SUPPLEMENTAL MATERIAL

METHODS

Interventions

Participants initially meeting the criteria required for partial success, but later found not to meet the negative factor (F)VIII inhibitor titre or normal FVIII recovery criterion, will have their treatment plan determined by the investigator.

Outcome measures

The rationale for the primary endpoint of the AKATSUKI study is as follows: Studies have hypothesised that persons with haemophilia A (PwHA) taking emicizumab prophylaxis typically have increased coagulation function and a milder haemophilia phenotype relative to baseline.[1 2] In PwHA with FVIII inhibitors, the activity of infused FVIII concentrates used in immune tolerance induction (ITI) therapy is neutralised or rapidly cleared; however, this activity could theoretically be partially restored when the FVIII inhibitor titre decreased.[3] Therefore, when concomitantly administering emicizumab and FVIII concentrates for ITI, potentially in addition to bypassing agents, the safety of the treatment regimen especially after eradication of FVIII inhibitors should be assessed for the risk of thromboembolic events, particularly given the potential for hypercoagulation.

Secondary outcomes

The definition of a bleeding event is as follows: An event will be considered a bleed if coagulation factors are administered to treat signs or symptoms of bleeding. A single bleeding episode starts with the first sign of a bleed and ends 72 h post-last injection; recurrent symptoms of bleeding at the same location, or repeated injections <72 h apart, will be considered related to the same bleed. Injections received >72 h after the preceding injection will be considered the first injection and, subsequently, a new bleed at the same location.

Matsushita T, *et al. BMJ Open* 2022; 12:e057018. doi: 10.1136/bmjopen-2021-057018

Bleed data will be categorised by spontaneous bleeds (defined as a bleed without an identifiable cause), traumatic bleeds (defined as a haemorrhage occurring secondary to an event such as trauma, strenuous activity, or overuse), joint bleeds and target joint bleeds (defined as a bleed in which ≥3 bleeds have occurred within 24 weeks prior to study enrolment). As per International Society on Thrombosis and Haemostasis definition, target joints will be defined as major joints in which ≥3 bleeding events occur over a 24-week period.[4] Documentation of bleeding episodes for 24 weeks prior to study enrolment will be required for the identification of target joints in participants aged <2 years.

Assessments

Sample preparation for measuring ITI response

Samples for central laboratory assay will be prepared by drawing a 4.5 mL blood sample into a blood collection tube containing 0.5 mL of 3.2% sodium citrate solution, mixing by inverting 5–6 times and then immediately centrifuging the sample in a cooling centrifuge for 15 min at 4 °C and 1700 G. The supernatant will then be pipetted into a designated container and cryostored. When drawing blood samples via a catheter or central venous access device (CVAD), a disposable tube must be used prior to sample collection to avoid potential contamination of the catheter/CVAD with isotonic sodium chloride solution used for cleaning or anticoagulant drug.

Ethics and dissemination

The study will be conducted in compliance with the principles of the Declaration of Helsinki, the Clinical Trials Act in Japan (Act No. 16 of 14 April 2017), the Enforcement Regulations of the Clinical Trials Act (MHLW Ordinance 17, 2018), the Ethical Guidelines for Medical and Health Research Involving Human Subjects (2017 Notification No. 1 of the Ministry of Education, Culture, Sports, Science and Technology and MHLW, 2017), other relevant guidelines and the approved study protocol.

Protocol amendments will be submitted by the Co-ordinating Principal Investigator for approval by the Certified Review Board (CRB). These changes will be implemented in the registered trial information and a notice of change submitted to the MHLW. The CRB and investigators will be notified of these changes.

Monitoring

To ensure the study is being conducted safely and in accordance with the approved protocol, independent monitoring will be performed biannually; the Periodic Monitoring Reports generated will contain information on recruitment status, participant eligibility, status of/reasons for treatment discontinuations, site-specific non-compliance and serious adverse events, adverse drug reactions and adverse events, and other study-/safety-related issues. For quality control and assurance purposes, periodic monitoring will be performed based on aggregated data entries in the electronic case report forms to ensure data are being collected appropriately. All study-related material will also be made available to independent quality assurance auditors, as required.

