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# Highlighted abstracts from the 60th Annual Meeting of the National Haemophilia Foundation, November 2008

### Extra-vascular clotting factor VIII protein protects against development of haemophilic synovitis in the presence of pre-existing anti-factor VIII inhibitor

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**Objective:** Progressive destruction of joints resulting from recurrent intra-articular haemorrhage represents the major morbidity resulting from haemophilia A or B. In addition to systemic clotting factor replacement, therapies localized to haemophilic joints may provide adjunctive protection. In a factor VIII$^{-/-}$ mice model, we investigated if extra-vascular delivery of recombinant human clotting factor VIII (rhFVIII) via intra-articular (IA) injection can prevent bleeding-induced joint damage, and also examined the possibility that IA delivery of FVIII carries greater risk of developing anti-rhFVIII inhibitor antibody.
**Methods:** FVIII$^{-/-}$ mice received rhFVIII by inserting a 30.5 G needle into the left knee joint, along with a range doses of FVIII(100, 25 and 5 IU kg$^{-1}$) in 5 μL, normal saline as the control. Comparison group received the same needle injury and intravenous (IV) rhFVIII (100, 25 and 5 IU kg$^{-1}$). 14 days after injury, both knee joints were collected for histological examination. To exclude the possibility that IA clotting factor was entering into circulation, mice received 100 IU kg$^{-1}$ rhFVIII IA, and FVIII activity was measured by aPTT. To see if IA rhFVIII delivery can carry greater risk of developing anti-FVIII antibody, mice were treated with a total dose of 300 IU kg$^{-1}$ rhFVIII over 10 days, either by IA or IV. 14 days after exposure, anti-FVIII was detected. After induction of anti-FVIII antibody by IV rhFVIII, mice were subjected either to needle puncture under coverage of bypassing agent (FEIBA) 100 IU kg$^{-1}$ or 100 IU kg$^{-1}$ IV rhFVIII, or needle puncture with 25 IU kg$^{-1}$ rhFVIII. Control mice received needle puncture with normal saline. Two weeks later, knee joints were collected for histological examination.
**Summary:** Mice receiving only saline at the time of needle puncture developed synovitis (mean score 5.0 ± 0.5). Mice treated with 25 IU kg$^{-1}$ IA rhFVIII developed better protection than mice treated with 100 IU kg$^{-1}$ IV rhFVIII (lower pathology score for IA, 0.733 ± 0.278 vs. IV 2.57 ± 1.70) and even better protection was achieved by the dose of 100IU IU kg$^{-1}$ IA (Pathology score of 0.25 ± 0.31). IA injection of 100 IU kg$^{-1}$ rhFVIII did not lead to increased circulating FVIII activity at any time point up to 48 h. In IV-treated mice, 100% of mice developed anti-FVIII antibody (8.06BU), while only 50% of mice developed anti-FVIII inhibitor at the lowest detection limit (0.61BU). In the presence of inhibitory antibody, only 46% of mice receiving IV FVIII survived the needle injury, 58% with FEIBA and 100% of mice survived with 25 IU kg$^{-1}$ FVIII IA injection. In the saline-injected control mice, needle injury led to a mean pathology score of 6.8. Neither IV FVIII nor FEIBA provided effective protection, with pathology scores of 6.3 and 5.4, respectively. Surprisingly, 25 IU kg$^{-1}$ IA rhFVIII produced a pathology score of only 1.7.
**Conclusion:** Extravascular rhFVIII in the joint space can contribute protection against bleeding-induced joint damage. Intra-articular rhFVIII delivery did not induce greater risk of inhibitory antibody formation in FVIII knockout mice than circulating factor VIII challenge; in fact, a lower incidence was observed. In the presence of anti-FVIII inhibitory antibodies, IA delivery of FVIII still can offer protection from bleeding-induced joint damage.

### Reduction of inhibitor titres by infusion of FVIII gene transduced tolerogenic dendritic cells in haemophilic mice

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**Background:** The development of anti-factor VIII (FVIII) inhibitory antibodies is a major complication of FVIII replacement therapy in the management of patients with haemophilia A. Evidence is accumulating that tolerogenic dendritic cells (tDCs) generated *in vitro* can induce regulatory T cells and promote durable antigen-specific tolerance *in vivo*.
**Objective:** In this study we used human FVIII transgene expressing tDCs to reduce inhibitor formation in haemophilic mice.
**Methods and results:** The tDCs were generated by cell sorting the CD11c$^{low}$CD45RB$^{high}$ population of cells resulting from culture of lineage negative bone marrow cells in media supplemented with IL-10 and the neural peptides VIP and PACAP38. Expression of co-stimulatory molecules CD40, CD80 and CD86 and MHC Class II was negative or low on these cells and they remain unactivated even after stimulation with lipopolysaccharide (LPS) or transduction by foamy virus (FV) vectors. Following transduction by a FV vector expressing a B domain deleted human FVIII transgene, 3–5 × 10$^5$ tDCs were infused by tail vein injection into Bal/c haemophilic mice (tDC-F8) weekly for two doses. Real-time PCR showed that the transduction efficiency of our FV vector in this population of tDCs was approximately 70%. Subsequently, mice were challenged with four weekly intravenous doses of 0.2 μg rhFVIII. Mice that received no cells (Neg-Ctrl) and mice that received an equivalent number of non-transduced tDCs (tDC-Ctrl) were used as controls. After immunization with rhFVIII, the spleen size, weight and total cell numbers in tDC-F8 mice were consistently lower than in Neg-Ctrl and tDC-Ctrl mice by approximately 50%. Flow cytometry assessment of CD4$^+$ splenocytes showed there were an increased number of cells expressing the regulatory T-cell markers FOXP3, CD25, CD103, CTLA4 and GITR in tDC-F8 treated mice. We observed a 60% and 61% reduction in the level of inhibitor titres from tDC-F8 mice compared with Neg-Ctrl and tDC-Ctrl mice. Splenic CD4$^+$ T cell proliferation in response to FVIII stimulation in cells from tDC-F8 mice was suppressed by approximately 90% compared with proliferation of cells from Neg-Ctrl and tDC-Ctrl mice. We also showed that pre-immunized mice treated with four infusions of hFVIII FV vector transduced tDCs had a reduction in their inhibitor titres by 54% ($P < 0.05$). No significant change in inhibitor titres were seen in untreated controls or mice given four doses of untransduced tDCs.
**Summary:** These data indicate that hFVIII gene transduced tolerogenic DCs are useful in decreasing inhibitory antibodies in haemophilic mice. More *in vivo* studies are in progress to confirm the antigen-specificity and durability of these effects. Our future studies will focus on isolating and characterizing the regulatory T cell populations induced by *in vivo* administration of transgene modified tDCs.

