nature medicine

Supplementary information

https://doi.org/10.1038/s41591-026-04636-8

Factor IX Padua AAV gene therapy in adolescents with hemophilia B: a phase 1 trial

In the format provided by the authors and unedited

Supplementary Appendix

Supplementary Methods

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Protocol

Supplementary Methods

BBM-H901 cassette sequence:

The BBM-H901 cassette had a fully synthesized smaller liver-specific promoter, LXP2.1. It drove expression of fully synthesized human FIX Padua gene. The FIX gene was optimized with the maximizing the usage of the more effective codons, and the CpG motifs were completely removed. The promoter and FIX gene were constructed into self-complementary AAV vector. The cassette sequence as followed:

AGGTTAATTTTTAAAAAGCAGTCAAAAGTCCAAGTGGCCCTTGGCAGCATTTACTC
TCTCTGTTTACTCTGGTTAATAATCTCAGGAGTACAAACATTCCAGATCCAGGTTAA
TTTTTAAAAAAAAGAGTGCTCTAGTTTTGCAATACAGGACATGCTATAAAAAGCGAA
GCGCGCGGTGGGCGGGGTTGAAGCTAACAAAGACCACGACGATATCACGGTCGTG
GTCTCAAAGAACAACAAACAACAAAGTCCGACTGAGAAGGTGAGTGGCGGGCCC
TGAGCTGGGGGGCGGGGGTGTTGGCTCTGGAGGCTGGGTCTGAGCGTAATTTTGC
ACCCCCGCGTCCCTGCAGGAGCCACCATGCAGAGGGTGAACATGATCATGGCTGA
GAGCCCTGGCCTGATCACCATCTGCCTGCTGGGCTACCTGCTGTCTGCTGAGTGCA
CTGTGTTCCTGGACCATGAGAATGCCAACAAGATCCTGAACAGGTGAGTATCTCAG
GGATCCAGACATGGGGATATGGGAGGTGCCTCTGATCCCAGGGCTCACTGTGGGTC
TCTCTGTTCACAGGCCCAAGAGATACAACTCTGGCAAGCTGGAGGAGTTTGTGCA
GGGCAACCTGGAGAGGGAGTGCATGGAGGAGAAGTGCAGCTTTGAGGAGGCCAG
GGAGGTGTTTGAGAACACTGAGAGGACCACTGAGTTCTGGAAGCAGTATGTGGAT
GGGGACCAGTGTGAGAGCAACCCCTGCCTGAATGGGGGCAGCTGCAAGGATGAC
ATCAACAGCTATGAGTGCTGGTGCCCCTTTGGCTTTGAGGGCAAGAACTGTGAGC
TGGATGTGACCTGCAACATCAAGAATGGCAGATGTGAGCAGTTCTGCAAGAACTC
TGCTGACAACAAGGTGGTGTGCAGCTGCACTGAGGGCTACAGGCTGGCTGAGAA
CCAGAAGAGCTGTGAGCCTGCTGTGCCATTCCCATGTGGCAGAGTGTCTGTGAGC
CAGACCAGCAAGCTGACCAGGGCTGAGGCTGTGTTCCCTGATGTGGACTATGTGA
ACAGCACTGAGGCTGAAACCATCCTGGACAACATCACCCAGAGCACCCAGAGCTT
CAATGACTTCACCAGGGTGGTGGGGGGGGAGGATGCCAAGCCTGGCCAGTTCCCC
TGGCAAGTGGTGCTGAATGGCAAGGTGGATGCCTTCTGTGGGGGCAGCATTGTGA
ATGAGAAGTGGATTGTGACTGCTGCCCACTGTGTGGAGACTGGGGTGAAGATCAC
TGTGGTGGCTGGGGAGCACAACATTGAGGAGACTGAGCACACTGAGCAGAAGAG
GAATGTGATCAGGATCATCCCCCACCACAACTACAATGCTGCCATCAACAAGTACA
ACCATGACATTGCCCTGCTGGAGCTGGATGAGCCCCTGGTGCTGAACAGCTATGTG
ACCCCCATCTGCATTGCTGACAAGGAGTACACCAACATCTTCCTGAAGTTTGGCTC
TGGCTATGTGTCTGGCTGGGGCAGGGTGTTCCACAAGGGCAGGTCTGCCCTGGTG
CTGCAGTACCTGAGGGTGCCCCTGGTGGACAGGGCCACCTGCCTGCTGAGCACCA

AGTTCACCATCTACAACAACATGTTCTGTGCTGGCTTCCATGAGGGGGGCAGGGA
CAGCTGCCAGGGGGACTCTGGCGGCCCCCATGTGACTGAGGTGGAGGGCACCAG
CTTCCTGACTGGCATCATCAGCTGGGGGGGAGGAGTGTGCCATGAAGGGCAAGTAT
GGCATCTACACCAAAGTCTCCAGATATGTGAACTGGATCAAGGAGAAGACCAAGC
TGACCTGATAAGCTAGCAGGCCTAATAAAGAGCTCAGATGCATCGATCAGAGTGTG
TTGGTTTTTTGTGT

AAV843 capsid sequence:

Liver tropic AAV capsid, AAV843 was screened from a AAV capsid gene shuffled library was as described before (Yang et al, Proc. Natl. Acad. Sci. USA 106(10):39A6 (2009) PMID: 19234115; Yang et al, Meth. Mol. Biol. 709:127 (2011) PMID: 21194025). The library was constructed from AAV serotypes 1, 2, 3B, 4, 6, 7, 8, and 9. The AAV843 protein sequence was as followed:

MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQQKQDDGRGLVLPGYKYLGP
FNGLDKGEPVNAADAAALEHDKAYDQQLQAGDNPYLRYNHADAEFQERLQEDTSF
GGNLGRAVFQAKKRVLEPLGLVEEGAKTAPGKKRPVEPSPQRSPDSSTGIGKKGQQPA
RKRLNFGQTGDSESVPDPQPLGEPPAAPSGVGPNTMASGGGAPMADNNEGADGVGN
ASGNWHCDSTWLGDRVITTSTRTWALPTYNNHLYKQISSASTGASNDNHYFGYSTPW
GYFDFNRFHCHFSPRDWQRLINNNWGFRPKRLNFKLFNIQVKEVTTNDGVTTIANNL
TSTVQVFSDSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYC
LEYFPSQMLRTGNNFTFSYTFEEVPFHSSYAHSQSLDRLMNPLIDQYLYYLNRTQNQS
GSAQNKDLLFSRGSPAGMSVQPKNWLPGPCYRQQRVSKTKTDNNNSNFTWTGASKY
NLNGRESIINPGTAMASHKDDKDKFFPMSGVMIFGKESAGASNTALDNVMITDEEEIK
ATNPVATERFGTVAVNLQSSSTDPATGDVHVMGALPGMVWQDRDVYLQGPIWAKIPH
TDGHFHPSPLMGGFGLKHPPPQILIKNTPVPANPPAEFSATKFASFITQYSTGQVSVEIE
WELQKENSKRWNPEVQYTSNYARSANVDFTVDNNGLYTEPRPIGTRYLTRPL

Table S1: Regimen of prophylactic prednisone in phase 1 study.

Time	Prednisone/prednisolone (mg/kg/d) when weight ≤80kg	Prednisone/prednisolone (mg/d) Weight > 80kg
-1 day	1	60
1 week	1	60
2 weeks	1	60
3 weeks	0.75	50
4 weeks	0.75	50
5 weeks	0.5	40
6 weeks	0.5	30
7 weeks	0.3	20
8 weeks	0.3	10

Note: The investigator may adjust and apply the hormonal taper schedule according to the clinical manifestations, test and examination indicators of the participants.

Table S2: Peptide list for Elispot

start	end	length	peptide	score	rank
316	325	10	KLFNIQVKEV	0.79427	0.08
681	690	10	SVEIEWELQK	0.773734	0.09
250	259	10	LPTYNNHLYK	0.713506	0.13
568	577	10	KATNPVATER	0.533178	0.26
6	15	10	YLPDWLEDNL	0.501999	0.26
614	623	10	YLQGPIWAKI	0.455924	0.3
275	284	10	FGYSTPWGYF	0.429631	0.24
535	544	10	FFPMSGVMIF	0.392805	0.26
705	714	10	NYARSANVDF	0.376639	0.28
406	415	10	RTGNNFTFSY	0.367515	0.47
50	59	10	YKYLGPFNGL	0.334406	0.32
91	100	10	LRYNHADAEF	0.330738	0.32
613	622	10	VYLQGPIWAK	0.324695	0.56
82	91	10	QLQAGDNPYL	0.298987	0.55
606	615	10	MVWQDRDVYL	0.274571	0.6
24	33	10	ALKPGAPKPK	0.273153	0.65
434	443	10	RLMNPLIDQY	0.265762	0.67
633	642	10	SPLMGGFGLK	0.257828	0.69
252	261	10	TYNNHLYKQI	0.254676	0.4
673	682	10	TQYSTGQVSV	0.231171	0.7
435	444	10	LMNPLIDQYL	0.230483	0.7
272	281	10	NHYFGYSTPW	0.191764	0.52
44	53	10	GLVLPGYKYL	0.189556	0.84
598	607	10	HVMGALPGMV	0.183622	0.87
662	671	10	EFSATKFASF	0.177018	0.55
509	518	10	KYNLNGRESI	0.168984	0.57
268	277	10	ASNDNHYFGY	0.160057	1.1
280	289	10	PWGYFDFNRF	0.156112	0.63
411	420	10	FTFSYTFEEV	0.153294	1.1
640	649	10	GLKHPPPQIL	0.145189	1.1

Supplementary Result

Figure S1 The vector shedding after gene therapy.

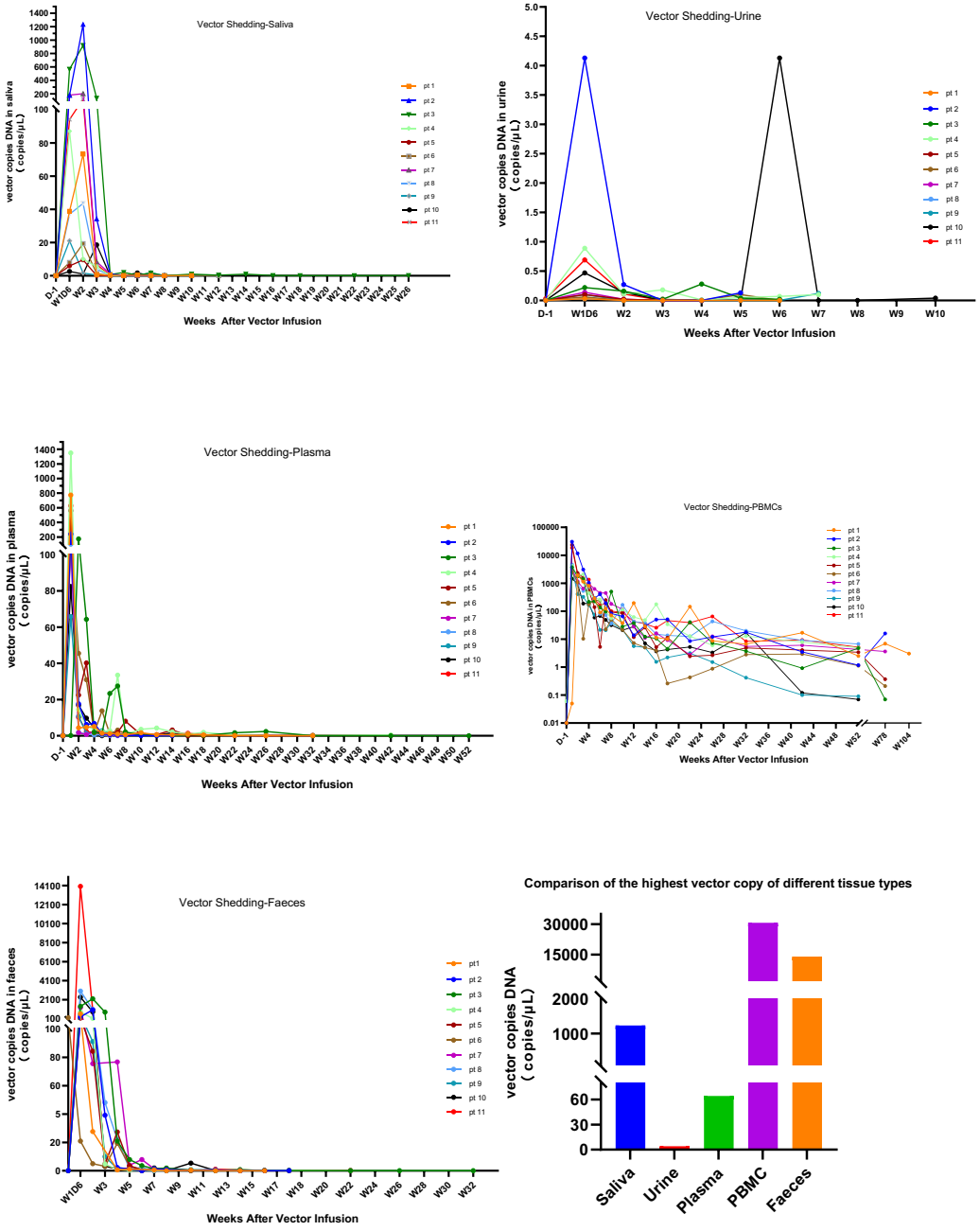


Figure S2 Elispot assay for participants 2, 4, 7, 8, 11

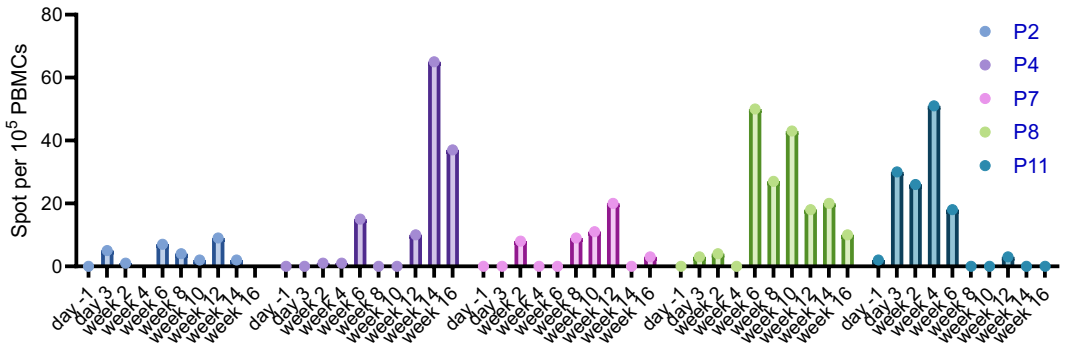


Figure S3

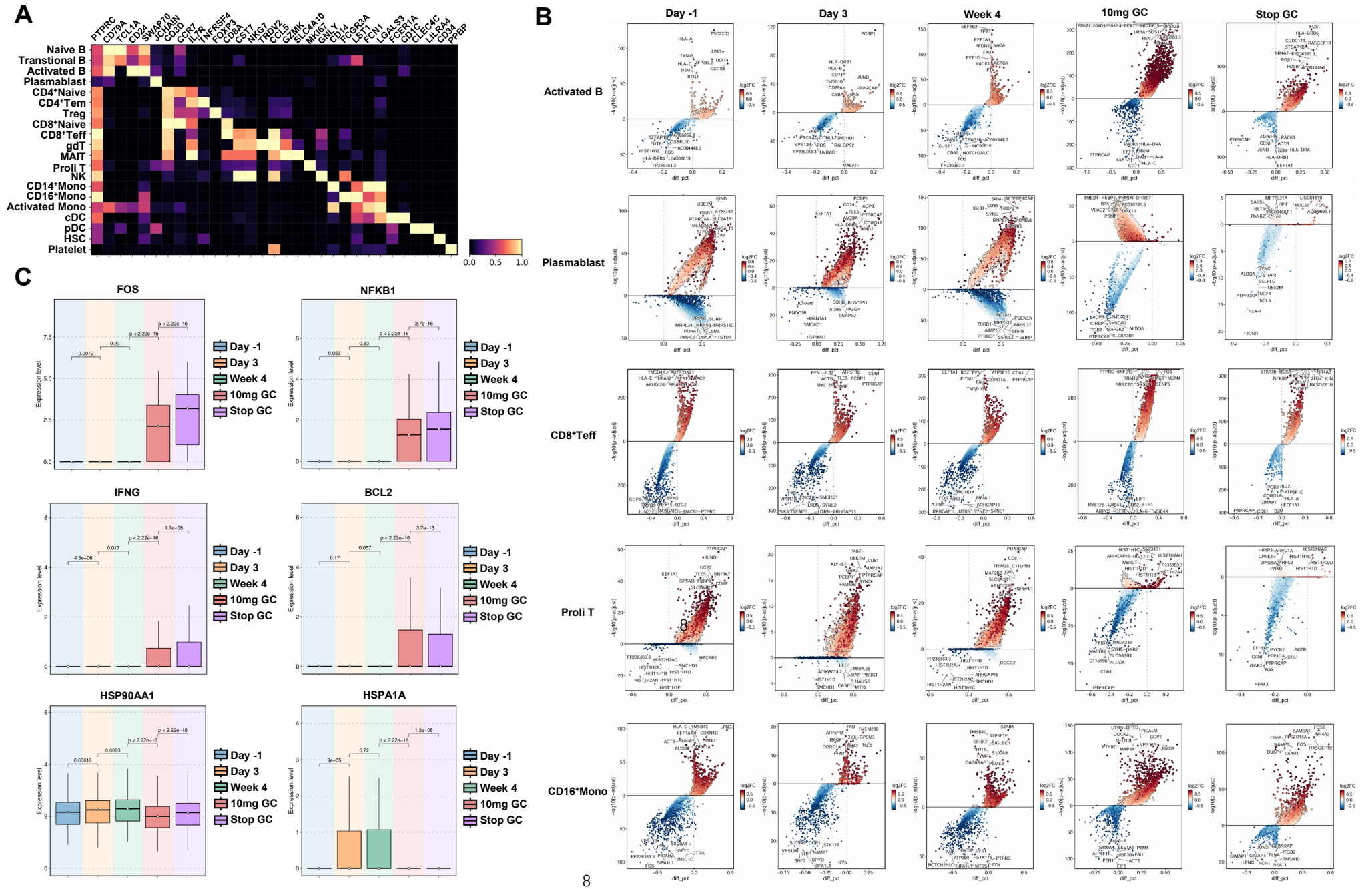

A
PTPRC
CD79A
TCL1A
CD24
SWAP70
JCHAIN
CD3D
CCR7
IL7R
TNFRSF4
FOXP3
CD8A
CST7
NKG7
TRDV2
CCL5
GZMK
SLC4A10
MKI67
GNLY
CD14
FCGR3A
LST1
FCN1
LGALS3
FCER1A
CD1C
CLEC4C
LILRA4
CD34
PPBP
Naive B
Transtional B
Activated B
Plasmablast
CD4⁺Naive
CD4⁺Tem
Treg
CD8⁺Naive
CD8⁺Teff
gdT
MAIT
Proli T
NK
CD14⁺Mono
CD16⁺Mono
Activated Mono
cDC
pDC
HSC
Platelet
0.0
0.5
1.0
B
Day -1
Day 3
Week 4
10mg GC
Stop GC
Activated B
Plasmablast
CD8⁺Teff
Proli T
CD16⁺Mono
C
FOS
NFKB1
IFNG
BCL2
HSP90AA1
HSPA1A
Expression level
Day -1
Day 3
Week 4
10mg GC
Stop GC

Figure S3 Single-cell transcriptomic characterization and expression dynamics of PBMC subpopulations. **a.** Heatmap showing the relative expression of canonical marker genes (columns) across the identified PBMC subpopulations (rows). *The color bar from dark purple to yellow represents scaled expression levels (0.0 to 1.0)*. **b.** Volcano plots illustrating differentially expressed genes in representative immune cell types (Activated B, Plasmablast, $CD8^+$Teff, Proli T, and $CD16^+$Mono) across different stages of treatment (Day -1, Day 3, Week 4, 10mg GC, and Stop GC). *The x-axis represents the difference in the fraction of cells expressing the gene (diff_pct), the y-axis represents −log10(adjusted P-value), and the color scale indicates the* log^2*(fold change)*. **c.** The box plots show the regularized expression levels of *FOS*, *NFKB1*, *IFNG*, *BCL2*, *HSP90AA1*, and *HSPA1A* in single cells (n = 3149 cells for Day -1, n = 1711 cells for Day 3, n = 1689 cells for Week 4, n = 4253 cells for 10mg GC, and n = 4082 cells for Stop GC). *In each box plot, the center line indicates the median, the box bounds represent the 25th and 75th percentiles (first and third quartiles), and whiskers extend to 1.5 times the interquartile range (IQR). Statistical significance was determined using the two-sided Wilcoxon rank-sum test; exact P-values are indicated.*

Figure S4

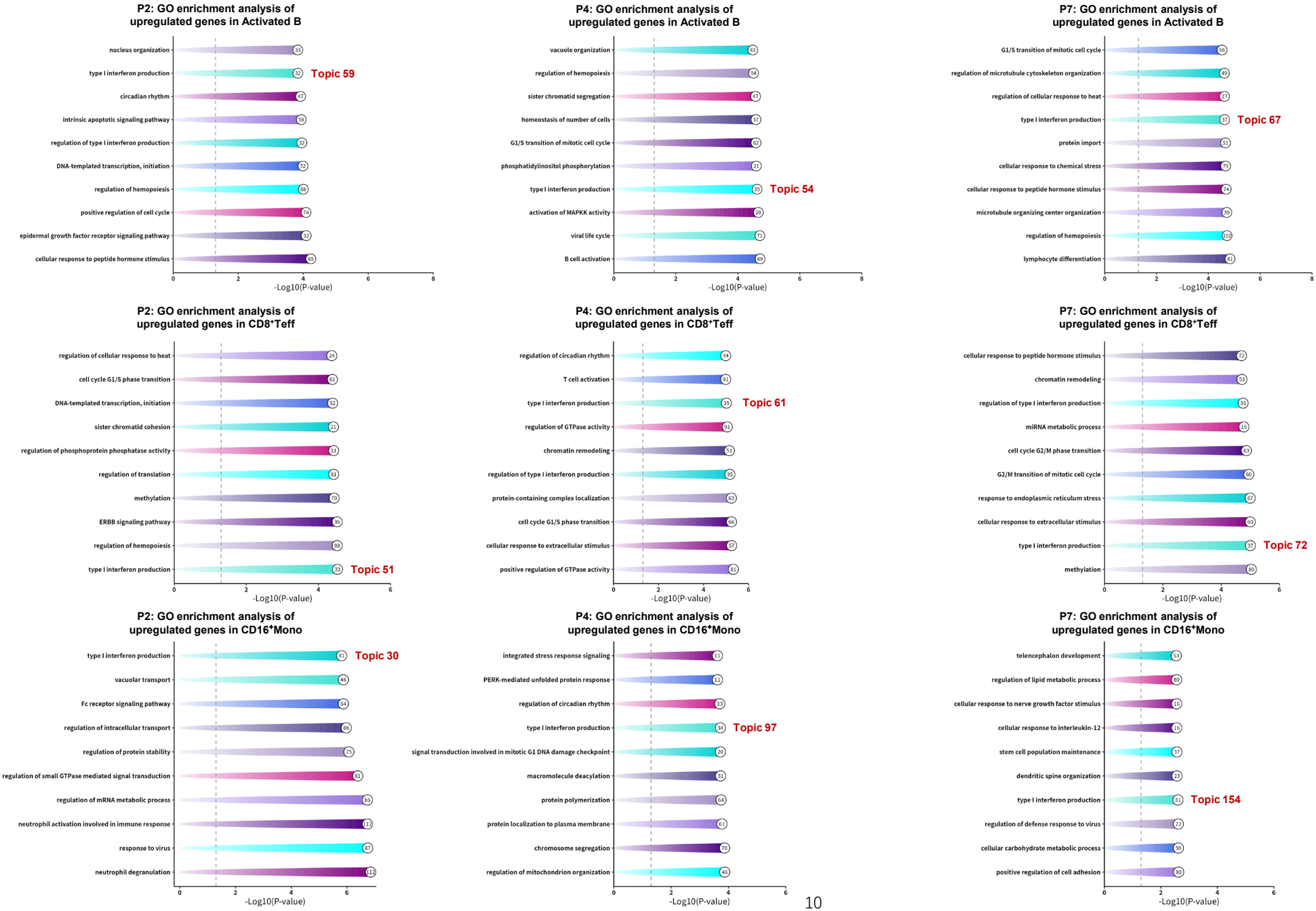
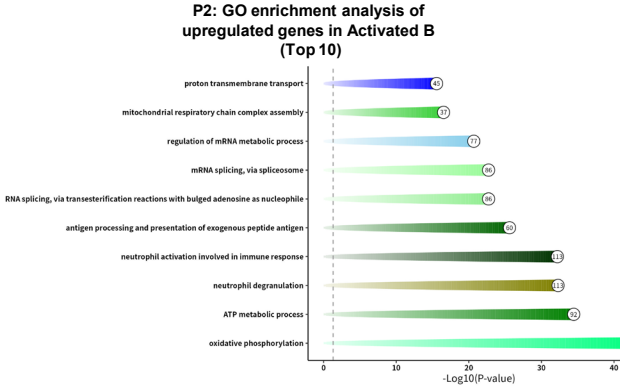
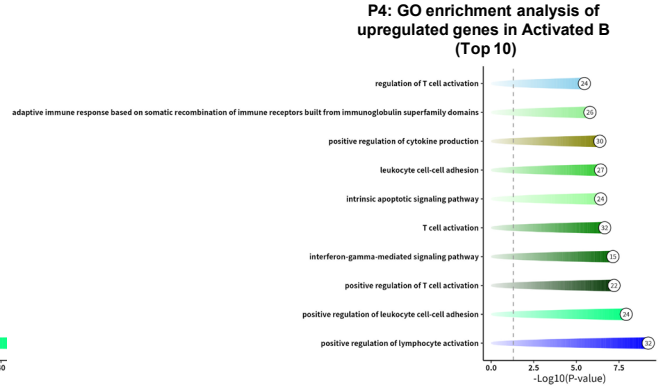
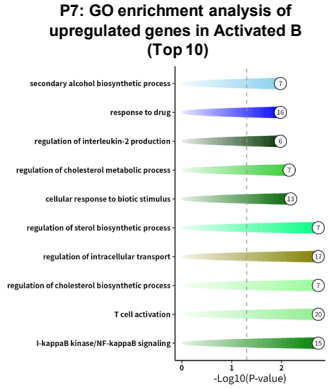

P2: GO enrichment analysis of upregulated genes in Activated B
nucleus organization 33
type I interferon production 32
circadian rhythm 47
intrinsic apoptotic signaling pathway 58
regulation of type I interferon production 32
DNA-templated transcription, initiation 52
regulation of hemopoiesis 88
positive regulation of cell cycle 74
epidermal growth factor receptor signaling pathway 32
cellular response to peptide hormone stimulus 85
Topic 59
-Log10(P-value)
P4: GO enrichment analysis of upregulated genes in Activated B
vacuole organization 43
regulation of hemopoiesis 94
sister chromatid segregation 47
homeostasis of number of cells 57
G1/S transition of mitotic cell cycle 62
phosphatidylinositol phosphorylation 21
type I interferon production 35
activation of MAPKK activity 20
viral life cycle 71
B cell activation 69
Topic 54
-Log10(P-value)
P7: GO enrichment analysis of upregulated genes in Activated B
G1/S transition of mitotic cell cycle 66
regulation of microtubule cytoskeleton organization 49
regulation of cellular response to heat 27
type I interferon production 37
protein import 51
cellular response to chemical stress 79
cellular response to peptide hormone stimulus 74
microtubule organizing center organization 39
regulation of hemopoiesis 102
lymphocyte differentiation 81
Topic 67
-Log10(P-value)
P2: GO enrichment analysis of upregulated genes in CD8+Teff
regulation of cellular response to heat 26
cell cycle G1/S phase transition 61
DNA-templated transcription, initiation 52
sister chromatid cohesion 21
regulation of phosphoprotein phosphatase activity 51
regulation of translation 91
methylation 70
ERBB signaling pathway 36
regulation of hemopoiesis 88
type I interferon production 33
Topic 51
-Log10(P-value)
P4: GO enrichment analysis of upregulated genes in CD8+Teff
regulation of circadian rhythm 34
T cell activation 91
type I interferon production 35
regulation of GTPase activity 91
chromatin remodeling 51
regulation of type I interferon production 35
protein-containing complex localization 63
cell cycle G1/S phase transition 66
cellular response to extracellular stimulus 57
positive regulation of GTPase activity 81
Topic 61
-Log10(P-value)
P7: GO enrichment analysis of upregulated genes in CD8+Teff
cellular response to peptide hormone stimulus 72
chromatin remodeling 53
regulation of type I interferon production 36
miRNA metabolic process 15
cell cycle G2/M phase transition 63
G2/M transition of mitotic cell cell cycle 60
response to endoplasmic reticulum stress 67
cellular response to extracellular stimulus 60
type I interferon production 37
methylation 80
Topic 72
-Log10(P-value)
P2: GO enrichment analysis of upregulated genes in CD16+Mono
type I interferon production 41
vacuolar transport 46
Fc receptor signaling pathway 64
regulation of intracellular transport 86
regulation of protein stability 75
regulation of small GTPase mediated signal transduction 81
regulation of mRNA metabolic process 80
neutrophil activation involved in immune response 112
response to virus 87
neutrophil degranulation 112
Topic 30
-Log10(P-value)
P4: GO enrichment analysis of upregulated genes in CD16+Mono
integrated stress response signaling 11
PERK-mediated unfolded protein response 11
regulation of circadian rhythm 33
type I interferon production 34
signal transduction involved in mitotic G1 DNA damage checkpoint 20
macromolecule deacylation 31
protein polymerization 64
protein localization to plasma membrane 61
chromosome segregation 70
regulation of mitochondrion organization 46
Topic 97
-Log10(P-value)
P7: GO enrichment analysis of upregulated genes in CD16+Mono
telencephalon development 53
regulation of lipid metabolic process 80
cellular response to nerve growth factor stimulus 16
cellular response to interleukin-12 16
stem cell population maintenance 37
dendritic spine organization 23
type I interferon production 31
regulation of defense response to virus 22
cellular carbohydrate metabolic process 59
positive regulation of cell adhesion 80
Topic 154
-Log10(P-value)

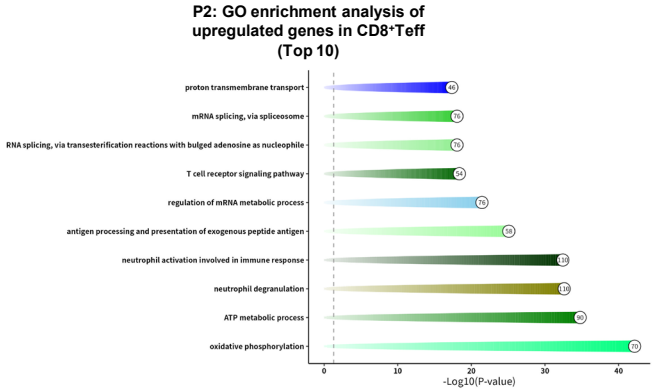
Figure S4 Gene Ontology (GO) enrichment analysis of upregulated genes across immune cell subpopulations in individual patients. *GO enrichment analysis of upregulated genes is shown for three major cell types: Activated B cells (top row), $CD8^+$ Teffcells (middle row), and $CD16^+$ Mono cells (bottom row), across three longitudinal patient samples: P2 (left column), P4 (middle column), and P7 (right column). The x-axis represents the statistical significance of enrichment expressed as −log10(P-value), The vertical dashed line indicates the significance threshold of P = 0.05. The numbers inside the circles at the end of each gradient bar represent the gene count (the number of upregulated genes enriched in the corresponding GO term). The red text annotations (e.g., Topic 59, Topic 54, etc.) highlight the specific biological pathways associated with type l interferon production.*

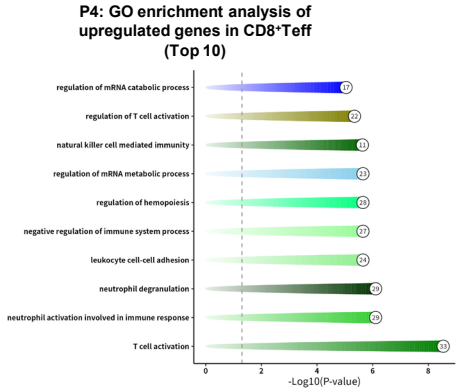
Figure S5

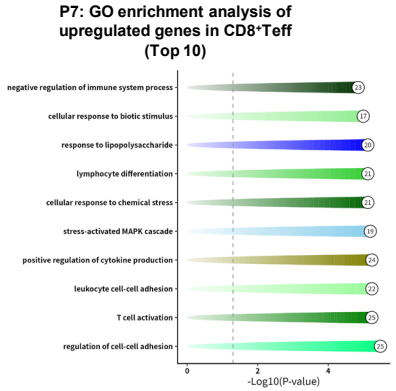

P2: GO enrichment analysis of upregulated genes in Activated B (Top 10)
proton transmembrane transport
mitochondrial respiratory chain complex assembly
regulation of mRNA metabolic process
mRNA splicing, via spliceosome
RNA splicing, via transesterification reactions with bulged adenosine as nucleophile
antigen processing and presentation of exogenous peptide antigen
neutrophil activation involved in immune response
neutrophil degranulation
ATP metabolic process
oxidative phosphorylation
-Log10(P-value)

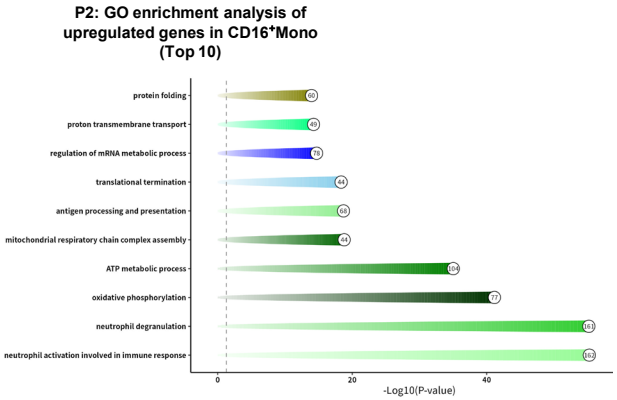

P4: GO enrichment analysis of upregulated genes in Activated B (Top 10)
regulation of T cell activation
adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains
positive regulation of cytokine production
leukocyte cell-cell adhesion
intrinsic apoptotic signaling pathway
T cell activation
interferon-gamma-mediated signaling pathway
positive regulation of T cell activation
positive regulation of leukocyte cell-cell adhesion
positive regulation of lymphocyte activation
-Log10(P-value)

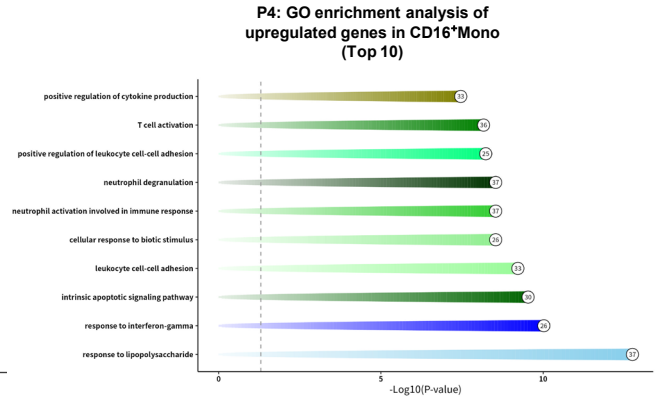

P7: GO enrichment analysis of upregulated genes in Activated B (Top 10)
secondary alcohol biosynthetic process
response to drug
regulation of interleukin-2 production
regulation of cholesterol metabolic process
cellular response to biotic stimulus
regulation of sterol biosynthetic process
regulation of intracellular transport
regulation of cholesterol biosynthetic process
T cell activation
I-kappaB kinase/NF-kappaB signaling
-Log10(P-value)


P2: GO enrichment analysis of upregulated genes in CD8⁺Teff (Top 10)
proton transmembrane transport
mRNA splicing, via spliceosome
RNA splicing, via transesterification reactions with bulged adenosine as nucleophile
T cell receptor signaling pathway
regulation of mRNA metabolic process
antigen processing and presentation of exogenous peptide antigen
neutrophil activation involved in immune response
neutrophil degranulation
ATP metabolic process
oxidative phosphorylation
-Log10(P-value)


P4: GO enrichment analysis of upregulated genes in CD8⁺Teff (Top 10)
regulation of mRNA catabolic process
regulation of T cell activation
natural killer cell mediated immunity
regulation of mRNA metabolic process
regulation of hemopoiesis
negative regulation of immune system process
leukocyte cell-cell adhesion
neutrophil degranulation
neutrophil activation involved in immune response
T cell activation
-Log10(P-value)


P7: GO enrichment analysis of upregulated genes in CD8⁺Teff (Top 10)
negative regulation of immune system process
cellular response to biotic stimulus
response to lipopolysaccharide
lymphocyte differentiation
cellular response to chemical stress
stress-activated MAPK cascade
positive regulation of cytokine production
leukocyte cell-cell adhesion
T cell activation
regulation of cell-cell adhesion
-Log10(P-value)


P2: GO enrichment analysis of upregulated genes in CD16⁺Mono (Top 10)
protein folding
proton transmembrane transport
regulation of mRNA metabolic process
translational termination
antigen processing and presentation
mitochondrial respiratory chain complex assembly
ATP metabolic process
oxidative phosphorylation
neutrophil degranulation
neutrophil activation involved in immune response
-Log10(P-value)


P4: GO enrichment analysis of upregulated genes in CD16⁺Mono (Top 10)
positive regulation of cytokine production
T cell activation
positive regulation of leukocyte cell-cell adhesion
neutrophil degranulation
neutrophil activation involved in immune response
cellular response to biotic stimulus
leukocyte cell-cell adhesion
intrinsic apoptotic signaling pathway
response to interferon-gamma
response to lipopolysaccharide
-Log10(P-value)

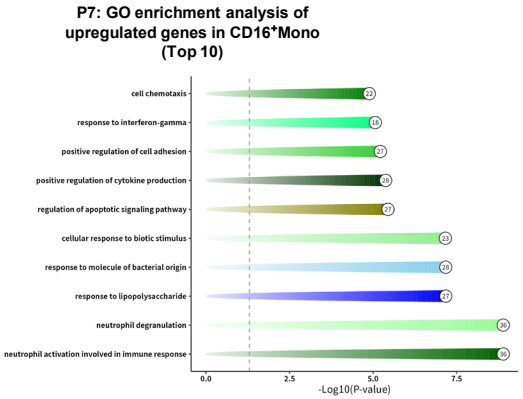

P7: GO enrichment analysis of upregulated genes in CD16⁺Mono (Top 10)
cell chemotaxis
response to interferon-gamma
positive regulation of cell adhesion
positive regulation of cytokine production
regulation of apoptotic signaling pathway
cellular response to biotic stimulus
response to molecule of bacterial origin
response to lipopolysaccharide
neutrophil degranulation
neutrophil activation involved in immune response
-Log10(P-value)

Figure S5 Top 10 enriched Gene Ontology (GO) terms of upregulated genes across immune cell subpopulations in individual patients. *GO enrichment analysis of upregulated genes is shown for three major cell types: Activated B cells (top row), $CD8^+$ Teff cells (middle row), and $CD16^+$ Mono cells (bottom row), across three longitudinal patient samples: P2 (left column), P4 (middle column), and P7 (right column). The x-axis represents the statistical significance of enrichment expressed as −log10(P-value), The vertical dashed line indicates the significance threshold of P = 0.05. The numbers inside the circles at the end of each gradient bar represent the gene count (the number of upregulated genes enriched in the corresponding GO term).*

Table S3 Adverse event in phase 1 study.

	Number of patients	Number of events
Adverse event related to BBM-H901		
Elevation of aminotransferase	1	2
Adverse event related to prednisone		
Elevation of white blood cell count	7	13
Elevation of neutrophil count	5	9
Rash	3	8
Decreased lymphocyte percentage	4	7
Decreased lymphocyte count	2	2
Nocturia	1	1
Elevation of lymphocyte percentage	1	1
Elevation of red blood cell count	1	1
Elevation of blood pressure	1	1
Discomfort after taking oral corticosteroids	1	1
Elevation of hemoglobin	1	1
Adverse event related to hemophilia		
Skin bruising	2	3
Synovitis in both elbows, knees, and ankles	1	1
urinary tract bleeding	1	1
Adverse event unrelated to treatment		
Cold	3	6
Hyperuricemia	1	3
Finger infection*	1	3
Acute bronchitis*	1	2
Fever	2	2
Upper respiratory tract infection	2	2
Acute gastritis	2	2
Gallbladder polyps	2	2
Chronic apical periodontitis**	1	1
Hyperkalemia	1	1
Sinus bradycardia	1	1
Hypertriglyceridemia	1	1
Rabies exposure	1	1
Papular rash	1	1
Eczema	1	1
Diarrhea	1	1
Gallstones	1	1
Hypercholesterolemia	1	1
Head trauma	1	1
Elevated cytokines	1	1
Avulsion fracture of the lateral margin of the right calcaneus	1	1
Traumatic bleeding of the left back	1	1
Left groin strain	1	1
Traumatic bleeding in the left groin area	1	1
Bruising in the left antecubital fossa	1	1
Elevation of bilirubin	1	1

*These AEs are grade 3 according to the CTCAE.

**This is the only SAE in this study, and unrelated to BBM-H901.

Table S4 Bone Density in phase 1 study.

	Before gene therapy			One year after gene therapy		
P1	Femoral neck (Z- score)	Lumbar 1-4 (Z- score)	Hip (Z- score)	Femoral neck (Z- score)	Lumbar 1-4 (Z- score)	Hip (Z- score)
	-2.3	-2.3	-2.4	-2.1	-2	-2.4
P2	Femoral neck (Z- score)	Lumbar 1-4 (Z- score)	Hip (Z- score)	Femoral neck (Z- score)	Lumbar 1-4 (Z- score)	Hip (Z- score)
	-2.1	-1.5	-2.1	-1.8	-1	-2
P3	Femoral neck (g/cm^2)	Lumbar 1-4 (g/cm^2)	Hip (g/cm^2)	Femoral neck (g/cm^2)	Lumbar 1-4 (g/cm^2)	Hip (g/cm^2)
	0.954	1.098	0.889	0.731	0.897	0.812
P4	Femoral neck (Z- score)	Lumbar 1-4 (Z- score)	Hip (Z- score)	Femoral neck (Z- score)	Lumbar 1-4 (Z- score)	Hip (Z- score)
	-1.2	1	-1.1	-0.9	0.4	-1
P5	Heel (Z- score)			Heel (Z- score)		
	-3			-3.06		
P6	Heel (Z- score)			Heel (Z- score)		
	-2.18			-2.09		
P7	Total (Z- score)			Total (Z- score)		
	-2.5			-2.8		
P8	Total (Z- score)			Total (Z- score)		
	-2.8			-2.1		
P9	Heel (Z- score)			Heel (Z- score)		
	-0.94			-0.39		
P10	Heel (Z- score)			Heel (Z- score)		
	NA			-1.48		
P11	Total (Z- score)			Total (Z- score)		
	0.4			0.7		

Table S5 Binding and Neutralizing Antibody Against AAV843 after Administration in Phase 1 study

	Screening		W1		W2		W4		W12		W26		W52		W78		W104		W130	
	Binding	Neutralizing	Binding	Neutralizing	Binding	Neutralizing	Binding	Neutralizing	Binding	Neutralizing	Binding	Neutralizing	Binding	Neutralizing	Binding	Neutralizing	Binding	Neutralizing	Binding	Neutralizing
1	<1:50	<1:1	< 1:50	1:16	< 1:50	1:2048	1:1600	1:2048	1:25600	1:512	1:12800	1:256	1:3200	1:64	1:3200	1:128	1:1600	1:128	1:3200	1:128
2	1:50	<1:1	1:1600	1:128	1:25600	1:1024	1:25600	1:1024	1:25600	1:1024	1:409600	1:4096	1:102400	1:2048	1:102400	1:2048	1:25600	1:4096	1:51200	1:2048
3	<1:50	<1:1	< 1:50	1:8	1:200	1:1024	1:50	1:1024	1:12800	1:256	1:25600	1:1024	1:25600	1:512	1:6400	1:512	1:12800	1:256	1:6400	1:256
4	<1:50	<1:1	< 1:50	1:16	1:6400	1:1024	1:12800	1:1024	1:25600	1:1024	1:25600	1:1024	1:25600	1:1024	1:12800	1:1024	1:25600	1:1024		
5	<1:50	<1:1	< 1:50	1:16	1:800	1:4096	1:25600	1:4096	1:102400	1:4096	1:51200	1:1024	1:51200	1:2048	1:25600	1:2048	1:51200	1:2048		
6	<1:50	<1:1	1:50	1:16	1:400	1:4096	1:3200	1:4096	1:51200	1:1024	1:25600	1:1024	1:12800	1:512	1:12800	1:512	1:12800	1:512		
7	<1:50	<1:1	< 1:50	1:16	1:3200	1:4096	1:6400	1:4096	1:102400	1:1024	1:51200	1:2048	1:51200	1:4096	1:51200	1:4096	1:51200	1:2048		
8	<1:50	<1:1	< 1:50	1:4	< 1:50	1:1024	1:6400	1:1024	1:51200	1:1024	1:51200	1:1024	1:25600	1:1024	1:12800	1:1024				
9	1:50	<1:1	1:3200	1:128	1:51200	1:1024	1:51200	1:4096	1:25600	1:512	1:25600	1:512	1:6400	1:256	1:6400	1:256				
10	<1:50	<1:1	1:25600	1:1024	1:102400	1:1024	1:51200	1:1024	1:25600	1:512	1:25600	1:512	1:6400	1:512	1:12800	1:512				
11	<1:50	<1:1	< 1:50	1:32	< 1:50	1:1024	1:1600	1:1024	1:25600	1:512	1:6400	1:512	1:6400	1:256	1:12800	1:512				

Protocol 1.0

Background of Study

Background and treatment of the disease

Hemophilia B is an X-linked recessive disorder of coagulation factor IX (FIX) deficiency in the blood. It is a bleeding disorder of coagulation dysfunction. The female carrier usually transmits the mutation gene to the male offspring, causing the latter to become hemophilia B patients. The severity of hemophilia B symptoms is related to the level of FIX in the body. The plasma FIX level of severe hemophilia B patients is less than 1% of normal people, so spontaneous bleeding occurs frequently, such as intra-articular hemorrhage, soft tissue hematoma, intra-abdominal hemorrhage, and cerebral hemorrhage, which eventually leads to severe arthropathy, chronic pain, and seriously affects the quality of life and even life span[1] of patients. The incidence of hemophilia is about 1/25000, and there is no racial or regional difference. According to the data reported by the Chinese Hemophilia Collaborative Group in 2019, there were 18,530 hemophilias registered in China, of which only 2447 were[2] hemophilia B.

At present, FIX replacement therapy is the main treatment for hemophilia B, which mainly includes recombinant FIX, blood-derived FIX and human prothrombin complex (hPCC). However, due to the short half-life in the body, frequent infusion is required to maintain the level of FIX and avoid the lesions of joints and other organs caused by repeated bleeding. Although the newly approved long-acting recombinant FIX product has a longer half-life, which prolongs the frequency of FIX prophylaxis to once a week and provides patients with a more convenient choice of medication, it still does not completely change the current situation that intravenous infusion of FIX biological products is difficult to maintain long-term high concentration of coagulation factor, and patients need to receive frequent injections. In addition, the price of FIX products is high, and the frequent infusion also puts a heavy economic burden on patients and their families, and regular prophylaxis is not popular. The World Hemophilia Federation (WFH) reported in 2018 that the proportion of children under 18 years old receiving regular prophylaxis of FIX products in China was 15%. In addition, patients with FIX products or hPCC need to have strict medication compliance to achieve the therapeutic effect, otherwise, the risk of bleeding will still increase if medication is omitted. Long-term use of blood products such as hPCC also carries the risk of infection with a variety of blood-borne diseases such as hepatitis B, hepatitis C, or HIV. Therefore, researchers at home and abroad have been looking for a way to "cure" hemophilia B, so that patients do not need to receive frequent injections of alternative treatment.

With the breakthroughs in gene and vector research, many gene therapies based on viral vectors (such as adeno-associated virus [AAV], lentivirus, etc.) carrying target genes have been

applied in experimental and clinical studies, and a variety of gene therapy drugs for rare diseases have been marketed.

AAV is a kind of single-stranded DNA virus, which is non-toxic and harmless, and can be used as a vector to carry therapeutic genes to achieve expression in vivo. The engineered AAV, which has removed all the genes of the virus itself, has better biological safety and has been used in gene therapy for more than 5000 genetic diseases and chronic diseases. At present, there are more than 300 clinical trials using AAV as a therapeutic vector, including gene therapy for hemophilia.

Hemophilia B has gradually become a hot field of gene therapy due to its following characteristics:

① Caused by single gene mutation, the pathogenic mechanism is clear;

② The short cDNA length of FIX could be easily carried by AAV vector.

③ A small increase in FIX level can significantly improve the symptoms of the disease. According to the existing clinical studies, increasing plasma FIX to more than 5% of the normal plasma level can significantly reduce the incidence of spontaneous bleeding events in critically ill patients.

④ The effective range of FIX treatment is wide (5%-100% of normal plasma level), and it is not necessary to strictly control the expression level of FIX.

⑤ The main expression organ of FIX is the liver, but a variety of tissue cells can correctly express and modify FIX, and secrete it into the blood;

⑥ Good animal models have been established, including large animal (hemophilia B dog) and small animal (hemophilia B mouse) models.

Gene therapy for hemophilia B is achieved by a single intravenous infusion of the FIX gene carried by AAV vector into target cells (mainly hepatocytes) to achieve long-term stable expression of normal and active FIX protein. At present, the data of clinical trials in Europe and the United States show that AAV is approaching maturity as a gene therapy vector for hemophilia, and is achieving the goal of "one-time treatment and long-term efficacy". However, predicting the treatment cost of millions of dollars per patient is not in line with the current medical status in China. Therefore, to accelerate the development of independent gene therapy drugs in China and reduce the cost of treatment is the ultimate goal of such products.

