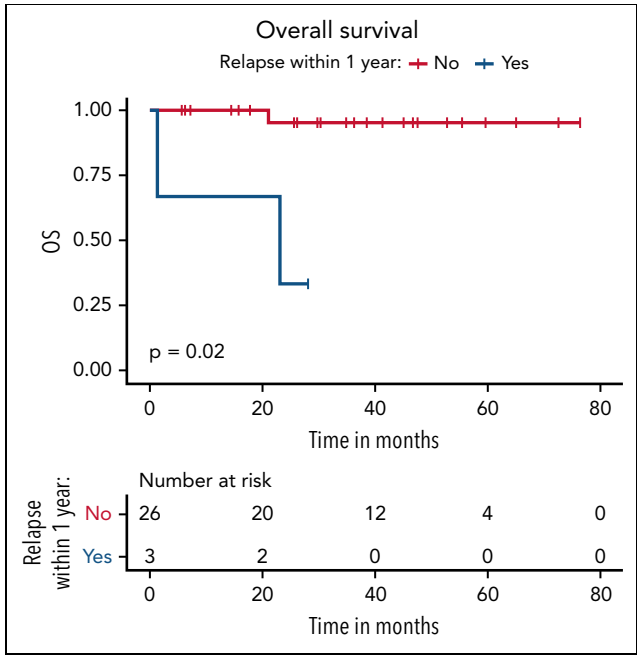

Landmark analysis after 1 year of vemurafenib therapy showed significantly higher overall survival (P = .02) of patients who had no relapse during the first year of treatment. See Figure 2B in the article by Handa et al, that begins on page 2663.

who are sensitive to BRAF inhibition and are therefore most likely to benefit from vemurafenib, as well as monitoring for emergence of resistant mutations, may aid rationalization of therapy. The data on resistant mutations were limited in this study, and this area merits further work. Although much can be learned from studying other BRAF-driven diseases, such as melanoma, the uniqueness of HCL means that observations in other diseases may not always be applicable, and mechanisms of resistance may be different.

Most patients with HCL can expect long remissions with PA monotherapy, but there is no sign of a plateau in progression-free survival curves, and alternative therapies are needed to ensure good long-term outcomes for all patients. It is also important to note that PAs, although well tolerated, are highly immunosuppressive drugs. The past 2 years of the COVID pandemic has driven changes in practice to maintain patient safety.[10] More patients with HCL have received a BRAF inhibitor in the frontline setting, and it is important to collect those data. The big question is whether BRAF inhibitors can come close to delivering the exceptionally long remissions seen after frontline PA, an unlikely possibility, given the relatively short remission (12-18 months) reported by Handa et al, albeit in the R/R setting. It is not yet clear which patients would benefit from combination therapies with novel agents at relapse or in the frontline setting, although several trials are in progress to address this question. The focus is now and will be on optimizing dose schedules and developing rational combination regimens to deliver deeper and more durable remissions and to overcome the mechanisms of disease resistance.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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GENE THERAPY

