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SOCIEDAD ESPAÑOLA DE TROMBOSIS Y HEMOSTASIA

ISTH International Society on Thrombosis and Haemostasis

Advanced course in Platelet Research

Murcia (Spain)

27-28 September, 2024

HMB-001: A BISPECIFIC ANTIBODY ACCUMULATING AND TARGETING ENDOGENOUS FVIIA TO ACTIVATED PLATELETS ENHANCES THROMBIN GENERATION AND FIBRIN FORMATION FOR SUBCUTANEOUS PROPHYLAXIS IN GLANZMANN THROMBASTHENIA

Prafull S. Gandhi, PhD

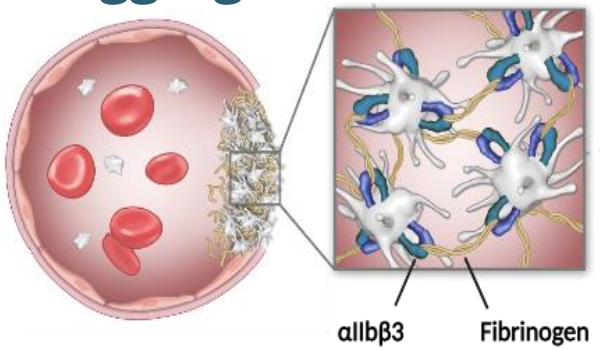
Hemab Therapeutics

Glanzmann Thrombasthenia

- Rare genetic **bleeding disorder** that disrupts platelet aggregation and clot formation
- Mutations in the *ITGA2B* and *ITGB3* genes render the GPIIb/IIIa* (fibrinogen) receptor absent or non-functional on platelets, **hindering formation of the platelet-fibrin mesh**
- **Frequent bleeding events** ranging from low volume epistaxis to life-threatening gastrointestinal hemorrhages[1]
  - Up to 2.17 bleeds/day with **nearly half of reported bleeding events requiring medical intervention** with tranexamic acid, platelet transfusions or recombinant FVIIa[2]
- The current standard of care for GT is reactive and on-demand, with **no approved therapies for primary prophylaxis.**

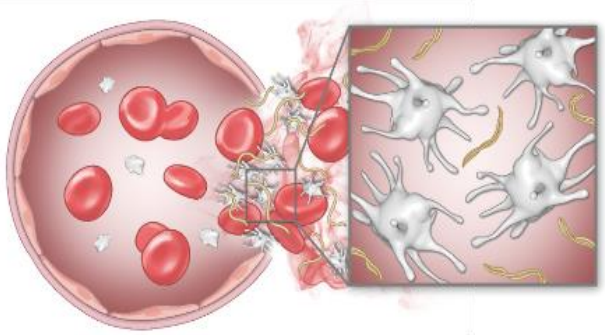
Healthy Platelet Aggregation

Fibrinogen binding to αIIbβ3 is required for normal platelet aggregation and haemostasis



Glanzmann Thrombasthenia

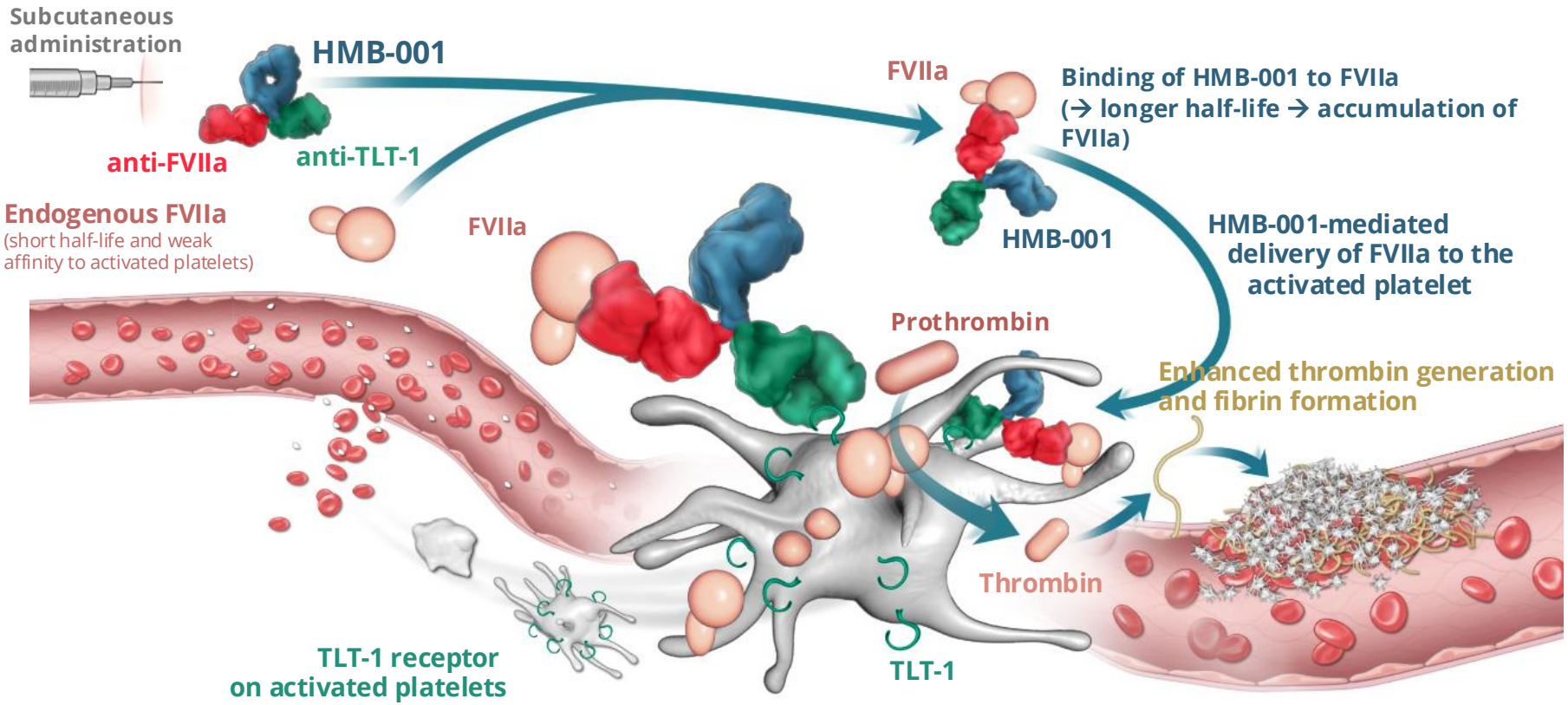
Deficiency of αIIbβ3 results in lack of fibrinogen-mediated bridging of platelets and a bleeding phenotype



[1]Khair K. Glanzmann's 360 Study [Speaker Abstracts]. *Haemophilia* (2023), 29: 5-13.2; 2. Rhea C. UK Bleed Diary [Poster]. *ISTH* (2024).
* Also known as glycoprotein (GP) IIb/IIIa

HMB-001 | A Novel Bispecific Antibody Targeting FVIIa & TLT-1

HMB-001 binds and accumulates endogenous FVIIa and, following vessel lesion, localises FVIIa to the surface of activated platelets



Objective:
Demonstrate impact of HMB-001 on FVIIa hemostatic activity in *ex vivo* models of GT and present preliminary data from ongoing FiH Phase 1/2 clinical studies in people with GT

Gandhi PS, et. al. Nature Cardiovasc Res. 2024;3:166-185.