BBM-H901 injection developed by Shanghai Xinzhi Medical Technology Co., Ltd. is an adeno-associated viral vector drug expressing human coagulation factor IX with independent intellectual property rights. It uses a self-developed AAV843 capsid with high liver targeting. Pada-fix mutant optimized by codon optimization program (GeneArt) for stable expression was carried,

which was a mutation at amino acid 338 of the wild type FIX protein, namely leucine instead of arginine. This mutation significantly increased FIX activity. This mutation has been widely used in several investigational gene therapy drugs. The use of the PADA-FIX mutant both enhances therapeutic efficacy and reduces the amount of gene vector used to minimize possible cellular immune responses. The mechanism of action of BBM-H901 injection is clear. Bbm-h901 injection shows good therapeutic effect in the mouse model of hemophilia B. It is suitable for the treatment of hemophilia B patients with endogenous FIX≤2 IU/ dL (≤2%).

Summary of preclinical studies

AAV843 capsid

The AAV gene transduction vector used in this study was a bioengineered AAV capsid. A library of AAV capsid protein mutants was constructed by DNA shuffling and selected in adult C57BL/6 mice to obtain a novel AAV capsid, AAV843, with high transfection potency in the liver, which greatly enhanced the efficiency of hepatocyte gene transduction in vivo, thereby minimizing the amount of gene vector and possible cellular immune response.

Hepatocyte-specific expression cassette

In this study, the FIX gene was optimized by GeneArt, and the TCG and CGT sequences in the FIX gene were removed without changing the amino acid sequence, so that it could be stably expressed. The padua mutation, which encodes a naturally occurring variant of FIX that is more active than the wild type, was used in the expression cassette to enhance FIX activity[3].

The natural intron length of the FIX gene is relatively large. To further increase the expression of the FIX gene, we synthesized small human introns and inserted them into the 5' untranslated region (5'UTR) and 5' coding region of the vector. The expression cassette was cloned into a mutant double-stranded AAV vector with deletion of the left inverted terminal repeat (ITR) D region. Finally, the adeno-associated viral expression cassette of FIX was obtained.

Brief introduction to preclinical animal studies

A single intravenous injection of BBM-H901 can shorten the activated partial thromboplastin time (APTT) and increase the FIX activity in hemophilia B model B-F9 KO mice, with significant long-term effect and dose-dependent relationship. In the tail vein transection test of hemophilia B model mice, a single intravenous injection of BBM-H901 injection for 8 weeks can significantly reduce the amount of bleeding after tail vein transection, and has obvious and long-term procoagulant effect.

In a safety pharmacology study on central nervous system function, a single intravenous

infusion of BBM-H901 injection had no effect on the central nervous system function of SD rats.

In the general toxicology study, after a single intravenous injection of BBM-H901 injection, the rats were observed for 13 weeks, and it was found that the high-dose group showed pharmacological changes in coagulation function at some time points. There were no abnormalities in hematology, blood biochemistry, immunology, histopathology and other related indexes in each dose group.

In summary, BBM-H901 injection, a gene vector drug independently developed by Shanghai Xinzhi Medical Technology Co., LTD., is an adeno-associated viral vector drug that uses a new AAV capsid with high liver targeting, and is obtained by optimizing the promoter and FIX gene to efficiently express FIX in the liver. The mechanism of action is clear. It shows a high level of transduction activity and therapeutic effect in preclinical in vivo and in vitro studies, with good safety.

Summary of similar international clinical studies

At present, gene therapy drugs based on rAAV technology have been widely used in genetic rare diseases, geriatric degenerative diseases and cancer. According to the data published in Clinical trials, more than 290 clinical trials have been published so far, involving more than 30 single-gene genetic diseases and more than 20 complex diseases, and most of the clinical trials are in the ongoing stage. In the world, five gene therapy drugs for familial lipoprotein lipase deficiency, hereditary retinal diseases and spinal muscular atrophy, aromatic L-amino acid decarboxylase deficiency, and hemophilia A have been published. Glybera, Luxturna (children and adults), Zolgensma (children under 2 years of age), Upstaza (children over 18 months of age), and Roctavian have been approved for marketing.

At present, the clinical studies of hemophilia B have been carried out first in people over 18 years old. The initial trials of gene therapy for hemophilia B with rAAV, which used a cytomegalovirus (CMV) enhancer/promoter and rAAV2 serotype (raAV2-CMV-F9-wt), delivered by skeletal-muscle injection, showed local safe, long-term expression of IX but not systemic, expression[4]. The favorable short - and long-term safety profile of this study led to the development of new liver-targeted products (the liver is a human FIX organ).

Because FIX expression is localized in the liver, a liver-specific promoter gene-therapy agent (rAAV2-hAAT-F9-WT) was developed at the Children's Hospital of Philadelphia and administered

through the hepatic artery to seven subjects. However, the presence of neutralizing antibodies severely suppressed FIX expression, with an initial level of 11% in one subject, but the duration of the expression level was limited to approximately 8 weeks, after which there was a gradual decline in FIX with transient asymptomatic increases in liver aminotransferase levels. Further studies showed that a cell-mediated immune response against the AAV capsid antigen destroyed the transduced hepatocytes, resulting in a decrease in the IX level and the development of transient abnormal liver function. These studies show that human hepatocytes can be transduced with the rAAV-2 vector with therapeutic levels of IX expression in vivo, although future studies in humans may require immunomodulation to achieve long-term stable expression[5].

In the third study, St. Jude Children's Hospital and University College London used rAAV8-LP1-F9-WT to treat[6,7] 10 hemophilia B subjects via a peripheral vein. "In this study, prednisone was administered immediately after the onset of abnormal liver function after the first treatment and demonstrated that the administration of this drug significantly counteracted the decrease in FIX expression." In addition, the study found that the abnormal liver function of the subjects was dose-dependent, with the number of subjects with abnormal liver function increasing as the dose increased.

The above studies provide reliable support for the further optimization of gene therapy drugs and clinical treatment strategies for hemophilia B.

Shire/Baxalta/Takeda conducted a phase I/II clinical trial using AAV8 carrying the FIX Padua variant (BAX335). BAX335 contains an expression cassette with liver-specific promoter elements. Three doses of vector were used to treat a total of eight subjects. The median peak value of FIX in the low (2×10^{11} vg/kg), middle (1×10^{12} vg/kg), and high (3×10^{12} vg/kg) dose groups was 3.5%, 12.0%, and 45.0%, respectively. One subject achieved sustained therapeutic FIX:C~20% without bleeding or replacement therapy for 4 years; In the others, FIX activity persisted for no more than 5 to 11 weeks. In contrast to the results of some previous studies, corticosteroid treatment did not counter the loss of FIX activity. The loss of gene expression may be due to the elicitation of an innate immune response[8].

In a phase I/II trial funded by Dimension Therapeutics, AAVrh10 serotype vector (DTX101) containing codon-optimized wild-type F9 cDNA was infused intravenously in six subjects at increasing doses (1.6×10^{12} vg/kg and 5×10^{12} vg/kg). Dimension Therapeutics ultimately decided to discontinue the trial because the phase I/II open-label clinical study did not show that the minimal goals for continued development or future commercialization would be met. The company continues to follow and closely monitor six hemophilia B subjects treated with DTX101 through a five-year extension study (NCT02971969).

The above study, part of the earlier exploration of AAV gene therapy for hemophilia B, provides a wealth of experience for subsequent studies. At present, Freeline's product FLT180a, Spark/Pfizer's product SPK-9001, and uniQure's product AMT-061 are also mutant FIX Padua AAV drugs in the world and are highly developed.

Freeline product FLT180a

The Freeline product FLT180a (AAV2/S3-HLP2-Ti-FIXco) uses a novel AAVS3 capsid that specifically transduces the FIX-Padua gene to hepatocytes. Phase I/II dose-finding study B-AMAZE (NCT03369444) involved 10 patients. After a single infusion of FLT180a at different doses (3.84×10^{11} vg/kg N=2, 6.4×10vg/kg N=2, 8.32×10^{11} vg/kg N=4, 1.28×10^{12} vg/kg N=2), all subjects had a dose-dependent increase in FIX activity. During a median follow-up time of 27.2 months (range: 19.1 to 42.4), sustained FIX activity was observed in all subjects except one subject who restarted FIX prophylaxis. As of September 20, 2021, 5 subjects had factor activity levels between 51% and 78%, 3 subjects had factor activity levels between 23% and 43%, and 1 subject reached 260%. A total of 287 aes were reported in 10 subjects, of which FLT180A-related aes were approximately 10% (29 aes), and immunosuppressant aes were approximately 24% (glucocorticoid-related aes were 35 aes, tacrolimus related aes were 36 aes). Adverse events related to FLT180a occurred in all three dose groups except two in the low-dose group. The most common adverse events related to FLT180a were in the presence of elevated liver aminotransferase levels. Other symptoms included fatigue/discomfort, high FIX level, muscle spasms/musculoskeletal pain/myalgia, dyspepsia/belching, decreased FIX level, pulmonary sepsis, drowsiness, and arteriovenous fistula thrombosis in subjects with high FIX activity level. A total of 17 serious adverse events occurred in 7 subjects, of which 12 were related to FLT180a, including elevated transaminase (9 times), arteriovenous fistula (1 time), decreased FIX activity (1 time), pulmonary sepsis (1 time), and immunosuppressant related 4 times. The main adverse events included epigastric pain (1), elevated amylase (1), elevated troponin (1), and tacrolimus toxicity (1)[9]. For safety reasons, based on the previous trial, Freeline continued to adjust the dosage and carried out the B-LIEVE study (NCT05164471) to explore the safe and effective key trial dose. At the 2022 International Society of Thrombosis and Hemostasis meeting, the latest data were announced: three subjects were enrolled at present, and the infusion dose was 7.7×10^{11} vg/kg, and the maximum infusion dose did not exceed 6.93×10^{13}vg (a dose of 90 kg body weight). Until May 23, 2022, the three subjects were followed up for 77 days, 56 days, and 36 days, respectively. FIX activity was 93 IU/dl, 92 IU/dl, and 80 IU/dl in 3 subjects, respectively. After infusion, there was no bleeding and no use of FIX products. Transaminase elevation occurred in all 3 subjects after infusion, and 2 of them needed hormone and tacrolimus intervention.

At present, FLT180a has not yet been registered in phase III clinical trials, and no marketing plan has been reported.

Pfizer /Spark product SPK-9001

The Pfizer /Spark Therapeutics product SPK-9001 was conducted in a hemophilia BI/II phase I/II clinical trial to investigate the safety and kinetics of an AAV vector expressing the FIX Padua mutant (SPK-9001). This vector was infused in a single intravenous dose of 5×10^{11} vg per kilogram and was accompanied by a 3-to 5-fold increase in the dose of a shell AAV for partial antibody binding to mitigate humoral immunity and enhance gene transduction of its product. A mean of ABR11.1 (range, 0-48) before administration and 2908 IU/kg (range, 0-8090 IU) of FIX product were used 1 week after infusion. FIX:C levels were detected and steady-state expression was achieved within 14 weeks. The 10 subjects who received SPK-9001 infusion had a mean FIX:C level of 33.7%±18.5% (range: 14.3% to 76.8%) over 52 weeks, and 8 of the 10 subjects completely discontinued FIX product replacement therapy. The mean ABR after infusion was 0.4 (0-4), and the mean FIX use was 49.2IU/kg (range: 0-376 IU). The infusion did not cause serious adverse events such as FIX inhibitor production or thrombosis. Two subjects had elevated alanine aminotransferase (ALT) levels and were treated [10]with steroids.

SPK-9001 was registered on clinical.gov in the global multi-center phase III clinical trial NCT03861273, planned to participate in 41 centers, distributed in the United States, European Union countries (France, Germany, Greece, Sweden), the United Kingdom, Australia, Brazil, Canada, Japan, Saudi Arabia, Turkey, and Taiwan China. Using the same dose as I/II, an international multicenter trial is planned to enroll 43 patients and observe efficacy and safety in an expanded sample size. The registration trial starts in July 2019, and no phase III data has been published, nor has it been reported in the marketing plan.

uniQure products AMT-060 and AMT-061

The phase I/II uniQure Biopharma study in hemophilia B used AAV5 as the delivery vector to express wild-type FIX (AMT-060) at doses of 5×10^{12}vg per kilogram and 2×10^{13}vg per kilogram, respectively. Ten subjects, 5 in each group, were enrolled, and all treated subjects showed stable FIX levels over 2.5 years of follow-up, with an increase in FIX:C to 4.4 IU/dl and a decrease in ABR from 9.8 to 4.6 (53%) in the low-dose cohort. In the high-dose cohort, the mean FIX activity increased to 6.9 IU/dL, and the ABR decreased from 3.0 to 0.9 (70%). In terms of safety, three subjects had elevated ALT levels and were treated with steroids within about 10 weeks after AAV injection, but there was no significant decrease[11] in FIX:C. The AMT-060 clinical trial showed a favorable safety profile, but efficacy was modest because it expressed wild-type FIX.

uniQure also developed an AAV5 viral vector with the FIX-Padua (AMT-061) cassette, which treated three subjects with AMT-061 at a dose of 2×10^{13}vg/kg. Reported week 26 data showed that mean FIX activity was 31% (23.9%-37.8%) at week 6 and increased to 47% (33.2%-57.0%) at week 26, with two subjects showing sustained FIX activity of >40%; In terms of safety, AMT-061 was well tolerated, with no clinically significant elevations in liver aminotransferase levels observed and no requirement for treatment-related corticosteroids[12]. On this basis, AMT-061 has been registered in a global multi-center phase III clinical trial (NCT03569891) in October 2018, with 35 research centers around the world. The countries and regions participating in the clinical research involve the United States, the European Union (Belgium, Denmark, Germany, Ireland, Italy, the Netherlands, Sweden), and the United Kingdom. At the 2022 European Hemophilia and Related Disorders Association annual meeting, uniQure presented the 6-month and 18-month data of a phase III clinical trial, which enrolled 54 subjects, all receiving 2×10^{13} vg/kg AMT-061 infusion. The mean FIX activity was 36.9 IU/dL at 6 months and 39.0 IU/dL at 18 months after a single dose of AMT-061 infusion. At 6 months after infusion, the annualized bleeding rate was reduced by 64% in all subjects and by 77% in the 7-to 18-month range. The drug was well tolerated, with no treatment-related serious adverse events, no reported FIX inhibition, and no correlation between safety-preexistent neutralizing antibody titers was observed.

uniQure has submitted a regulatory application to the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA), which has accepted the application, but no approval has been reported.

In summary, Freeline's FLT180a, Spark's Spark 9001, uniQure's AMT-061, and Xinzhi's BBM-H901 are the most advanced AAV drug development companies and products in hemophilia B. In different rAAV clinical trials, In different raAV clinical trials, the mutant FIX Padua did not show significant immunogenicity or spontaneous thrombosis in humans.

Summary of clinical study of BBM-H901 injection

An investigator-initiated clinical trial (IIT) of BBM-H901 (NCT04135300) was initiated in 2019, and the study was approved by the Ethics Committee of Institute of Hematology and Blood Diseases, Chinese Academy of Medical Sciences. This was a single-center, single-arm, open-label study of adeno-associated viral vector-expressed human coagulation factor IX gene therapy in patients with hemophilia B. We evaluated the safety, tolerability, and pharmacokinetics of a single intravenous infusion of BBM-H901 in patients with hemophilia B and endogenous factor IX activity levels of 2 IU/dl or less.

Data, published in the Lancet Haematology International Journal on May 19, 2022, showed[13] that 10 subjects were treated with 5×10^{12} vg/kg and followed for a cumulative period of 705 weeks after infusion (time range: During a median follow-up of 58 weeks, the mean FIX:C was 36.93 ±20.49 IU/dl, no serious adverse events occurred, and no FIX inhibitors were detected. Nine subjects had no bleeding events after infusion, and only one subject had a non-serious old bleeding event within 2 years of follow-up. The mean FIX:C level remained above 30IU/dl. Two subjects had asymptomatic aminotransferase elevations associated with decreased FIX:C levels, but the mean FIX:C level remained higher than 10 IU per deciliter.

BBM-H901 injection is also the first intravenous AAV gene therapy drug for hemophilia approved to enter the registered clinical trial in China. The first registered study adult subject was successfully administered in December 2021, and the trial is currently progressing smoothly.

Benefit/risk assessment

This study was designed to evaluate the safety, tolerability, and efficacy of BBM-H901 injection in the treatment of patients with hemophilia B. The expected benefits mainly include a reduction in the rate of bleeding and the frequency and volume of exogenous FIX protein products needed for on-demand or prevention of bleeding, a reduction in the number of target joints, an improvement in the quality of life of patients, the possibility of lifelong cure through a one-time infusion of genetic drugs, and a reduction in the economic burden of patients. AAV gene therapy can be used to prevent bleeding in children under 18 years old as early as possible, which may avoid joint deformity, improve the quality of life of children, and enable them to return to normal study and life.

The expected risks include allergic reactions caused by AAV vector gene product infusion,

the development of inhibitors, the risk of thrombosis when FIX activity increased to ≥150%, the elevation of liver transaminase caused by immune response, and bleeding due to lack of efficacy.

Objectives of the study

Main Objectives:

- To evaluate the safety and tolerability of a single intravenous infusion of BBM-H901 in male hemophilia B patients (12 years ≤ age <18 years) with endogenous factor IX (FIX) activity ≤2 IU/dL (i.e.

Secondary objectives:

- To evaluate the pharmacokinetics of PADA-FIX and adeno-associated viral (AAV) vectors after a single intravenous infusion of BBM-H901 in hemophilia B patients (12 years ≤ age <18 years) with endogenous FIX activity ≤2%.
- To evaluate the efficacy of a single intravenous infusion of BBM-H901 in patients with hemophilia B (12 years ≤ age <18 years) with endogenous FIX activity ≤2%.

Objectives of the long-term follow-up phase:

- To evaluate the long-term efficacy and safety of a single intravenous infusion of BBM-H901 injection.

Study DESIGN

Overall design

This study was a single-center, single-arm, open-label design to investigate the safety, tolerability, pharmacokinetics, and efficacy of a single intravenous infusion of BBM-H901 in patients with hemophilia B (12 years ≤ age <18 years) with endogenous factor IX (FIX) activity ≤2 IU/dL (i.e., ≤2%).

We planned to enroll 9 evaluable subjects, who were divided into 3 age groups (group 1, group 2, and group 3) according to the age from the oldest to the youngest, and were enrolled from the oldest to the youngest. Group 1 will include 3 subjects aged <18 years and ≥16 years; Group 2 included 3 subjects aged <16 years and ≥14 years; Group 3 will include 3 subjects aged <14 years and ≥12 years.

First, the BBM-H901 injection infusion study was conducted for the subjects in group 1. One sentinel subject was set up to complete at least 2 weeks of observation after administration, and the researcher comprehensively judged whether to continue the treatment of the other 2 subjects in the group.

The last patient in group 1 could be evaluated 10 weeks after the completion of the study, and SRC would make a comprehensive judgment on whether to enter the study in group 2. One sentinel subject was also set up in group 2, who completed at least 2 weeks of observation after drug administration. The investigator comprehensively judged whether to continue the treatment of the other 2 subjects in the group.

The last patient in group 2 could be evaluated 10 weeks after the completion of the study, and the investigator comprehensively judged whether to enter the study in group 3. One sentinel subject in group 3 was observed for at least 2 weeks after administration, and SRC comprehensively judged whether to continue the treatment of the other 2 subjects in the group.

The last evaluable subject in group 3 completed the 10-week follow-up, and the safety and tolerability were comprehensively judged by SRC. The drug will not be continued in other age groups.

Participants who complete the 52-week assessment will continue to be followed for 5 years to obtain long-term assessment data.

Participants were followed for approximately 5 years after enrollment in the study, including

a screening period of up to 4 weeks (start of immunomodulatory therapy on day -7, baseline examination on day -1), a follow-up period for the first 52 weeks after infusion (weekly visits at D3, D6, weeks 2-8, and 6, respectively), and a follow-up period of up to 5 weeks after infusion. Every 2 weeks from week 10 to week 18, every 4 weeks from week 22 to week 26, every 10 weeks from week 32 to week 42 to week 52, and a long-term follow-up period beyond week 52 (every 6 months) for an additional 4 years.

Study END POINTS

Primary endpoints:

（1） The incidence of dose-limiting toxicity (DLT) events determined by the safety data review Committee (SRC) within 10 weeks after infusion of BBM-H901 injection;

（2） The incidence of adverse events and serious adverse events during the first 52 weeks after BBM-H901 infusion;

（3） Changes in liver function (including alanine aminotransferase [ALT] and aspartate aminotransferase [AST]) before and after treatment within 52 weeks after BBM-H901 infusion.

Secondary endpoints:

（1） Pharmacokinetic parameters of Padua-FIX and rAAV vectors after BBM-H901 infusion:

- Pada-fix activity level, including peak activity level, reached steady state or average activity level within 52 weeks after BBM-H901 infusion.
- AAV vector shedding in plasma, urine, feces, saliva and peripheral mononuclear cells (PBMCs) within 52 weeks after BBM-H901 infusion;

（2） Efficacy end points for BBM-H901 injection, including:

- The annualized bleeding rate (ABR) within 52 weeks after BBM-H901 injection infusion (including spontaneous bleeding, traumatic bleeding, joint bleeding, etc.);
- FIX activity level at each visit within 52 weeks after BBM-H901 infusion;
- The number and volume of other FIX protein products (including recombinant and plasma-derived FIX protein products, such as recombinant human coagulation factor IX injection, prothrombin complex, etc.) infused within 52

weeks after BBM-H901 injection;

- The number of target joints within 52 weeks after BBM-H901 infusion;
- The number of joint bleeding within 52 weeks after BBM-H901 injection infusion;
- The proportion of subjects without bleeding events within 52 weeks after BBM-H901 injection infusion;
- BBM - H901 injection infusion after 4 weeks, 12 weeks, 26 weeks, 52 phase for baseline joint health score, arthritis and ultrasonic score and the change of the quality of life, with hemophilia assessment scale, including: Hemophilia joint health score (HJHS), hemophilia early oa ultrasonic testing score (HEAD-US-C) and Canadian hemophilia children prognosis and quality of life assessment tool (CHO-KLAT);

（3） Other safety end points for BBM-H901 injection:

- Incidence of FIX inhibitor (based on Bethesda method or Nijmegen corrected Bethesda method) within 52 weeks after BBM-H901 infusion;
- AAV capsid-neutralizing antibody and capsid-binding antibody titers;
- There were clinically significant changes in vital signs, physical examination, clinical laboratory tests, 12-lead electrocardiogram, and alpha-fetoprotein levels within 52 weeks after BBM-H901 infusion.

Long-term follow-up endpoints:

Long-term follow-up end points after BBM-H901 infusion included, but were not limited to:

- ABR (including spontaneous bleeding, traumatic bleeding);
- Padua-fix activity;
- The number and number of other FIX protein products (including recombinant and plasma-derived FIX protein products) infused per year;
- The annualized number of bleeding events of different types, including spontaneous bleeding, traumatic bleeding, bleeding without treatment, etc.
- Number of target joints;
- Joint bleeding frequency;

- rAAV vector viral load;
- The incidence of adverse events and serious adverse events;
- FIX inhibitor incidence;
- rAAV neutralizing antibody and capsid-binding antibody titers;
- There were clinically significant changes in vital signs, physical examination, clinical laboratory tests, 12-lead electrocardiogram and alpha-fetoprotein (AFP).

Basis for dose selection

Most of the clinical studies of adeno-associated virus gene therapy for hemophilia B in the world have selected the dose between 5×10^{10}~2×10^{13} vg/kg, and the expression and activity of FIX factor increased with the increase of the dose. But because of difference of AAV capsid use and restructuring FIX expression element of different lead to the different treatment effect. FIX gene expression levels ranged from 5% to 40%. In the IIT studies and registered clinical studies of BBM-H901 injection in the treatment of patients with hemophilia B≥18 years old, 5×10^{12} vg/kg was used as the starting dose. According to the above data, considering that the age of the subjects in this study ranged from ≥12 years old to < 18 years old, which was the characteristics of older children, 5×10^{12} vg/kg was also selected as the dose used in this clinical study. The actual infusion volume of the subjects was calculated according to their body weight.

Definition of dose-limiting toxicity

The severity of adverse events will be evaluated and graded according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), version 5.0 (v5.0). DLT defined as BBM - at 10 weeks after H901 infusion (happened, the researchers determine the following adverse events associated with BBM - H901 injection:

（1） Non-hematologic toxicities:

- ≥ grade 3 total bilirubin elevation;
- Grade 3 elevation of alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST), which could not return to baseline after 4 weeks of immunosuppressive therapy;
- Grade 4 elevated ALT and/or AST;
- Grade 3 or 4 anaphylaxis, such as bronchospasm and immediate anaphylaxis;
- Pada-fix activity ≥150% and investigator-judged thrombotic events related to

BBM-H901 injection (excluding grade 4 infusion site thrombophlebitis);

- QTcF interval prolongation (absolute value >500 ms or prolongation >60 ms from baseline);
- Grade 3 vomiting or diarrhea that persists for >3 days despite adequate medical therapy;
- Grade 4 (life-threatening) vomiting or diarrhea, regardless of the duration of the event;
- Other nonhematologic toxicities of grade ≥4.

（2） Hematologic toxicities:

- Grade 4 neutropenia lasting >5 days (neutrophil count $<0.5 \times 10^9$/L);
- Grade 3 febrile neutropenia (neutrophil count $<1.0\times10^9$ per liter with a single temperature of >38.3 °C or a sustained temperature of ≥38 °C for more than 1 hour);
- Grade 4 thrombocytopenia (platelet count $<25.0\times10^9$/L) or grade 3 thrombocytopenia (platelet count $<50.0 \times10^9$/L) with bleeding;
- Grade 4 anemia.

（3） Any other clinically significant or unacceptable toxicity that was deemed DLT by the sponsor or investigator.

The following are not DLTS:

- Hair loss of any grade;
- Isolated laboratory test changes of any grade with no clinical sequelae or clinical significance.

Safety data review Board

A safety data review committee (SRC) will be established and is planned to be composed of investigators and other relevant experts. Any unexpected situation during the study will be discussed and decided by the SRC. The safety, tolerability and efficacy data obtained were discussed through teleconsultation, web conference and email feedback, and opinions on whether to enroll the next dose or whether to suspend the study were given.

Study outcome measures

Safety and tolerability indicators included DLT, adverse events, serious adverse events,

vital signs, physical examination, clinical laboratory tests (blood routine test, blood biochemistry test, urine routine test, coagulation function test), 12-lead electrocardiogram, FIX inhibitor test, AAV capsid neutralizing antibody and capsid binding antibody test, etc.

Pharmacokinetic parameters of BBM-H901 injection: Samples were collected at the specified sampling points and assessed for Padua-FIX activity levels, FIX antigen levels, and analysis of AAV viral vector shedding.

Efficacy Measures:

1) ABR (including spontaneous bleeding, traumatic bleeding, joint bleeding, etc.);
2) Padua -FIX activity level;
3) The number and volume of other FIX protein products (including recombinant and plasma-derived FIX protein products, such as recombinant human coagulation factor IX injection, prothrombin complex);
4) Number of target joints;
5) Hemophilia assessment related scales (HJHS, HEAD-US-C and CHO-KLAT).

Study suspension/early termination

The ethics committee and investigators have the right to suspend or prematurely terminate part of the clinical study or the entire clinical study.

If such a decision is made by the ethics committee, the investigator should immediately report it to the clinical research institution with detailed written explanations.

If the decision was made by the investigator, the investigator should immediately report it to the clinical research institution and the ethics committee and provide detailed written explanations.

Causes of study suspension/early termination include, but are not limited to:

- New information leading to a judgment of risk benefit against studying AAV gene therapy agents, for example, as a result of:
  - Deaths that were judged by the investigators to be related to the BBM-H901 injection occurred during the study period;
  - When the following occurred during the study and because the subject could not tolerate BBM-H901 injection:
    a） Grade 3 or 4 toxic effects (excluding elevated aminotransferase levels) that were deemed by the investigator to be related to the BBM-H901 injection, according to

the NCI CTCAE v5.0, such as bronchospasm, immediate anaphylaxis, and other adverse events.

b） Grade 3 elevations in aminotransferase levels (including ALT and/or AST) that were investigator-judged to be related to BBM-H901 injection according to NCI CTCAE v5.0 and that did not return to baseline after 4 weeks of immunosuppressive therapy;

c） Grade 4 aminotransferase elevations (including ALT and/or AST) that were investigator-judged to be related to BBM-H901 injection, according to NCI CTCAE v5.0;

d） Pada-fix activity level ≥150% and BBM-H901 injection-related thrombotic events after vector infusion (except grade 4 infusion site thrombophlebitis);

- There was a lack of efficacy of the gene carrier agent, either in this study or in other studies;
- The occurrence of a significant previously unknown adverse effect or an unexpected known adverse effect of high severity or incidence;
- Other adverse safety findings, including clinical examination and nonclinical findings;

- It is not reasonable to continue the aforementioned study for medical or ethical reasons;
- It is unlikely that the study will be completed within an acceptable time frame due to difficulties in enrolling subjects;
- Suspension or termination due to regulatory requirements.

Definition of end of study

The end of the study was defined as the completion of the last scheduled early termination.

Study flow chart

Study flow chart1

Project	Screening period		Administration period	Follow-up Period 1																				Long-term follow-up
				1 week (±1 day)		2 to 18 weeks (±2 days)													22 to 52 weeks (±7 days)					Every 6 months ±14 days/early withdrawal
Time (weeks)	4 weeks	-1 day	0 days	1		2	3	4	5	6	7	8	10	12	14	16	18		22	26	32	42	52/ Early exit	
				Day 3	Day 6																			
Informed consent form	X																							
Entry/exclusion criteria audit	X	X																						
Demographic characteristics	X																							
History and treatment of hemophilia B	X																							
Other medical history and treatment history	X																							
Physical examination	X	X			X	X		X		X		X		X		X			X	X	X	X	X	X
Height, weight	X	X																						
Vital signs	X	X	X		X	X		X		X		X		X		X			X	X	X	X	X	X
Blood routine, blood biochemistry, urine routine	X	X			X	X		X		X		X		X		X			X	X	X	X	X	X
ALT, AST		X		X			X		X		X													
Coagulation function	X	X			X	X		X		X		X		X		X			X	X	X	X	X	X
Infectious disease screening	X																							

Project	Screening period		Administration period	Follow-up Period 1																			Long-term follow-up
				1 week (±1 day)		2 to 18 weeks (±2 days)													22 to 52 weeks (±7 days)					Every 6 months ±14 days/early withdrawal
Time (weeks)	4 weeks	-1 day	0 days	1		2	3	4	5	6	7	8	10	12	14	16	18	22	26	32	42	52/ Early exit	
				Day 3	Day 6																		
Genetic testing for hemophilia	X																						
A 12-lead ECG	X	X	X										X						X			X	X
Blood type	X																						
Immunomodulatory therapy	←										→												
BBM-H901 injection infusion			X																				
FIX protein product infusion log fill in	←																						→
Bleeding assessment	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Target joint count assessment	X	X																	X			X	X
Abdominal B ultrasound	X																		X			X	X
FIX activity assay	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FIX inhibitor detection	X	X				X		X				X		X		X			X	X	X	X	X
Scale assessment		X						X						X					X			X	
AAV capsid neutralizing antibody assay	X	X			X	X		X						X					X			X	X
AAV capsid-binding antibody assay	X	X			X	X		X						X					X			X	X

Project	Screening period		Administration period	Follow-up Period 1																		Long-term follow-up
				1 week (±1 day)		2 to 18 weeks (±2 days)												22 to 52 weeks (±7 days)					Every 6 months ±14 days/early withdrawal
Time (weeks)	4 weeks	-1 day	0 days	1		2	3	4	5	6	7	8	10	12	14	16	18	22	26	32	42	52/ Early exit	
				Day 3	Day 6																		
Alpha-fetoprotein	X																		X			X	X
Samples of PBMCs, plasma, saliva, urine, and stool were collected		X			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse events	←																					→	X
Combined medications	←																					→	X

Note: ALT: alanine aminotransferase; AST: aspartate aminotransferase; FIX: coagulation factor IX; AAV: adeno-associated virus; PBMCs: peripheral blood mononuclear cells

1. Additional visits (including those not included in the flow chart) and/or additional unscheduled visits may be made based on actual safety and efficacy assessments, including but not limited to: Liver transaminase, FIX activity, FIX antigen, blood lipids (total cholesterol [TC], high density lipoprotein ([HDL], low density lipoprotein [LDL], very low density lipoprotein [VLDL], triglyceride [TG]); Coagulation function, thrombin generation assay (TGA), hepatitis B DNA quantification, hepatitis C RNA quantification, serum interferon, lymphocyte subsets (B/T/NK), etc.

2. The information of all subjects participating in the screening, including the reasons for screening failure, was collected.

3. 96 hours before you BBM - H901 injection infusion or five half-life (will be subject to the elders) products and shall not use other FIX proteins in the plasma concentrations. Study drugs could not be thawed without confirmation from the investigator regarding washout criteria for the subject.

4. Participants demographic information collection (including gender, age, nationality, birth status);

5. The subject's history of hemophilia (including date of diagnosis, severity, genotype, human leukocyte antigen [HLA, if available], previous use of FIX products (including recombinant and plasma sources, etc.) was collected. Such as human recombinant coagulation factor IX, including injection, prothrombin complex type continuous infusion (on-demand, prevention, etc.), dosage regimen, dosage, infusion times], bleeding times, etc.), determine retrospectively recent 26 weeks, 52 weeks of bleeding times (including spontaneous hemorrhage, traumatic hemorrhage, don't need to FIX the product treatment of bleeding). Record as faithfully as possible, and estimate if

necessary.

6. Other history and history of treatment include: history, family history, and current history of allergies, surgery and surgical plan, smoking, drinking, eating habits, family planning, vaccination; 3 months prior to screening history of blood donation, to participate in clinical trials, in addition to this study disease of other drugs and treatment, and so on and so forth.

7. Physical examination: only screening period for regular physical examination, including skin/mucosa, lymph nodes, the head, neck, chest, abdomen, musculoskeletal/limbs, nerve/spirit, only for the rest of the visit signs/symptoms leading medical examination. Investigators could increase the frequency of examination based on safety concerns.

8. Height and weight were measured only at screening and weight only at the remaining visits. The body weight measured on day -1 was used to calculate the dose of BBM-H901 injection.

9. Vital signs: including blood pressure, body temperature, pulse, breathing rate, the subjects seat resting at least 5 min, the measurement of vital signs. In addition to routine visits for vital signs, vital signs were measured on day 0 before the injection of FIX protein products. If vital signs exceeded the normal range or had clinically significant changes and were deemed by the investigator to require repeat measurements, two additional measurements were required, with an interval of approximately 2 minutes between each measurement. Investigators could increase the frequency of measurements on the basis of safety concerns.

10. Blood routine, biochemical and blood routine urine: if within 7 days before the BBM - H901 infusion carried out by the laboratory tests, the first 1 day can undertake no longer; If blood biochemical tests are required on day-1, ALT, AST, and GGT on day-1 may not be repeated. Investigators could increase the frequency of testing during the trial on the basis of safety concerns.

11. Coagulation function: including plasma prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), plasma fibrinogen (FIB), D-dimer. If this test is performed within 7 days prior to BBM-H901 infusion, it may not be performed again before the infusion of BBM-H901 injection on day-1. Investigators could increase the frequency of testing based on safety concerns.

12. Infectious disease inspection include: hepatitis b virus check (HBsAg and HBV DNA quantitative detection) and hepatitis c virus check (HCV - Ab, HCV - RNA quantitative detection) and HCV viral load (if any), human immunodeficiency virus (HIV) antibody (HIV - Ab) inspected, syphilis antibodies.

13. For genotypes known subjects, such as can always obtain genotype for report, the screening period can no longer detect genotype; For unknown genotype of subjects, the sample drawn in screening for analysis.

14. 12-lead ECG: The 12-lead ECG should be performed in the supine position after the subject has rested in the supine position for at least 5 min. Electrocardiograms were obtained for heart rate, PR interval, QRS interval, uncorrected QT interval, and QTc interval corrected with Fridericia's formula (QTcF). When abnormal ecg measurements

(including QTcF abnormalities, such as QTcF > 500 ms or increase relative to baseline > 60 ms) or by the judgment of abnormal clinical significance, an extra two ecg measurement, repeated measurement should be at least 2 min interval. Dosing the 12-lead electrocardiogram in BBM - H901 within 1 hour after infusion of fluids. The frequency of testing could be increased appropriately during the study because of safety concerns.

15. Prophylactic use of oral glucocorticoids (prednisone or prednisolone) was to be used 1 week before and for 8 weeks after infusion of BBM-H901. Specific treatment in this research plan.

16. Infusion logs: signed informed consent during the start to the end of the study subjects FIX protein product usage, including the cause, commencement date, type of treatment (on-demand, prevention, and continuous infusion, etc.), frequency and dose.

17. Assessment of target joint number: The history of target joints within 52 weeks before screening was collected, and the number of target joints was evaluated within 52 weeks after infusion. Definition for six consecutive months target joint, individual joint happened three times or more than 3 times of spontaneous bleeding. If the joint bleeding 2 or less time for 12 months, then the joint is no longer regarded as target joints. Investigators could increase the frequency of examinations based on safety concerns.

18. Abdominal ultrasound showed liver, gallbladder, pancreas, spleen and kidney. Investigators may increase the frequency of ultrasound examination according to safety considerations.

19. FIX is required for the process of activity evaluation subjects were suspended after accepting BBM - H901 injection infusion FIX protein product preventive treatment, until the researchers suggest that recovery; Such as hemorrhage occurs in the process of research subjects by the researchers determine need on-demand use FIX protein product, in the assessment of FIX activity should guarantee meet FIX protein product before at least 96 hours or five half-life of longer (to) the washout period. FIX activity levels were detected by two methods in one-stage method (HisM platform, Actin FSL aPTT reagent and Wofin platform, HemosIL SynthASil reagent).

20. FIX inhibitor: adopting the Bethesda test or Nijmegen improved Bethesda method testing inhibitor.

21. Collection of PBMCs, plasma, saliva, urine, and feces samples: samples were collected for vector shedding detection, and quantitative polymerase chain reaction (qPCR) was used to detect virus titers in plasma, PBMC, saliva, urine, and feces. Until three consecutive test results were negative.

22. Adverse events: signed informed consent from the book and began to collect adverse events, until the 52 weeks after the treatment + 7 days for follow-up of patients with early exit or start other gene medication (shall be subject to occur earlier), until all the adverse events were adverse events or restoring, stable or disease, or the event can be explained, or patients died, or lost to follow-up. Only adverse events related to the study drug were collected in the long-term follow-up phase, which could be followed up to 5 years/early withdrawal.

23. Concomitant medications and concomitant therapies during the screening period and the study-treatment period were recorded until 52 ±7 weeks after the dose was

administered or until early withdrawal from follow-up or initiation of another genetic agent, whichever occurred first. During the long-term follow-up period, information on medication use related to hemophilia treatment was collected only.

Study Subjects

Inclusion Criteria

To be eligible for the study, all of the following criteria were met:

1. Subjects and/or their legal guardians were fully aware of the purpose, nature, methods and possible adverse reactions of the study, volunteered to be subjects, and signed informed consent.
2. The age and sex requirements were as follows: 12 years old ≤ patient age <18 years old, male;
3. If the patient is clinically diagnosed with hemophilia B and the endogenous FIX activity level is ≤2 IU/dL (≤2%), if the FIX activity level >2% at screening is caused by insufficient elution of FIX protein products, it can be retested after full elution.
4. Treatment exposure days (EDs) of receiving any recombinant and plasma-derived FIX protein products ≥75;
5. The titer of anti-BBM-H901 capsid neutralizing antibody was ≤1:4, and the titer of capsid-binding antibody was ≤1:200.
6. A bleeding event and/or injection of FIX products (both recombinant and plasma-derived) within 12 weeks prior to screening was listed in the subject's medical record;
7. No history of allergic reactions or allergies related to the administration of FIX or intravenous human immunoglobulin;
8. No FIX inhibitors and no previous history of having a FIX inhibitor after FIX product 75EDs; There were no clinical signs or symptoms of decreased response to FIX product infusion;
9. Obtain acceptable laboratory values during screening and before dosing (Day-1) :
    a) Hemoglobin ≥11 g/dL or ≥ the upper limit of normal for peers
    b) Platelet count ≥100×10^9/L
    c) AST and ALT≤1.5 times the upper limit of normal

d) Total bilirubin ≤1.5 times upper limit of normal

e) eGFR≥60 ml/ min

10. For older underage subjects and their legal guardians, they should be clearly aware of the contraceptive requirements, avoid sexual life or use reliable contraceptive measures.

11. The subjects and their legal guardians have the willingness to participate in the clinical research of "gene therapy", and can be followed up regularly. During the follow-up period, the subjects can accurately complete the diary of the subjects, and family members or caregivers can help the subjects to carry out the study.

Exclusion Criteria

Exclusion from the study was required if any of the following criteria were met:

1. Positive for hepatitis B surface antigen (HBsAg) or hepatitis B virus DNA (HBV-DNA), positive for hepatitis C virus antibody (HCV-Ab) or hepatitis C virus RNA (HCV-RNA). Subjects with a history of hepatitis B or C were considered as negative if they had two previous sample collection with an interval of at least 3 months.
2. Receiving antiviral therapy for hepatitis B or C;
3. Have coagulopathy other than hemophilia B;
4. Use of any systemic immunosuppressive agent other than glucocorticoids and investigator-recommended immunosuppressive agents within 30 days before screening; Known allergic to immunosuppressants such as glucocorticoids and tacrolimus;
5. A history of vaccination within 30 days before screening or a planned vaccination during immunomodulatory therapy;
6. Underlying liver disease, such as a previous diagnosis of portal hypertension, splenomegaly, hepatic encephalopathy, or liver fibrosis of stage ≥3; Or nodules or cysts detected by B-ultrasound, or elevated alpha-fetoprotein detected by laboratory tests, etc. These abnormal conditions are judged by the investigator to be clinically significant and the subject is not suitable for the study.
7. Planned surgical procedures for the 52-week study period after the infusion of BBM-H901

were determined;

8. The presence of an acute/chronic infection that might increase the risk of participating in the trial, or the presence of another chronic medical condition that the investigator judged to be unsuitable for participating in the study;
9. Had received gene therapy before screening, had received an additional investigational agent within 4 weeks before screening, or within five half-lives of the trial agent, whichever was longer;
10. Use of any botanicals (herbal supplements or traditional Chinese medicine of plant, mineral, or animal origin) that may affect liver function within 4 weeks before or during the study, except topical medications; Or Chinese herbal medicine that may affect the study in the judgment of the investigator;
11. The subject has a history of life-threatening bleeding such as intracranial hemorrhage, including if the subject has gastric ulcers that present a risk of severe gastrointestinal bleeding;
12. Have a major medical condition or other condition considered by the investigator to be inappropriate for study participation;
13. A family member of a site staff member who is directly involved in the conduct of the study, a family member of a site staff member who is otherwise supervised by the investigator, or a family member of an employee of a related company who is directly involved in the conduct of the study.

Repeat testing and screening

Repeat testing could be performed if some of the results of the screening period were abnormal and clinically relevant and were deemed to be necessary in the assessment of the investigator. Repeat testing was permitted only once if no treatment was taken for the abnormal value during the screening period, and the corresponding data were recorded. If screening failure was confirmed, information on the subjects who participated in the screening, including the reasons for the screening failure, should be collected.

Subjects who failed the first screening and met the protocol requirements after treatment could be re-screened. After re-screening, if the subjects met the inclusion and exclusion criteria

specified in the protocol, they could be enrolled in the study.

Participants who underwent rescreening were required to sign a new informed consent form, assign a new screening number, and undergo screening again.

Subject replacement

If a subject fails to complete the required 10-week observation period for non-tolerability reasons, subject replacement may be considered.

Drop-out of subjects

4.5.1 Withdrawal criteria

All subjects may withdraw from the study at any stage on their own. At the same time, the subject should withdraw from the study if the following conditions occur during the study and it is medically necessary for the subject to discontinue the study, specifically:

1) If an intolerable adverse event/serious adverse event occurs and the investigator judges that the risks of continuing to participate in the study outweigh the benefits, the participant must withdraw from the study and take appropriate treatment measures at the same time.
2) If the patient's condition deteriorated after the study treatment, according to the investigator's judgment, the patient needed to receive prohibited treatment in the study and should be withdrawn from the study.
3) The researchers judged that the compliance of the subjects was poor, which affected the evaluation of the research results.
4) Participants were asked to withdraw from the study by the investigator for any medical reason.
5) Major protocol deviations;
6) Subjects meeting exclusion criteria (new or confirmed).

All withdrawn subjects should retain their full original data. To clear out research or revoke agree but did not attend study visits and conditions are not clear of the subjects, the researchers should take various forms as far as possible to contact the subjects, such as telephone, SMS asked reason, at least three representations and continue to follow-up research efforts when subjects failed to return after can only be claimed to have participants lost to follow-up, The measures taken to contact subjects (such as contact date, method, etc.) should be recorded.

Subjects who withdraw due to intolerable reasons should be treated appropriately by the investigator.

4.5.2 Management of dropouts

For subjects who discontinue the study early, the date and reason for discontinuation must be recorded in the corresponding electronic Case report Form (eCRF). Subjects who withdraw early (except where replacement is permitted) will not be replaced by a new subject, and once withdrawn from the study, the subject cannot be re-enrolled in the study.

To ensure the safety of the subjects, all pre-existing or new adverse events and abnormal laboratory measures at the time of withdrawal will be followed until clinically cured, stable status, or pretreatment status (at the investigator's discretion, The status is not likely to be resolved because of the subject's illness), baseline level (if the baseline level is known), subject loss to follow-up (e.g., no more information is available or subject/caregiver refuses to provide information), or death.

Study Medications

Basic information about the study drug

Generic name: BBM-H901 injection

English name: BBM-H901 Injection

Product name: not available

Specifications: 8.0×10^{12}vg (2mL)/bottle

Storage: -80°C±10°C

Labeling of drug preparations

The study drug was AAV vector BBM-H901 injection in 8.0×1012vg (2mL) per vial and had to be labeled. The study drug was labeled with the standard text "for clinical trial use" and included drug number, protocol number, and product name.

The receipt, distribution and storage of investigational drugs

Of this study was to use drugs by clinical research unit personnel responsible for research in drug storage, management, counting, distribution and recycling, bottle is disposable supplies, left in the bottle of infusion after any study drug should not be used for another object. All researchers should refer to the BBM - H901 injection, manual researchers understand vehicle handling, preparation, management and disposal of specific instructions, and according to the plan calls for use in the clinical research within the framework of the study drug. In accordance with the requirements for clinical research unit for studying drug reception, distribution and return the file record. At the end of the study, all unused or partially used medications and their packaging will be returned by the investigator to the provider for destruction and recorded on the Medication distribution and recycling form.

Dosing regimens

BBM - H901 injection should be - 80 ° C + 10 ° C for preservation, out on the day of infusion, natural melting at room temperature, weight according to the subjects, computing BBM - H901 injection dosage required (5×10^{12} vg/kg). Under the medical ward, at a rate of 2.5 mL/min, by complete disposable intravenous injection pump.

The researchers before ready for dose of the subjects, should be carefully read product manual instructions; Researchers preparing infusions should use universal precautions and personal protective equipment; Use sterile techniques for handling; Thaw frozen medication at room

temperature prior to use.

Immunomodulatory Therapy

According to the guidelines issued by the American Association for the Study of Liver Diseases (AASLD) and the data of SJ-UCL study, in the absence of other causes, if the liver transaminase (ALT and/or AST) >1.5 times the upper limit of normal or persistently higher than the baseline level after gene vector infusion during the follow-up period; Or under no FIX inhibitors of evidence, a carrier of Padua - FIX activity levels drop, can use glucocorticoid (such as prednisone/prednisone dragon) treatment, the researchers allowed and according to the laboratory parameters and/or the subjects' tolerance to adjust.

Prevention of sex hormone is adopted in this research, a week before the infusion BBM - H901 injection of eight weeks after the start and infusion prophylactic use of oral corticosteroids (prednisone or prednisone dragon). The initial regimen of oral glucocorticoids during the study period is shown in table 2. The recommended hormone-reduction schedules after prophylactic hormone use are shown in table 3.

Table 2 Initial Treatment with oral Glucocorticoids

Timing	Prednisone/prednisolone (mg/kg/ day)
-1 week	1
1 week	1
2 weeks	1 *

Notes:

Corticosteroid approximately 1 mg/kg/ day (orally [PO], once daily [qd]) is recommended as the starting dose in week 1 unless the investigator uses a different regimen based on the patient's history.

* At the investigator's discretion, 1 mg/kg/ day (PO, qd) could be extended for an additional week, i.e., to week 2, if patients had no hormone-related adverse effects.

Table 3 Recommended hormone reduction schedule

Timing	Prednisone/prednisolone (mg/kg/ day)
3 weeks	0.75
4 weeks	0.75
5 weeks	0.5
6 weeks	0.5 * *

7 weeks	0.3
8 weeks	0.3

Note: ** Maintained at 0.5 mg/kg/ day until transaminases (ALT and/or AST) returned to baseline, then tapered to 0.3 mg/kg/ day.

Concomitant medications

Concomitant medications including any therapeutic and preventive medications (including symptomatic treatment information, prescription drugs, over-the-counter drugs, natural extracts, traditional Chinese medicine, nutritional supplements, etc.) from the time the patient signed the informed consent form until the safety follow-up after the end of study treatment (52 weeks of follow-up/early withdrawal [±7 days]) should be recorded in the patient's medical record and reported on the eCRF.

Best supportive care was allowed during the study. Concomitant use of other medications that may induce or aggravate clinical study symptoms is not recommended and should be monitored when necessary.

5.6.1 Medications that are allowed to be combined

Drugs that do not affect the efficacy and safety evaluation of gene carrier drugs in the treatment of hemophilia B can be used. However, the investigator should make a detailed record in the eCRF concomitant medication/concomitant therapy page, including the reason for the medication/treatment, the administration/treatment method, and the start and end time of the medication/treatment.