Comment on Batty et al, page 2672

Lifelong gene therapy in dogs with hemophilia A

Steven W. Pipe | University of Michigan

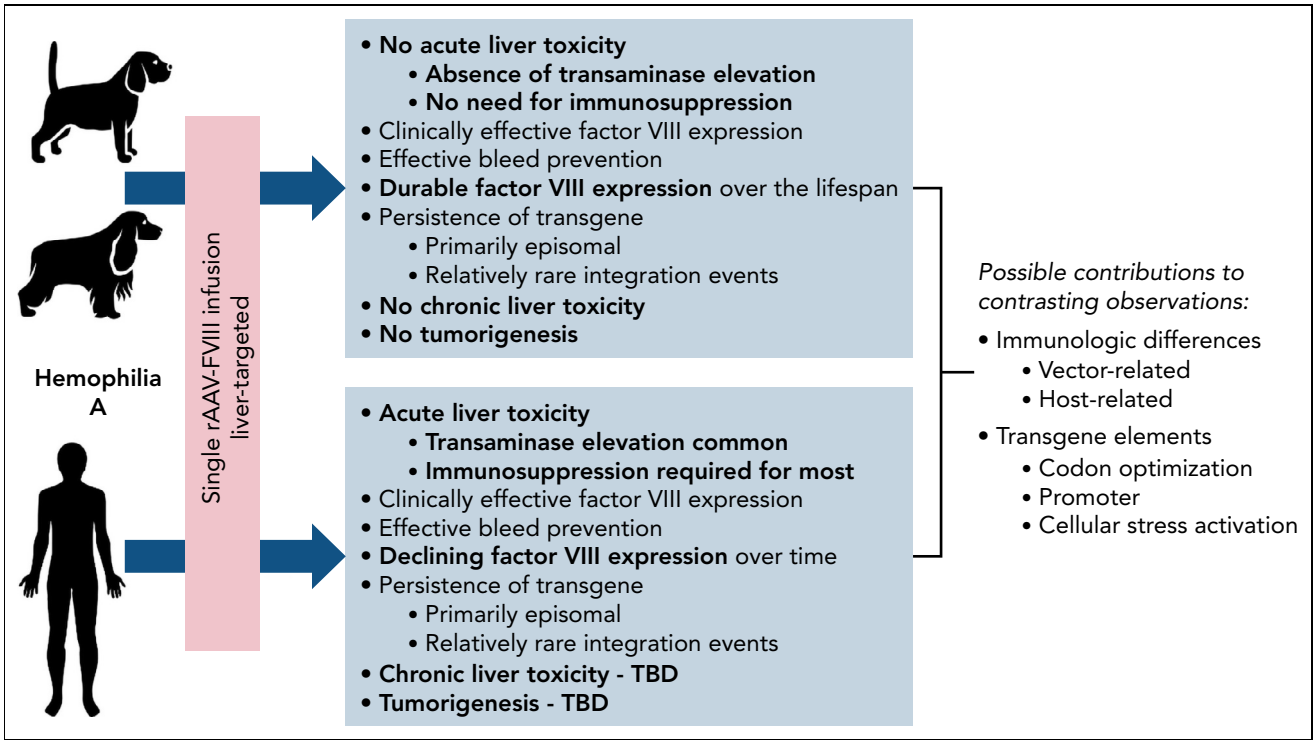
In this issue of *Blood*, Batty et al[1] report stable lifelong factor VIII (FVIII) expression in the liver of dogs with hemophilia A after a single dose of adeno-associated viral vector (AAV) gene therapy with durable efficacy and reassuring insights on the lack of adverse impact on liver health.

Dogs serve as loyal companions, helpers, even lifesavers, and also work side-by-side with us as protectors and coworkers in the field. I've had two dogs in my life: a beagle and an Irish setter, both of whom embraced their roles as loyal companions. However, for a hemophilia investigator, these 2 particular breeds also highlight their coworker role of being "man's best friend."

Animal models have been critical to the development of novel hemophilia therapeutics. Whether through naturally occurring or engineered mutations, they inform dosing, efficacy, and safety before human trials. The first hemophilia animal model, established in an Irish setter in 1947 at the University of North Carolina Chapel Hill, replicates the bleeding phenotype of severe hemophilia A and has been used continuously over 7 decades. Another colony of dogs with hemophilia A was established at Queens University in Canada in 1980 from affected miniature schnauzers and spaniels and was then expanded to include beagles.[2] Notably, the basis for the FVIII deficiency in both colonies was an intron 22 gene inversion analogous to that found in ~40% of humans with hemophilia A. The safety and efficacy of replacement therapy with plasma-derived and then recombinant FVIII was first established in dogs. Novel therapies that were not well-tolerated in this model did not progress to human testing.

Animal models have been critical in evaluating strategies to deliver transgenes to tissue targets for expressing missing proteins.[3] These approaches often use viral vectors to deliver the complementary DNA to the nucleus of the host cell where, depending on the vector chosen, it integrates into the chromosomal DNA or remains primarily episomal with only relatively rare integrations. Mouse models of hemophilia A engineered via knockout methods have been invaluable because of large litters in the mouse population, short generation time, and well-controlled efficacy measures. However, those models fail to mirror many aspects of human disease, and there are limits to longitudinal studies of long duration. Primates are closer in size for scaling issues and can more faithfully inform relevant human tissue physiology, but no genetic models replicate the hemophilia phenotype. Once again, dogs with hemophilia have proven to be excellent models to assess the short- and long-term safety and efficacy of gene therapy for hemophilia.