Overview of Pre-clinical and Clinical Studies

Pre-clinical Studies

1 *In Vitro* Studies

FVIIa localization on activated human platelets

- Flow cytometry to demonstrate HMB-001's ability to localize FVIIa to activated platelets via TLT-1

HMB-001 activity

- Platelet aggregation assay using GT platelets to show effect on fibrin-dependent aggregation

Fibrin formation

- Quantified by microfluidic flow chamber with collagen-coated surface in GT whole blood

2 *In Vivo* Studies

HMB-001 PK/PD

- Normocoagulant cynomolgus monkeys
- FVIIa quantification as a measure of HMB-001 PD

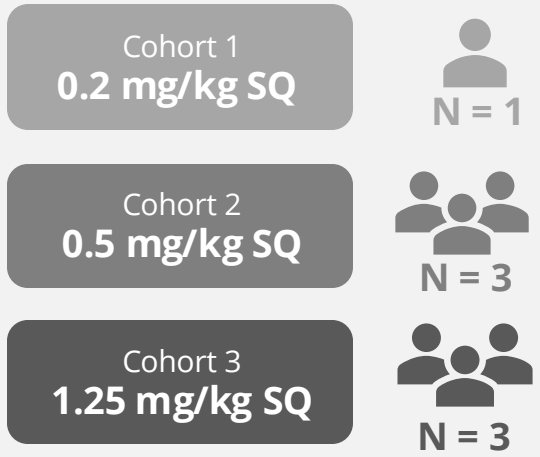
Hemostatic efficacy assessment in *ex vivo* studies

- HMB-001 potentiates FVIIa activity in platelet rich plasma Thrombin generation (PRP-TG) *ex vivo* model

First-in-Human Phase 1/2 Study[1]

3 Phase 1, Single Ascending Dose study

- HMB-001 PK ELISA
- HMB-001 PD:
  - FVIIa clot activity assay, Stago
  - Total FVII(a) ELISA, Stago
- Safety and tolerability
- Identify optimal dosing levels and intervals for Phase 2



PK, pharmacokinetics | PD, pharmacodynamics

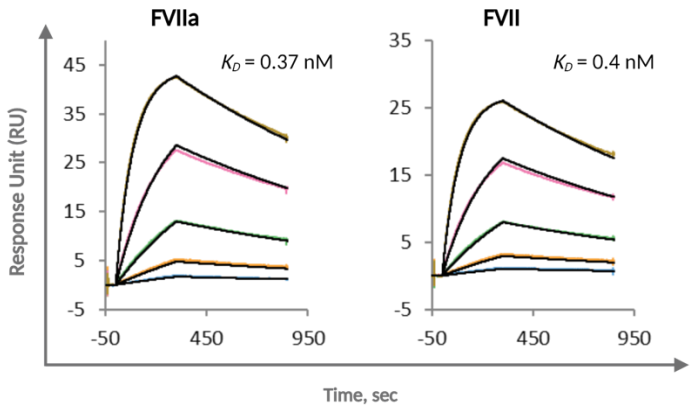
1. NCT06211634

HMB-001 binds to FVIIa in a neutral manner

Key Properties of FVIIa and FVII

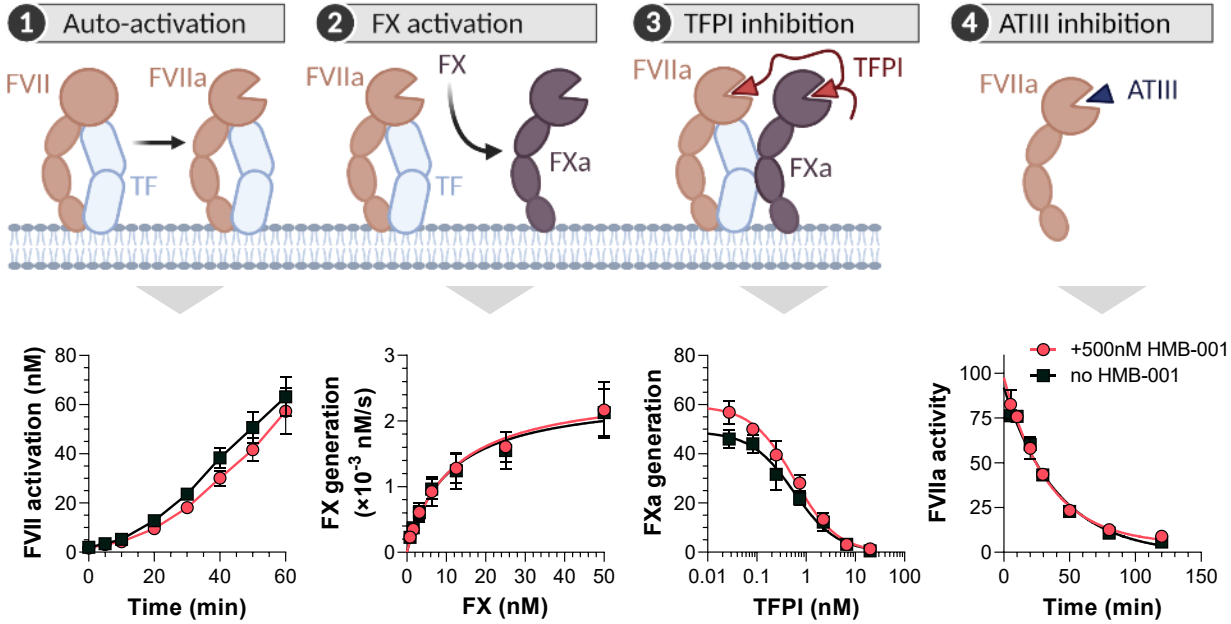
- Coagulation FVII circulates in blood as an inactive zymogen (FVII; 9 nM) and as an active protease (FVIIa; 70 pM)[1,2]
- PK studies with FVII and FVIIa demonstrate comparable half-lives with a FVIIa half-life of 2–3 h[3,4]
- FVIIa displays weak binding affinity (Kd = 1.2 µM) to activated platelets[5]

HMB-001 binds equally well to FVIIa and FVII



HMB-001 binding does not affect key physiological functions of FVIIa and FVII

- FVIIa:TF complex is a key initiator of the coagulation cascade
  - Activates coagulation substrates FVII, FX, and FIX, and
  - Inhibited by TFPI and Antithrombin III (ATIII)



1. Wildgoose, P., et. al., Blood 1992; 80, 25–28. 2. Morrissey, J.H., et. al., Blood 1993; 81, 734–744. 3. Mathijssen N.C.J., et. al., Thromb Res. 2013; 132(2):256-62. 4. Tiede, A. et. al., J. Thrombosis and Haemostasis. 2011; 9, 1191–1199. 5. Kjalke, M., et. al., 2007; J. Thrombosis and Haemostasis 5, 774–780.

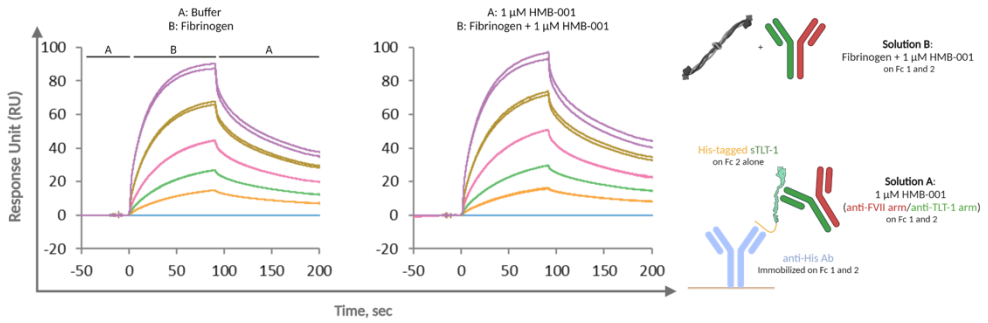
HMB-001 does not influence key platelet properties

Key properties of TLT-1

- Membrane-bound protein - extracellular immunoglobulin variable (IgV) domain (1–110), stalk peptide (111–147), transmembrane segment (148–169) and C-terminal cytoplasmic domain (170–296)[1]
- Present exclusively in intracellular pools of resting platelets and megakaryocytes
- Upon platelet activation, TLT-1 is redistributed from α-granules to the platelet surface[2,3]
- Binds weakly to fibrinogen[4]
- Proteolytically cleaved and shedded from the activated platelets[5]