After receiving the BBM-H901 infusion, the subject will be asked to withhold prophylaxis with the FIX protein product until recommended by the investigator; If a subject has bleeding during the study and is deemed by the investigator to need to use FIX protein products on an on-demand basis, the subject can be infused with FIX protein products on an on-demand basis. The use of FIX protein products (product name, reason, start and end date, type (on-demand, prophylactic, continuous infusion, etc.), frequency and dose will be recorded in the infusion log. A washout period of at least 96 hours or 5 half-lives (whichever is longer) of the FIX product has been met before the evaluation of FIX activity and FIX antigen is performed.

5.6.2 Prohibited medications and lifestyle restrictions after treatment

During the study period (until 52 weeks after infusion), drugs that affect the efficacy and safety evaluation of gene vector drugs in hemophilia B were prohibited, including but not limited

to:

1) Ibuprofen and acetylsalicylic acid (aspirin). Use other non-steroidal anti-inflammatory drugs with caution (at the discretion of the investigator);
2) Blood products such as red blood cells, platelets and fresh frozen plasma for the treatment of hemophilia B;
3) Other investigational drugs (not limited to hemophilia B indications);
4) Other gene therapy products;
5) Other immunosuppressive agents, with the exception of protocol-permitted glucocorticoids and alternative immunosuppressive therapy considered by the investigator.

If there is a medical indication for use, the investigator considers it necessary to keep a record of the use of prohibited drugs, including but not limited to the date of use, dose, and reason for treatment.

In addition, participants were required to abstain from alcohol consumption for the entire duration of the study, including the long-term follow-up period.

Study Procedures

The study included the screening period, the dosing period, the first 52-week postinfusion follow-up period, and the long-term follow-up period (up to 5 years postinfusion).

Screening period (up to 4 weeks)

Subjects will be screened after the clinical study protocol has been approved by the ethics committee of the clinical research unit. The investigator is responsible for explaining the purpose, methods, benefits and potential risks of this clinical study to each subject, and obtaining the informed consent signed by the subject. In any study or research related required before operation, program or assessment, must sign a consent form. After subjects sign the informed consent form, they are given a study screening number. Screening procedures were performed within 4 weeks before subjects received the study drug.

During screening, if bleeding occurred and an infusion of the FIX protein product was required, as judged by the investigator, a washout period of at least 96 hours or five half-life periods of the FIX protein product, whichever was longer, was completed by day 0.

The following items were required to be completed during the screening period:

1) Sign informed consent;
2) Review of entry/exclusion criteria;
3) Demographic characteristics were recorded;
4) The medical history and treatment history of hemophilia B were recorded.
5) Other medical history and treatment history were recorded.
6) Routine physical examination;
7) Height and weight measurement;
8) Vital signs measurement;
9) Blood routine, urine routine and blood biochemical examination;
10) Coagulation function test;
11) Infectious disease screening;
12) Genetic testing for hemophilia: for subjects with known genotype, genotype testing is not required during the screening period if a previously obtained genotype report is available; For subjects with unknown genotypes, samples will be drawn for analysis at screening;
13) A 12-lead ECG;

14) Blood type;
15) Immunomodulatory therapy: subjects who met the inclusion criteria and did not meet any exclusion criteria as assessed by the investigator could be treated with glucocorticoid prophylaxis starting 1 week before the planned infusion of BBM-H901 injection according to the actual situation of the subjects.
16) FIX protein product infusion log fill;
17) Bleeding assessment;
18) Target joint count assessment;
19) Abdominal B-ultrasound;
20) FIX activity assay;
21) FIX inhibitor detection;
22) AAV capsid neutralizing antibody detection;
23) AAV capsid-binding antibody detection;
24) Alpha-fetoprotein detection;
25) Adverse events;
26) Concomitant medications.

Screening period (day -1)

Subjects initially confirmed to be eligible for study inclusion were assessed for the following items 1 day before BBM-H901 infusion (i.e., day -1) :

1) Review of entry/exclusion criteria;
1) Oriented physical examination;
2) Body weight measurement;
3) Vital signs;
4) Blood routine, blood biochemistry, urine routine;
5) ALT and AST: if blood biochemical tests are required at this visit, these tests may not be repeated;
6) Coagulation function test;
7) 12-lead electrocardiogram examination;
8) Immunomodulatory therapy;
9) FIX protein product infusion log filling;

10) Bleeding assessment;
11) Target joint count assessment;
12) FIX activity test: the elution period of other FIX protein products of at least 96 hours or 5 half-lives (whichever is longer) should be satisfied before the evaluation of FIX activity;
13) FIX inhibitor testing;
14) Hemophilia related scale assessment;
15) AAV capsid neutralizing antibody detection;
16) AAV capsid-binding antibody detection;
17) PBMC, plasma, saliva, urine and stool samples were collected for vector shedding test.
18) Adverse events;
19) Concomitant medications were used.

Dosing period (day 0)

Infusion of **BBM-H901 injection:** A single intravenous infusion of AAV vector BBM-H901 injection was performed under medical supervision according to the dosing protocol in Section 5.4. An additional 24 hours of observation after the end of the infusion was recommended for monitoring and evaluation at protocol-specified time points. The following items were to be completed on day 0, as described in the study flow chart.

1) Vital signs: Vital signs were measured before the infusion of BBM-H901, and investigators could increase the frequency of inspections during and after the infusion according to safety concerns.
2) BBM-H901 injection infusion;
3) 12-lead ECG examination: within 1 hour after BBM-H901 injection infusion;
4) Immunomodulatory therapy;
5) Fill in the FIX protein product infusion log;
6) Bleeding assessment;
7) Adverse events;
8) Concomitant medications.

Follow-up Period (Weeks 1-52)

The subjects returned to the clinical research unit at the specified time after the drug administration for follow-up examination and evaluation.

1) Guided physical examination: day 6, week 2, 4, 6, 8, 12, 16, 22, 26, 32, 42, 52 / early withdrawal. Investigators may increase the frequency of physical examination according to safety considerations.
2) Vital signs: on day 6, week 2, 4, 6, 8, 12, 16, 22, 26, 32, 42, 52 / early withdrawal, investigators could increase the frequency of examination according to safety considerations;
3) Blood routine, blood biochemistry, and urine routine;
4) ALT, AST: on the 3rd day, 3rd, 5th and 7th week after BBM-H901 infusion, investigators can increase the frequency of examination according to safety considerations;
5) Blood coagulation function;
6) 12-lead electrocardiogram examination: 10, 26, 52 weeks, the researchers can increase the inspection frequency based on security considerations;
7) Immunomodulatory therapy: prophylactic steroid therapy was used until 8 weeks after the infusion of BBM-H901 injection. If elevated transaminase was found at any other time, steroid therapy was determined by the investigator according to the actual situation of the subject.
8) Fill in the FIX protein product infusion log;
9) Bleeding assessment: day 3, day 6, day 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 18, 22, 26, 32, 42, 52 weeks/early withdrawal;
10) The number of target joints was evaluated at week 26 and 52 / early withdrawal;
11) Abdominal ultrasound: 26, 52 weeks/early withdrawal;
12) Pada-fix activity test: Days 3 and 6 after BBM-H901 injection infusion, weeks 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 18, 22, 26, 32, 42, 52 / premature withdrawal, Pada-fix activity should be assessed before ensuring that a washout period of at least 96 hours or 5 half-lives of the FIX protein product, whichever is longer, has been met;
13) FIX inhibitor testing: week 2, 4, 8, 12, 16, 26, 32, 42, 52 / early withdrawal;
14) Hemophilia related scale assessment: 4, 12, 26, 52 weeks/early withdrawal;
15) AAV capsid neutralizing antibody detection: 6 days, 2 weeks, 4 weeks, 12 weeks, 26 weeks and 52 weeks after infusion of BBM-H901 / premature withdrawal;
16) Combining the AAV capsid antibody detection: BBM - H901 injection 6 days after drug

infusion, 2, 4, 12, 26, 52 weeks/early exit;

17) Alpha-fetoprotein (AFP) detection: 26, 52 weeks/early withdrawal;
18) To detect carrier off PBMC, plasma, saliva, urine, stool samples collection: BBM - H901 injection 6 days after drug infusion, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 18, 22, 26, 32, 42, 52 weeks/early exit; Note: if the research process in the three consecutive test results were negative, the subsequent can no longer to collect the samples;
19) Adverse events;
20) Concomitant medications were used.

Long-term follow-up (from week 52 to 5 years)

After 52 weeks (±7 days) of follow-up, participants will enter the long-term follow-up period, and they will be followed up every 6 months (±14 days). The specific examination items or follow-up items are as follows.

1) Investigator-directed physical examination: every 6 months after the week 52 visit until 5 years after BBM-H901 infusion/early withdrawal, investigators may increase the frequency of examination according to safety concerns;
2) Vital signs: every 6 months after the week 52 visit until 5 years after BBM-H901 infusion/early withdrawal, investigators could increase the frequency of examinations according to safety concerns;
3) Blood routine, urine routine, and blood biochemical tests: every 6 months after the week 52 visit until 5 years after the infusion of BBM-H901 / early withdrawal, investigators could increase the frequency of testing according to safety concerns.
4) Coagulation tests;
5) 12-lead electrocardiography: every 6 months after the week 52 visit until 5 years after the infusion of BBM-H901 or early withdrawal, investigators could increase the frequency according to safety concerns;
6) The FIX protein product infusion log was completed;
7) Bleeding assessment: every 6 months after the 52 week visit until the 5th year after BBM-H901 infusion/early withdrawal;
8) Target number of joint assessment: 52 weeks after the visit, every 6 months to BBM - H901 injection infusion after 5 years/early exit;

9) Abdominal ultrasound: every 6 months after the 52nd week visit, until the 5th year after BBM-H901 injection infusion/early withdrawal;
10) Pada-fix activity testing: every 6 months after the week 52 visit until 5 years after BBM-H901 infusion/early withdrawal, a washout period of at least 96 hours or 5 half-life periods of the FIX protein product, whichever is longer, should be met before assessment of PADA-FIX activity;
11) FIX inhibitor testing: every 6 months after the week 52 visit until 5 years after BBM-H901 infusion/early withdrawal;
12) AAV capsid neutralizing antibody testing: every 6 months after the week 52 visit until 5 years after BBM-H901 infusion/early withdrawal;
13) AAV capsid-binding antibody testing: every 6 months after the week 52 visit until 5 years after BBM-H901 infusion/early withdrawal;
14) Alpha-fetoprotein (AFP) testing: every 6 months after the week 52 visit until 5 years after BBM-H901 infusion/early withdrawal;
15) PBMC, plasma, saliva, urine, and stool samples for vector shedding testing (if appropriate) were collected every 6 months after the week 52 visit until 5 years after BBM-H901 infusion/early withdrawal;
16) Adverse events (aes);
17) Concomitant medications.

Unscheduled visits and additional tests

Additional unscheduled visits during the study were at the discretion of the investigators on the basis of clinical needs (e.g., clinically significant laboratory abnormalities, adverse events, etc.). During the unscheduled visits, the corresponding examinations and adverse events should be faithfully recorded.

In addition to the examination items specified in the protocol, additional evaluation items may be added if necessary at the discretion of the investigator based on the actual situation of the subject, including but not limited to: Liver transaminase, FIX activity, FIX antigen, blood lipids (total cholesterol [TC], high density lipoprotein [HDL], low density lipoprotein [LDL], very low density lipoprotein [VLDL], triglyceride [TG]); Coagulation function, thrombin generation assay (TGA), hepatitis B DNA quantification, hepatitis C RNA quantification, serum interferon,

lymphocyte subsets (B/T/NK), etc. Additional tests and adverse events should be faithfully recorded.

Collection and testing of biological Samples

Methods of collecting and processing biological samples

Blood samples were collected from:

Blood samples were collected during the study at the following designed time points (marked as "time 0" when the infusion of BBM-H901 was started) :

Blood samples were collected for corresponding analysis according to the blood collection points specified in the flow chart of the protocol study, and the actual time of collection was recorded as follows. Samples specific operation refer to biological sample collecting and processing related standard operating procedure or operation manual.

1) FIX activity test: Screening (including BBM - H901 injection before infusion. 1 day), the BBM - H901 injection 3 and 6 days after drug infusion, At weeks 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 18, 22, 26, 32, 42, 52, and every 6 months after the week 52 visit to the 5th year after the BBM-H901 infusion or early withdrawal;
2) FIX inhibitor testing: screening period, weeks 2, 4, 8, 12, 16, 26, 32, 42, 52, and every 6 months after the week 52 visit until 5 years after BBM-H901 infusion or early withdrawal;
3) AAV capsid neutralizing antibody detection: screening (including BBM - H901 injection before infusion. 1 day), BBM - H901 injection 6 days after drug infusion, 2, 4, 12, 26, 52 weeks, and after 52 weeks visit every 6 months to 5 years after the BBM - H901 injection infusion, or exits in advance;
4) AAV capsid-binding antibody testing: screening period (1 day before infusion), day 6, weeks 2, 4, 12, 26, and 52 after infusion, and every 6 months after the week 52 visit until 5 years after infusion or early withdrawal;
5) Collection of PBMC and plasma samples for vector shedding assays: Screening period the first 1 day, BBM - H901 injection 6 days after drug infusion, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 18, 22, 26, 32, 42, 52 weeks, and every 6 months after 52 weeks visit to BBM - 5 years after the H901 injection infusion, or exit ahead of time. Note: If three consecutive test results are negative during the course of the study, the sample may not be collected in the subsequent period.

After whole blood specimen collection in accordance with the corresponding inspection units

to provide biological sample collecting and processing related standard operating procedure or operation manual, and according to FIX activity and FIX inhibitor, AAV capsid neutralization antibodies, as well as the combination of AAV capsid, PBMC analysis different requirements analysis, such as centrifugal and/or save, The corresponding biological samples processing and storage records were made.

Collection of stool, urine and saliva samples:

According to the flow chart of this solution sampling time point of feces, urine and saliva samples collection, carrier for loss analysis, specific as follows.

1) Collection of saliva, urine and stool samples for vector shedding test: Screening period the first 1 day, BBM - H901 injection 6 days after drug infusion, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 18, 22, 26, 32, 42, 52 weeks, and every 6 months after 52 weeks visit to BBM - 5 years after the H901 injection infusion, or exits in advance; Note: If the test result is negative for three consecutive times during the study, the sample can not be collected in the subsequent period.

Feces, urine, saliva samples according to the test units to provide biological samples after collecting and processing the relevant standard operating procedures or operating manual save, and make corresponding biological sample processing and warehousing storage record.

Labels of biological samples

In biological samples before the sampling tube and two unification of freeze pipe number, and paste special printing labels, tags include project code, research category, type of sample, the subjects number (ID), sampling, sampling points, detection/backup sample information such as date and time. Sample label at the actual using label in clinical center, specific see sample inspection units to provide biological sample collecting and processing related standard operating procedure or operation manual.

Transportation of biological samples

If the samples need to be transported to an out-of-hospital laboratory for testing, the sample test tubes (including blood, stool, urine, and saliva samples) will be transported to the testing unit through the logistics cold chain. Before transportation, a sufficient amount of dry ice should be added to the sample packaging box to ensure that the whole sample transportation process is under freezing conditions (≤-60°C), and the sample should be sent to the biological sample testing unit in time under freezing conditions, and the temperature should be monitored throughout the process, and the corresponding records should be provided. The testing unit is responsible for the storage of blood, feces, urine and saliva samples according to the relevant standard operating procedures or operation manuals for the collection and processing of biological samples.

As the conditions of biological sample processing may change, the requirements for biological sample collection, processing, storage and transportation shall be subject to the relevant standard operating procedures or operation manuals for biological sample collection and processing.

Determination and analysis of biological samples

Detection unit sample analysis planning, use of a validated, biological sample analysis method of testing analysis of biological samples, issue the biological analysis report.

Coagulation factor activity and adeno-associated virus vector viral load evaluation

Padua-ix activity

Padua-FIX activity and its changes were evaluated by plasma Padua-FIX activity level, including peak activity level, steady-state activity level, or mean activity level.

FIX activity levels were measured by two methods in one-stage method (SYSMEX platform, Actin FSL aPTT reagent and Werfen platform, HemosIL SynthASil reagent). Actin FSL method was mainly used for efficacy and safety evaluation, while SynthASil method was only used for methodological comparison.

Adeno-associated viral vector viral load

AAV vector viral loads in plasma, saliva, urine, and feces of the subjects were used for vector shedding changes (plasma, urine, feces, saliva, and PBMCs).

Carrier off mainly through quantitative polymerase reaction (qPCR) method, in different time periods before and after injection collected saliva, urine, feces and plasma biological samples, through total genomic DNA extraction, and through the match the viral genome primers extensions, to detect the virus in the fluid sample drops, evaluation after injection of the virus loads in the fluid dynamic change.

Efficacy Evaluation

Efficacy evaluation indicators include:

1) Bleeding times (including spontaneous bleeding, traumatic bleeding, joint bleeding, etc.);
2) Blood samples were collected for Padua-FIX activity analysis.
3) The number and volume of other FIX protein products (including recombinant and plasma-derived FIX protein products) transfused;
4) Number of target joints;
5) Hemophilia assessment related scales (HJHS, HEAD-US-C and CHO-KLAT).

Safety Evaluation

Safety and tolerability evaluation indicators

The safety and tolerability evaluation indicators included adverse events/serious adverse events (including DLT), physical examination, vital signs, 12-lead electrocardiogram, clinical

laboratory tests (blood routine, blood biochemistry, urine routine, coagulation function), FIX inhibitor test, AAV capsid neutralizing antibody and capsid binding antibody, etc.

Procedures for safety evaluation

Physical examination

Physical examination items include: skin/mucosa, lymph nodes, head, neck, chest, abdomen, skeletal muscle/limbs, and nerves/spirit.

Physical examinations were performed according to the schedule specified in the protocol. Investigators could increase the frequency of physical examinations according to safety concerns.

Height and Weight

Height and weight were measured and recorded during the screening period, and body mass index (BMI) was calculated as BMI= weight (kg)/height^2 (m^2). The body weight measured on day -1 will be used to calculate the dose of BBM-H901 injection administered.

Vital signs

Vital signs include blood pressure (systolic and diastolic), pulse, temperature, and respiratory rate. The vital signs were measured while the subjects were seated and resting for at least 5 min. If the vital signs exceeded the normal range or the changes were clinically significant, and the investigators considered that repeated measurements were necessary, two additional measurements were required, with an interval of approximately 2 minutes between each measurement. The results of each measurement should be recorded.

Vital-sign acquisition was allowed within the time window specified in the protocol. When the time of vital signs measurement conflicted with the time of blood sample collection, vital signs were measured before the specified time of blood sample collection.

Investigators could increase the frequency of testing according to safety concerns.

A 12-lead electrocardiogram

Subjects took a 12-lead ECG measurement after resting in the supine position for at least 5 min. Heart rate (HR), PR interval, QRS interval, QT interval, and QT interval corrected according to Fridericia's formula (QTcF) were recorded. When ECG measurements were abnormal (including QTcF abnormalities, such as QTcF>500 ms or an increase from baseline of >60 ms) or were judged by the investigator to be clinically significant, two additional ECG measurements were required, with an interval of at least 2 minutes. The results of each ECG measurement, whether it was abnormal, and its clinical significance were recorded.

For two consecutive measurements of QTcF of more than 500 msec or an increase from

baseline of more than 60 msec, close monitoring (e.g., electrocardiography every hour) and appropriate action were at the discretion of the investigator.

When there was a conflict between the timing of electrocardiography and other study procedures (e.g., vital signs, blood sample collection), electrocardiography was performed first, followed by vital signs, to ensure that the blood sample was collected at the specified time.

Investigators could increase the frequency of examinations according to safety concerns.

Clinical Laboratory Testing

Clinical laboratory tests include blood routine tests, blood biochemistry tests, urine routine tests, and coagulation function tests. For clinically significant abnormal clinical laboratory tests, repeat tests should be arranged as soon as possible, and try to be performed within 7 days. Clinical laboratory abnormalities that were judged by the investigators to be clinically significant and met the criteria for adverse events or serious adverse events were reported as adverse events or serious adverse events and were followed closely until recovery to normal, baseline levels (if known), stable status (if the abnormalities were not clinically significant), loss to follow-up, or death. Every time the results of the measurement, if abnormal, and shall be recorded in the clinical significance.

Blood, urine, and other samples were collected for clinical laboratory tests at protocol-specified visit times. If relevant laboratory tests (except blood biochemistry) have been performed within 7 days before the infusion of BBM-H901 injection, it is not necessary to repeat the tests on day-1. Investigators could increase the frequency of testing based on safety concerns.

Factor IX inhibitors

The Bethesda assay or the modified Bethesda assay by Nijmegen was used and expressed in Bethesda units (BU). Adequate methodological validation of the assay should be performed before sample testing. If the inhibitor test result of BBM-H901 injection was negative before infusion, and the inhibitor test result was positive after infusion, the blood sample of the subject should be collected for reexamination and confirmation, and the sampling time point should be recorded in the SAE report.

Inhibitor production was defined as Bethesda inhibitor titer ≥0.6 BU/mL. The threshold was defined as ≥0.6 BU/mL for a "low titer" inhibitor and >5 BU/mL for a "high titer" inhibitor.

Adeno-associated virus capsid neutralizing antibody, adeno-associated virus capsid binding antibody

The principle of AAV capsid neutralizing antibody detection is to block AAV infection by

different dilutions of the antibody in serum, and to evaluate the level of serum antibody by detecting the gene expression level in AAV infected cells with a microplate reader.

AAV capsid-binding antibodies measure the amount of immunoglobulin G (IgG) in serum that binds to the AAV capsid by means of enzyme-linked immunosorbent assay.

Adverse events

Every effort must be made to remain vigilant for possible adverse events (AEs) throughout the study. If an AE occurs, the safety of the subject should be considered first. If necessary, appropriate medical intervention should be provided.

At the time of signing the informed consent form (ICF), the name and telephone number of the study site staff had to be reported to each subject for reporting of AE and medical emergencies.

Any subject presenting with an AE will be treated according to local guidelines.

10.3.1 Definitions

Serious predose events:

A serious predose event was any event that occurred after the subject signed the ICF but met the criteria for serious adverse event (SAE) reporting before the study medication was administered.

Adverse events:

An adverse event (AE) is any adverse, undesired or unplanned medical event, regardless of causality, occurring in a subject participating in a clinical study, including signs (including clinically evident abnormal laboratory tests), symptoms, or events temporarily related to the use of an AAV gene therapy drug. An AE will include the following conditions:

- Not present before the infusion of the AAV gene therapy drug, but present after the infusion of the gene therapy drug;
- Which is present before the gene vector infusion but is more severe or increased in frequency after the gene vector infusion.

Serious adverse events:

Serious adverse event (SAE) refers to adverse medical events such as death, life-threatening, permanent or severe disability or loss of function, the need for hospitalization or prolonged hospitalization of the subject, and congenital anomalies or birth defects after the subject receives the trial drug.

The SAE definition	**Guiding Principles**
Presence of death	AE was the immediate or primary cause of death in the subject.

Life-threatening	The occurrence of adverse events brings immediate risk of death to the subjects. After excluding those serious progress may lead to the adverse events of death, such as: no drug-induced hepatitis liver failure happens.
Permanent or severe disability or loss of function	Any cause injury, damage or destroy the function of the subjects, physiology, or the two both, physical activity, or adverse events in the quality of life.
The subject requires hospitalization or prolonged hospitalization	Any first admission to a health care facility (even within 24 hours) meets this criterion, excluding: Rehabilitation institutions, hospice care institutions and temporary medical care (such as home care patients), skilled nursing facilities, nursing homes and conventional er treated within 24 hours, routine hospital physical examination, outpatient hospital and on the day of surgery, disease progression itself lead to prolonged hospitalization and was in the hospital.
Adverse medical events, such as congenital anomalies or birth defects	The offspring of the subject has congenital abnormalities and malformations.
Other important medical events	Medical and scientific judgment must be used to decide whether to expedite reporting of other conditions. For example, a critical medical event may not immediately endanger life, death, or hospitalization, but it is generally considered to be serious if medical action is required to prevent one of these conditions. Such as important treatment in the emergency room or home anaphylactic bronchospasm, not evil fluid to pledge in hospital or convulsion, drug dependence and addiction. **For this study, the following events were considered medically significant and were reported as SAEs:** • Any death related to gene transduction technology therapy during the study period; • Grade III-IV toxicity, such as anaphylaxis, elevated transaminase, related or likely related to the study vector in any subject; • Any subjects related to gene therapy techniques or may be related to the treatment of immunosuppressive therapy, 8 weeks failed to improve or alleviate III - IV hepatic transaminases associated; • Any subjects in connection with or may be related to gene therapy technology level IV (> 10 times), transaminase lifts;

	• Carrier at any time before and after the infusion may or must be related to malignant tumor.

Arrangements in advance, the selected program or routine treatment is not regarded as SAE requiring hospital treatment; The study site must record all of the following:

- Pre-scheduled, elective, or routinely scheduled treatments (or scheduled on a waiting list) were recorded before the subject's consent to participate in the study was obtained;
- Prescheduled, elective, or routinely scheduled treatments that were present before the study and that did not deteriorate or progress before the subject agreed to participate in the study;
- Prescheduled, elective, or routinely planned treatment was the sole reason for intervention or hospitalization.

10.3.2 3 Monitor and record events

Serious pre-treatment events:

After signing the ICF, but before gene vector therapy, serious pretreatment events experienced by subjects will be recorded on the SAE form and reported to the SRC and Ethics Committee within 24 hours of the event being discovered by study site staff.

Adverse events:

All adverse events were collected from the time the ICF was signed until 52 ±7 days after the dose was administered or until early withdrawal from follow-up or the patient started another genetic drug, whichever occurred first. All adverse events were followed until the event or recovery, or stable disease, or the event could be explained, or the patient died or was lost to follow-up. Only adverse events related to the study drug were collected in the long-term follow-up phase, which could be followed up to 5 years/early withdrawal.

Serious adverse events:

Regardless of the severity of the event or relationship to the study treatment, any SAEs experienced by the subject from the day of signing the ICF should be recorded on the SAE form. The SAEs must be reported to clinical research unit designees, as specified in the study.

When a subject completes the study or stops the study, any SAEs will be followed up by the investigator until the event is resolved, stabilized, or returned to baseline status.

Must be at the scene of the research staff know the SAE the formal notification within 24 hours of ethics committee. It is the investigator's responsibility to ensure that SAE reporting information and procedures are properly used and followed.

The leading causes of death event should be recorded in the proper CRF and report. All causes

of death must be reported as SAEs. Researchers should make every effort to obtain a death certificate and the autopsy report, and send it to the ethics committee.

10.3.3 Relevance Judgment

In the assessment of adverse events with the test with the causal relationship between drugs, should be considered one of the important factors include:

a) Temporal relationship to drug administration: Events should occur after the administration of the drug. The length of time from exposure to an event should be evaluated in the clinical context of the event.
b) (to invoke) and after the drug was stopped again (to be) reaction of the medicine, should be combined with relevant events in patients with common clinical course assessment to inspire or motivate after reaction.
c) Basic diseases, combined disease, concurrent illness: should be combined with the current treatment of diseases and patients may be suffering from other diseases of the natural history and course evaluation of each event.
d) Merger or drug treatment should check the patient when the adverse events are taking other drugs or therapy, to determine whether it could lead to this event.
e) Known types of reactions to this drug class (clinical/preclinical)
f) Physical/psychological stress exposure: Stress exposure may induce adverse changes in the patient and provide a more plausible explanation for the event.
g) Research and treatment of pharmacology and pharmacokinetics: research and treatment must be taken into account comprehensively the pharmacokinetics characteristics (absorption, distribution, metabolism and excretion) and the pharmacodynamics of individual patients.

Researchers according to the adverse events with the test judging principle analysis of correlation between adverse events and the correlation of drug, is divided into five types, namely "sure about, is likely to be relevant, may be relevant, has nothing to do, must not", researchers must in terms of medical record.

The specific criteria are as follows:

	Definitely related	**Probably related**	**Possibly related**	**Probably unrelated**	**Unrelated**

STUDY DRUGS IN A REASONABLE CHRONOLOGICAL ORDER	+	+	+	+	-
KNOWN TYPES OF DRUG REACTIONS	+	+	+	-	-
THE REACTION LESSENS OR DISAPPEARS AFTER STOPPING THE DRUG	+	+	±	±	-
THE REACTION RECURRED AFTER READMINISTRATION	+	?	?	?	-
SUBJECT ILLNESS COULD NOT BE EXPLAINED	+	+	-	±	-

Note: + affirmative - negative ± probable? Unknown

"Definitely related", "probably related", and "possibly related" were taken as the total number of adverse reactions of the trial drugs, and the incidence of adverse reactions was calculated accordingly. It is worth noting that the Chinese regulatory interpretation of "may not be related" as a case in which an association cannot be ruled out.

If a possibility cannot be fully met against the above criteria, it is recommended to adopt the conservative principle or the unfavorable new drug principle, that is, if the judgment result is between "may be relevant" and "may not be relevant", it should be judged as "may be relevant" or "may not be relevant" in the case of insufficient information.

10.3.4 severity

Investigators graded the severity of AE according to CTCAE 5.0 criteria as follows:

- **Mild (grade I) :** asymptomatic or mild; Clinically or diagnostically only; No treatment is required.
- **Moderate (grade II) :** requires smaller, local, or non-invasive treatments; And age-appropriate limitations in instrumental activities of daily living*.

* Refers to instrumental activities of daily living as cooking, buying clothing, using the

telephone, managing money, etc.

- **Severe (Class III) :** severe or medically important but not immediately life-threatening; Leading to or prolonging hospitalization; Disabling; Limitation of physical activities of daily living**.

  * * the rational daily life activity refers to the bath, wear strip, eat, wash one's hands, medication, etc., not lying in bed.

- **Life-threatening (Level IV) :** life-threatening; Requiring emergency treatment.
- **Death (Grade V) :** AE related death.

10.3.5 Process for dealing with special circumstances

Bleeding events and suspected bleeding events

All bleeding events and suspected bleeding events, regardless of the need for exogenous FIX treatment, should be recorded in the subject diary and on the eCRF designated bleeding events page. Bleeding events and suspected bleeding events will generally not be recorded as aes. Unless the event meet the SAE standard definition of one or more (whether or not finalized for bleeding events, whether for hemophilia disease itself should end or exogenous FIX treatment).

Overdose

Too much is to give participants than expected any dose drug doses. An overdose is not counted as an AE; However, all excess should be recorded on the SAE form, and report within 24 hours to the SRC and ethics committee. An overdose should be reported even if it does not result in an AE. Too much do not need to record for adverse events, but excessive symptoms should be as adverse events recorded in the electronic case report; Dosage information is recorded on the CRF.

death

All deaths during the study period, regardless of association with the study drug, must be recorded as serious adverse events and reported to the ethics committee and the collaborator within 24 hours of being made aware of them.

Deaths should be considered outcomes rather than independent events. The cause of death was or ending events or disease should be as a single concept of medical records. The leading causes of death event can also be in the follow-up report further perfect information. Terms "sudden death" is only used to describe the following situation: on or before the original heart disease subjects without heart disease, the acute onset of symptoms within 1 hour after all of a

sudden, unexpected death, and assume that died because of heart disease; Or death, unsubstantiated, within 24 hours after the subject was last seen with stable vital signs. If the cause of death is unknown and the report is still unable to determine the cause of death, should be recorded as "unexplained death". "If the cause of death is subsequently identified (e.g., after an autopsy)," unexplained death "should be replaced with the established cause.

Investigators were required to provide the ethics committee and collaborators with other required information, such as autopsy reports and final medical reports.

10.3.6 Duties of the investigator

The responsibilities of a researcher include the following:

- According to the requirements of collection time monitoring and record, including the SAE AE;
- Determine the relationship and severity of AE/SAE events;
- Determine the date of occurrence and resolution of the AE/SAE event;
- Fill out an SAE form for each serious event and report it to the ethics committee within 24 hours of the study site staff being aware of the event;
- Actively and steadily track SAE follow-up information. Follow-up information must be reported to the ethics committee using an SAE form within 24 hours of the study site staff becoming aware of new information;
- Ensure that all AE and SAE report support subjects medical records in the file;
- If applicable, in accordance with the requirements of local law to local SAE and ethical committee report.

Data Management

Data traceability, case report form completion and handover

ECRF data are derived from the source data and source files, personnel designated by the researchers and the researchers to fill in, need to ensure that information integrity and accuracy. In the event of any errors that need to be corrected, modifications should be made in accordance with the eCRF completion instructions. ECRF should be timely submit through the network to fill in after the completion of the electronic data collection (EDC) system, EDC data in the system through on-the-spot verification source data, data managers audit, question and so on processing after confirmed without question, before the data lock, researchers need to be electronic signature confirmation.

Design and establishment of the database

Electronic Data Capture System (EDC) was used for data collection, and the database was established by the CRO data department appointed by the clinical research unit. Database to login system, data input, modify, or delete data such as trace.

Data entry and modification

Data recorded in the Electronic Case Report Form (eCRF) should come from source files and be guaranteed to be consistent with the source data. All the source files should be kept clear and clean, can ensure the data accurate identification. Study visit record copy of the permanent will be deemed to be the source file, used to record data selected subjects. Data from source documents, such as study medical records, should be entered into the eCRF by entry personnel in a timely and accurate manner. Data administrator in eCRF data check, found the question in the form of a question table through clinical examiner asked researchers, data managers data modification and answered by the researchers confirmed that the question list again when necessary.

Data review

At the end of the data entry and verification, by the data administrator, principal investigator for checking data, statistical analysis, personnel, and completes the analysis of the crowd at the end of the definition and judgment.

Coding of data

Data managers are responsible for medical coding. Past medical history, concomitant medications, and adverse events were coded.

Past medical history and adverse events were coded with the use of the latest edition of the International Dictionary for Medical Use (MedDRA), and concomitant medications were coded

with the use of the latest version of the WHO DD (World Health Organization Pharmacodictionary).

Coding process, any due to improper medical terms provide, inaccurate, fuzzy and cause cannot be encoded data problems, researchers will, in the form of data question please check and confirm.

In front of the database locking, data managers report to the clinical research unit sends medical coding and through clinical research unit audit.

Data Lock

When the following conditions are met, can lock the data.

1) All data have been entered into the database;

2) All questions have been resolved;

3) The analytic population has been defined and judged.

At the end of the study, the database was locked by the principal investigator, statistical analysts, and others after data checks confirmed that the database had been created correctly. In principle, no further changes to the locked database were allowed. If the problem found after the data lock is confirmed to be unlocked, it will be corrected after unlocking according to the data management unlocking process. Written records of the application for unlocking records and the unlocking correction content shall be kept.

Statistical Analysis

Sample size estimation

No formal statistical hypotheses were involved in this study. Approximately 9 patients were planned to be enrolled (the number of patients enrolled could be adjusted according to efficacy and safety).

Analysis Set

Full analysis set (FAS) : all cases treated with BBM-H901 injection. Socio-demographic characteristics variables, disease-related variables, vector shedding, FIX activity, and efficacy indicators were analyzed based on the analysis set.

Safety analysis set (SS) : the set of all patients who received BBM-H901 injection and could be evaluated for safety. Safety indicators were analyzed based on this analysis set.

Statistical Analysis Methods

12.3.1 Generic analysis

The final sample size for statistical analysis will be determined by the actual number of patients enrolled.

Descriptive statistics were generally performed for the measurement data, including the number of cases (n), arithmetic Mean (Mean), standard deviation (SD), Median (Median), minimum (Min) and maximum (Max). The number of cases (n) and percentage (%) were generally counted for count data. Appropriate statistical tables and figures were used to summarize the data. The number of subjects enrolled and the cases dropped out were described, and the baseline characteristics of the enrolled cases were analyzed by descriptive statistics.

Due to the small number of subjects, the results of single cases should be combined with professional analysis.

12.3.2 Demographic and baseline characteristics

List and statistics of baseline characteristics based on SS.

Baseline was defined as the result of a subject's last measurement or examination before receiving an infusion.

Demographic variables and baseline characteristics of SS (e.g. : physical examination, vital signs, laboratory tests, etc.) were tabulated and descriptive statistics were performed.

12.3.3 Padua-FIX activity and AAV vector viral load analysis

Padua-FIX activity and AAV vector viral load data were tabulated and statistically analyzed based on FAS.

Age groups, planned blood sampling sites, and tabulated and descriptive statistics for Padua-FIX activity levels and AAV vector viral load were performed, as well as tabulated for individual subject data. Padua-FIX concentration-time curves were plotted according to the actual time of blood collection. Mean and median Padua-FIX concentration-time plots were plotted according to the planned time of blood collection.

At the end of the study, information on different age groups of subjects, planned blood collection sites, FIX activity, AAV vector viral load was summarized descriptively, and individual subject data were tabulated.

12.3.4 Safety Analyses

Tabulated and descriptive statistics of safety and tolerability indicators based on SS, The incidence of adverse events, vital signs, physical examination, clinical laboratory tests (blood routine test, urine routine test, blood biochemistry test, coagulation function test), vector shedding analysis, 12-lead electrocardiogram, FIX inhibitor test, AAV capsid neutralizing antibody, AAV capsid binding antibody and other results and their changes from baseline were collected.

Adverse events were coded and categorized under System Organ Class and preferred term, according to the most recent version of MedDRA.

All adverse events will be tabulated by occurrence, summarized by system organ class, preferred term, and dose group. The incidence of each adverse event will be summarized by system organ classification, severity (according to NCI CTCAE v5.0), and association with the use of BBM-H901 injection.

Physical examination and clinical laboratory abnormalities will be summarized by time point.

Baseline values and changes in key safety data (e.g., liver-function tests) were tabulated at each time point.

All adverse events, serious adverse events, adverse events leading to drug discontinuation, and adverse events related to BBM-H901 injection were summarized separately.

12.3.5 Efficacy Analysis

Effectiveness analysis was conducted based on FAS, and the data of effectiveness analysis were summarized descriptively.

Descriptive statistics were used for ABR within 52 weeks of the subjects. ABR was calculated using the following formula:

ABR before infusion = number of bleeding events that occurred before infusion of BBM-H901 injection ÷ duration of observation before infusion ×365.25 days per year, with 1 decimal place reserved.

ABR after infusion = number of bleeding events after infusion of BBM-H901 injection ÷

duration of observation after infusion ×365.25 days per year, with 1 decimal place reserved for the result.

Similar analyses were performed for other efficacy measures, if applicable.

For categorical measures, two-sided 95% confidence intervals were calculated with the use of the exact probability method, where applicable.

Detailed statistical analysis methods will be described in the final statistical analysis plan.

Analysis Report

The final analysis of this study will be conducted after the last subject has completed 52 weeks of follow-up and a summary report will be written; After the last subject in the whole study completed an additional 4 years of long-term follow-up, all follow-up information was summarized and the supplementary report was completed.

Management of Clinical Studies

Statement

This clinical study will be conducted in accordance with the protocol and the CRO's standard operating procedures, These practices are designed to ensure that the study adheres to the Declaration of Helsinki, the E6 Guidelines for Good Clinical issued by the International Council for Harmonisation (ICH), Technical requirements for the Registration of Medicinal Products for Human Use Practice, the Good Clinical Practice (GCP) issued by the National Medical Products Administration (NMPA), and the requirements of the Drug Clinical Research regulations were implemented.

Ethics section

This study was designed and prepared in accordance with the World Medical Association Declaration of Helsinki with the rights and welfare of patients in mind. The principal investigator or investigator of the clinical study explained the purpose of the study and all potential possibilities to the patient. Patients who voluntarily agreed to participate in the clinical study and signed the informed consent form were eligible for the study.

Clinical research investigators and investigators participating in the study should correctly understand and be familiar with the study plan, and be able to prepare measures in advance. Clinical investigators are required to comply with the Declaration of Helsinki, ICH E6 Guidelines for Good Clinical Practice, NMPA GCP and relevant regulations when conducting clinical research.

Major researchers and staff involved in the study should comply with the requirements of the research plan on the content of the scientifically in order to study the technical level of the identified.

According to national policies and regulations, researchers are required to provide relevant research documents to the ethics committee.

Prior to the initiation of clinical research, approval from the ethics committee and relevant regulatory authorities must be obtained.

Amendments to the study protocol should be submitted to the ethics committee for approval.

In the course of a clinical study, investigators are required to report any serious adverse events or unexpected adverse events related to clinical study safety that may affect the safety of subjects and the conduct of the study to the ethics committee according to regulations.

The ethics committee should be informed of the end of the study.

Written informed consent

The investigator (as required by the applicable regulations) or a person authorized and responsible for the investigator should fully inform the subject of all questions related to the study, including written information approved by the ethics committee. The investigator should inform the subject about the study to the maximum extent possible in a language or terminology that the subject can understand.

Prior to participation in the study, participants or their legal representative should co-sign a face-to-face informed consent form (name and date) with the investigator from whom the participants discussed informed consent. Investigators should provide participants with a copy of the signed informed consent form.

Informed consent forms used by investigators should be reviewed and approved by the sponsor before they are submitted to the ethics committee for review.

Modification of the clinical study protocol

After the approval of the ethics committee, if the protocol needs to be revised, a protocol amendment note should be prepared and signed by the principal investigator. After the protocol revision, it should be reviewed by the ethics committee or put on record before implementation.

Protocol deviations

Protocol Deviation is defined as any intentional or unintentional deviation from and non-compliance with the treatment protocols, examinations, or data collection procedures prescribed in an approved study protocol. In general, protocol deviation was not allowed. Unless there is an emergency medical situation that really requires a change or deviation from the protocol. The researchers or researchers designated personnel need to record and explain plan details and reasons of deviation. Deviations from the protocol were described in the clinical summary report.

When serious protocol violations occur, they should be evaluated. The clinical unit had the option to stop the trial early if necessary.

Data preservation

All clinical research data should be properly preserved with traceability and confidentiality guaranteed. According to GCP requirements, investigators are required to maintain clinical study data for at least 5 years after the end of the clinical study.

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Appendix

Appendix I Clinical Laboratory Tests

Blood biochemical tests Total bilirubin, direct bilirubin, indirect bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein, albumin, lactate dehydrogenase (LDH), creatine kinase isoenzyme, sodium (Na), potassium (K), chloride (Cl), magnesium (Mg), phosphate, calcium (Ca), carbonic acid Hydrogen salts, glucose, blood urea nitrogen (BUN) or urea, serum creatinine, blood lipids (including total cholesterol, triglyceride, high density lipoprotein, low density lipoprotein, polar density lipoprotein) Blood lipid levels were measured only when indicated during the screening period and the study.	**Routine blood tests** White blood cell count (WBC) and classification, red blood cell count (RBC), hemoglobin concentration (HGB), hematocrit (HCT), and platelet count (PLT)
Routine urinalysis Urine protein, white blood cell, red blood cell, urine specific gravity (SG), pH, urine glucose (U-GLU), ketone body (U-KET), urine occult blood (urinalysis), urine bilirubin, urobilinogen	**Coagulation function** Plasma prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), plasma fibrinogen (FIB), D-dimer
Infectious disease Screening Hepatitis B virus test (HBsAg, HBV-DNA quantitative test), hepatitis C virus test (HCV-Ab, HCV-RNA quantitative test), human immunodeficiency virus antibody test (anti-HIV-Ab), syphilis antibody test	**Alpha fetoprotein**
Blood type	/

Other laboratory tests

Hemophilia genes	Clotting factor IX (FIX) activity levels
Vector shedding (including peripheral blood mononuclear cells, plasma, saliva, urine, feces)	FIX Inhibitor
Adeno-associated virus (AAV) capsid neutralizing antibody	AAV capsid-binding antibodies

Appendix II Hemophilia Joint Health Score (HJHS) Scale

The HJHS2.1 provides the total score (maximum score = 124, with higher scores indicating worse scores), joint specific scores, and global gait scores.

Appendix III HEAD-US-C System for the Early Ultrasonographic Assessment of Bone and Joints in Hemophilia

The HEAD-US scale includes 3 items (0-8 points): synovial hyperplasia, cartilage destruction, and bone destruction. According to the needs of clinical diagnosis, treatment and follow-up, on the basis of the three items of HEAD-US scale, two indicators of disease activity in acute bleeding stage, including synovial fluid exudation and synovial vascular proliferation, were added in HEAD-US-C, and the grades were assigned according to the Melchiorre scoring scale. The joint effusion/blood collection and synovial neovascularization were scored semi-quantitatively (0-3 grades according to the amount of effusion/blood collection, corresponding to an ultrasound score of 0-3 points; 0-2 grades according to the distribution of synovial blood flow signals in the power Doppler sampling frame, corresponding to an ultrasound score of 0-2 points).

Appendix IV Canadian Quality of Life Assessment Tool for Children with Hemophilia (CHO-KLAT)

The Canadian Quality of Life Assessment Tool for Children with Hemophilia (CHO-KLAT) is a hemophilia-specific, child-centered measure of healthy quality of life. The CHO-KLAT consists of 35 question items that can be completed in 10 to 15 minutes. The CHO-KLAT includes two versions: self-report by children and self-report by parents. The child version is suitable for hemophilia boys aged 7-18 years, while the parent version is suitable for younger children (4-8 years).

The CHO-KLAT scale includes 35 items in 8 dimensions, namely treatment (7 items), physical health (3 items), family (3 items), future (1 item), feelings (10 items), understanding hemophilia (1 item), others and friends (4 items), and control of one's own life (6 items). Scale using Likert type 5 rating one incident frequency over the past four weeks, from "never" to "often".

The sum of scores on eight domains is a composite score, with higher scores indicating better QoL.

Appendix 5 Possible adverse events and treatment plans of the study drugs

BBM-H901 injection is an adeno-associated virus (AAV) vector drug expressing human coagulation factor IX (FIX), which was independently developed by Shanghai Xinzhi Medical Technology Co., LTD. In order to control the safety risk of subjects and ensure the interests of subjects, it is necessary to closely observe the subjects in the process of this study, including adverse complaints, physical examination and laboratory abnormalities, etc. According to the data of preclinical research, investigator-initiated clinical research and the safety data of similar drugs in clinical research, the BBM-H901 injection is a safe and effective drug. Adverse events that may occur after BBM-H901 injection infusion and their treatment plans are as follows.

1. Treatment measures to prevent abnormal liver function that cannot be controlled by sex hormones

Elevated transaminase levels are a sign of severe liver damage, and these subjects are prone to progress to severe liver injury called drug-induced liver injury (DILI). Liver function should be closely monitored in studies, especially in subjects with transaminases elevated more than 3 times the upper limit of normal. Potential DILI was evaluated according to the criteria of abnormal elevation of transaminase (AST and ALT) and total bilirubin in Hay's rule. The threshold of abnormal laboratory tests in subjects with potential DILI depends on the individual baseline values and underlying diseases of the subjects. Further evaluation should be performed according to Hay's law to clarify the cause of abnormal laboratory values. The subject should return to the study center as soon as possible, preferably within 48 hours after the result is known, in case of any suspected abnormal liver-function test results. The evaluation of cases of abnormal liver function should include laboratory testing, imaging studies, a detailed history, concomitant medications, and any tests and assessments that the investigator deems necessary. Regardless of whether the cause of the abnormal liver function is clear, this DILI case should be reported as a serious adverse event and treated/managed by the investigator accordingly according to local clinical practice.

Prophylactic or symptomatic treatment was used throughout the study:

According to the guidelines issued by the American Association for the Study of Liver Diseases (AASLD) and SJ-UCL, in the absence of other causes, if the liver transaminase (ALT and/or AST) was >1.5 times the upper limit of normal or persistently higher than the baseline level during the follow-up period after gene vector infusion; Or in the case of no FIX inhibitor, a carrier of Padua - FIX activity levels drop, can use glucocorticoid (such as prednisone dragon/prednisone)

treatment, allow researchers adjust tolerance of laboratory parameters and/or subjects glucocorticoid treatment.

2. Management of bleeding events due to lack of efficacy

This drug is under development, and its efficacy and safety have yet to be verified. There may be a lack of efficacy in some subjects. If there is a lack of efficacy, the subjects can freely choose whether to withdraw from the study. If there are symptoms, signs and/or important medical events caused by lack of efficacy, the corresponding treatment/treatment will be carried out according to the clinical judgment of the investigator, and the symptoms, signs and/or important medical events should be reported to the safety department and regulatory authorities as adverse events (AE) or serious adverse events (SAE) according to the local regulatory requirements.

The production of inhibitors often leads to bleeding events due to the lack of efficacy of drugs for the treatment of hemophilia B. Therefore, the changes of coagulation indexes and bleeding events should be closely monitored throughout the clinical research. Through ward rounds, follow-up, timely tracking of laboratory test results and other measures, FIX can be timely detected and given throughout the prevention or on-demand treatment to ensure the safety of the subjects. At the same time, special attention should be paid to the formation of inhibitors, and detailed follow-up information, such as FIX drug dosage, type, activity recovery rate, half-life, inhibitor, etc., should be submitted. For the risk of bleeding, the following measures will be taken:

Throughout the clinical study, patients will be closely monitored for changes in coagulation indicators and bleeding, and FIX protein products will be given prophylaxis or treatment as needed if necessary:

（1）. Blood routine tests were performed during the screening period, follow-up period, long-term follow-up and before leaving the group, including white blood cell count (WBC) and classification, red blood cell (RBC), hemoglobin (HGB), hematocrit (HCT) and platelet count (PLT).

（2）. Coagulation function tests including plasma prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), plasma fibrinogen (FIB) and D-dimer were performed in the screening period, follow-up period, long-term follow-up period and before leaving the group.

（3）. FIX protein product infusion log: from the screening period to the end of the study, the use of FIX protein products (prophylaxis and/or on-demand treatment) was recorded, including the reason of treatment (such as bleeding site, frequency and cause, etc.), start and end date, type (on-demand, prophylaxis, continuous infusion, etc.), frequency and dose.

3. Management measures of thrombus

This AAV-based gene therapy product is under development. In addition to the safety events observed in the preclinical and human studies that have been completed, there are still unknown other safety events that may occur, such as thrombosis, etc. Any safety events in this study that meet the definition of adverse events/serious adverse events determined by the investigators should be reported in accordance with regulatory requirements. Any safety events in this study should be reported according to the definition of adverse events/serious adverse events as judged by the investigators.