The ideal gene therapy for hemophilia A would achieve long-term efficacy after a single treatment with no toxicity from the vector, the transgene, or the FVIII protein. The platform that has advanced the farthest in clinical development is recombinant AAVs (rAAVs) packaged with engineered FVIII transgenes that target the liver to achieve sufficient FVIII expression to correct the bleeding phenotype.[4] The best risk:benefit ratio is seen with rAAVs, given their derivation from nonpathogenic replication-defective viruses and the ability to transduce post-mitotic cells in vivo that exhibit high tissue targeting to the liver. Clinical trials have produced clinically relevant expression of FVIII. Expression declines over time,[5-7] but it has resulted in reduction of bleeding episodes, elimination of the need for prophylactic FVIII replacement therapy, and improvements in quality of life for at least 5 years after a single infusion event. Safety concerns include asymptomatic liver toxicity manifested by increased transaminases that occur from shortly after the infusion and continue for up to a year or more after dosing. This toxicity is thought to result from immune responses to the vector capsid and has been treated with immunosuppression for resolution and salvage of transgene expression, although other potential mechanisms such as cellular stress from



Comparison of observations in dogs vs humans after AAV gene therapy in hemophilia A. TBD, to be determined through long-term follow-up studies.

overexpression of the transgene have been hypothesized. Given the high variability of FVIII expression, also seen in all animal models including dogs, high doses (up to 6×10^{13} genome copies per kilogram) have been required to achieve sufficient efficacy. Thus, with quadrillions of vector particles infused, even at a 0.01% rate of integration, millions of integrations can be expected within liver. Accordingly, there is a need for models to evaluate the long-term risks related to insertional mutagenesis and other unintended consequences.

Batty et al now report on 8 dogs with severe hemophilia A treated with a single dose of rAAV containing a canine FVIII; the follow-up was 8 to 12 years, the longest for any animal model to date. Six dogs achieved durable FVIII expression (median FVIII activity one-stage, 12.7%; chromogenic, 7.2%) with accompanying improvement in annualized bleed rates to near zero. Notably, the FVIII transgene was detected postmortem primarily in the liver in all dogs. All FVIII messenger RNA was derived solely from the liver because of the specificity of the transgene promoter elements. Importantly, the liver showed no chronic changes (fibrosis or cirrhosis) or malignancy. The investigators did observe nodular hyperplasia in the liver, which is a common idiopathic finding seen in aging dogs. Nguyen et al[8] reported on rAAV-canine FVIII treatment of dogs from the University of North Carolina Chapel Hill that also showed durable FVIII expression. Their integration analysis characterized 1741 integration events and some expanded cell clones, but there was no evidence of altered liver function or tumorigenesis.

No model was able to replicate all of the observations from the clinical trials (see figure). For example, none of the dogs exhibited signs of acute liver toxicity within the first year after rAAV treatment as observed in a high proportion of human patients. No immunologic profiling assessments were conducted in this dog study to evaluate for capsid-directed cellular immune responses, although these will be important features of future prospective experiments. Understanding why the dogs are showing durable expression over a lifespan compared with the waning expression in humans will be critical to moving this field toward a curative gene therapy approach in the future.

Overall, these observations should provide reassurance that the rAAV platform is demonstrating a long-term safety profile consistent with the rationale that was posited to move this into human clinical trials. The hemophilia and research community should greatly appreciate the coworker role of these dogs—man's best friend indeed.

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IMMUNOBIOLOGY AND IMMUNOTHERAPY

Comment on Freiwan et al, page 2684

Selecting CD7$^-$ T cells for CAR T-cell therapy

Maria Themeli | Amsterdam University Medical Centers (location Vrije Universiteit)

In this issue of *Blood*, Freiwan et al demonstrate the feasibility of using selected, naturally occurring CD7$^-$ T cells to generate CD7-targeting chimeric antigen receptor (CAR) T cells without CD7-directed fratricide and show that CAR^{CD7-} T cells have favorable biological characteristics.[1] The potential of exploiting CD7$^-$ T cells can facilitate the manufacturing of CD7 CAR T cells for T-cell acute lymphoblastic leukemia (T-ALL). In addition, the authors report that beyond T-ALL targeting, CD19-specific CAR^{CD7-} T cells show improved immunotherapeutic properties leading to better antitumor function, and thus, CD7$^-$ T cells are an interesting effector population for CAR T-cell therapy of hematological malignancies.

The overlapping antigen expression between healthy T cells and cancer cells limits the development of CAR T-cell therapy for T-cell–derived malignancies. CD7 is one of the most attractive target antigens since it is highly expressed in T-ALL blasts and T-cell lymphomas. However, its expression on normal T cells results in fratricide of CD7 CAR T cells, which compromises the successful and efficient product manufacturing. Several strategies have been proposed to mitigate the self-elimination of CD7 CAR T cells. These mainly include the disruption of the *CD7* gene using gene editing methods (CRISPR-Cas9 or base editing)[2-4] or the cytoplasmic sequestration of CD7 protein using a protein expression blocker.[5] Both these approaches rely on additional genetic modifications, which