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ORIGINAL ARTICLE

Low-dose immune tolerance induction alone or with immunosuppressants according to prognostic risk factors in Chinese children with hemophilia A inhibitors

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Abstract

Background: In developing countries, children with hemophilia A (HA) with high-titer inhibitor and poor immune tolerance induction (ITI) prognostic risk(s) cannot afford the recommended high- or intermediate-dose ITI.

Objectives: To determine the efficacy of low-dose ITI (plasma-derived factor VIII [FVIII]/von Willebrand factor at 50 FVIII IU/kg every other day) by itself (ITI-alone) or combined with immunosuppressants rituximab and prednisone (ITI-IS) in children with HA with high-titer inhibitor.

Methods: All enrolled patients had pre-ITI inhibitor ≥10 BU. We used ITI-alone if inhibitor titer was <40 BU pre-ITI and during ITI, and ITI-IS if titer was ≥100 BU (historic) or ≥40 BU (pre- or during ITI) or if the patient was nonresponsive on ITI-alone.

Results: Fifty-six children were analyzable, with median historic peak inhibitor titer 48.0 BU and followed for median 31.4 months. Overall, 35 (62.5%) achieved phase 2 success with negative inhibitor and normal FVIII recovery. The phase 2 success rate was 95% for the 20 patients receiving ITI-alone. For the 36 patients receiving ITI-IS, the phase 2 success rate was 44.4%, but would increase to 63.6% if the 14 patients with historic peak inhibitor titer ≥100 BU (and having phase 2 success rate of only 14.3%) were excluded. One patient developed repeated infection after IS treatment.

Relapse occurred in 11.4% (4/35) patients with phase 2 success associated with rapid ITI dose reduction or irregular post-ITI FVIII prophylaxis. Our strategy reduced the cost from high-dose ITI by 74% to 90%.

Conclusion: The use of low-dose ITI with or without immunosuppressants according to ITI prognostic risk(s) is a clinically and economically feasible strategy for eradicating inhibitors in children with HA, particularly for those with historic peak inhibitor titer <100 BU.

KEYWORDS
hemophilia A, high-titer inhibitor, immune tolerance induction, low-dose, rituximab

Essentials

- High-titer inhibitor with poor risk tends to be treated with high- or intermediate-dose immune tolerance induction (ITI).
- Cost is a limiting factor, allowing only low-dose ITI, which shows poor efficacy in poor-risk patients.
- Low-dose ITI-alone or with the immunosuppressant strategy we reported gave a >60% normal factor VIII recovery rate.
- This strategy reducing cost by 74% to 90% is feasible in economic constraint areas in the world.

1 | INTRODUCTION

Neutralizing antibodies (inhibitors) against factor VIII (FVIII) develop in approximately 30% of previously untreated patients with severe hemophilia A (SHA) exposed to FVIII, with the highest-risk period within the first 20 exposure days.[1] About 20% of children with SHA will have a high-titer inhibitor and are in need of eradication treatment.[2,3] Inhibitors render FVIII replacement therapy ineffective, increasing the risk of morbidity and mortality. Immune tolerance induction (ITI) with repeated administration of FVIII is recommended as the only method for inhibitor eradication and would achieve 60% to 80% overall success rates.[4,5]

Not all ITI regimens are equally efficacious in all patients with inhibitors. Although the International ITI (I-ITI) study showed that high-dose and low-dose ITI regimens had similar tolerization rates in patients with high-titer inhibitors, those on the low-dose regimen took longer for tolerization and had a higher bleeding rate.[6] Their studied patients all had good ITI prognostic risk as ITI was not started until the inhibitor titer had fallen to <10 BU.[6] Clinicians tend to use high- and intermediate-dose ITI regimen for patients with high-titer inhibitor with poor ITI prognostic risk(s),[7-9] and use low-dose ITI regimens for patients with low-titer inhibitor without poor ITI prognostic risk.[10,11] Indeed, in patients with high-titer inhibitors and poor ITI prognostic risk(s), the success rate of low-dose ITI was only 26.3% to 33.0%.[12,13] Some studies, however, showed von Willebrand factor (VWF)-containing plasma-derived FVIII (pdFVIII/VWF) concentrates (instead of recombinant FVIII [rFVIII] products) would improve the ITI success rate.[7,14,15] This observation was also supported by an animal study suggesting that VWF attenuates FVIII memory immune response in HA mice.[16] Addition of immunosuppressant (IS) agents to ITI regimens has also been shown to improve the inhibitor eradication efficiency in 50% to 75% of patients who failed ITI previously.[17,18]

As one of the developing countries with economic constraints, high- and intermediate-dose ITI are unaffordable in China. We developed a low-dose ITI strategy using pdFVIII/VWF (at lower cost than that of recombinant products) for children with high-titer inhibitor, adding IS for those patients with additional predefined poor ITI-prognostic risk(s) (see Section 2.2). In a pilot study, we saw promising results of low-dose ITI.[18] The aim of this study was to demonstrate effectiveness of low-dose ITI in a large prospective cohort of patients.

2 | MATERIAL AND METHODS

2.1 | Study design and participants

This single-center prospective cohort study (ClinicalTrials.gov: NCT03598725) was conducted at the Beijing Children's Hospital (BCH) Hemophilia Comprehensive Care Center in China. This study was approved by the ethics review board of BCH. Informed consent was obtained from one parent or a legal guardian of each enrolled child.