HMB-001 does not affect TLT-1 binding to fibrinogen

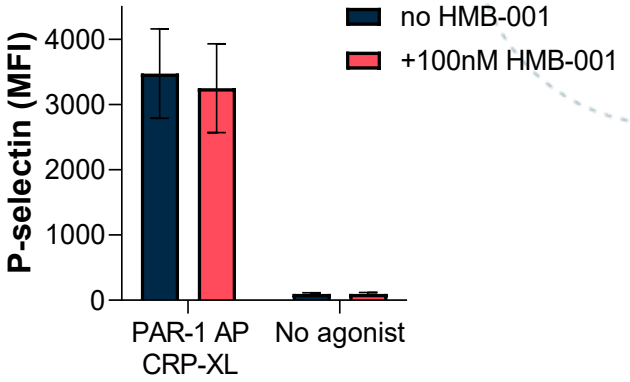
$K_{D,\ sTLT\text{-}1}$ = 2.3 nM



HMB-001 does not affect platelet activation and aggregation

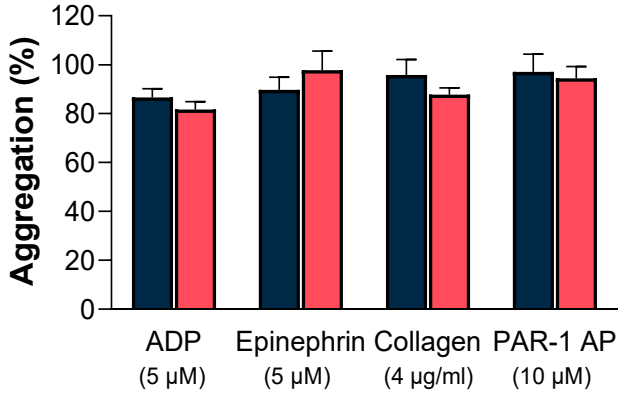
1 Platelet activation

- Exposure of normal whole blood to platelet activator ± 100nM HMB-001
- After 20 min, P-selectin exposure was quantified by FACS (mean ± SD, n = 3)



2 Platelet aggregation

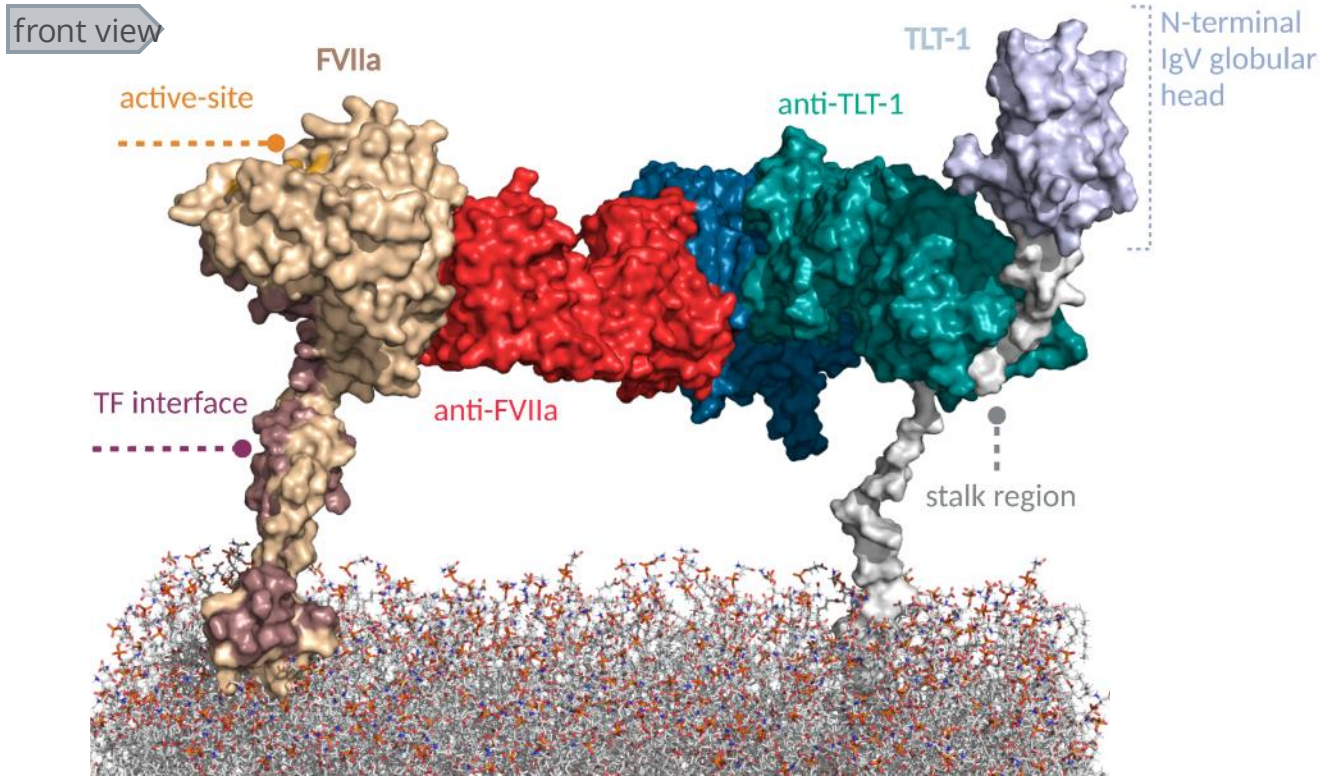
- Aggregation of platelet rich plasma in presence of platelet activator ± 100nM HMB-001
- Max amplitude at 1 hr (mean ± SD, n = 3)

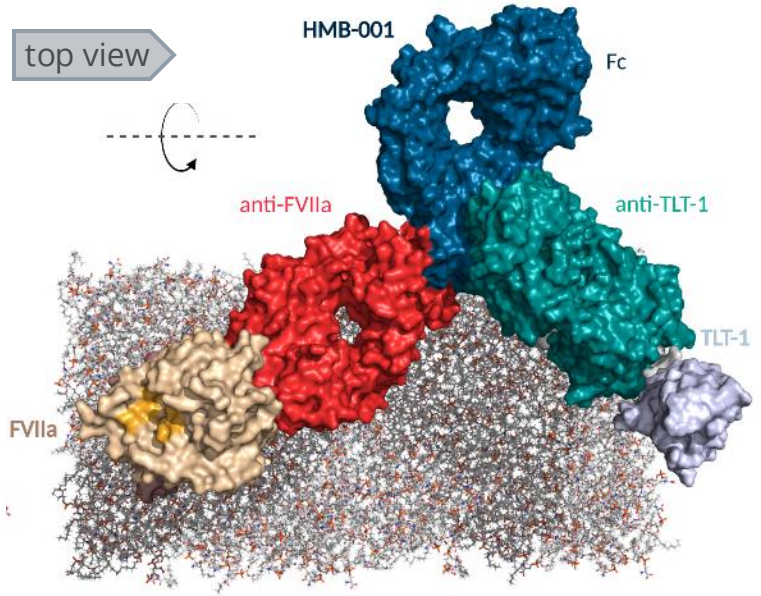


1.Gattis, J. L. et. al., JBC. 2006; 281, 13396–13403. 2. Washington, A. V. et. al., Blood. 2004; 104, 1042–1047. 3. Smith, C. W. et. al., Blood Adv. 2018; 2, 2072–2078. 4. Washington, A. V. et. al., J. Clin. Invest. 2009; 119, 1489–1501. 5. Branfield, S. & Washington, A. V. Platelets 2021; 32, 753–760.

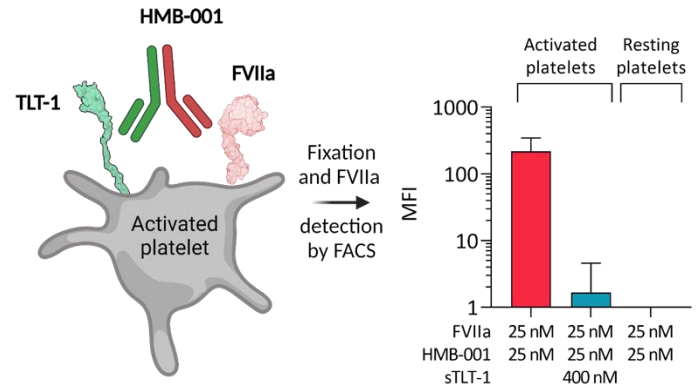
HMB-001 promotes FVIIa localization on the activated platelets

Complex structure	Resolution	PDB
HMB-001 anti-FVIIa Fab:FVIIa:sTF	3.5 Å	8CN9
HMB-001 anti-TLT-1 Fab:TLT-1 stalk peptide	1.5 Å	8CHE



