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GENE THERAPY FOR COAGULATION DISORDERS: CURRENT STATE AND FUTURE PERSPECTIVES

Kunisheva Aziza Ruzibayevna

The Republican specialized scientific practical medical center for hematology, hematologist, research fellow

Email: kunishevaaziza@gmail.com

Abstract. *Inherited bleeding disorders, particularly hemophilia A and B, represent significant global health challenges affecting approximately 400,000 individuals worldwide. Recent advances in gene therapy have revolutionized treatment paradigms, with multiple adeno-associated virus (AAV)-based therapies demonstrating sustained clotting factor expression following single administration. This review synthesizes current clinical evidence, regulatory approvals, safety profiles, and implementation challenges, while proposing strategic recommendations for resource-limited settings. Clinical trials demonstrate factor level increases from <1% to 5-50% of normal, with 40-70% of recipients achieving zero bleeding episodes. However, significant barriers including pre-existing anti-AAV antibodies (20-60% of candidates), high costs ($2-3 million per patient), manufacturing constraints, and uncertainty about lifelong durability temper enthusiasm. For developing nations, strategic preparation through registry development, molecular diagnostics infrastructure, and international collaboration represents a pragmatic pathway toward eventual implementation.*

Keywords: *gene therapy, hemophilia, bleeding disorders, AAV vectors, factor VIII, factor IX, healthcare implementation*

Hemophilia A and B, caused by deficiencies in coagulation factors VIII and IX respectively, affect approximately 1 in 5,000 male births globally. These X-linked disorders result in spontaneous bleeding episodes, particularly hemarthroses leading to progressive joint destruction, chronic pain, and significant disability. Traditional management relies on lifelong prophylactic factor replacement therapy via frequent intravenous infusions, imposing substantial treatment burden and healthcare costs.

Gene therapy represents a paradigm shift, offering potential for sustained endogenous factor production following single administration. The rationale is compelling: these are monogenic disorders with well-characterized defects, modest factor level increases (1-5% to >5%) dramatically reduce bleeding, and clotting factors are secreted proteins not requiring cell-specific expression. This review examines the current state of gene therapy for bleeding disorders, analyzing clinical outcomes, safety profiles, regulatory landscape, and implementation considerations for diverse healthcare settings.

Hemophilia A results from mutations in the F8 gene (Xq28), encoding a 2,351 amino acid protein. Over 2,000 mutations have been identified, with the intron 22 inversion occurring in 45% of severe cases. The large F8 cDNA (approximately 9 kb) exceeds standard AAV packaging capacity (4.7 kb), necessitating B-domain deleted, codon-optimized constructs.

Hemophilia B arises from F9 gene mutations (Xq27.1-27.2), encoding a 461 amino acid protein. The compact F9 cDNA (1.5 kb) fits readily within AAV vectors, facilitating gene therapy development. Over 1,000 F9 mutations have been documented, predominantly missense mutations affecting factor IX synthesis or function.

AAV vectors have emerged as the predominant platform for hemophilia gene therapy due to efficient non-dividing cell transduction, long-term episomal transgene expression, low immunogenicity, and established safety profiles. Serotypes AAV2, AAV5, AAV8, and liver-specific variant AAV-LK03 demonstrate efficient hepatocyte transduction. Hepatocyte targeting capitalizes on the liver's natural role in producing coagulation factors.

Primary AAV limitations include packaging capacity constraints, pre-existing neutralizing antibodies (excluding 20-60% of candidates depending on serotype), and potential immune responses against capsid or transgene products. Alternative platforms including lentiviral vectors for ex vivo hematopoietic stem cell modification and emerging CRISPR-Cas9 gene editing approaches are under investigation but remain earlier in clinical development.

Hemophilia B has been the focus of extensive clinical development due to the compact F9 gene facilitating vector design. Landmark studies by Nathwani et al. demonstrated sustained factor IX expression extending over 13 years following AAV8-mediated gene transfer, with median levels of 3.1% at five years,

elimination of prophylactic replacement in most participants, and acceptable safety profiles.