A total of 74 participants meeting the eligibility criteria were enrolled consecutively. The eligibility criteria included boys under 14 years of age with severe or moderate HA (FVIII < 0.05 IU/mL), and inhibitor titer ≥10 BU at the start of ITI. Exclusion criteria included prior inhibitor eradication attempts, use of IS to treat other disease(s), or failure to provide informed consent.

Our center developed the treatment regimen and performed the regular follow-up as well as inhibitor testing/monitoring. The children carried out the treatment in the local medical units or by home

infusion. Enrollment was between September 2016 and August 2019. Data analysis was performed in February 2021.

2.2 | Treatment regimens

All patients received domestic intermediate-purity pdFVIII/VWF products from various Chinese manufacturers as available at local hospitals at 50 FVIII IU/kg every other day (low-dose ITI-alone regimen). None of the patients required a central venous catheter. IS (rituximab and prednisone) was added (low-dose ITI-IS regimen) in patients with additional predefined poor ITI prognostic risk(s) as follows: (i) ITI-IS was used up front, in patients with historic peak inhibitor titer ≥100 BU[19] and/or inhibitor titer ≥40 BU at ITI initiation[20]; and (ii) patients on ITI alone were switched to ITI-IS if the inhibitor titer during ITI increased to ≥40 BU[20] or if the inhibitor decline during ITI was <20% over the first 3 months after initial peak inhibitor titer during ITI.

Rituximab dosage was 375 mg/m^2 (maximum 600 mg) weekly for 4 weeks. Prednisone dosage was 2 mg/kg (maximum 60 mg) daily for 1 month, then tapered over 3 months. Intravenous immunoglobulin (IVIG) replacement therapy (200 mg/kg monthly for 6 months) was given to patients receiving rituximab for infection prophylaxis. Once the patient had achieved negative inhibitor plus normal FVIII recovery (phase 2 success, per definition in section 2.3), the FVIII dose would be reduced slowly to ≤30 IU/kg two to three times a week for continuing prophylaxis.

Records of bleeding episodes were collected from the patient's bleed diary at each clinical visit. Breakthrough bleedings during ITI when the inhibitor titer was ≥2 BU were treated with domestically manufactured nonactivated prothrombin complex concentrate (PCC) at 50 IU/kg every 8 to 12 hours or recombinant activated factor VII (rFVIIa) at 90 μg/kg every 4 to 6 hours.[21] Nondomestic PCC and activated PCC are not licensed in China. pdFVIII/VWF (50 IU FVIII/kg) was used effectively when the inhibitor titer had lowered to <2 BU. PCC prophylaxis (40–50 IU/kg every other day) was used for inhibitor patients who ever had episode(s) of life-threatening bleeding.

2.3 | Definitions of ITI outcomes

1. Success (based on response phases, as adapted from the international ITI study)[6]:
   a. Phase 1 success: achieving inhibitor elimination (to inhibitor titer <0.6 BU at two consecutive visits >1 week apart).
   b. Phase 2 success: achieving (a) plus normal FVIII recovery (≥66% of expected) >1 month after achieving phase 1 success.
   c. Phase 3 success: achieving (b) plus normal FVIII half-life (≥6 hours) >1 month after achieving phase 2 success, that is, tolerization.
2. Failure
   a. Patients on any ITI regimen not achieving phase 1 success at the time of data analysis.
   b. In patients taking ITI-IS, if at 6 months after starting rituximab, the inhibitor titer had not declined by at least 20% or if the absolute inhibitor titer remained ≥100 BU.
3. Relapse: recurrence of inhibitor titer ≥1.0 BU after achieving any of the success phases.

2.4 | Coagulation assay

FVIII clotting activity was determined using a one-stage clotting assay. The titer of the inhibitor was measured using Nijmegen modification Bethesda titer assay.[22] During ITI, inhibitor titer was initially monitored every 1 to 2 weeks until a downward trend was evident after the initial peak from early repeated FVIII exposure, then monthly until normal recovery was achieved, and thereafter every 3 monthly.

FVIII recovery was estimated after injection of a single dose of pdFVIII/VWF (50 FVIII IU/kg) given after a 48 to 72 hours washout period. The FVIII half-life was calculated based on the method described by Bjorkman et al[23] infusing pdFVIII/VWF (50 FVIII IU/kg) after a 72 hours washout period, followed by recording FVIII coagulation activity at 15 to 30 minutes and 1, 9, 24, and 48 hours after infusion.

All clotting factor and inhibitor assays including those for recovery and half-life studies were performed on samples obtained at the study center. We did not use samples transported from local centers because of specimen quality concerns.

2.5 | Statistics

Categorical variables, expressed as frequencies and percentage values, were compared by chi-square or Fisher's exact test. Continuous variables, expressed as median values and ranges, were compared by the Student's *t* test (for normal distribution) or the Mann-Whitney *U* test (for nonnormal distribution). Kaplan-Meier curves were compared with log-rank test. The reported *P* value are two-sided and value <.05 were considered to be statistically significant. All statistical analyses were performed using SPSS, version 22.0 (IBM Corp., Armonk, NY, USA).