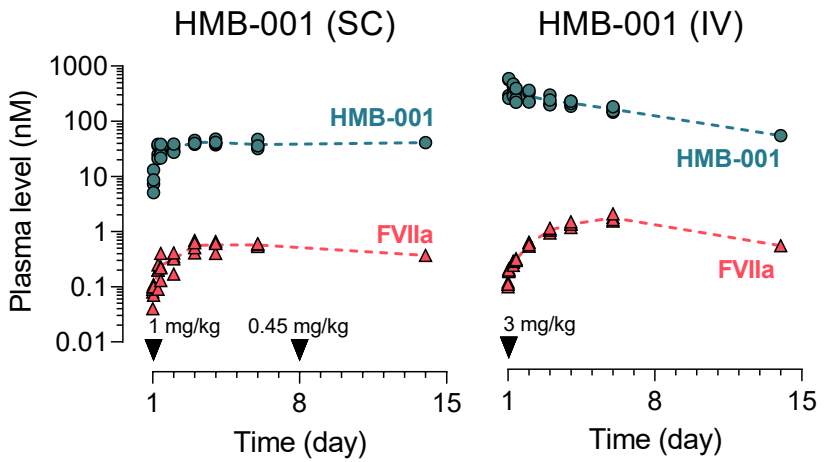


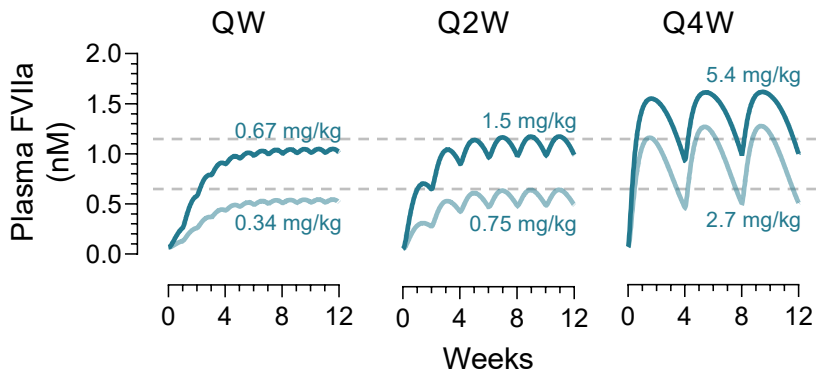
Activated platelet delivery of FVIIa by HMB-001



HMB-001 leads to accumulation of FVIIa in NHP and potentiates FVIIa activity in ex vivo studies

Accumulation of endogenous FVIIa





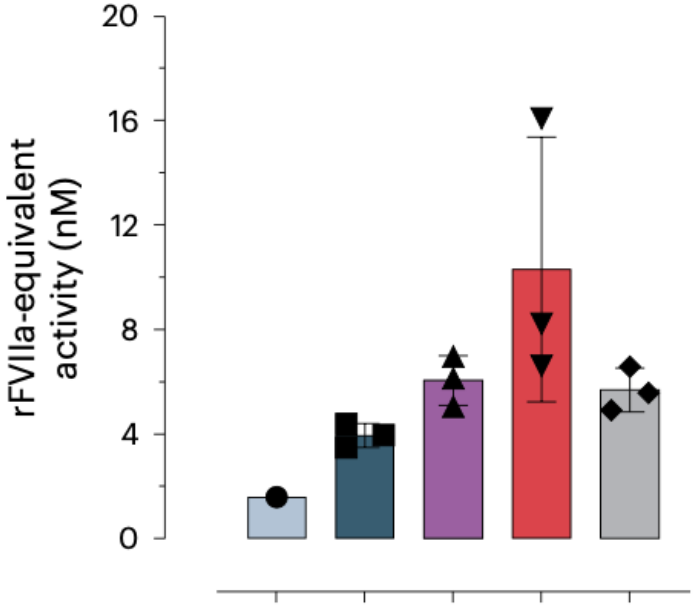
PK in Cynomolgus Monkey Methods

- Study in healthy NHP (cynomolgus monkey)
- SC/IV administration of HMB-001 (n = 4)
- Measurement of HMB-001 (ELISA) and FVIIa (FVIIa:clot assay)

Predicted PK in Humans Methods

- Population PK/PD model describing HMB-001 and FVIIa based on PK in NHP
- Allometric scaling applied to simulate multiple-dose scenarios in the human setting

HMB-001 Potentiates FVIIa Activity in PRP-Thrombin Generation

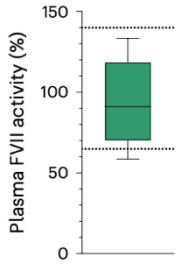


Clinical scenario	1	2	3	4	5
FVIIa (nM)	0.2	0.5	1.0	1.4	1.8
Total FVII(a) (nM)	14.9	18.0	20.9	23.0	26.6
HMB-001 (nM)	27.6	58.7	116.0	191.7	863.0

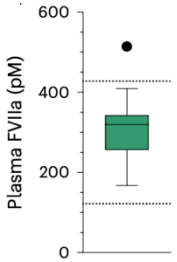
HMB-001 improves FVIIa-dependent platelet aggregation

Retained FVII activity, FVIIa levels and TLT-1 in GT samples

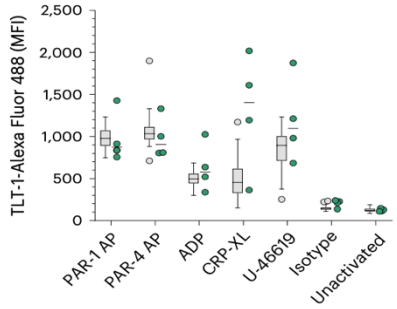
Total FVII activity



FVIIa levels



TLT-1 expression

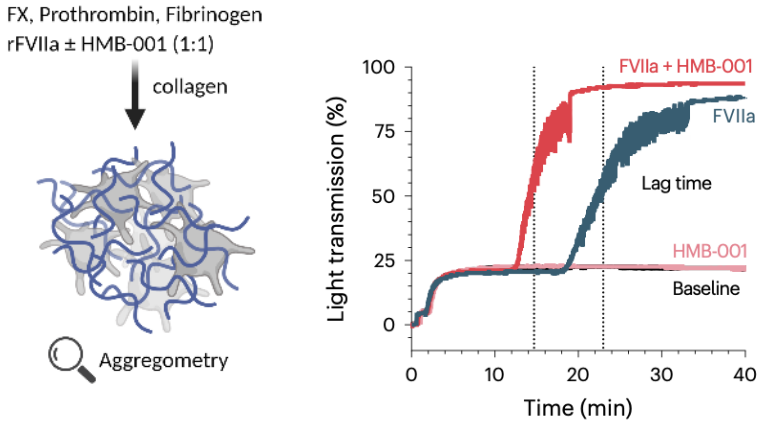


Study design

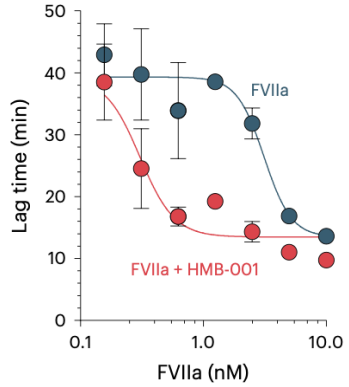
- Plasma FVII activity and plasma FVIIa levels in GT blood samples (n = 13)
- TLT-1 expression upon platelet activation using FACS (n = 4)

HMB-001 potentiates FVIIa activity by 10-fold in platelet aggregation assay using GT platelets in a TLT-1 dependent manner

