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Safety, efficacy, and immunogenicity of a novel IgG degrading enzyme (KJ103): results from two randomised, blinded, phase 1 clinical trials

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The approved intravenous adeno-associated virus (AAV) therapies are limited by the widespread prevalence of pre-existing anti-AAV antibodies in the general population, which are known to restrict patients' ability to receive gene therapy and limit transfection efficacy in vivo. To address this challenge, we have developed a novel recombinant human immunoglobulin G degrading enzyme KJ103, characterized by low immunogenicity and clinical value for the elimination of anti-AAV antibodies in gene transfer. Herein, we conducted two randomized, blinded, placebo-controlled, single ascending dose Phase I studies in China and New Zealand, to evaluate the pharmacokinetics, pharmacodynamics, safety and immunogenicity of KJ103 in healthy volunteers. The results confirmed that KJ103 rapidly reduced IgG and maintained plasma IgG at low levels for one week. Dose of KJ103 ranging from 0.01 to 0.40 mg/kg had a favorable safety and tolerability profile across diverse ethnic and gender groups. KJ103 demonstrated a lower incidence of pre-existing anti-drug antibodies (ADAs) compared to currently approved human IgG degrading enzyme Imlifidase, with most induced ADAs predominantly reverting to baseline six months after administration. These properties are ideal for the management of immune disorders, rejection responses, and immunotherapies where pre-existing antibodies can reduce efficacy. Furthermore, we tested AAV2 neutralizing antibodies to confirm the potential utility of KJ103 in enhancing gene therapy.

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INTRODUCTION

Immunoglobulin G (IgG) constitutes a significant fraction of human serum, accounting for approximately 10–20% of plasma proteins [1]. IgG is essential for normal immune function in the body [2]. IgG antibodies against viruses and bacteria are produced following infection or vaccination; however, they can also be elicited against human antigens, such as blood group antigens, Human Leukocyte Antigen (HLA) and tissue-specific antigens, due to blood transfusions, pregnancy, or idiopathic reasons [3]. Additionally, even benign antibodies occasionally interfere with the efficacy of contemporary therapeutic interventions. An example of this challenge is encountered in organ transplantation, where positive donor-specific alloantibody (DSA) is a contra-indication to conventional kidney transplantation [3]. Recent case involves adeno-associated virus (AAV) capsids for intravenous (i.v.) delivery gene therapy. Based on different serotypes of AAV vector, four of the five approved recombinant AAV i.v. drugs (Roctavian [4], Hemgenix [5], Elevidys [6], Zolgensma [7] and Beqvez [8]) are affected by different seroprevalence of pre-existing anti-AAV antibodies in the general population. These antibodies are known to diminish in vivo transfection efficacy of AAV vectors, thereby constraining the eligibility of patients for these products [9, 10]. The current clinical solution of anti-AAV antibodies is limited to plasmapheresis, which is both costly and inefficient [11].

In recent years, the IgG degrading enzyme Imlifidase (IdeS) has been used for conditions requiring the depletion of pre-existing antibodies, leading to a breakthrough for DSA-positive renal failure patients [12]. However, IdeS, derived from *Streptococcus pyogenes*, is subject to the limitation of high immunogenicity. Antibodies against IdeS are present in over 90% of the population. A screening study of 130 participants found that only 10 of them had anti-IdeS antibody concentrations below the detection limit (2.0 mg/L). The median level of pre-existing anti-IdeS antibodies was 6.1 mg/L (range 2.0–78.0 mg/L), with the 80th percentile 15 mg/L [13]. These anti-IdeS antibodies may increase the risk of hypersensitivity/infusion-related reactions against IdeS [14, 15]. Moreover, evidence suggests that neutralizing antibodies are able to weaken the activity of IdeS [13, 16]. To date, there are no reports indicating that IdeS can sufficiently facilitate the clearance of pre-existing antibodies in the clinical practice of AAV-based gene therapy.

To reduce risk associated with immunogenicity, IdeE (an IgG-degrading enzyme of *S. equi* ssp. *equi*) was selected from a species that typically does not infect humans. The development of the

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next-generation product, KJ103, was based on physicochemical analysis of IdeE's structure and activity. A library of IdeE mutants was constructed, and KJ103 was identified through screening by the criteria of more stable physicochemical properties [17]. The results of in vitro studies have proved that KJ103 can efficiently cleave human IgG at the hinge region, and the results from animal studies indicated that KJ103 has a good safety and efficacy profile. KJ103 is expected to be a potential agent for desensitization of all IgG subclasses. To further assess KJ103, Phase I studies were conducted in volunteers in China and New Zealand. In addition to overall safety and efficacy, the capability of KJ103 to clear AAV neutralizing antibodies (NAbs) has been validated. These studies support further therapeutic clinical trials of KJ103.

MATERIALS AND METHODS

Study design

Two similar randomized, blinded, placebo-controlled, single ascending-dose phase I studies were conducted independently in China (Identifier: NO. ChiCTR2300075920) and New Zealand (Identifier: NO. NCT05274659). The studies were designed to evaluate the safety, tolerability, pharmacokinetic profile, pharmacodynamic profile, and immunogenicity of KJ103 in volunteers.

The study protocols and all amendments were approved by the local Ethics Committee of each center (Suzhou Municipal Hospital, New Zealand Clinical Research), and the studies were conducted in compliance with the Declaration of Helsinki and the international standards of Good Clinical Practice. Written informed consent was obtained from all volunteers prior to any study-related procedures. The studies were reported according to the 2022 update to the Consolidated Standards of Reporting Trials (CONSORT) 2010 statement.

Five dose levels were chosen to explore the safety and tolerability of KJ103, ranging from 0.01–0.40 mg/kg (Table 1). The first 0.01 mg/kg group enrolled 2 volunteers to receive KJ103. The remaining groups enrolled 8 volunteers, with 6 volunteers randomly allocated by computer to receive KJ103 and 2 volunteers allocated to receive placebo. Sentinel dosing was employed (except the first dose level), in which two volunteers were dosed with KJ103 or placebo on Day 1 in order to monitor potential acute reactions. The remaining 6 volunteers dosed ≥24 h after the sentinel dose, once the Investigator completed the safety assessment of the first two volunteers. The Investigator was blinded to the group allocation during the experiment or when assessing the safety profile. Volunteers were followed-up for a total of 2 months after dosing.

The safety and tolerability were observed during the dose-limiting toxicity (DLT) observation period. The DLT observation period in China was 7 days, while in New Zealand it was 14 days. If none of the volunteers experienced an event meeting the dose escalation termination criteria (≥ grade 3), the study proceeded to the next dose level. A safety review committee was set with the responsibility to make decisions on whether to proceed to the next dose level through obtained pharmacokinetics (PK), pharmacodynamics (PD) and safety data once the DLT observation period finished in each group.

Participants

In these studies, eligible participants were selected according to the major inclusion criteria: healthy male or female participants aged between 18 to 55 years; body mass index (BMI) between 18 and 30 kg/m^2. The participants were excluded according to the following criteria: clinically significant immunodeficiency (including but not limited to immunoglobulin A deficiency); history of tuberculosis; positive screening for HBcAb, HCV antibodies, HIV antibodies, and syphilis antibodies; allergy to KJ103 or its excipients; participation in other clinical trials; pregnant or nursing women; blood loss or donation of >400 mL in the 3 months before enrollment; alcohol or drug abuse; clinically significant abnormalities in vital signs, 12-lead electrocardiogram (ECG) or laboratory tests.

To mitigate the increased risk of opportunistic infection post-KJ103 administration, all participants received oral prophylactic antibiotics (Cefuroxime Axetil Tablets, or Amoxicillin and Clavulanate Potassium Tablets) starting on the day of administration and continuing until Day 28 or until IgG levels were at least 6.0 g/L. If grade 2 or higher infusion-related reactions occurred in the first two dose groups, participants in the 3/4/5 dose group would receive preventive medications for infusion-related reactions, such as glucocorticoids or antihistamines (Cetirizine Dihydrochloride Tablets or Promethazine Hydrochloride Injection) as needed.

Assessment of safety

All adverse events that emerged during the studies were assessed and graded according to the National Cancer Institute Common Standard Terminology for Adverse Events (NCI-CTCAE), version 5.0. Parameters included in evaluations were vital signs, physical examinations, 12-ECG, clinical laboratory tests (hematology, urinalysis, biochemistry, coagulation function, etc.), clinical adverse and serious adverse events (AEs, SAEs).

Pharmacokinetics. Blood samples were collected at the predetermined time points for PK evaluation. For Cohorts 1 and 2, blood samples were taken at pre-dose, 1 min before infusion completion, and at the exit visit. For Cohorts 3–5, blood samples were taken at pre-dose, 1 min before infusion completion, 5 min, 45 min, 6 h, 24 h, 48 h, 72 h, and 144 h after infusion and at study termination in cases of early withdrawal. Plasma KJ103 levels were measured using a validated enzyme-linked immunosorbent assay (ELISA) method at JOINN Laboratories (Beijing,China).