3 | RESULTS

3.1 | Characteristics of patients

A total of 74 patients were screened for enrollment eligibility. Of these, a total of 18 were excluded/withdrawn (7 declined ITI and 11 unable to follow the ITI protocol or were lost to follow-up during ITI) causing an exclusion/dropout rate of 24.3% (Figure 1). Data from 56 patients who completed the study and followed for a median 31.4 (range, 18.6-53.3) months were analyzable. Their median age at ITI initiation was 4.0 (range, 0.8-13.2) years, and their median historic

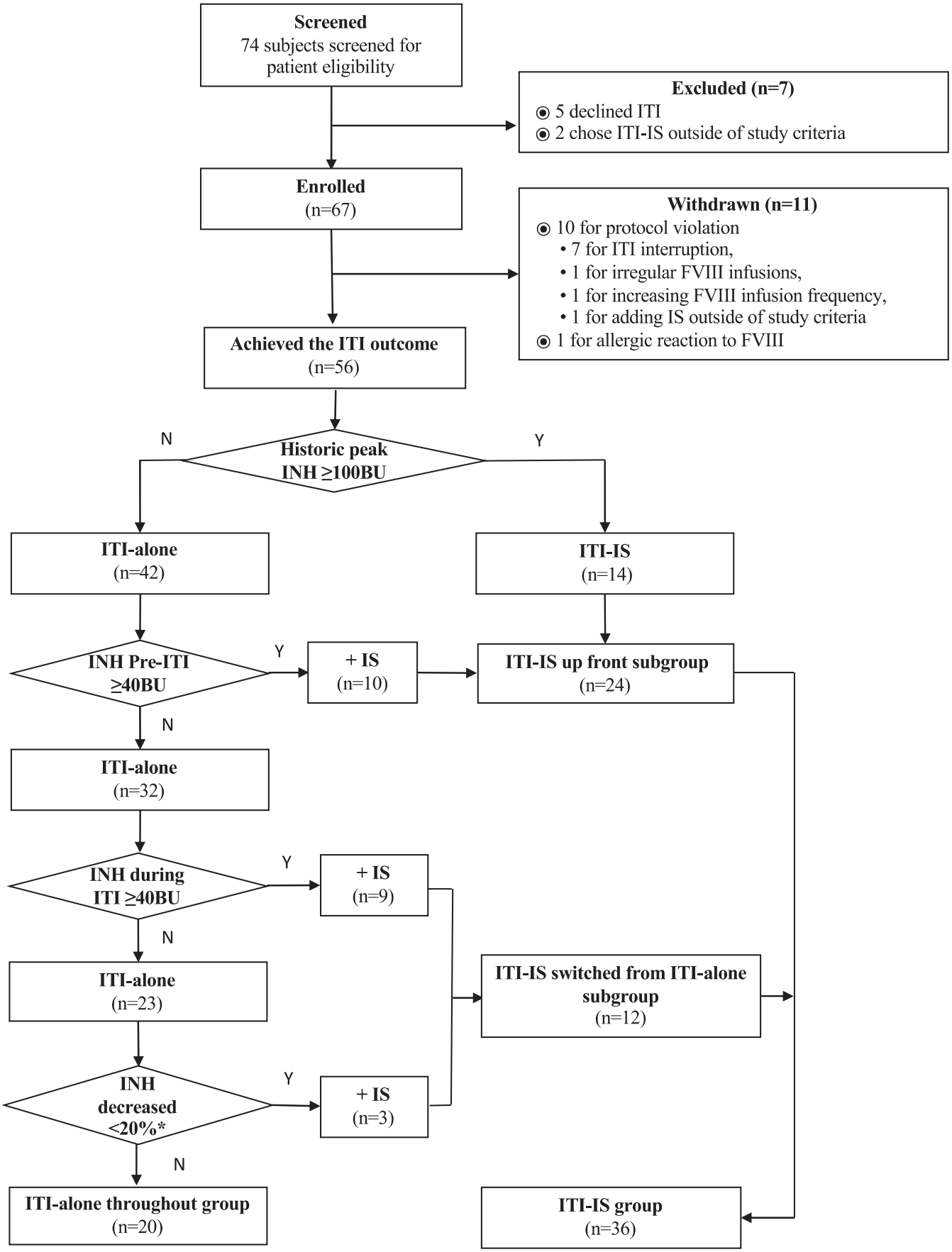

FIGURE 1 Flowchart of patients enrolled in the study. *INH decreased <20% over the first 3 months after initial peak inhibitor titer during ITI. INH, inhibitor; ITI, immune tolerance induction; IS, immunosuppressants

peak inhibitors titer was 48.0 (range, 10.1-416.0) BU. *F8* mutations are available in 53 patients, being null mutation (intron 22 or 1 inversions, large deletions, frameshift, nonsense, conserved splicing site mutation)[24] in 48 (90.5%) patients, nonnull mutations (missense, nonconserved splicing mutations)[24] in 3 (5.7%) patients, and not detectable in 2 (3.8%) patients (Table 1).

3.2 | ITI outcome

3.2.1 | Overall cohort

At the analysis time point, 38 of 56 (67.9%) patients achieved phase 1 success in median 9.4 (range, 2.1-25.1) months, 35 (62.5%) achieved phase 2 success in median 11.5 (range, 3.5-29.9) months (Table 2).