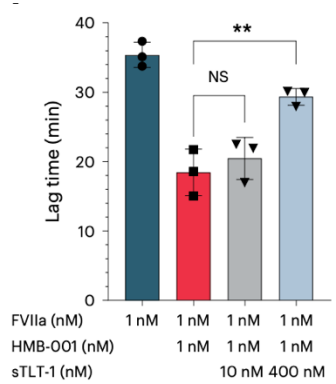
Aggregation of GT platelets



Concentration-response



HMB-001 potentiation is TLT-1 dependent



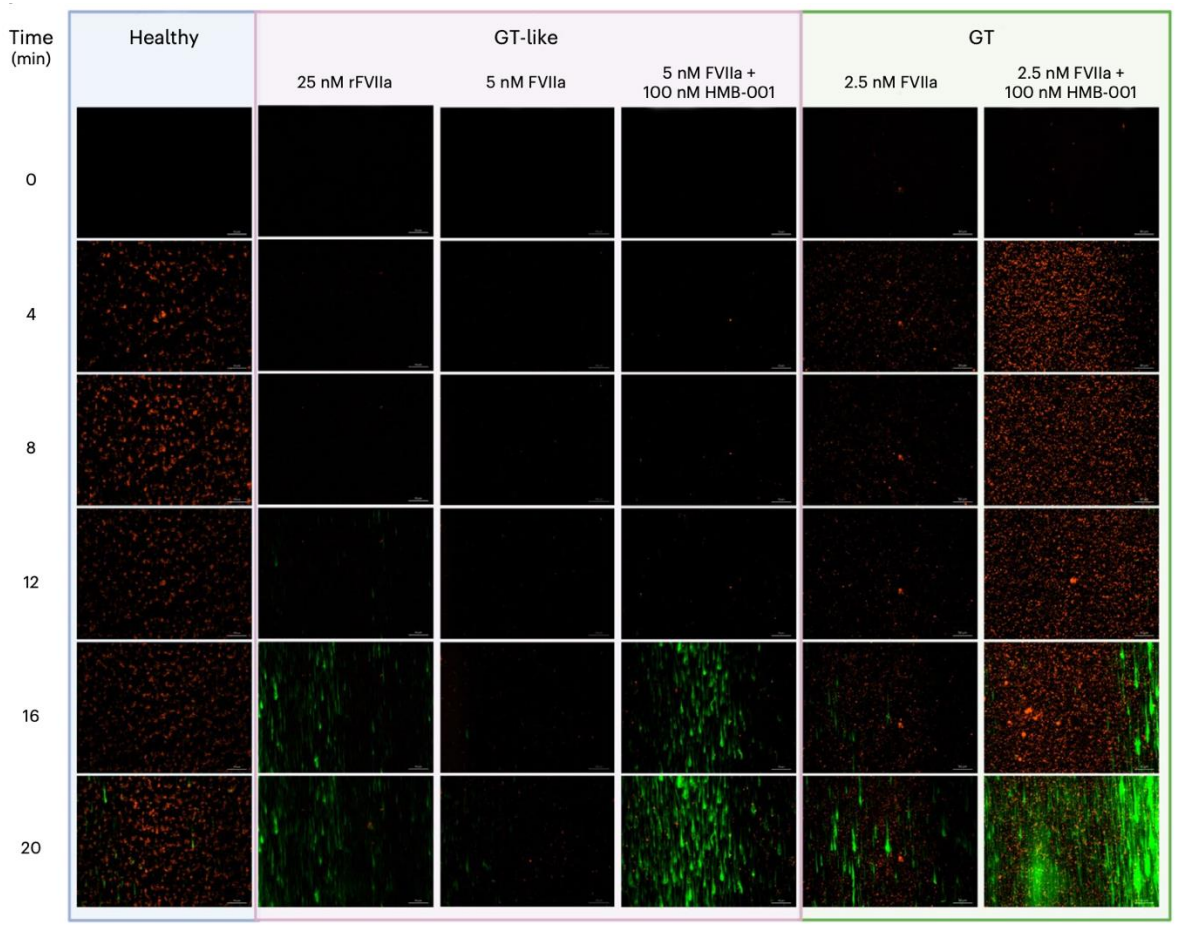
Study design

- GT platelets for platelet aggregation assay. GT-like platelets for dose-response and TLT-1 effect assay. GT-like: GPIIb/IIIa-inhibited (RGDW) normal human platelets
- Platelet activation by collagen in presence of FX, Prothrombin, Fibrinogen and rFVIIa ± HMB-001 (equimolar concentration)
- Aggregation monitored by light transmission aggregometry. Reported as lag-time = time to half maximum aggregation (mean ± SD, n = 3)

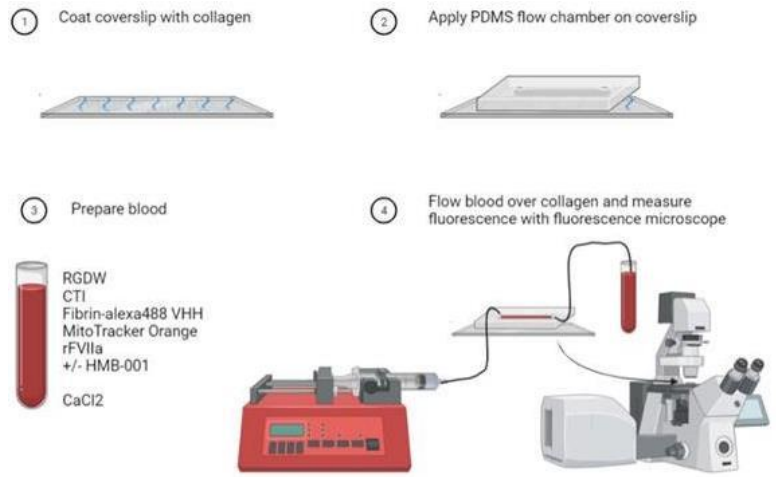
Ref: Lisman et. al., *Blood*, 2004

HMB-001 enhances FVIIa-mediated fibrin deposition on adhered platelets in *ex vivo* flow model of GT

HMB-001 potentiates FVIIa-mediated fibrin deposition in flow model using human whole blood and GT platelets



Flow model assay setup



***Ex vivo flow model* study design**

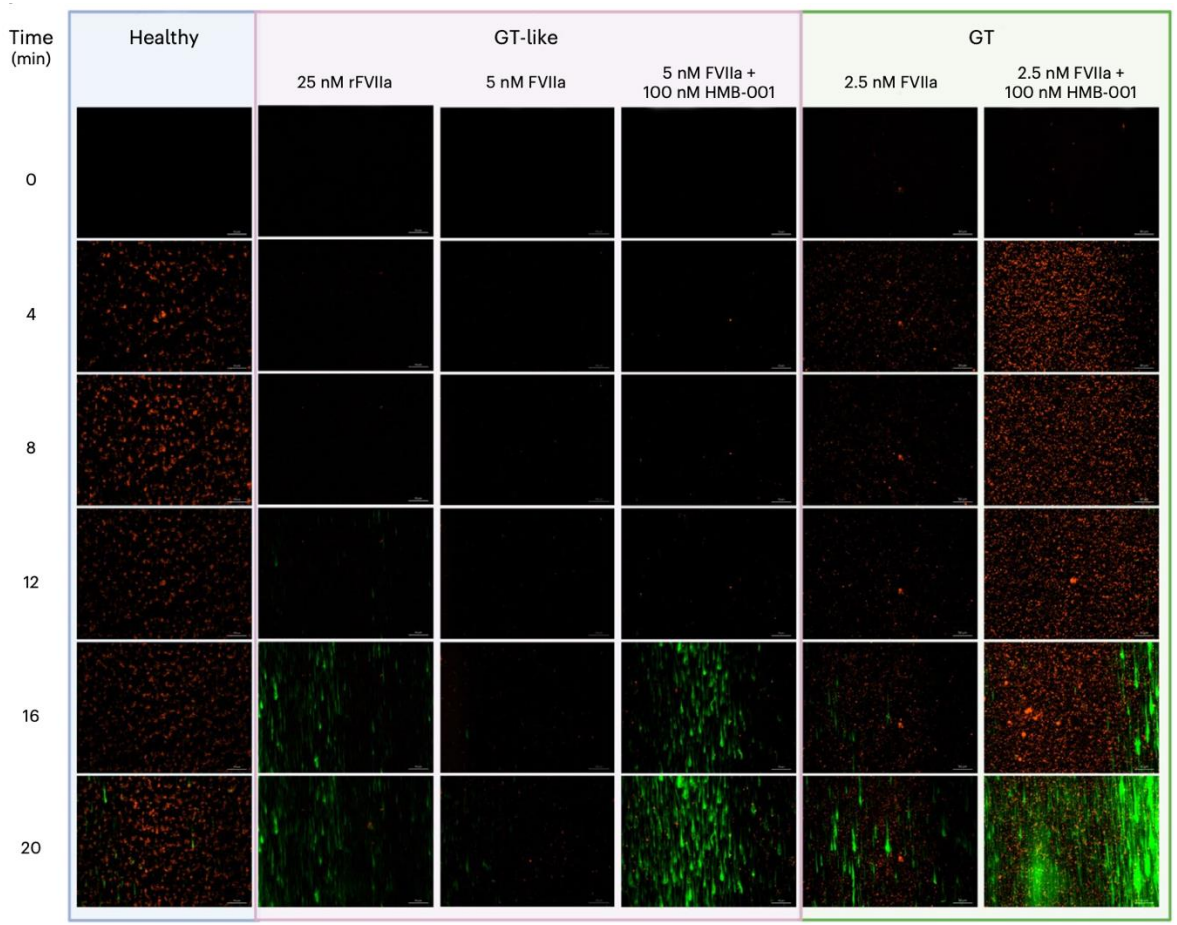
- Coverslips coated with bovine type I collagen. Polydimethylsiloxane (PDMS) parallel plate microfluidic device washed and blocked with 1% BSA. Coverslips attached to the PDMS device and mounted in a confocal microscope.
- Platelets labelled with MitoTracker™ Orange. Human citrated whole blood, supplemented with AF488-conjugated anti-fibrin, CTI ± 0 – 25 nM rFVIIa or 0 – 100 nM HMB-001 and recalcified. For GT-like, αIIbβ3 blocked with 500 µM D-RGDW and blood pulled through the flow chamber with a syringe pump at a shear rate of 300 s^{-1}.
- Snap shots taken at 20x magnification with an interval of 20 seconds to monitor platelet adhesion and fibrin formation in real time for 20 minutes.