REFERENCES

1. Shima M, Hanabusa H, Taki M, et al. Factor VIII-mimetic function of humanized bispecific antibody in hemophilia A. *N Engl J Med* 2016;374(21):2044–53.
2. Yoneyama K, Schmitt C, Kotani N, et al. A Pharmacometric approach to substitute for a conventional dose-finding study in rare diseases: Example of phase III dose selection for emicizumab in hemophilia A. *Clinical pharmacokinetics* 2018;57(9):1123–34.
3. DiMichele D. The North American Immune Tolerance Registry: Contributions to the thirty-year experience with immune tolerance therapy. *Haemophilia* 2009;15:320-28.
4. Blanchette VS, Key NS, Ljung LR, et al. Definitions in hemophilia: communication from the SSC of the ISTH. *J Thromb Haemost* 2014;12(11):1935–39.

SUPPLEMENTAL TABLE 1 Assessment schedule for PwHA with FVIII inhibitors receiving emicizumab prophylaxis under ITI therapy*

	Screening†	Week 1 (enrolment to first emicizumab study dose, +7 days)	Week 5 (±7 days)	Week 9 (±7 days)	Week 13 (±7 days)	Week 17 and every 4 weeks thereafter (±7 days)	Week 25 and every 12 weeks thereafter (±28 days)	Week 49 and every 48 weeks thereafter (±28 days)	Week 193‡ (±28 days)	Safety follow-up visit‡ (±28 days)
Informed consent	X									
Inclusion and exclusion criteria	X									
Medical history and demographic data	X									
Weight	X				X		X		X	X
FVIII inhibitor titre§	X	X	X	X	X	X			X	X
FVIII activity‖	X	X	X	X	X	X			X	X
FVIII recovery¥	X	X	X	X	X	X	X	X	X	X
FVIII half-life**		X	X	X	X	X	X	X	X	X
Laboratory tests††		←	—	—	—	—	—	—	—	→
Emicizumab plasma concentration‡‡		X	X	X	X	X	X	X	X	X

Haemo-QoL-SF and adapted INHIB-QoL questionnaires[§§]	X						X	X	
Bleeds and drugs used[‖‖]	X	X	X	X	X	X	X	X	X
AEs[¥¥]	X	X	X	X	X	X	X	X	X

The permissible time window for assessments between screening and enrolment is ≤28 days.

*For assessments scheduled on, or after, the date of the next study visit following investigator confirmation of ITI partial success, see Table 2.

[†]Screening results obtained prior to acquisition of informed consent can be used in lieu of retesting provided that they are considered medically appropriate by the investigator.

[‡]Participants who discontinue FVIII concentrates for ITI therapy will be requested to undergo early termination assessments at the next scheduled study visit following their last treatment. Participants who discontinue emicizumab will be requested to attend a follow-up assessment scheduled at 24 weeks after the last emicizumab dose.

[§]FVIII inhibitors will be centrally measured using anti-idiotypic antibodies to emicizumab. However, results measured by the study site after spiking the sample solution with anti-idiotypic antibodies to emicizumab can be used at screening provided they were obtained within 8 weeks before enrolment. Results of screening assays can also be used as the Week 1 results, provided the interval between screening and Week 1 does not exceed 7 days. Blood samples for central laboratory analysis can also be collected at unscheduled timepoints during the study at the investigator's discretion. FVIII inhibitor assay samples will be collected after the 48-h washout period following the last dose of FVIII concentrate (or after 72 h when using extended half-life FVIII concentrates).

[‖]FVIII activity will be centrally measured using anti-idiotypic antibodies to emicizumab. Results of screening assays can also be used as the Week 1 results provided the interval between screening and Week 1 does not exceed 7 days. Blood samples for central laboratory analysis can also be collected at unscheduled timepoints during the study at the clinical discretion of the investigator. FVIII activity assay samples will be collected after the 48-h washout period following the last dose of FVIII concentrate (or after 72 h when using extended half-life FVIII concentrate).

