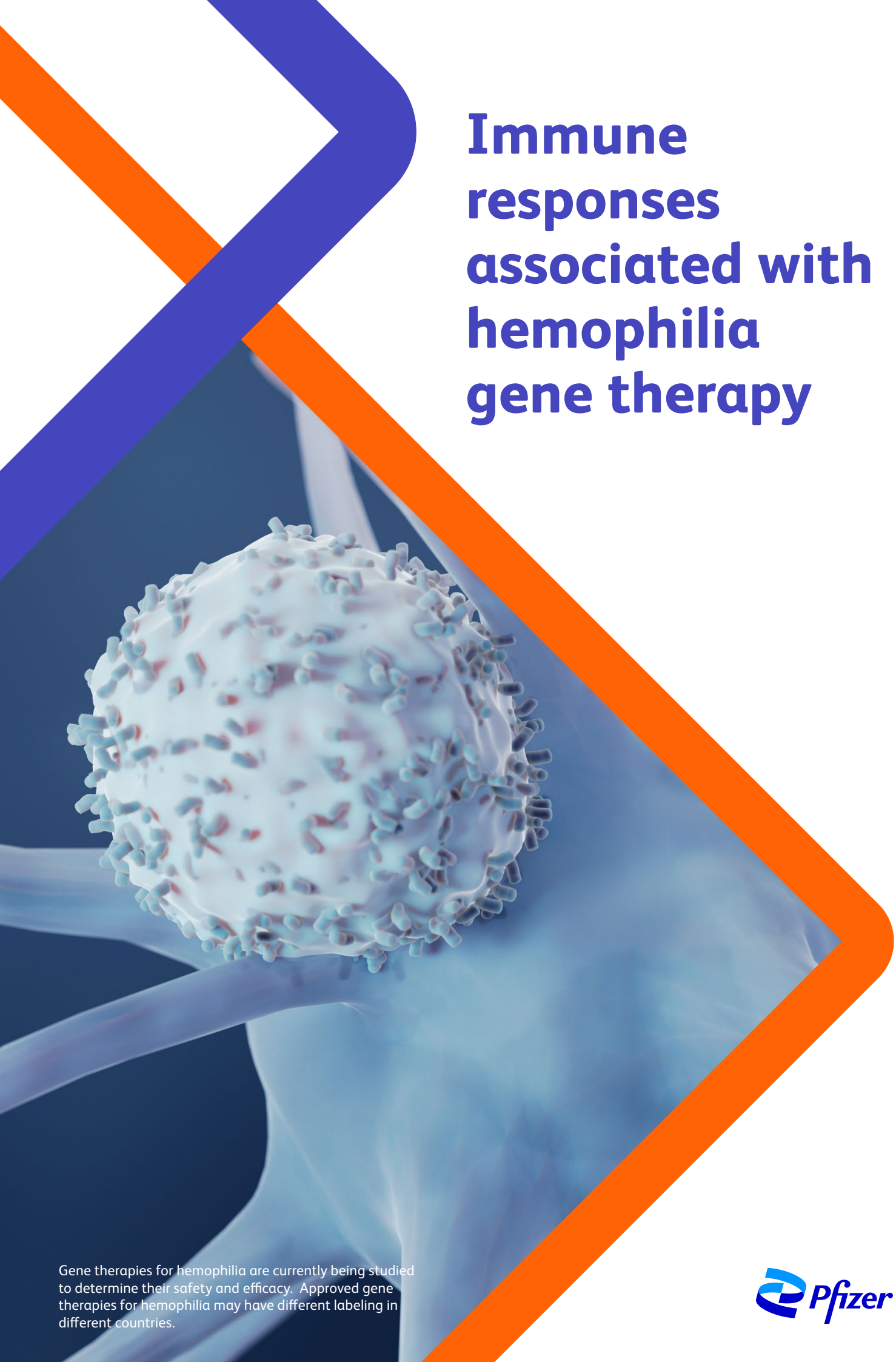
Immune responses associated with hemophilia gene therapy



Gene therapies for hemophilia are currently being studied to determine their safety and efficacy. Approved gene therapies for hemophilia may have different labeling in different countries.

