Development and Optimization of a Fully Human FVIII-Mimetic Bispecific Antibody for Patients with Hemophilia A

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Introduction. Recently, a range of novel therapies has been developed to improve treatment options for patients with hemophilia A. One approach is to generate a humanised bispecific antibody (BiAb) that can act as a Factor VIII (FVIII) mimetic. Taking advantage of Kymab’s IntelliSelect® antibody discovery platform (Figure 1A), we describe the selection and optimization of a fully-human FVIII-mimetic common light chain (CLC) BiAb, KY1049. KY1049 can efficiently catalyse the generation of Factor Xa (FXase assay), normalise the activated partial thromboplastin time (aPTT assay) and thrombin generation (TGA assay) in FVIII-depleted human plasma (Figure 1B). Factor X (FX) and Factor X (F-X) binding antibodies were generated by immunizing IntelliSelect® Transgenic mice, which contain the full human immunoglobulin heavy and light chain repertoires. Combinations of isolated F-X and F-X-specific arms were co-expressed as 2-heavy-2-light-chain (2H2L) BiAbs. Purified 2H2L BiAbs were screened using a high-throughput chromogenic FXase assay (Figure 2A). The light chain of a lead F-X arm was chosen to generate transgenic mice expressing this bespoke common light chain (CLC) as the sole functional light chain in the IntelliSelect® Transgenic background. By immunizing these CLC transgenic mice with F-X, F-X-binding antibodies whose heavy chains paired with the CLC were identified. The heavy chains of these F-X-binding antibodies were co-expressed with the heavy and light chains of the chosen F-X arm to generate CLC BiAbs. Biologically active BiAbs were identified by functional assays, and chosen for further optimization (Figure 2B). Kymab’s IntelliSelect® Bioinformatics platform (Figure 3A), coupled with site-specific mutagenesis (Figure 3B) was applied to optimize the lead BiAb (Figure 4A). After optimization, KY1049 was purified by ion-exchange chromatography (Figure 4B), and functionally characterized using aPTT (Figure 4C) and TGA assays (Figure 4D). The characteristics of KY1049 make it a promising therapeutic candidate for the treatment of hemophilia A.

Figure 1. IntelliSelect® Bispecifics - A Robust Combinatorial Platform to Generate FVIII-Mimetic Bispecific Antibodies. A. IntelliSelect® Bispecifics, together with IntelliSelect® Transgenic, IntelliSelect® Screening, and IntelliSelect® Bioinformatics platforms were applied to develop a fully-human, naturally-paired common light chain BiAb. B. Activities of the BiAbs were validated using clinically relevant hemostatic assays.

Figure 2A. Primary FXase Screening Identifies Common Light Chain (CLC). IntelliSelect® Transgenic mice were immunised with F-Xa and F-X. Antigen-specific B cells were then isolated by single cell sorting. Variable regions of anti-F-Xa and anti-F-X monospecific antibodies were recombined into BiAb expression constructs, and expressed as 2H2L BiAbs with each heavy chain paired with its own cognate light chain. More than 8,000 2H2L BiAbs with different combinations of F-Xa and F-X arms were expressed, purified and screened in the FXase assay to identify an anti-F-Xa common light chain (CLC) for KY1049.

Figure 2B. Generating Common Light Chain Bispecific Antibodies. IntelliSelect® Bispecifics CLC mice can produce antibodies using the full human heavy chain repertoire, but with the one selected light chain exclusively. CLC mice were immunised with F-X. More than 400 CLC BiAbs were expressed, purified and screened using a chromogenic FXase assay to identify lead CLC BiAbs.

Figure 3. IntelliSelect® Bioinformatics Optimization of Lead CLC Bispecific Antibodies. A. Diverse antibody repertoires generated from F-Xa or F-X immunisations can be clustered into different B cell families based on the sequence similarities. This allows the reconstruction of the evolution history of key clones identified in the primary screen. Related clones in the same family can be further investigated. B. Deep mining of single cell and bulk NGS data from same mouse, coupled with a minimal mutagenesis strategy, was used to identify variants with elevated hemostatic activity.

Figure 4A. Progression of FVIII-Mimetic Activity of KY1049. A. Using the IntelliSelect® Bispecifics discovery platform, a fully-human CLC BiAb was efficiently optimized. B. KY1049 can be purified using a routine purification process in line with Chemistry, Manufacturing And Controls (CMC) standards. After Protein A capture, ion-exchange chromatography was applied to separate the desired F-Xa/F-X heterodimer from the two homodimers (F-Xa/F-Xa and F-X/F-X) using a salt gradient elution. A high percentage of F-Xa/F-X heterodimer was achieved using transiently and stably transfected CHO cells (data not shown). C. The hemostatic activity of KY1049 was assessed initially by a high-throughput chromogenic FXase assay (data not shown) and subsequently verified by aPTT. A reduction in the clotting time was observed in FVIII-depleted human plasma supplemented with different concentrations of KY1049. D. A dose-dependent increase in thrombin generation was observed in FVIII-depleted human plasma supplemented with KY1049.

Conclusion. 1) KY1049, a potent FVIII-mimetic BiAb, was developed and optimised using Kymab’s IntelliSelect® platforms. KY1049 demonstrates robust ex vivo efficacy and represents a promising future therapeutic in patients with hemophilia A. 2) IntelliSelect® Bispecifics platform is now being applied to develop other BiAb targets at Kymab.