A Fully-Human Bispecific Antibody Functionally Rescues Factor VIII Deficiency Ex Vivo

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KY1049 – A Factor VIII Mimetic Bispecific Antibody

KY1049 is a fully-human common light-chain bispecific antibody (BiAb) discovered using the IntelliSelect® Bispecific platform – part of Kymab's IntelliSelect® suite of technologies. KY1049 simultaneously binds coagulation factor IXa (F.IXa) and factor X (F.X) on the surface of platelets, bringing both coagulation factors into close proximity. This coincident binding stimulates the F.IXa catalysed activation of F.X resulting in restoration of the coagulation cascade in the absence of factor VIII (F.VIII) **(Fig. 1 A / B)**. Here we outline the derivation and optimisation of KY1049 using high-throughput screening. We demonstrate KY1049's ability to restore hemostatic function in F.VIII immunodepleted and hemophilia A patient plasma with and without inhibitory allo-antibodies. KY1049 has recently achieved Development Candidate (DC) nomination and is expected to enter clinical trials in 2021/22.

Figure 1

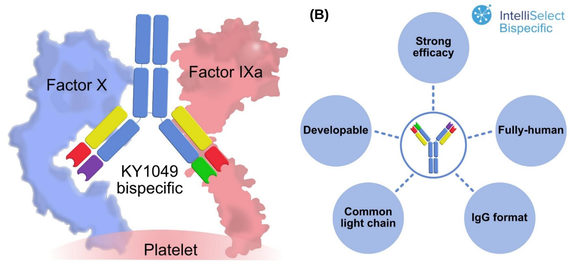

Figure 1: Reconstitution of the FXase Complex in Patient with HA. (A) Patients with hemophilia A (HA) lack Factor VIII and are unable to form the FXase complex. KY1049 is a functional mimetic for F.VIII, allowing a KY1049-F.IXa-F.X complex to form on the surface of platelets. (B) Properties of KY1049 include strong potency, good developability profile and naturally selected common light chain (CLC).

IntelliSelect® Bispecific Platform

Figure 2

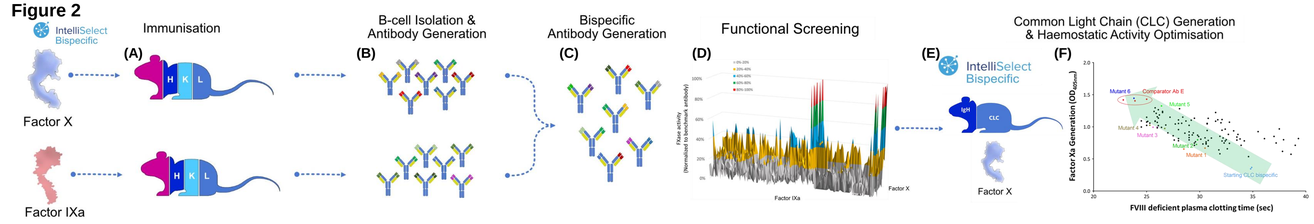

Figure 2: Derivation of KY1049 from IntelliSelect® Bispecific Platform. Multi-dimensional discovery workflow resulting in the generation of KY1049.

IntelliSelect® Transgenic mice were immunised with F.IXa or F.X and antigen-specific B-cells were isolated. **(Fig. 2 A)**. Variable regions of anti-F.IXa and anti-F.X monospecific antibodies were reformatted for BiAb expression **(Fig. 2 B/C)**. More than 8,000 BiAbs with different combinations of F.IXa and F.X arms were screened using an in vitro FXase assay, identifying an anti-F.IXa common light chain (CLC) **(Fig. 2 D)**. IntelliSelect® Bispecifics CLC mice can produce antibodies using the full human heavy chain repertoire, but with only the one selected light chain. CLC mice were immunised with F.X and more than 400 CLC BiAbs were screened in a FXase assay to identify lead CLC BiAbs **(Fig. 2 E)**. The molecule was iteratively optimized to enhance haemostatic activity **(Fig. 2F)**. Manufacturing was co-optimised for KY1049 resulting in favourable expression, purification and analytical profiles.

KY1049 Purification

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 (cIEX) was applied to separate the desired F.IXa/F.X heterodimer from the two homodimers (F.IXa/F.IXa and F.X/F.X) using a step-wise salt elution **(Fig. 3 A / B)**. The purity of the F.IXa/F.X heterodimer was determined by analytical HPLC and shown to be greater than 95 % **(Fig. 3 C)**. Further developability processing has achieved > 3.5 g/L heterodimer, with significant room for further optimisation. Cell lines for KY1049 have been generated, demonstrating enrichment for F.IXa/F.X heterodimer during cell line development (CLD) selection.

Figure 3

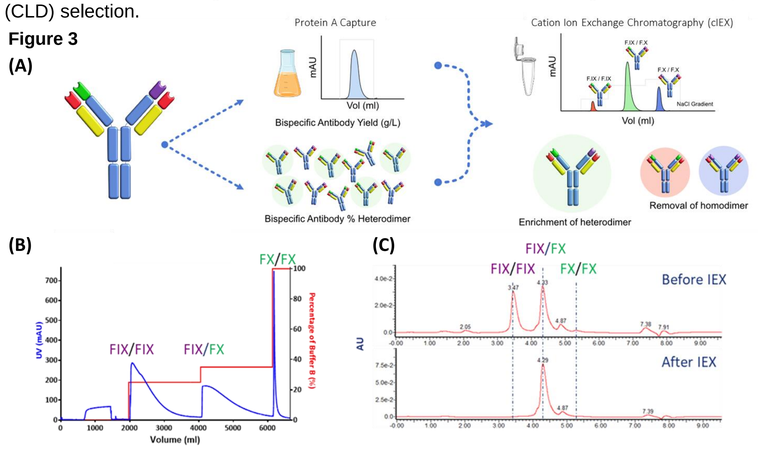

Figure 3: KY1049 Purification Profile. (A) Two-step purification strategy for KY1049 to achieve highly pure F.IXa/F.X heterodimer from a heterogenous mixture following expression. (B) Purification development has confirmed Protein A binding and hetero/homodimer baseline separation by cIEX in a scalable manner. (C) Analytical HPLC analysis of purified F.IX/F.X heterodimer confirms successful separation of KY1049 heterodimer from homodimer contaminants.