### Immunogenetics of factor VIII inhibitor development

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**Objective:** The purpose of our study is to investigate the hypothesis that polymorphisms in immunoregulatory genes and/or the factor VIII gene are partly responsible for recognition of therapeutic factor VIII as 'foreign', thereby provoking inhibitor antibodies. As a critical first step in our study we have set out to determine the minimal number of single nucleotide polymorphisms (SNPs) in immunoregulatory genes and the factor VIII gene that define the relevant DNA haplotypes seen in the Caucasian population (CEU) as shown in the HapMap project.
**Methods:** We searched the CEU dataset in the HapMap genetic database (release 23a, March 8, 2008) as a source for SNPs with potential to define haplotypes 'tag SNPs' in the genes for IL1-beta, IL4, IL10, IL13, interferon gamma, TNF-alpha, TGF-beta, zinc alpha-2 glycoprotein I, and the coagulation factor VIII gene. These genes were selected based on their central role in regulation of the immune response, previously published correlations with factor VIII inhibitor risk, or results of studies of factor VIII immunogenicity in animals. Using the Haploview programme we surveyed SNPs in each gene, plus approximately 20 kb of DNA 5′ and 3′ to each gene to assess potential regulatory elements that might influence gene expression. Tag SNPs were picked for each gene at various levels of coverage, based on correlation coefficients ($r2$) of >0.8, >0.9, and =1.0.
**Summary:** Our analysis indicates that the total number of tag SNPs required to study the nine genes of interest, with $r2$ values of > 0.8, > 0.9, or = 1.0, is on the order of 96, 138 and 146, respectively. Thus, a study of these proposed candidate genes is feasible with currently available SNP ascertainment based on multiplex PCR amplification/extension and MALDI-TOF analysis.
**Conclusions:** With approximately 150 SNPs (at $r2$ = 1.0) we will be able to determine association between factor VIII inhibitor development and any of seven immunoregulatory genes and two plasma proteins, including the coagulation factor VIII gene. We have extracted genomic DNA from peripheral blood leukocytes obtained from >900 caucasian haemophilia A patients who participated in the Multicenter Haemophilia Cohort Studies I & II, and are beginning to ascertain these SNPs for their association with risk for development of inhibitors to factor VIII in haemophilia A patients.

### Recurrence of inhibitor after orthotopic liver transplantation in severe haemophilia A

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**Objective:** Orthotopic liver transplantation (OLT) treats both hepatitis C-associated cirrhosis and haemophilia A with factor VIII activity increasing into the normal range in most patients. However, the results of OLT in haemophilia patients with inhibitors have been mixed. We report the follow-up factor VIII values after OLT in an inhibitor patent.
**Methods:** A middle-aged white male with severe haemophilia A, who had been on immune tolerance for >10 years for a high-responding factor VIII inhibitor underwent OLT. Factor VIII activity and inhibitor titres were followed over the next 9 months.
**Summary:** The patient had received factor VIII 40 u kg$^{-1}$ three times/week for many years, which suppressed inhibitor titres to <2 BU. Higher doses of factor had been used to support him through surgery with good results. At the time of OLT, his inhibitor titre was 0.7BU. He was treated prior to transplant surgery with 116 u kg$^{-1}$ with smaller doses every 2–4 h with monitoring during the operation. In the next 24 h he required another 300 u kg$^{-1}$ of factor VIII to maintain activity between 61–122%. On postop days 1 and 2 he received 46 and 35 u kg$^{-1}$ to maintain mean activity of about 50%. Around Day 6 his requirement for factor VIII increased to 70 u kg$^{-1}$ daily to maintain the same levels. He was treated for possible acute liver rejection with high dose Solu–Medrol and his requirements for factor VIII decreased so that it was discontinued on day 13 postop. Over the next month, his factor VIII activity increased from 57–91% and he had no bleeding complications. Immune suppression was achieved with tacrolimus, mycophenolate and prednisone, the latter being discontinued about 1 month post OLT. After a peak of factor VIII activity at one month, his level decreased so that it was 52% and 37% at 4 and 6 months, respectively, despite the continuous use of immunosuppression for the OLT. Seven months after transplant, the patient underwent a procedure

 