Single-dose PK parameters of KJ103 were estimated using non-compartmental analysis, including C_{max}, T_{max}, $AUC_{0\text{-}t}$, $AUC_{0\text{-}\infty}$, λ_z, $t_{1/2}$, $MRT_{0\text{-}\infty}$, CL, and V_z. Concentration data were tabulated, and descriptive statistics were performed for grouped planned blood sampling times. The average plasma concentration-time curves of the volunteers in different dose groups (0.12 mg/kg, 0.25 mg/kg and 0.40 mg/kg) were plotted.

Pharmacodynamics. For the purpose of PD assessment, blood samples were obtained according to the following schedule. For Cohort 1, samples were collected at pre-dose, 6 h after the infusion, and at the exit visit. For Cohorts 2–5, samples were collected at pre-dose, 1 min before infusion completion, 5 min, 20 min, 45 min, 1 h, 1.5 h, 2 h, 3 h, 6 h, 24 h, 48 h, 72 h, 144 h, Days 14, 21, 28, and 63 after infusion and at study termination in cases of early withdrawal. Plasma IgG levels of the volunteers were measured by ELISA at JOINN Laboratories (Beijing,China), with the lower limit of ELISA detection being 0.40 g/L. The levels of single-chain IgG molecules (scIgG), F $(ab')_2$ and Fc were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) at JOINN Laboratories (Beijing,China).

Population pharmacokinetic model and PK/PD models

The estimation method for population pharmacokinetic (PopPK) modeling was the first-order conditional estimation method (FOCEI) considering the η-ε interaction.

Table 1. The dose design and participant allocation of KJ103 in single ascending-dose studies.

Dose group	Dose	No. of healthy participants (China, SHBJ-2021-002)		No. of healthy participants (New Zealand, SHBJ-2021-001)	
		KJ103	Placebo (Normal Saline)	KJ103	Placebo (Normal Saline)
1	0.01 mg/kg	2	0	2	0
2	0.04 mg/kg	6	2	6	2
3	0.12 mg/kg	6	2	6	2
4	0.25 mg/kg	6	2	6	2
5	0.40 mg/kg	6	2	6	2
Total		26	8	26	8

The inter-individual variability (IIV) was modeled as an exponential model (Eq. 1):

$$P_i = P_{TV} \times \exp(\eta_i) \quad (1)$$

where P_{TV} is the typical value of the PK parameter, P_i is the individual parameter value, and η_i is the inter-individual variation for P_{TV} with a normal distribution of mean of 0 and variance of ω^2.

The following models, additive (Eq. 2), proportional (Eq. 3) and combined additive and proportional (Eq. 4) and logarithmic (Eq. 5) model, were investigated for residual variability (RV):

$$Y_{obs.ij} = Y_{pred.ij} + \varepsilon_{ij.1} \quad (2)$$

$$Y_{obs.ij} = Y_{pred.ij} \times (1 + \varepsilon_{ij.1}) \quad (3)$$

$$Y_{obs.ij} = Y_{pred.ij} \times (1 + \varepsilon_{ij.1}) + \varepsilon_{ij.2} \quad (4)$$

$$Y_{obs.ij} = \mathrm{LOG}(Y_{\mathrm{pred.ij}}) + \varepsilon_{\mathrm{ij.1}} \quad (5)$$

where $Y_{obs,ij}$ and $Y_{pred,ij}$ are the observed and predicted concentrations in i^{th} volunteer and at j^{th} sampling time point, respectively. $\varepsilon_{ij,1}$ and $\varepsilon_{ij,2}$ are normal distributions with mean of 0 and variances of $\sigma_{ij,1}{}^2$ and $\sigma_{ij,2}{}^2$, respectively.

Covariates included gender, age, ethnicity, body weight, body mass index, baseline alanine transaminase (ALT), post-administration anti-drug antibody (ADA), baseline IgG, were assessed by step-wise forward ($P < 0.05$) selection and backward elimination ($P < 0.001$). The PopPK models were evaluated by goodness-of-fit plots, visual predictive checks and bootstrap resampling procedures.

IgG level was selected as the effect index for exposure-response (E-R) analysis of KJ103. Individual KJ103 blood concentration data was obtained using a Bayesian posterior estimation method through the final PopPK model. The PK/PD model of KJ103 concentration and IgG level was explored through an effect-compartment model or an indirect response model. The covariate screening method and model evaluation method are the same as PopPK.

Anti-drug antibody

Blood samples were collected at the following time points for ADA assessment: pre-dose, 24 h, 48 h, 72 h, 144 h, and on Days14, 21, 28, 63 and Day 180 post-infusion or in cases of early withdrawal. Anti-KJ103 antibody in human serum were measured using a validated bridging ELISA method at JOINN Laboratories (Beijing,China). The data reproducibility and quality control adhered to White Paper of Anti-drug Antibody Validation Testing and Reporting Harmonization [18].

Statistical analysis

All statistical analyses were performed using SAS 9.4 except for the calculation of PK parameters which were made using Phoenix WinNonlin 8.2. Enumeration data and grade data were described by the number of cases and percentage. NONMEM (Version 7.5) and its tool software Wings for NONMEM (nm753) and Perl Speaks NONMEM (Version 5.3.0) were used to build models and simulations. R (Version 4.1.2) was used for collating and analyzing data sets, statistical analyses, modeling, mapping, and construction of virtual populations.

AAV2 NAb assay

The assay was based on AAV2 transduction inhibition. Briefly, on day 1, 96-well plates were seeded with HEK293 (Cellverse, Cat: icell-h086, STR-authenticated, mycoplasma-resistant culture) 1.4×10^5 cells per well. An AAV2-Luc vector was then diluted in Opti-MEM I(1x) Reduced Serum Medium (Gibco, Cat: 51985-034) and incubated with two-fold serial dilutions of the serum samples for 1 h at 37 °C. Subsequently, the serum-vector mixtures were added to the cells at a multiplicity of infection of 500. Cells incubated with two-fold gradient dilution of the anti-AAV2 intact particle Mouse monoclonal antibody (Progen, cat: 61055) and rAAV-CMV-Luc served as positive controls ; cells incubated with seronegative serum and rAAV-CMV-Luc served as negative controls. After 48 h, cells were lysed and luciferase activity was measured on SpectraMax i3 (Molecular Devices). Luciferase expression was quantified as relative light units (RLUs) per second. The anti-AAV2 NAb titer was defined as the lowest serum dilution that inhibited AAV2 transduction by at least 50% compared with the positive control. The data reproducibility and quality control adhered to White Paper of Neutralizing Antibody Validation Testing and Reporting Harmonization [19].

ELISA-based F(ab')$_2$ assay

Standard F(ab')$_2$ sample was purified from KJ103-digested IVIg with CaptureSelect™ CH1-XL kit (Thermo Fisher Scientific, Cat: 1943462005). The levels of F(ab')$_2$ in plasma were quantified using a sandwich ELISA. Briefly, 96-well microplates (Sigma-Aldrich) were coated with Anti hinge antibody (AHA-2095-scFv) binding tail of F(ab')$_2$ at 3 µg/mL in 100 µL coating buffer and incubated overnight at 4 °C. AHA-2095-scFv was a rabbit monoclonal antibody specifically targeting the IdeS-generated F(ab')$_2$ fragments [20]. After coating, plates were washed, blocked for 1 h at room temperature and samples were added to each well in duplicate. An 8-point standard curve was created by spiking in standard F(ab')$_2$ into PBST (with 1% BSA) at known concentrations ranging from 160 to 5 ng/ml in duplicate wells. Samples were added to wells at a volume of 100 µl and incubated for 1 h at 37 °C. After sample incubation, plates were washed and incubated with a monoclonal Mouse-anti-Human IgG Fab Antibody (Genscript, Cat: A01855) at a dilution of 1:5,000 (100 µl). Plates were washed and a chromogenic signal was generated by adding 100 µl of TMB substrate to each well, followed by quenching with 100 µl of 1 N sulfuric acid. Absorbance was measured using SpectraMax i3 (Molecular Devices) at 450 nm; (Fab)2 antigen levels were extrapolated from the standard curve. The data reproducibility and quality control adhered to ICH guideline M10 on bioanalytical method validation and study sample analysis.

RESULTS

Demographic characteristics

In these two independent but similarly designed studies, a total of 68 volunteers (China $n = 34$; New Zealand $n = 34$) were included. In the study conducted in China, 100% of the volunteers were of Asian ethnicity and males accounted for 85.3%. In the New Zealand study, most of the volunteers were Caucasian (58.8%), and females accounted for 79.4% of all. With the exception of sex and ethnicity, all other baseline characteristics were similar between both studies (Table 2).

Safety and tolerability

Of the total 68 volunteers across both studies, 52 received KJ103 and the remainder placebo. During the study, there were no DLTs or SAEs, and no treatment-emergent adverse events (TEAEs) or treatment-related adverse events (TRAEs) leading to study termination. All the predicted dose levels were escalated steadily and all volunteers received the proposed dose with one exception. One participant in New Zealand, receiving 0.40 mg/kg, experienced a self-limited infusion reaction leading to early termination of infusion, attributable to the protocol deviation of no pre-medication.