Etranacogene dezaparvovec received FDA approval in 2022, representing a milestone as the first approved gene therapy for hemophilia B. Phase 3 trial data demonstrated mean factor IX activity of 36.9% at 18 months, reduction in annualized bleeding rate from 4.19 to 1.51 events, with 54% of participants experiencing zero bleeding episodes. These outcomes established gene therapy as a viable clinical option for eligible patients.

Gene therapy development for hemophilia A faces additional challenges due to F8 gene size. Strategies employing B-domain deleted FVIII, codon optimization, and bioengineered variants with enhanced expression have enabled clinical translation. Valoctocogene roxaparvovec received FDA approval in 2023 based on phase 3 data demonstrating mean FVIII activity of 42.5 IU/dL at year 2, annualized bleeding rate reduction from 4.8 to 1.2 events, with 53.8% of participants experiencing zero treated bleeds.

Additional hemophilia A candidates remain in clinical development, employing various vector designs and dosing strategies. Long-term follow-up continues to accumulate, providing insights into durability, late adverse effects, and factors influencing therapeutic outcomes.

Meta-analyses demonstrate substantial clinical benefits following gene therapy. Factor level achievement ranges from 5-50% of normal for hemophilia B and 10-50% for hemophilia A, with significant inter-individual variability

influenced by vector dose, participant characteristics, immune status, and vector design. Annualized bleeding rate reductions of 60-95% compared to pre-treatment rates on prophylaxis have been reported, with 40-70% of recipients achieving zero bleeding episodes during follow-up extending 1-5 years.

Quality of life improvements across multiple domains including physical health, emotional well-being, and treatment satisfaction have been documented using validated instruments. The elimination of frequent intravenous infusions represents a transformative outcome for individuals accustomed to lifelong intensive treatment regimens.

Durability represents a critical determinant of gene therapy value. Clinical data with follow-up extending 5-15 years demonstrate sustained transgene expression in many recipients, though individual variability is evident. The longest follow-up from the 2011 hemophilia B trial shows sustained factor IX expression beyond 13 years in several participants, suggesting many recipients may achieve benefit for decades.

Factors associated with expression loss include immune responses, liver disease progression, and potentially hepatocyte turnover. Some participants demonstrate gradual factor level decline over time, with mechanisms incompletely understood. The inability to re-dose with the same AAV serotype due to neutralizing antibody development means expression loss may necessitate resumption of conventional therapy without gene therapy re-treatment options using the same vector.

Transient aminotransferase elevations represent the most common clinically significant adverse event, occurring in 20-50% of recipients typically within 3-4 months post-infusion. These elevations reflect T cell-mediated immunity against AAV capsid epitopes presented on transduced hepatocytes. Prompt corticosteroid administration (prednisone 0.5-1 mg/kg/day) successfully manages most cases, preserving transgene expression.

Pre-existing anti-AAV neutralizing antibodies exclude 20-60% of potential candidates from treatment with specific serotypes. Antibody screening during eligibility assessment is mandatory. Development of inhibitors against transgene products appears rare in clinical experience to date, though most trials enrolled previously treated patients with established immune tolerance.

Supraphysiologic factor levels, particularly for factor IX with its longer half-life, raise theoretical thrombotic concerns. Several trials documented factor IX levels exceeding 100% of normal in subsets of participants, particularly early post-treatment. Rare thrombotic events including deep vein thrombosis and pulmonary embolism have been reported, though causality remains uncertain. Monitoring protocols include factor level assessment with planned interventions for persistently elevated levels.

AAV-associated hepatocellular carcinoma in preclinical animal studies raised concerns about long-term oncogenic risk. Clinical experience extending beyond 10 years has not identified increased cancer risk, though follow-up may be

insufficient for definitive conclusions. Regulatory agencies mandate 15-year safety surveillance including periodic imaging and tumor marker assessment.

Current protocols exclude substantial proportions of bleeding disorder populations through stringent eligibility criteria including pre-existing anti-AAV antibodies, inhibitor history, liver disease, age restrictions, and comorbidities. These restrictions limit accessibility to a subset who might otherwise benefit.

