Raising Factor VIII's Half-Life to the Next Level

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INTRODUCTION

The half-life of Factor VIII (FVIII) is a critical parameter for defining the frequency of injections along with the FVIII trough levels in patients. Thus, the half-life of FVIII directly correlates with the patient's burden for a treatment. As currently approved FVIII products do not provide mean half-lives of more than 20 hours, it is one of the major issues of modern FVIII replacement therapy.[1]

In this study, we aimed for the development of a novel recombinant FVIII with von-Willebrand-Factor-independent half-life prolongation by endogenous albumin-binding, while this molecule was to maintain the characteristics of a FVIII wild-type after its activation.

MATERIALS & METHODS

Albumin-binding domains (ABD) were incorporated into a single chain FVIII molecule construct. Human cells were transfected with several FVIII-ABD variants and supernatants were screened for FVIII expression and activity. The most promising FVIII-ABD variants were produced, purified, and investigated for von-Willebrand-Factor binding potency as well as for thrombin-mediated activation. Half-life was assessed by pharmacokinetic studies in hemophilia A mice and in albumin-deficient mice which express the human instead of the murine neonatal Fc receptor. The *in vivo* functionality was investigated in hemophilia A mice using a tail transection assay determining bleeding time and blood loss after a standardized cut.

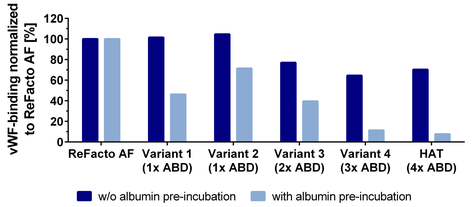
CONCLUSION

Here we present a next generation recombinant FVIII molecule with four albumin-binding domains incorporated (Hemophilia A Therapeutic ≙ HAT) resulting in a 4-fold half-life extension in a close-to-human model while preserving the wild type characteristics of activated FVIII with full *in vivo* functionality.

DEVELOPMENT OF HAT (HEMOPHILIA A THERAPEUTIC)

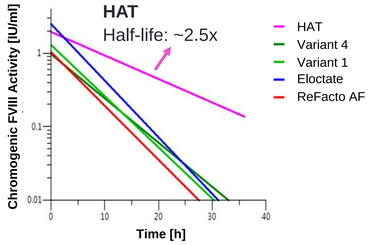
The screening of various FVIII-ABD fusion molecules demonstrated good expression in a human cell line and *in vitro* functionality of several candidates. An increasing number of ABDs and their respective positions in FVIII-ABD candidates led to a reduced binding to vWF (Fig.1), known to be the limiting parameter of previous half-life extension approaches of FVIII.[2]

Fig. 1: The von-Willebrand-Factor (vWF) binding of certain FVIII-ABD fusion variants in relation to ReFacto AF is demonstrated either in the absence of human albumin or with a 30 min pre-incubation with human albumin using physiological concentrations.



The inverse correlation between half-life extension and vWF-binding was in line with pharmacokinetic (PK) results in hemophilia A mice (Fig. 2). The most promising candidate was designated as Hemophilia A Therapeutic (HAT), incorporating four albumin binding domains, and demonstrating full *in vitro* functionality. An *in silico* model of HAT is shown in Fig. 3.

Fig. 2: Pharmacokinetic study in hemophilia A mice (B6;129S-F8tm1Kaz/J). 200 IU/kg bodyweight were injected i.v. and chromogenic FVIII activity was measured 0.5, 4, 8, 12, and 20 h post injection. A non-compartmental analysis was performed for half-life calculations. n= 12 mice/construct.



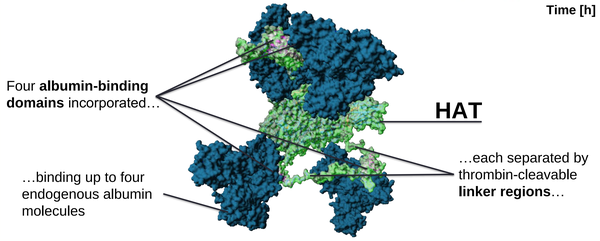


Fig. 3: *In silico* homology model of Hemophilia A Therapeutic (HAT, green) binding four endogenous albumin molecules (blue).

CHARACTERIZATION OF HAT

As murine albumin has a much shorter half-life compared with human albumin, hemophilia A mice are not an ideal model for PK investigations of HAT. Thus, an albumin-deficient, human FcRn α-chain-expressing mouse model was used demonstrating a 4-fold longer half-life of HAT compared with ReFacto AF, even with only 1% human albumin in the formulation (Fig. 4).

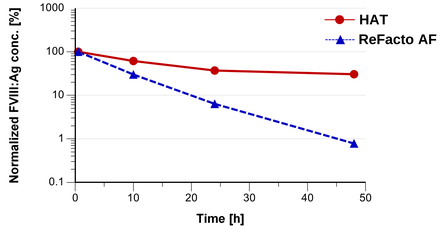
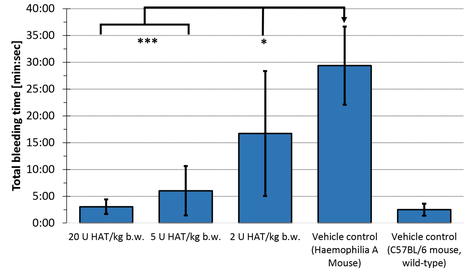


Fig. 4: Pharmacokinetic study in albumin-deficient, human FcRn α-chain-expressing mice (B6.Cg-*Alb*^{em12Mvw} *Fcgrt*^{tm1Dcr} Tg(FCGRT)32Dcr/MvwJ). 200 IU/kg bodyweight were administered i.v. and FVIII:Antigen levels were measured 0.5, 10, 24, and 48 h post injection. Half-life was calculated by non-compartmental analysis. n= 5 mice/construct.

The *in vivo* efficacy of HAT was demonstrated in a dose-dependent manner using hemophilia A mice for the tail vein transection assay. A dose of 20 U/kg bodyweight corresponding to approx. 10% of normal FVIII levels, resulted in bleeding times similar to wild type mice (Fig. 5).

Fig. 5: *In vivo* efficacy demonstrated in hemophilia A mice using the tail vein transection assay. Indicated doses of HAT or control were administered i.v. and the total bleeding time was monitored after a standardized cut into the left lateral tail vein. n = 8 mice/group; *: p<0.05; ***: p<0.001



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