A total of 27 volunteers (79.4%) experienced 44 TEAEs in the Chinese study. 23 volunteers' TEAEs (23/34, 67.6%) were grade 1, with fewer cases (4/34, 11.8%) being grade 2, and none grade 3 or

Table 2. Demographic characteristics of trial participants.

Demographic	Category/ Statistics	Phase I - China ($N = 34$)	Phase I - New Zealand ($N = 34$)
Sex			
Male	*n* (%)	29 (85.3)	7 (20.6)
Female	*n* (%)	5 (14.7)	27 (79.4)
Age (years)	Mean	32.1	33.1
Ethnicity			
Asian	*n* (%)	34(100)	3 (8.8)
Caucasian	*n* (%)	0	20(58.8)
Other	*n* (%)	0	11 (32.4)

Table 3. Listing of TRAEs in two studies.

PT (Severity of AE)	Site	0 ~ 24 h	Day 2 ~ 7	Day 8 ~ 14	Day 15 ~ 21	Day 22 ~ 63	Relationship to Study Medication	Action Taken with Study Treatment	Outcome
0.01 mg/kg	China		Oral mucositis (Grade 1)				Possible	None	Resolved
			Limbs pain (Grade 1)				Probable	None	Resolved
0.04 mg/kg	China		Lymph node pain (Grade 1)				Possible	None	Resolved
	New Zealand			Nasal congestion (Grade 1)			Possible	None	Resolved
0.12 mg/kg	China		Diarrhea (Grade 1)				Possible	None	Resolved
					Elevated alanine aminotransferase (Grade 1)		Probable	None	Resolved
					Elevated aspartate aminotransferase (Grade 1)		Probable	None	Resolved
	New Zealand		Fatigue (Grade 1)				Possible	None	Resolving
0.25 mg/kg	China		Bacterial infections (Grade 1)				Probable	None	Resolved
			Elevated alanine aminotransferase (Grade 1)				Probable	None	Resolved
			Elevated blood uric acid (Grade 1)				Possible	None	Resolved
			Elevated gamma-glutamyl transpeptidase (Grade 1)				Possible	None	Resolved
						Decreased lymphocyte count (Grade 2)	Possible	None	Resolved
	New Zealand				Vulvovaginal candidiasis (Grade 1)		Possible	None	Resolved
			Limb discomfort				Possible	None	Resolved

Table 3. continued

PT (Severity of AE)	Site	0 ~ 24 h	Day 2 ~ 7	Day 8 ~ 14	Day 15 ~ 21	Day 22 ~ 63	Relationship to Study Medication	Action Taken with Study Treatment	Outcome
			(Grade 1)						
			Diarrhea				Possible	None	Resolved
			(Grade 2)						
			Rash				Possible	None	Resolved
			(Grade 1)						
0.40 mg/kg	New Zealand	Infusion related reaction (Grade 2)					Definite	Medication	Resolved
			Deranged liver blood tests[a]				Probable	None	Resolved
			(Grade 3)						

a: The alanine aminotransferase, alkaline phosphatase and aspartate aminotransferase levels of a subject from the 0.40 mg/kg dose group in New Zealand was measured clinically significant. The results are as follows: alanine aminotransferase (Normal range:0 ~ 45 U/L) is 228 U/L on day 7, 134 U/L on day 11, and 110 U/L on day 21; alkaline phosphatase (Normal range:40 ~ 110 U/L) is 137 U/L on day 7, 127 U/L on day 11, and 118 U/L on day 21; aspartate aminotransferase (Normal range:0 ~ 45 U/L) is 167 U/L on day 7, 78 U/L on day 11, and 68 U/L on day 21.

above. A total of 9 volunteers (9/34, 26.5%) had 11 TRAEs in this study. Most of them (8/34, 23.5%) were grade 1, and only 1 volunteer (1/34, 2.9%) had a grade 2 TRAE (decreased lymphocyte count). No volunteers in the 0.40 mg/kg group experienced TRAE.

A total of 30 volunteers (88.2%) experienced 79 TEAEs in the New Zealand study. 26 (26/34, 76.5%) volunteers' TEAEs were grade 1; 2 cases (2/34, 5.9%) were grade 2, and 2 volunteers (2/34, 5.9%) had a grade 3 AE (one volunteer at 0.04 mg/kg developed dental caries that was assessed as unlikely to be related to the test drug, and another participant at 0.40 mg/kg developed deranged liver blood tests that were assessed as probably related to the test drug, both of whom recovered). A total of 8 participants (8/34, 23.5%) reported 8 cases of TRAEs.

Combining the above results, 19 AEs observed in 17 of the 68 participants were classified as related (i.e. possible, probable or definite), as presented in Table 3. The most common AE related to KJ103 was elevated alanine aminotransferase (ALT). The primary concern with IgG-degrading enzymes is the risk of infection. In this regard, the following results affirm KJ103's safety. One case of grade 1 infectious illness (suspected bacterial infection) occurred in the 0.25 mg/kg group in China, and was determined by the Investigator as probably related to KJ103. One case of grade 1 vulvovaginal candidiasis occurred in the 0.25 mg/kg group in New Zealand, and was determined possibly related to KJ103. There was no severe infection events related to KJ103 (Table 3).

Pharmacodynamics

Compared to the placebo, IgG showed mild degradation at dose of 0.01 mg/kg of KJ103. With increasing dose, a sharper "drop off" of IgG level was observed in a dose-dependent manner. A greater reduction in IgG after administration of KJ103 at a dose of 0.12 mg/kg to Asian volunteers in both studies. In both the 0.25 mg/kg and 0.40 mg/kg group, IgG levels reached their lowest point within 6 h of administration, falling by over 90%, and remained consistently below 70% of normal levels for the first week after dosing. After the initial fall ward, IgG levels gradually increased after one week of administration. IgG recovered to near or above baseline levels within 1 to 2 months post-dose in most volunteers. It is worth noting that dose levels of KJ103 did not affect the rate and extent of IgG recovery, only the initial "drop off". These results indicate that the IgG changes related to dosing with KJ103 were consistent across different ethnicities and demonstrate a favorable pharmacological effect (Fig. 1).

Exploratory pharmacodynamics analysis

Based on the mechanism of action of KJ103, the digested fragments of scIgG, F $(ab')_2$ and Fc were also analyzed by SDS-PAGE. SDS-PAGE results were consistent with ELISA findings. Compared with the placebo group, in which there was no change, all the samples from KJ103 groups showed an increase in fragment concentration, rising in proportion to the corresponding decrease in intact IgG. All volunteers in the 0.04 mg/kg group and some volunteers in the 0.12 mg/kg group did not undergo complete IgG cleavage. Volunteers in the 0.25 mg/kg and 0.40 mg/kg groups experienced rapid, efficient, and complete cleavage of most IgG into $F(ab')_2$ and Fc fragments by KJ103 within 45 min - 6 h and 20 min - 6 h post-dosing, respectively. Combining the SDS-PAGE results, the IgG signal obtained 6 h post-dosing by ELISA primarily originates from scIgG. $F(ab')_2$ and Fc fragments decreased to the baseline levels within 7 days after dosing. Within one to two weeks, all volunteers demonstrated newly synthesized full-length IgG. Within 1 to 2 months post-dosing, IgG recovered to near or above baseline levels (Fig. 2).

Pharmacokinetics

After a single i.v. infusion of KJ103, the overall blood drug concentrations of volunteers in the 0.01 mg/kg and 0.04 mg/kg dose groups were below the detection limit, as a result of low