For gene products for the treatment of hemophilia B, when the FIX activity after gene expression is increased to 150% or more, there may be a risk of thrombosis, and thrombotic events need to be closely monitored and reported. Specific measures include:

Blood coagulation parameters were closely monitored throughout the clinical study to predict possible thrombosis:

（1）. Coagulation function tests including PT, APTT, TT, FIB and D-dimer were performed during the screening period, the follow-up period, the long-term follow-up period and before leaving the group to monitor closely and find the possible risk of thrombosis in time.

（2）. Blood routine tests were performed during the screening period, follow-up period, long-term follow-up and before leaving the group, including white blood cell count (WBC) and classification, red blood cell (RBC), hemoglobin (HGB), hematocrit (HCT) and platelet count (PLT).

In case of abnormal laboratory tests that may cause thrombosis, relevant preventive treatment can be carried out. At the same time, for the existing thrombosis, the corresponding symptomatic treatment measures should be taken in time, including the use of anticoagulants and thrombolytic therapy.

4. Allergic reactions and other adverse reactions

There have been no reports of allergic reactions or anaphylaxis during AAV-FIX vector infusions. Allergy-type reactions, including anaphylaxis, have been reported for all FIX products. Although rare, the occurrence of these events is often closely related to the presence of FIX inhibitors. Subjects should be informed of the early signs and symptoms of allergy, including urticaria, generalized urticaria, angioedema, chest distress, dyspnea, wheezing, syncope, hypotension, tachycardia, and so on. If such events occur at home, the subject should be instructed to seek immediate medical attention.

5. Measurement of serum levels of interferon secretion

AAV may activate part of the immune response and induce the production of interferon. Clinical observation should be strengthened during the study. If the subject is suspected to have symptoms related to immune response after the infusion of BBM-H901 injection, the level of interferon secretion can be detected by the investigator's judgment.

6. Treatment of inhibitors

During the treatment of hemophilia B, the probability of inhibitor production is very low, and there are no clear guidelines for the treatment of hemophilia B inhibitor production. However, peripheral immune tolerance can be achieved through long-term regular and frequent administration of coagulation factors and immune tolerance induction therapy (ITI).

7. Genotoxicity

So far, the virus titers in the semen of 10 subjects in the IIT study have been determined. The results showed that all subjects had reduced to the background level on day 56 after treatment.

Because of the use of viral vector-based gene therapy, there may be a risk of vertical germline transmission of vector DNA. Therefore, inclusion criteria 10 stipulated that "older minor subjects and their legal guardians should be clearly aware of the contraceptive requirements, avoid sexual activity or use reliable contraceptive methods."

8. Long-term safety risks of gene therapy

Gene therapy achieves therapeutic effect by inducing permanent or long-term changes in the body, which may increase unpredictable risks such as late side effects. Although no serious adverse events associated with AAV gene therapy drugs for hemophilia B have been reported, due to the

particularity of gene therapy, after the main follow-up period of 52 weeks after infusion, according to the requirements of the "Technical Guidelines for long-term follow-up clinical research of gene therapy Products" (trial) issued by the Center for Drug Evaluation (CDE) of National Medical Products Administration, The long-term follow-up period was set up for an additional 4 years, with a total follow-up period of 5 years after infusion to observe long-term safety.

PROTOCOL 3.0

Protocol Title	**Title:** A Single-arm, Open-label, Single-dose Clinical Study to Evaluate the Safety, Tolerability, and Efficacy of BBM-H901 Injection in Patients with Hemophilia B (Aged 12 to Below 18)
Study Objectives	**Primary objective:** • To evaluate the safety and tolerability of a single intravenous infusion of BBM-H901 injection in male hemophilia B patients (≥ 12 years and < 18 years) with endogenous coagulation factor IX (FIX) activity levels ≤ 2 IU/dL (i.e., ≤ 2%). **Secondary objectives:** • To evaluate the pharmacokinetics (PK) profile of Padua-FIX and adeno-associated virus (AAV) vectors in hemophilia B patients (≥ 12 years and < 18 years) with endogenous FIX activity levels ≤ 2% after a single intravenous infusion of BBM-H901 injection; • To evaluate the efficacy of a single intravenous infusion of BBM-H901 injection in hemophilia B patients (≥ 12 years and < 18 years) with endogenous FIX activity levels ≤ 2%. **Study objectives of the long-term follow-up:** • To evaluate the long-term efficacy and safety of a single intravenous infusion of BBM-H901 injection.
Version/Date	V3.0/Oct. 16, 2023
Investigational Product	Generic name: BBM-H901 Injection Strength: 8.0×10^{12} vg (2 mL)/vial Storage: Store at −80 °C ± 10 °C.
Indication	Hemophilia B (congenital FIX deficiency)
Study Endpoints	**Primary endpoints:** 1. Incidence of dose-limiting toxicity (DLT) events, as determined by the Safety Review Committee (SRC), within 10 weeks after BBM-H901 infusion; 2. Incidence of adverse events (AEs) and serious adverse events (SAEs) within 52 weeks after BBM-H901 infusion; 3. Changes in liver function (including alanine aminotransferase [ALT] and aspartate aminotransferase [AST]) within 52 weeks after BBM-H901 infusion from before treatment. **Secondary endpoints:** 1. PK parameters of Padua-FIX and AAV vectors after BBM-H901 infusion: • Padua-FIX activity levels within 52 weeks after BBM-H901 infusion, including peak activity level, steady-state activity level or mean activity level; • Changes in AAV vector shedding [plasma, urine, feces, saliva, and peripheral blood mononuclear cells (PBMCs)] within 52 weeks after BBM-H901 infusion; 2. Efficacy endpoints for BBM-H901 injection, including: • Annualized bleeding rate (ABR) within 52 weeks after BBM-H901 infusion (including spontaneous bleeding, traumatic bleeding, and joint bleeds); • FIX activity levels in each visit period within 52 weeks after BBM-H901 infusion;

	• Number and volume of infusions of other FIX protein products (including recombinant and plasma-derived FIX protein products, such as recombinant human coagulation factor IX for injection and prothrombin complex concentrate) within 52 weeks after BBM-H901 infusion; • Number of target joints within 52 weeks after BBM-H901 infusion; • Number of joint bleeds within 52 weeks after BBM-H901 infusion; • Proportion of subjects without bleeding events within 52 weeks after BBM-H901 infusion; • Changes in joint health scores, joint ultrasound scores, and quality of life at weeks 4, 12, 26, and 52 after BBM-H901 infusion from baseline measured using relevant scales, which include Hemophilia Joint Health Score (HJHS), Hemophilic Early Arthropathy Detection with UltraSound in China (HEAD-US-C), and Canadian Hemophilia Outcomes - Kids Life Assessment Tool (CHO-KLAT); 3. Other safety endpoints for BBM-H901 injection: • Incidence of FIX inhibitors (detected by the Bethesda method or the Nijmegen-Bethesda) within 52 weeks after BBM-H901 infusion; • Titers of neutralizing antibody against and binding antibody to AAV capsid; • Clinically significant changes in vital signs, physical examination, clinical laboratory test values, 12-lead electrocardiogram (ECG), and alpha-fetoprotein within 52 weeks after BBM-H901 infusion; • Cellular immune status of the body before and after AAV vector infusion assessed by single-cell RNA sequencing analysis; • Changes in bone mineral density before and after treatment in adolescent subjects. **<u>Long-term follow-up endpoints:</u>** Long-term follow-up endpoints after BBM-H901 infusion (52 weeks to 5 years after infusion) include but are not limited to: • ABR (including spontaneous bleeding and traumatic bleeding); • Padua-FIX activity; • Number and volume of infusions of other FIX protein products (including recombinant and plasma-derived FIX protein products, such as recombinant human coagulation factor IX for injection and prothrombin complex concentrate) per year; • Annualized bleeding rate of different bleeding events: including spontaneous bleeding, traumatic bleeding, and bleeding requiring no treatment; • Number of target joints; • Frequency of joint bleeds; • Viral load of rAAV vector; • Incidence of AEs and SAEs; • Incidence of FIX inhibitors; • Titers of neutralizing antibody against and binding antibody to AAV capsid; • Clinically significant changes in vital signs, physical examination, clinical laboratory test values, 12-lead ECG, and alpha-fetoprotein; • Changes in bone mineral density before and after treatment in adolescent subjects.

Dose-limiting Toxicity (DLT)	The severity of AEs will be evaluated and graded according to the (US) National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 (v5.0). DLTs are defined as the following AEs that occur within 10 weeks after BBM-H901 infusion and are related to BBM-H901 injection as determined by the investigator: （1） Non-hematological toxicities: • ≥ Grade 3 total bilirubin increase; • Grade 3 ALT and/or AST increase that cannot return to baseline levels after 4 weeks of immunosuppressive therapy; • Grade 4 ALT and/or AST increase; • Grade 3 or 4 allergic reactions, such as bronchospasm and immediate hypersensitivity; • Thrombosis events (other than grade 4 thrombophlebitis at the infusion site) related to BBM-H901 injection as determined by the investigator with Padua-FIX activity levels ≥ 150%; • Prolonged QTcF interval (absolute value > 500 ms or prolongation > 60 ms from baseline); • Grade 3 vomiting or diarrhea lasting >3 days after adequate drug treatment; • Grade 4 (life-threatening) vomiting or diarrhea, irrespective of the duration of the event; • Other ≥ grade 4 non-hematological toxicities. (2) Hematological toxicities: • Grade 4 neutrophil count decrease (neutrophil count < 0.5×10^9/L) lasting > 5 days; • Grade 3 febrile neutropenia (neutrophil count < 1.0×10^9/L with temperature of > 38.3 °C once or a sustained temperature of ≥ 38 °C for more than 1 h); • Grade 4 platelet count decreased (platelet count < 25.0×10^9/L) or grade 3 platelet count decreased (platelet count < 50.0×10^9/L) with bleeding; • Grade 4 anemia. (3) Any other clinically significant or unacceptable toxicities determined as DLTs by the sponsor or investigator. The following cases are not defined as DLTs: • Any grade of alopecia; • Any grade of isolated laboratory changes without clinical sequelae or clinical significance.
Study Design	This study is designed as a single-center, single-arm, open-label study to investigate the safety, tolerability, PK profile, and efficacy of a single intravenous infusion of BBM-H901 injection in hemophilia B patients (≥ 12 years and < 18 years) with FIX activity levels ≤ 2 IU/dL (i.e., ≤ 2%). Nine evaluable subjects are planned to be enrolled successively into three age groups (group 1, group 2, and group 3) in descending order of age. Three subjects aged < 18 years and ≥ 16 years will be enrolled in group 1; 3 subjects aged < 16 years and ≥ 14 years will be

	enrolled in group 2; 3 subjects aged < 14 years and ≥ 12 years will be enrolled in group 3. BBM-H901 infusion will be first performed in subjects in group 1. One sentinel subject will be set up to complete at least 2 weeks of observation after administration, and the investigator will comprehensively determine whether to continue the treatment for the other 2 subjects in this group. After the last evaluable subject in group 1 completes the 10-week follow-up after administration, the SRC will comprehensively determine whether to enter the study for group 2. One sentinel subject will also be set up in group 2 to complete at least 2 weeks of observation after administration, and the investigator will comprehensively determine whether to continue the treatment for the other 2 subjects in this group. After the last evaluable subject in group 2 completes the 10-week follow-up after administration, the SRC will comprehensively determine whether to enter the study for group 3. One sentinel subject will be set up in group 3 to complete at least 2 weeks of observation after administration, and the investigator will comprehensively determine whether to continue the treatment for the other 2 subjects in this group. After the last evaluable subject in group 3 completes the 10-week follow-up, the SRC will comprehensively determine the safety and tolerability. Administration at other age groups will not be continued. Subjects who complete the 52-week assessments will continue to be followed up for 5 years after administration to obtain long-term assessment data.
Safety Review Committee (SRC)	A Safety Review Committee (SRC), which is planned to be composed of investigators and other relevant experts, will be established for this study. Unexpected circumstances that arise during the course of the study will be discussed by the SRC. The available safety, tolerability, and efficacy data for subjects will be discussed in the forms of conference call, online meeting, and email feedback, and the comments on whether to continue the enrollment in the next age group and whether to suspend the study will be given.
Dosing Regimen	BBM-H901 injection should be stored at −80 °C ± 10 °C, removed on the day of infusion, and naturally thawed at room temperature. The required dose of BBM-H901 injection (5×10^{12} vg/kg) should be calculated based on the body weight of the subject. Under medical care, one single intravenous infusion is done via injection pump at a rate of 2.5 mL/min.
Study Period	Subjects will be followed up for a total of approximately 5 years after enrollment in the study, including a maximum 4-week screening period (starting immunomodulatory therapy on day −1, and baseline examination on day −1), a post-infusion 52-week follow-up period (follow-up on D3, D6, weekly at weeks 2–8, every 2 weeks at weeks 10–18, every 4 weeks at weeks 22–26, at week 32, and every 10 weeks at weeks 42–52), and a long-term follow-up period for additional 4 years after 52 weeks (every 6 months).
Study Subjects/Sample Size	Hemophilia B patients (≥ 12 years and < 18 years) with endogenous FIX activity levels ≤ 2 IU/dL (i.e., ≤ 2%) are chosen for study, and about 9 patients are planned to be enrolled (the specific number of enrolled patients may be adjusted according to the efficacy and safety).

Inclusion Criteria	Subjects will be enrolled in this clinical study only if they meet all of the following criteria: 1. Subjects and/or their legal guardians fully understand the purpose, nature, method and possible adverse reactions of the study and sign the informed consent form (ICF), and the subjects are voluntarily participating in the study; 2. Requirements for age, gender, and body weight are as follows: Patients aged ≥ 12 years and < 18 years, male, body weight of 45 kg and above for patients aged ≥ 16 years and < 18 years, and body weight of 40 kg and above for patients aged ≥ 12 years and < 16 years; 3. Patients clinically diagnosed as hemophilia B with endogenous FIX activity levels ≤ 2 IU/dL (≤ 2%); if FIX activity level > 2% at screening is caused by insufficient elution of FIX protein products, the test can be repeated once after sufficient elution; 4. Titers of neutralizing antibody against and binding antibody to BBM-H901 capsid ≤ 1:4 and 1:200, respectively; 5. A history of bleeding events and/or injection of a FIX product (including recombinant and plasma-derived) within 12 weeks prior to screening in the subject's medical record; 6. No history of allergic reactions or allergy related to the administration of FIX or intravenous human immunoglobulin; 7. Patients who have received any recombinant and plasma-derived FIX products for 75 exposure days (EDs) with negative FIX inhibitors and have no clinical signs or symptoms of decreased response to FIX products after infusion at screening; or patients who have a positive history of FIX inhibitors but have turned negative for two years after immune tolerance induction (ITI) therapy and received FIX products for a cumulative total of 100 EDs during negative period (ineligible for those who have not been treated with ITI for previous positive inhibitor, or those who have positive result recurred after post-ITI negative conversion). 8. Acceptable laboratory test values obtained during screening and prior to administration (Day −1): a) Hemoglobin ≥ 11 g/dL or ≥ upper limit of normal (ULN) for peers b) Platelets ≥ 100×10^9/L c) AST and ALT ≤ 1.5 × ULN d) Total bilirubin ≤ 1.5 × ULN e) Estimated glomerular filtration rate (eGFR) ≥ 60 mL/min 9. Older underage subjects and their statutory guardians should clearly understand the requirements for contraception and know that subjects must avoid sexual intercourse or use reliable contraception methods; 10. Good compliance. Subjects and their statutory guardians are willing to participate in the "gene therapy" clinical study, able to be followed-up regularly, and able to accurately complete the subject diary during the follow-up period with the assistance from family members or caregivers.

Exclusion Criteria	Subjects will be excluded from the clinical study if they meet any of the following criteria: 1. Positive for hepatitis B surface antigen (HBsAg), hepatitis B virus deoxyribonucleic acid (HBV-DNA), or hepatitis C virus ribonucleic acid (HCV-RNA). Subjects with a history of hepatitis B or C should have two previous sample collections at an interval of at least 3 months and are considered negative if negative results are obtained for the above indicators at the two assessments; 2. Receiving antiviral therapy for hepatitis B and C; 3. Patients with other coagulation disorders except hemophilia B; 4. Patients taking any other systemic immunosuppressive agents within 30 days prior to screening except glucocorticoids and immunosuppressive agents recommended by the investigator; patients allergic to glucocorticoids, tacrolimus, and other immunosuppressive agents; 5. Patients with a history of vaccination within 30 days prior to screening, or a vaccination plan during immunomodulatory therapy; 6. Patients with the following conditions are not suitable for this study: potential liver diseases, such as previous diagnosis of portal hypertension, splenomegaly, hepatic encephalopathy or liver fibrosis ≥ stage 3; nodules and cysts found by B ultrasound, or alpha-fetoprotein increase found by laboratory tests, which are determined to be clinically significant by the investigator; 7. Patients with scheduled surgery during the 52-week study period after BBM-H901 infusion; 8. Presence of acute/chronic infection that may be at an increased risk due to participation in the study, or other chronic diseases that may make the subject unsuitable for participation in the study as determined by the investigator; 9. Patients who have received gene therapy prior to screening or other investigational products within 4 weeks prior to screening or within 5 half-lives of the investigational product (whichever is longer); 10. Intolerable anaphylaxis to prior treatment with FIX, or nephrotic syndrome on prior treatment with FIX; 11. Use of any plant preparation (herbal supplements or traditional Chinese medicines derived from plants, minerals or animals) that may affect liver function within 4 weeks prior to the study or during the study, except for drugs for topical use; Chinese herbal medicines that may affect the study as determined by the investigator; 12. Life-threatening bleeding such as intracranial hemorrhage within 2 years before enrollment, or sequelae after previous intracranial hemorrhage and severe hemorrhage, or with potential causes of gastrointestinal bleeding, including but not limited to gastrointestinal ulcers;; 13. Subjects with serious diseases or other conditions that the investigator deems unsuitable for study participation; 14. Family members of study site staff directly involved in the implementation of the study, family members of study site staff who are otherwise supervised by the investigator, or family members of employees of related companies directly involved in the implementation of the study.

Withdrawal Criteria and Handling of Dropouts	All subjects and/or their statutory guardians have the right to withdraw from the study at any time. At the same time, the subject should withdraw from the study if the investigator considers study discontinuation necessary for the subject from a medical point of view due to the following conditions during the study: • Occurrence of intolerable AEs/SAEs, posing risks outweighing benefits if the subject continues to participate in the study, as determined by the investigator. In this case, the subject must withdraw from the study and be treated with appropriate measures; • Worsened condition after investigational product injection, for which treatments prohibited in this study are required as determined by the investigator. In this case, the subject should withdraw from the study; • Poor compliance, as determined by the investigator, that may affect the evaluation of study results; • Subject withdrawal from the study requested by the investigator for any medical reason; • Serious protocol deviation; • Subjects meeting exclusion criteria (new or confirmed). All original data should be retained for all withdrawn subjects. For subjects who do not participate in the study visit without a clear statement of withdrawal from the study or withdrawal of consent and whose status are unclear, the investigator should contact the subject as far as possible by various means such as telephone and SMS, and ask for the reasons. Loss to follow-up is declared if the subject still fails to perform a return visit after at least three negotiations for follow-up visit and study continuation have been made. Measures taken to contact the subject (such as contact date and method) should be well documented. For subjects who withdraw from the study due to intolerability, the investigator should take appropriate treatment measures. For subjects who prematurely discontinue from the study, the date and reason for discontinuation must be documented. A subject who prematurely withdraws will not be replaced by a new subject, and cannot enter the study again once he/she withdraws. To ensure subject safety, all existing or new AEs and laboratory abnormalities at the time of withdrawal will be followed up until clinical recovery, stable disease, pretreatment status (a condition that, in the opinion of the investigator, is not likely to resolve due to the subject's disease), return to baseline level (if the baseline level is known), loss to follow-up (e.g., no further information is available or the subject/caregiver refuses to provide information), or death.
Study Suspension/Early Termination	The Ethics Committee or the investigator has the right to suspend or prematurely terminate part of the clinical study or the entire clinical study. Reasons for study suspension/early termination include but are not limited to: • New information leads to a risk-benefit judgment unfavorable to the investigational AAV gene therapy drug, for example, due to: – Deaths related to BBM-H901 injection occur during the study period as judged by the investigator;

	– The following conditions that occur during the study and are due to the subjects' inability to tolerate BBM-H901 injection: a） Grade 3 or 4 toxicity (except for transaminase increased) related to BBM-H901 injection as determined by the investigator according to NCI CTCAE v5.0, such as bronchospasm and immediate hypersensitivity; b） Grade 3 transaminase increased (including ALT and/or AST) related to BBM-H901 injection as determined by the investigator according to NCI CTCAE v5.0 that cannot be recovered to baseline after 4 weeks of immunosuppressive therapy; c） Grade 4 transaminase increased (including ALT and/or AST) related to BBM-H901 injection as determined by the investigator according to NCI CTCAE v5.0; d） Thrombosis events (other than grade 4 thrombophlebitis at the infusion site) related to BBM-H901 injection after vector infusion and Padua-FIX activity levels ≥ 150%; – Lack of efficacy of investigational gene vector drug, either in this or other studies; – Occurrence of a significant previously unknown adverse reaction or an unexpected known adverse reaction of a high severity or incidence rate; – Other unfavorable safety findings, including clinical examination and non-clinical findings; • Medical or ethical reasons making continuation of the above study unreasonable; • Difficulty in subject enrollment making the study unlikely to be completed within an acceptable time range; • Suspension or termination as required by regulatory authorities.
Immunomodulatory Therapy	In this study, prophylactic steroids will be used, that is, oral glucocorticoids (prednisone or prednisolone) will be prophylactically used from Day −1 before BBM-H901 infusion to eight weeks after infusion. Specific prophylactic glucocorticoid dosing regimens and recommended steroid reduction plans are detailed in Section 5.5 of the text. By referring to the guidelines issued by the American Association for the Study of Liver Diseases (AASLD), the data of SJ-UCL study, and the management experience for this product in previous studies, in the context of no other etiologies, if the following conditions are observed during the follow-up period after gene vector infusion: • Liver aminotransferase (ALT and/or AST) > 1.5 × baseline or ALT and/or AST above the ULN; • Or in the absence of evidence of FIX inhibitor production, a continuous decrease in the vector Padua-FIX activity; • Requirement for initiating immunomodulatory therapy comprehensively determined by the investigator based on clinical laboratory parameters and clinical assessment; Glucocorticoids (e.g., prednisone/prednisolone) may be used for treatment. If glucocorticoids are not effective, the investigator may consider other alternative or

	combination therapies with other immunosuppressive agents (e.g., tacrolimus). For specific initial dosing regimen and dose reduction plan of glucocorticoids, please refer to the prophylactic steroid dosing regimen. The application can be adjusted by the investigator according to the clinical manifestations, test, and examination indicators of subjects.
Evaluation Indicators	**Safety evaluation:** During the study, any AEs that occur in all subjects during the study will be observed and recorded, including abnormalities in clinical symptoms, physical examination, vital signs, clinical laboratory tests (hematology, blood biochemistry, urinalysis, and coagulation test), 12-lead ECG, FIX inhibitor test, and vector shedding. AEs and clinical laboratory abnormalities will be graded using the NCI CTCAE v5.0, and their correlation with BBM-H901 injection will be determined. Safety and tolerability indicators: AEs/SAEs (including DLT), clinical symptoms, physical examination, vital signs, clinical laboratory tests (hematology, blood biochemistry, urinalysis, and coagulation test), 12-lead ECG, FIX inhibitor test, AAV capsid neutralizing antibody and capsid-binding antibody test, assessment of cellular immunity by single cell sequencing, and bone density examination. **PK parameters of BBM-H901 injection:** Samples will be collected at the specified sampling time points to assess changes in Padua-FIX activity levels and AAV viral loads. **Efficacy evaluation:** 1. ABR (including spontaneous bleeding, traumatic bleeding, and joint bleeds); 2. Padua-FIX activity level; 3. Number and volume of infusions of other FIX protein products (including recombinant and plasma-derived FIX protein products, such as recombinant human coagulation factor IX for injection and prothrombin complex concentrate); 4. Number of target joints; 5. Relevant scales for hemophilia assessment (HJHS, HEAD-US-C, and CHO-KLAT).
Methods for Collection and Processing of Biological Samples	**Blood sample collection**: Blood samples for corresponding analyses will be collected according to the time points of the protocol, and the actual sampling time will be documented. For specific operation of sample collection, please refer to relevant standard operating procedures or operation manual for biological sample collection and processing. After collection, whole blood samples will be centrifuged and/or stored according to relevant standard operating procedures or operation manual for biological sample collection and processing, and the requirements for different analyses, such as Padua-FIX activity, FIX inhibitor, neutralizing antibody against and binding antibody to AAV capsid, and PBMC analyses, with the processing and warehousing records of biological samples well documented. **Fecal, urine, and saliva sample collection:** Fecal, urine, and saliva samples will be collected for vector shedding analysis according to the sampling time points for corresponding samples in the protocol. After collection, fecal, urine, and saliva samples will be stored according to the relevant standard operating

	procedures or operation manual for biological sample collection and processing, with the processing and warehousing records of biological samples well documented.

Statistical Analysis	Measurement data will be generally summarized using descriptive statistics, including number of subjects (n), arithmetic mean (mean), standard deviation (SD), median, minimum (min), and maximum (max). Enumeration data will be generally summarized by number of subjects (n) and percentage (%). The data will be summarized using appropriate statistical tables and graphs. The descriptive statistical analysis will be performed on the number of enrolled subjects, dropouts, and baseline characteristics of enrolled subjects. **Analysis sets:** Full analysis set (FAS): All subjects who receive BBM-H901 injection. Sociodemographic characteristics variables, disease-related variables, vector shedding, FIX activity, and efficacy indicators will be analyzed based on this analysis set. Safety analysis set (SS): All subjects who receive BBM-H901 injection and are evaluable for safety. Safety indicators will be analyzed based on this analysis set. **Safety analysis:** Based on SS, tabulation and descriptive statistics will be performed for safety and tolerability indicators, including the incidence of various AEs, vital signs, physical examination, clinical laboratory test, FIX inhibitor test, thrombosis test, vector shedding analysis, neutralizing antibody against and binding antibody to AAV capsid, 12-lead ECG results, bone density, and assessment of cellular immunity by single cell sequencing and their changes from baseline. AEs will be coded and classified by "system organ class (SOC)" and "preferred term (PT)" according to the Medical Dictionary for Regulatory Activities (MedDRA, the latest version). All AEs will be tabulated by occurrence and summarized by SOC, PT, and dose group. The incidence of each AE will be summarized by SOC, severity (as per NCI CTCAE v5.0), and correlation with the use of BBM-H901 injection. Physical examinations and abnormal clinical laboratory test values will be summarized by time point. Baseline values of and changes in critical safety data (e.g., liver function test results) at each time point will be listed. All AEs, SAEs, AEs leading to discontinuation, and investigational product-related AEs will be summarized separately. **Analysis on Padua-FIX activity and AAV viral loads:** Padua-FIX activity and AAV viral load data will be listed and statistically analyzed based on the FAS. Padua-FIX activity levels and AAV viral loads will be tabulated and subjected to descriptive statistics by age group and scheduled blood sampling time points, and individual subject data will be tabulated. Individual Padua-FIX concentration-time profiles will be plotted according to the actual blood sampling time points. Mean and median Padua-FIX concentration-time profiles will be plotted according to the scheduled blood sampling time points. At the end of the study, information on different age groups of subjects, the scheduled blood sampling time points, FIX activity, and AAV viral loads will be summarized descriptively, and individual subject data will be listed. **Efficacy analysis:** Efficacy analyses will be performed based on the FAS, and the efficacy analysis data will be

	summarized descriptively. ABRs within 52 weeks will be presented with descriptive statistics. Similar analyses will be performed for other efficacy indicators, if applicable. Categorical indicators will be calculated using Fisher's exact test, with two-sided 95% confidence intervals provided, if applicable. **Analysis report:** The final analysis of this study will be conducted and a summary report will be prepared after the last subject completes the 52-week/early withdrawal follow-up. After the last subject in the entire study completes the 5-year long-term follow-up/early withdraws, all follow-up data will be summarized to complete a supplementary report.

ABBREVIATIONS

Abbreviation	Definition
AAV	Adeno-associated Virus
AASLD	Guidelines Issued by the American Association for the Study of Liver Diseases
ABR	Annualized Bleeding Rate
AE	Adverse Event
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
APTT	Activated Partial Thromboplastin Time
AST	Aspartate Aminotransferase
BMI	Body Mass Index
BU	Bethesda Unit
BUN	Urea Nitrogen
Ca	Calcium
CHO-KLAT	Canadian Hemophilia Outcomes - Kids Life Assessment Tool
CI	Confidence Interval
Cl	Chlorine
CTCAE	Common Terminology Criteria for Adverse Events
DILI	Drug-induced Liver Injury
DNA	Deoxyribonucleic Acid
SRC	Safety Review Committee
DLT	Dose-limiting Toxicity
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
eGFR	Estimated Glomerular Filtration Rate
EDC	Electronic Data Capture
FAS	Full Analysis Set
FIB	Fibrinogen
FIX	Coagulation Factor IX
GCP	Good Clinical Practice
HBsAg	Hepatitis B Surface Antigen
HBV	Hepatitis B Virus
HCT	Hematocrit
HCV	Hepatitis C Virus
HCV-Ab	Antibody to Hepatitis C Virus
HEAD-US-C	Hemophilic Early Arthropathy Detection with UltraSound in China
HIV-Ab	Human Immunodeficiency Virus Antibody
HGB	Hemoglobin
HJHS	Hemophilia Joint Health Score
IB	Investigator's Brochure
ICH	The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use

Abbreviation	Definition
IIT	Investigator-initiated Clinical Study
ITI	Immune Tolerance Induction
ITR	Inverted Terminal Repeat
K	Potassium
LDH	Lactate Dehydrogenase
Max	Maximum
Mean	Arithmetic Mean
MedDRA	Medical Dictionary for Regulatory Activities
Median	Median
Mg	Magnesium
Min	Minimum
MTD	Maximum tolerance dose
NCI	(US) National Cancer Institute
NHPS	Nonhuman Primates
NMPA	National Medical Products Administration
PBMC	Peripheral Blood Mononuclear Cell
PCT	Patent Cooperation Treaty
PLT	Platelet Count
PT	Prothrombin Time
QoL	Quality of Life
qPCR	Quantitative Polymerase Chain Reaction
QTcF	QT Interval Corrected by Fridericia Formula
RBC	Red Blood Cell Count
RNA	Ribonucleic Acid
SAE	Serious Adverse Event
SD	Standard Deviation
SG	Urine Specific Gravity
SOP	Standard Operating Procedure
SS	Safety Analysis Set
TGA	Thrombin Generation Assay
TT	Thrombin Time
U-GLU	Urine Glucose
U-KET	Urine Ketone
WBC	White Blood Cell Count
WHO DD	World Health Organization Drug Dictionary

STUDY BACKGROUND

Disease Background and Current Treatment Status

Hemophilia B, an X-linked recessive genetic disease resulting in coagulation factor IX (FIX) deficiency in blood, is a bleeding disorder due to coagulation dysfunction. Generally, female carriers may pass on the mutated gene to male offspring, causing the latter to become hemophilia B patients. The severity of hemophilia B symptoms is related to FIX levels in the body; the plasma FIX content in critically ill patients is less than 1% of that in normal people, so spontaneous bleeding occurs frequently, such as intra-articular bleeding, soft tissue hematoma, abdominal bleeding, and cerebral bleeding, which may eventually lead to severe arthropathy and chronic pain. These resulting conditions may seriously compromise patients' quality of life or even life span[1]. The incidence of hemophilia is approximately 1/25,000 with no ethnic or regional differences. According to the data reported by the Hemophilia Treatment Center Collaboration Network of China (HTCCNC) in 2019, 18,530 hemophilia patients were registered in China, of whom 2447 were with hemophilia B[2].

Replacement therapy of exogenous FIX supplementation is currently the main way for the treatment of hemophilia B, which mainly includes recombinant FIX, blood-borne FIX, and human prothrombin complex concentrate (hPCC). However, the short half-lives of these drugs render repeated infusion necessary to maintain FIX levels and avoid recurrent bleeding causing lesions in joints and other organs. Although the newly approved long-acting recombinant FIX product provides a more convenient dosing option for patients as it has a longer half-life and reduces the frequency of prophylactic FIX to once a week, it still does not fundamentally change the current status that it is difficult to maintain long-term high concentrations of coagulation factor by intravenous infusion of FIX biological products, and patients still need frequent injections. Nonetheless, FIX products are expensive, and frequent infusions bring a heavy economic burden for patients and their families. Therefore, regular prophylaxis is not widespread. According to the report issued by the World Federation of Hemophilia (WFH) in 2018, the proportion of patients aged < 18 years who were receiving regular prophylaxis with FIX products in China was 15%. In addition, when FIX product or hPCC is used for prophylaxis, patients need to be strictly compliant to achieve the therapeutic effect, otherwise missing medication will still increase the risk of bleeding; long-term use of blood products such as hPCC also has the risk of infection with various blood-borne diseases such as hepatitis B, hepatitis C or HIV. Therefore, researchers in China and abroad have been trying hard to "cure" hemophilia B, so as to free patients from frequent injections of replacement therapy.

With the breakthrough in gene and vector research, many gene therapies based on viral vectors (such as AAV and lentivirus) carrying target genes have been applied in experimental and clinical studies, and several gene therapy drugs for rare diseases have been marketed.

AAV is a single-stranded DNA virus that itself is non-toxic and harmless and can be used as a vector to carry therapeutic genes to achieve expression *in vivo*. The engineered AAV has all genes of the virus itself removed, showing better bio-safety, and has been applied to gene therapies for more than 5000 genetic diseases and chronic diseases. At present, more than 300 clinical trials have used AAV as a therapeutic vector, including gene therapy for hemophilia.

Hemophilia B has gradually become a hot field of gene therapy due to its following characteristics:

1) It is caused by single gene mutation, with clear pathogenesis;
2) The short cDNA length of FIX facilitates carriage by AAV vectors;
3) A small increase in FIX level can significantly improve disease symptoms. According to available clinical study reports, an increase in plasma FIX to more than 5% of normal plasma can significantly reduce the incidence of spontaneous bleeding events in critically ill patients;
4) The effective range of FIX treatment is wide (reaching 5%–100% of normal plasma), thus strict control of FIX expression level is not required;
5) FIX is mainly expressed in liver, but it can be correctly expressed and modified by a variety of tissue cells and secreted into the blood;
6) Model animals are well-established, including large animal (hemophilia B in dogs) and small animal (hemophilia B in mice) models.

Gene therapy for hemophilia B, by a single intravenous infusion of FIX gene carried by AAV vectors to target cells (mainly hepatocytes), achieves long-term stable expression of normal active FIX protein for the purpose of treating hemophilia B. At present, the data from clinical studies in Europe and the United States showed that the use of AAV as a gene therapy vector for hemophilia is approaching maturity and is achieving the goal of "one treatment, long-term effectiveness". However, the predicted treatment cost of millions of dollars per patient is not in line with the current medical status in China. Therefore, accelerating the independent research and development of gene therapy drugs in China and reducing treatment cost are the ultimate goals of such products.

BBM-H901 injection, developed by Shanghai Xinzhi BioMed Co., Ltd. with independent intellectual property rights, is an adeno-associated virus vector drug expressing human coagulation factor IX. It uses an independently developed highly liver-targeted AAV843 capsid and carries a stably expressed padua-FIX mutant optimized by codon optimization program (GeneArt). This mutant has a mutation at amino acid 338 of wild-type FIX protein, where leucine replaces arginine. This mutation significantly increases FIX activity and has been widely used in several gene therapy drugs under development. The use of padua-FIX mutant both enhances therapeutic effect and reduces gene vector dosage, thus minimizing possible cellular

immune responses. BBM-H901 injection, with a clear mechanism of action, has shown a good therapeutic effect in mouse models with hemophilia B, and is clinically intended for the treatment of hemophilia B patients with endogenous FIX ≤ 2 IU/dL (≤ 2%).

Summary of Preclinical Studies

AAV843 capsid

The AAV gene transduction vector used in this study is a bioengineered AAV capsid. A library of AAV capsid protein mutants is constructed using the DNA shuffling technology and screened from adult C57BL/6 mice to obtain a new AAV capsid, AAV843, with high transfectivity in hepatocytes, greatly enhancing the *in vivo* transduction efficiency of the gene in hepatocytes so as to minimize the consumption of gene vector and probable cellular immune response.

Hepatocyte-specific expression cassette

In this study, the FIX gene is optimized using GeneArt, and without changing the amino acid sequence, the TCG and CGT sequences in the FIX gene are removed to achieve a stable expression. The padua mutation, where leucine replaces arginine at amino acid 338, results in a naturally occurring FIX mutant that is more active than wild-type and can increase FIX activity levels[3].

The natural intron of the FIX gene is relatively long. In order to further increase the FIX gene expression, small human introns are synthesized in this study and inserted into the 5' untranslated region (5' UTR) and 5' coding region of the vector. The expression cassette is also cloned within a mutant double-stranded AAV vector with a deletion in the left inverted terminal repeat (ITR) D region of AAV to finally obtain a FIX AAV expression cassette.

Introduction to preclinical animal studies

The single intravenous administration of BBM-H901 injection to B-F9 KO mice, a hemophilia B model, shortened activated partial thromboplastin time (APTT) and increased FIX activity in B-F9 KO mice, a hemophilia B model, in a significant long-acting and dose-dependent manner. In the tail vein transection bleeding test in hemophilia B model mice, single intravenous injection of BBM-H901 injection to model mice could significantly reduce the amount of bleeding after tail vein transection at 8 weeks after administration, indicating a significant and long-acting procoagulant effect.

In a safety pharmacology study on central nervous system function, BBM-H901 injection did not affect the central nervous system function of SD rats after single intravenous infusion.

In the general toxicology study, during the 13-week observation after single intravenous injection of BBM-H901 injection to SD rats, it was noted that the coagulation parameters of animals in the high-dose group showed pharmacology-related changes at some time points. Other relevant

indicators such as hematology, blood biochemistry, immunology, and histopathology did not show abnormality in each dose group.

In summary, BBM-H901 injection, a gene vector drug independently developed by Shanghai Xinzhi BioMed Co., Ltd., is an AAV vector drug obtained by optimizing the promoter and FIX gene with high-efficiency expression of FIX in the liver using a brand-new high-liver-targeted AAV capsid, with clear mechanism of action. It shows excellent transduction activity and therapeutic effect in preclinical *in vivo* and *in vitro* studies, with good safety profile.

Summary of Similar International Clinical Studies

At present, gene therapy drugs based on the rAAV technology have been widely used in hereditary rare diseases, senile degenerative diseases, and cancer. According to the data published from Clinical Trial, more than 290 clinical trials have been published so far, involving more than 30 monogenic diseases and more than 20 complex diseases, and most clinical trials are ongoing. Worldwide, 5 gene therapy drugs for the treatment of familial lipoprotein-lipase deficiency, hereditary retinal disorder and spinal muscular atrophy, aromatic L-amino acid decarboxylase deficiency, and hemophilia A, i.e., Glybera, Luxturna (children and adults), Zolgensma (children under 2 years of age), Upstaza (children over 18 months of age), and Roctavian, have been successively approved for marketing.

Current hemophilia B clinical studies are all first conducted in people > 18 years of age. In the initial study on rAAV gene treatment of hemophilia B, cytomegalovirus (CMV) enhancer/promoter and rAAV2 serotype (rAAV2-CMV-F9-WT) were used for skeletal muscle injection, exhibiting safe, long-term local expression of FIX, but systemic permanent expression failed[4]. Thanks to good short-term and long-term safety in this study, new products with liver as target organ have been developed successively (liver is a FIX-generating organ).

As FIX is expressed in liver, the Children's Hospital of Philadelphia (CHOP) developed a liver-specific promoter gene therapy (rAAV2-hAAT-F9-WT), which was administered to 7 subjects via hepatic artery. However, the presence of neutralizing antibody prominently disrupted the expression of FIX in subjects. The initial FIX level in 1 subject reached 11%, but the FIX expression level was limited to 8 weeks or so, and later, FIX level decreased gradually, associated with transient asymptomatic hepatic transaminitis. Further research showed that cell-mediated immune response to AAV capsid antigen disrupted the transduced hepatocytes, leading to decreased FIX level and transient hepatic insufficiency. The above studies demonstrate that rAAV-2 vector is able to transduce human hepatocytes such that a desired expression level of FIX for treatment could be achieved. However, in future clinical studies, immunomodulation may be needed to enable long-term stable expression[5].

In the third study, St. Jude Children's Research Hospital (SJCRH) and University College London (UCL) treated 10 subjects with hemophilia B using rAAV8-LP1-F9-WT via peripheral vein[6, 7]. In this study, prednisone was used immediately after hepatic function abnormality due to initial therapy, and it was demonstrated that the use of this drug was able to notably antagonize the decline in FIX expression. Furthermore, it was also found in this study that, hepatic function abnormality in the subject was dose dependent, as more subjects experienced hepatic insufficiency with increasing dose.

The above studies provide sound support for further optimization of gene therapy drugs and clinical treatment strategy for hemophilia B.

Shire/Baxalta/Takeda conducted a phase I/II clinical study using AAV8 carrying FIX-Padua mutant (BAX335). BAX335 contains an expression cassette of liver-specific promoter element. A total of 8 subjects were treated with 3 dose levels of the vector. The subjects in the low-dose group (2×10^{11} vg/kg), medium-dose group (1×10^{12} vg/kg), and high-dose group (3×10^{12} vg/kg) had median maximum FIX levels of 3.5%, 12.0%, and 45.0%, respectively. Persistent therapeutic FIX:C level up to 20% was achieved in one subject, without hemorrhage or replacement treatment over 4 years; while in other subjects, FIX activity durations were not longer than 5–11 weeks. In contrast to the findings of some previous studies, corticosteroid therapy could not antagonize the loss of FIX activity. The absence of FIX gene expression may be due to challenge of intrinsic immune response[8].

In the phase I/II clinical trial sponsored by Dimension Therapeutics, AAVrh10 serotype vector (DTX101) was provided to 6 subjects, which contained codon-optimized wildtype F9 cDNA and was administered by intravenous infusion at ascending doses (1.6×10^{12} vg/kg and 5×10^{12} vg/kg). Finally, Dimension Therapeutics decided to terminate this clinical trial, as this phase I/II open-label clinical trial did not show the feasibility of the minimum goal of continuous development or commercialization in the future. Through a five-year expansion study (NCT02971969), this company continued to track and monitor closely 6 subjects with hemophilia B receiving DTX101 therapy.

The above studies are earlier exploration efforts of AAV gene therapy for hemophilia B and offer valuable experience for subsequent research. At present, early international developers of FIX-Padua AAV drugs (as BBM-H901 from Shanghai Xinzhi BioMed Co., Ltd.) include FLT180a from Freeline, SPK-9001 from Spark/Pfizer, and AMT-061 from uniQure.

FLT180a from Freeline

FLT180a (AAV2/S3-HLP2-Ti-FIXco) from Freeline employs novel AAVS3 capsid capable of transducing specifically FIX-Padua gene to hepatocytes. In the phase I/II dose-finding study B-AMAZE (NCT03369444), 10 subjects were enrolled to receive single infusion of different doses

of FLT180a (3.84×10^{11} vg/kg, N = 2; 6.4×10^{11} vg/kg, N = 2; 8.32×10^{11} vg/kg, N = 4; and 1.28×10^{12} vg/kg, N = 2), respectively; dose-dependent increases in FIX activity were noted in all the subjects, and within a median follow-up period of 27.2 months (range: 19.1–42.4 months), persistent FIX activity was observed in all subjects other than one subject who restarted prophylactic FIX therapy. As of Sep. 20, 2021, the factor activity levels in 5 subjects varied between 51% and 78%, the factor activity levels in 3 subjects between 23% and 43%, and the factor activity level in 1 subject reached 260%. A total of 287 AEs were reported in 10 subjects, among which FLT180a-related AEs accounted for about 10% (29 AEs), and immunosuppressant-related AEs for about 24% (35 AEs were glucocorticoid-related and 36 AEs were tacrolimus-related). FLT180a-related AEs occurred in 3 dose groups except for 2 subjects in the low-dose group. Hepatic transaminitis was the most common FLT180a-related AE; 13 events of transaminitis occurred in 8 subjects. Other FLT180a-related AEs included fatigue/discomfort, FIX level increased, muscle spasm/musculoskeletal pain/muscle pain, dyspepsia/belching, FIX level decreased, pulmonary sepsis, somnolence, and arteriovenous fistula thrombosis in subjects with higher FIX activity level. A total of 17 SAEs occurred in 7 subjects, among which, 12 SAEs were FLT180a-related, consisting of transaminitis (9 events), arteriovenous fistula (1 event), FIX activity decreased (1 event), and pulmonary sepsis (1 event), while 4 SAEs were immunosuppressant-related, consisting of epigastric pain (1 event), amylase increased (1 event), troponin increased (1 event), and tacrolimus toxicity (1 event)[9]. For safety concern, Freeline continued to modify dosage based on the previous study and carried out B-LIEVE study (NCT05164471) in order to explore a safe effective critical dose. On International Society on Thrombosis and Haemostasis (ISTH) 2022 Congress, the latest data were published as follows: 3 subjects had been enrolled to date, and a dose level of 7.7×10^{11} vg/kg was infused; the maximum infusion dose was 6.93×10^{13} vg (based on a body weight of 90 kg); as of May 23, 2022, 3 subjects had been followed up for 77 days, 56 days, and 36 days, respectively. For these 3 subjects, FIX activity was assayed using the one-stage method, and the measured FIX activity levels were 93 IU/dL, 92 IU/dL, and 80 IU/dL, respectively. No bleeding occurred after infusion, no FIX product was used, and transaminitis occurred in all 3 subjects after infusion, among which 2 subjects had to be intervened with steroids and tacrolimus.

Currently, FLT180a has not been registered for any phase III clinical trial, nor has there been any report on the marketing plan.

Pfizer/Spark's SPK-9001

Pfizer/Spark Therapeutics's SPK-9001 is currently undergoing phase I/II clinical trials for hemophilia B to determine the safety and kinetics of the AAV vector expressing FIX Padua mutant (SPK-9001). The vector is administered via a single intravenous infusion at a dose of 5×10^{11} vg/kg, combined with the injection of empty AAV capsids at a dose 3-5 times higher, which

bind to some antibodies to mitigate humoral immunity and enhance gene transduction. The mean ABR was 11.1 (range: 0–48) and mean FIX use was 2908 IU/kg (range: 0–8090 IU) before vector administration. FIX:C levels were detected one week after vector infusion, and steady-state expression was reached within 14 weeks. The mean level of FIX:C in the 10 subjects who received SPK-9001 infusion was 33.7% ± 18.5% (14.3%–76.8%) during 52 weeks, and 8 of 10 subjects completely stopped the use of replacement FIX products. The mean ABR was 0.4 (range: 0–4) and mean FIX use was 49.2 IU/kg (range: 0–376 IU) after vector administration. The infusion did not result in any serious adverse events such as FIX inhibitor formation or thrombosis. Two subjects had elevated alanine aminotransferase (ALT) and received steroid therapy[10].

A global multicenter phase III clinical trial (NCT03861273) of SPK-9001 has been registered on clinical.gov, and the trial planned to include 41 participating sites across the United States, European Union countries (France, Germany, Greece, and Sweden), the United Kingdom, Australia, Brazil, Canada, Japan, Saudi Arabia, Turkey, and Taiwan region of China. Using the same dose as in the phase I/II trial, a total of 45 subjects were enrolled in an international multicenter trial to determine the efficacy and safety with an expanded sample size. The trial was registered in Jul. 2019. The results of Pfizer's SPK-9001 phase III study published on its official website on Dec. 29, 2022 showed that the mean ABR was 4.43 in subjects before infusion and 1.3 in subjects after infusion. There has been no marketing progress report.

uniQure's AMT-060 and AMT-061

In uniQure Biopharma's phase I/II trial in hemophilia B patients, an AAV5 vector containing wild-type FIX gene (AMT-060) was used at doses of 5×10^{12} vg/kg and 2×10^{13} vg/kg. Ten subjects were enrolled in the study, 5 in each group. The treated subjects showed stable FIX levels during the 2.5-year follow-up. In the low-dose group, FIX:C increased to 4.4 IU/dL, and ABR decreased from 9.8 to 4.6 (53%). In the high-dose group, the mean FIX activity increased to 6.9 IU/dL, and ABR decreased from 3.0 to 0.9 (70%). In terms of safety, 3 subjects had elevated ALT levels within 10 weeks after the AAV infusion and received steroid therapy. However, no significant decrease in FIX:C was detected[11]. The clinical trial showed that AMT-060 had a positive safety profile, but the efficacy was not significant due to the expression of wild-type FIX.

uniQure also developed an AAV5 vector with a FIX-Padua gene cassette (AMT-061) and treated 3 subjects with 2×10^{13} vg/kg of AMT-061. The reported 26-week data showed that at week 6, the mean FIX activity was 31% (23.9%–37.8%), which increased to 47% (33.2%–57.0%) at week 26, with 2 subjects exhibiting sustained FIX activity of > 40%. As for safety, AMT-061 demonstrated good tolerability, with no clinically significant elevations in liver transaminases, and no use of treatment-related corticosteroids[12]. Based on these results, a global multicenter phase III clinical trial (NCT03569891) for AMT-061 was registered in Oct. 2018. The trial

included 35 sites worldwide, and the countries and regions participating in this trial included the United States, the European Union (Belgium, Denmark, Germany, Ireland, Italy, the Netherlands, and Sweden), and the United Kingdom. At the 2022 Annual Congress of the European Association for Hemophilia and Allied Disorders (EAHAD), uniQure announced 6-month and 18-month data from the phase III clinical trial, which treated 54 subjects with 2×10^{13} vg/kg of AMT-061 infusion. The results showed that the subjects had a mean FIX activity of 36.9 IU/dL at 6 months and 39.0 IU/dL at 18 months following a single AMT-061 infusion. At 6 months after the infusion, all subjects had a 64% reduction in their annualized bleeding rate. Within 7–18 months post-infusion, the reduction was 77%. The drug demonstrated good tolerability with no reported treatment-related serious adverse events, no FIX inhibition, and no observed correlation between safety and pre-existing neutralizing antibody titers.

uniQure has submitted regulatory applications to the US Food and Drug Administration (FDA) and European Medicines Agency (EMA), which have now been approved for marketing by the FDA on Nov. 22, 2022 and by the EMA on Feb. 20, 2023

Overall, the leading companies and products in the development of AAV drugs for hemophilia B include Freeline's FLT180a, Spark's Spark-9001, uniQure's AMT-061 and BBM's BBM-H901 Injection. In different rAAV clinical trials, the FIX-Padua mutant has not shown significant immunogenicity or resulted in the formation of spontaneous thrombosis in human body.