Ref: Neeves KB et. al., *JTH* 2008

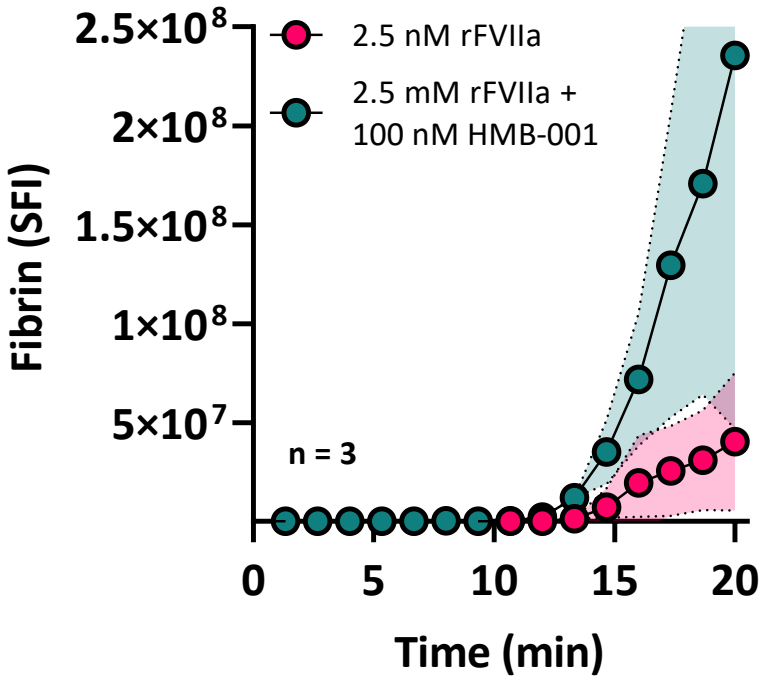
GT-like platelets were made by adding 500 µM $_{D}$-RGDW peptide

HMB-001 enhances FVIIa-mediated fibrin deposition on adhered platelets in *ex vivo* flow model of GT

HMB-001 potentiates FVIIa-mediated fibrin deposition in flow model using human whole blood and GT platelets



Potentiation confirmed using whole blood from GT patients



GT-like platelets were made by adding 500 µM $_{D}$-RGDW peptide

Overview of Pre-clinical and Clinical Studies

Pre-clinical Studies

1 *In Vitro* Studies

FVIIa localization on activated human platelets

- Flow cytometry to demonstrate HMB-001's ability to localize FVIIa to activated platelets via TLT-1

HMB-001 activity

- Platelet aggregation assay using GT platelets to show effect on fibrin-dependent aggregation

Fibrin formation

- Quantified by microfluidic flow chamber with collagen-coated surface in GT whole blood

2 *In Vivo* Studies

HMB-001 PK/PD

- Normocoagulant cynomolgus monkeys
- FVIIa quantification as a measure of HMB-001 PD

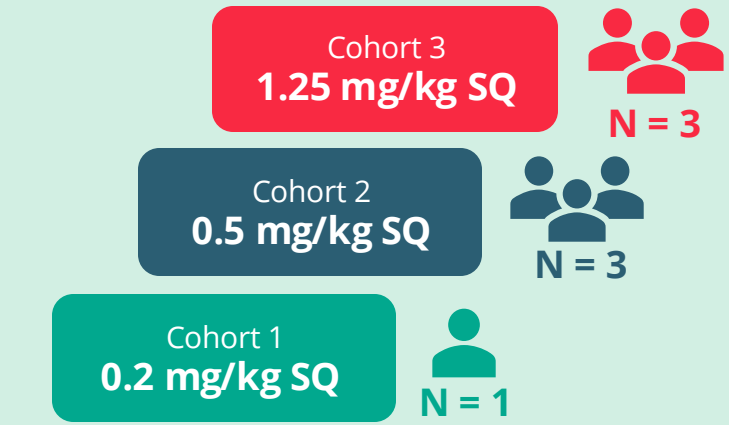
Hemostatic efficacy assessment in *ex vivo* studies

- HMB-001 potentiates FVIIa activity in platelet rich plasma Thrombin generation (PRP-TG) *ex vivo* model

First-in-Human Phase 1/2 Study[1]

3 Phase 1, Single Ascending Dose study

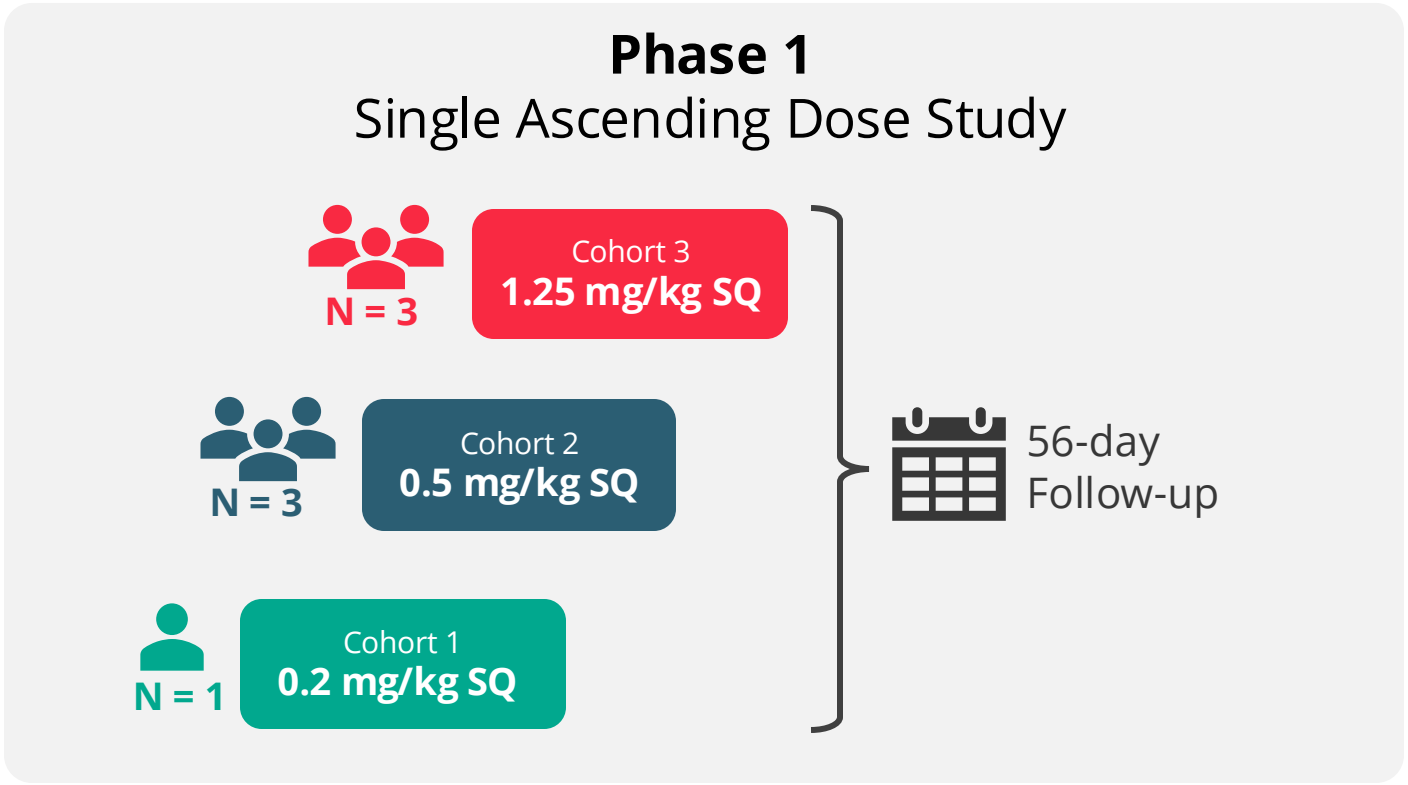
- HMB-001 PK ELISA
- HMB-001 PD:
  - FVIIa clot activity assay, Stago
  - Total FVII(a) ELISA, Stago
- Safety and tolerability
- Identify optimal dosing levels and intervals for Phase 2