[¥]In the event of two consecutive negative FVIII inhibitor assay results (<0.6 BU/mL) at screening and at the next study site visit with an interval of ≥2 weeks, FVIII recovery will be assayed until it is confirmed to be normal (ie, two consecutive assays at an interval of ≥2 weeks showing ≥66% of predicted value). Samples for FVIII recovery assay will be collected 15–30 min pre-/post-FVIII concentrate administration and after a washout period of 48 h from the last dose of 50 IU/kg FVIII concentrate (or 72 h when

using extended half-life FVIII concentrates). However, FVIII recovery is not a mandatory screening test if the result of screening FVIII inhibitor titre testing is confirmed to be ≥0.6 BU/mL; moreover, results measured by the study site can be used at screening provided that they were obtained within 8 weeks before enrolment. Blood samples for central laboratory analysis can also be collected at unscheduled timepoints during the study at the investigator's discretion.

**In the event of two consecutive negative FVIII inhibitor assay results (<0.6 BU/mL) at an interval of ≥2 weeks, FVIII recovery will be assayed until it is confirmed to be normal (ie, two consecutive assays at an interval of ≥2 weeks showing ≥66% of predicted value). Samples for FVIII half-life measurements will be collected 15–30 min and at 1, 2, 4, 6, 24 and 48 h pre-/post-FVIII concentrate administration and after a washout period of 48 h from the last dose of 50 IU/kg FVIII concentrate (or 72 h when using extended half-life FVIII concentrates). Additional sampling timepoints will be established at 72 and 96 h after administration for patients receiving extended half-life FVIII concentrates. Blood samples for central laboratory analysis can also be collected at unscheduled timepoints during the study at the investigator's discretion.

††Laboratory tests will be performed if aPCC or FVIII concentrates were administered, and will include the following variables: PT, APTT, D-dimer, fibrin, FDP, LDH, platelets and creatinine. Tests can also be performed at unscheduled timepoints during the study at the investigator's discretion.

‡‡Plasma emicizumab concentrations measured using a post-authorisation assay program will be reported in the eCRF if the investigator makes a clinical assessment that the participant may have developed antibodies to emicizumab after exhibiting signs of reduced therapeutic response.

§§Participants aged 8–17 years at study enrolment will be instructed to complete the Haemo-QoL-SF questionnaire and all caregivers will be instructed to complete the adapted INHIB-QoL questionnaire using a web application programme. If a participant has difficulty inputting responses to the questionnaires into the device, the caregiver may enter the response as given by the participant.

‖‖The participant or caregiver will record any bleeds requiring treatment with coagulation factors and all doses of emicizumab and other blood coagulation factor concentrates using the web application programme. Information on procedure-/surgery-related bleeds will be recorded in the eCRF or ePRO device.

¥¥Participants will be required to inform the investigator about any AEs at each study visit; AEs will be reported in the eCRF by the investigator.

AE, adverse event; aPCC, activated prothrombin complex concentrate; APTT, activated partial thromboplastin time; BU, Bethesda unit; eCRF, electronic case report form; ePRO, electronic participant-reported outcome; FDP, fibrin and fibrinogen degradation products; FVIII, factor VIII; Haemo-QoL-SF, haemophilia-specific Quality of Life Short Form; INHIB-QoL, Inhibitor-specific Quality of Life with Aspects of Caregiver Burden; ITI, immune tolerance induction; LDH, lactate dehydrogenase; PT, prothrombin time; PwHA, person with haemophilia A.

SUPPLEMENTAL TABLE 2 Assessment schedule for PwHA with FVIII inhibitors receiving emicizumab prophylaxis immediately after ITI therapy

	Immediately after ITI		**Every 48 weeks after first emicizumab dose (±28 days)**	**Week 193 after first emicizumab dose (±28 days)**	**Discontinuation (±28 days)**	**Safety follow-up visit† (±28 days)**
	Week 1* (±28 days)	**Week 13 and every 12 weeks thereafter (±28 days)**				
Weight	X	X		X	X	
FVIII inhibitor titre‡	X	X		X	X	
FVIII activity§	X	X		X	X	X
FVIII recovery‖	X	X	X	X	X	X
FVIII half-life¥	X	X	X	X	X	X
Laboratory tests**	◄	———	———	———	———	►
Plasma emicizumab concentration††	X	X	X	X	X	X
Haemo-QoL-SF and adapted INHIB-QoL questionnaires‡‡			X	X	X	
Bleeds and drugs used§§	X	X	X	X	X	X
AEs‖‖	X	X	X	X	X	X

The permissible time window for assessments between screening and enrolment is ≤28 days.