Of the 35 patients who achieved phase 2 success (19 on ITI alone, 16 on ITI-IS), 24 declined FVIII half-life testing, which required 6 blood samples over 48 hours. Of the 11 (6 on ITI alone, 5 on ITI-IS) tested, all had normal FVIII half-life (median, 7.8 hours) in median 16.1 (range, 6.2-40.2) months. This gave an "apparent" total phase 3 success rate of only 19.6% (11/56). However, we anticipate that the "real" overall phase 3 success rate to be rather higher had the remaining 24 patients in phase 2 success also had a FVIII half-life study done.

3.2.2 | "ITI-alone" throughout group

Twenty patients received "ITI-alone" throughout. Nineteen (95%) achieved phase 1 success at median 6.9 (range, 2.7-24.3) months, and all 20 achieved phase 2 success at median 9.4 (range, 4.1-25.8) months. Six patients had FVIII half-life performed, and all achieved phase 3 success (Table 2).

3.2.3 | Patients receiving low-dose "ITI-IS" (ITI-IS group)

Thirty-six of 56 (64.3%) patients received an ITI-IS regimen (either up front or switched from ITI-alone), 16 of 36 (44.4%) achieved phase 2 success in median 13.6 (range, 3.5-29.9) months.

ITI-IS up front subgroup

Twenty-four of 36 (66.7%) patients received ITI-IS up front either for having historic peak inhibitors ≥100 BU (n = 14), or for pre-ITI inhibitor titer ≥40 BU (n = 10). Of these 24 patients, 11 (45.8%) achieved phase 1 success in median 10.0 (range, 2.1-11.0) months and 8 (33.3%) achieved phase 2 success in median 13.6 (range, 3.5-13.6) months. Three phase 2 success patients had FVIII half-life performed and all achieved phase 3 success.

ITI-IS switched from ITI-alone subgroup

Twelve of 36 (33.3%) patients were switched from ITI-alone to ITI-IS during ITI, 9 (75%) for having a peak inhibitor titer ≥40 BU during ITI and in 3 (25%) because the inhibitor titer failed to decline by >20% over the first 3 months after initial peak inhibitor titer during ITI (Figure 1). Eight (67%) patients achieved phase 1 success at median 9.7 (range, 5.1-25.1) months, all also achieved phase 2 success in median 11.9 (range, 6.9-29.9) months (Table 2).

Influence of high historic inhibitor titer (≥100 BU) on ITI success rate

The success rate of the 14 patients with a historic peak inhibitor titer ≥100 BU (all treated with ITI-IS) was very low; only 4 (28.6%) achieved phase 1 success, and 2 (14.3%) achieved phase 2 success. If these 14 patients were removed from the ITI-IS treatment group, the phase 2 success rates would be increased from 62.5% (35/56) to 78.6% (33/42) for the entire study cohort, from 44.4% (16/36) to 63.6% (14/22) for the ITI-IS group, and from 33.3% (8/24) to 60% (6/10) for the ITI-IS up front patient subgroup.

3.2.4 | Outcome comparison between ITI treatment groups

The three treatment groups (ITI-alone throughout, ITI-IS up front, ITI-IS switched groups) had significant different rates ($P < .001$) and time ($P = .03$) to phase 2 success (Table 2). Patients receiving ITI-IS up front took a longer time to phase 2 success than those receiving ITI alone throughout ($P = .02$) and those switched to ITI-IS midcourse during ITI ($P = .09$) (Figure 2).

3.3 | Treated-breakthrough bleedings and adverse events

A total of 206 treated breakthrough bleeding episodes were recorded in 48 of 56 (85.7%) patients during ITI. The median treated bleeding rate in time per month was 0.33 (range, 0-1.86) during ITI compared to 0.67 (range, 0-5.33) before ITI, representing a significant reduction of 72.9% ($P = .002$). Among the 18 patients who failed ITI, the median treated bleeding rate during ITI was higher, at 0.53 (range, 0.09-1.86) time/month, not significantly different from the median 0.71 (range, 0.08-2.08) time/month before ITI.

Rituximab infusion-related side effects like rash and nausea, which could be resolved and subsequently prevented by antihistamine drugs, were reported in 10 of 36 (27.8%) patients in the ITI-IS group. Only a 1-year-old patient developed severe infection manifested as continuous cough, fever, and diarrhea from the third day to the eighth week following the first dose of rituximab, requiring treatment with cephalosporin antibiotics.

3.4 | Relapse

Overall, 7 of the 38 patients (18.4%) who had achieved at least phase 1 success relapsed. These included all 3 who had phase 1 success

TABLE 1 Demographics and clinical characteristics of the 56 evaluable children with hemophilia A with high-titer inhibitors treated with low-dose ITI-alone throughout or ITI-IS regimens