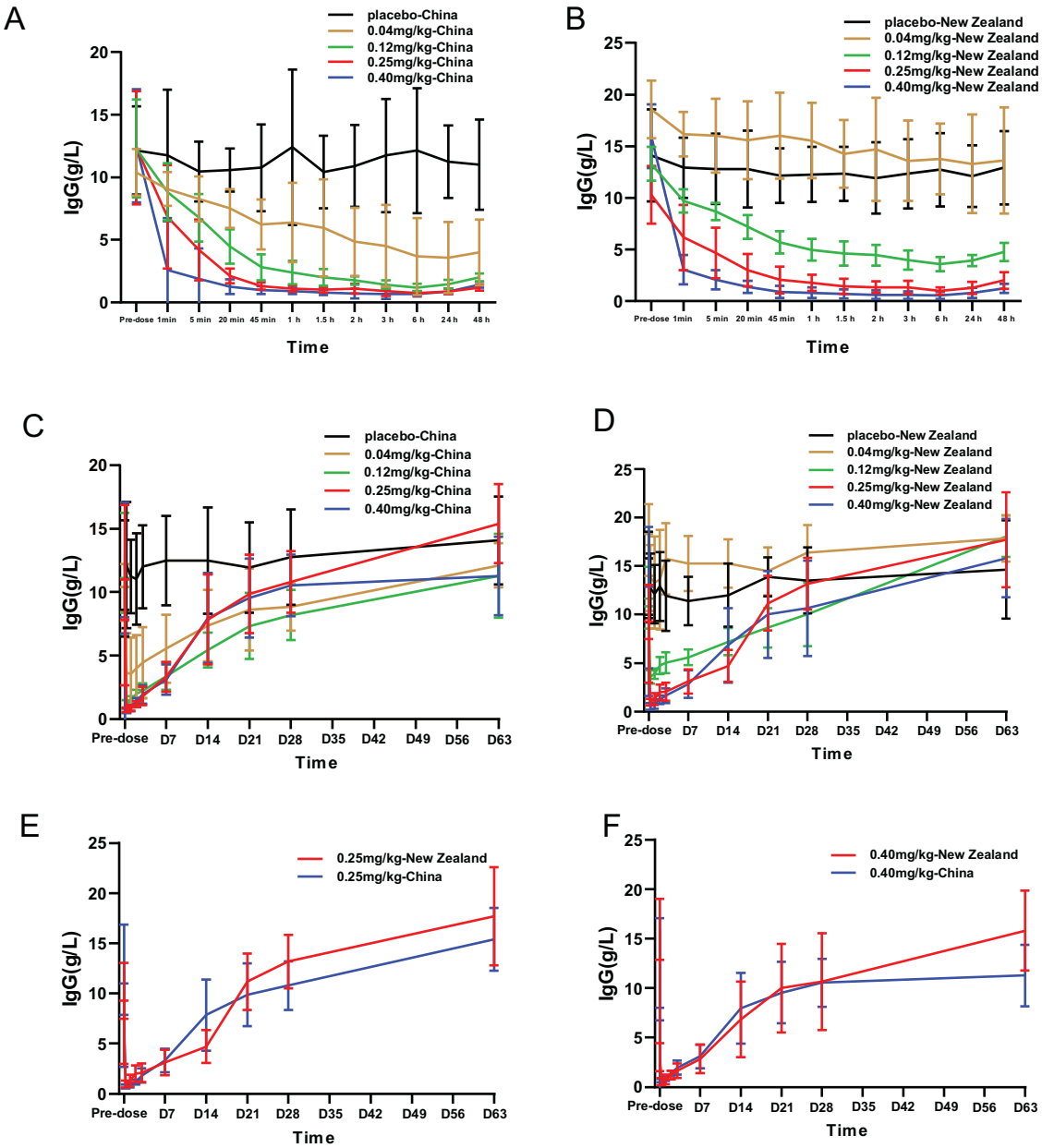

Fig. 1 Quantitative pharmacodynamics analysis by ELISA showed rapid degradation of IgG (Dose group 3–5). A Mean IgG values for each dose group at each visit within 48 h after dosing in China. **B** Mean IgG values for each dose group at each visit within 48 h after dosing in New Zealand. **C** Mean IgG values for each dose at each visit within D63 after dosing in China. **D** Mean IgG values for each dose at each visit within D63 after dosing in New Zealand. **E** Mean IgG values for 0.25 mg/kg dose group at each visit within D63 after dosing. **F** Mean IgG values for 0.40 mg/kg dose group at each visit within D63 after dosing.

exposure and therefore low blood concentration of the administered drug. PK concentration results showed good reproducibility of PK curves in the dose range of 0.12 mg/kg to 0.40 mg/kg for all dose groups. The mean KJ103 concentration peaked immediately after administration, reaching the plateau phase during which the distribution and metabolism of the drug achieved equilibrium in the body, followed by slow elimination of the drug. Most of the KJ103 was eliminated within 24 h after administration (Fig. 3).

After a single i.v. infusion of KJ103 in Chinese volunteers, the median T_{max} of 0.12 mg/kg, 0.25 mg/kg and 0.40 mg/kg dose groups were 0.333 h, 0.333 h and 0.583 h, respectively. C_{max} (Mean ± SD) were 2.208 ± 0.4985 mg/L, 5.142 ± 0.2922 mg/L and 9.200 ± 1.3049 mg/L, respectively. $AUC_{0\text{-}t}$ (Mean ± SD) were 28.405 ± 29.6554 h*mg/L, 43.114 ± 21.7201 h*mg/L and 170.261 ± 176.5502 h*mg/L, respectively. After excluding abnormal PK data, the average $t_{1/2}$ of each dose group was 6.260 h, 4.862 h and 81.648 h, respectively. $t_{1/2}$ showed a non-linear increase with the increase of dose.

After the New Zealand volunteers received a single i.v. infusion of KJ103, excluding the volunteer in the 0.40 mg/kg dose group who interrupted the dose due to an infusion reaction, the median T_{max} of 0.12 mg/kg, 0.25 mg/kg and 0.40 mg/kg dose groups were 0.4583 h, 0.3333 h and 0.5833 h, respectively. C_{max} (Mean ± SD) were 2.8135 ± 0.5216 mg/L, 6.5245 ± 0.9994 mg/L and 10.0124 ± 2.6712 mg/L, respectively. $AUC_{0\text{-}t}$ (Mean ± SD) were 45.2515 ± 47.9476 h*mg/L, 101.9423 ± 113.8636 h*mg/L and 61.9188 ± 29.7180 h*mg/L, respectively. After excluding abnormal PK data, the average $t_{1/2}$ of each dose group was 11.3978 h, 2.2629 h and 8.4130 h, respectively (Table 4).

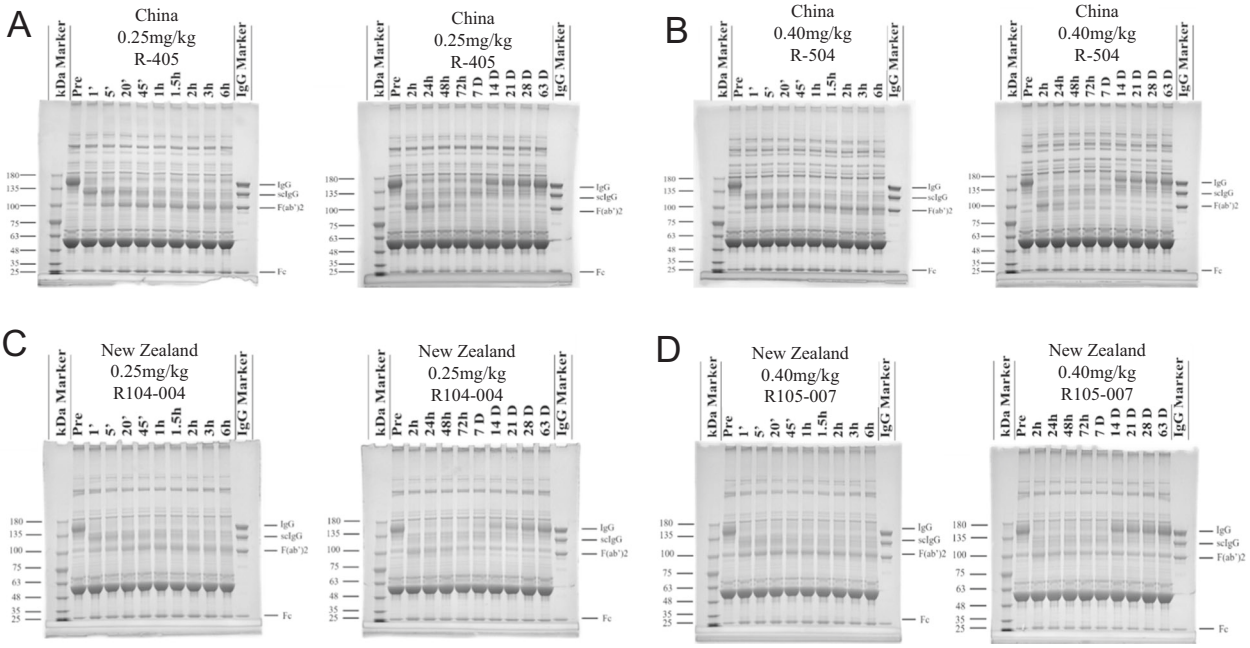

Fig. 2 Exploratory pharmacodynamics (scIgG, F (ab')$_2$ and Fc) analysis by SDS-PAGE. A Subject R-405 in China received 0.25 mg/kg BW KJ103 and the exploratory indicators showed changes over time on SDS-PAGE. **B** Volunteer R-504 in China received 0.40 mg/kg BW KJ103 and the exploratory indicators showed changes over time on SDS-PAGE. **C** Volunteer R104-004 in New Zealand received 0.25 mg/kg BW KJ103 and the exploratory indicators showed changes over time on SDS-PAGE. **D** Volunteer R105-007 in New Zealand received 0.40 mg/kg BW KJ103 and the exploratory indicators showed changes over time on SDS-PAGE.

