(2021). Exosome-mediated stable epigenetic repression of HIV-1. Nat. Commun. *12*, 5541. https://doi.org/10.1038/s41467-021-25839-2.

7. Fu, Z., Zhang, X., Zhou, X., Ur-Rehman, U., Yu, M., Liang, H., Guo, H., Guo, X., Kong, Y., Su, Y., et al. (2021). In vivo self-assembled small RNAs as a new generation of RNAi therapeutics. Cell Res. *31*, 631–648. https://doi.org/10.1038/s41422-021-00491-z.

8. Villamizar, O., Waters, S.A., Scott, T., Grepo, N., Jaffe, A., and Morris, K.V. (2021). Mesenchymal Stem Cell exosome delivered Zinc Finger Protein activation of cystic fibrosis transmembrane conductance regulator. J. Extracell. Vesicles *10*, e12053. https://doi.org/10.1002/jev2.12053.

9. Shrivastava, S., and Morris, K.V. (2021). The multifunctionality of exosomes; from the garbage bin of the cell to a next generation gene and cellular therapy. Genes *12*, 173. https://doi.org/10.3390/genes12020173.

10. Staufer, O., Hernandez Bucher, J.E., Fichtler, J., Schroter, M., Platzman, I., and Spatz, J.P. (2022). Vesicle induced receptor sequestration: mechanisms behind extracellular vesicle-based protein signaling. Adv. Sci. *9*, e2200201. https://doi.org/10.1002/advs.202200201.

# Worldwide use of factor IX Padua for hemophilia B gene therapy

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https://doi.org/10.1016/j.ymthe.2022.06.002

It is a bittersweet coincidence the Xue et al. published online their successful adeno-associated virus (AAV) gene therapy trial for Chinese men with hemophilia B (HB) utilizing the high-specific-activity factor IX (FIX) variant Padua in *Lancet Hematology*[1] almost on the same day that the co-discoverer of FIX-Padua,[2] Valder R. Arruda, passed away unexpectedly after a brief illness. Their report augments a growing list of promising gene therapy products for HB, all using FIX-Padua,[3,4] including at least three in phase 3. In part because of his early experiences practicing hematology in his native Brazil, Dr. Arruda was always interested in addressing the 80% of the worldwide hemophilia population outside of Western countries that have limited access to treatments;[5] thus, the phenotypic cure of at least 9 out of 10 non-Western HB subjects expressing FIX-Padua after gene therapy is a well-timed testament to his contributions to the field of gene therapy.

FIX-Padua was identified as a rare X-linked thrombophilia in a collaboration between Dr. Arruda's laboratory in Philadelphia and Dr. Paolo Simioni's team in Padua, Italy.[2] The two collaborators had met 10 years prior when they were both post-docs in Leiden, the Netherlands, which was typical of Dr. Arruda's ability to mix long-term friendships and science with colleagues from around the world. The proband had a spontaneous deep vein thrombosis and was found to have an 8-fold increase in FIX activity but a normal FIX antigen level. Dr. Arruda and his collaborators demonstrated that the single amino-acid substitution R338L in FIX-Padua resulted in an 8-fold increased specific activity compared with wild-type FIX. Further, they showed that the R338L substitution was present in the three affected members of the proband's family and was not present in two non-affected members.

The Arruda laboratory[6–8] and others[9] subsequently demonstrated that the hyperactivity of FIX-Padua could enhance HB gene therapy in preclinical models. This was especially exciting because of the growing recognition of the limitations of escalating AAV vector doses, especially the AAV-capsid cellular immune response.[10] The capsid immune response is AAV vector dose dependent and is most frequently clinically manifested as an asymptomatic hepatotoxicity but can result in the complete loss of transgene FIX.[10] The high-specific activity of FIX-Padua offered a strategy to achieve high FIX activity levels while maintaining lower vector doses—if safety considerations of using a variant transgene could be addressed.

To this end, the Arruda laboratory also demonstrated that FIX-Padua had comparable immunogenicity and thrombogenicity with wild-type FIX in experiments that spanned protein biochemical assays to large-animal studies,[6–8,11] including the highly compelling demonstration that a HB dog with a pre-existing antibody to wild-type FIX could be tolerized after liver-directed AAV gene therapy with FIX-Padua.[7] This result strongly supported the lack of immunogenicity concerns for FIX-Padua. These studies set the stage for the current widespread use of FIX-Padua in clinical gene therapy for HB.

Xue et al. treated 10 Chinese patients with HB with a novel AAV vector (BBM-H901). BBM-H901 has an engineered liver-tropic capsid with 98% identity with AAV6 and AAV1 and uses a synthesized LXP2.1 promoter, which is described as liver specific. LXP2.1 drives the expression of a codon-optimized, but CpG depleted, FIX-Padua gene. The vector was manufactured with triple-plasmid transfections of mammalian cells and purified to <20% empty particles.