Group	All patients	ITI-alone throughout group	ITI-IS group	
			ITI-IS up front subgroup	ITI-IS switched from ITI-alone subgroup
N (%)	56 (100.0)	20 (35.7)	24 (42.9)	12 (21.4)
Hemophilia A severity, n (%)				
Severe	51 (91.1)	19 (95.0)	22 (91.7)	10 (83.3)
Moderate	5 (8.9)	1 (5.0)	2 (8.3)	2 (16.7)
Number of patients tested *F8* mutations, n (%)	53	20	23	10
Null mutation*	48 (90.5)	16 (80.0)	23 (100)	9 (90.0)
Nonnull mutation**	3 (5.7)	2 (10.0)	0	1 (10.0)
No mutation detectable	2 (3.8)	2 (10.0)	0	0
Estimated exposure days at inhibitor diagnosis, median (range, IQR)	28.0 (5.0-200.0, 15.0-50.0)	30.0 (10.0-117.0, 18.0-50.0)	22.5 (5.0-200.0, 11.8–46.0)	24.5 (8.0-200.0, 16.5-53.8)
Age at inhibitor diagnosis, yr, median (range, IQR)	2.5 (0.5-11.0, 1.3-5.3)	2.9 (0.6-7.9, 1.7-5.4)	1.9 (0.5-11.0, 1.1-5.3)	2.7 (1.2-9.1, 2.1-5.3)
Age at ITI initiation, yr, median (range, IQR)	4.0 (0.8-13.2, 2.5–6.7)	3.8 (0.8-13.2, 2.4-7.2)	4.6 (0.8-12.1, 1.9-6.7)	3.7 (2.2-11.9, 2.7-7.5)
Time interval between inhibitor diagnosis and ITI initiation, mo, median (range, IQR)	11.6 (0-75.0, 1.0-29.5)	6.5 (0-75.0, 0.4-30.3)	14.0 (0-56.0, 1.3-31.8)	5.0 (0-61.0, 2.6-20.8)
Historic peak inhibitor, BU, median (range, IQR)	48.0 (10.1-416.0, 23.1-98.4)	23.8 (10.1-75.0, 17.2-37.3)	101.3 (47.4-416.0, 71.5-208.0)	29.7 (15.7-64.0, 21.5-37.8)
Pre-ITI inhibitor titer, BU, median (range, IQR)	30.1 (10.1-416.0, 16.8-63.5)	16.0 (10.1-33.8, 10.9-23.2)	73.1 (25.3-416.0, 48.2-193.0)	21.6 (10.3-35.8, 16.2-31.9)
Peak inhibitor during ITI, BU, median (range, IQR)	49.3 (6.0-665.0, 15.2-126.3)	10.9 (6.0-38.1, 8.2-17.2)	125.8 (18.4-665.0, 76.8-258.6)	60.8 (27.8-275.2, 39.7-109.0)
Monthly bleeding rate				
Pre-ITI, median (range, IQR)	0.67 (0-5.33, 0.42-1.42)	0.67 (0.25-5.33, 0.44-1.46)	0.75 (0.10-2.50, 0.46-1.00)	0.88 (0-5.00, 0.21-2.88)
During ITI, median (range, IQR)	0.33 (0-1.86, 0.13-0.52)	0.32 (0-1.50, 0.08-0.43)	0.48 (0-1.86, 0.22-0.75)	0.21 (0-0.66, 0.08-0.47)

Note: p-value: comparison between ITI-alone vs ITI-IS.

Abbreviations: BU, Bethesda Unit; IQR, Inter-Quartile Range; IS, immunosuppressants; ITI, immune tolerance induction.

*Intron 22 or 1 inversions, large deletions, frameshift, nonsense, and conserved splicing mutations.; **Missense, nonconserved splicing mutations.

only, and 4 of 35 (11.4%) who achieved at least phase 2 success (including 2 of the 11 [18.2%] in phase 3 success).

3.4.1 | For the patients who achieved only phase 1 success

Three children in the "ITI-IS up front" subgroup relapsed, with their inhibitor titer increasing to 1.0 to 2.0 BU. One relapsed at 11 months into the post-ITI FVIII prophylaxis phase while infusing FVIII irregularly. He reestablished phase 1 success upon strict adherence to the FVIII prophylaxis regimen. The other two relapsed 6 months after completing ITI-IS. Each was given one additional rituximab dose (375 mg/m^2). One achieved phase 2 success again, while the other continued to have low-titer inhibitor over 10.4 months at the time of data analysis.

3.4.2 | For the patients achieving at least phase 2 success

Following at least phase 2 success, while in the FVIII prophylaxis phase, relapse occurred in 3 of the 19 children in the ITI-alone group (respectively, at 2.3, 4.2, and 7.9 months after phase 2 success) and 1 of 16 in the ITI-IS group (at 36.6 months after phase 2 success). These 4 relapses included 2 of the 6 children already in phase 3 success following treatment with ITI-alone. One of the four relapses were attributed to taking FVIII irregularly, two when FVIII dose was

TABLE 2 Outcomes using the low-dose ITI with or without IS strategy according ITI-prognostic risk factors