Summary of Clinical Studies for BBM-H901 Injection

For BBM-H901 injection, an investigator-initiated clinical trial (IIT) (NCT04135300) was launched in 2019, which was approved by the Ethics Committee of Institute of Hematology, Chinese Academy of Medical Sciences. This is a single-center, single-arm, open-label clinical trial to evaluate the safety, tolerability, and pharmacokinetics of a single intravenous infusion of BBM-H901 injection (gene therapy with AAV vectors expressing human FIX) in hemophilia B patients with endogenous FIX activity levels ≤ 2 IU/dL.

The trial data published on Lancet Hematology on May 19, 2022 showed the following results[13]: 10 subjects were treated with 5×10^{12} vg/kg and after infusion followed up for a total of 705 weeks (range: 50–117 weeks), the mean FIX:C value was 36.93 ± 20.49 IU/dL in a median of 58 weeks of follow-up, no serious adverse events occurred, and no FIX inhibitors were detected; 9 subjects had no bleeding events after infusion, only 1 subject had a non-serious old bleeding event during two years of follow-up, and his mean FIX:C level remained above 30 IU/dL. Two subjects experienced asymptomatic transaminase elevations with concomitant FIX:C decreases, but the mean FIX:C levels remained above 10 IU/dL.

On Oct. 27, 2022, NEJM published an online article[14] indicating that hemophilia B subjects treated with BBM-H901 injection successfully underwent total knee arthroplasty 16 months after infusion of BBM-H901 injection. This represents the world's first successful total knee arthroplasty without coagulation factor infusion after gene therapy for hemophilia. This case

suggests that *in vivo* FIX Padua after treatment with BBM-H901 injection has a good hemostatic effect for major surgery requiring high hemostatic capacity.

BBM-H901 injection is also the first intravenous hemophilia AAV gene therapy drug to enter the registered clinical trial in China. Its applicant has successfully completed the initial administration in a registered study in adult subjects in Dec. 2021. At present, the trial is underway as scheduled.

Benefit/Risk Assessment

This is a clinical study to evaluate the safety, tolerability, and efficacy of BBM-H901 injection in the treatment of hemophilia B. The expected benefits mainly include the following: reducing the bleeding rate, reducing the frequency and amount of infusion of exogenous FIX protein products as needed or required to prevent bleeding, reducing the number of target joints, improving the quality of life of patients, achieving a lifelong cure through one-time gene drug infusion, reducing the economic burden of patients, etc. Early use of AAV gene therapy to prevent bleeding in minors < 18 years of age may avoid joint deformities, improve the quality of life of minors, and help them to return to normal study and life.

The expected risks mainly include allergic reactions caused by infusion of AAV vector gene products, production of inhibitors, possible thrombosis risk when FIX activity is increased to ≥ 150%, elevated liver transaminases caused by immune response, bleeding caused by lack of efficacy, etc.

STUDY OBJECTIVES

Primary objective:

- To evaluate the safety and tolerability of a single intravenous infusion of BBM-H901 injection in hemophilia B male subjects (≥ 12 years and < 18 years) with endogenous coagulation factor IX (FIX) activity levels ≤ 2 IU/dL (i.e., ≤ 2%).

Secondary objectives:

- To evaluate the pharmacokinetics (PK) profile of Padua-FIX and adeno-associated virus (AAV) vectors in hemophilia B patients (≥ 12 years and < 18 years) with endogenous FIX activity levels ≤ 2% after a single intravenous infusion of BBM-H901 injection;
- To evaluate the efficacy of a single intravenous infusion of BBM-H901 injection in hemophilia B patients (≥ 12 years and < 18 years) with endogenous FIX activity levels ≤ 2%.

Study objectives of the long-term follow-up:

- To evaluate the long-term efficacy and safety of a single intravenous infusion of BBM-H901 injection.

STUDY DESIGN

Overall Design

This study is designed as a single-center, single-arm, open-label study to investigate the safety, tolerability, PK profile, and efficacy of a single intravenous infusion of BBM-H901 injection in hemophilia B patients (≥ 12 years and < 18 years) with FIX activity levels ≤ 2 IU/dL (i.e., ≤ 2%).

Nine evaluable subjects are planned to be enrolled successively into three age groups (group 1, group 2, and group 3) in descending order of age. Three subjects aged < 18 years and ≥ 16 years will be enrolled in group 1; 3 subjects aged < 16 years and ≥ 14 years will be enrolled in group 2; 3 subjects aged < 14 years and ≥ 12 years will be enrolled in group 3.

BBM-H901 infusion will be first performed in subjects in group 1. One sentinel subject will be set up to complete at least 2 weeks of observation after administration, and the investigator will comprehensively determine whether to continue the treatment for the other 2 subjects in this group.

After the last evaluable subject in group 1 completes the 10-week follow-up after administration, the SRC will comprehensively determine whether to enter the study for group 2. One sentinel subject will also be set up in group 2 to complete at least 2 weeks of observation after administration, and the investigator will comprehensively determine whether to continue the treatment for the other 2 subjects in this group.

After the last evaluable subject in group 2 completes the 10-week follow-up after administration, the investigator will comprehensively determine whether to enter the study for group 3. One sentinel subject will be set up in group 3 to complete at least 2 weeks of observation after administration, and the SRC will comprehensively determine whether to continue the treatment for the other 2 subjects in this group.

After the last evaluable subject in group 3 completes the 10-week follow-up, the SRC will comprehensively determine the safety and tolerability. Administration at other age groups will not be continued.

Subjects who complete the 52-week assessments will continue to be followed up for 5 years after administration to obtain long-term assessment data.

Subjects will be followed up for a total of approximately 5 years after enrollment in the study, including a maximum 4-week screening period (starting immunomodulatory therapy on day −1, and baseline examination on day −1), a post-infusion 52-week follow-up period (follow-up on D3, D6, weekly at weeks 2–8, every 2 weeks at weeks 10–18, every 4 weeks at weeks 22–26, at week 32, and every 10 weeks at weeks 42–52), and a long-term follow-up period for additional 4 years after 52 weeks (every 6 months).

A schematic of the study design is shown below.

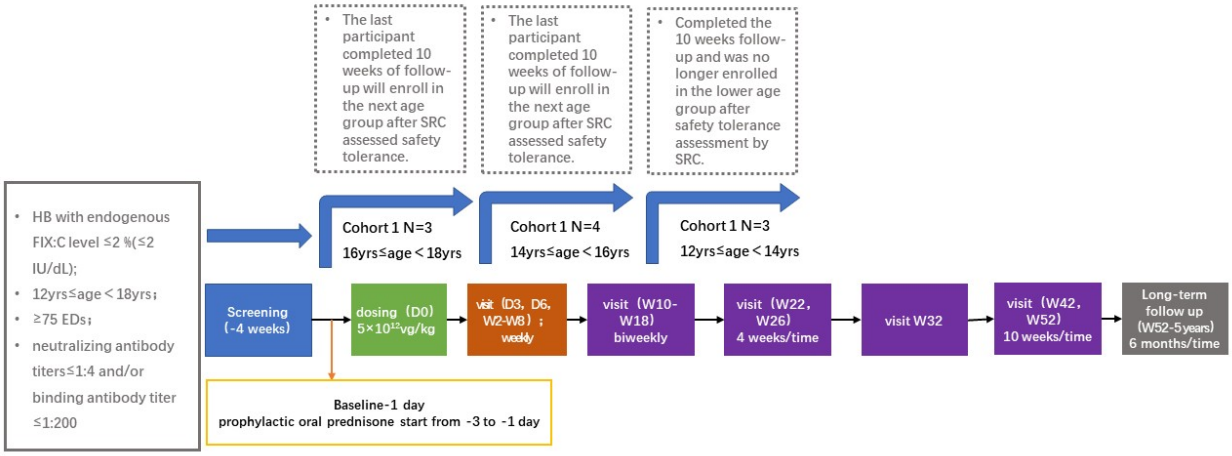

Figure 1. Schematic of study design

Study Endpoints

Primary endpoints:

（1） Incidence of dose-limiting toxicity (DLT) events, as determined by the Safety Review Committee (SRC), within 10 weeks after BBM-H901 infusion;

（2） Incidence of adverse events (AEs) and serious adverse events (SAEs) within 52 weeks after BBM-H901 infusion;

（3） Changes in liver function (including alanine aminotransferase [ALT] and aspartate aminotransferase [AST]) within 52 weeks after BBM-H901 infusion from before treatment.

Secondary endpoints:

（1） PK parameters of Padua-FIX and rAAV vectors after the infusion of BBM-H901 injection:

- Padua-FIX activity levels within 52 weeks after BBM-H901 infusion, including peak activity level, steady-state activity level or mean activity level;
- Changes in AAV vector shedding [plasma, urine, feces, saliva, and peripheral blood mononuclear cells (PBMCs)] within 52 weeks after BBM-H901 infusion;

（2） Efficacy endpoints for BBM-H901 injection, including:

- Annualized bleeding rate (ABR) within 52 weeks after BBM-H901 infusion (including spontaneous bleeding, traumatic bleeding, and joint bleeds);
- FIX activity levels at each visit within 52 weeks after the infusion of BBM-H901

injection;

- Number and volume of infusions of other FIX protein products (including recombinant and plasma-derived FIX protein products, such as recombinant human coagulation factor IX for injection and prothrombin complex concentrate) within 52 weeks after BBM-H901 infusion;
- Number of target joints within 52 weeks after BBM-H901 infusion;
- Number of joint bleeds within 52 weeks after BBM-H901 infusion;
- Proportion of subjects without bleeding events within 52 weeks after BBM-H901 infusion;
- Changes in joint health score, joint ultrasound score, and quality of life from baseline to 4 weeks, 12 weeks, 26 weeks, and 52 weeks after the infusion of BBM-H901 injection using the following scales related to hemophilia assessment: Hemophilia Joint Health Score (HJHS), Hemophilic Early Arthropathy Detection with UltraSound in China (HEAD-US-C), and Canadian Hemophilia Outcomes-Kids' Life Assessment Tool (CHO-KLAT);

（3） Other safety endpoints for BBM-H901 injection:

- Incidence of FIX inhibitors (detected by the Bethesda method or the Nijmegen-Bethesda) within 52 weeks after BBM-H901 infusion;
- Titers of neutralizing antibody against and binding antibody to AAV capsid;
- Clinically significant changes in vital signs, physical examination, clinical laboratory test values, 12-lead electrocardiogram (ECG), and alpha-fetoprotein within 52 weeks after the infusion of BBM-H901 injection;
- Cellular immune status of the body before and after AAV vector infusion assessed by single-cell RNA sequencing analysis;
- Changes in bone mineral density before and after treatment in adolescent subjects.

Long-term follow-up endpoints:

The long-term follow-up endpoints after the infusion of BBM-H901 injection include but are not limited to:

- ABR (including spontaneous bleeding and traumatic bleeding);
- Padua-FIX activity;
- The number and volume of infusions of other FIX protein products (including

recombinant and plasma-derived FIX protein products) per year;

- Annualized bleeding rate of different bleeding events: including spontaneous bleeding, traumatic bleeding, and bleeding requiring no treatment;
- Number of target joints;
- Frequency of joint bleeds;
- Viral load of rAAV vector;
- Incidence of AEs and SAEs;
- Incidence of FIX inhibitors;
- rAAV neutralizing antibody titer and capsid-binding antibody titer;
- Clinically significant changes in vital signs, physical examination, clinical laboratory test values, 12-lead ECG, and alpha-fetoprotein (AFP);
- Changes in bone mineral density before and after treatment in adolescent subjects.

Rationale for Dose Selection

The dose used in clinical studies on AAV gene therapy worldwide for hemophilia B mostly ranged from 5×10^{10} to 2×10^{13} vg/kg. FIX expression and activity increase with dose. However, differences in AAV capsid and the use of different recombinant FIX expression elements have resulted in varying treatment outcomes, with levels of FIX gene expression ranging from 5% to 40%. A starting dose of 5×10^{12} vg/kg was used in the ongoing IIT study and registration clinical studies of BBM-H901 injection in patients ≥ 18 years of age with hemophilia B. With reference to the above data, in consideration that the age range of subjects in this study is ≥ 12 years and < 18 years, which is characteristic of older children, 5×10^{12} vg/kg is also selected as the dose for this clinical study. The actual infusion volume received by the subjects will be calculated based on their body weight.

Definition of Dose-Limiting Toxicity

The severity of AEs will be evaluated and graded according to the (US) National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 (v5.0). DLTs are defined as the following AEs that occur within 10 weeks after BBM-H901 infusion and are related to BBM-H901 injection as determined by the investigator:

（1） Non-hematological toxicities:

- ≥ Grade 3 total bilirubin elevation;
- Grade 3 ALT and/or AST increase that cannot return to baseline levels after

4 weeks of immunosuppressive therapy;

- Grade 4 ALT and/or AST increase;
- Grade 3 or 4 allergic reactions, such as bronchospasm and immediate hypersensitivity;
- Thrombosis events (other than grade 4 thrombophlebitis at the infusion site) related to BBM-H901 injection as determined by the investigator with Padua-FIX activity levels ≥ 150%;
- Prolonged QTcF interval (absolute value > 500 ms or prolongation > 60 ms from baseline);
- Grade 3 vomiting or diarrhea lasting >3 days after adequate drug treatment;
- Grade 4 (life-threatening) vomiting or diarrhea, irrespective of the duration of the event;
- Other ≥ grade 4 non-hematological toxicities.

（2） Hematological toxicities:

- Grade 4 neutrophil count decrease (neutrophil count < 0.5×10^9/L) lasting > 5 days;
- Grade 3 febrile neutropenia (neutrophil count < 1.0×10^9/L with transient body temperature > 38.3 °C or persistent body temperature ≥ 38 °C for more than 1 h);
- Grade 4 platelet count decreased (platelet count < 25.0×10^9/L) or grade 3 platelet count decreased (platelet count < 50.0×10^9/L) with bleeding;
- Grade 4 anemia.

（3） Any other clinically significant or unacceptable toxicities determined as DLTs by the sponsor or investigator.

The following cases are not defined as DLTs:

- Any grade of alopecia;
- Any grade of isolated laboratory changes without clinical sequelae or clinical significance.

Safety Review Committee

A Safety Review Committee (SRC), which is planned to be composed of investigators and other relevant experts, will be established for this study. Unexpected circumstances that arise during

the course of the study will be discussed by the SRC. The available safety, tolerability, and efficacy data for subjects will be discussed in the forms of conference call, online meeting, and email feedback, and the comments on whether to continue the enrollment in the next dose group and whether to suspend the study will be given.

Study Evaluation Indicators

Safety and tolerability indicators: DLT, AEs, SAEs, vital signs, physical examination, clinical laboratory tests (hematology, blood biochemistry, urinalysis, coagulation function), 12-lead ECG, FIX inhibitor test, AAV capsid neutralizing antibody and capsid-binding antibody test, bone density, and assessment of cellular immunity by single cell sequencing.

Pharmacokinetic parameters for BBM-H901 injection: The analysis of Padua-FIX activity levels, FIX antigen levels, and AAV viral vector shedding will be assessed on samples collected at defined sampling points.

Efficacy endpoints:

1) ABRABR (including spontaneous bleeding, traumatic bleeding, joint bleeding, etc.);
2) Padua-FIX activity level;
3) Number and volume of infusions of other FIX protein products (including recombinant and plasma-derived FIX protein products, such as recombinant human coagulation factor IX for injection and prothrombin complex concentrate);
4) Number of target joints;
5) Relevant scales for hemophilia assessment (HJHS, HEAD-US-C, and CHO-KLAT).

Study Suspension/Early Termination

The Ethics Committee or the investigator has the right to suspend or prematurely terminate part of the clinical study or the entire clinical study.

- If the Ethics Committee makes the above decision, the investigator should immediately report to the clinical study site and provide a detailed written description.
- If the investigator makes the above decision, the investigator should immediately report to the clinical study site and the Ethics Committee and provide a detailed written description.

Reasons for study suspension/early termination include but are not limited to:

- The new information leads to an unfavorable judgment of the risk and benefit of the study for AAV gene therapy drug, for example, due to:
  - Deaths related to BBM-H901 injection occur during the study period as judged by

the investigator;

- The following situations occur during the study and are due to the subjects' inability to tolerate BBM-H901 injection:

  a） Grade 3 or 4 toxicity (except for transaminase increased) related to BBM-H901 injection as determined by the investigator according to NCI CTCAE v5.0, such as bronchospasm and immediate hypersensitivity;

  b） Grade 3 transaminase increased (including ALT and/or AST) related to BBM-H901 injection as determined by the investigator according to NCI CTCAE v5.0 that cannot be recovered to baseline after 4 weeks of immunosuppressive therapy;

  c） Grade 4 transaminase increased (including ALT and/or AST) related to BBM-H901 injection as determined by the investigator according to NCI CTCAE v5.0;

  d） Thrombosis events (other than grade 4 thrombophlebitis at the infusion site) related to BBM-H901 injection after vector infusion and Padua-FIX activity levels ≥ 150%;

- Lack of efficacy of investigational gene vector drug, either in this or other studies;
- Occurrence of a significant previously unknown adverse reaction or an unexpected known adverse reaction of a high severity or incidence rate;
- Other unfavorable safety findings, including clinical examination and non-clinical findings;

- Medical or ethical reasons making continuation of the above study unreasonable;
- Difficulty in subject enrollment making the study unlikely to be completed within an acceptable time range;
- Suspension or termination as required by regulatory authorities.

Definition of the End of Study

The end of the entire study is defined as when the last subject completes the last scheduled visit, unless the study is prematurely terminated for reasons described in the protocol.

Schedule of Activities

Item[1]	Screening period[2]		Dosing period[3]	Follow-up period 1																					Long-term follow-up
				Week 1 (± 1 day)		Weeks 2–18 (± 2 days)												Weeks 22–52 (± 7 days)					Every 6 months ± 14 days/ early withdrawal
Time (weeks)	Week 4	Day −1	Day 0	1		2	3	4	5	6	7	8	10	12	14	16	18	22	26	32	42	52/early withdrawal	
				Day 3	Day 6																		
Informed consent form	X																						
Review of the inclusion/exclusion criteria	X	X																					
Demographic characteristics[4]	X																						
Medical and treatment history of hemophilia B[5]	X																						
Other medical and treatment history[6]	X																						
Physical examinations[7]	X	X			X	X		X		X		X		X		X		X	X	X	X	X	X
Height, weight[8]	X	X																	X			X	X
Vital signs[9]	X	X	X		X	X		X		X		X		X		X		X	X	X	X	X	X
Hematology, blood biochemistry, and urinalysis[10]	X	X			X	X		X		X		X		X		X		X	X	X	X	X	X
ALT, AST		X		X			X		X		X												
Coagulation function[11]	X	X			X	X		X		X		X		X		X		X	X	X	X	X	X

Item[1]	**Screening period**[2]		**Dosing period**[3]	**Follow-up period 1**																				**Long-term follow-up**
				Week 1 (± 1 day)		**Weeks 2–18 (± 2 days)**													**Weeks 22–52 (± 7 days)**					**Every 6 months ± 14 days/ early withdrawal**
Time (weeks)	**Week 4**	**Day −1**	**Day 0**	**1**		**2**	**3**	**4**	**5**	**6**	**7**	**8**	**10**	**12**	**14**	**16**	**18**	**22**	**26**	**32**	**42**	**52/early withdrawal**	
				Day 3	**Day 6**																		
Infectious disease screening[12]	X																						
Genetic testing for hemophilia[13]	X																						
12-lead ECG[14]	X	X	X										X						X			X	X
Blood typing	X																						
Immunomodulatory therapies[15]		←																					
Infusion of BBM-H901 injection			X																				
Completion of FIX protein product infusion log[16]	←																					→	
Bleeding assessment	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Target joint count assessment[17]	X	X																	X			X	X
Abdominal B ultrasound[18]	X																		X			X	X
DXA for bone density		X																				X	Once every 12 months
FIX activity test[19]	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Item[1]	**Screening period**[2]		**Dosing period**[3]	**Follow-up period 1**																			**Long-term follow-up**
	Week 4	**Day −1**	**Day 0**	**Week 1 (± 1 day)**		**Weeks 2–18 (± 2 days)**												**Weeks 22–52 (± 7 days)**					**Every 6 months ± 14 days/ early withdrawal**
Time (weeks)				**1**		**2**	**3**	**4**	**5**	**6**	**7**	**8**	**10**	**12**	**14**	**16**	**18**	**22**	**26**	**32**	**42**	**52/early withdrawal**	
				Day 3	**Day 6**																		
FIX inhibitor test[20]	X	X				X		X				X		X		X			X	X	X	X	X
Scale assessment		X						X						X					X			X	
AAV capsid neutralizing antibody test	X	X			X	X		X						X					X			X	X
AAV capsid binding antibody test	X	X			X	X		X						X					X			X	X
AFP	X																		X			X	X
PBMCs, plasma, saliva, urine, stool sample collection[21]		X			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Single-cell sequencing of peripheral blood cells[22]																							
AEs[23]	◄																					►	X
Concomitant medications[24]	◄																					►	X

Notes: ALT: alanine aminotransferase; AST: aspartate aminotransferase; FIX: coagulation factor IX; AAV: adeno-associated virus; PBMCs: peripheral blood mononuclear cells

1. Additional visit items (including those not included in the schedule of activities) and/or additional unscheduled visits may be performed according to the actual safety and efficacy assessments, including but not limited to: liver transaminases, FIX activity, FIX antigen, blood lipids (total cholesterol [TC], high-density lipoprotein [HDL], low-density lipoprotein [LDL], very low-density lipoprotein [VLDL], triglycerides [TG]); coagulation function, thrombin generation assay (TGA), hepatitis B deoxyribonucleic acid quantification, hepatitis C ribonucleic acid quantification, serum interferon, lymphocyte subsets (B/T/NK cells), etc.
2. Collect the information of all subjects who participate in the screening, including the reasons for screening failure.
3. Do not use other FIX protein products and plasma concentrates within 96 h or 5 half-lives (whichever is longer) prior to the infusion of BBM-H901 injection. The investigational product should not be thawed until confirmation by the investigator regarding subject washout criteria.
4. Collect demographic data of subjects (including gender, age, ethnicity, and fertility status);
5. Collect the subject's hemophilia history (including date of diagnosis, severity, genotype, human leukocyte antigen [HLA, if any], previously used FIX products [including recombinant and plasma-derived, such as recombinant human coagulation factor IX injection, prothrombin complex, etc., type (on-demand, prophylaxis, continuous infusion, etc.), dosing regimen, dose, number of infusions], number of bleeding, etc.), and the number of bleeding (including spontaneous bleeding, traumatic bleeding, and bleeding not requiring FIX product treatment) in the last 26 and 52 weeks will be retrospectively determined. Record as truthfully as possible and estimate if necessary.
6. Other medical history and treatment history include: past and present medical history, family medical history, allergic history, surgical history and surgical plan, smoking, drinking, dietary habits, fertility plan, vaccination; history of blood donation within 3 months before screening, any participation in clinical trials, other medications and treatments other than those for the disease under study.
7. Physical examination: Only routine physical examination will be performed during the screening period, including skin/mucosa, lymph nodes, head, neck, chest, abdomen, musculoskeletal/extremities, and neurological system/psychiatric conditions. Only symptoms/vital signs-specific physical examination will be performed for other visits. The investigator may increase the frequency of tests based on safety considerations.
8. Height and weight: Only body weight will be measured on day −1, which will be used to calculate the dose of BBM-H901 injection, and height and weight will be measured at other visits.
9. Vital signs: including blood pressure, body temperature, pulse rate, and respiratory rate. Vital signs will be measured after the subject has rested for at least 5 min in sitting position. In addition to the routine visit points for vital signs, vital signs will be measured before injection of FIX protein products on day 0. If vital signs are beyond the normal range or the change is clinically significant and the investigator considers that a repeat measurement is required, 2 additional measurements will be taken approximately 2 min apart. The investigator may increase the frequency of tests based on safety considerations.
10. Hematology, blood biochemistry, and urinalysis: If the above laboratory tests are performed within 7 days before BBM-H901 infusion, they may not be performed on day −1; if blood biochemistry tests are required on day −1, the ALT, AST and GGT may not be repeated on day −1. During the trial, the investigator may increase the frequency of tests based on safety considerations.
11. Coagulation function: including plasma prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), plasma fibrinogen (FIB), and D-dimer. If this test is performed within 7 days prior to BBM-H901 infusion, it may not be performed prior to the infusion of BBM-H901 injection on day −1. The investigator may increase the frequency of tests based on safety considerations.
12. Infectious disease tests include: hepatitis B virus test (HBsAg, HBV-DNA quantitative detection), hepatitis C virus test (HCV-Ab, HCV-RNA quantitative detection), HCV viral load (if any), human immunodeficiency virus antibody (anti-HIV-Ab) test, and syphilis antibody test.
13. For subjects with known genotype, if the previously obtained genotype report is available, genotype test may not be performed at screening; for subjects with unknown genotype, samples will be collected at screening for analysis.
14. 12-lead ECG: The 12-lead ECG should be performed in supine position after the subject has rested for at least 5 min in supine position. ECG indicators

include heart rate, PR interval, QRS interval, uncorrected QT interval, and Fridericia-corrected QTc interval (QTcF). When an ECG measurement is abnormal (including an abnormal QTcF, e.g., QTcF > 500 ms or an increase from baseline > 60 ms) or is judged clinically significant by the investigator, 2 additional ECG measurements will be performed at least 2 min apart. The 12-lead ECG on the day of dosing will be performed within 1 h after the infusion of BBM-H901 injection. During the study, the frequency of tests may be increased as appropriate based on safety considerations.

15. In this study, prophylactic steroids will be used, that is, oral glucocorticoids (prednisone or prednisolone) will be prophylactically used from Day −1 before BBM-H901 infusion to eight weeks after infusion. See the study protocol for the specific treatment regimen.
16. Infusion log: The use of the FIX protein product will be recorded from the signing of informed consent to the end of the study, including the reason for treatment, start and end dates, type (on-demand, prophylaxis, continuous infusion, etc.), frequency, and doses.
17. Target joint count assessment: The subject's history of target joint disease will be collected within 52 weeks before screening, and the number of target joints will be assessed within 52 weeks after infusion. The target joint is defined as the occurrence of 3 or more spontaneous bleeding in a single joint in a consecutive 6-month period. If a joint bleeds ≤ 2 times in a consecutive 12-month period, the joint is no longer considered a target joint. The investigator may increase the frequency of tests based on safety considerations.
18. Abdominal B ultrasound: liver, gallbladder, pancreas, spleen, and bilateral kidneys. The investigator may increase the frequency of tests based on safety considerations.
19. During the assessment of FIX activity, subjects are required to suspend the prophylactic treatment with the FIX protein product after receiving the infusion of BBM-H901 injection until dose resumption is recommended by the investigator; if the subject develops bleeding during the study and requires on-demand use of FIX protein product as judged by the investigator, the washout period of at least 96 h or 5 half-lives (whichever is longer) for the FIX protein product should be ensured before the assessment of FIX activity. FIX activity levels will be measured using two methods of the one-stage methods (Sysmex platform, Actin FSL aPTT reagent, and Werfen platform, HemosIL SynthASil reagent).
20. FIX Inhibitor: Inhibitor detection is performed using the Bethesda assay or Nijmegen-modified Bethesda assay.
21. Collection of PBMCs, plasma, saliva, urine, and feces samples: Collect samples for vector shedding detection, and detect the titer of viral particles in plasma, PBMCs, saliva, urine, and feces using quantitative polymerase chain reaction (qPCR) until the results of three consecutive tests are negative.
22. Single-cell sequencing of peripheral blood cells: Single-cell RNA sequencing analysis method will be used to assess the cellular immune status of the body before and after AAV vector infusion and the impact of glucocorticoids on the immune status, without sampling from all subjects. The recommended sampling points are as follows: on day −1 before vector infusion, on day 3 after vector infusion, and at week 4 after vector infusion; and when transaminases increase by 1.5–2 times from the baseline value, when coagulation factors decrease sharply after stabilization, and at 2 weeks after steroid withdrawal; if the interval between blood sampling points is close, the investigator may combine the blood sampling points based on the actual situation.
23. AEs: AEs will be collected from the time of signing the informed consent form to 52 weeks ± 7 days of follow-up after drug administration, early withdrawal, or initiation of another gene drug treatment (whichever occurs earlier), and all AEs will be followed up until recovery of AEs, or stable disease, or interpretation of the AEs, subjects' death, or loss to follow-up. Only investigational product-related AEs will be collected during the long-term follow-up period and may be followed for up to 5 years/until early withdrawal.
24. Concomitant medications and treatments will be recorded during the screening period and study treatment until 52 weeks ± 7 days after drug administration, early withdrawal from follow-up, or initiation of another gene drug treatment, whichever occurs earlier. Only medications related to hemophilia therapy will be collected during the long-term follow-up period.

STUDY SUBJECTS

Inclusion Criteria

Subjects will be enrolled in this clinical study only if they meet all of the following criteria:

1. Subjects and/or their legal guardians fully understand the purpose, nature, method and possible adverse reactions of the study and sign the informed consent form (ICF), and the subjects are voluntarily participating in the study;
2. Requirements for age, gender, and body weight are as follows: Patients aged ≥ 12 years and < 18 years, male, body weight of 45 kg and above for patients aged ≥ 16 years and < 18 years, and body weight of 40 kg and above for patients aged ≥ 12 years and < 16 years;
3. Patients clinically diagnosed as hemophilia B with endogenous FIX activity levels ≤ 2 IU/dL (≤ 2%); if FIX activity level > 2% at screening is caused by insufficient elution of FIX protein products, the test can be repeated once after sufficient elution;
4. Titers of neutralizing antibody against and binding antibody to BBM-H901 capsid ≤ 1:4 and 1:200, respectively;
5. A history of bleeding events and/or injection of a FIX product (including recombinant and plasma-derived) within 12 weeks prior to screening in the subject's medical record;
6. No history of allergic reactions or allergy related to the administration of FIX or intravenous human immunoglobulin;
7. Patients who have received any recombinant and plasma-derived FIX products for 75 exposure days (EDs) with negative FIX inhibitors and have no clinical signs or symptoms of decreased response to FIX products after infusion at screening; or patients who have a positive history of FIX inhibitors but have turned negative for two years after immune tolerance induction (ITI) therapy and received FIX products for a cumulative total of 100 EDs during negative period (ineligible for those who have not been treated with ITI for previous positive inhibitor, or those who have positive result recurred after post-ITI negative conversion).
8. Acceptable laboratory test values obtained during screening and prior to administration (Day −1):
    a) Hemoglobin ≥ 11 g/dL or ≥ upper limit of normal (ULN) for peers
    b) Platelets ≥ 100×10^9/L
    c) AST and ALT ≤ 1.5 × ULN
    d) Total bilirubin ≤ 1.5 × ULN
    e) Estimated glomerular filtration rate (eGFR) ≥ 60 mL/min

9. Older underage subjects and their statutory guardians should clearly understand the requirements for contraception and know that subjects must avoid sexual intercourse or use reliable contraception methods;
10. Good compliance. Subjects and their statutory guardians are willing to participate in the "gene therapy" clinical study, able to be followed-up regularly, and able to accurately complete the subject diary during the follow-up period with the assistance from family members or caregivers.

Exclusion Criteria

Subjects will be excluded from the clinical study if they meet any of the following criteria:

1. Positive for hepatitis B surface antigen (HBsAg), hepatitis B virus deoxyribonucleic acid (HBV-DNA), or hepatitis C virus ribonucleic acid (HCV-RNA). Subjects with a history of hepatitis B or C should have two previous sample collections at an interval of at least 3 months and are considered negative if negative results are obtained for the above indicators at the two assessments;
2. Receiving antiviral therapy for hepatitis B and C;
3. Patients with other coagulation disorders except hemophilia B;
4. Patients taking any other systemic immunosuppressive agents within 30 days prior to screening except glucocorticoids and immunosuppressive agents recommended by the investigator; patients allergic to glucocorticoids, tacrolimus, and other immunosuppressive agents;
5. Patients with a history of vaccination within 30 days prior to screening, or a vaccination plan during immunomodulatory therapy;
6. Patients with the following conditions are not suitable for this study: potential liver diseases, such as previous diagnosis of portal hypertension, splenomegaly, hepatic encephalopathy or liver fibrosis ≥ stage 3; nodules and cysts found by B ultrasound, or alpha-fetoprotein increase found by laboratory tests, which are determined to be clinically significant by the investigator;
7. Patients with scheduled surgery during the 52-week study period after BBM-H901 infusion;
8. Presence of acute/chronic infection that may be at an increased risk due to participation in the study, or other chronic diseases that may make the subject unsuitable for participation in the study as determined by the investigator;
9. Patients who have received gene therapy prior to screening or other study drugs within 4 weeks prior to screening or within 5 half-lives of the investigational product (whichever is longer);

10. Intolerable anaphylaxis to prior treatment with FIX, or nephrotic syndrome on prior treatment with FIX;
11. Use of any plant preparation (herbal supplements or traditional Chinese medicines derived from plants, minerals or animals) that may affect liver function within 4 weeks prior to the study or during the study, except for drugs for topical use; Chinese herbal medicines that may affect the study as determined by the investigator;
12. Life-threatening bleeding such as intracranial hemorrhage within 2 years before enrollment, or sequelae after previous intracranial hemorrhage and severe hemorrhage, or with potential causes of gastrointestinal bleeding, including but not limited to gastrointestinal ulcers;
13. Subjects with serious diseases or other conditions that the investigator deems unsuitable for study participation;
14. Family members of study site staff directly involved in the implementation of the study, family members of study site staff who are otherwise supervised by the investigator, or family members of employees of related companies directly involved in the implementation of the study.

Retest and Re-screen

If some test results during the screening period are abnormal and clinically significant, retest can be performed if it is necessary as judged by the investigator; retest is allowed only once during the screening period when no therapeutic measures are taken for the abnormal results, and corresponding records should be made. If screening failure is confirmed, the information of subjects participating in the screening should be collected, including the reasons for screening failure.

Subjects who fail the first screening and meet the requirements of the protocol after treatment may be re-screened. After re-screening, if the subjects meet the inclusion and exclusion criteria specified in the protocol, they can be enrolled in this study.

Subjects who are re-screened should re-sign the informed consent form and be assigned a new screening number for re-screening.

Replacement of Subjects

If a subject fails to complete the specified 10-week observation due to reasons other than the drug intolerability, replacement of the subject may be considered.

Subject Dropouts

4.5.3 Withdrawal criteria

All subjects are free to withdraw from the study at any time. At the same time, the subject should withdraw from the study if the investigator considers study discontinuation necessary for the subject from a medical point of view due to the following conditions during the study:

1) Occurrence of intolerable AEs/SAEs, posing risks outweighing benefits if the subject continues to participate in the study, as determined by the investigator. In this case, the subject must withdraw from the study and be treated with appropriate measures;
2) Worsened condition after the study treatment, for which treatments prohibited in this study are required as determined by the investigator. In this case, the subject should withdraw from the study;
3) Poor compliance, as determined by the investigator, that may affect the evaluation of study results;
4) Subject withdrawal from the study requested by the investigator for any medical reason;
5) Serious protocol deviation;
6) Subjects meeting exclusion criteria (new or confirmed).

All original data should be retained for all withdrawn subjects. For subjects who do not participate in the study visit without a clear statement of withdrawal from the study or withdrawal of consent and whose status are unclear, the investigator should contact the subject as far as possible by various means such as telephone and SMS, and ask for the reasons. Loss to follow-up is declared if the subject still fails to perform a return visit after at least three negotiations for follow-up visit and study continuation have been made. Measures taken to contact the subject (such as contact date and method) should be well documented.

For subjects who withdraw due to intolerability, the investigator should take appropriate therapeutic measures.

4.5.4 Handling of dropouts

For subjects who prematurely discontinue the study, the date and reason for discontinuation must be recorded on corresponding eCRF. A subject who prematurely withdraws (except in cases where replacement is allowed as described in Section 4.4) will not be replaced by a new subject and will not be enrolled into the study again once he/she withdraws from the study.

To ensure subject safety, all existing or new AEs and laboratory abnormalities at the time of withdrawal will be followed up until clinical recovery, stable disease, pretreatment status (a condition that, in the opinion of the investigator, is not likely to resolve due to the subject's disease), return to baseline level (if the baseline level is known), loss to follow-up (e.g., no further information is available or the subject/caregiver refuses to provide information), or death.

STUDY MEDICATION

General Information of Investigational Product

Generic name: BBM-H901 Injection

Trade name: None at the moment

Strength: 8.0×10^{12} vg (2 mL)/vial

Storage: Store at −80 °C ± 10 °C

Labeling of Drug Product

The investigational product is AAV vector-based BBM-H901 injection. The strength is 8.0×10^{12} vg (2 mL)/vial and labeling is required. The packaging label of the investigational product is labeled with the standard text "exclusively for clinical trials" and also contains the drug number, protocol number, product name, and other information.

Receipt, Dispensing, and Storage of Investigational Product

The investigational product will be kept, managed, counted, distributed, and recycled by dedicated personnel in the clinical study site. The investigational product bottle is a disposable one. Any investigational product left in the bottle after infusion should not be used for another subject. All investigators should refer to the BBM-H901 Injection Investigator's Brochure for specific instructions on the handling, preparation, management, and disposal of the vector and use the investigational product within the framework of this clinical study as required by the protocol. The receipt, dispensing, and return of the investigational products will be documented according to the requirements of the clinical study site. At the end of the study, all unused or partially used drugs and packages will be returned to the drug supplier by the investigator for destruction, and recorded in the drug dispensing and retrieval form.

Dosing Regimen

BBM-H901 injection should be stored at −80 °C ± 10 °C, removed on the day of infusion, and naturally thawed at room temperature. The required dose of BBM-H901 injection (5×10^{12} vg/kg) should be calculated based on the body weight of the subject. Under medical care, one single intravenous infusion is done via injection pump at a rate of 2.5 mL/min.

Study staff should read the instructions in the product manual carefully before preparing the dose for injection to subjects; study staff preparing the infusion should use universal precautions and personal protective equipment; operate with aseptic techniques; and thaw frozen drug at room temperature before use.

Immunomodulatory Therapy

In this study, prophylactic steroids will be used, that is, oral glucocorticoids (prednisone or prednisolone) will be prophylactically used from Day −1 before BBM-H901 infusion to eight weeks after infusion. The initial regimen of oral glucocorticoids during the study and taper schedule after prophylactic steroid treatment are shown in the table below.

By referring to the guidelines issued by the American Association for the Study of Liver Diseases (AASLD), the data of SJ-UCL study, and the management experience for this product in previous studies, in the context of no other etiologies, if the following conditions are observed during the follow-up period after gene vector infusion:

- Liver aminotransferase (ALT and/or AST) > 1.5 × baseline or ALT and/or AST above the ULN;
- Or in the absence of evidence of FIX inhibitor production, a continuous decrease in the vector Padua-FIX activity;
- Requirement for initiating immunomodulatory therapy comprehensively determined by the investigator based on clinical laboratory parameters and clinical assessment;

Glucocorticoids (e.g., prednisone/prednisolone) may be used for treatment. If glucocorticoids are not effective, the investigator may consider other alternative or combination therapies with other immunosuppressive agents (e.g., tacrolimus). For specific initial dosing regimen and dose reduction plan of glucocorticoids, please refer to the prophylactic steroid dosing regimen. The application can be adjusted by the investigator according to the clinical manifestations, test, and examination indicators of subjects.

Recommended initial regimen of prophylactic oral glucocorticoids

Time	Prednisone/prednisolone (mg/kg/day) For body weight < 60 kg	Prednisone/prednisolone (mg/day) For body weight ≥ 60 kg
Week −1	1	60
Week 1	1	60
Week 2	1*	60

Notes:

Corticosteroids are recommended at a starting dose of approximately 1 mg/kg/day (for body weight < 60 kg) or 60 mg/day (for body weight ≥ 60 kg) orally once daily for the first week, unless the investigator has used a different regimen based on the subject's medical history.

* At the discretion of the investigator, 1 mg/kg/day (for body weight < 60 kg) or 60 mg/day (for body weight ≥ 60 kg) orally once daily may be extended for an additional week, i.e., to week 2, if the subject has no steroid-related adverse effects.

Recommended hormonal taper schedule

Time	Prednisone/prednisolone (mg/kg/day) For body weight < 60 kg	Prednisone/prednisolone (mg/day) For body weight ≥ 60 kg
Week 3	0.75	50
Week 4	0.75	50
Week 5	0.5*	40
Week 6	0.5	30
Week 7	0.3	20
Week 8	0.3	10

Note: The investigator may adjust and apply the hormonal taper schedule according to the clinical manifestations, test and examination indicators of the subjects.

* Steroid-responsive application can be maintained at 0.5 mg/kg/day until transaminases (ALT and/or AST) return to baseline, followed by tapering.

Concomitant Medications

Concomitant medications include any therapeutic and prophylactic drugs (including symptomatic treatment, prescription drugs, over-the-counter drugs, natural extracts, traditional Chinese medicines, nutritional supplements, etc.) that are administered from the signing of the informed consent form to the safety follow-up visit after the end of study treatment (52 weeks of follow-up/early withdrawal [± 7 days]), which should be recorded in the subject's medical records and reported in the eCRF.

The best supportive care is allowed during the study. Other drugs that may induce or aggravate symptoms in clinical studies are not recommended to be used concomitantly, and subjects should be closely monitored if such drugs have to be used.

5.6.3 Allowed concomitant medications

Drugs that do not affect the evaluation of the efficacy and safety of gene vector drugs in the treatment of hemophilia B can be used. However, the investigator should record the detailed information in the concomitant medication/treatment profile in the eCRF, and specify the reason for medication/treatment, administration/treatment method and start and stop time.

Subjects will be required to interrupt the prophylactic treatment with FIX protein product after receiving the infusion of BBM-H901 injection until dose resumption is recommended by the investigator; if a subject develops bleeding during the study requiring on-demand use of FIX protein product at the investigator's discretion, the subject may receive the on-demand infusion of FIX protein product, with the use conditions [product name, reason, start and end dates, type (on-demand, prophylaxis, continuous infusion, etc.), frequency, and doses] recorded in the infusion log. A washout period of at least 96 h or 5 half-lives (whichever is longer) of the FIX product should be assured prior to FIX activity and FIX antigen assessment.

5.6.4 **Prohibited medications and lifestyle restrictions after treatment**

Drugs that affect the evaluation of the efficacy and safety of gene vector drugs for the treatment of hemophilia B are prohibited during the study (until 52 weeks after infusion), including but not limited to:

1) Ibuprofen and acetylsalicylic acid (aspirin). Use other non-steroidal anti-inflammatory drugs with caution (based on the actual situation of the subjects as judged by the investigator);
2) Treatment of hemophilia B with blood products such as red blood cells, platelets, and fresh frozen plasma;
3) Other study drugs (not limited to hemophilia B);
4) Other gene therapy products;
5) Other immunosuppressive agents (except for protocol-allowed glucocorticoids and alternative immunosuppressive therapies suggested by the investigator).

In case of any medical indication, if the investigator considers that it is necessary to use prohibited drugs, relevant records should be made for their use, including but not limited to the date of use, dose, and reason for treatment.

In addition, in terms of lifestyle, subjects are required to stop drinking alcohol throughout the study, including the long-term follow-up period.

STUDY PROCEDURES

The study includes a screening period, a dosing period, a follow-up period for the first 52 weeks after infusion, and a long-term follow-up period (up to 5 years after infusion).

Screening Period (Up To 4 Weeks)

After the clinical study protocol is approved by the Ethics Committee affiliated to the clinical study site, subjects will be screened. The investigator is responsible for explaining the objectives, methods, benefits and potential risks of this clinical study to each subject and obtaining the subject's signed informed consent form. The informed consent form must be signed before any study-required or study-related operations, procedures, or assessments are performed. After the subject has signed the informed consent form, the study screening number will be obtained. Screening procedures will be performed within 4 weeks before the subject receives the investigational product.

At screening, if the subject experiences bleeding and requires the infusion of FIX protein product at the discretion of the investigator, a washout period of at least 96 h or 5 half-lives, (whichever is longer) for FIX protein product is required prior to day 0.

The items to be completed during the screening period are as follows and are detailed in the Schedule of Activities above.

1) Sign the informed consent form;
2) Review the inclusion/exclusion criteria;
3) Record demographic characteristics;
4) Record the medical and treatment history of hemophilia B;
5) Record other medical and treatment history;
6) Routine physical examinations;
7) Height and weight measurement;
8) Vital sign measurement;
9) Hematology, urinalysis, and blood biochemistry;
10) Coagulation function test;
11) Infectious disease screening;
12) Hemophilia gene assay: For subjects with known genotype, if the previously obtained genotype report is available, genotype test may not be performed at screening; for subjects with unknown genotype, samples will be collected at screening for analysis;
13) 12-lead ECG;
14) Blood typing;
15) Completion of FIX protein product infusion log;
16) Bleeding assessment;
17) Target joint count assessment;
18) Abdominal B ultrasound;
19) FIX activity test;
20) FIX inhibitor test;
21) AAV capsid neutralizing antibody test;
22) AAV capsid binding antibody test;
23) AFP test;
24) Adverse events;
25) Concomitant medications.

Screening Period (Day −1)

Subjects who are preliminarily deemed eligible to participate in the study should complete the following assessments 1 day before the infusion of BBM-H901 (i.e., day −1):

1) Review the inclusion/exclusion criteria;
2) Specific physical examinations;
3) Body weight measurement;
4) Vital signs;
5) Hematology, blood biochemistry, and urinalysis for specific test indicators): If these laboratory tests are performed within 7 days before BBM-H901 infusion, they may not be performed on day −1;
6) ALT and AST: Blood biochemistry may not be repeated if it is required at this visit;
7) Coagulation function test for specific test indicators): If this test is performed within 7 days before BBM-H901 infusion, it may not be performed on day −1;
8) 12-lead ECG;
9) Immunomodulatory therapy;
10) Completion of FIX protein product infusion log;
11) Bleeding assessment;
12) Target joint count assessment;
13) FIX activity test: A washout period of at least 96 h or 5 half-lives (whichever is longer) of the other FIX protein product should be assured prior to FIX activity assessment;
14) FIX inhibitor test;
15) Hemophilia-related scale assessment;
16) AAV capsid neutralizing antibody test;
17) AAV capsid binding antibody test;
18) Collection of PBMCs, plasma, saliva, urine, and feces samples for vector shedding test;
19) Adverse events;
20) Concomitant medications.

Dosing Period (Day 0)

Infusion of BBM-H901 injection: AAV vector-based BBM-H901 injection will be administered via a single intravenous infusion under medical care according to the dosing regimen in Section 5.4. It is advisable to continue to stay in the hospital for 24-h observation after the end of infusion and to undergo monitoring and assessments at the time points specified in the protocol. The items to be completed on day 0 are as follows.

1) Vital signs: Vital signs will be measured before the infusion of BBM-H901 injection, and the investigator may increase the frequency of tests during and after infusion based on safety considerations.
2) Infusion of BBM-H901 injection;
3) 12-lead ECG: within 1 h after the infusion of BBM-H901 injection;
4) Immunomodulatory therapy;
5) Completion of FIX protein product infusion log;
6) Bleeding assessment;
7) Adverse events;
8) Concomitant medications.

Follow-Up Period (Weeks 1–52)

Subjects should return to the clinical study site to complete follow-up examinations and assessments at given time points after dosing.

1) Specific physical examination: On day 6, weeks 2, 4, 6, 8, 12, 16, 22, 26, 32, 42, and 52/early withdrawal, the investigator may increase the frequency of tests based on safety considerations;
2) Height and weight: At week 26, week 52/early withdrawal, and the investigator may increase the frequency of examination based on safety considerations;
3) Vital signs: On day 6, weeks 2, 4, 6, 8, 12, 16, 22, 26, 32, 42, and 52/early withdrawal, the investigator may increase the frequency of tests based on safety considerations;
4) Hematology, blood biochemistry, and urinalysis: On day 6, weeks 2, 4, 6, 8, 10, 12, 16, 22, 26, 32, 42, and 52/early withdrawal, the investigator may increase the frequency of tests based on safety considerations;
5) ALT and AST: On day 3 and weeks 3, 5, and 7 after the infusion of BBM-H901 injection, the investigator may consider increasing the frequency of tests based on safety considerations;

6) Coagulation test: On day 6, weeks 2, 4, 6, 8, 12, 16, 22, 26, 32, 42, and 52/early withdrawal, the investigator may increase the frequency of tests based on safety considerations;

7) 12-lead ECG: At weeks 10, 26, and 52, the investigator may increase the frequency of tests based on safety considerations;

8) Immunomodulatory therapy: Steroid prophylactic treatment will be used until 8 weeks after the infusion of BBM-H901 injection. If transaminase elevation is found at any other time, steroid therapy will be considered by the investigator according to the actual condition of the subjects;

9) Completion of FIX protein product infusion log;

10) Bleeding assessments: days 3 and 6, weeks 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 18, 22, 26, 32, 42, and 52/early withdrawal;

11) Target joint count assessment: weeks 26 and 52/early withdrawal;

12) Abdominal B ultrasound: weeks 26 and 52/early withdrawal;

13) DXA for bone density: day -1, week 52/early withdrawal

14) Padua-FIX activity test: days 3 and 6, weeks 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 18, 22, 26, 32, 42, and 52/early withdrawal after the infusion of BBM-H901 injection; a washout period of at least 96 h or 5 half-lives (whichever is longer) of the FIX protein product should be assured prior to the FIX activity assessment;

15) FIX inhibitor test: weeks 2, 4, 8, 12, 16, 26, 32, 42, and 52/early withdrawal;

16) Hemophilia-related scale assessments: weeks 4, 12, 26, and 52/early withdrawal;

17) AAV capsid neutralizing antibody test: day 6, weeks 2, 4, 12, 26, and 52 after the infusion of BBM-H901 injection/early withdrawal;

18) AAV capsid binding antibody test: day 6, weeks 2, 4, 12, 26, and 52 after the infusion of BBM-H901 injection/early withdrawal;

19) AFP test: weeks 26 and 52/early withdrawal;

20) Collection of PBMCs, plasma, saliva, urine, and feces samples for vector shedding test: day 6, weeks 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 18, 22, 26, 32, 42, and 52 after the infusion of BBM-H901 injection/early withdrawal. Note: If the results of three consecutive tests are negative during the study, the sample may not be collected later;

21) Adverse events;

22) Concomitant medications.