KY1049 Thrombin Generation

Coagulation is driven by the formation of thrombin, the key clotting factor responsible for converting fibrinogen to fibrin. Thrombin Generation Assay (TGA) characterises the formation of thrombin in real-time. TGA analysis reveals that KY1049 demonstrates greater potency at lower concentrations compared to a sequence identical analogue (SIA) of emicizumab using F.VIII immunodepleted plasma **(Fig. 4 A)**. Moreover, KY1049 is able to rescue prolonged clotting in severe hemophilia A patients with and without inhibitors **(Fig. 4B)**, demonstrating again greater potency at lower concentrations compared to emicizumab.

Figure 4

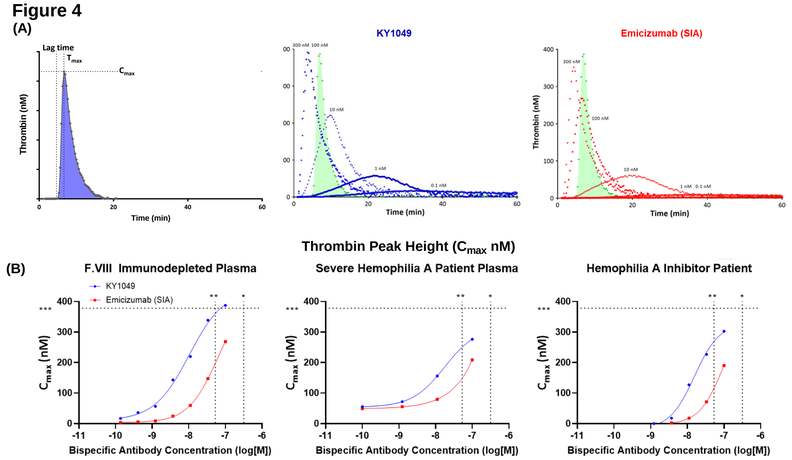

Figure 4: KY1049 Thrombin Generation Assay (TGA). (A) TGA thrombogram dose response for KY1049 (blue) and emicizumab (red). Far right – thrombin maximal peak height dose response compared to emicizumab. (B) TGA Cmax profiles for KY1049 and emicizumab in different plasma samples. *A median annualized bleeding rate (ABR) of 0 achieved at emicizumab steady state trough plasma concentration ≥ 45 µg/ml. ** Minimum KY1049 dosing concentration required to achieve the same potency as 45 µg/ml emicizumab. *** Cmax achieved using normal plasma.

KY1049 Rescues Clotting Ex Vivo

Activated partial thromboplastin time (aPTT) measures time taken for plasma to clot and is used to diagnose patients with hemophilia A. The clotting profile for KY1049 was compared to a sequence identical analogue (SIA) of emicizumab. The ability of KY1049 to rescue the observed clotting defect was investigated in F.VIII immunodepleted plasma and severe hemophilia A patients with and without inhibitory allo-antibodies against endogenously administered F.VIII (inhibitor patients). Such inhibitor patients are refractory to F.VIII therapy and represent an at-need patient population. KY1049 is able to dose-dependently rescue the clotting defect observed in the above mentioned plasma samples similar to emicizumab.

Figure 5

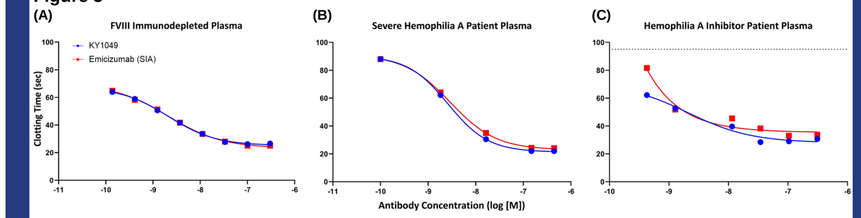

Figure 5: KY1049 Rescues Clotting Ex Vivo in Activated Partial Thromboplastin Time Assay (aPTT). aPTT clotting time dose response for KY1049 (blue) compared to a sequence identical analogue (SIA) of emicizumab (red) in F.VIII immunodepleted plasma **(A)**, severe hemophilia A patient plasma **(B)** and severe inhibitor hemophilia A patient plasma (70 BU) **(C)**.

Conclusion

KY1049 is a factor VIII mimetic bispecific antibody originating from Kymab's IntelliSelect® Bispecific antibody platform. A fully-human common light chain bispecific IgG antibody, KY1049 exhibits strong potency in ex-vivo studies. KY1049 shows favourable efficacy at reduced dosing levels compared to a sequence identical analogue (SIA) of emicizumab. Manufacturing process development for KY1049 demonstrates good expression, purification and analytical profiles. Clinical trial commencement is planned for 2021/22.