IN_VIVO CHARACTERIZATION OF NOVEL GENETICALLY ENGINEERED INACTIVATION RESISTANT COAGULATION FACTOR VIII. CD Thornburg, X Deng, HZ Miao, L Palmer, RJ Kaufman, SW Pipe, University of Michigan, Ann Arbor, MI

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Hemophilia A results from a qualitative or quantitative deficiency of coagulation factor VIII (FVIII). Thrombin (IIa)-activated FVIII (FVIIIa) is a heterotrimer of subunits A1/A2/light chain and rapidly inactivates due to spontaneous dissociation of the A2-domain or proteolytic cleavage. Inactivation resistant FVIII (IR8) is a novel genetically engineered factor VIII protein that has enhanced in vitro stability after IIa activation due to resistance to spontaneous A2-domain dissociation and proteolytic inactivation. IR8 protein was derived from stably-transfected CHO cells and has previously demonstrated effective hemostasis in the Chapel Hill Hemophilia A dog model. The specific activity of IR8 (78,235 mU/ml) was markedly higher than FVIII wild-type (WT) (1766 U/mg). We hypothesized that IR8 could provide effective hemostasis at much lower doses of protein compared to FVIII WT. We studied this in a murine model of hemophilia A (FVIII exon 16 knockout). Hemophilia A mice were injected via tail vein with either IR8 or FVIII WT protein to obtain 0-100% correction of FVIII activity. Blood loss was quantitated over 20 min after the tail was transected at a diameter of 2 mm. The mean blood loss (µl/kg/min) from 17 mice injected with FVIII WT was 528 at 0-5% correction, 682 at 5-10% correction and 183 at 50-100% correction. The mean blood loss from 12 mice injected with IR8 was 574 at 0-5% correction, 184 at 5-10% correction, 217 at 10-50% and 121 at 50-100%. Therefore, IR8’s markedly increased specific activity enabled control of tail cut induced bleeding superior to FVIII WT at a 20-fold reduced amount of protein infused. We also tested the efficacy of IR8 expressed in vivo. Five hemophilia A mice were injected with 100ug of IR8 within a mammalian DNA expression plasmid via hydrodynamic tail vein injection. Expression was confirmed by FVIII activity analysis. In all five mice, FVIII activity was corrected into the normal range from 545-1038 mU/ml. Mice expressing IR8 had complete correction of tail cut induced bleeding. IR8’s hemostatic efficacy, high specific activity and resistance to inactivation make it an interesting and potentially useful novel FVIII therapeutic for FVIII replacement or as part of gene therapy applications for patients with hemophilia A.