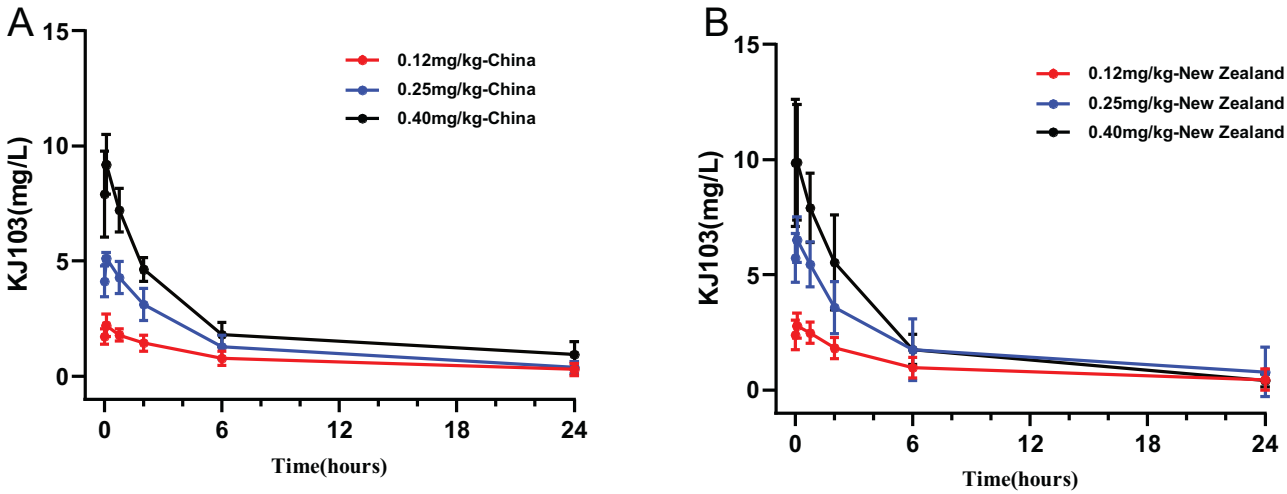

Fig. 3 Pharmacokinetics of KJ103 in serum. A The concentration of 0.12–0.40 mg/kg BW KJ103 in the blood of volunteers in China varies over time. **B** The concentration of 0.12–0.40 mg/kg BW KJ103 in the blood of volunteers in New Zealand varies over time.

Based on the analysis of PK parameters using a non-compartmental model, KJ103 exhibited characteristics of rapid distribution and slow elimination in volunteers in China and New Zealand. The exposure of KJ103 in various dosage groups demonstrated a non-linear increase with dosage escalation.

PopPK and PK/PD models

Based on clinical trial data from both studies, we established a KJ103 population pharmacokinetic (PopPK) model to explore the covariates affecting PopPK parameters (Table S1). We found that only body weight affects the clearance of KJ103. For a participant with body weight at the 10th~90th percentile of the study population relative to the median body weight in the study population, the C_{max} and $AUC_{0\sim168}$ varied from -29% ~ 26% (Table 5). Other covariates such as region, ethnicity and gender did not impact PopPK parameters ($P > 0.05$). The results also indicate similar PK characteristics of populations between China and New Zealand.

The optimal PopPK model was a two-compartment model with first-order elimination. The population typical values (RSE%) of CL, V_c, V_p and Q were 0.162 L/h (13.6), 3.23 L/h (4.3), 14.2 L/h (18.5) and 0.591 L/h (5.0), respectively. The results of the prediction-corrected visual predictive check (pcVPC) are presented in Fig. 4. The median, upper and lower 5th percentiles of the observed values were mostly contained within the 95% confidence intervals of the predicted values. Additionally, the predicted interval encompassed most of the observed values, indicating a good predictive performance of the model.

Table 4. Summary of PK parameters.

PK Parameter (Unit)	Statistics	China			New Zealand		
		0.12 mg/kg (*N* = 6)	0.25 mg/kg (*N* = 6)	0.40 mg/kg (*N* = 6)	0.12 mg/kg (*N* = 6)	0.25 mg/kg (*N* = 6)	0.40 mg/kg (*N* = 6)
C_{max} (mg/L)	Mean (SD)	2.208 (0.4985)	5.142 (0.2922)	9.200 (1.3049)	2.8135 (0.5216)	6.5245 (0.9994)	8.5279 (4.3509)
T_{max} (h)	Median	0.333	0.333	0.583	0.4583	0.3333	0.5833
$AUC_{0\text{-}t}$ (h*mg/L)	Mean (SD)	28.405 (29.6554)	43.114 (21.7201)	170.261 (176.5502)	45.2515 (47.9476)	101.9423 (113.8636)	56.4783 (29.7341)
$AUC_{0\text{-}\infty}$ (h*mg/L)	Mean (SD)	14.426 (9.9368)	28.566 (12.7335)	310.104 (368.8912)	27.3255 (26.0003)	19.4597 (1.9367)	55.8364 (20.6792)
λz (1/h)	Mean (SD)	6.260 (6.1106)	4.862 (2.5096)	81.648 (107.5154)	0.1144 (0.1389)	0.1571 (0.1649)	0.1155 (0.1445)
$t_{1/2}$ (h)	Mean (SD)	6.260 (6.1106)	4.862 (2.5096)	81.648 (107.5154)	11.3978 (13.3972)	2.2629 (0.1476)	8.4130 (8.0801)
$MRT_{0\text{-}\infty}$ (h)	Mean (SD)	7.956 (7.8214)	5.714 (2.7533)	196.187 (268.7298)	14.7852 (17.5298)	2.9775 (0.2253)	8.9927 (7.8506)
CL (L/h/kg)	Mean (SD)	0.011 (0.0075)	0.011 (0.0063)	0.004 (0.0052)	0.0079 (0.0054)	0.0129 (0.0014)	0.0079 (0.0027)
Vz (L/kg)	Mean (SD)	0.065 (0.0283)	0.059 (0.0076)	0.113 (0.0661)	0.0592 (0.0197)	0.0423 (0.0054)	0.0836 (0.0710)

A total of 48 volunteers were included in the PK/PD analyses, using IgG levels as the effect indicator. Individual volunteers' KJ103 blood concentration data were obtained using the PopPK final model using Bayesian a posteriori estimation to explore the PK/PD model of KJ103 concentration and IgG level (Table S2). The relationship between KJ103 concentration and IgG level was described by an effector chamber model. The results revealed that IgG began to decline after the administration of KJ103 at a dose of 0.25 mg/kg, and was maintained near the nadir and close to the lower limit of detection after 5–19 h. The IgG recovered to more than 1 g/L after 36 h of administration, and more than 2 g/L after 96 h, and stayed lower than 4 g/L for 7 days. Analyses of covariates showed that gender influenced the IC50 (concentration of KJ103 when IgG level reaches half of its maximum inhibitory effect), whereas different ethnicities had no effect on the PK/PD model. The results of the prediction-corrected visual predictive check (pcVPC) are presented in Fig. 5. The median and upper and lower 5th percentiles of the observed values were mostly contained within the 95% confidence intervals of the predicted values. Additionally, the predicted interval encompassed most of the observed values, indicating a good predictive performance of the model.

Simulation of IgG change levels after a single administration of 0.25 mg/kg in male and female typical volunteers showed that the overall trend of IgG change in males and females was similar. IgG started to decline after administration, approaching near the trough value after 5–24 h, and then recovered slowly (Fig. 6). However, the IgG trough value was 0.66 g/L higher in females than in males, and females recovered faster than males, although both sexes were lower than 5 g/L for 7 days. Simulating IgG trough levels after a single dose in the range of 0.01 to 0.40 mg/kg in male volunteers shows that the IgG trough levels decrease with increasing dosage (Fig. 7). At 0.25 mg/kg of KJ103, enzymatic digestion of IgG is more than 90% effective and reaches a plateau. The IgG levels at 0.25 mg/kg dosage remained at a low level within a week under various conditions.

Anti-drug antibody

The pre-existing ADA positivity rate for all enrolled volunteers was 33.82% (23/68), and the median value of pre-existing ADA titers was 0 (range: 0 to 1:429.61). There were no significant differences in the proportion and titer of pre-existing ADA among volunteers of different ethnicities enrolled in China and New Zealand (Fig. 8). The low proportion of pre-existing ADA in volunteers indicated that KJ103 has a low immunogenic background in the population. The relatively low levels of anti-KJ103 antibodies support the safety and efficacy of KJ103 administration. Only one volunteer from the 0.40 mg/kg group in New Zealand experienced an infusion reaction post-administration attributable to the failure to use prophylactic medication as per protocol. No infusion reactions occurred in the remaining participants from both studies.

Among volunteers in the China cohort, the median baseline ADA value was 0 (titer range: 0 ~ 1: 429.61); on the 7th day after KJ103 administration, the median ADA titer was 0 (range: 0 ~ 1: 408.25); on day 14 post-dose, ADA levels were near peak, with a median ADA value of 1:1,041.97 (range: 0 to 1:144,433.38). Two months after administration, the median ADA titer for all KJ103 users was 1: 605.89 (range: 1: <10 ~ 1: 10,989.27). Six months after administration, the median ADA titer for all KJ103 users was 1:524.77 (range: 0 ~ 1: 4,921.42) (Table 6).

In the New Zealand cohort, the median baseline ADA titer was 0 (range: 0 ~ 1: 242.58); on the 7th day after KJ103 administration, the median ADA titer was 0 (range: 0 ~ 1: 246.78); on day 14 post-dose, ADA levels were near peak, with a median ADA value of 1:257.50 (range 0, 1: 20,855.78). Subsequently, ADA levels gradually declined, and after two months of administration, the median ADA titer for all KJ103 users was 1: 158.64 (range: 0 ~ 1: 7171.39), and the median value of ADA titer at 6 months after administration was 1:214.35 (range: 0 ~ 1: 5,782.43) (Table 6). After

Table 5. The effect of body weight on exposure parameters of KJ103.