Long-Term Follow-Up (Week 52 to Year 5)

After the subjects complete the 52-week (± 7 days) follow-up after infusion, they will enter the long-term follow-up period, which will be performed every 6 months (± 14 days). The specific test items or follow-up items are as follows.

1) Specific physical examination: every 6 months after the week 52 visit until 5 years after the infusion of BBM-H901 injection/early withdrawal; the investigator may increase the frequency of tests based on safety considerations;
2) Height and weight: every 6 months after the week 52 visit until 5 years after the infusion of BBM-H901 injection/early withdrawal; the investigator may increase the frequency of tests based on safety considerations;
3) Vital signs: every 6 months after the week 52 visit until 5 years after the infusion of BBM-H901 injection/early withdrawal; the investigator may increase the frequency of tests based on safety considerations;
4) Hematology, urinalysis, and blood biochemistry: every 6 months after the week 52 visit until 5 years after the infusion of BBM-H901 injection/early withdrawal; the investigator may increase the frequency of tests based on safety considerations;
5) Coagulation function test: every 6 months after the week 52 visit until 5 years after the infusion of BBM-H901 injection/early withdrawal; the investigator may increase the frequency of tests based on safety considerations;
6) 12-lead ECG: every 6 months after the week 52 visit until 5 years after the infusion of BBM-H901 injection/early withdrawal; the investigator may increase the frequency of tests based on safety considerations;
7) Completion of FIX protein product infusion log;
8) Bleeding assessment: every 6 months after the week 52 visit until 5 years after the infusion of BBM-H901 injection/early withdrawal;
9) Target joint count assessment: every 6 months after the week 52 visit until 5 years after the infusion of BBM-H901 injection/early withdrawal;
10) Abdominal B ultrasound: every 6 months after the week 52 visit until 5 years after the infusion of BBM-H901 injection/early withdrawal;
11) DXA for bone density: every 12 months after week 52 visit, to 5 years after BBM-H901 injection infusion/early withdrawal;
12) Padua-FIX activity test: every 6 months after the week 52 visit until 5 years after the infusion of BBM-H901 injection/early withdrawal; a washout period of at least 96 h or

5 half-lives (whichever is longer) of the FIX protein product should be assured prior to the FIX activity assessment;

13) FIX inhibitor test: every 6 months after the week 52 visit until 5 years after the infusion of BBM-H901 injection/early withdrawal;
14) AAV capsid neutralizing antibody test: every 6 months after the week 52 visit until 5 years after the infusion of BBM-H901 injection/early withdrawal;
15) AAV capsid binding antibody test: every 6 months after the week 52 visit until 5 years after the infusion of BBM-H901 injection/early withdrawal;
16) AFP test: every 6 months after the week 52 visit until 5 years after the infusion of BBM-H901 injection/early withdrawal;
17) Collection of PBMCs, plasma, saliva, urine, and feces samples for vector shedding test (if applicable): every 6 months after the week 52 visit until 5 years after the infusion of BBM-H901 injection/early withdrawal;
18) Adverse events;
19) Concomitant medications.

Unscheduled Visits and Additional Tests

During the study, the investigator may decide whether to add unscheduled visits as clinically indicated (e.g., clinically significant laboratory abnormalities and adverse events). During unscheduled visits, corresponding tests and adverse events should be faithfully recorded.

In addition to the test items specified in this protocol, if necessary, the investigator may decide to add additional evaluation items according to the actual condition of the subject, including but not limited to: liver transaminases, FIX activity, FIX antigen, blood lipids (total cholesterol [TC], high-density lipoprotein [HDL], low-density lipoprotein [LDL], very low-density lipoprotein [VLDL], triglycerides [TG]); coagulation function, thrombin generation assay (TGA), hepatitis B deoxyribonucleic acid quantification, hepatitis C ribonucleic acid quantification, serum interferon, lymphocyte subsets (B/T/NK cells), etc. Additional tests and adverse events should be faithfully recorded.

COLLECTION AND TEST OF BIOLOGICAL SAMPLES

Methods for Collection and Processing of Biological Samples

Blood sample collection:

Blood samples (the start of infusion of BBM-H901 injection will be recorded as "time 0") will be collected at the following time points during the study:

Blood samples for corresponding analysis will be collected according to the blood collection time points specified in the Schedule of Activities of this protocol, and the actual collection time will be recorded, as shown below. For specific operation of sample collection, please refer to relevant standard operating procedures or operation manual for biological sample collection and processing.

1) FIX activity test: screening period (including 1 day before the infusion of BBM-H901 injection), days 3 and 6, weeks 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 18, 22, 26, 32, 42, and 52 after the infusion of BBM-H901 injection, and every 6 months after the week 52 visit until 5 years after the infusion of BBM-H901 injection, or early withdrawal;
2) FIX inhibitor test: screening period, weeks 2, 4, 8, 12, 16, 26, 32, 42, and 52, and every 6 months after the week 52 visit until 5 years after the infusion of BBM-H901 injection, or early withdrawal;
3) AAV capsid neutralizing antibody test: screening period (including 1 day before the infusion of BBM-H901 injection), day 6, weeks 2, 4, 12, 26, and 52 after the infusion of BBM-H901 injection, and every 6 months after the week 52 visit until 5 years after the infusion of BBM-H901 injection, or early withdrawal;
4) AAV capsid binding antibody test: screening period (including 1 day before the infusion of BBM-H901 injection), day 6, weeks 2, 4, 12, 26, and 52 after the infusion of BBM-H901 injection, and every 6 months after the week 52 visit until 5 years after the infusion of BBM-H901 injection, or early withdrawal;
5) Collection of PBMCs and plasma samples for vector shedding test: day −1 of screening period, day 6, weeks 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 18, 22, 26, 32, 42, and 52 after the infusion of BBM-H901 injection, and every 6 months after the week 52 visit until 5 years after the infusion of BBM-H901 injection, or early withdrawal. Note: If the results of three consecutive tests are negative during the study, the sample may not be collected later.

The collected whole blood specimens will be centrifuged and/or stored according to related standard operating procedures or operating manual for the collection and processing of biological samples provided by the corresponding testing facility as well as analytical requirements for FIX

activity, FIX inhibitors, neutralizing antibody against and binding antibody to AAV capsid, and PBMCs analysis, with the processing and warehousing of corresponding biological samples documented.

Fecal, urine, and saliva sample collection:

Feces, urine, and saliva samples will be collected at the sampling time points specified in the Schedule of Activities of this protocol for vector shedding analysis as detailed in the following.

1) Collection of saliva, urine, and feces samples for vector shedding test: day −1 of screening period, day 6, weeks 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 18, 22, 26, 32, 42, and 52 after the infusion of BBM-H901 injection, and every 6 months after the week 52 visit until 5 years after the infusion of BBM-H901 injection, or early withdrawal. Note: If the results of three consecutive tests are negative during the study, the sample may not be collected later;

After collection, fecal, urine, and saliva samples will be stored according to the relevant standard operating procedures or operation manual for biological sample collection and processing provided by the corresponding testing facility, with the processing and warehousing records of biological samples well documented.

Biological Sample Labeling

Before biological sample collection, the sampling tubes and two cryotubes will be uniformly numbered, and a special printed label will be pasted, which includes the project code, study group, sample type, subject ID, sampling time point, test/backup sample, sampling date and time, and other information. The sample label is subject to the label actually used in the clinical site. See the relevant standard operating procedures or operating manual for the collection and processing of biological samples provided by the corresponding testing facility for details.

Biological Sample Transportation

If the samples need to be transported to a third-party laboratory for testing, the sample test tubes (including blood, feces, urine, and saliva samples) will be transported to the testing facility through the cold chain logistics. Prior to transportation, it is necessary to add sufficient dry ice to the sample packing box, so as to ensure that the whole sample transportation process is under frozen condition (≤ −60 °C). The sample packing box should be timely sent to the biological sample testing facility in frozen condition under whole-process temperature monitoring, and corresponding records should be provided. The testing facility is responsible for preserving the blood, feces, urine, and saliva samples to be tested according to the relevant standard operating procedures or operating manual for the collection and processing of biological samples.

Since the processing conditions of biological samples may change, in terms of requirements related to the collection, processing, storage, and transportation of biological samples, the relevant standard operating procedures or operating manual for the collection and processing of biological samples shall prevail.

Determination and Analysis of Biological Samples

The testing facility is to develop the sample analysis plan, use the validated analytical method of biological samples to test and analyze the biological samples, and issue the bioanalytical report.

EVALUATION OF COAGULATION FACTOR ACTIVITY AND AAV VIRAL LOADS

Coagulation Factor Padua-IX Activity

Plasma Padua-FIX activity levels from subjects will be used to evaluate the Padua-FIX activity and its changes, including the peak activity level, steady-state activity level or mean activity level.

FIX activity levels will be measured using two methods of the one-stage methods (Sysmex platform, Actin FSL aPTT reagent, and Werfen platform, HemosIL SynthASil reagent). The Actin FSL method is mainly used for the efficacy and safety evaluation, while the SynthASil method is only used for methodological comparison.

AAV Vector Viral Loads

AAV viral loads in plasma, saliva, urine, and feces of subjects will be used for testing vector shedding changes (plasma, urine, feces, saliva, and PBMCs).

Vector shedding test is mainly performed using quantitative polymerase chain reaction (qPCR). Biological samples of saliva, urine, feces, and plasma are collected at different time points before and after injection. Total genomic DNA is extracted and amplified by primers matching the viral genome to detect the virus titer in humour samples and evaluate the dynamic changes of viral load in humour after injection.

EFFICACY EVALUATION

Efficacy evaluation indicators include:

1) Bleeding frequency (including spontaneous bleeding, traumatic bleeding, joint bleeding, etc.);
2) Analysis of the Padua-FIX activity level by blood sampling;
3) The number and volume of infusions of other FIX protein products (including recombinant and plasma-derived FIX protein products);
4) Number of target joints;
5) Relevant scales for hemophilia assessment (HJHS, HEAD-US-C, and CHO-KLAT).

SAFETY AND TOLERABILITY EVALUATION

Safety and Tolerability Evaluation Indicators

Safety and tolerability evaluation indicators include AEs/SAEs (including DLT), physical examinations, vital signs, 12-lead ECG, clinical laboratory tests (hematology, blood chemistry, urinalysis, and coagulation function test), FIX inhibitor test, neutralizing antibody against and binding antibody to AAV capsid, bone density, and assessment of cellular immunity by single cell sequencing.

Safety Evaluation Procedures

10.2.1 Physical examinations

Physical examinations include skin/mucosa, lymph nodes, head, neck, chest, abdomen, skeletal muscle/extremities, and nerves/mental status.

Physical examinations will be performed at protocol-specified time points. The investigator may increase the frequency of tests based on safety considerations.

10.2.2 Body height and weight

During the screening period, the body height and weight will be measured and documented, and body mass index (BMI) will be calculated (BMI = body weight (kg)/body height^2 (m^2)). Only body weight will be measured on day -1, which will be used to calculate the dose of BBM-H901 injection, and height and weight will be measured at other visits.

10.2.3 Vital signs

Vital signs include blood pressure (systolic and diastolic blood pressures), pulse, body temperature, and respiratory rate. Vital signs of subjects in a sitting position will be measured after subjects are at rest in a sitting position for at least 5 min. If vital signs are beyond the

normal range or the change is clinically significant and the investigator considers that a repeat measurement is required, 2 additional measurements will be taken approximately 2 min apart. Each measurement will be documented.

Vital signs will be examined within the protocol-specified time windows. If the vital sign examination time conflicts with the blood sample collection time, vital signs will be examined before the specified blood sample collection time.

The investigator may increase the frequency of tests based on safety considerations.

10.2.4 12-lead ECG

Subjects will undergo 12-lead ECG after rest in the supine position for at least 5 min. Heart rate, PR interval, QRS interval, QT interval, and QT-interval as corrected by Fridericia's formula (QTcF) will be documented. When an ECG measurement is abnormal (including an abnormal QTcF, e.g., QTcF > 500 ms or an increase from baseline > 60 ms) or is judged clinically significant by the investigator, 2 additional ECG measurements will be performed at least 2 min apart. Each measurement should be documented, indicating abnormal or not and its clinical significance.

If two consecutive measurements of QTcF are > 500 ms or prolong > 60 ms from baseline, whether subjects will be closely monitored (e.g., ECG at an interval of 1 h) and whether appropriate measures will be taken will be determined by the investigator.

If ECG conflicts with other study procedures (e.g., vital sign examination, blood sample collection), it should be performed first, followed by vital sign examination, and make sure that blood samples be collected at given time points.

The investigator may increase the frequency of tests based on safety considerations.

10.2.5 Clinical laboratory tests

Clinical laboratory tests include hematology, blood chemistry, urinalysis, and coagulation function test. Clinically significant abnormal laboratory test indicators should be re-tested when possible, preferably within 7 days. Clinical indicator abnormalities deemed clinically significant by the investigator should be reported as AEs or SAEs if they meet the criteria for AEs or SAEs, and such abnormalities should be closely observed and followed up until return to normal or baseline level (if baseline is known), stable disease (if abnormalities are not clinically significant), loss to follow-up, or death. Each measurement should be documented, indicating abnormal or not and its clinical significance.

Blood and urine samples will be collected at protocol-specified visits for clinical laboratory tests. If relevant laboratory indicator tests (except blood chemistry) are performed within 7 days before infusion of BBM-H901 injection, such tests will not be performed anew on day −1. The investigator may increase the frequency of tests based on safety considerations.

10.2.6 Dual-energy X-ray absorptiometry for bone density:

DXA should be used to detect the bone mineral density. It is recommended that the evaluation site be consistent before and after administration, and the same hospital be selected as far as possible if detection equipment is not available at study site. The bone mineral density of specific examination site and its clinical significance should be collected.

10.2.7 Coagulation factor IX (FIX) inhibitor

The inhibitor will be tested using the Bethesda assay or Nijmegen-Bethesda assay, and the results will be presented with Bethesda unit (BU). Prior to sample test, the test method will be sufficiently validated. If the inhibitor test result is negative before and positive after infusion of BBM-H901 injection, the blood samples should be recollected from subjects for retest and confirmation, and the sample collection time points should be documented in the SAE report.

Inhibitor formation is defined as Bethesda inhibitor titer ≥ 0.6 BU/mL. The threshold is defined as ≥ 0.6 BU/mL for "low-titer" inhibitors and > 5 BU/mL for "high-titer" inhibitors.

10.2.8 Neutralizing antibody against and binding antibody to AAV capsid

The test principle of neutralizing antibody against AAV capsid is that the antibody level in serum can be evaluated by detecting the gene expression level in AAV-infected cells using a microplate reader via the blockage of AAV infection by the antibodies in serum diluted at different ratios.

The test principle of binding antibody to AAV capsid is that the amount of immunoglobulin G (IgG) that can bind to AAV capsid in serum can be evaluated by enzyme-linked immunosorbent assay.

10.2.9 Single-cell RNA sequencing

Single-cell RNA sequencing will be used to assess the cellular immunity of the body before and after infusion of AAV and the effect of glucocorticoids on cellular immunity, with no need to collect samples from all subjects. The recommended sampling points are as follows: on day −1 before vector infusion, on day 3 after vector infusion, and at week 4 after vector infusion; and when transaminases increase by 1.5–2 times from the baseline value, when coagulation factors decrease sharply after stabilization, and at 2 weeks after steroid withdrawal; if the interval between blood sampling points is close, the investigator may combine the blood sampling points based on the actual situation.

Adverse Event

During the study period, caution should be exercised against potential AEs. In case of an AE, the top priority should be given to subject safety. If necessary, appropriate medical interventions should be provided.

When the informed consent form (ICF) is signed, each subject should be informed of the names and contact numbers of personnel at the study site to facilitate the reporting of AEs and medical emergencies.

Subjects who develop an AE will be treated under local guidance.

10.3.7 Definition

Serious pre-dose event:

A serious pre-dose event is any event meeting SAE reporting criteria that occurs after subjects sign the ICF and prior to use of the investigational product.

Adverse event:

An AE is any untoward, unexpected, or unintended medical occurrence, including signs (e.g., clinically significant abnormal laboratory tests), symptoms, or events temporally associated with the use of AAV-based gene therapy drugs, in subjects participating in a clinical study, regardless of causality. The criteria for AEs are shown below:

- An AE does not occur before infusion of AAV gene therapy drugs, but occurs after infusion of gene therapy drugs;
- An AE occurs before gene vector infusion, and is more severe or its incidence increases following gene vector infusion.

Serious adverse event:

An SAE is any untoward medical occurrence that results in death, is life-threatening, results in persistent or significant disability or incapacity, requires hospitalization or prolonged hospitalization, causes a congenital anomaly/birth defect after subjects receive the investigational product.

SAE definition	Guidelines
Death	The AE is the immediate or primary cause of a subject's death.
Life-threatening	The occurrence of an AE causes a subject to be at immediate risk of death. AEs that may cause death after severe progression (e.g., drug-induced hepatitis causing no hepatic failure) are excluded.
Persistent or significant disability/incapacity	Any AE that results in an injury, impairs or disrupts a subject's functional and/or physiological structure(s), physical activities, or quality of life.
Subjects requires hospitalization or prolonged hospitalization	Any first hospitalization in a medical institution (even if the length of hospital stay is ≤ 24 h) complies with this criterion, with the exception of any hospitalization in rehabilitation institutions, hospice institutions, respite care institutions (e.g. patients requiring in-home care), skilled nursing facilities, and sanatorium, general emergency admission, routine hospitalization for physical examinations, outpatient observation within 24 h and same-day surgery, and progressive disease-related hospitalization and prolonged hospitalization.
Congenital anomalies or birth defects	The offspring of a subject has a congenital anomaly or malformation.
Other important medical events	Medical and scientifically reasonable judgments must be exercised in deciding whether expedited reporting is appropriate for other events, i.e., important medical events that may not be immediately life-threatening or result in death or hospitalization may be considered serious if medical interventions are necessary to prevent any of the above events. Examples include major treatment in the emergency room or allergic bronchospasm at home, cachexia or convulsion requiring no hospitalization, and drug dependence or addiction. **In this study, the following events are considered medically important and must be reported as SAEs:** • Any death related to gene transduction treatment during the study period; • Grades III–IV toxicities related or possibly related to the study vector in any subjects, such as anaphylaxis and transaminase elevations; • Stages III–IV liver transaminase elevations related to gene therapy or immunosuppressive therapy, but fail to be improved or resolved after 8-week treatment; • Grade IV (> 10 folds) transaminase elevations in any subjects that are related to or possibly related to gene therapy; • Malignancies that are possibly or definitely related to vector infusion at any time after vector infusion.

Pre-scheduled and selected procedures or routine treatments requiring hospitalization are not considered SAEs; the following contents must be documented at the study site:

- Arrangements for hospitalization before a subject's consent to participate in the study, or pre-scheduled, selective, routinely scheduled treatments (or scheduled on a waiting list) should be documented;
- Pre-scheduled, selective, or routinely scheduled hospitalization occur before the study, and no disease worsens or progresses before a subject agrees to participate in the study;
- Pre-scheduled, selective, or routinely scheduled treatments are the sole causes of interventions or hospitalization.

10.3.8 **Monitoring and documentation of events**

Serious pre-treatment event:

Serious pre-treatment events (after ICF signing and before gene vector therapy) in subjects will be documented on the SAE form, and will be reported to the SRC and Ethics Committee within 24 h after such events are identified by personnel at the study site.

Adverse event:

AEs will be collected from the time of signing ICF to 52 weeks ± 7 days after drug administration, premature withdrawal, or start of other gene drug therapy (whichever occurs earlier), and all AEs will be followed up until recovery of AEs, stable disease, interpretation of the AEs, patients' death, or loss to follow-up. Only investigational product-related AEs will be collected during the long-term follow-up period and may be followed for up to 5 years/until early withdrawal.

Serious adverse event:

Any SAEs experienced by a subject starting from the day of signing the ICF should be documented on the SAE form, regardless of the severity of the events or their relationship to study treatment. SAEs must be reported to the designated personnel at the clinical study site according to the provisions of the study.

When a subject completes the study or discontinues participation in the study, all SAEs will be followed up by the investigator until the events are resolved, become stable, or return to baseline status.

The Ethics Committee must be formally notified of SAEs within 24 h after the personnel at the study site learn of the SAEs. The investigator should ensure that SAE reporting information is properly used and SAE reporting procedures are properly followed.

Events leading to death should be documented on the appropriate CRF and reported. All causes of death must be reported as SAEs. The investigator should exert every effort to obtain the death certificate and autopsy report and send them to the Ethics Committee.

10.3.9 **Correlation judgment**

When the causality between AEs and the investigational product is assessed, important factors to be considered include:

a) Temporal relationship to drug administration: Events should occur after administration. The length of time from drug exposure to event occurrence should be assessed in the clinical context of the event.

b) Reactions after drug withdrawal (dechallenge) and re-administration (rechallenge): Patients should be assessed for reactions after dechallenge or rechallenge based on the common clinical disease course of related event.

c) Underlying diseases, comorbidities, and concurrent illness: Each event should be evaluated based on the natural medical history and course of the treated illness and other diseases that patients may develop.

d) Concomitant medications or treatments: Other medications or treatments the patients are receiving when an AE occurs should be identified to determine whether such medications or treatments cause the AE.

e) Known reactions (clinical/pre-clinical) to the gene vector drugs

f) Physical/psychological stress exposure: Stress exposure, which may induce adverse changes in patients, provides a more plausible explanation for the events.

g) Pharmacology and pharmacokinetics of study treatment: The pharmacokinetic properties (absorption, distribution, metabolism, and excretion) as well as the pharmacodynamics in individual patients should be taken into consideration.

The investigator will analyze the correlation between AEs and the investigational product, and such correlation is classified into 5 categories, namely, "definitely related, probably related, possibly related, unlikely related, and unrelated". The investigator must document such correlation using medical terms.

The criteria are detailed below:

	Definitely related	**Probably related**	**Possibly related**	**Unlikely related**	**Unrelated**
A reasonable temporal relationship with administration of the investigational product	+	+	+	+	-
Known reactions to investigational product	+	+	+	-	-
Reactions abate or disappear after drug withdrawal	+	+	±	±	-
Reactions recur after rechallenge	+	?	?	?	-
Uninterpretable using subjects' diseases	+	+	-	±	-

Note: + Yes - No ± Possible ? Unknown

"Definitely related", "probably related", and "possibly related" represent investigational product-related adverse reactions, and the incidence of adverse reactions is calculated accordingly. It is noteworthy that the regulatory authorities in China interpret "unlikely related" as a situation where the correlation cannot be ruled out.

If a possible correlation dose not fully correspond to the above criteria, the use of the conservative principle or unfavorable principle for the new drug is recommended, namely, if the judgment result is between "possibly related" and "unlikely related", "possibly related" should be determined, or "unlikely related" should be determined in the absence of sufficient information.

10.3.10 **Severity**

The investigator will classify the severity of AEs according to CTCAE 5.0:

- **Mild (Grade I):** Asymptomatic or mild symptoms; clinical or diagnostic findings only; intervention not indicated.
- **Moderate (Grade II):** Minimal, local, or non-invasive intervention required; limiting age-appropriate instrumental activities of daily living*.

* Instrumental activities of daily living refer to cooking, shopping for clothes, using a telephone, and managing money matters.

- **Severe (Grade III):** Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disability; limiting self-care activities of daily living**.

** Self-care activities of daily living refer to bathing, dressing and undressing, dining, washing, and medication use without being bedridden.

- **Life-Threatening (Grade IV):** Life-threatening; urgent intervention indicated.
- **Death (Grade V):** Death related to AE.

10.3.11 **Procedures for managing special situations**

Bleeding and suspected bleeding events

All bleeding and suspected bleeding events, regardless of the need for treatment with exogenous FIX, should be documented in the subject diary and on the bleeding event page in the eCRF. Generally, bleeding and suspected bleeding events will not be documented as AEs unless such events meet one or more of the criteria in the SAE definition (whether it is finally determined to be a bleeding event, whether it is a disease outcome for hemophilia, or whether treatment with exogenous FIX is required).

Overdose

Overdose is any dose administered to a subject in excess of the intended dose. Overdose is not considered an AE; however, all overdoses should be documented on the SAE form, and should be reported to the SRC and Ethics Committee within 24 h. Overdose should be reported even if it does not lead to an AE. Overdose should not be documented as an AE, yet overdose-related symptoms should be documented as an AE in the electronic case report; dose information should be documented on the CRF.

Death

All deaths in the study, regardless of correlation to the investigational product, must be documented as SAEs, and should be reported to the Ethics Committee and project partner within 24 h after the deaths are learned of.

Death should be considered an outcome rather than a stand-alone event. Event or disease that induces or causes death should be documented as a single medical concept. Information pertaining to events leading to deaths may be further improved in follow-up reports. The term "sudden death" is used to describe the following conditions only: A subject with pre-existing or no pre-existing heart disease dies suddenly and accidentally within 1 h of the onset of acute symptoms, with the cause of death presumed to be the heart disease; or a subject dies, without confirmation of death, within 24 h after the subject is last seen (his/her vital signs are stable at that time). If the cause of death is unknown and cannot be determined at the time of reporting, "unexplained death" should be recorded. If the cause of death is subsequently ascertained (e.g., after autopsy), "unexplained death" should be replaced with an established cause of death.

The investigator should provide the Ethics Committee and project partner with other necessary materials, such as autopsy report and final medical report.

10.3.12 Investigator's responsibilities

The investigator's responsibilities include the following:

- Monitor and document AEs including SAEs as per the collection time requirements;
- Determine the relationship to and severity of AEs/SAEs;
- Determine the dates of occurrence and resolution of AEs/SAEs;
- Fill out an SAE form for each serious event, and report each event to the Ethics Committee within 24 h after the personnel at the study site learn of such event;
- Actively and steadily follow up subsequent SAE information. Report subsequent information to the Ethics Committee using the SAE form within 24 h after the personnel at the study site learn of new information;
- Ensure that all AE and SAE reports are consistent with the documentation in subjects' medical records;
- Report SAEs to the local regulatory authority and Ethics Committee as per local regulatory requirements if applicable.

DATA MANAGEMENT

Data Traceability and Completion and Submission of Case Report Form

The data in eCRF, derived from source data and source documents, will be filled in by the investigator or the investigator's designee, and completeness and accuracy of the information should be ensured. Errors should be corrected according to the instructions for completion of eCRF. The completed eCRF should be timely submitted to the electronic data capture (EDC) system via internet. After the data in the EDC system are verified against source data on site and reviewed by the data manager and all queries are addressed, the investigator should provide an electronic signature for confirmation prior to data lock.

Design and Establishment of Database

In this study, data will be captured using the EDC system, and the database will be established by the data department of CRO designated by the clinical study site. The database should enable the management of data traces, such as system login, data entry, modification, or deletion.

Data Entry and Modification

Data recorded in the eCRF should be derived from source documents and be identical with the source data. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Permanent copies of study visit records will be considered as source documents and used to record data of enrolled subjects. The personnel responsible for data entry should timely and accurately enter the data in source documents (e.g., medical records in the study) into the eCRF. The data manager will verify the data in the eCRF, and the clinical research associate will ask the investigator for all identified queries using a query form. The data manager will modify and validate the data based on the investigator's answers and may submit a query form anew if necessary.

Data Review

After the completion of data entry and verification, the data manager, principal investigator, and statistical analysts will jointly review the data and then define and judge analysis sets.

Data Coding

The data manager is responsible for medical coding. Coded contents include past medical history, concomitant medications, and AEs.

Past medical history and AEs will be coded according to the latest version of the medical dictionary for regulatory activities (MedDRA), whereas concomitant medications will be coded according to the latest version of World Health Organization Drug Dictionary (WHO DD).

In the coding process, any data, which cannot be coded due to provision of inappropriate, inaccurate, and vague medical terms, will be verified and validated by the investigator in the form of data query.

Prior to database lock, the medical coding report will be sent by the data manager to the clinical study site, and should pass the review by the clinical study site.

Data Lock

The data can be locked if the following criteria are met.

1) All data have been entered into the database;
2) All queries have been addressed;
3) Analysis sets have been defined and judged.

At the end of the study, the principal investigator and statistical analysts will jointly lock the database after the data verification committee confirms that the database has been correctly established. In principle, no modifications should be made to the locked database. Issues identified after data lock that require unlock after confirmation will be unlocked according to the data management and unlocking procedures to conduct amendments. The application for unlocking and the post-unlocking amendments should be documented in written form.

STATISTICAL ANALYSIS

Sample Size Estimation

No formal statistical hypotheses are involved in this study. This study intends to enroll 9 subjects (the number of subjects enrolled may be adjusted based on the efficacy and safety).

Analysis Sets

Full analysis set (FAS): All subjects who receive BBM-H901 injection. Sociodemographic characteristics variables, disease-related variables, vector shedding, FIX activity, and efficacy indicators will be analyzed based on this analysis set.

Safety analysis set (SS): All subjects who receive BBM-H901 injection and are evaluable for safety. Safety indicators will be analyzed based on this analysis set.

Statistical Analysis Methods

12.3.6 General analysis

The final sample size used for statistical analysis will be determined based on the actual number of patients enrolled.

Measurement data will be generally summarized using descriptive statistics, including number of subjects (n), arithmetic mean (mean), standard deviation (SD), median, minimum (min), and maximum (max). Enumeration data will be generally summarized by number of subjects (n) and percentage (%). The data will be summarized using appropriate statistical tables and graphs. The descriptive statistical analysis will be performed on the number of enrolled subjects, dropouts, and baseline characteristics of enrolled subjects.

Due to the small number of subjects, the results for a single subject should be analyzed based on specialty.

12.3.7 **Demographic and baseline characteristics**

Baseline characteristics will be tabulated and statistically analyzed based on SS.

Baseline is defined as the last measurement or test result before a subject receives the infusion.

Based on SS, demographic variables and baseline characteristics (e.g., physical examinations, vital signs, and laboratory tests) will be tabulated and subjected to descriptive statistics.

12.3.8 **Analysis of Padua-FIX activity and AAV viral loads**

Padua-FIX activity and AAV viral load data will be listed and statistically analyzed based on the FAS.

Padua-FIX activity levels and AAV viral loads will be tabulated and subjected to descriptive statistics by age group and scheduled blood sampling time points, and individual subject data will be tabulated. Individual Padua-FIX concentration-time profiles will be plotted according to the actual blood sampling time points. Mean and median Padua-FIX concentration-time profiles will be plotted according to the scheduled blood sampling time points.

At the end of the study, information on different age groups of subjects, the scheduled blood sampling time points, FIX activity, and AAV viral loads will be summarized descriptively, and individual subject data will be listed.

12.3.9 **Safety analysis**

Based on SS, safety and tolerability indicators will be tabulated and subjected to descriptive statistics, including the results for incidence of various AEs, vital signs, physical examinations, clinical laboratory tests (hematology, urinalysis, blood biochemistry, and coagulation function), vector shedding analysis, 12-lead ECG, FIX inhibitor test, neutralizing antibody against and binding antibody to AAV capsid and their changes from baseline.

AEs will be coded and classified using SOC and PT according to the latest version of MedDRA.

All AEs will be tabulated by occurrence and summarized by SOC, PT, and dose group. The incidence of each AE will be summarized by SOC, severity (as per NCI CTCAE v5.0), and correlation with the use of BBM-H901 injection.

Physical examinations and abnormal clinical laboratory test values will be summarized by time point.

Baseline values of and changes in critical safety data (e.g., liver function test results) at each time point will be listed.

All AEs, SAEs, AEs leading to drug withdrawal, and BBM-H901 injection-related AEs should be separately summarized.

12.3.10 **Efficacy analysis**

Efficacy analysis will be performed based on FAS, and the efficacy analysis data will be summarized descriptively.

ABRs within 52 weeks will be presented with descriptive statistics. The ABR is calculated as follows:

Pre-infusion ABR = Number of bleeding events occurring before infusion of BBM-H901 injection ÷ Pre-infusion duration of observation × 365.25 days/year, with the result retained to 1 decimal place.

Post-infusion ABR = Number of bleeding events occurring after infusion of BBM-H901 injection ÷ Post-infusion duration of observation × 365.25 days/year, with the result retained to 1 decimal place.

Similar analyses will be performed for other efficacy indicators, if applicable.

Categorical indicators will be calculated using Fisher's exact test, with two-sided 95% confidence intervals provided, if applicable.

The statistical analysis methods will be detailed in the final statistical analysis plan.

Analysis Report

After the last subject completes the 52-week follow-up in this study, the final analysis will be performed, and the final report will be prepared; after the last subject completes an additional 4-year long-term follow-up, all follow-up information will be summarized, and a supplementary report will be completed.

MANAGEMENT OF CLINICAL STUDY

Statement

This clinical study will be conducted in accordance with the protocol and SOP of the CRO, which aim to ensure that this study is conducted in compliance with the "Declaration of Helsinki", E6 "Guidelines for Good Clinical Practice" issued by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), and

"Good Clinical Practice" (GCP) and pharmaceutical clinical study regulations issued by National Medical Products Administration (NMPA).

Ethics

The study is designed and prepared based on the World Medical Association's Declaration of Helsinki and taking into account the rights and welfare of patients. The principal investigator or investigator of the clinical study will explain the study objectives and all potential possibilities to the patients, and only patients who voluntarily agree to participate in the clinical study and sign the ICF will become subjects.

The investigator of this clinical study and personnel participating in the study should correctly understand and familiarize themselves with the study plan and get well prepared. The investigator of this clinical study must conduct the clinical study in accordance with "Declaration of Helsinki", ICH E6 "Guidelines for Good Clinical Practice", GCP issued by NMPA, and related regulations.

The principal investigator and personnel participating in the study should follow the requirements defined in the study protocol, and should scientifically conduct the study based on the currently accepted technical level.

According to national policies and regulations in China, the investigator should provide the Ethics Committee with study-related documents.

Prior to the start of the clinical study, approval must be obtained from the Ethics Committee and related regulatory authorities.

The amendment to the study protocol should be submitted to the Ethics Committee for review and approval.

In case of any SAEs or unexpected AEs that are related to the clinical study safety and may affect the safety of subjects and the conduct of the study in the clinical study, the investigator should report such events to the Ethics Committee as per regulatory requirements.

The Ethics Committee should be informed of the end of the study.

Informed Consent Form

The investigator (as per related regulatory requirements) or responsible personnel designated by the investigator should fully inform subjects of all study-related issues, including the written information concerning approval by the Ethics Committee. The investigator should inform subjects of study-related information to the greatest extent in a language or terms understandable to subjects.

Prior to participation in the study, subjects or their legally acceptable representatives should discuss with the investigator and sign the written ICF (name and date) together. The investigator

should provide subjects with a copy of the signed ICF.

Before being submitted to the Ethics Committee for review, the ICF used by the investigator should be reviewed and approved by the sponsor.

Amendment to Clinical Study Protocol

If the protocol, approved by the Ethics Committee, needs to be amended, a protocol amendment description should be prepared and signed by the principal investigator. The amended protocol should be executed only after review or filing by the Ethics Committee.

Protocol Deviation

Protocol deviation is any intentional or unintentional deviation from and noncompliance with the approved study protocol-specified treatment procedures, tests, or data collection procedures. Generally, no protocol deviations are acceptable. Protocol modifications or deviations will only be acceptable when there is a medical emergency. The investigator or investigator's designee should document and explain protocol deviations and their causes. Protocol deviations should be described in the final clinical report.

In case of a major protocol violation, it should be assessed. The study site may prematurely terminate the study if necessary.

Data Retention

All clinical study data should be properly retained to maintain data traceability and confidentiality. According to the requirements of GCP, the investigator should retain the clinical study dossier until at least 5 years after the termination of the clinical study.

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APPENDICES

Appendix I Clinical Laboratory Tests

Blood biochemistry Total bilirubin, direct bilirubin, indirect bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein, albumin, lactate dehydrogenase (LDH), creatine kinase isoenzyme, sodium (Na), potassium (K), chloride (Cl), magnesium (Mg), phosphate, calcium (Ca), bicarbonate, glucose, blood urea nitrogen (BUN) or urea, serum creatinine, and blood lipids (including total cholesterol, triglycerides, high density lipoprotein, low density lipoprotein, and very-low-density lipoprotein) Specifically, blood lipid levels will be measured only when indicated during the screening and study periods.	**Hematology** White blood cell (WBC) count and differential count, red blood cell (RBC) count, hemoglobin (HGB) concentration, hematocrit (HCT), and platelet (PLT) count
Urinalysis Urinary protein, WBC, RBC, urine specific gravity (SG), pH, urine glucose (U-GLU), ketone bodies (U-KET), urinary occult blood (urine microscopy), urine bilirubin, and urobilinogen	**Coagulation function** Plasma prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), plasma fibrinogen (FIB), and D-dimer
Infectious disease screening Hepatitis B virus test (quantitative detection of HBsAg and HBV-DNA), hepatitis C virus test (quantitative detection of HCV-Ab and HCV-RNA), human immunodeficiency virus antibody (anti-HIV-Ab) test, and syphilis antibody test	**AFP**
Blood typing	/

Other Laboratory Tests

Genetic test for hemophilia	Coagulation factor IX (FIX) activity level
Vector shedding (including peripheral blood mononuclear cells, plasma, saliva, urine, and stool)	FIX inhibitor
Neutralizing antibody against AAV capsid	Binding antibody to AAV capsid
Single-cell RNA sequencing	

Appendix II Hemophilia Joint Health Score (HJHS)

HJHS 2.1 provides the total score (maximum score = 124, with a higher score indicating worse joint health), specific joint scores, and a global gait score.

Assessment No.: Evaluator Name:_____

Hemophilia Joint Health Score Worksheet 2.1

Subject ID No.:_____ Date of Evaluation: _____
MM/DD/YYYY

Swelling None (N), Puffy (P), Spongy (S), Tense (T) **Landmarks:** Visible (V); Partially Visible (PV); Not Visible (NV) Palpable (P); Non Palpable (NP)	**Left elbow** □ N □ P □ S □ T □ V □ PV □ NV □ P □ NP	**Right elbow** □ N □ P □ S □ T □ V □ PV □ NV □ P □ NP	**Left knee** □ N □ P □ S □ T □ V □ PV □ NV □ P □ NP	**Right knee** □ N □ P □ S □ T □ V □ PV □ NV □ P □ NP	**Left ankle** □ N □ P □ S □ T □ V □ PV □ NV □ P □ NP	**Right ankle** □ N □ P □ S □ T □ V □ PV □ NV □ P □ NP
Score	□	□	□	□	□	□
	0 = No swelling 1 = Mild – appears, feels slightly swollen: landmarks visible 2 = Moderate - looks swollen, feels spongy: some landmarks partly obscured 3 = Severe – looks very swollen; is tense: bony landmarks fully obscured					
Comments: Please provide any comments in the space provided: (If necessary, may note circumference in cm)						
Duration of swelling Note number of months Please checkmark one □ Patient Report □ Parent Report □ Reported from chart □ Other:_____	**Left elbow** ____ months	**Right elbow** ____ months	**Left knee** ____ months	**Right knee** ____ months	**Left ankle** ____ months	**Right ankle** ____ months
Score	☐	☐	☐	☐	☐	☐
	0 = No swelling or < 6 months 1 = ≥ 6 months					

Revised on Sep. 13, 2021

Subject ID No.:_____ **Physiotherapist Name:** _____

Assessment No.:_____ **Date:**_____
MM/DD/YYYY

Time:_____

Hemophilia Joint Health Score 2.1 - Score Summary Table

	Left elbow		Right elbow		Left knee		Right knee		Left ankle		Right ankle	
Swelling		□ NE		□ NE		□ NE		□ NE		□ NE		□ NE
Duration (swelling)		□ NE		□ NE		□ NE		□ NE		□ NE		□ NE
Muscle atrophy		□ NE		□ NE		□ NE		□ NE		□ NE		□ NE
Crepitus on motion		□ NE		□ NE		□ NE		□ NE		□ NE		□ NE
Flexion loss		□ NE		□ NE		□ NE		□ NE		□ NE		□ NE
Extension loss		□ NE		□ NE		□ NE		□ NE		□ NE		□ NE
Joint pain		□ NE		□ NE		□ NE		□ NE		□ NE		□ NE
Strength		□ NE		□ NE		□ NE		□ NE		□ NE		□ NE
Total score of joint												

Sum of total scores of joint + ☐

Global gait evaluation ☐
(□ NE item) Listed in Gait

HJHS total score = ☐

NE = Non-evaluable

Swelling

0 = No swelling
1 = Mild
2 = Moderate
3 = Severe

Duration

0 = No swelling or < 6 months
1 = ≥ 6 months

Muscle atrophy

0 = None
1 = Mild
2 = Severe

Joint pain

0 = No pain through active range of motion
1 = No pain through active range of motion; only pain on gentle overpressure or palpation
2 = Pain through active range of motion

Crepitus on motion

0 = None
1 = Mild
2 = Severe

Flexion loss

Contralateral side:
0 = < 5°
1 = 5°–10°
2 = 11°–20°
3 = > 20°

Normal value

0 = Within normal limits
1 = 1°–4°
2 = 5°–10°
3 = > 10°

Extension loss

Contralateral side:
0 = < 5°
1 = 5°–10°
2 = 11°–20°
3 = > 20°

Normal value

0 = Within normal limits
1 = 1°–4°
2 = 5°–10°
3 = > 10°

Muscle strength (Daniels & Worthingham's scale)

Within available ROM

0 = Holds test position against gravity with maximum resistance (grade: 5)
1 = Holds test position against gravity with moderate resistance (but breaks with maximal resistance) (grade: 4)
2 = Holds test position against gravity with minimal resistance (grade: 3+), or holds test position against gravity (grade: 3)
3 = Able to partially complete ROM against gravity (grade: 3/2+), or able to move through ROM gravity eliminated (grade: 2), or through partial ROM gravity eliminated (grade: 2-)
4 = Trace (grade: 1) or no muscle contraction (grade: 0)
NE = Non-evaluable

Overall gait (walking, stairs, running, and hopping on 1 leg)

0 = All skills are within normal limits.
1 = One skill is not within normal limits
2 = Two skills are not within normal limits
3 = Three skills are not within normal limits
4 = No skills are within normal limits
NE = Non-evaluable

Note: Attach to the instruction manual and worksheet necessary for executing HJHS

General Notes:

_____
_____
_____

Revised on Sep. 13, 2021

Appendix III The Hemophilia Early Arthropathy Detection with Ultrasound in China (HEAD-US-C) Scale

The HEAD-US scale includes three items (scores ranging from 0 to 8): synovial hyperplasia, cartilage destruction, and bone destruction. HEAD-US-C, according to the needs of clinical diagnosis, treatment, and follow-up, has two additional indicators of disease activity in the acute bleeding phase, joint effusion, and synovial vascular hyperplasia, on the basis of the three items of HEAD-US scale, and gives grading assignment with reference to Melchiorre scoring scale to semi-quantitatively score joint effusion/hemarthrosis and synovial neovascularization (i.e., categorized into Grades 0–3 according to the amount of effusion/hemarthrosis, which corresponds to the ultrasound scores of 0–3 respectively, and Grades 0–2 according to the distribution of synovial blood flow signals in the power Doppler sampling frame, which corresponds to the ultrasound scores of 0–2 respectively).

Item	HEAD-US Score	HEAD-US-C Score
Joint effusion		
None		0
Small amount		1
Moderate amount		2
Large amount		3
Synovial blood flow		
None		0
Blood flow signal within the ROI < 3		1
Blood flow signal within the ROI ≥ 3/dendritic blood flow signal		2
Synovial hyperplasia		
None	0	0
Mild/Moderate	1	1
Severe	2	2
Cartilage		
Normal	0	0
Loss of articular cartilage on target surface < 25%	1	1
Loss of articular cartilage on target surface ≤ 50%	2	2
Loss of articular cartilage on target surface > 50%	3	3
Complete loss of articular cartilage on target surface	4	4
Bone		
Normal	0	0
Mild subchondral bone irregularity with/without small periarticular osteophytes	1	1
Marked subchondral bone irregularity and/or significant periarticular osteophyte formation	2	2

Note: "ROI" refers to region of interest

Appendix IV Canadian Hemophilia Outcomes - Kids' Life Assessment Tool (CHO-KLAT)

Appendix V Possible Adverse Events in the Application of Investigational Product and Their Treatment Plans

BBM-H901 injection is an adeno-associated virus (AAV) vector drug expressing human coagulation factor IX (FIX), independently developed by Shanghai Xinzhi BioMed Co., Ltd. In order to control the safety risks to subjects and ensure the benefits of subjects, it is necessary to closely observe the conditions of subjects during this study, including adverse chief complaints, physical examination, and laboratory abnormalities. Based on preclinical study data and the safety data from clinical studies initiated by investigators and clinical studies of similar drugs, the possible adverse events that may occur after infusion of BBM-H901 injection and their treatment plans are as follows.

9. Management of abnormal hepatic function with no response to prophylactic steroids

Transaminases increased is a predictor of severe liver injury. These subjects are prone to progress to severe liver injury, known as drug-induced liver injury (DILI). Hepatic function should be closely monitored during studies, especially in subjects with transaminases increased exceeding 3 × ULN. Potential DILI shall be evaluated based on the criteria for abnormal increases of aminotransferase (AST and ALT) and total bilirubin in Hy's law. The threshold for laboratory abnormalities in potential DILI subjects depends on the baseline values and pre-existing diseases of individual subjects, and further evaluations based on Hy's law are required to determine the causes of laboratory abnormalities. Subjects should return to the study site as soon as possible, preferably within 48 h of knowing any suspected abnormalities in hepatic function tests. Evaluation of abnormal hepatic function should include laboratory tests, imaging examinations, detailed medical history, concomitant medications, and any tests and evaluations deemed necessary by the investigator. The case of DILI should be reported as a serious adverse event regardless of whether the cause of abnormal hepatic function is clear, and appropriately treated/managed by the investigator according to the local clinical practice.

Prophylactic or symptomatic treatment will be given throughout the study:

According to the guidelines issued by the American Association for the Study of Liver Diseases (AASLD) and SJ-UCL, in the absence of other causes, if the liver aminotransferase (ALT and/or AST) is > 1.5 × ULN or persistently higher than the baseline level in the follow-up period after gene vector infusion, or in the absence of FIX inhibitors, if the vector Padua-FIX activity is decreased, then glucocorticoids (e.g., prednisolone/prednisone) can be used for treatment. The investigator is allowed to adjust the glucocorticoid regimen according to the laboratory parameters and/or the subject tolerance.

10. Management of bleeding events caused by poor response

BBM-H901 is under development, and its efficacy and safety have yet to be verified, so poor response may be observed in some subjects. If so, the subjects can freely choose whether to withdraw from the study, and the post-withdrawal treatment regimen will be discussed and determined jointly by the investigator and the subjects. If symptoms, signs, and/or important medical events are caused by poor response, the investigator will perform appropriate management/treatment according to the clinical judgment, and report the symptoms, signs, and/or important medical events as adverse events (AEs) or serious adverse events (SAEs) to the safety department and regulatory authorities according to the local regulatory requirements.

The production of inhibitors often leads to bleeding events due to poor response to drugs for the treatment of hemophilia B. Therefore, the changes in coagulation indicators and bleeding events should be closely monitored by ward rounds, follow-up, timely tracking of laboratory test results, and other measures throughout the clinical study, and FIX prophylaxis or on-demand treatment can be given to ensure the safety of subjects. Moreover, special attention should be paid to the formation of inhibitors, and detailed follow-up information including FIX drug dose, type, activity, recovery, half-life, and inhibitors should be submitted. The following measures will be taken for bleeding risk:

The changes in coagulation indicators and bleeding should be closely monitored throughout the clinical study, and prophylaxis or on-demand treatment with FIX protein products should be given timely if necessary:

（1）. Hematology: Hematological tests, including white blood cell count (WBC) and differential count, red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), and platelet count (PLT), will be performed in the screening period, in the follow-up period, during long-term follow-up, and before withdrawal;

（2）. Coagulation function: Coagulation function tests, including plasma prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), plasma fibrinogen (FIB), and D-dimer, will be performed in the screening period, in the follow-up period, during long-term follow-up, and before withdrawal;

（3）. FIX protein product infusion log: The use of FIX protein products (prophylaxis and/or on-demand treatment) will be recorded from screening period to the end of the study, including the reason for treatment (e.g., bleeding site, frequency, and reason), start and end date, type (on-demand, prophylaxis, continuous infusion, etc.), frequency, and dose.

11. Management of thrombosis

BBM-H901 is a gene therapy product with AAV as the vector under development. In addition to the safety events observed in the completed preclinical and human studies, there may be other unknown safety events, such as thrombosis. Any safety event that occurs during this study and satisfies the definition of AE/SAE as judged by the investigator should be reported in accordance with regulatory requirements and appropriately managed/treated by the investigator in accordance with the local clinical practice.