PK, pharmacokinetics | PD, pharmacodynamics

1. NCT06211634

Overview of First-in-Human, Phase 1/2, Clinical Study



Phase 2
Multiple Ascending Dose Study

Multiple Dose Cohorts

3 months treatment

Phase 2b
Extension Study

Recommended Phase 2b Dose/Regimen TBD

9 months treatment

12 months of treatment

Study Objective:
Evaluate PK, PD (FVIIa clot activity assay, Stago and Total FVII(a) antigen ELISA, Stago) safety, and tolerability to identify optimal dosing levels and intervals for Phase 2 study

First-in-Human, Phase 1, Single Ascending Dose Study

Single Ascending Dose Study

7 patients

3 cohorts

56 days follow-up

Multiple Ascending Dose Study

Now enrolling

Adverse Events (AEs)*

- No AEs/serious AEs events related to HMB-001
- Majority of AEs were mild or moderate
- No dose-limiting toxicities
- No thromboembolic events

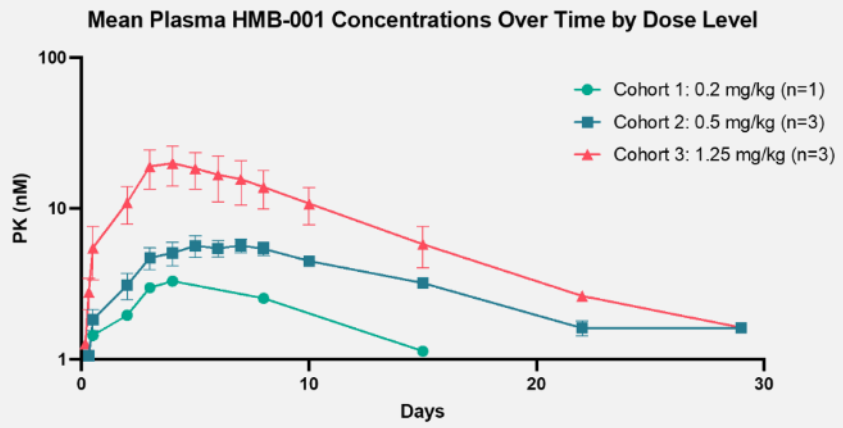
*Adverse event grouping based on MedDRA-coded terms, excluding bleed events

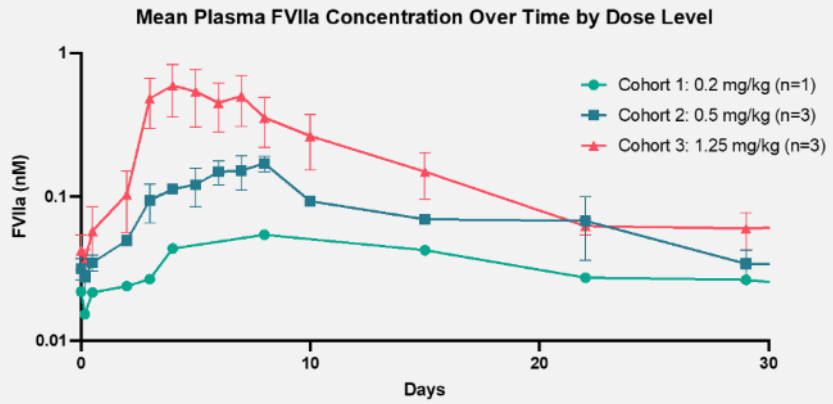
Coagulation

- No clinically significant changes in fibrinogen and PT/APTT
- Single Ascending Dose Study
  - Transient change in platelet count
  - Non-clinically significant elevation in D-dimer, not associated with clinical signs or symptoms
- Multiple Ascending Dose Study:
  - Non-clinically significant changes in platelet counts which resolve over time

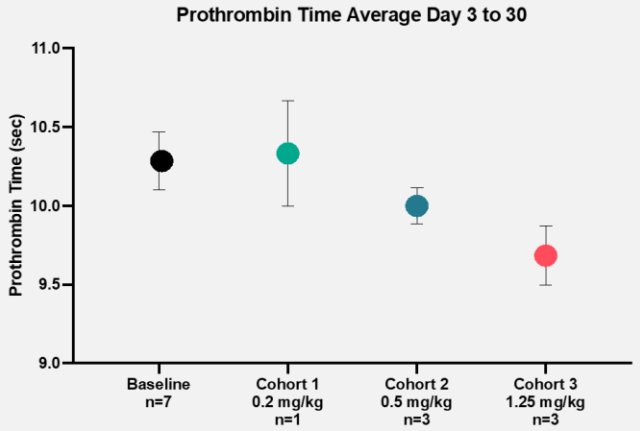
PK/PD and Coagulation Results support HMB-001 Mechanism of Action (MoA)

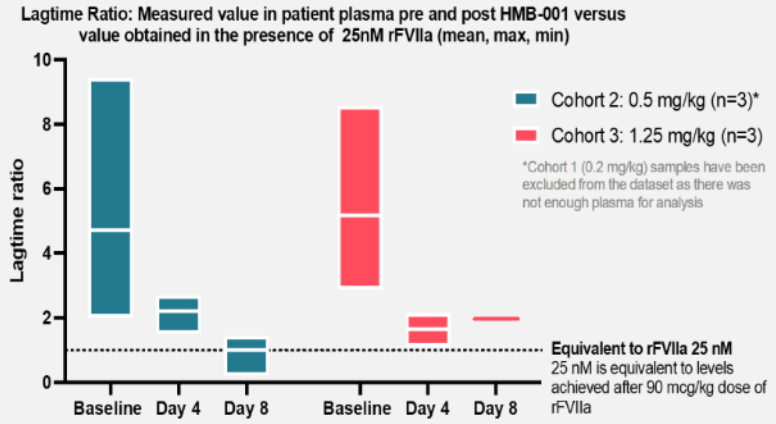
Pharmacokinetics & Pharmacodynamics





Coagulation Activity





Initial Bleed Data

- Reduction in bleed rate
- High proportion of bleed free days at peak HMB-001 concentrations

Data extract: 16Nov2023. Data are from an ongoing study and are not considered final.