			ITI-IS ($N = 36$)					
				(b1) ITI-IS up front subgroup ($N = 24$)		(b2) ITI-IS switched from ITI-alone subgroup ($N = 12$)		
Group	All patients ($N = 56$)	(a) ITI-alone throughout ($N = 20$)	(b) All ITI-IS patients ($N = 36$)	Historic peak inhibitor ≥100 BU (n = 14)	Inhibitor at ITI initiation ≥40 BU (n = 10)	Poor response to ITI-alone regimen (n = 3)	Peak inhibitor during ITI ≥40 BU ($n = 9$)	*p*-value [between groups (a) and (b)]
Achieving negative inhibitor (phase 1 success), n (% of group total)	38 (67.9)	19 (95.0)	19 (52.8)	4 (28.6)	7 (70.0)	2 (66.7)	6 (66.7)	0.002
Time to negative inhibitor, mo, median (range)***	9.4 (2.1-25.1)	6.9 (2.7-24.3)	10.0 (2.1-25.1)	-	9.9 (2.1-11.0)	9.7 (6.7-9.7)	9.3 (5.1-25.1)	0.104
Relapse in phase 1 success, n (%)	7 (18.4)	3 (15.8)	4 (21.1)	2 (50.0)	1 (14.3)	0	1 (16.7)	0.655
Achieving FVIII recovery ≥66% (phase 2 success), n (% of group total)	35 (62.5)	19 (95.0)	16 (44.4)	2 (14.3)	6 (60.0)	2 (66.7)	6 (66.7)	<0.001
Time to FVIII recovery ≥66%, mo, median (range)***	11.5 (3.5-29.9)	9.4 (4.1-25.8)	13.6 (3.5-29.9)	-	11.5 (3.5-13.6)	11.3 (8.7-11.3)	12.3 (6.9-29.9)	0.029
FVIII recovery (% of expected), median (range)	78.8 (54.2-112.6)	82.0 (58.2-110.5)	74.0 (54.2-112.6)	73.0 (54.2-93.1)	77.5 (62.3-112.6)	87.0 (75.0-98.9)	71.2 (68.3-102.4)	0.513
Relapse in phase 2 success, n (%)	4 (11.4)	3 (15.8)	1 (6.3)	0	0	0	1 (16.7)	0.497
FVIII half-life ≥6 h (phase 3 success**), n (% of group total)	11 (19.6*)	6 (30.0*)	5 (13.9*)	1 (7.1*)	2 (20.0*)	1 (33.3*)	1 (11.1*)	-
Time to FVIII half-life ≥6 h, mo, median (range)***	16.1 (6.2-40.2)	17.0 (6.4-40.2)	11.1 (6.2-27.9)	11.1	6.2 (6.2-7.9)	27.8	16.1	-
FVIII half-life, h, median (range)	7.8 (6.3-15.5)	9.9 (6.5-15.5)	7.8 (6.3-11.0)	7.0	7.0 (6.3-7.8)	11.0	7.8	-
Relapse in phase 3 success, n (%)	2 (18.2)	2 (33.3)	0	0	0	0	0	-
Failure, n (%)	18 (32.1)	1 (5.0)	17 (47.2)	10 (71.4)	3 (30.0)	1 (33.3)	3 (33.3)	-

Abbreviations: FVIII, factor VIII; IQR, Inter-Quartile Range; IS, immunosuppressants; ITI, immune tolerance induction.

*Apparent rate based only on a total of 11 children with normal FVIII recovery (phase 2 success) consenting to FVIII half-life study (6 in the ITI-alone throughout group and 5 in the ITI-IS group). The true rate is not available since 24 other patients (13 in the ITI-alone throughout group and 11 in the ITI-IS group) who achieved normal FVIII recovery did not consent to have FVIII half-life testing.; **In this study, phase 1 success = when inhibitor becomes <0.6 BU; phase 2 success = when FVIII recovery reached ≥66% expected; phase 3 success = when FVIII half-life reached ≥6 h.; ***Time from start of ITI to time of the respective success.

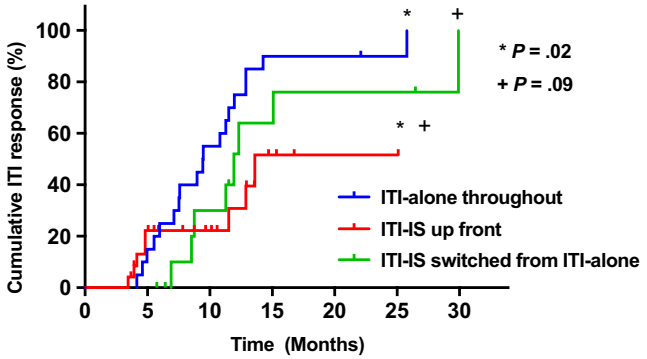

FIGURE 2 Time to phase 2 success by treatment group. Kaplan-Meier plot shows the time to phase 2 success (FVIII recovery ≥66% of expected). The time to phase 2 success was significant different among the three treatment groups ($P = 0.03$). ITI, immune tolerance induction; IS, immunosuppressants

reduced rapidly from 50 to 30 IU/kg over a 2-month period, while the fourth occurred after receiving four vaccinations over a 2-week period. All four children reestablished phase 2 success upon repeating the original ITI regimen followed by FVIII prophylaxis.

3.5 | Cost and consumption analysis

Average consumption cost (per kg body weight) was calculated based on the median number of treatment doses consumed to achieve phase 2 success. This included the cost of FVIII and rituximab for ITI, PCC for treatment of breakthrough bleed, and IVIG for infection prevention in IS patients. The average cost (per kilogram of body weight) was ¥19 600.2 (US$2985.1) for the ITI-alone throughout group, and ¥29 763.7 (US$4533.0) for the ITI-IS group (Table 3). Among the expenditure, pdFVIII/VWF accounted for 93.8% to 98.5% of the cost, rituximab for 3.8%, and PCC for breakthrough bleeding treatment for 1.5% to 1.7%. Not factored into the cost calculation was prednisone (for IS, very inexpensive in China) as well as the rare prophylactic use of PCC (in very few patients with inconsequential average cost spread out to the whole patient cohort). Compared to the expenditure for high-dose ITI,[25] our cost for the ITI-alone group was lower by 82.8% (when using domestic pdFVIII/VWF) to 90.5% (if using rFVIII). For the ITI-IS group, our cost was lower by 73.8% (when using domestic pdFVIII/VWF) to 85.5% (if using rFVIII) (Table 3).

