Prevention of Factor VIII inhibitor formation by ATX-F8-117, an antigen specific immunotherapy, in humanized HLA-DRB1*1501 mice

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Introduction
Haemophilia A (HA) is a blood clotting disorder caused by genetic deficiencies in the Factor VIII (FVIII) gene. HA is inherited with an X-linked recessive inheritance pattern and affects about 1:5000 males. Severe forms of HA result in spontaneous bleeding episodes particularly in joints and muscles. The main treatment is FVIII replacement therapy, using either plasma derived or recombinant products. Patients receiving FVIII replacement therapy are at risk of developing alloantibody responses to FVIII, so-called FVIII inhibitors, rendering the FVIII replacement therapy ineffective. FVIII tolerance can be elicited through immune tolerance induction (ITI) protocols in some patients but it is a costly desensitization therapy with limited success. Long-term eradication of inhibitors in HA patients could be achieved by antigen-specific immunotherapy as formation of FVIII inhibitors is T cell dependent. Here, we report a peptide-based antigen specific immunotherapy designed to specifically re-establish immune tolerance to FVIII.

The Apitope® Technology

- **Apitopes**: Soluble, synthetic peptides based on the human sequence which mimic the naturally processed T cell epitopes but do not require processing by antigen-presenting cells (= antigen-processing independent epitopes).
  - Peptides are identified using a proprietary discovery process unique to Apitope® comprising HLA-DR transgenic (Tg) mice technology, human PBMC cultures and in silico prediction tools.
- **Mode of action**: Soluble apitopes® bind to empty MHC receptors and selectively trigger activation of IL-10+ regulatory T cells which suppress pathogenic T helper cells
  - Soluble apitopes® do not induce inflammation and are safe and well-tolerated in clinical trials.

Apitope® ATX-F8-117 peptide cocktail down-regulates T cell response to human FVIII

**A** Dose escalation: 3 increasing + 3 top doses in 2 weeks

-15 -13 -11 -9 -8 -6 -4 0 10

Termination

Priming: Parental peptides in CFA s.c.

**B**

- LN
- Spleen

100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5 0

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**Figure 2**: HLA-DRB1*1501 Tg mice were treated with Apitope® ATX-F8-117 peptide cocktail or control peptide prior to immunization with the parental peptides in CFA as indicated (A). Ten days after immunization, spleens and draining lymph nodes were isolated and re-stimulated with graded concentrations of recombinant human FVIII (rhFVIII) or protein purified derivative (PPD) as an immunization control. Proliferation is determined by [3H]-thymidine incorporation 72h after start of culture and shown as stimulation index (SI) +/- SEM (SI = cpm of culture with antigen/without antigen) (B). Significance by two-tailed Mann-Whitney test: * p<0.05; ** p<0.01; *** p<0.001

Apitope® ATX-F8-117 comprises two apitopes® P1 and P17 for tolerance induction to human FVIII

**Figure 1**: Domain organization of the human FVIII (reference: Ngo et al., Structure 2008) and position of the identified apitopes®

Apitope® ATX-F8-117 Prevents Generation of FVIII inhibitors by 96%

**A** Dose escalation: 3 increasing + 3 top doses in 2 weeks

Dose stabilisation: Top dose continued weekly

-14 -12 -10 -8 -6 -4 -2 0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36

**B** Priming: 1µg mFVIII in PBS s.c.

**Figure 3**: HLA-DRB1*1501 Tg mice were treated prophylactically with Apitope® ATX-F8-117 or PBS according to a dose escalation scheme as indicated (A). Animals were then primed weekly with 1µg rhFVIII and given a concurrent treatment with Apitope® ATX-F8-117 at top dose. On day 56, total anti-FVIII IgG antibodies were determined using an anti-rhFVIII radioimmunoassay and expressed in percent of the total amount of added radioactivity i.e. % bound/total (kB/T) +/- SEM. FVIII inhibitors were determined using a modified Bethesda assay and shown as mean +/- SEM (B). Significance by two-tailed Mann-Whitney test: **p<0.001, ns = not significant.

Conclusion

- Using the Apitope® technology, we identified two FVIII immune dominant peptides in immunized human leukocyte antigen HLA-DRB1*1501 Tg mice that do not require processing by antigen-presenting cells.

- The combination of these two FVIII apitopes® (ATX-F8-117) administered according to a dose escalation protocol, induced T cell tolerance towards FVIII in HLA-DRB1*1501 Tg mice.

- Treatment with ATX-F8-117 significantly reduced FVIII inhibitor formation in a FVIII neutralizing antibody mouse model.

- ATX-F8-117 efficiently regulates the anti-FVIII T cell and B cell antibody responses, specifically the generation of FVIII inhibitors, in HLA-DRB1*1501 Tg mice showing great promise for peptide-based antigen-specific immunotherapy in inhibitor positive HA patients.

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