AAV production using mammalian cell culture systems yields limited vector quantities with substantial batch-to-batch variability and rigorous quality control requirements. Current manufacturing capacity can produce vector for hundreds to low thousands of patients annually, far below global hemophilia populations of 400,000 individuals. Scalability constraints will limit availability for decades absent significant manufacturing innovations.

Approved gene therapies cost $2-3 million per patient, representing among the highest prices for any therapeutic. While pharmacoeconomic models suggest potential cost-effectiveness over 10-20 year horizons if durability is sustained, upfront costs create substantial barriers for healthcare systems, payers, and patients. Access remains limited primarily to high-income countries, with vast disparities excluding populations in low- and middle-income countries where disease burden is greatest.

Published data derive predominantly from carefully selected clinical trial populations with intensive monitoring. Real-world effectiveness and safety in broader populations remain incompletely characterized. Post-approval registries

are collecting clinical practice data, but follow-up duration is limited. Comparative effectiveness among products has not been formally evaluated through head-to-head trials.

Next-generation vector engineering aims to improve transduction efficiency, reduce immunogenicity, and overcome pre-existing immunity through capsid modification and novel serotypes. CRISPR-Cas9 and base editing technologies enable targeted genetic correction rather than transgene addition, offering potential advantages including restoration of endogenous regulation and permanent correction. Bioengineered clotting factors with enhanced expression or activity may improve outcomes.

Extension to pediatric populations, individuals with pre-existing liver disease, and those with inhibitor histories represents important expansion opportunities. Gene therapy development for von Willebrand disease and rare factor deficiencies continues, though clinical translation lags behind hemophilia due to additional biological complexities and smaller patient populations.

Global access initiatives including technology transfer, tiered pricing, voluntary licensing, and simplified approaches suitable for resource-limited settings represent critical priorities. Addressing the disparity between availability in high-income settings and inaccessibility for the majority of the global bleeding disorder population constitutes both a scientific and ethical imperative.

Based on this comprehensive analysis, three strategic recommendations are proposed for Uzbekistan to prepare for eventual gene therapy implementation:

1. Establish a National Bleeding Disorders Registry: Create a centralized electronic database documenting all patients with hemophilia and other coagulopathies, including disease severity, inhibitor status, complications, and treatment patterns. Collaborate with the World Federation of Hemophilia to integrate into global registries, enabling benchmarking and resource planning.

2. Develop Molecular Diagnostics Infrastructure: Equip national medical centers with gene sequencing capabilities for F8, F9, and VWF genes. Train specialists in molecular diagnostics and establish a national mutation database. Implement genetic counseling services for affected families, enabling precise diagnosis necessary for future treatment candidacy assessment.

3. Build Capacity through International Partnerships: Partner with WFH and humanitarian programs to improve access to current therapies including modern clotting factors. Establish a specialized hemophilia treatment center with multidisciplinary expertise. Develop regulatory frameworks for gene therapy oversight. Participate in international clinical trials and explore regional cooperation with Central Asian countries for shared access to expensive technologies.

These recommendations acknowledge current resource limitations while establishing foundational infrastructure enabling future participation in gene therapy advances as global accessibility improves.

In conclusion, gene therapy for bleeding disorders represents one of the most significant advances in hematology, with regulatory approvals establishing it

as standard care for eligible patients in high-resource settings. Clinical evidence demonstrates substantial benefits including sustained factor expression, dramatic bleeding rate reductions, and quality of life improvements. However, significant challenges including eligibility restrictions, manufacturing constraints, extraordinary costs, and uncertain long-term durability temper enthusiasm.

For resource-limited settings including Uzbekistan, immediate gene therapy implementation is impractical, but strategic preparation through registry development, molecular diagnostics infrastructure, and international collaboration will position the healthcare system to participate as these technologies mature and accessibility expands globally. The investments required for this preparation simultaneously improve current clinical care, representing a pragmatic pathway toward equitable, world-class treatment for bleeding disorders.

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