For a gene therapy product for the treatment of hemophilia B, an increase in FIX activity to 150% or above after gene expression indicates a potential risk of thrombosis, so thrombotic events need to be closely monitored and reported. Specific measures include:

Subjects should be closely monitored for changes in coagulation indicators to predict potential thrombosis throughout the clinical study:

（1）. Coagulation function: Coagulation function tests, including PT, APTT, TT, FIB, and D-dimer, will be performed in the screening period, in the follow-up period, during long-term follow-up, and before withdrawal, and the potential risk of thrombosis should be monitored closely and discovered in time;

（2）. Hematology: Hematological tests, including white blood cell count (WBC) and differential count, red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), and platelet count (PLT), will be performed in the screening period, in the follow-up period, during long-term follow-up, and before withdrawal.

In case of any laboratory abnormality that may cause thrombosis, relevant prophylactic treatment may be given. Moreover, for existing thrombi, appropriate symptomatic treatment should be given in time, including anticoagulants and thrombolytic therapy.

12. Anaphylaxis and other adverse reactions

There have been no reports of anaphylaxis during AAV-FIX vector infusion. Anaphylactic type reactions, including anaphylaxis, have been reported for all FIX products. Although rare, these events are often closely related to the presence of FIX inhibitors. Subjects should be informed of the early signs and symptoms of allergic symptoms, including urticaria, generalized urticaria, angioedema, chest tightness, dyspnea, wheezing, syncope, hypotension, and tachycardia. Subjects should be instructed to see a doctor immediately if such an event occurs at home.

13. Determination of serum level of interferon

AAV may activate part of immune response and induce the production of interferon after entering the human body. Clinical observation should be strengthened during the study. If a subject is suspected with symptoms related to immune response after the infusion of BBM-H901 injection, the level of interferon secretion can be determined at the discretion of the investigator.

14. Management of inhibitors

Inhibitors are rarely produced during the treatment of hemophilia B, so there are no clear guidelines for the management of inhibitors produced during the treatment of hemophilia B. However, peripheral immune tolerance can be achieved by long-term regular and frequent administration of coagulation factors and immune tolerance induction (ITI).

15. Genotoxicity

At present, the determination of viral titer in semen of 10 subjects has been completed in the IIT. The results showed that the viral titer of all subjects decreased to background levels on day 56 post-treatment.

Due to the use of viral vector-based gene therapy, there may be a risk of vertical germline transmission of vector DNA. Therefore, Inclusion Criterion 10 states: "Older underage subjects and their statutory guardians should clearly understand the requirements for contraception and know that subjects must avoid sexual intercourse or use reliable contraception methods".

16. Long-term safety risk of gene therapy

Gene therapy achieves therapeutic effects by causing permanent or long-term changes in the human body. These changes persist in the body for a long time and may increase unpredictable risks such as delayed adverse reactions. There have been no reports of serious adverse events associated with AAV gene therapy drugs for hemophilia B. However, due to the particularity of gene therapy, long-term follow-up of another 4 years is set outside of the 52-week main follow-up period after infusion, according to the requirements of *Technical Guidelines for Long-term Follow-up in Clinical Studies of Gene Therapy Products* (Trial) issued by the Center for Drug Evaluation (CDE) of National Medical Products Administration. Thus, follow-up will last for 5 years after infusion to observe the long-term safety.

Appendix VI Protocol Change Records

Version no./date	Section with change	Content	Reason for change
Version 1.0/Sep. 16, 2022	Not applicable (N/A)	The version submitted for project approval and ethics review	Not applicable (N/A)
Version 2.0/Oct. 31, 2022	Synopsis and in-text Section 4.1: inclusion criterion 2	The version revised according to ethics comments	According to ethics comments, the requirement of "body weight ≥ 50 kg" was added in inclusion criterion 2.
Version 2.1/Feb. 22, 2023	Synopsis and in-text Section 4.1: inclusion criterion 2	Inclusion criterion 2 was revised: According to the body weight data of patients in different age groups, "body weight ≥ 50 kg" was changed to "body weight of 45 kg and above for patients aged ≥ 16 years and < 18 years, and body weight of 40 kg and above for patients aged ≥ 12 years and < 16 years".	The revision was made as per investigators' recommendations
Version 2.1/Feb. 22, 2023	Synopsis and in-text Section 4.2: exclusion criterion 1	Revised exclusion criterion 1: The requirement that patients with positive hepatitis C antibody should be excluded at enrollment was removed. Only patients with positive hepatitis C virus RNA will be excluded.	After communicating with the investigator, it was considered that hepatitis C antibody may be positive for a long time while hepatitis C RNA is negative, in which case there is no viral replication, so this unreasonable restriction condition was adjusted.
Version 2.1/Feb. 22, 2023	Synopsis and in-text Section 5.5: Immunomodulatory Therapy	The administration start time of prophylactic steroids was changed from "1 week before infusion" to "day −1 before infusion", and the initial dosing regimen was established based on body weight as "prednisone/prednisolone 1 mg/kg/d for subjects with body weight < 60 kg; prednisone/prednisolone 60 mg/d for subjects with body weight ≥ 60 kg"; the dose reduction regimen was adjusted accordingly based on the initial dosing regimen.	The revisions were made to minimize the dose of steroids taken by subjects.
Version 2.1/Feb. 22, 2023	In-text Section 3.1: Schematic of study design in Overall Design	In the schematic of study design, "1 sentinel subject is followed up for at least 2 weeks after drug administration, and the other 2 subjects can continue to be enrolled after the SRC has assessed the safety and tolerability" was changed to "1 sentinel subject is followed up for at least 2 weeks after drug administration, and the other 2 subjects can continue to be enrolled after the investigator has assessed the safety and tolerability"	To be consistent with the text.
Version 2.1/Feb. 22, 2023	Synopsis, in-text Section 3.9 "Schedule of Activities", in-text Section 10.2.8 "Single-cell RNA	"Cellular immune status of the body before and after AAV vector infusion assessed by single-cell RNA sequencing analysis" was added.	Single-cell RNA sequencing was added to assess cellular immune status before and after AAV vector infusion as recommended by the investigator.

Version no./date	Section with change	Content	Reason for change
	sequencing"		
Version 2.1/Feb. 22, 2023	In-text Section 1: STUDY BACKGROUND	The latest information of this study and similar studies was updated	Information was updated according to actual situation
Version 2.1/Feb. 22, 2023	In-text Section 14 "REFERENCES": Reference 14	Reference 14 was added	Information was updated according to actual situation
Version 3.0/Oct. 16, 2023	Synopsis and text: inclusion criterion 4	Deleted the original inclusion criterion 4 and incorporated it into original inclusion criterion 8 (now inclusion criterion 7): Deleted original inclusion criterion 4 "Exposure days (EDs) to any recombinant and plasma-derived FIX protein product treatment ≥ 75;" due to duplication of content and incorporated it into inclusion criterion 7.	Adjustment of repeated content
Version 3.0/Oct. 16, 2023	Synopsis and text: exclusion criterion 8	Revised original inclusion criterion 8 (now inclusion criterion 7): **Removed original inclusion criterion 8** "No FIX inhibitor, and no past medical history of FIX inhibitor after 75EDs of FIX product; no clinical signs or symptoms of reduced response after the infusion of FIX product" **and revised it as the current inclusion criterion 7** "Patients who have received any recombinant and plasma-derived FIX products for 75 exposure days (EDs) with negative FIX inhibitors and have no clinical signs or symptoms of decreased response to FIX products after infusion at screening; or patients who have a positive history of FIX inhibitors but have turned negative for two years after immune tolerance induction (ITI) therapy and received FIX products for a cumulative total of 100 EDs during negative period (ineligible for those who have not been treated with ITI for previous positive inhibitor, or those who have positive result recurred after negative conversion due to ITI);"	At the discretion of the investigator, the current inclusion criterion 7 was modified to better screen patients with a history of FIX inhibitors.
Version 3.0/Oct. 16, 2023	Synopsis and text: exclusion criterion 10	Added exclusion criterion 10 **Added exclusion criterion 10** "Intolerable anaphylaxis to prior treatment with FIX, or nephrotic syndrome on prior treatment with FIX;"	Added exclusion criterion 10 to exclude patients with high risk factors for FIX use as recommended by the investigator.
Version 3.0/Oct. 16, 2023	Synopsis and text: inclusion criterion 8	Revised exclusion criterion 11: **Deleted the original exclusion criterion 11** "Subjects with a history of life-threatening bleeding such as intracranial hemorrhage, including those with gastric ulcer posing a risk of severe gastrointestinal bleeding;" **and revised it as current exclusion criterion 12** "Life-threatening bleeding such as intracranial hemorrhage within 2 years before enrollment, or sequelae after previous intracranial hemorrhage and severe hemorrhage, or with potential causes of gastrointestinal bleeding, including but not limited to gastrointestinal ulcers;"	As recommended by the investigator, the original exclusion criterion 11 was modified to better screen subjects with previous severe bleeding, so as to ensure the safety of subjects.

Version no./date	Section with change	Content	Reason for change
Version 3.0/Oct. 16, 2023	Synopsis and text: Evaluation Indicators	Added safety evaluation indicator for bone mineral density	Added the adolescent growth and development indicator — bone mineral density
Version 3.0/Oct. 16, 2023	Table 1. Schedule of activities, in-text Section 6 "STUDY PROCEDURES"	Added bone density test at day –1, week 52/early withdrawal, and once every 12 months in long term follow-up	Added the adolescent growth and development indicator — bone mineral density
Version 3.0/Oct. 16, 2023	Table 1. Schedule of activities, in-text Section 6 "STUDY PROCEDURES"	Added height and weight measurements at week 26, week 52/early withdrawal, and once every 6 months in long term follow-up	Added adolescent growth and development indicator — height and weight

Statistical Analysis Plan 1.0

1 Overview of the Experiment

BBM-H901 injection, developed by Shanghai Xinzhi BioMed Co., Ltd. with independent intellectual property rights, is an adeno-associated virus vector drug expressing human coagulation factor IX. It uses an independently developed highly liver-targeted AAV843 capsid and carries a stably expressed padua-FIX mutant optimized by codon optimization program (GeneArt). This mutant has a mutation at amino acid 338 of wild-type FIX protein, where leucine replaces arginine. This mutation significantly increases FIX activity and has been widely used in several gene therapy drugs under development. The use of padua-FIX mutant both enhances therapeutic effect and reduces gene vector dosage, thus minimizing possible cellular immune responses. BBM-H901 injection, with a clear mechanism of action, has shown a good therapeutic effect in mouse models with hemophilia B, and is clinically intended for the treatment of hemophilia B patients with endogenous FIX ≤ 2 IU/dL (≤ 2%). This document presents the Statistical Analysis Plan (SAP) for the analysis of the data collected from the BBM001-IIT1002 study ("A Single-Arm, Open-Label, Single-dose Clinical Study to Evaluate the Safety, Tolerability, and Efficacy of BBM-H901 Injection in Patients with Hemophilia B [Aged 12 to Below 18]"). This SAP summarizes the study design, objectives and provides definitions and statistical methods for the analysis of data collected from the study.

The analyses described in this SAP are based on trial protocol BBM001-IIT1002, version number 3.0 (10/16/2023).

2 Research Objective and Endpoints

2.1 Research Objective

Primary objective:

- To evaluate the safety and tolerability of a single intravenous infusion of BBM-H901 injection in hemophilia B male subjects (≥ 12 years and < 18 years) with endogenous coagulation factor IX (FIX) activity levels ≤ 2 IU/dL (i.e., ≤ 2%).

Secondary objectives:

- To evaluate the pharmacokinetics (PK) profile of Padua-FIX and adeno-associated virus (AAV) vectors in hemophilia B patients (≥ 12 years and < 18 years) with endogenous FIX activity levels ≤ 2% after a single intravenous infusion of BBM-H901 injection;

- To evaluate the efficacy of a single intravenous infusion of BBM-H901 injection in hemophilia B patients (≥ 12 years and < 18 years) with endogenous FIX activity levels ≤ 2%.

Study objectives of the long-term follow-up:

- To evaluate the long-term efficacy and safety of a single intravenous infusion of BBM-H901 injection.

2.2 Endpoints

Primary endpoints:

（1） Incidence of dose-limiting toxicity (DLT) events, as determined by the Safety

Review Committee (SRC), within 10 weeks after BBM-H901 infusion;

（2） Incidence of adverse events (AEs) and serious adverse events (SAEs) within 52 weeks after BBM-H901 infusion;

（3） Changes in liver function (including alanine aminotransferase [ALT] and aspartate aminotransferase [AST]) within 52 weeks after BBM-H901 infusion from before treatment.

Secondary endpoints:

（1） PK parameters of Padua-FIX and rAAV vectors after the infusion of BBM-H901 injection:

- Padua-FIX activity levels within 52 weeks after BBM-H901 infusion, including peak activity level, steady-state activity level or mean activity level;
- Changes in AAV vector shedding [plasma, urine, feces, saliva, and peripheral blood mononuclear cells (PBMCs)] within 52 weeks after BBM-H901 infusion;

（2） Efficacy endpoints for BBM-H901 injection, including:

- Annualized bleeding rate (ABR) within 52 weeks after BBM-H901 infusion (including spontaneous bleeding, traumatic bleeding, and joint bleeds);
- FIX activity levels at each visit within 52 weeks after the infusion of BBM-H901 injection;
- Number and volume of infusions of other FIX protein products (including recombinant and plasma-derived FIX protein products, such as recombinant human coagulation factor IX for injection and prothrombin complex concentrate) within 52 weeks after BBM-H901 infusion;
- Number of target joints within 52 weeks after BBM-H901 infusion;
- Number of joint bleeds within 52 weeks after BBM-H901 infusion;
- Proportion of subjects without bleeding events within 52 weeks after BBM-H901 infusion;
- Changes in joint health score, joint ultrasound score, and quality of life from baseline to 4 weeks, 12 weeks, 26 weeks, and 52 weeks after the infusion of BBM-H901 injection using the following scales related to hemophilia assessment: Hemophilia Joint Health Score (HJHS), Hemophilic Early Arthropathy Detection with UltraSound in China (HEAD-US-C), and Canadian Hemophilia Outcomes-Kids' Life Assessment Tool (CHO-KLAT);

（3） Other safety endpoints for BBM-H901 injection:

- Incidence of FIX inhibitors (detected by the Bethesda method or the Nijmegen-Bethesda) within 52 weeks after BBM-H901 infusion;
- Titers of neutralizing antibody against and binding antibody to AAV capsid;
- Clinically significant changes in vital signs, physical examination, clinical laboratory test values, 12-lead electrocardiogram (ECG), and alpha-fetoprotein within 52 weeks after

the infusion of BBM-H901 injection;

- Cellular immune status of the body before and after AAV vector infusion assessed by single-cell RNA sequencing analysis;

- Changes in bone mineral density before and after treatment in adolescent subjects.

Long-term follow-up endpoints:

The long-term follow-up endpoints after the infusion of BBM-H901 injection include but are not limited to:

- ABR (including spontaneous bleeding and traumatic bleeding);

- Padua-FIX activity;

- The number and volume of infusions of other FIX protein products (including recombinant and plasma-derived FIX protein products) per year;

- Annualized bleeding rate of different bleeding events: including spontaneous bleeding, traumatic bleeding, and bleeding requiring no treatment;

- Number of target joints;

- Frequency of joint bleeds;

- Viral load of rAAV vector;

- Incidence of AEs and SAEs;

- Incidence of FIX inhibitors;

- rAAV neutralizing antibody titer and capsid-binding antibody titer;

- Clinically significant changes in vital signs, physical examination, clinical laboratory test values, 12-lead ECG, and alpha-fetoprotein (AFP);

- Changes in bone mineral density before and after treatment in adolescent subjects.

3 Trial Design

3.1 Overall Design

This study is designed as a single-center, single-arm, open-label study to investigate the safety, tolerability, PK profile, and efficacy of a single intravenous infusion of BBM-H901 injection in hemophilia B patients (≥ 12 years and < 18 years) with FIX activity levels ≤ 2 IU/dL (i.e., ≤ 2%).

Nine evaluable subjects are planned to be enrolled successively into three age groups (group 1, group 2, and group 3) in descending order of age. Three subjects aged < 18 years and ≥ 16 years will be enrolled in group 1; 3 subjects aged < 16 years and ≥ 14 years will be enrolled in group 2; 3 subjects aged < 14 years and ≥ 12 years will be enrolled in group 3.

BBM-H901 infusion will be first performed in subjects in group 1. One sentinel subject will be set

up to complete at least 2 weeks of observation after administration, and the investigator will comprehensively determine whether to continue the treatment for the other 2 subjects in this group. After the last evaluable subject in group 1 completes the 10-week follow-up after administration, the SRC will comprehensively determine whether to enter the study for group 2. One sentinel subject will also be set up in group 2 to complete at least 2 weeks of observation after administration, and the investigator will comprehensively determine whether to continue the treatment for the other 2 subjects in this group.

After the last evaluable subject in group 2 completes the 10-week follow-up after administration, the investigator will comprehensively determine whether to enter the study for group 3. One sentinel subject will be set up in group 3 to complete at least 2 weeks of observation after administration, and the SRC will comprehensively determine whether to continue the treatment for the other 2 subjects in this group.

After the last evaluable subject in group 3 completes the 10-week follow-up, the SRC will comprehensively determine the safety and tolerability. Administration at other age groups will not be continued.

Subjects who complete the 52-week assessments will continue to be followed up for 5 years after administration to obtain long-term assessment data.

Subjects will be followed up for a total of approximately 5 years after enrollment in the study, including a maximum 4-week screening period (starting immunomodulatory therapy on day −1, and baseline examination on day −1), a post-infusion 52-week follow-up period (follow-up on D3, D6, weekly at weeks 2–8, every 2 weeks at weeks 10–18, every 4 weeks at weeks 22–26, at week 32, and every 10 weeks at weeks 42–52), and a long-term follow-up period for additional 4 years after 52 weeks (every 6 months).

3.2 Sample Size Considerations

No formal statistical hypotheses are involved in this study. This study intends to enroll 9 subjects (the number of subjects enrolled may be adjusted based on the efficacy and safety).

4. Methods of Statistical Analysis

Unless otherwise specified, for each efficacy and safety endpoint, the data will be summarized and analyzed separately for subgroups of (1)16-year old≤ age <18-year old; (2)14-year old ≤ age <16-year old; and (3) 12-year old≤ age <14-year old. Results based on the combined data will also be provided.

4.1 Data Quality Assurance

All tables, graphs and data listings that will be included in the statistical analysis report will be independently verified for consistency and completeness in accordance with our standard operating procedures.

4.2 General Considerations

4.2.1 Generic Analysis Methods

1) Descriptive Statistics

Unless otherwise stated, the following will be provided as descriptive statistical summary for different types of data:

- Continuous variables: number of subjects (n), mean (mean), standard deviation (SD), median (median), minimum (min) and maximum (max) values, and confidence intervals (CI) (when appropriate).
- Categorical Variables: frequency counts, percentages of total for each category, and 95% Clopper-Pearson confidence intervals for percentages (if needed and when appropriate).

2) Number of Decimal Places

Decimal places are reserved according to the following rules, unless otherwise specified.

Parameter Name	**Targets**	**Specification**
Descriptive Statistics	Individual Subject Data	In accordance with raw data
	N, Missing	Zero decimal place
	Mean, Median	Maximum number of decimal places in the raw data + 1, up to three decimal places
	SD, SE	Maximum number of decimal places in the raw data + 2, up to four decimal places
	Min, Max, Range	Maximum number of decimal places for raw data, up to three decimal places
	Percentage (%)	One decimal place
Statistical Estimator	Mean Difference, Confidence Interval (CIs), Test statistics	Two decimal places
	P value ≥ 0.001	Three decimal places
	P value < 0.001	Displayed as "<0.001"

4.2.2 Definitions

1) Baseline

Baseline is defined as the day prior to infusion of BBM-H901 Injection (Day-1), if not otherwise specified.

If there is interference in the Day-1 day FIX Padua data (>2%) due to the use of exogenous FIX, the Screening Period FIX Padua activity data will be used as the baseline.

Note: If the baseline FIX activity is <1%, the baseline FIX activity will be calculated as 1%.

Baseline levels of the following efficacy endpoints are defined as values assessed within 52 weeks prior to signing of the ICF:

- Number and volume of infusions of other FIX protein products (including recombinant and plasma-derived FIX protein products, such as recombinant human coagulation factor IX for injection and prothrombin complex concentrate) within 52 weeks after BBM-H901 infusion
- Number of joint bleeds within 52 weeks after BBM-H901 infusion
- Proportion of subjects without bleeding events within 52 weeks after BBM-H901 infusion

Unless otherwise specified, the "baseline" level of other indicators is defined as the last non-missing measurement/assessment prior to infusion.

2) End of Trial

The end of the entire study is defined as the last subject completing the last scheduled visit, unless the study is terminated earlier for reasons outlined in Section 3.8 of the protocol.

3) Study Days

The day of infusion will be used as the reference date and to calculate time intervals between the day of infusion and the date of any event or evaluation during the trial period.

If the event date occurs prior to infusion, the number of study days (in days) = event date - reference date;

If the event date occurs on or after the day of infusion, the number of study days (in days) = event date - reference date + 1.

4) Conversion of Months and Years

Month = days/30.4375, year = (days)/365.25, rounded to 1 decimal place.

5) Medical Coding

In this study, medical coding was done for past and present medical history (MH), adverse events (AE, including serious adverse events), and prior/concomitant non-pharmacologic therapy (CT) using MedDRA version 26.1 or higher version.

Prior/concomitant medications (CM), FIX product medication history, FIX protein product infusion log (FIXD), prior plasma product infusion (PLA), and plasma product infusion (PIN) will be coded using WHODrug Global (B3) C [V2023SEP] or higher version.

6) Prior and Concomitant Therapy

Prior and concomitant therapy will be determined using the following rules, respectively:

Prior treatment is defined as treatment with an end time prior to signing of the ICF, including prior medication versus prior non-pharmacologic therapy.

Concomitant therapy is defined as drug or non-pharmacologic therapy that started before signing of the ICF and ended on or after the day of signing of the ICF, or drug or non-pharmacologic therapy that started on or after the day of signing the ICF, including concomitant medication and concomitant non-pharmacologic therapy.

7) Adverse Events

An AE is any untoward, unexpected, or unintended medical occurrence, including signs (e.g., clinically significant abnormal laboratory tests), symptoms, or events temporally associated with the use of AAV-based gene therapy drugs, in subjects participating in a clinical study, regardless of causality. The criteria for AEs are shown below:

- An AE does not occur before infusion of AAV gene therapy drugs, but occurs after infusion of gene therapy drugs;
- An AE occurs before gene vector infusion, and is more severe or its incidence

increases following gene vector infusion. Adverse events occurring during treatment

- Adverse Events Occurring During Treatment

Treatment-Emergent Adverse Event (TEAE) is defined as an AE that occurs at the start of first dosing or thereafter until the end of the study (EOS).

- Study Drug-Related Adverse Event

Adverse events related to study drug are defined as adverse events for which the "causal relationship to study drug" on the EDC Adverse Events page is categorized as "definitely related, probably related, possibly related, undetermined" and missing.

- Adverse Events Related to Hormone Use

Adverse events related to hormone use are defined as adverse events "related to hormone use" on the EDC Adverse Events page.

8) Analysis Window

For post-baseline visits, when analyzing by visit, statistical analyses will be performed by scheduled visit time point, and unscheduled visit data will not be included (however, unscheduled visit data will be included in the analyses related to the most clinically significant laboratory abnormality). Test results performed due to clinical indications and unscheduled visits will be provided through listings.

9) Missing Data and Outliers

If not otherwise specified, all analyses are based on observational data, except for certain event dates, imputation of missing data will not be considered; potential outliers will be discussed at the data review meeting to determine the handling method. If there are values of "＞", "＜", "≤", and "≥" in the data, they will be presented with the mathematical operation symbols in listings, and will be calculated using value without the mathematical operation symbols during summary statistical analysis.

For missing date information:

If the start/end date of prior and concomitant therapy is missing, prior or concomitant treatment will be categorized according to the rule stated in section 7.2.

If the start date of the adverse event is missing, the start date for TEAE will be imputed according to the rule stated in section 7.3 to define the TEAE .

When calculating the duration of AE, if the start date or end date of the adverse event is missing, the duration is treated as Missing.

10) Analysis Software

All report results will be analyzed using SAS 9.4 or higher version.

4.3 Data Analysis Sets

Full Analysis Set (FAS): all subjects treated with BBM-H901 injection. Socio-demographic characteristic variables, disease-related variables, AAV vector shedding, FIX Padua activity, and efficacy endpoints will be analyzed based on this analysis set.

Per protocol Set (PPS): a subset of the FAS. Refers to all subjects enrolled and sufficiently compliant to the study protocol, with adherence to planned treatment, available assessment results of primary endpoint, and without important protocol deviation potentially impacting efficacy evaluation. Supplemental analyses of efficacy endpoints will be performed based on this analysis set.

Safety Analysis Set (SS): all subjects enrolled, treated with BBM-H901 injection and with documented post-treatment safety evaluation results. Safety endpoints will be analyzed based on

this analysis set.
The inclusion of individual cases in each analysis data set will be determined at the data review meeting.

4.4 Study Subjects

4.4.1 Subject Disposition

The following will be summarized: the number and proportion of screened subjects, screen failures and corresponding reasons, subjects who passed the screening, subjects who completed the 52-week follow-up and subjects withdrawal from the trial. Reasons for early withdrawal will be analyzed.
Listing of subjects who terminated the study and failed screening will be provided, respectively.
Based on subjects who passed screening, the number and proportion of subjects in the full analysis set, per protocol set, and safety analysis set will be summarized, and listing of subjects who did not enter each analysis set will be provided.

4.4.2 Protocol Deviations

The number and proportion of subjects with major protocol deviations will be summarized, and listing of all protocol deviations will be provided.
Major protocol deviations, as well as any defined procedures taken to exclude subjects or affected data from a particular analysis, will be specified in the protocol deviation description document.

4.4.3 Demographic and Baseline Characteristics

Based on the FAS, the following demographic information, and baseline characteristics will be descriptively summarized.

- Demographic information (age, ethnicity, height, weight, BMI, blood type)
- Baseline clinical characteristics (family history of hemophilia B, allergy history, dietary habits, history of smoking and alcohol consumption, type of treatment prior to baseline, number of subjects with and without target joints, baseline level of endogenous FIX activity)
- History of hemophilia B (time from first diagnosis to infusion, severity of first diagnosis, number of bleeds in the 52 weeks prior to ICF signing, ABR within 52-week prior to ICF signing)

Time from first diagnosis to infusion (months) = (date of infusion - date of first diagnosis + 1)/30.4375.
Past medical history/present medical history will be summarized as the number and proportion of subjects according to System Organ Class (SOC) and Preferred Term (PT), respectively.
Past medications, history of FIX product use, and past plasma product transfusions will be summarized as the number and proportion of subjects according to ATC2 and preferred names (PN), respectively.
Listings will be provided for demographic information, baseline clinical characteristics, smoking history, alcohol consumption history, history of hemophilia B, past/present medical history, past medications, history of FIX product medications, past plasma product transfusions, history of other past treatments, past bleeding, and surgical plans.

4.4.4 Concomitant Therapy

Based on SS, concomitant medications will be summarized according to ATC2 and PN, and

concomitant nonpharmacological therapies will be summarized according to SOC and PT. Listings of concomitant medications and non-drug therapies will be provided.

4.4.5 Drug Exposure and Compliance

Based on the FAS, the relative infusion dose, actual infusion dose, study duration (days) of BBM-H901 Injection and hormone and immunomodulatory therapy use will be summarized.

Note: Relative Infusion Dose (%) = Actual Dose/Scheduled Dose.

Study Duration (days) during the Main Study Period = (Date of Completion of the Main Study - Date of BBM-H901 Infusion) + 1.

Note: Date of completion of main study: date of completing the planned data collection within 52 weeks of BBM-H901 infusion

Based on the FAS, FIX protein product infusion, plasma product infusion and hormone and immunomodulatory therapy will be summarized on number and proportion of subjects, number of times used, dose used, and number of days used according to ATC2 and Preferred Names (PN).

Listings of FIX protein product infusions, plasma product infusions, hormone and immunomodulatory therapy, and BBM-H901 injection infusions will be provided

4.5 Safety Analysis

If not otherwise specified, safety analysis will be performed based on SS.

4.5.1 DLT Events

Based on SS, the number of DLT cases and proportion will be summarized by age group and the corresponding PT.

4.5.2 Adverse Events

Severity of adverse events will be defined according to the CTCAE (version 5.0 or latest version).

1. The number of subjects, proportion and incidence of AEs will be summarized by age group and for combined data according to the following classification:

- All AE
- AE with CTCAE ≥ 3
- All SAEs
- AE associated with hormone use
- TEAE
- TEAE with CTCAE ≥ 3
- Serious adverse event during treatment (TESAE)
- Study drug-related TEAE
- TEAE leading to infusion suspension
- TEAE leading to delay in dosing
- TEAE leading to discontinuation of medication
- TEAE leading to death

2. The following AEs will be categorized by System Organ Class (SOC) and Preferred Term (PT) and severity of the adverse event (any level, ≥3, 3, 4, 5), the number and proportion of subjects will be summarized by age group, and the adverse event under each SOC and PT category will be listed in descending order based on the aggregated number of cases of any level. When

aggregated by severity, only the most severe occurrence of the same SOC or PT in the same subject will be counted:

- All AEs
- All SAEs
- AE associated with hormone use
- TEAE
- TESAE
- Study drug-related TEAE
- TEAE with CTCAE ⩾3
- TEAE leading to infusion suspension
- TEAE leading to delay in medication administration
- TEAE leading to discontinuation of medication
- TEAE leading to death

3. Based on the SS, the number and proportion of death, as well as the cause of death will be summarized by age group.

4. Listings of all AEs, SAEs, DLTs, TEAEs, TESAEs related to study drug, TEAEs with CTCAE ⩾ 3, TEAEs leading to discontinuation of treatment, TEAEs leading to death, and deaths will be provided.

4.5.3 ALT and AST

The number and proportion of subjects with abnormal aminotransferase concentrations (ALT and/or AST) after BBM-H901 infusion will be summarized. The number and proportion of subjects with abnormal aminotransferase concentrations and received prophylactic and on-demand immunomodulatory therapy will be summarized, respectively. The number of days on treatment and dose of prophylactic and on-demand immunomodulatory therapy used will be summarized.

Individual AST and ALT concentration-time curves will be plotted by actual time and will be marked with reference lines for the duration of hormonal and immunomodulatory therapy use and abnormal cut-off values of aminotransferase concentration.

Note: Abnormal ALT and/or AST values are defined as any ALT and/or AST test value above the upper limit of normal (50 U/L) or 1.5 times the baseline value after BBM-H901 infusion. Prophylactic Immunomodulatory Therapy and On-Demand Immunomodulatory Therapy categories will be determined by the investigator.

Listings of AST and ALT abnormal test results with clinical significance will be provided.

4.5.4 FIX Inhibitors

Based on SS, the number and proportion of subjects with FIX inhibitors will be summarized by age group and for combined data, and listing of test results on FIX inhibitors will be provided.

4.6 Efficacy Analysis

4.6.1 ABR within 52 weeks after BBM-H901 infusion

- Variable: ABR within 52 weeks after BBM-H901 infusion

The formula for calculating ABR, is as follows:

ABR after infusion = Number of bleeding events after BBM-H901 Injection infusion ÷ Effective observation duration after infusion (days) × (52 weeks * 7 days)/year, and the result is retained in 1 decimal place.

- Primary analysis: The efficacy analysis on ABR will be based on FAS. A negative binomial regression model will be used to analyze the ABR within 52 weeks post-infusion to estimate the mean ABR and its 95% confidence interval. Number of bleeding episodes will be descriptively summarized. Bleeding events happened in three consecutive days will be counted as one bleeding episode. If the convergence of negative binomial regression model is questionable and the model fit may be not valid (i.e. due to small sample size), the poisson regression model will be applied instead.

- Intercurrent events and strategies:

1) Spontaneous bleeding, traumatic bleeding, or other unspecified causes of on-demand prophylactic treatment (FIX expressed): a treatment policy strategy will be used, i.e., the impact duration from the alternative treatment will be deducted from the effective observation time (for each use, a 5-half-life period, i.e., 96 hours, will be deducted; or, if the interval between two treatments is less than 4 days, the overlapping time portion will be counted only once), and bleeding events happened during the replacement therapy will still be used to calculate the ABR.

2) Death: the while on treatment strategy will be used and data after death will not be included in the analysis.

3) Alternative treatment applied due to FIX non-expression (i.e., treatment failure): a composite variable strategy will be used. The mean ABR of the general prophylaxis population (20.0) will be used to impute the ABR within 52 weeks after infusion. FIX non-expression is defined as: Mean FIX Padua activity failed to reach 5% during the period from the time of the BBM-H901 injection infusion to the time of the subject's first use of the FIX protein product.

4) Restarting regular preventive FIX replacement therapy: During the period between infusion of BBM-H901 and the restart of regular preventive FIX replacement therapy, the observed bleeding episodes and number of on-demand FIX replacement therapy (will be used to exclude non-risk exposure time) will be integrated to calculate the ABR. The value of 20 will be used for ABR imputation after restarting regular preventive FIX replacement therapy.

-Supplementary Analysis: Supplementary analyses will be performed based on PPS and using the same analysis method with the primary analysis.

-Subgroup analyses will be performed on ABR for patients with different baseline characteristics.

Characteristic	subgroup
Type of treatment before baseline	Treatment as needed; preventive treatment; other
Combination of target joints before treatment vs. no target joint combination	Combined target joints; without combined target joints

Baseline levels of endogenous FIX activity	⩽ 1%; 1% < baseline level of endogenous FIX activity ⩽ 2%

4.6.2 FIX activity levels at each visit within 52 weeks after the infusion of BBM-H901 injection

- Variable: change from baseline in FIX Padua activity level over 52 weeks after BBM-H901 injection infusion
- Primary analysis: Based on the FAS, provide summary of baseline FIX Padua activity, FIX Padua activity at Day 3, Week 12, Week 26 and Week 52 post-infusion, and the change of FIX Padua activity at Week 52 post-infusion from baseline, measured using the Actin FSL assay. The Wilcoxon signed rank test will be used to analyze the difference between the FIX Padua activity level at Week 52 post-infusion and the baseline level.
- Intercurrent events and strategies:

1) Spontaneous bleeding, traumatic bleeding, or other on-demand, regular, or prophylactic treatment of unspecified cause (FIX expressed) and alternative treatment due to FIX unexpressed (i.e., treatment failure): treatment policy strategy will be applied, and analysis will be performed based on actual observed data.

2) Death: the while on treatment strategy will be used and data after death will not be included in the analysis.

- Supplementary analysis: Supplementary analyses will be performed based on PPS and using the same analysis method with the primary analysis.

4.6.3 Number and volume of infusions of other FIX protein products (including recombinant and plasma-derived FIX protein products, such as recombinant human coagulation factor IX for injection and prothrombin complex concentrate) within 52 weeks after BBM-H901 infusion

- Variable: change from baseline in the number and volume of other FIX protein products (including recombinant and plasma-derived FIX protein products, such as recombinant human coagulation factor IX injection and thrombospondin complex, etc.) infused during the 52-week period after BBM-H901 injection infusion;
- Primary analysis: Based on the FAS, provide summary of the number of infusions and total volume of other FIX protein products (including recombinant and plasma-derived FIX protein products, such as recombinant human coagulation factor IX injection, plasminogen complex, etc.) infused within 52 weeks post-infusion and the difference from baseline. Wilcoxon signed-rank test will be used to analyze the difference from baseline.
- Intercurrent events and strategies:

1) Spontaneous bleeding, traumatic bleeding, or other as-needed, regular, or prophylactic therapy of unspecified cause (FIX expressed) and use of alternative therapy due to FIX unexpressed (i.e., treatment failure): treatment policy strategy will be applied, and analysis will be performed based on actual observed data.

2) Death: the while on treatment strategy will be used and data after death will not be included in the analysis.

- Supplementary analysis: Supplementary analyses will be performed based on PPS and using the same analysis method with the primary analysis

4.6.4 Number of target joints within 52 weeks after BBM-H901 infusion

- Variable: change from baseline in the number of target joints at Week 52 after BBM-H901 injection infusion;
- Primary analysis: based on the FAS, summarizes the number of target joints within 52 weeks before and 52 weeks after injection infusion, and the difference between the number of target joints within 52 weeks before and 52 weeks after infusion. The Wilcoxon signed rank test will be used to analyze the difference in the number of target joints during the 52 weeks before and 52 weeks after the infusion.
- Intercurrent events and strategies:

1) Spontaneous bleeding, traumatic bleeding, or other as-needed, regular, or prophylactic treatment of unspecified cause (FIX expressed) and alternative treatment due to FIX unexpressed (i.e., treatment failure): treatment policy strategy will be applied, and analysis will be performed based on actual observed data.

2) Death: the while on treatment strategy will be used and data after the event will not be included in the analysis.

- Supplementary analysis: Supplementary analyses will be performed based on PPS and using the same analysis method with the primary analysis

4.6.5 Number of joint bleeds within 52 weeks after BBM-H901 infusion

- Variable: change from baseline in the number of joint bleeds during the 52 weeks after BBM-H901 injection infusion
- Primary analysis: based on the FAS, the number of joint bleeds within 52 weeks before and 52 weeks after injection infusion will be summarized, as well as the difference between the number of joint bleeds within 52 weeks before and 52 weeks after infusion. The Wilcoxon signed rank test will be used to analyze the difference in the number of joint bleeds during the 52 weeks before and 52 weeks after the infusion.
- Intercurrent events and strategies:

1) Spontaneous bleeding, traumatic bleeding, or other on-demand, regular, or prophylactic treatment of unspecified cause (FIX expressed) and alternative treatment due to FIX unexpressed (i.e., treatment failure): treatment policy strategy will be applied, and analysis will be performed based on actual observed data.

2) Death: the while on treatment strategy will be used and data after the event will not be included in the analysis.

- Supplementary analysis: Supplementary analyses will be performed based on PPS and using the same analysis method with the primary analysis

4.6.6 Proportion of subjects without bleeding events within 52 weeks after BBM-H901 infusion

- Variable: Proportion of subjects without bleeding events within 52 weeks after BBM-H901 injection infusion;
- Primary Analysis: Based on the FAS, the proportion of subjects free of bleeding events within 52

weeks after BBM-H901 Injection infusion will be calculated based on actual observed values, and descriptive statistics (number, percentage) will be presented in tabular form.
- Intercurrent events and strategies: the treatment policy strategy will be applied to all Intercurrent events, missing data will not be imputed, and the analysis will be performed on actual observed values.
- Supplementary analyses: Supplementary analyses will be performed based on PPS and using the same analysis method with the primary analysis

4.6.7 Changes in joint health score and joint ultrasound score from baseline to 4 weeks, 12 weeks, 26 weeks, and 52 weeks after the infusion of BBM-H901 injection using the following scales related to hemophilia assessment: Hemophilia Joint Health Score (HJHS) and Hemophilic Early Arthropathy Detection with Ultrasound in China (HEAD-US-C)
- Variables: changes in HJHS and HEAD-US-C scores from baseline to Week 4, Week 12, Week 26 and Week 52 after BBM-H901 infusion
- Primary Analysis: Based on the FAS, each scale score and change from baseline will be summarized through descriptive statistics (median, minimum, maximum, etc.) by scheduled visits and baseline. The analysis of this efficacy endpoint is based on actual observations, missing data will not be imputed, and the analysis of this endpoint will not include hypothesis testing.
- Intercurrent events and strategies: the treatment policy strategy will be applied to all intercurrent events, missing data will not be imputed, and the analysis will be performed on actual observed values.
- Supplementary analyses: Supplementary analyses will be performed based on PPS and using the same analysis method with the primary analysis

4.6.8 Proportion of subjects discontinuing treatment with exogenous FIX products within 52 weeks of BBM-H901 injection infusion
- Variable: proportion of subjects discontinuing treatment with exogenous FIX products within 52 weeks of BBM-H901 injection infusion
- Primary Analysis: Based on the FAS, the proportion of subjects discontinuing treatment with exogenous FIX products within 52 weeks after BBM-H901 injection infusion will be calculated. The analysis will be performed based on actual observed values and descriptive statistics (number, percentage) will be presented in tabular form. This efficacy endpoint will not be analyzed through hypothesis testing.
- Intercurrent events and strategies: the treatment policy strategy will be applied to all intercurrent events, missing data will not be imputed, and the analysis will be performed on actual observed values.
- Supplementary analyses: Supplementary analyses will be performed based on PPS and using the same analysis method with the primary analysis

4.7 Pharmacokinetic/vector shedding analysis
Descriptive statistics based on FAS will be provided and plotted for FIX Padua activity levels and AAV vector viral loads in PBMC, plasma, saliva, urine and semen samples. Concentration-time curves will be plotted for individual (according to the actual time of blood sample collection), mean and median (according to the planned time of blood sample collection), respectively. The mean and

median FIX Padua concentration-time curves of different assays will be plotted according to the planned time of blood collection. Peak and mean FIX Padua activity levels will be calculated and descriptive statistical analysis will be provided. The number of cases of AAV vector shedding, the shedding rate and the time (in days) to shedding in PBMC, plasma, saliva, urine and semen samples will be summarized through descriptive statistics, and the Kaplan-Meier curves will be presented to describe the time to shedding since infusion.

Note: Vector shedding was defined as occurrence of three consecutive negative vector shedding test results. Days to shedding was defined as the time from infusion of BBM-H901 Injection to the first of three negative carrier shedding results.

Listings of individual subject data of FIX Padua activity, AAV vector shedding, AAV vector viral loads and AAV cellular immunity will be provided.

5. Statistical Analysis Schedule and Study Reporting Plan

The statistical analyses described in this SAP and the corresponding study reports are for the analysis and reporting of the main study phase trial data collected up to the 52-week follow-up of the last subject in the confirmatory study phase, excluding the long-term follow-up data collected during this period.

Additional analyses will be performed and analytical reports will be developed after the last subject completes an additional 4 years of long-term follow-up.

6. References

None

7 Appendix

7.1 Appendix 1 Schedule of activities

Item[1]	**Screening period**[2]		**Dosing period**[3]	**Follow-up period 1**																			**Long-term follow-up**
				Week 1 (± 1 day)		**Weeks 2–18 (± 2 days)**												**Weeks 22–52 (± 7 days)**					**Every 6 months ± 14 days/ early withdrawal**
Time (weeks)	**Week 4**	**Day −1**	**Day 0**	**1**		**2**	**3**	**4**	**5**	**6**	**7**	**8**	**10**	**12**	**14**	**16**	**18**	**22**	**26**	**32**	**42**	**52/early withdrawal**	
				Day 3	**Day 6**																		
Informed consent form	X																						
Review of the inclusion/exclusion criteria	X	X																					
Demographic characteristics[4]	X																						
Medical and treatment history of hemophilia B[5]	X																						
Other medical and treatment history[6]	X																						
Physical examinations[7]	X	X			X	X		X		X		X		X		X		X	X	X	X	X	X
Height, weight[8]	X	X																	X			X	X
Vital signs[9]	X	X	X		X	X		X		X		X		X		X		X	X	X	X	X	X
Hematology, blood biochemistry, and urinalysis[10]	X	X			X	X		X		X		X		X		X		X	X	X	X	X	X

Item[1]	**Screening period**[2]		**Dosing period**[3]	**Follow-up period 1**																				**Long-term follow-up**
				Week 1 (± 1 day)		**Weeks 2–18 (± 2 days)**													**Weeks 22–52 (± 7 days)**					**Every 6 months ± 14 days/ early withdrawal**
Time (weeks)	**Week 4**	**Day −1**	**Day 0**	**1**		**2**	**3**	**4**	**5**	**6**	**7**	**8**	**10**	**12**	**14**	**16**	**18**	**22**	**26**	**32**	**42**	**52/early withdrawal**	
				Day 3	**Day 6**																		
ALT, AST		X		X			X		X		X												
Coagulation function[11]	X	X			X	X		X		X		X		X		X		X	X	X	X	X	X
Infectious disease screening[12]	X																						
Genetic testing for hemophilia[13]	X																						
12-lead ECG[14]	X	X	X										X						X			X	X
Blood typing	X																						
Immunomodulatory therapies[15]		◄		———		———	———	———	———	———	———												
Infusion of BBM-H901 injection			X																				
Completion of FIX protein product infusion log[16]	◄			———		———	———	———	———	———	———	———	———	———	———	———	———	———	———	———	———	———	
Bleeding assessment	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Target joint count assessment[17]	X	X																	X			X	X

Item[1]	**Screening period**[2]		**Dosing period**[3]	**Follow-up period 1**																				**Long-term follow-up**
				Week 1 (± 1 day)		**Weeks 2–18 (± 2 days)**												**Weeks 22–52 (± 7 days)**					**Every 6 months ± 14 days/ early withdrawal**
Time (weeks)	**Week 4**	**Day −1**	**Day 0**	**1**		**2**	**3**	**4**	**5**	**6**	**7**	**8**	**10**	**12**	**14**	**16**	**18**	**22**	**26**	**32**	**42**	**52/early withdrawal**	
				Day 3	**Day 6**																		
Abdominal B ultrasound[18]	X																		X			X	X
DXA for bone density		X																				X	Once every 12 months
FIX activity test[19]	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FIX inhibitor test[20]	X	X				X		X				X		X		X			X	X	X	X	X
Scale assessment		X						X						X					X			X	
AAV capsid neutralizing antibody test	X	X			X	X		X						X					X			X	X
AAV capsid binding antibody test	X	X			X	X		X						X					X			X	X
AFP	X																		X			X	X
PBMCs, plasma, saliva, urine, stool sample collection[21]		X			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Single-cell sequencing of																							

Item[1]	**Screening period**[2]		**Dosing period**[3]	**Follow-up period 1**																		**Long-term follow-up**
				Week 1 (± 1 day)		**Weeks 2–18 (± 2 days)**												**Weeks 22–52 (± 7 days)**					**Every 6 months ± 14 days/ early withdrawal**
Time (weeks)	**Week 4**	**Day −1**	**Day 0**	**1**		**2**	**3**	**4**	**5**	**6**	**7**	**8**	**10**	**12**	**14**	**16**	**18**	**22**	**26**	**32**	**42**	**52/early withdrawal**	
				Day 3	**Day 6**																		
peripheral blood cells[22]																							
AEs[23]	←																				→		X
Concomitant medications[24]	←																				→		X

Notes: ALT: alanine aminotransferase; AST: aspartate aminotransferase; FIX: coagulation factor IX; AAV: adeno-associated virus; PBMCs: peripheral blood mononuclear cells

1. Additional visit items (including those not included in the schedule of activities) and/or additional unscheduled visits may be performed according to the actual safety and efficacy assessments, including but not limited to: liver transaminases, FIX activity, FIX antigen, blood lipids (total cholesterol [TC], high-density lipoprotein [HDL], low-density lipoprotein [LDL], very low-density lipoprotein [VLDL], triglycerides [TG]); coagulation function, thrombin generation assay (TGA), hepatitis B deoxyribonucleic acid quantification, hepatitis C ribonucleic acid quantification, serum interferon, lymphocyte subsets (B/T/NK cells), etc.

2. Collect the information of all subjects who participate in the screening, including the reasons for screening failure.

3. Do not use other FIX protein products and plasma concentrates within 96 h or 5 half-lives (whichever is longer) prior to the infusion of BBM-H901 injection. The investigational product should not be thawed until confirmation by the investigator regarding subject washout criteria.

4. Collect demographic data of subjects (including gender, age, ethnicity, and fertility status);

5. Collect the subject's hemophilia history (including date of diagnosis, severity, genotype, human leukocyte antigen [HLA, if any], previously used FIX products [including recombinant and plasma-derived, such as recombinant human coagulation factor IX injection, prothrombin complex, etc., type (on-demand, prophylaxis, continuous infusion, etc.), dosing regimen, dose, number of infusions], number of bleeding, etc.), and the number of bleeding (including spontaneous bleeding, traumatic bleeding, and bleeding not requiring FIX product treatment) in the last 26 and 52 weeks will be retrospectively determined. Record as truthfully as possible and estimate if necessary.

6. Other medical history and treatment history include: past and present medical history, family medical history, allergic history, surgical history and surgical plan, smoking, drinking, dietary habits, fertility plan, vaccination; history of blood donation within 3 months before screening, any participation in clinical trials, other medications and treatments other than those for the disease under study.

7. Physical examination: Only routine physical examination will be performed during the screening period, including skin/mucosa, lymph nodes, head, neck, chest, abdomen, musculoskeletal/extremities, and neurological system/psychiatric conditions. Only symptoms/vital signs-specific physical examination will be performed for other visits. The investigator may increase the frequency of tests based on safety considerations.

8. Height and weight: Only body weight will be measured on day −1, which will be used to calculate the dose of BBM-H901 injection, and height and weight will be measured at other visits.

9. Vital signs: including blood pressure, body temperature, pulse rate, and respiratory rate. Vital signs will be measured after the subject has rested for at least 5 min in sitting position. In addition to the routine visit points for vital signs, vital signs will be measured before injection of FIX protein products on day 0. If vital signs are beyond the normal range or the change is clinically significant and the investigator considers that a repeat measurement is required, 2 additional measurements will be taken approximately 2 min apart. The investigator may increase the frequency of tests based on safety considerations.

10. Hematology, blood biochemistry, and urinalysis: If the above laboratory tests are performed within 7 days before BBM-H901 infusion, they may not be performed on day −1; if blood biochemistry tests are required on day −1, the ALT, AST and GGT may not be repeated on day −1. During the trial, the investigator may increase the frequency of tests based on safety considerations.

11. Coagulation function: including plasma prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), plasma fibrinogen (FIB), and D-dimer. If this test is performed within 7 days prior to BBM-H901 infusion, it may not be performed prior to the infusion of BBM-H901 injection on day −1. The investigator may increase the frequency of tests based on safety considerations.

12. Infectious disease tests include: hepatitis B virus test (HBsAg, HBV-DNA quantitative detection), hepatitis C virus test (HCV-Ab, HCV-RNA quantitative detection), HCV viral load (if any), human immunodeficiency virus antibody (anti-HIV-Ab) test, and syphilis antibody test.

13. For subjects with known genotype, if the previously obtained genotype report is available, genotype test may not be performed at screening; for subjects with unknown genotype, samples will be collected at screening for analysis.

