



# Canine factor VII deficiency as a test-platform to evaluate FVII gene therapy in humans

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The review of our article titled “Sustained correction of FVII deficiency in dogs using AAV-mediated expression of zymogen FVII” (1) by Drs. Bernardi and Pinotti (2) condenses basic biochemical and clinical data involving coagulation Factor VII. The authors highlight the physiological role played by tissue factor complexed with factor VII (FVII) or activated FVII in the initiation of coagulation and their involvement in pathological states such as thrombosis and cancer. They also provide very insightful views on human FVII deficiency and its specifics relative to other bleeding defects. Importantly, they discuss the concept of gene therapy for human monogenic coagulation defects. Within this framework, they present their comments on our article that demonstrated long-term expression of FVII following gene transfer in a large animal model of FVII deficiency.

In contrast to Factor VIII or Factor IX deficiency (hemophilia A or B, respectively), FVII deficiency has attracted limited research aimed to evaluate gene therapy approaches using animal models *in vivo*. Experiments with adeno-associated virus (AAV) to deliver and express FVII in a mouse model of FVII deficiency showed sustained efficacy (>100% expression levels); however, when the same experiments were performed in wild type non-human primates, the observed FVII expression levels following gene transfer quickly declined to a plateau level of ~7% (3), below what is considered therapeutic (10–15%). These data, albeit promising, do not provide an obvious case for the therapeutic potential of gene therapy for FVII deficiency. Clearly, the success of investigational therapies for genetic disorders strongly relies on the availability of

appropriate animal models that mimic the human disease. Although hemophilic dogs have been instrumental in the development of gene-based approaches for hemophilia (4) that have already entered the clinical phase, unfortunately, no efficacy or safety data for FVII deficiency exist in a large animal model of the disorder.

We decided to use gene delivery of a FVII transgene *in vivo* to address several limitations of prior efforts and formed the basis of our work reviewed by Drs. Bernardi and Pinotti (2). Specifically, we wanted to (I) use a large animal model of FVII deficiency that mimics the mutation profile of the human disorder and (II) obtain long-term efficacy and safety data, including potential detrimental effects in animal health or immunological responses. We had previously described dogs with FVII deficiency due to a missense mutation (Gly96 to Glu, G96E) that resulted in very low FVII activity (5). Expanding that work, we subsequently confirmed that the particular FVII mutation results in low circulating FVII antigen. As most patients have low circulating FVII antigen and activity (type I mutation), the FVII G96E dogs represented the majority of human FVII mutation types and were therefore ideal for evaluating novel treatments, including gene therapy. Based on our previous experience expressing various forms of FVII in hemophilic mice and dogs via AAV gene transfer (6-8), we utilized AAV to deliver *in vivo* a FVII transgene under the control of a liver specific promoter/enhancer. In particular, we chose to use the canine version of FVII, for two reasons. First, we wanted to ensure that the expressed FVII therapeutic protein would be optimally functional within the context of the canine coagulation system (versus

using a human FVII transgene). In this way, coagulation and all other physiological processes that involve canine FVII (or its interaction[s] with other proteins) would likely remain uncompromised. Second, using a canine FVII transgene would exclude species incompatibilities as a reason for any observed immunologic responses towards the transgene product.

Following canine FVII transgene delivery via AAV serotype 8 (AAV8) administration, we observed that FVII deficient dogs receiving AAV at 6E11, 2E12 or 4.95E13 vector genomes (vg)/kg exhibited a dose-dependent increase in circulating FVII levels to 15, 29 or 770% normal, respectively. Several important conclusions drawn from this work should be pointed out. First, the elevated FVII levels in the treated dogs were stable and long-term. As a matter of fact, FVII expression in the dog receiving 4.95E13 vg/kg was the longest ever reported in gene therapy experiments for FVII deficiency (2.6 years). Second, the obtained FVII levels following gene transfer were at or above what is considered the therapeutic threshold for the disorder. Third, the recent hemophilia B gene therapy clinical trial has suggested an upper acceptable limit in the AAV8 vector dose ( $\leq 2E12$  vg/kg) administered in humans (9,10). In our study, the 6E11 vg/kg dose resulted in stable circulating FVII levels at the therapeutic threshold; this AAV dose would be considered acceptable in humans.

No detrimental effects were observed in terms of animal physiology throughout the study, including evidence of a perturbed coagulation system. We did, however, observe an immunologic response to FVII in the dog that received the highest AAV8 dose. Nonetheless, this immunologic response was transient, not inhibitory and did not affect the final plateau FVII levels reached in that dog, a rather astonishing 770% of normal. Of course, such expression levels would not be necessary for an effective therapy for FVII deficiency. Importantly, the dogs that achieved FVII levels at the therapeutic range (but below 100%) did not show any immune responses against FVII. Notably, the exceptionally high FVII levels in the dog that received the highest AAV dose, albeit derived from a single animal, suggest for the first time that sustained and very high FVII levels do not appear to be detrimental to animal health.

In conclusion, I believe that we achieved the goals we set forth prior to initiating our work using AAV-based gene delivery of FVII as a therapeutic approach for FVII deficiency. We demonstrated sustained and therapeutic efficacy at AAV vector doses acceptable in humans and provided essential safety data using a large animal model

that represents the majority of human FVII mutation types. Therefore, I believe that our work makes a good case for gene therapy for human FVII deficiency and I hope that it will stimulate further efforts for novel therapies for this rare disorder.

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## Footnote

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*Ethical Statement:* The author is accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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