	50th (67.8 kg)	10th (55.1 kg)	90th (86.1 kg)	10th/50th	90th/50th
C_{max} (ng/mL)	5098.1	4155.7	6427.6	0.82	1.26
$AUC_{0\text{-}168h}$ (h*ng/mL)	81095.3	93113.4	57493.2	1.15	0.71

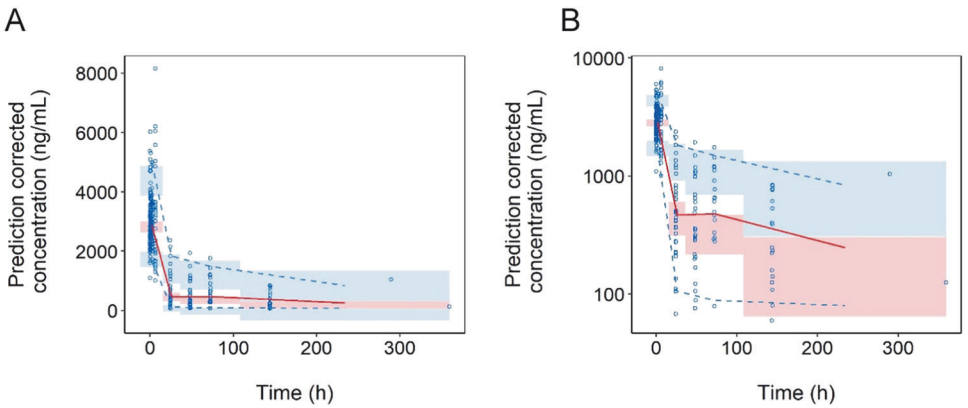

Fig. 4 PopPK final model pcVPC. Blue hollow point: measured value; Blue line: 5th and 95th quantiles of measured values; Red line: median measured value; Blue-shaded intervals: 95% prediction intervals of 5th and 95th quantiles predicted by the model; Red shaded interval: 95% prediction interval of the median predicted by the model. **A** Prediction corrected concentrations of KJ103 in the blood of healthy population (linear). **B** Prediction corrected concentrations of KJ103 in the blood of healthy population (logarithm).

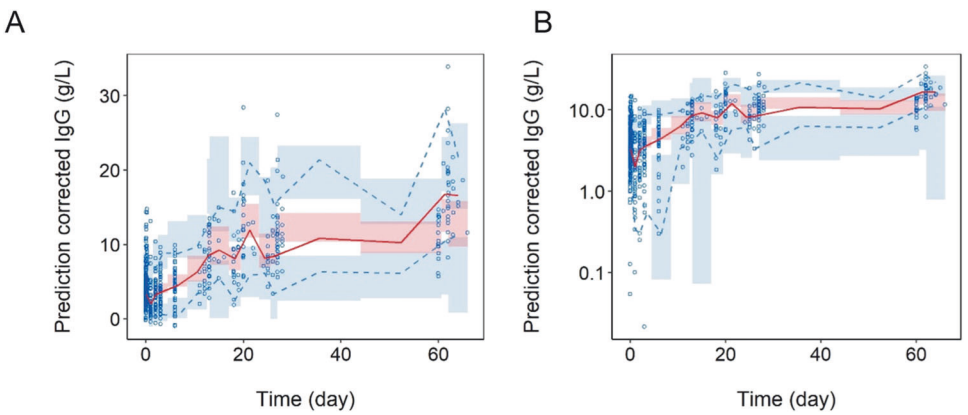

Fig. 5 PK/PD final model pcVPC. Blue hollow point: measured value; Blue line: 5th and 95th quantiles of measured values; Red line: median measured value; Blue-shaded intervals: 95% prediction intervals of 5th and 95th quantiles predicted by the model; Red shaded interval: 95% prediction interval of the median predicted by the model. **A** Prediction corrected IgG in the blood of healthy population (linear). **B** Prediction corrected IgG in the blood of healthy population (logarithm).

2 weeks and 2 months of KJ103 administration, Chinese volunteers showed a wider range of ADA titer change in the 0.25 mg/kg and 0.40 mg/kg dose groups than New Zealand volunteers. There were no significant differences in the median values and ranges of change in ADA titers among participants in the two countries in each of the dose groups prior to KJ103 administration, and at 1 week and at 6 months after KJ103 administration.

The majority of volunteers in the two studies showed ADA changes beginning on Day 14 after KJ103 administration, peaking at approximately two weeks and then gradually declining. After 6 months of KJ103 administration, 56.86% (29/51) of volunteers' ADA were back to baseline levels (Fig. 9). Although there are significant individual differences in the development of immunogenic responses, in general, immunogenic responses appear to be dose-related.

Pre-existing AAV2 NAbs reduced by KJ103

It is reported that AAV2 exposure is high in Chinese population [21]. Therefore, AAV2 NAbs titers were examined in clinical samples from China to investigate the ability of KJ103 to remove pre-existing AAV NAbs. Another research indicates that NAb titers greater than 1:100 significantly increased innate immune responses in gene therapy [22]. Using 1:100 as the threshold, the percentage of pre-existing AAV2 NAbs before KJ103 administration was 73.5% (25/34), which was similar to that reported in the literature. Ten volunteers with pre-administration titers greater than 1:100 were selected from the 0.25 mg/kg and 0.40 mg/kg groups with fully cleavage of IgG. Their AAV2 NAb levels were estimated before and after administration of KJ103. Figure 10A–C displays the titration changes within 21 days for each individual. The re-grouping was based on the differences in pre-dose titers. Following one dose of 0.25 or 0.40 mg/kg KJ103, all pre-dose titers

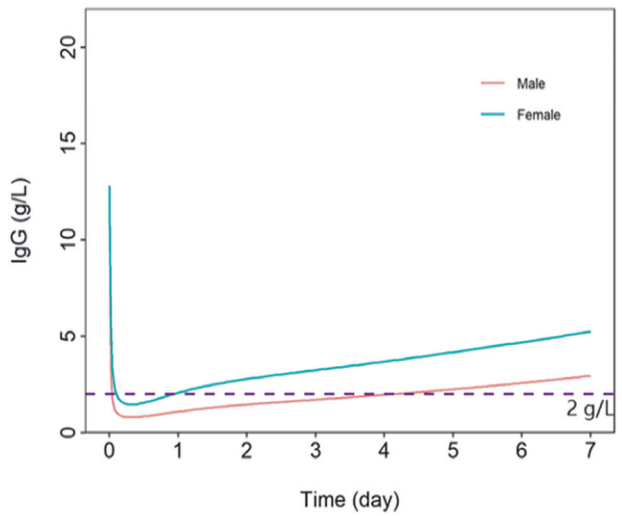

Fig. 6 Characteristics of simulated IgG changes over time after administration of different sexes. Simulation of 8.5–19.4 g/L baseline IgG range (10th-90th baseline IgG level of the included population), the change of IgG decline trough after a single dose of 0.25 mg/kg in typical male volunteers; Dots and solid lines represent predicted trough IgG values. The dashed line represents IgG=1 g/L.

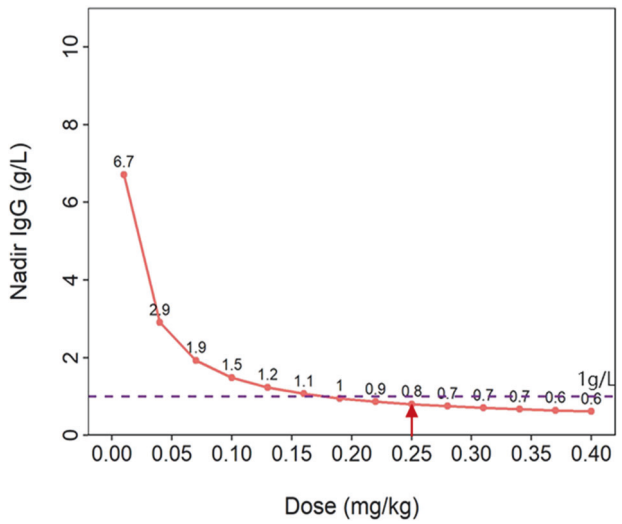

Fig. 7 Characteristics of trough IgG values after administration of simulated different doses. The change of trough value of IgG in typical volunteers after a single dose of 0.01–0.40 mg/kg was simulated, and the baseline median IgG value of the included population was 12.8 g/L. Dots and solid lines represent predicted trough IgG values. The dashed line represents IgG=1 g/L.