Conclusions

HMB-001 Mechanism of Action (MoA)

- Prolonged endogenous FVIIa half-life resulting in its accumulation
- Effectively targets FVIIa to activated platelets leading to improved FVIIa hemostatic activity in static and flowing blood conditions
- Potentially applicable to other bleeding disorders beyond GT

Phase 1, Single Ascending Dose study in people with GT

- Favorable safety and tolerability profile; no HMB-001-related adverse events and no thrombotic events
- PK data support less frequent dosing of HMB-001
- PD data shows dose-dependent accumulation of endogenous FVIIa with associated decrease in Prothrombin time and support HMB-001 MoA
- Enhanced thrombin generation parameters validate potentiation of FVIIa activity mediated by HMB-001 in the presence of activated platelets

HMB-001 shows promise as a subcutaneous prophylactic treatment for people with GT

Acknowledgements

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- **Hemab Therapeutics**: Gandhi P, Malladi R, Amin J, Østergaard H, Bonde A, Rea C, Sørensen B, Nagilla P, Gosnell A, Vogel J, Skands A, Rasmussen C
- This study was sponsored by Hemab Therapeutics

- **The authors thank the study participants, their families, the investigators and study site personnel**
- **Queen Mary**: Sivapalaratnam S

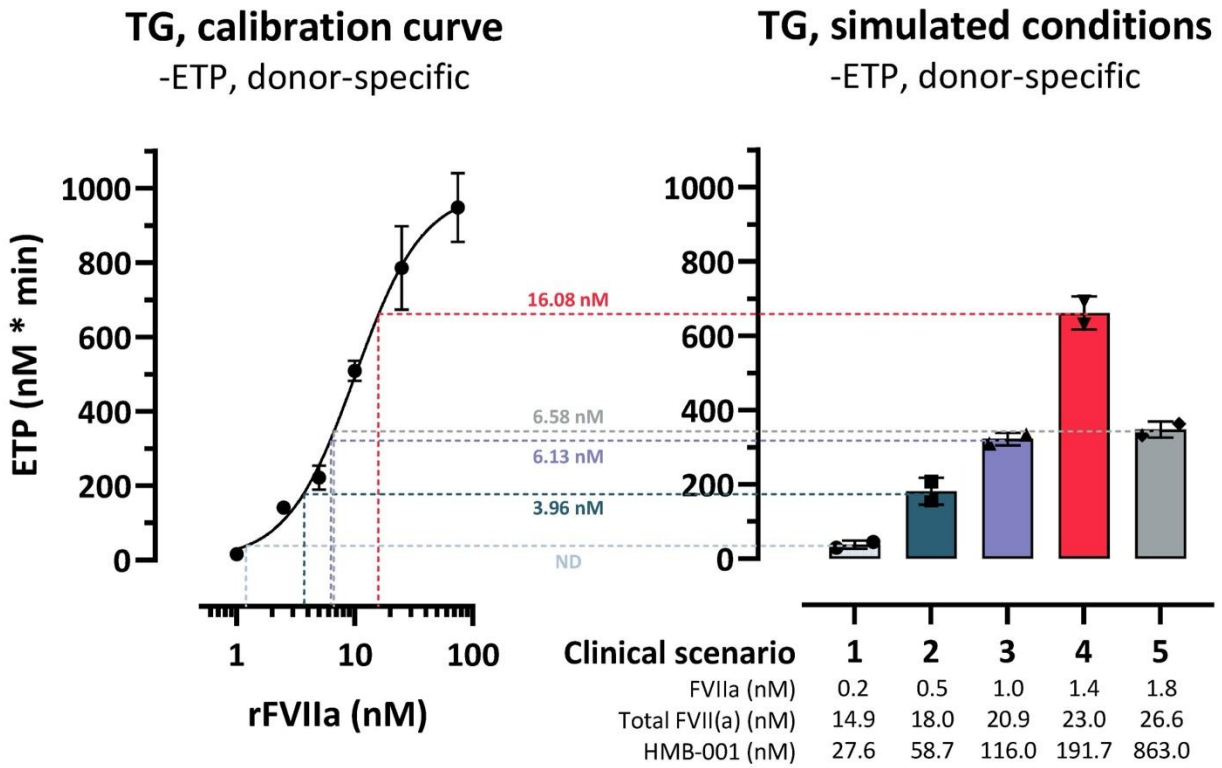
Phase 2 of HMB-001

now enrolling individuals with Glanzmann thrombasthenia

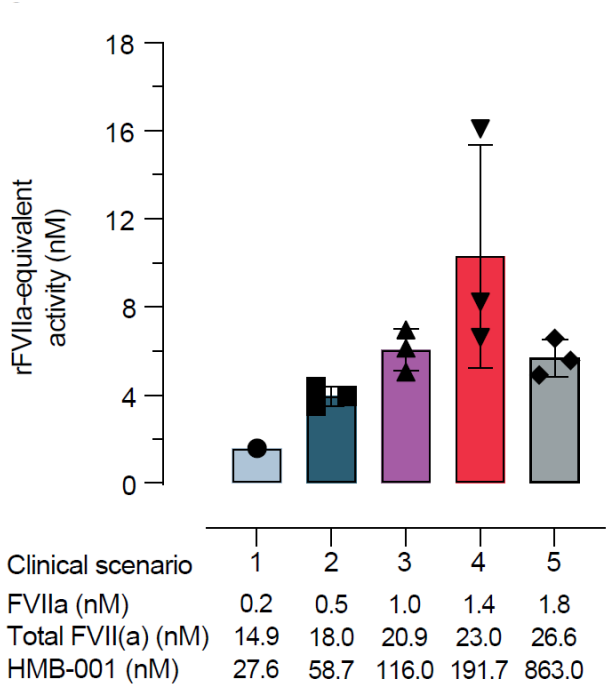
Country	Site
United Kingdom	The Royal London Hospital
	Richmond Pharmacology
	Royal Free London
	Queen Elizabeth Hospital Birmingham
	Leeds Teaching Hospitals
France	AP-HP Hopital Necker
	AP-HP Hopital Bicetre
	AP-HM - Hopital de la Timone
Italy	IRCCS Ca' Granda Maggiore Hospital
	Careggi University Hospital
Belgium	University Hospital Leuven
Netherlands	University Medical Center Utrecht

HMB-001 mediated FVIIa activity potentiation as evaluated by PRP-TG assay

A. PRP-TG assay

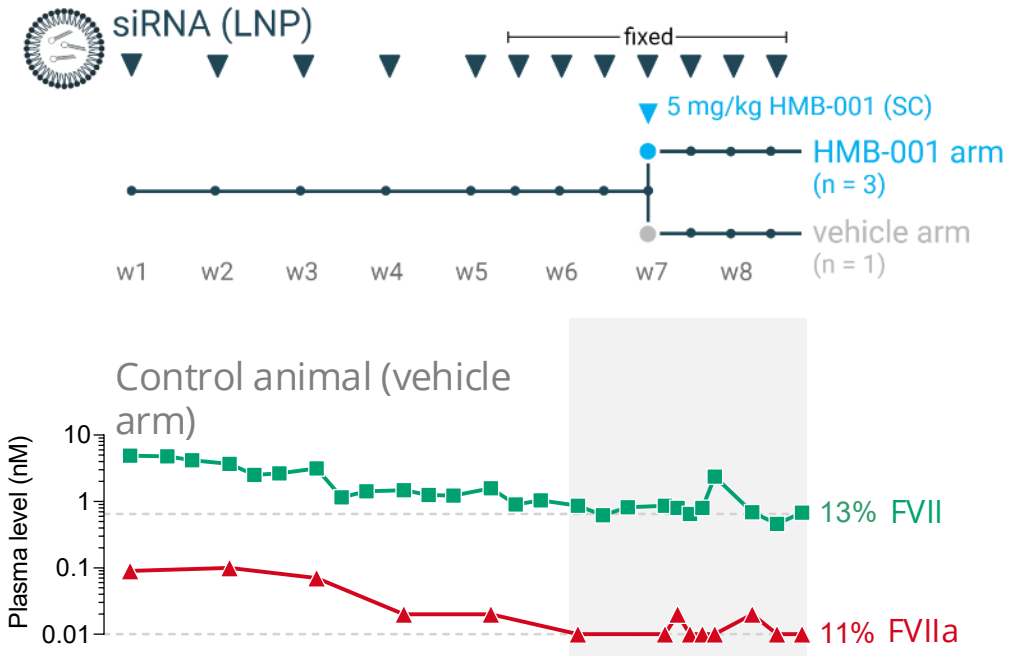


B. rFVIIa-equivalent activity



HMB-001 Accumulates Endogenous FVII/FVIIa under *in vivo* Conditions Mimicking FVII deficiency

siRNA knock-down of FVII/FVIIa in cynomolgus monkey



Study design

- siRNA (XL10-LNP)-mediated knock-down of endogenous FVII in cynomolgus monkey
- At stable knock-down: SC dosing of HMB-001 at 5 mg/kg with siRNA treatment maintained
- Measurement of HMB-001 [ELISA], FVII [ELISA], FVIIa [FVIIa:clot] throughout study

Accumulation of FVII/FVIIa by HMB-001 within normal range