In a single-center phase 1 study, BBM-H901 was administered intravenously at a dose of

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$5 \times 10^{12}$ vg/kg. This dose is intermittent between those employed in previously successful AAV trials using FIX-Padua: $5 \times 10^{11}$ vg/kg in George et al.[3] and $2 \times 10^{13}$ vg/kg in von Drygalski et al.[4] Though as Xue et al. note, the total capsid dose (empty capsids plus full vectors) of BBM-H901 is similar to the dose in George et al. In Xue et al., participants were required to have AAV neutralizing antibody (NAb) titers $\leq$1:4; again, this NAb inclusion criteria is similar to George et al., who also evaluated a bioengineered AAV capsid[3], but notably distinct from von Drygalski et al., who evaluated an AAV5 vector.[4] In a stark illustration of the worldwide treatment gap for HB, the Chinese subjects were treated mostly with plasma-derived prothrombin complex concentrate prior to gene therapy compared with the more-targeted HB-specific FIX concentrates used prior to gene therapy in trials that were conducted in Western countries.[3,4]

In addition to the novel vector components, this trial incorporated several features aimed at better addressing and understanding the limitations imposed by the immune response against AAV vectors. These features included the use of prophylactic immunosuppression with oral steroids starting a full week prior to vector administration, the use of antioxidant and hepatoprotective agents initiated reactively once there was evidence of asymptomatic hepatotoxicity, and single-cell RNA sequencing of circulating cells to help decipher the immune response against AAV.

BBM-H901 was generally well-tolerated with only grade $\leq$2 adverse events to the vector or immunosuppression. There was no immune response against the FIX-Padua protein consistent with all previous studies. With an average follow up of more than a year, the mean FIX activity was 37% normal, as determined by an ellagic-acid-based one-stage assay. This result is comparable to the level reported by George et al. of 34% (n = 10) and by von Drygalski et al. of 47% (n = 3), though the silica-based assay used in the latter study outputs a higher activity for FIX-Padua than the assays used in the other studies.[12] The FIX activity in Xue et al. appears very stable between years 1 and 2. As would be expected for near-curative FIX activity levels, 9 out of the 10 BBM-H901 recipients had zero bleeds and did not need additional hemostatic treatments, including a head laceration requiring sutures. The single recipient that received additional hemostatic treatments had a confusing clinical picture, and it is ambiguous if he had new bleeding or an exacerbation of a chronic injury.

Despite prophylactic steroids, at least one subject had a capsid-directed cellular immune response with alanine transaminase (ALT) elevations, decreased FIX, and a positive ELISPOT; this was managed effectively with a second course of steroids. Other subjects had isolated elevated ALT levels and separately positive ELISPOTs, though these were of unclear significance. The incidence of a capsid cellular immune response of at least 1 out of 10 recipients in Xue et al. is similar to the 2 out of 10 reported by George et al. where steroids were initiated reactively. Intriguingly, Xue et al. were able to bifurcate their last seven subjects into an inferior cohort (FIX < 40%, n = 3) and satisfactory cohort (FIX $\geq$ 40%, n = 4). They report that the inferior cohort, compared with the satisfactory cohort, had higher cumulative ALT levels as well as evidence of increased T cell activation, proliferation, differentiation, and inflammatory cytokine secretion. Though limited by the small number in each cohort, these results suggest that the some of the heterogeneity of transgene-product levels may be due to anti-AAV immune responses that are undetectable by current laboratory monitoring.

This last result further supports Dr. Arruda's approach of using FIX-Padua to enhance the potency of HB gene therapy to achieve therapeutically relevant FIX activity levels with the lowest possible vector dose. Indeed, other novel vectors with alternative capsids and promoters—all utilizing FIX-Padua—are in also development, with a focus on expanding gene therapy to patients with HB worldwide.[13] That patients with HB around the world are now benefiting from FIX-Padua is one of the lasting legacies of Valder Arruda's career.