*Assessments to be performed on the date of the next scheduled study visit following investigator confirmation of partial success. If body weight was measured ≤28 days before transition to post-ITI prophylaxis, this measurement can be used as the Week 1 measurement.

†Participants who discontinue from emicizumab prophylaxis will be requested to attend a follow-up assessment 24 weeks after the last dose of emicizumab.

‡FVIII inhibitors will be centrally measured using anti-idiotypic antibodies to emicizumab. Blood samples for central laboratory analysis can also be collected at unscheduled timepoints during the study at the investigator's discretion. FVIII inhibitor assay samples will be collected after the 48-h washout period following the last dose of FVIII concentrate (or after 72 h when using extended half-life FVIII concentrates).

§FVIII activity will be centrally measured using anti-idiotypic antibodies to emicizumab. Blood samples for central laboratory analysis can also be collected at unscheduled timepoints during the study at the investigator's discretion. FVIII activity assay samples will be collected after the 48-h washout period following the last dose of FVIII concentrate (or after 72 h when using extended half-life FVIII concentrates).

‖Blood samples for FVIII recovery measured during the study at the investigator's discretion. Samples for FVIII recovery assay will be collected 15–30 min pre-/post-FVIII concentrate administration and after a washout period of 48 h from the last dose of FVIII concentrate (or 72 h when using extended half-life FVIII concentrates).

¥Blood samples for FVIII half-life measurement will be drawn during the study at the investigator's discretion, and will be collected 15–30 min and at 1, 2, 4, 6, 24 and 48 h pre-/post-FVIII concentrate administration and after a washout period of 48 h from the last dose of FVIII concentrate (or 72 h when using extended half-life FVIII concentrates). Additional sampling timepoints will be established at 72 and 96 h after administration for participants receiving extended half-life FVIII concentrates.

**Laboratory tests will be performed at the investigator's discretion, and will include the following variables: PT, APTT, D-dimer, FDP, LDH, platelets and creatinine.

††Plasma emicizumab concentrations measured using a post-authorisation assay program will be reported in the eCRF if the investigator makes a clinical assessment that the participant may have developed antibodies to emicizumab after exhibiting signs of reduced therapeutic response.

‡‡Participants aged 8–17 years at study enrolment will be instructed to complete the Haemo-QoL-SF questionnaire and all caregivers will be instructed to complete the adapted INHIB-QoL questionnaire using a web application programme. If a participant has difficulty inputting responses to the questionnaires into the device, the caregiver may enter the response as given by the participant. The questionnaire scheduled at Week 193 may be omitted if no more than 28 days has elapsed from the questionnaires performed in Weeks 1 or 49, or every 48 weeks thereafter following ITI.

§§The participant or caregiver will record any bleeds requiring treatment with coagulation factors and all doses of emicizumab and other blood coagulation factor concentrates using the web application programme. Information on procedure-/surgery-related bleeds will be recorded in the eCRF or ePRO device.

‖‖Participants will be required to inform the investigator about any AEs at each study visit; AEs will be reported in the eCRF by the investigator.

AE, adverse event; APTT, activated partial thromboplastin time; eCRF, electronic case report form; ePRO, electronic participant-reported outcome; FVIII, factor VIII; FDP, fibrin and fibrinogen degradation products; Haemo-QoL-SF, haemophilia-specific Quality of Life Short Form; INHIB-QoL, Inhibitor-specific Quality of Life with Aspects of Caregiver Burden; ITI, immune tolerance induction; LDH, lactate dehydrogenase; PT, prothrombin time; PwHA, person with haemophilia A.