14. 12-lead ECG: The 12-lead ECG should be performed in supine position after the subject has rested for at least 5 min in supine position. ECG indicators include heart rate, PR interval, QRS interval, uncorrected QT interval, and Fridericia-corrected QTc interval (QTcF). When an ECG measurement is abnormal (including an abnormal QTcF, e.g., QTcF > 500 ms or an increase from baseline > 60 ms) or is judged clinically significant by the investigator, 2 additional ECG measurements will be performed at least 2 min apart. The 12-lead ECG on the day of dosing will be performed within 1 h after the infusion of BBM-H901 injection. During the study, the frequency of tests may be increased as appropriate based on safety considerations.

15. In this study, prophylactic steroids will be used, that is, oral glucocorticoids (prednisone or prednisolone) will be prophylactically used from Day −1 before BBM-H901 infusion to eight weeks after infusion. See the study protocol for the specific treatment regimen.

16. Infusion log: The use of the FIX protein product will be recorded from the signing of informed consent to the end of the study, including the reason for treatment, start and end dates, type (on-demand, prophylaxis, continuous infusion, etc.), frequency, and doses.

17. Target joint count assessment: The subject's history of target joint disease will be collected within 52 weeks before screening, and the number of target joints will be assessed within 52 weeks after infusion. The target joint is defined as the occurrence of 3 or more spontaneous bleeding in a single joint in a consecutive 6-month period. If a joint bleeds ≤ 2 times in a consecutive 12-month period, the joint is no longer considered a target joint. The investigator may increase the frequency of tests based on safety considerations.

18. Abdominal B ultrasound: liver, gallbladder, pancreas, spleen, and bilateral kidneys. The investigator may increase the frequency of tests based on safety considerations.

19. During the assessment of FIX activity, subjects are required to suspend the prophylactic treatment with the FIX protein product after receiving the infusion of BBM-H901 injection until dose resumption is recommended by the investigator; if the subject develops bleeding during the study and requires on-demand use of FIX protein product as judged by the investigator, the washout period of at least 96 h or 5 half-lives (whichever is longer) for the FIX protein product should be ensured before the assessment of FIX activity. FIX activity levels will be measured using two methods of the one-stage methods (Sysmex platform, Actin FSL aPTT reagent, and Werfen platform, HemosIL SynthASil reagent).

20. FIX Inhibitor: Inhibitor detection is performed using the Bethesda assay or Nijmegen-modified Bethesda assay.

21. Collection of PBMCs, plasma, saliva, urine, and feces samples: Collect samples for vector shedding detection, and detect the titer of viral particles in plasma, PBMCs, saliva, urine, and feces using quantitative polymerase chain reaction (qPCR) until the results of three consecutive tests are negative.

22. Single-cell sequencing of peripheral blood cells: Single-cell RNA sequencing analysis method will be used to assess the cellular immune status of the body before and after AAV vector infusion and the impact of glucocorticoids on the immune status, without sampling from all subjects. The recommended sampling points are as follows: on day −1 before vector infusion, on day 3 after vector infusion, and at week 4 after vector infusion; and when transaminases increase by 1.5–2 times from the baseline value, when coagulation factors decrease sharply after stabilization, and at 2 weeks after steroid withdrawal; if the interval between blood sampling points is close, the investigator may combine the blood sampling points based on the actual situation.

23. AEs: AEs will be collected from the time of signing the informed consent form to 52 weeks ± 7 days of follow-up after drug administration, early withdrawal, or initiation of another gene drug treatment (whichever occurs earlier), and all AEs will be followed up until recovery of AEs, or stable disease, or interpretation of the AEs, subjects' death, or loss to follow-up. Only investigational product-related AEs will be collected during the long-term follow-up period and may be followed for up

to 5 years/until early withdrawal.

24. Concomitant medications and treatments will be recorded during the screening period and study treatment until 52 weeks ± 7 days after drug administration, early withdrawal from follow-up, or initiation of another gene drug treatment, whichever occurs earlier. Only medications related to hemophilia therapy will be collected during the long-term follow-up period.

7.2 Appendix 2 Prior and Concomitant Therapy Determination

If the prior and concomitant therapy start/end dates are incomplete, determine the type of therapy by the available information as much as possible:

1) If the end date is earlier than the date of signing of the ICF, it will be defined as a prior therapy; if the end date is later than the date of signing of the ICF, it will be defined as a concomitant therapy.

2) If cannot be determined based on the end date, the therapy will be defined as a concomitant therapy.

7.3 Appendix 3 Adverse Event Missing Date Imputation Rules

If the adverse event start date was incomplete, the start date will be imputed to determine the AE period:

Start date	Filling rules
Completely missing	1. The end date is earlier than the infusion date: impute the start date using the earliest date in the same year of the end date.
	2. The end date is later than the infusion date or cannot be determined: impute the end date using the infusion date.
Partially missing	1. The start date is earlier than the infusion date, impute the start date using the earliest date in the same year/month of the start date.
	2. The start date is in the same year/month as the infusion date: if the end date is before e the infusion, impute the start date using the earliest date of the same year/month; if the end date is after the infusion or cannot be determined, impute the start date using the infusion date.
	3. The start date is later than the infusion date, impute the start date using the earliest date of the same year/month of the start date

Statistical Analysis Plan 2.0

1. Overview of the Experiment

BBM-H901 injection, developed by Shanghai Xinzhi BioMed Co., Ltd. with independent intellectual property rights, is an adeno-associated virus vector drug expressing human coagulation factor IX. It uses an independently developed highly liver-targeted AAV843 capsid and carries a stably expressed padua-FIX mutant optimized by codon optimization program (GeneArt). This mutant has a mutation at amino acid 338 of wild-type FIX protein, where leucine replaces arginine. This mutation significantly increases FIX activity and has been widely used in several gene therapy drugs under development. The use of padua-FIX mutant both enhances therapeutic effect and reduces gene vector dosage, thus minimizing possible cellular immune responses. BBM-H901 injection, with a clear mechanism of action, has shown a good therapeutic effect in mouse models with hemophilia B, and is clinically intended for the treatment of hemophilia B patients with endogenous FIX ≤ 2 IU/dL (≤ 2%). This document presents the Statistical Analysis Plan (SAP) for the analysis of the data collected from the BBM001-IIT1002 study ("A Single-Arm, Open-Label, Single-dose Clinical Study to Evaluate the Safety, Tolerability, and Efficacy of BBM-H901 Injection in Patients with Hemophilia B [Aged 12 to Below 18]"). This SAP summarizes the study design, objectives and provides definitions and statistical methods for the analysis of data collected from the study.

The analyses described in this SAP are based on trial protocol BBM001-IIT1002, version number 3.0 (10/16/2023).

2. Research Objective and Endpoints

2.1 Research Objective

Primary objective:

- To evaluate the safety and tolerability of a single intravenous infusion of BBM-H901 injection in hemophilia B male subjects (≥ 12 years and < 18 years) with endogenous coagulation factor IX (FIX) activity levels ≤ 2 IU/dL (i.e., ≤ 2%).

Secondary objectives:

- To evaluate the pharmacokinetics (PK) profile of Padua-FIX and adeno-associated virus (AAV) vectors in hemophilia B patients (≥ 12 years and < 18 years) with endogenous FIX activity levels ≤ 2% after a single intravenous infusion of BBM-H901 injection;

- To evaluate the efficacy of a single intravenous infusion of BBM-H901 injection in hemophilia B patients (≥ 12 years and < 18 years) with endogenous FIX activity levels ≤ 2%.

Study objectives of the long-term follow-up:

- To evaluate the long-term efficacy and safety of a single intravenous infusion of BBM-H901 injection.

2.2 Endpoints

Primary endpoints:

（1） Incidence of dose-limiting toxicity (DLT) events, as determined by the Safety

Review Committee (SRC), within 10 weeks after BBM-H901 infusion;

（2） Incidence of adverse events (AEs) and serious adverse events (SAEs) within 52 weeks after BBM-H901 infusion;

（3） Changes in liver function (including alanine aminotransferase [ALT] and aspartate aminotransferase [AST]) within 52 weeks after BBM-H901 infusion from before treatment.

Secondary endpoints:

（1） PK parameters of Padua-FIX and rAAV vectors after the infusion of BBM-H901 injection:

- Padua-FIX activity levels within 52 weeks after BBM-H901 infusion, including peak activity level, steady-state activity level or mean activity level;
- Changes in AAV vector shedding [plasma, urine, feces, saliva, and peripheral blood mononuclear cells (PBMCs)] within 52 weeks after BBM-H901 infusion;

（2） Efficacy endpoints for BBM-H901 injection, including:

- Annualized bleeding rate (ABR) within 52 weeks after BBM-H901 infusion (including spontaneous bleeding, traumatic bleeding, and joint bleeds);
- FIX activity levels at each visit within 52 weeks after the infusion of BBM-H901 injection;
- Number and volume of infusions of other FIX protein products (including recombinant and plasma-derived FIX protein products, such as recombinant human coagulation factor IX for injection and prothrombin complex concentrate) within 52 weeks after BBM-H901 infusion;
- Number of target joints within 52 weeks after BBM-H901 infusion;
- Number of joint bleeds within 52 weeks after BBM-H901 infusion;
- Proportion of subjects without bleeding events within 52 weeks after BBM-H901 infusion;
- Changes in joint health score, joint ultrasound score, and quality of life from baseline to 4 weeks, 12 weeks, 26 weeks, and 52 weeks after the infusion of BBM-H901 injection using the following scales related to hemophilia assessment: Hemophilia Joint Health Score (HJHS), Hemophilic Early Arthropathy Detection with UltraSound in China (HEAD-US-C), and Canadian Hemophilia Outcomes-Kids' Life Assessment Tool (CHO-KLAT);

（3） Other safety endpoints for BBM-H901 injection:

- Incidence of FIX inhibitors (detected by the Bethesda method or the Nijmegen-Bethesda) within 52 weeks after BBM-H901 infusion;
- Titers of neutralizing antibody against and binding antibody to AAV capsid;
- Clinically significant changes in vital signs, physical examination, clinical laboratory test values, 12-lead electrocardiogram (ECG), and alpha-fetoprotein within 52 weeks after

the infusion of BBM-H901 injection;

- Cellular immune status of the body before and after AAV vector infusion assessed by single-cell RNA sequencing analysis;

- Changes in bone mineral density before and after treatment in adolescent subjects.

Long-term follow-up endpoints:

The long-term follow-up endpoints after the infusion of BBM-H901 injection include but are not limited to:

- ABR (including spontaneous bleeding and traumatic bleeding);

- Padua-FIX activity;

- The number and volume of infusions of other FIX protein products (including recombinant and plasma-derived FIX protein products) per year;

- Annualized bleeding rate of different bleeding events: including spontaneous bleeding, traumatic bleeding, and bleeding requiring no treatment;

- Number of target joints;

- Frequency of joint bleeds;

- Viral load of rAAV vector;

- Incidence of AEs and SAEs;

- Incidence of FIX inhibitors;

- rAAV neutralizing antibody titer and capsid-binding antibody titer;

- Clinically significant changes in vital signs, physical examination, clinical laboratory test values, 12-lead ECG, and alpha-fetoprotein (AFP);

- Changes in bone mineral density before and after treatment in adolescent subjects.

3. Trial Design

3.1 Overall Design

This study is designed as a single-center, single-arm, open-label study to investigate the safety, tolerability, PK profile, and efficacy of a single intravenous infusion of BBM-H901 injection in hemophilia B patients (≥ 12 years and < 18 years) with FIX activity levels ≤ 2 IU/dL (i.e., ≤ 2%).

Nine evaluable subjects are planned to be enrolled successively into three age groups (group 1, group 2, and group 3) in descending order of age. Three subjects aged < 18 years and ≥ 16 years will be enrolled in group 1; 3 subjects aged < 16 years and ≥ 14 years will be enrolled in group 2; 3 subjects aged < 14 years and ≥ 12 years will be enrolled in group 3.

BBM-H901 infusion will be first performed in subjects in group 1. One sentinel subject will be set

up to complete at least 2 weeks of observation after administration, and the investigator will comprehensively determine whether to continue the treatment for the other 2 subjects in this group. After the last evaluable subject in group 1 completes the 10-week follow-up after administration, the SRC will comprehensively determine whether to enter the study for group 2. One sentinel subject will also be set up in group 2 to complete at least 2 weeks of observation after administration, and the investigator will comprehensively determine whether to continue the treatment for the other 2 subjects in this group.

After the last evaluable subject in group 2 completes the 10-week follow-up after administration, the investigator will comprehensively determine whether to enter the study for group 3. One sentinel subject will be set up in group 3 to complete at least 2 weeks of observation after administration, and the SRC will comprehensively determine whether to continue the treatment for the other 2 subjects in this group.

After the last evaluable subject in group 3 completes the 10-week follow-up, the SRC will comprehensively determine the safety and tolerability. Administration at other age groups will not be continued.

Subjects who complete the 52-week assessments will continue to be followed up for 5 years after administration to obtain long-term assessment data.

Subjects will be followed up for a total of approximately 5 years after enrollment in the study, including a maximum 4-week screening period (starting immunomodulatory therapy on day −1, and baseline examination on day −1), a post-infusion 52-week follow-up period (follow-up on D3, D6, weekly at weeks 2–8, every 2 weeks at weeks 10–18, every 4 weeks at weeks 22–26, at week 32, and every 10 weeks at weeks 42–52), and a long-term follow-up period for additional 4 years after 52 weeks (every 6 months).

3.2 Sample Size Considerations

No formal statistical hypotheses are involved in this study. This study intends to enroll 9 subjects (the number of subjects enrolled may be adjusted based on the efficacy and safety).

4. Methods of Statistical Analysis

Unless otherwise specified, for each efficacy and safety endpoint, the data will be summarized and analyzed separately for subgroups of (1)16-year old≤ age <18-year old; (2)14-year old ≤ age <16-year old; and (3) 12-year old≤ age <14-year old. Results based on the combined data will also be provided.

4.1 Data Quality Assurance

All tables, graphs and data listings that will be included in the statistical analysis report will be independently verified for consistency and completeness in accordance with our standard operating procedures.

4.2 General Considerations

4.2.1 Generic Analysis Methods

1) Descriptive Statistics

Unless otherwise stated, the following will be provided as descriptive statistical summary for different types of data:

- Continuous variables: number of subjects (n), mean (mean), standard deviation (SD), median (median), minimum (min) and maximum (max) values, and confidence intervals (CI) (when appropriate).
- Categorical Variables: frequency counts, percentages of total for each category, and 95% Clopper-Pearson confidence intervals for percentages (if needed and when appropriate).

2) Number of Decimal Places

Decimal places are reserved according to the following rules, unless otherwise specified.

Parameter Name	**Targets**	**Specification**
Descriptive Statistics	Individual Subject Data	In accordance with raw data
	N, Missing	Zero decimal place
	Mean, Median	Maximum number of decimal places in the raw data + 1, up to three decimal places
	SD, SE	Maximum number of decimal places in the raw data + 2, up to four decimal places
	Min, Max, Range	Maximum number of decimal places for raw data, up to three decimal places
	Percentage (%)	One decimal place
Statistical Estimator	Mean Difference, Confidence Interval (CIs), Test statistics	Two decimal places
	P value ≥ 0.001	Three decimal places
	P value < 0.001	Displayed as “<0.001”

4.2.2 Definitions

1) Baseline

Baseline is defined as the day prior to infusion of BBM-H901 Injection (Day-1), if not otherwise specified.

If there is interference in the Day-1 day FIX Padua data (>2%) due to the use of exogenous FIX, the Screening Period FIX Padua activity data will be used as the baseline.

Note: If the baseline FIX activity is <1%, the baseline FIX activity will be calculated as 1%.

Baseline levels of the following efficacy endpoints are defined as values assessed within 52 weeks prior to signing of the ICF:

- Number and volume of infusions of other FIX protein products (including recombinant and plasma-derived FIX protein products, such as recombinant human coagulation factor IX for injection and prothrombin complex concentrate) within 52 weeks after BBM-H901 infusion
- Number of joint bleeds within 52 weeks after BBM-H901 infusion
- Proportion of subjects without bleeding events within 52 weeks after BBM-H901 infusion

Unless otherwise specified, the "baseline" level of other indicators is defined as the last non-missing measurement/assessment prior to infusion.

2) End of Trial

The end of the entire study is defined as the last subject completing the last scheduled visit, unless the study is terminated earlier for reasons outlined in Section 3.8 of the protocol.

3) Study Days

The day of infusion will be used as the reference date and to calculate time intervals between the day of infusion and the date of any event or evaluation during the trial period.

If the event date occurs prior to infusion, the number of study days (in days) = event date - reference date;

If the event date occurs on or after the day of infusion, the number of study days (in days) = event date - reference date + 1.

4) Conversion of Months and Years

Month = days/30.4375, year = (days)/365.25, rounded to 1 decimal place.

5) Medical Coding

In this study, medical coding was done for past and present medical history (MH), adverse events (AE, including serious adverse events), and prior/concomitant non-pharmacologic therapy (CT) using MedDRA 28.0 zh_CN for the main phase (data collected up to 52 weeks post infusion) and MedDRA_27_1_Chinese for the long-term follow-up phase (data collected after 52 weeks post infusion).

Prior/concomitant medications (CM), FIX product medication history, FIX protein product infusion log (FIXD), prior plasma product infusion (PLA), and plasma product infusion (PIN) will be coded using WHODrug 20250301 zh_CH for the main phase (data collected up to 52 weeks post infusion) and GLOBALB3Sep23_CHS for the long-term follow-up phase (data collected after 52 weeks post infusion).

6) Prior and Concomitant Therapy

Prior and concomitant therapy will be determined using the following rules, respectively:

Prior treatment is defined as treatment with an end time prior to signing of the ICF, including prior medication versus prior non-pharmacologic therapy.

Concomitant therapy is defined as drug or non-pharmacologic therapy that started before signing of the ICF and ended on or after the day of signing of the ICF, or drug or non-pharmacologic therapy that started on or after the day of signing the ICF, including concomitant medication and concomitant non-pharmacologic therapy.

7) Adverse Events

An AE is any untoward, unexpected, or unintended medical occurrence, including signs (e.g., clinically significant abnormal laboratory tests), symptoms, or events temporally associated with the use of AAV-based gene therapy drugs, in subjects participating in a clinical study, regardless of

causality. The criteria for AEs are shown below:

- An AE does not occur before infusion of AAV gene therapy drugs, but occurs after infusion of gene therapy drugs;
- An AE occurs before gene vector infusion, and is more severe or its incidence increases following gene vector infusion. Adverse events occurring during treatment
- Adverse Events Occurring During Treatment

Treatment-Emergent Adverse Event (TEAE) is defined as an AE that occurs at the start of first dosing or thereafter until the end of the study (EOS).

- Study Drug-Related Adverse Event

Adverse events related to study drug are defined as adverse events for which the "causal relationship to study drug" on the EDC Adverse Events page is categorized as "definitely related, probably related, possibly related, undetermined" and missing.

- Adverse Events Related to Hormone Use

Adverse events related to hormone use are defined as adverse events "related to hormone use" on the EDC Adverse Events page.

8) Analysis Window

For post-baseline visits, when analyzing by visit, statistical analyses will be performed by scheduled visit time point, and unscheduled visit data will not be included (however, unscheduled visit data will be included in the analyses related to the most clinically significant laboratory abnormality). Test results performed due to clinical indications and unscheduled visits will be provided through listings.

9) Missing Data and Outliers

If not otherwise specified, all analyses are based on observational data, except for certain event dates, imputation of missing data will not be considered; potential outliers will be discussed at the data review meeting to determine the handling method. If there are values of "＞", "＜", "≤", and "≥" in the data, they will be presented with the mathematical operation symbols in listings, and will be calculated using value without the mathematical operation symbols during summary statistical analysis.

For missing date information:

If the start/end date of prior and concomitant therapy is missing, prior or concomitant treatment will be categorized according to the rule stated in section 7.2.

If the start date of the adverse event is missing, the start date for TEAE will be imputed according to the rule stated in section 7.3 to define the TEAE .

When calculating the duration of AE, if the start date or end date of the adverse event is missing, the duration is treated as Missing.

10) Analysis Software

All report results will be analyzed using SAS 9.4 or higher version.

4.3 Data Analysis Sets

Full Analysis Set (FAS): all subjects treated with BBM-H901 injection. Socio-demographic characteristic variables, disease-related variables, AAV vector shedding, FIX Padua activity, and efficacy endpoints will be analyzed based on this analysis set.

Safety Analysis Set (SS): all subjects enrolled, treated with BBM-H901 injection and with documented post-treatment safety evaluation results. Safety endpoints will be analyzed based on

this analysis set.

The inclusion of individual cases in each analysis data set will be determined at the data review meeting.

4.4 Study Subjects

4.4.1 Subject Disposition

The following will be summarized: the number and proportion of screened subjects, screen failures and corresponding reasons, subjects who passed the screening, subjects who completed the 52-week follow-up and subjects withdrawal from the trial. Reasons for early withdrawal will be analyzed.

Listing of subjects who terminated the study and failed screening will be provided, respectively.

Based on subjects who passed screening, the number and proportion of subjects in the full analysis set, per protocol set, and safety analysis set will be summarized, and listing of subjects who did not enter each analysis set will be provided.

4.4.2 Demographic and Baseline Characteristics

Based on the FAS, the following demographic information, and baseline characteristics will be descriptively summarized.

- Demographic information (age, ethnicity, height, weight, BMI, blood type)
- Baseline clinical characteristics (family history of hemophilia B, allergy history, dietary habits, history of smoking and alcohol consumption, type of treatment prior to baseline, number of subjects with and without target joints, baseline level of endogenous FIX activity)
- History of hemophilia B (time from first diagnosis to infusion, severity of first diagnosis, number of bleeds in the 52 weeks prior to ICF signing, ABR within 52-week prior to ICF signing)

Time from first diagnosis to infusion (months) = (date of infusion - date of first diagnosis + 1)/30.4375.

Past medical history/present medical history will be summarized as the number and proportion of subjects according to System Organ Class (SOC) and Preferred Term (PT), respectively.

Past medications, history of FIX product use, and past plasma product transfusions will be summarized as the number and proportion of subjects according to ATC2 and preferred names (PN), respectively.

Listings will be provided for demographic information, baseline clinical characteristics, smoking history, alcohol consumption history, history of hemophilia B, past/present medical history, past medications, history of FIX product medications, past plasma product transfusions, history of other past treatments, past bleeding, and surgical plans.

4.4.3 Concomitant Therapy

Based on SS, concomitant medications will be summarized according to ATC2 and PN, and concomitant nonpharmacological therapies will be summarized according to SOC and PT. Listings of concomitant medications and non-drug therapies will be provided.

4.4.4 Drug Exposure and Compliance

Based on the FAS, the relative infusion dose, actual infusion dose, study duration (days) of BBM-H901 Injection and hormone and immunomodulatory therapy use will be summarized.

Note: Relative Infusion Dose (%) = Actual Dose/Scheduled Dose.

Study Duration (days) during the Main Study Period = (Date of Completion of the Main Study - Date of BBM-H901 Infusion) + 1.

Note: Date of completion of main study: date of completing the planned data collection within 52 weeks of BBM-H901 infusion

Based on the FAS, FIX protein product infusion, plasma product infusion and hormone and immunomodulatory therapy will be summarized on number and proportion of subjects, number of times used, dose used, and number of days used according to ATC2 and Preferred Names (PN).

Listings of FIX protein product infusions, plasma product infusions, hormone and immunomodulatory therapy, and BBM-H901 injection infusions will be provided

4.5 Safety Analysis

If not otherwise specified, safety analysis will be performed based on SS.

4.5.1 Adverse Events

Severity of adverse events will be defined according to the CTCAE (version 5.0 or latest version).

1. The number of subjects, proportion and incidence of AEs will be summarized by age group and for combined data according to the following classification:

- All AE
- AE with CTCAE ≥ 3
- All SAEs
- AE associated with hormone use
- TEAE
- TEAE with CTCAE ≥ 3
- Serious adverse event during treatment (TESAE)
- Study drug-related TEAE
- TEAE leading to infusion suspension
- TEAE leading to delay in dosing
- TEAE leading to discontinuation of medication
- TEAE leading to death

2. The following AEs will be categorized by System Organ Class (SOC) and Preferred Term (PT) and severity of the adverse event (any level, ≥3, 3, 4, 5), the number and proportion of subjects will be summarized by age group, and the adverse event under each SOC and PT category will be listed in descending order based on the aggregated number of cases of any level. When aggregated by severity, only the most severe occurrence of the same SOC or PT in the same subject will be counted:

- All AEs
- All SAEs
- AE associated with hormone use
- TEAE
- TESAE
- Study drug-related TEAE
- TEAE with CTCAE ≥3
- TEAE leading to infusion suspension

- TEAE leading to delay in medication administration
- TEAE leading to discontinuation of medication
- TEAE leading to death

3. Based on the SS, the number and proportion of death, as well as the cause of death will be summarized by age group.

4. Listings of all AEs, SAEs, DLTs, TEAEs, TESAEs related to study drug, TEAEs with CTCAE ⩾ 3, TEAEs leading to discontinuation of treatment, TEAEs leading to death, and deaths will be provided.

4.5.2 ALT and AST

The number and proportion of subjects with abnormal aminotransferase concentrations (ALT and/or AST) after BBM-H901 infusion will be summarized. The number and proportion of subjects with abnormal aminotransferase concentrations and received prophylactic and on-demand immunomodulatory therapy will be summarized, respectively. The number of days on treatment and dose of prophylactic and on-demand immunomodulatory therapy used will be summarized.

Individual AST and ALT concentration-time curves will be plotted by actual time and will be marked with reference lines for the duration of hormonal and immunomodulatory therapy use and abnormal cut-off values of aminotransferase concentration.

Note: Abnormal ALT and/or AST values are defined as any ALT and/or AST test value above the upper limit of normal (50 U/L) or 1.5 times the baseline value after BBM-H901 infusion. Prophylactic Immunomodulatory Therapy and On-Demand Immunomodulatory Therapy categories will be determined by the investigator.

Listings of AST and ALT abnormal test results with clinical significance will be provided.

4.5.3 FIX Inhibitors

Based on SS, the number and proportion of subjects with FIX inhibitors will be summarized by age group and for combined data, and listing of test results on FIX inhibitors will be provided.

4.6 Efficacy Analysis

4.6.1 ABR after BBM-H901 infusion

- Variable: ABR within 52, 78 and 104 weeks after BBM-H901 infusion

The formula for calculating ABR, is as follows:

ABR after infusion = Number of bleeding events after BBM-H901 Injection infusion ÷ Effective observation duration after infusion (days) × (52 weeks * 7 days)/year, and the result is retained in 1 decimal place.

- Primary analysis: The efficacy analysis on ABR will be based on FAS. A negative binomial regression model will be used to analyze the ABR within 52 weeks post-infusion to estimate the mean ABR and its 95% confidence interval. Number of bleeding episodes will be descriptively summarized. Bleeding events happened in three consecutive days will be counted as one bleeding episode. If the convergence of negative binomial regression model is questionable and the model fit may be not valid (i.e. due to small sample size), the poisson regression model will be applied instead.

- Intercurrent events and strategies:

1) Spontaneous bleeding, traumatic bleeding, or other unspecified causes of on-demand prophylactic treatment (FIX expressed): a treatment policy strategy will be used, i.e., the impact duration from the alternative treatment will be deducted from the effective observation time (for each use, a 5-half-life period, i.e., 96 hours, will be deducted; or, if the interval between two treatments is less than 4 days, the overlapping time portion will be counted only once), and bleeding events happened during the replacement therapy will still be used to calculate the ABR.

2) Death: the while on treatment strategy will be used and data after death will not be included in the analysis.

3) Alternative treatment applied due to FIX non-expression (i.e., treatment failure): a composite variable strategy will be used. The mean ABR of the general prophylaxis population (20.0) will be used to impute the ABR within 52 weeks after infusion. FIX non-expression is defined as: Mean FIX Padua activity failed to reach 5% during the period from the time of the BBM-H901 injection infusion to the time of the subject's first use of the FIX protein product.

4) Restarting regular preventive FIX replacement therapy: During the period between infusion of BBM-H901 and the restart of regular preventive FIX replacement therapy, the observed bleeding episodes and number of on-demand FIX replacement therapy (will be used to exclude non-risk exposure time) will be integrated to calculate the ABR. The value of 20 will be used for ABR imputation after restarting regular preventive FIX replacement therapy.

-Subgroup analyses will be performed on ABR for patients with different baseline characteristics.

Characteristic	subgroup
Type of treatment before baseline	Treatment as needed; preventive treatment; other
Combination of target joints before treatment vs. no target joint combination	Combined target joints; without combined target joints
Baseline levels of endogenous FIX activity	≤1%; 1% < baseline level of endogenous FIX activity≤2%

4.6.2 FIX activity levels after the infusion of BBM-H901 injection

- Variable: change from baseline in FIX Padua activity level at each visit after BBM-H901 injection infusion
- Primary analysis: Based on the FAS, provide summary of baseline FIX Padua activity, FIX Padua activity at Day 3, Week 12, Week 26, Week 52, Week 78 and Week 104 post-infusion, and the change of FIX Padua activity at Week 52 post-infusion from baseline, measured using the Actin FSL assay. The Wilcoxon signed rank test will be used to analyze the difference between the FIX Padua activity level at Week 52 post-infusion and the baseline level.
- Intercurrent events and strategies:

1) Spontaneous bleeding, traumatic bleeding, or other on-demand, regular, or prophylactic treatment of unspecified cause (FIX expressed) and alternative treatment due to FIX unexpressed (i.e., treatment failure): treatment policy strategy will be applied, and analysis will be performed based on actual observed data.

2) Death: the while on treatment strategy will be used and data after death will not be included in the analysis.

4.6.3 Number and volume of infusions of other FIX protein products (including recombinant and plasma-derived FIX protein products, such as recombinant human coagulation factor IX for injection and prothrombin complex concentrate) after BBM-H901 infusion

- Variable: change from baseline in the number and volume of other FIX protein products (including recombinant and plasma-derived FIX protein products, such as recombinant human coagulation factor IX injection and thrombospondin complex, etc.) infused during the 52-week period and the 53 to 104-week period after BBM-H901 injection infusion;
- Primary analysis: Based on the FAS, provide summary of the number of infusions and total volume of other FIX protein products (including recombinant and plasma-derived FIX protein products, such as recombinant human coagulation factor IX injection, plasminogen complex, etc.) infused within 52 weeks post infusion and within 53 to 104-week post infusion. The changes in number and volume of other FIX protein products use from baseline will be calculated, and Wilcoxon signed-rank test will be used to analyze the difference between the baseline and within 52 weeks post injection infusion.
- Intercurrent events and strategies:

1) Spontaneous bleeding, traumatic bleeding, or other as-needed, regular, or prophylactic therapy of unspecified cause (FIX expressed) and use of alternative therapy due to FIX unexpressed (i.e., treatment failure): treatment policy strategy will be applied, and analysis will be performed based on actual observed data.

2) Death: the while on treatment strategy will be used and data after death will not be included in the analysis.

4.6.4 Number of target joints after BBM-H901 infusion

- Variable: change from baseline in the number of target joints at Week 52, 78 and 104 after BBM-H901 injection infusion;
- Primary analysis: based on the FAS, summarizes the number of target joints at baseline (within 52 weeks before the infusion) and 52, 78 and 104 weeks after injection infusion, and the change in

number of target joints from baseline at Week 52, 78 and 104 after infusion. The Wilcoxon signed rank test will be used to analyze the difference in the number of target joints between baseline and 52 weeks after the infusion.

- Intercurrent events and strategies:

1) Spontaneous bleeding, traumatic bleeding, or other as-needed, regular, or prophylactic treatment of unspecified cause (FIX expressed) and alternative treatment due to FIX unexpressed (i.e., treatment failure): treatment policy strategy will be applied, and analysis will be performed based on actual observed data.

2) Death: the while on treatment strategy will be used and data after the event will not be included in the analysis.

4.6.5 Number of joint bleeds after BBM-H901 infusion

- Variable: change from baseline in the number of joint bleeds during the 52-week period and the 53 to 104-week period after BBM-H901 injection infusion
- Primary analysis: based on the FAS, the number of joint bleeds at baseline (within 52 weeks before injection infusion), during the 52-week post injection and the 53 to 104-week post injection infusion will be summarized, as well as the change from baseline. The Wilcoxon signed rank test will be used to analyze the difference in the number of joint bleeds between baseline and within 52 weeks after the injection infusion.
- Intercurrent events and strategies:

1) Spontaneous bleeding, traumatic bleeding, or other on-demand, regular, or prophylactic treatment of unspecified cause (FIX expressed) and alternative treatment due to FIX unexpressed (i.e., treatment failure): treatment policy strategy will be applied, and analysis will be performed based on actual observed data.

2) Death: the while on treatment strategy will be used and data after the event will not be included in the analysis.

4.6.6 Proportion of subjects without bleeding events after BBM-H901 infusion

- Variable: Proportion of subjects without bleeding events within 52, 78 and 104 weeks after BBM-H901 injection infusion;
- Primary Analysis: Based on the FAS, the proportion of subjects free of bleeding events within 52 ,78 and 104 weeks after BBM-H901 Injection infusion will be calculated based on actual observed values, and descriptive statistics (number, percentage) will be presented in tabular form.
- Intercurrent events and strategies: the treatment policy strategy will be applied to all Intercurrent events, missing data will not be imputed, and the analysis will be performed on actual observed values.

4.6.7 Changes in joint health score, joint ultrasound score and quality of life from baseline to 4 weeks, 12 weeks, 26 weeks and 52 weeks after the infusion of BBM-H901 injection using the following scales related to hemophilia assessment: Hemophilia Joint Health Score (HJHS) , Hemophilic Early Arthropathy Detection with Ultrasound in China (HEAD-US-C) and Canadian Hemophilia Outcomes-Kids' Life Assessment Tool (CHO-KLAT).

- Variables: changes in HJHS and HEAD-US-C scores from baseline to Week 4, Week 12, Week 26, Week 52, Week 78 and Week 104 after BBM-H901 infusion

- Primary Analysis: Based on the FAS, each scale score and change from baseline will be summarized through descriptive statistics (median, minimum, maximum, etc.) by scheduled visits and baseline. The analysis of this efficacy endpoint is based on actual observations, missing data will not be imputed, and the analysis of this endpoint will not include hypothesis testing.
- Intercurrent events and strategies: the treatment policy strategy will be applied to all intercurrent events, missing data will not be imputed, and the analysis will be performed on actual observed values.

4.6.8 Proportion of subjects discontinuing treatment with exogenous FIX products after BBM-H901 injection infusion

- Variable: proportion of subjects discontinuing treatment with exogenous FIX products within 52, 78 and 104 weeks of BBM-H901 injection infusion
- Primary Analysis: Based on the FAS, the proportion of subjects discontinuing treatment with exogenous FIX products within 52,78 and 104 weeks after BBM-H901 injection infusion will be calculated. The analysis will be performed based on actual observed values and descriptive statistics (number, percentage) will be presented in tabular form. This efficacy endpoint will not be analyzed through hypothesis testing.
- Intercurrent events and strategies: the treatment policy strategy will be applied to all intercurrent events, missing data will not be imputed, and the analysis will be performed on actual observed values.

4.7 Pharmacokinetic/vector shedding analysis

Descriptive statistics based on FAS will be provided and plotted for FIX Padua activity levels and AAV vector viral loads in PBMC, plasma, saliva, urine and semen samples. Concentration-time curves will be plotted for individual (according to the actual time of blood sample collection), mean and median (according to the planned time of blood sample collection), respectively. The mean and median FIX Padua concentration-time curves of different assays will be plotted according to the planned time of blood collection. Peak and mean FIX Padua activity levels will be calculated, and descriptive statistical analysis will be provided. The number of cases of AAV vector shedding, the shedding rate and the time (in days) to shedding in PBMC, plasma, saliva, urine and semen samples will be summarized through descriptive statistics, and the Kaplan-Meier curves will be presented to describe the time to shedding since infusion.

Note: Vector shedding was defined as occurrence of three consecutive negative vector shedding test results. Days to shedding was defined as the time from infusion of BBM-H901 Injection to the first of three negative carrier shedding results.

Listings of individual subject data of FIX Padua activity, AAV vector shedding, AAV vector viral loads and AAV cellular immunity will be provided.

5. Statistical Analysis Schedule and Study Reporting Plan

The statistical analyses described in this SAP and the corresponding study reports are for the analysis and reporting of the main study phase (including data collected up to the 52-week follow-up of the last subject in the confirmatory study phase) and the long-term follow-up phase (including data collected up to the data cut-off date at the time of analysis).

Additional analyses will be performed and analytical reports will be developed after the last subject

completes an additional 4 years of long-term follow-up.

6. References

None

7. Appendix

7.1 Appendix 1 Schedule of activities

Item[1]	**Screening period**[2]		**Dosing period**[3]	**Follow-up period 1**																			**Long-term follow-up**
				Week 1 (± 1 day)		**Weeks 2–18 (± 2 days)**												**Weeks 22–52 (± 7 days)**					**Every 6 months ± 14 days/ early withdrawal**
Time (weeks)	**Week 4**	**Day −1**	**Day 0**	**1**		**2**	**3**	**4**	**5**	**6**	**7**	**8**	**10**	**12**	**14**	**16**	**18**	**22**	**26**	**32**	**42**	**52/early withdrawal**	
				Day 3	**Day 6**																		
Informed consent form	X																						
Review of the inclusion/exclusion criteria	X	X																					
Demographic characteristics[4]	X																						
Medical and treatment history of hemophilia B[5]	X																						
Other medical and treatment history[6]	X																						
Physical examinations[7]	X	X			X	X		X		X		X		X		X		X	X	X	X	X	X
Height, weight[8]	X	X																	X			X	X
Vital signs[9]	X	X	X		X	X		X		X		X		X		X		X	X	X	X	X	X
Hematology, blood biochemistry, and urinalysis[10]	X	X			X	X		X		X		X		X		X		X	X	X	X	X	X

Item[1]	**Screening period**[2]		**Dosing period**[3]	**Follow-up period 1**																				**Long-term follow-up**
				Week 1 (± 1 day)		**Weeks 2–18 (± 2 days)**													**Weeks 22–52 (± 7 days)**					**Every 6 months ± 14 days/ early withdrawal**
Time (weeks)	**Week 4**	**Day −1**	**Day 0**	**1**		**2**	**3**	**4**	**5**	**6**	**7**	**8**	**10**	**12**	**14**	**16**	**18**	**22**	**26**	**32**	**42**	**52/early withdrawal**	
				Day 3	**Day 6**																		
ALT, AST		X		X			X		X		X												
Coagulation function[11]	X	X			X	X		X		X		X		X		X		X	X	X	X	X	X
Infectious disease screening[12]	X																						
Genetic testing for hemophilia[13]	X																						
12-lead ECG[14]	X	X	X										X						X			X	X
Blood typing	X																						
Immunomodulatory therapies[15]		◄		———		———	———	———	———	———	———												
Infusion of BBM-H901 injection			X																				
Completion of FIX protein product infusion log[16]	◄			———		———	———	———	———	———	———	———	———	———	———	———	———	———	———	———	———	———	
Bleeding assessment	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Target joint count assessment[17]	X	X																	X			X	X

Item[1]	**Screening period**[2]		**Dosing period**[3]	**Follow-up period 1**																				**Long-term follow-up**
				Week 1 (± 1 day)		**Weeks 2–18 (± 2 days)**												**Weeks 22–52 (± 7 days)**					**Every 6 months ± 14 days/ early withdrawal**
Time (weeks)	**Week 4**	**Day −1**	**Day 0**	**1**		**2**	**3**	**4**	**5**	**6**	**7**	**8**	**10**	**12**	**14**	**16**	**18**	**22**	**26**	**32**	**42**	**52/early withdrawal**	
				Day 3	**Day 6**																		
Abdominal B ultrasound[18]	X																		X			X	X
DXA for bone density		X																				X	Once every 12 months
FIX activity test[19]	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FIX inhibitor test[20]	X	X				X		X				X		X		X			X	X	X	X	X
Scale assessment		X						X						X					X			X	
AAV capsid neutralizing antibody test	X	X			X	X		X						X					X			X	X
AAV capsid binding antibody test	X	X			X	X		X						X					X			X	X
AFP	X																		X			X	X
PBMCs, plasma, saliva, urine, stool sample collection[21]		X			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Single-cell sequencing of																							

Item[1]	**Screening period**[2]		**Dosing period**[3]	**Follow-up period 1**																		**Long-term follow-up**
				Week 1 (± 1 day)		**Weeks 2–18 (± 2 days)**												**Weeks 22–52 (± 7 days)**					**Every 6 months ± 14 days/ early withdrawal**
Time (weeks)	**Week 4**	**Day −1**	**Day 0**	**1**		**2**	**3**	**4**	**5**	**6**	**7**	**8**	**10**	**12**	**14**	**16**	**18**	**22**	**26**	**32**	**42**	**52/early withdrawal**	
				Day 3	**Day 6**																		
peripheral blood cells[22]																							
AEs[23]	←																				→		X
Concomitant medications[24]	←																				→		X

Notes: ALT: alanine aminotransferase; AST: aspartate aminotransferase; FIX: coagulation factor IX; AAV: adeno-associated virus; PBMCs: peripheral blood mononuclear cells

1. Additional visit items (including those not included in the schedule of activities) and/or additional unscheduled visits may be performed according to the actual safety and efficacy assessments, including but not limited to: liver transaminases, FIX activity, FIX antigen, blood lipids (total cholesterol [TC], high-density lipoprotein [HDL], low-density lipoprotein [LDL], very low-density lipoprotein [VLDL], triglycerides [TG]); coagulation function, thrombin generation assay (TGA), hepatitis B deoxyribonucleic acid quantification, hepatitis C ribonucleic acid quantification, serum interferon, lymphocyte subsets (B/T/NK cells), etc.

2. Collect the information of all subjects who participate in the screening, including the reasons for screening failure.

3. Do not use other FIX protein products and plasma concentrates within 96 h or 5 half-lives (whichever is longer) prior to the infusion of BBM-H901 injection. The investigational product should not be thawed until confirmation by the investigator regarding subject washout criteria.

4. Collect demographic data of subjects (including gender, age, ethnicity, and fertility status);

5. Collect the subject's hemophilia history (including date of diagnosis, severity, genotype, human leukocyte antigen [HLA, if any], previously used FIX products [including recombinant and plasma-derived, such as recombinant human coagulation factor IX injection, prothrombin complex, etc., type (on-demand, prophylaxis, continuous infusion, etc.), dosing regimen, dose, number of infusions], number of bleeding, etc.), and the number of bleeding (including spontaneous bleeding, traumatic bleeding, and bleeding not requiring FIX product treatment) in the last 26 and 52 weeks will be retrospectively determined. Record as truthfully as possible and estimate if necessary.

6. Other medical history and treatment history include: past and present medical history, family medical history, allergic history, surgical history and surgical plan, smoking, drinking, dietary habits, fertility plan, vaccination; history of blood donation within 3 months before screening, any participation in clinical trials, other medications and treatments other than those for the disease under study.

7. Physical examination: Only routine physical examination will be performed during the screening period, including skin/mucosa, lymph nodes, head, neck, chest, abdomen, musculoskeletal/extremities, and neurological system/psychiatric conditions. Only symptoms/vital signs-specific physical examination will be performed for other visits. The investigator may increase the frequency of tests based on safety considerations.

8. Height and weight: Only body weight will be measured on day −1, which will be used to calculate the dose of BBM-H901 injection, and height and weight will be measured at other visits.

9. Vital signs: including blood pressure, body temperature, pulse rate, and respiratory rate. Vital signs will be measured after the subject has rested for at least 5 min in sitting position. In addition to the routine visit points for vital signs, vital signs will be measured before injection of FIX protein products on day 0. If vital signs are beyond the normal range or the change is clinically significant and the investigator considers that a repeat measurement is required, 2 additional measurements will be taken approximately 2 min apart. The investigator may increase the frequency of tests based on safety considerations.

10. Hematology, blood biochemistry, and urinalysis: If the above laboratory tests are performed within 7 days before BBM-H901 infusion, they may not be performed on day −1; if blood biochemistry tests are required on day −1, the ALT, AST and GGT may not be repeated on day −1. During the trial, the investigator may increase the frequency of tests based on safety considerations.

11. Coagulation function: including plasma prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), plasma fibrinogen (FIB), and D-dimer. If this test is performed within 7 days prior to BBM-H901 infusion, it may not be performed prior to the infusion of BBM-H901 injection on day −1. The investigator may increase the frequency of tests based on safety considerations.

12. Infectious disease tests include: hepatitis B virus test (HBsAg, HBV-DNA quantitative detection), hepatitis C virus test (HCV-Ab, HCV-RNA quantitative detection), HCV viral load (if any), human immunodeficiency virus antibody (anti-HIV-Ab) test, and syphilis antibody test.

13. For subjects with known genotype, if the previously obtained genotype report is available, genotype test may not be performed at screening; for subjects with unknown genotype, samples will be collected at screening for analysis.

14. 12-lead ECG: The 12-lead ECG should be performed in supine position after the subject has rested for at least 5 min in supine position. ECG indicators include heart rate, PR interval, QRS interval, uncorrected QT interval, and Fridericia-corrected QTc interval (QTcF). When an ECG measurement is abnormal (including an abnormal QTcF, e.g., QTcF > 500 ms or an increase from baseline > 60 ms) or is judged clinically significant by the investigator, 2 additional ECG measurements will be performed at least 2 min apart. The 12-lead ECG on the day of dosing will be performed within 1 h after the infusion of BBM-H901 injection. During the study, the frequency of tests may be increased as appropriate based on safety considerations.

15. In this study, prophylactic steroids will be used, that is, oral glucocorticoids (prednisone or prednisolone) will be prophylactically used from Day −1 before BBM-H901 infusion to eight weeks after infusion. See the study protocol for the specific treatment regimen.

16. Infusion log: The use of the FIX protein product will be recorded from the signing of informed consent to the end of the study, including the reason for treatment, start and end dates, type (on-demand, prophylaxis, continuous infusion, etc.), frequency, and doses.

17. Target joint count assessment: The subject's history of target joint disease will be collected within 52 weeks before screening, and the number of target joints will be assessed within 52 weeks after infusion. The target joint is defined as the occurrence of 3 or more spontaneous bleeding in a single joint in a consecutive 6-month period. If a joint bleeds ≤ 2 times in a consecutive 12-month period, the joint is no longer considered a target joint. The investigator may increase the frequency of tests based on safety considerations.

18. Abdominal B ultrasound: liver, gallbladder, pancreas, spleen, and bilateral kidneys. The investigator may increase the frequency of tests based on safety considerations.

19. During the assessment of FIX activity, subjects are required to suspend the prophylactic treatment with the FIX protein product after receiving the infusion of BBM-H901 injection until dose resumption is recommended by the investigator; if the subject develops bleeding during the study and requires on-demand use of FIX protein product as judged by the investigator, the washout period of at least 96 h or 5 half-lives (whichever is longer) for the FIX protein product should be ensured before the assessment of FIX activity. FIX activity levels will be measured using two methods of the one-stage methods (Sysmex platform, Actin FSL aPTT reagent, and Werfen platform, HemosIL SynthASil reagent).

20. FIX Inhibitor: Inhibitor detection is performed using the Bethesda assay or Nijmegen-modified Bethesda assay.

21. Collection of PBMCs, plasma, saliva, urine, and feces samples: Collect samples for vector shedding detection, and detect the titer of viral particles in plasma, PBMCs, saliva, urine, and feces using quantitative polymerase chain reaction (qPCR) until the results of three consecutive tests are negative.

22. Single-cell sequencing of peripheral blood cells: Single-cell RNA sequencing analysis method will be used to assess the cellular immune status of the body before and after AAV vector infusion and the impact of glucocorticoids on the immune status, without sampling from all subjects. The recommended sampling points are as follows: on day −1 before vector infusion, on day 3 after vector infusion, and at week 4 after vector infusion; and when transaminases increase by 1.5–2 times from the baseline value, when coagulation factors decrease sharply after stabilization, and at 2 weeks after steroid withdrawal; if the interval between blood sampling points is close, the investigator may combine the blood sampling points based on the actual situation.

23. AEs: AEs will be collected from the time of signing the informed consent form to 52 weeks ± 7 days of follow-up after drug administration, early withdrawal, or initiation of another gene drug treatment (whichever occurs earlier), and all AEs will be followed up until recovery of AEs, or stable disease, or interpretation of the AEs, subjects' death, or loss to follow-up. Only investigational product-related AEs will be collected during the long-term follow-up period and may be followed for up

to 5 years/until early withdrawal.

24. Concomitant medications and treatments will be recorded during the screening period and study treatment until 52 weeks ± 7 days after drug administration, early withdrawal from follow-up, or initiation of another gene drug treatment, whichever occurs earlier. Only medications related to hemophilia therapy will be collected during the long-term follow-up period.

7.2 Appendix 2 Prior and Concomitant Therapy Determination

If the prior and concomitant therapy start/end dates are incomplete, determine the type of therapy by the available information as much as possible:

1) If the end date is earlier than the date of signing of the ICF, it will be defined as a prior therapy; if the end date is later than the date of signing of the ICF, it will be defined as a concomitant therapy.

2) If cannot be determined based on the end date, the therapy will be defined as a concomitant therapy.

7.3 Appendix 3 Adverse Event Missing Date Imputation Rules

If the adverse event start date was incomplete, the start date will be imputed to determine the AE period:

Start date	Filling rules
Completely missing	1. The end date is earlier than the infusion date: impute the start date using the earliest date in the same year of the end date. 2. The end date is later than the infusion date or cannot be determined: impute the end date using the infusion date.
Partially missing	1. The start date is earlier than the infusion date, impute the start date using the earliest date in the same year/month of the start date. 2. The start date is in the same year/month as the infusion date: if the end date is before e the infusion, impute the start date using the earliest date of the same year/month; if the end date is after the infusion or cannot be determined, impute the start date using the infusion date. 3. The start date is later than the infusion date, impute the start date using the earliest date of the same year/month of the start date

Revision Record

version number	commencement date	Main revisions
1.0	May 30, 2025	new document
2.0	August 31, 2025	Added analysis plan for long-term follow-up data. Added analysis plan for data collected using the Canadian Hemophilia Outcomes-Kids' Life Assessment Tool (CHO-KLAT)