## REFERENCES

1. Xue, F., Li, H., Wu, X., Liu, W., Zhang, F., Tang, D., Chen, Y., Wang, W., Chi, Y., Zheng, J., et al. (2022). Safety and activity of an engineered, liver-tropic adeno-associated virus vector expressing a hyperactive Padua factor IX administered with prophylactic glucocorticoids in patients with haemophilia B: a single-centre, single-arm, phase 1, pilot trial. Lancet Haematol. https://doi.org/10.1016/S2352-3026(22)00113-2.
2. Simioni, P., Tormene, D., Tognin, G., Gavasso, S., Bulato, C., Iacobelli, N.P., Finn, J.D., Spiezia, L., Radu, C., and Arruda, V.R. (2009). X-linked thrombophilia with a mutant factor IX (factor IX Padua). N. Engl. J. Med. *361*, 1671–1675. https://doi.org/10.1056/NEJMoa0904377.
3. George, L.A., Sullivan, S.K., Giermasz, A., Rasko, J.E.J., Samelson-Jones, B.J., Ducore, J., Cuker, A., Sullivan, L.M., Majumdar, S., Teitel, J., et al. (2017). Hemophilia B gene therapy with a high-specific-activity factor IX variant. N. Engl. J. Med. *377*, 2215–2227. https://doi.org/10.1056/NEJMoa1708538.
4. Von Drygalski, A., Giermasz, A., Castaman, G., Key, N.S., Lattimore, S., Leebeek, F.W.G., Miesbach, W., Recht, M., Long, A., Gut, R., et al. (2019). Etranacogene dezaparvovec (AMT-061 phase 2b): normal/near normal FIX activity and bleed cessation in hemophilia B. Blood Adv. *3*, 3241–3247. https://doi.org/10.1182/bloodadvances.2019000811.
5. Skinner, M.W. (2012). WFH: closing the global gap–achieving optimal care. Haemophilia *18*, 1–12. https://doi.org/10.1111/j.1365-2516.2012.02822.x.
6. Finn, J.D., Nichols, T.C., Svoronos, N., Merricks, E.P., Bellenger, D.A., Zhou, S., Simioni, P., High, K.A., and Arruda, V.R. (2012). The efficacy and the risk of immunogenicity of FIX Padua (R338L) in hemophilia B dogs treated by AAV muscle gene therapy. Blood *120*, 4521–4523. https://doi.org/10.1182/blood-2012-06-440123.
7. Crudele, J.M., Finn, J.D., Siner, J.I., Martin, N.B., Niemeyer, G.P., Zhou, S., Mingozzi, F., Lothrop, C.D., Jr., and Arruda, V.R. (2015). AAV liver expression of FIX-Padua prevents and eradicates FIX inhibitor without increasing thrombogenicity in hemophilia B dogs and mice. Blood *125*, 1553–1561. https://doi.org/10.1182/blood-2014-07-588194.
8. French, R.A., Samelson-Jones, B.J., Niemeyer, G.P., Lothrop, C.D., Jr., Merricks, E.P., Nichols, T.C., and Arruda, V.R. (2018). Complete correction of hemophilia B phenotype by FIX-Padua skeletal muscle gene therapy in an inhibitor-prone dog model. Blood Adv. *2*, 505–508. https://doi.org/10.1182/bloodadvances.2017015313.
9. Cantore, A., Nair, N., Della Valle, P., Di Matteo, M., Màtrai, J., Sanvito, F., Brombin, C., Di Serio, C., D'Angelo, A., Chuah, M., et al. (2012). Hyperfunctional coagulation factor IX improves the

efficacy of gene therapy in hemophilic mice. Blood *120*, 4517–4520. https://doi.org/10.1182/blood-2012-05-432591.

10. Manno, C.S., Pierce, G.F., Arruda, V.R., Glader, B., Ragni, M., Rasko, J.J.E., Ozelo, M.C., Hoots, K., Blatt, P., Konkle, B., et al. (2006). Successful transduction of liver in hemophilia by AAV-Factor IX and limitations imposed by the host immune response. Nat. Med. *12*, 342–347. https://doi.org/10.1038/nm1358.

11. Samelson-Jones, B.J., Finn, J.D., George, L.A., Camire, R.M., and Arruda, V.R. (2019). Hyperactivity of factor IX Padua (R338L) depends on factor VIIIa cofactor activity. JCI Insight *5*, 128683. https://doi.org/10.1172/jci.insight.128683.

12. Robinson, M.M., George, L.A., Carr, M.E., Samelson-Jones, B.J., Arruda, V.R., Murphy, J.E., Rybin, D., Rupon, J., High, K.A., and Tiefenbacher, S. (2021). Factor IX assay discrepancies in the setting of liver gene therapy using a hyperfunctional variant factor IX-Padua. J. Thromb. Haemost. *19*, 1212–1218. https://doi.org/10.1111/jth.15281.

13. Kumar, S.R.P., Xie, J., Hu, S., Ko, J., Huang, Q., Brown, H.C., Srivastava, A., Markusic, D.M., Doering, C.B., Spencer, H.T., et al. (2021). Coagulation factor IX gene transfer to non-human primates using engineered AAV3 capsid and hepatic optimized expression cassette. Mol. Ther. Methods Clin. Dev. *23*, 98–107. https://doi.org/10.1016/j.omtm.2021.08.001.