4 | DISCUSSION

In this study, we determined the efficacy of low-dose ITI when VWF-containing pdFVIII alone or with IS was used in Chinese children with hemophilia A with high-titer inhibitor and having immediate pre-ITI inhibitor titer ≥10 BU. Low-dose ITI-alone without IS was used for patients with historic peak inhibitor titer <100 BU and immediate pre-ITI titer of 10 to 40 BU. ITI-IS was used instead if the historic peak inhibitor titer was ≥100 BU and/or the immediate pre-ITI titer ≥40 BU. Patients originally on ITI-alone would be switched to ITI-IS should the peak inhibitor titer during ITI rose to ≥40 BU or if the titer during ITI did not decline by 20% within the first 3 months after initial peak inhibitor titer during ITI.

Of all analyzable patients, the rate of phase 2 success (achieving negative inhibitor and normal FVIII recovery) was 62.5% (35/56). This was lower than the 74.2% (49/66) of patients achieving the equivalent phase 1/phase 2 successes in the I-ITI study.[6] This may not be surprising given that we included patients with historic inhibitor titer ≥200 BU and pre-ITI inhibitor titer ≥10 BU, both considered exclusion criteria in the I-ITI study.[6] The median historic peak inhibitor titer in our patients was 48 BU as opposed to 22 BU in the I-ITI study. That historic peak inhibitor titer may influence the success rate has been previously reported.[19,26] This is also evident in our own study, in that those with the titer ≥100 BU had poorer outcome even if ITI-IS was used up front. Excluding the 14 patients with historic peak inhibitor titer ≥100 BU would have improved the phase 2 success rate (from 44.4% to 63.6% for patients in the ITI-IS group and from 62.5% to 78.6% for the whole cohort).

In our study, the median 13.6 months taken for patients having higher ITI prognostic risk and treated with ITI-IS to achieve phase 2 success was similar to that for patients with lower ITI prognostic risk in the low-dose arm of the I-ITI study (not using IS).[5] Our whole cohort even took slightly shorter time (11.5 months). Thus, our low-dose ITI strategy did improve the outcome of patients with higher ITI-prognostic risk(s). The median time to phase 2 success using the ITI-IS regimen (13.6 months) was, however, longer than that using the ITI-alone regimen (9.4 months), reflecting the fact that those treated with ITI-IS had higher ITI prognostic risk.

One major limitation of this study is that only 11 of the 35 patients achieving phase 2 success consented to have FVIII half-life evaluation. The reality in China is that these multiple blood sampling over 48 hours represent an out-of-pocket cost burden to the family with economic constraints. In addition to the test cost, many of our patients lived a distance away from Beijing. There was therefore an added cost for transportation and accommodation in Beijing (eg, hotel), plus up to 2 days away from work for the parent(s). The apparent rate of phase 3 success (tolerization with normal FVIII half-life >6 hours) was therefore quite low, being 11 of 56 (19.6%). However, we contend that the real rate of phase 3 success would have been higher. All 11 patients with phase 2 success when tested had normal FVIII half-life, suggesting that a good proportion of the remaining 24 phase 2 successes would likely also have a normal FVIII half-life if they were also tested, increasing the real rate of phase 3 success. Obviously, without testing, we cannot assume any of our phase 2 success patients to have achieved immune tolerance. Another limitation is the relatively high exclusion/withdrawal rate of 24.3% (18 patients) (Figure 1). Of these 18 patients, 5 were excluded because they could not afford the considerable out-of-pocket cost (beyond medical insurance) for ITI (that included cost of concentrates/blood products, medications, and monitoring tests), and therefore declined to start, 8 were withdrawn because they had problems financing the

TABLE 3 Cost of different ITI protocols (per kilogram of body weight) from ITI initiation to phase 2 success (inhibitor titer <0.6 BU, FVIII recovery ≥66% expected)

	Low-dose ITI alone	Low-dose ITI-IS (rituximab)	High-dose ITI[6] (pdFVIII/VWF)	High-dose ITI[6] (rFVIII)
ITI regimen (FVIII IU/kg)	50/QOD	50/QOD	100/Q12h	100/Q12h
Median time to phase 2 success, mo	9.4	13.6	6.9	6.9
Cost of FVIII concentrate per ITI course	¥19 299.4 (US$2939.3)	¥27 922.5 (US$4252.6)	¥113 332.5 (US$17260.5)	¥205 677.5 (US$31 324.7)
Mean bleeds/mo	0.32	0.37	0.28	0.28
PCC dose (IU/kg) × n doses per bleed	50.0 × 2 doses	50.0 × 2 doses	85.0 × 2 doses[25]	85.0 × 2 doses[25]
Cost of PCC per ITI course	¥300.8 (US$45.8)	¥503.2 (US$76.6)	¥328.4 (US$50.0)	¥328.4 (US$50.0)
Cost of IS per ITI course	-	¥1050.0 (US$159.9)	-	-
Cost of IVIG (infection prophylaxis) during 6 months after starting rituximab	-	¥288.0 (US$43.9)	-	-
Total cost per kg per ITI course	¥19 600.2 (US$2985.1)	¥29 763.7 (US$4533.0)	¥113 660.9 (US$17 310.6)	¥206 005.9 (US$31 374.7)