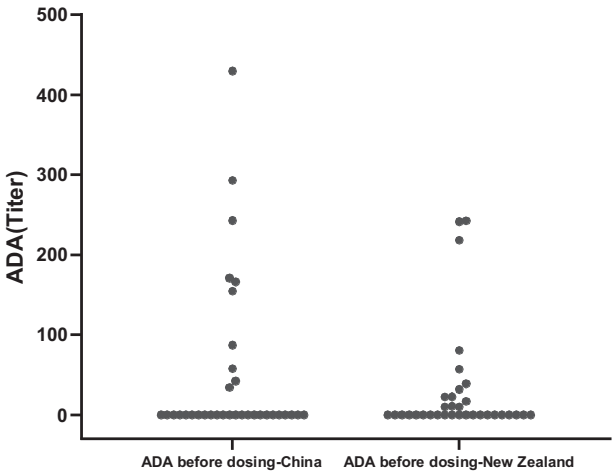

Fig. 8 ADA titers of volunteers before drug administration. Human serum samples were analyzed using a validated bridging ELISA method. Among the 34 healthy volunteers randomised in China, the pre-existing ADA positivity rate was 29.41% (10/34), and the median value of pre-existing ADA titer was 0 (range: 0 to 1:429.61). Among the 34 healthy volunteers randomised in New Zealand, the pre-existing ADA positivity rate was 38.2% (13/34), and the median value of pre-existing ADA titer was 0 (range: 0 to 1:242.58). No differences were found in pre-existing ADA positivity or titers between populations.

Table 6. Changes in ADA.

Visits	Statistics	China	New Zealand
Pre-dose	*N*	34	34
	Median	0	0
	Min,Max	0, 1:429.61	0, 1:242.58
D7	*N*	26	26
	Median	0	0
	Min,Max	0, 1:408.25	0, 1:246.78
D14	*N*	26	26
	Median	1:1041.97	1:257.50
	Min,Max	0, 1:144433.38	0, 1:20855.78
D63	*N*	24	25
	Median	1:605.89	1:158.64
	Min,Max	<1:10, 1:10989.27	<1:10, 1:7171.39
D180	*N*	25	25
	Median	1:524.77	1:214.35
	Min,Max	0, 1:4921.42	0, 1:5782.43

less than 1:1000 were reduced to below the 1:100 cutoff. Pre-dose titers ranging from 1:1000 to 1:2000 decreased close to the cutoff, whereas titers above 1:2000 did not reach it. The maximal degree of changes across individuals was similar, all achieving about 90% reduction when the lowest titers were observed at day 3 or 4. It indicates that one dose of KJ103 above 0.25 mg/kg can generally increase the threshold of NAb titer for AAV-based gene therapy by approximately 10-fold irrespective of the initial titer. For individuals with high titers, a 90% reduction still leaves the titers above the threshold. In such cases, a second dose of KJ103 may be required to further decrease the titers.

To estimate how KJ103 changed AAV2 NAbs titers and the possibility of a second dose, we then tested the changes of $F(ab')_2$ from these ten volunteers (Fig. 10D). Degradation of IgG by KJ103 resulted in rapid production of $F(ab')_2$. $F(ab')_2$ was gradually metabolized and almost cleared by day 7. During the first two days, AAV2 NAb titers declined more slowly than total IgG, probably because of neutralizing activity of newly produced and unmetabolized $F(ab')_2$. IgG levels gradually increased since day 2, whereas AAV2 NAb titers kept low from day 2 to day 4, leaving an ideal time window for gene therapy. Additionally, if the initial titer is too high, the lowest titer would still exceed the permissible threshold for gene therapy within this time window. A second dose of KJ103 could be considered

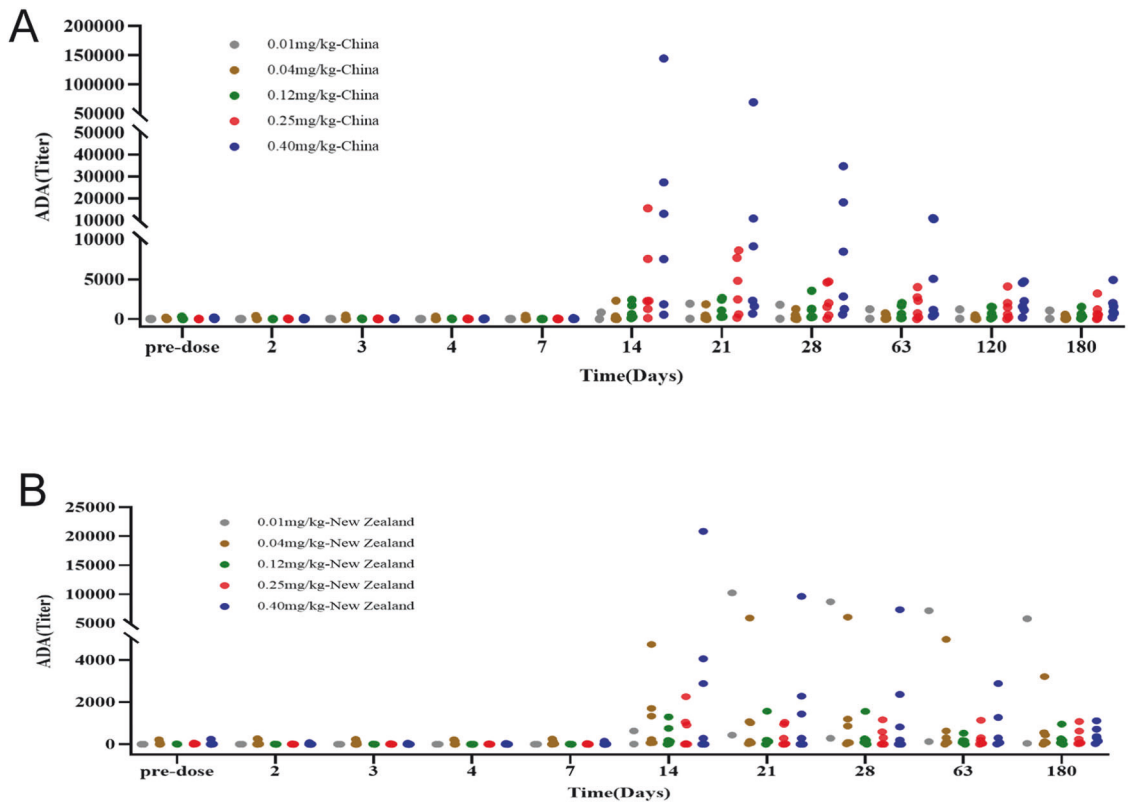

Fig. 9 Changes in ADA of volunteers. Comparison of ADA titers before and after administration of different doses of KJ103. **A** Changes in ADA titers in Chinese volunteers before and after receiving different doses of KJ103 administration (2 in 0.01 mg/kg group, 6 in other groups each). **B** Changes in ADA titers in New Zealand volunteers before and after receiving different doses of KJ103 administration (2 in 0.01 mg/kg group, 6 in other groups each).

to degrade remaining IgG and suppress IgG recovery. The strategy could help stabilize or extend the time window, as well as further expand the population eligible for gene therapy.

DISCUSSION

The most commonly used viral vectors for in vivo gene therapy are based on AAV. However, NAbs against AAV vectors are prevalent in humans, impeding transduction and posing a major limitation to AAV-based gene therapy [23]. Furthermore, for individuals eligible for gene therapy, the potential for complement activation continues to be a major safety concern. In vitro experiments prove that high-dose i.v. AAV infusion or high exposure to AAV empty capsids leads to antibody-dependent activation of the complement system in human plasma [24]. Evidences from ongoing clinical trials also suggest that high doses of AAV significantly increase complement activation. Some participants in these studies developed severe and life-threatening inflammatory responses that were likely secondary to the activation of the complement system [25–29].

IgG-degrading enzyme demonstrates clinical potential in the field of gene therapy for reducing NAb titers and mitigating complement toxicity simultaneously. It was confirmed that IgG-degrading enzyme reduced AAV NAb levels from human plasma samples in vitro, including plasma from prospective gene therapy trial participants [30]. These results provide a potential solution to overcome NAbs against AAV-mediated gene therapy and inhibit antibody-dependent activation of the complement system. Apart from plasma IgG, it inhibits the B cell Receptor (BCR) mediated cell signal by degrading IgG of BCR, transiently preventing memory B cell response to antigenic stimulation and their transition into antibody-producing cells [31]. This may help to create a time window long enough for safer and more efficient gene therapy administration.

Although IgG-degrading enzymes have therapeutically applied in many fields, the immunogenicity of biologics cannot be ignored. Pre-existing ADAs may increase the risk of infusion reactions and hypersensitivity reactions during i.v. delivery of biologics [32, 33]. The widespread presence of pre-existing anti-IdeS antibodies limits clinical applications of IdeS, and can potentially compromise drug efficacy [13]. During clinical studies, individuals with anti-IdeS IgG titers exceeding 15 mg/L were excluded from the research, and therefore not all populations can receive IdeS treatment [13]. These shortcomings limit the application of IdeS in i.v. administration of AAV-based gene therapy.

