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A fully-human bispecific antibody for the treatment of hemophilia A

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Background: Loss of blood coagulation Factor VIII (F.VIII) results in the bleeding disorder, hemophilia A. The current standard of care for hemophilia A patients routinely involves replacement of F.VIII either as prophylaxis or on-demand. However, approximately 30 % of hemophilia A patients taking F.VIII develop inhibitory antibodies against the replacement F.VIII, blocking its action. Additionally, venous infusion of F.VIII is burdensome especially in young patients. Recently, a novel treatment for hemophilia A involving the humanized bispecific antibody (BiAb) Hemlibra, which mimics the action of F.VIII, was developed. Successful approval of Hemlibra provides new therapeutic options for patients.

Aims: Integrating Kymab's IntelliSelect® Transgenic and IntelliSelect® Bispecific platforms, we sought to develop a fully-human F.VIII common light chain (CLC) bispecific antibody that can mimic the action of F.VIII *ex vivo*.

Methods: Kymab IntelliSelect® Transgenic mice were immunized with Factor IX (F.IX) or Factor X (F.X) and isolated arms were combinatorially expressed as 2-heavy-2-light (2H2L) BiAbs. Functionally active BiAbs were identified using a high-throughput chromogenic FXase assay. We identified a F.IX arm which demonstrated high FXase activity when paired with a variety of different F.X arms. To generate a common light chain (CLC) BiAb, we focused on a CLC BiAb based on the light chain isolated from this promiscuous F.IX arm. To identify Factor X antibodies able to pair with the F.IX CLC, IntelliSelect® transgenic mice solely expressing the isolated F.IX CLC were generated, immunized with F.X and F.X specific antibodies recovered. The isolated F.X arms were then expressed as CLC BiAbs, together with the heavy and light chains of the selected F.IX arm and re-screened by functional assays. Antibody sequences of biologically active BiAbs were further optimized using deep mining of next generation sequencing (NGS) data using Kymab's IntelliSelect® Bioinformatics platform. Coupled with a minimal mutagenesis strategy, this optimization identified variants with elevated hemostatic activity resulting in a lead BiAb, KY1049. KY1049 was purified using Protein A and cation ion-exchange chromatography (cIEX). The purified BiAb was then characterized using a combination of *in vitro* and *ex vivo* hemostatic assays including chromogenic FXase (FXase), activated partial thromboplastin time (aPTT) and thrombin generation assay (TGA).

Results: More than 8000 2H2L BiAbs were initially screened, followed by the analysis of more than 400 CLC F.X arms by chromogenic FXase to identify functionally active CLC BiAbs. Further optimization to iteratively and combinatorially optimize the F.IX/F.X BiAb was carried out resulting in a potent BiAb, KY1049. KY1049 demonstrates a dose-dependent reduction in clotting time (aPTT) and an increase in thrombin generation (TGA), thereby functionally restoring the hemostatic activity of F.VIII-depleted plasma. KY1049 can be purified using standard purification processes and is identified as a single species by mass spectrometry. KY1049 simultaneously binds F.IX and F.X by surface plasmon resonance (SPR).

Summary/Conclusion: KY1049, developed using Kymab's IntelliSelect® Bispecific platform, is a potent F.VIII mimetic BiAb with comparable hemostatic activities to a sequence-identical analogue of Hemlibra. KY1049 is a fully-human F.VIII mimetic CLC BiAb in which both heavy chains naturally bind a cognate CLC.