz. L. Rostaing reports receiving honoraria from Fresenius Medical and Novartis; reports research with BMS and Hansa; and reports having an advisory or leadership role with Bristol Myers Scibb, Novartis, and HansaBiopharma. M. Segelmark reports being a consultant for AstraZeneca, Hansa Biopharma, and Vifor Pharma; and reports receiving research funding from Hansa Biopharma and reports receiving research grants from Ingrid Asp research foundation. N. Kamar reports being a consultant for Astellas, Neovii, and Novartis; reports receiving research funding from Astellas, Neovii, and Novartis; reports receiving honoraria from AbbVie, Astellas, Biotest, CSL Behring, Chiesi, Merck Sharp and Dohme, Neovii, Novartis Pharma, Sanofi, Sandoz, Shire, and Takeda; reports having patents or royalties with UpToDate; and reports having an advisory or leadership role with Astellas, Biotest, Merck Sharp and Dome, Novartis, and Takeda. V. Tesar reports being a consultant for Alexion, Boehringer-Ingelheim, Calliditas, Eli Lilly, Fresenius Medical Care, Novartis, Omeros, Otsuka, Pfizer, Sanofi, Swixx BioPharma, Hansa Biopharma and Travere; reports receiving honoraria for consultancy from Alexion, Boehringer-Ingelheim, Calliditas, Fresenius Medical Care, Novartis, Omeros, and Travere; and reports having an advisory or leadership role as a member of B. Braun, Calliditas, Fresenius Medical Care, Novartis, Omeros, and Travere. S. McAdoo reports consultancy with GSK and Vifor, and honoraria from Rigel Pharmaceuticals, ThermoFisher Scientific, and Celltrion. All remaining authors have nothing to disclose.

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AUTHOR CONTRIBUTIONS

M. Segelmark conceptualized the study; C. Elfving, C. Kjellman, M. Segelmark, and F. Uhlin were responsible for the data curation; M. Segelmark and F. Uhlin were responsible for the formal analysis; M. Segelmark was responsible for the funding acquisition; I. Bajema, A. Bruchfeld, E. Daugas, N. Kamar, A. Kronbichler, A. Lionet, S. McAdoo, J. Molne, M. Mysilvecek, C. Rafat, L. Rostaing, E. Sonesson, M. Segelmark, I. Soveri, W. Szpirt, V. Tesar, and F. Uhlin were responsible for the investigation; I. Bajema, A. Bruchfeld, C. Elfving, A. Fernström, A. Kronbichler, J. Molne, M. Segelmark, E. Sonesson, and F. Uhlin were responsible for the methodology; M. Segelmark and F. Uhlin were responsible for the project administration; A. Bruchfeld, E. Daugas, A. Fernström, N. Kamar, C. Kjellman, A. Kronbichler, A. Lionet, L. Rostaing, M. Segelmark, E. Sonesson, I. Soveri, W. Szpirt, C. Rafat, V. Tesar, and F. Uhlin were responsible for the resources; M. Segelmark and F. Uhlin provided supervision; M. Segelmark, E. Sonesson, and F. Uhlin were responsible for the validation; M. Segelmark and F. Uhlin were responsible for the visualization; M. Segelmark wrote the original draft; and I. Bajema, A. Bruchfeld, E. Daugas, C. Elfving, A. Fernström, N. Kamar, C. Kjellman, A. Kronbichler, A. Lionet, S. McAdoo, J. Molne, M. Mysilvecek, C. Rafat, L. Rostaing, W. Szpirt, E. Sonesson, I. Soveri, V. Tesar, and F. Uhlin reviewed and edited the manuscript.

SUPPLEMENTAL MATERIAL

This article contains the following supplemental material online at http://jasn.asnjournals.org/lookup/suppl/doi:10.1681/ASN.2021111460/-/DCSupplemental.

Supplemental Table 1. Criteria for inclusion and exclusion.
Supplemental Table 2. Steroid dosing.
Supplemental Table 3. PLEX in relation to anti-GBM levels.
Supplemental Table 4. Putative prognostic factors.
Supplemental Table 5. Pharmacokinetic analyses.

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