Here, we report that KJ103, an IdeE variant with IgG-cleaving activity similar to IdeS, exhibits a low prevalence rate and low titers of pre-existing ADAs in the population. In clinical trials of IdeS, anti-IdeS antibodies were present in all participants at baseline, and there was an observed rise in ADAs from day 7 of the dosing regimen. ADA levels then peaked at approximately 19.6 times baseline at about two weeks, and then declined progressively, reaching approximately 16.6 times baseline ADA concentrations after two months of dosing [10]. In comparison, the pre-existing anti-KJ103 antibodies positivity rate for all enrolled participants was 33.82% (23/68), and the median value of pre-existing antibodies titers was 0 (range: 0 to 1:429.61). ADAs appeared and peaked at approximately two weeks after KJ103 administration, with a median ADA titer in positive participants that was 10.8 times that of the baseline positive participants, and then declined progressively, reaching a titer of approximately 4.7 times that of the baseline in positive participants after two months of dosing. The maximum dose of IdeS applied in humans was

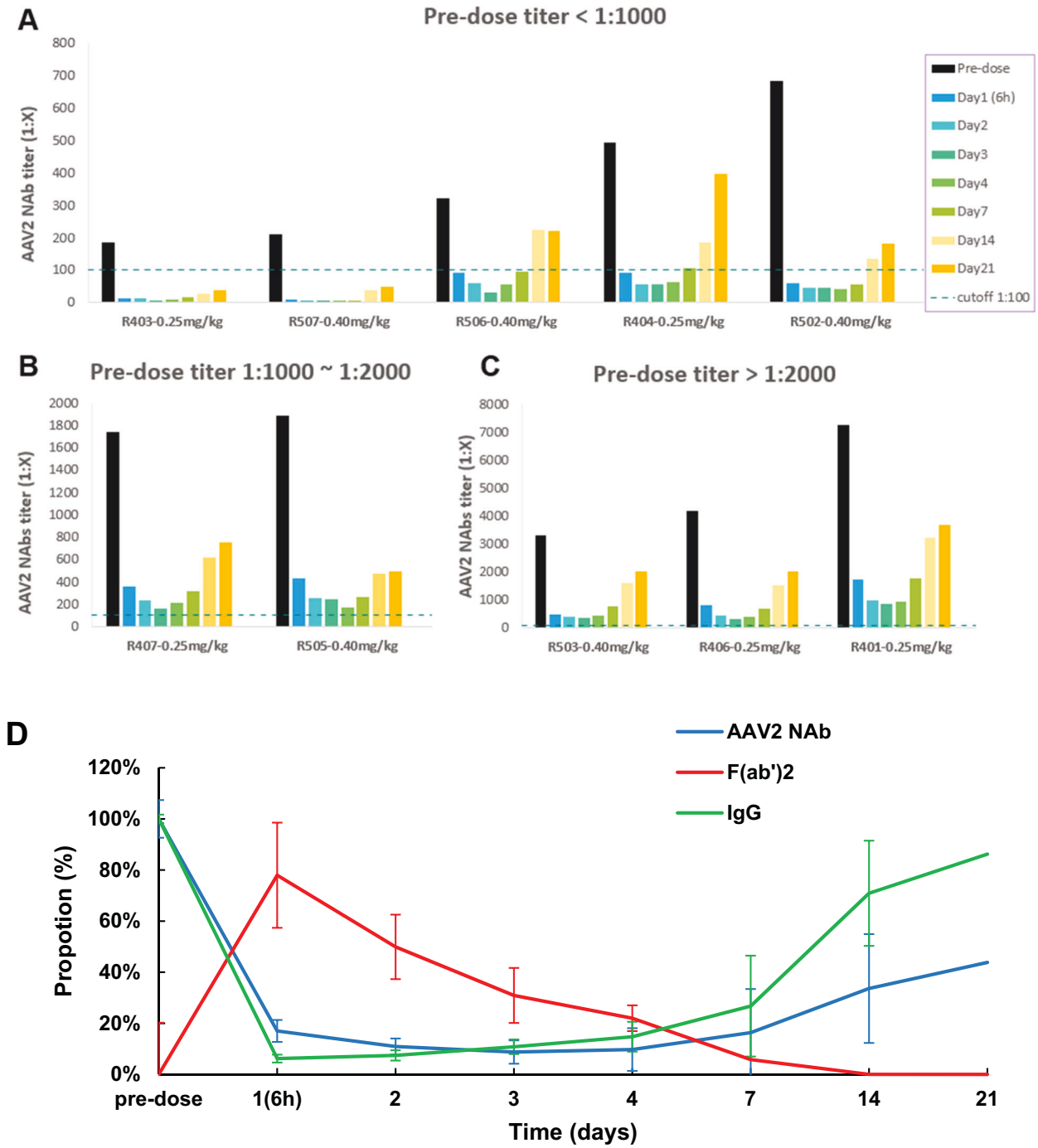

Fig. 10 KJ103 reduced pre-existing AAV2 NAb activity. The titers of AAV2 Nab before and after administration were individually shown, grouped by their pre-dose titer as <1:1000 (**A**), 1:1000 ~ 1:2000 (**B**) and >1:2000 (**C**). The naming convention "R+number" represents the codes for different volunteers, with the corresponding dose indicated by the suffix. **D** The temporal variation of NAb, $F(ab')_2$ and IgG levels in the selected volunteers. Details regarding the selection and grouping referred to in the main text.

0.25 mg/kg, whereas the maximum dose of KJ103 first applied in humans was 0.40 mg/kg dose, with an excellent safety and tolerability profile across all dose groups of KJ103. ADA emergence post-KJ103 administration occurred later than for IdeS, with lower titers and a shorter duration to return to baseline levels, highlighting the advantage of KJ103.

We observed no events meeting the dose-escalation termination criteria during the DLT observation period in all participants. Most TEAEs and TRAEs were graded as grade 1 or 2, with a few at grade 3. Safety profiles of TRAEs for KJ103 were overall comparable to IdeS [34]. No severe infection events occurred during either study.

Post-administration, KJ103 exhibited a dose-dependent reduction in IgG levels in volunteers. We observed a greater reduction in IgG after administration of KJ103 at a dose of 0.12 mg/kg to Asian participants in both studies. The average IgG levels for volunteers in China and New Zealand were 11.67 g/L and 14.63 g/L, respectively, while the average levels of pre-existing anti-KJ103 antibodies were not significantly different. Our PK/PD models indicated that gender influences efficacy. A meta-analysis

involving 28 studies indicated that factors such as being Caucasian, smoking, or using corticosteroids tend to decrease IgG levels, whereas the use of probiotics, hypertension, or acute psychological stress tends to elevate IgG levels [35]. Overall, differences in pharmacodynamics between the 0.12 mg/kg group in the two trials were caused by differences in baseline IgG levels. We infer that these differences may be due to gender disparities, but we do not rule out racial differences. Additionally, utilizing the PopPK model, we simulated various gender and baseline IgG levels and found their impact on efficacy to be negligible at 0.25 mg/kg dose.

Both trials demonstrate exceptional safety, tolerability and IgG cleavage efficiency of KJ103. The 0.25 mg/kg dose of KJ103 efficiently, rapidly, and specifically enzymatically cleaved human IgG and maintained a low serum IgG level for one week. The promising safety and tolerability of KJ103 was indicated by low positive rates and titers of pre-existing ADA in the population, and rapid return to baseline ADA levels within six months in about half of participants post-administration. The trials show that KJ103 avoids IdeS shortcomings, with a higher safe dose, wide safety window, low pre-existing antibody ratio and titer, and a broader application population of fields such as organ transplantation and AAV-based gene therapy [36]. In particular, the characteristics allow for short-interval repeated administration without issues of safety and loss of efficacy due to ADA.

Taken AAV2 as a representative, we have conducted tests and confirmed that a single dose of KJ103 can reduce AAV2 NAbs by approximately 90% unbiased of pre-dose NAb titers. To our knowledge, it is the first report of rescue strategy that can elevate the enrollment threshold of AAV-based gene therapy by at least one order of magnitude. For individuals with particularly high levels of NAbs, further reduction is required. Due to the low prevalence and low titers of pre-existing anti-KJ103 antibodies in the population, it is possible to administer KJ103 twice in humans at short intervals without significant safety concerns. Extra dose of KJ103 could potentially delay recovery of IgG levels in the body, allow sufficient time for the clearance of degraded $F(ab')_2$ fragments and maintain low IgG levels. According to the changes in NAbs and $F(ab')_2$ depicted in Fig. 10, the appropriate window for second administration ranges from 3 to 7 days.

Based on the above results and mechanisms, we believed that KJ103 could rescue treatment ineligibility due to AAV NAbs and prevent complement-mediated immune reactions leading to treatment failure in AAV-based gene therapy.

DATA AVAILABILITY

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AUTHOR CONTRIBUTIONS

MC, RK, ZW analyzed the data and wrote the article. XL, QG, CC, ZZ, YY, KL, MM, YL substantially implemented the survey and consolidated the data. All authors critically revised the article and approved the final manuscript.

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COMPETING INTERESTS

ZW, ZZ, YL are employees of Shanghai Bao Pharmaceuticals Co., Ltd, and own stock/other equities. Other authors declare no competing interests.

ETHICAL APPROVAL

Both phase 1 clinical trials of KJ103 were approved separately by the ethics committee of the "New Zealand Clinical Research" (Protocol no. SHBJ-2021-001) and "Suzhou Municipal Hospital" (Protocol no. SHBJ-2021-002). All subjects signed written informed consent before undergoing any study-specific procedures.

ADDITIONAL INFORMATION

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