Note: Cost calculation algorithm reference to our pilot study[30] and based on median number of treatment doses (n) up until phase 2 success (including FVIII, rituximab, PCC for treatment of breakthrough bleeds) × Unit or milligram(s) cost × Units per kilogram per dose. Cost calculation of IVIG was based on 6 months use dosage (mg/kilogram body-weight) × cost/milligram. Not included are: the cost of (i) rFVIIa (for breakthrough bleeds treatment) and (ii) PCC (for bleed prophylaxis) both used only in very few patients with inconsequential average cost for the groups, and (iii) prednisone (for IS) which is inexpensive in China with inconsequential cost.

Abbreviations: IS, immunosuppressants; ITI, immune tolerance induction; IVIG, intravenous immunoglobulin; PCC, prothrombin complex concentrate; pdFVIII/VWF, plasma derived FVIII/von Willebrand factor; rFVIII, recombinant FVIII.

ITI program continuously, leading to ITI interruption (n = 7) or irregular dosing (n = 1). In China, medical insurance coverage rates vary in different regions depending on their economic development. Patients in economically less developed regions had problems affording even low-dose ITI.

The risk of relapse following ITI success ranged between 0% and 12.5% according to a 2013 analysis on cohort studies and registers.[27] Relapse was reported as ≈15% (15-year follow-up) in the North American Immune Tolerance registry,[28] 6.8% (9.1-year follow-up) in the Grifols ITI study,[29] and 13.0% (1-year follow-up) in the I-ITI study.[6] Our cohort follow-up for a median 2.8 years, having a comparable overall relapse rate of 11.4% in those achieving phase 2 success. However, our relapse rate of 18.2% in the 11 patients with proven phase 3 success was higher. This might likely be a consequence of the limited sample size. Given that many of the phase 2 successes would potentially have phase 3 success were they tested for FVIII half-life (as indicated earlier), we speculate that the overall relapse rate for "real" phase 3 success could be lower. Of note is that the relapse rate of our patients with phase 2 success on ITI-IS was quite low, at 6.3%, compared to that of the IS-containing regimens reported from the United Kingdom (3/6 or 50%) and by Antun et al[28] (4/5 or 80%). Among our proven phase 3 successes, two relapses occurred in the six treated with ITI alone, but no relapse in the five treated with IS. However, the numbers are too small to make a statement on the relative outcome merit of IS. Some of our relapses occurred as the FVIII dosage was rapidly decreased or infusions were interrupted, suggesting that rapid reduction in ITI dose or nonadherence with regular infusions represented risk for relapse. The guidelines from the United Kingdom emphasized that FVIII tapering should be attempted in patients with poor ITI prognostic risk until the FVIII half-life is >7 hours, and dose reduction should then be undertaken cautiously.[10]

The cost of our low-dose ITI regimen until phase 2 success was 74% to 90% lower than that for high-dose ITI[6,25] and confirms the finding in our earlier pilot economic study.[30] This low-dose ITI alone or with IS strategy is affordable for children with HA with high-titer inhibitor in China with economic constraint, and by extension also in other regions with developing economies.

There are other limitations in the studies, in addition to the small number of patients having half-life studies to confirm toleration, discussed earlier. In many patients, the baseline treatment data before we started their ITI were acquired retrospectively from their local referral centers or verbally from the parents; the latter also may have the problem of recall bias. FVIII recovery studies were usually delayed after the inhibitor titer had become negative, given that these patients are mostly from out of town at a long distance, making the apparent time to achieve phase 2 success longer than real. Our sample size is limited, especially for relapse evaluation.

5 | CONCLUSION

In conclusion, our strategy to stratify treatment with low-dose ITI-alone and low-dose ITI-IS according to their ITI prognostic risk(s) was safe, with satisfactory efficacy rate in inhibitor elimination in children with hemophilia A with high-titer inhibitors. The efficacy was particularly good for those with historic peak inhibitor titer <100 BU. Compared to the expenditure for high-dose ITI, our cost

was lower by 90% for patients with lower ITI prognostic risk in the ITI-alone group and by 74% for the patients with higher ITI prognostic risk in the ITI-IS group. This will be a much more affordable ITI regimen for China and other regions with economic constraint.

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AUTHOR CONTRIBUTIONS

RW contributed to the study design and preparation of the manuscript; ZL wrote the manuscript and analyzed the data; ZC, GL, and XC reviewed the article; WY and KH performed the research; XW, GL, and YZ performed literature searches. SC provided data analysis. M-CP provided input on study design and data analysis as well as critical and detailed revision of the manuscript. All of the authors had full access to the data and participated in the design of the analysis, discussion of results, and revising the draft manuscript.

RELATIONSHIP DISCLOSURE

The authors declare no conflicts or bias of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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