Pfizer

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> Synopsis

In order to protect itself against bacteria, viruses and other foreign or harmful substances, the human body naturally elicits an immune response.[1] With gene therapy, both the capsid and the transgene components of the vector may be seen as "foreign" by the immune system, potentially triggering a cascade of cellular events that lead to an immune response.[2] In the humoral immune response, neutralizing antibodies can bind to the recombinant AAV (rAAV) vector and block its entry into target cells, and may prevent transduction.[3,4] Humoral immunity can also result in a hypersensitive immune response following re-exposure to a pathogen that poses health risks to patients.[5] Cellular immune responses can result in elimination of transduced cells and may prevent the establishment of a stable population of cells producing the therapeutic protein.[3,4] Both humoral and cellular adaptive immune responses to rAAV gene therapy vectors can be influenced by prior exposure to wild-type AAV from which the vector was engineered.[2] Therefore, both pre-existing immunity or an immune response to the gene therapy vector (capsid or transgene) are important considerations for the efficacy of hemophilia gene therapy.[2,3]

The information included in this brochure is accurate as of January 2023. Please visit www.genetherapyscience.com for further information and check back regularly for updates.

Gene therapies for hemophilia are currently being studied to determine their safety and efficacy. Approved gene therapies for hemophilia may have different labeling in different countries.

Immunological considerations for gene therapy

- Recombinant adeno-associated virus (rAAV) vectors are a commonly used vector for hemophilia gene therapies under clinical investigation[2]
- While considerable research efforts have been applied to enhance transduction efficiency by engineering the capsid and expression cassette, vector immunogenicity (which reflects the interactions of the rAAV vector with the host immune system) remains a challenge due to the natural responses of the recipient's immune system[6]
- In order to begin to understand the immune response to rAAV gene therapy and to consider how this can be managed, it is important to understand the complex, interweaving mechanisms of the immune response and its orchestration

The immune response: multiple lines of defense

- Immune responses may arise when the recipient's immune system encounters vector components (capsid or transgene) and/or resulting products (e.g., dsRNA, transgene product) and recognizes them as "foreign"[2]
- The type and speed of the response depends on whether the recipient has had prior exposure to a similar AAV[2,4]

Innate immunity

In the **absence of prior exposure** to a pathogen, the innate immune system acts as the **first line of defense** against pathogens[7–9]
Innate immune cells and proteins recognize and respond to "foreign" material during the first **hours and days** of exposure[7]
No specificity toward "foreign" material and not based on immunologic memory[4,7]

Creates an inflammatory environment and signals to cells of the adaptive immune system[8]

Adaptive immunity

Humoral immune response

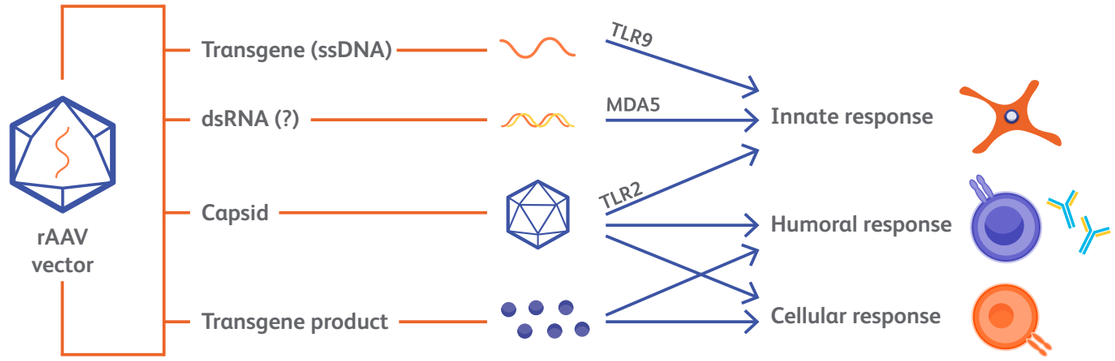
Release of neutralizing antibodies (NAbs) by B cells that will recognize and bind to the foreign particle, preventing its entry into cells[4,10,11]

Cellular immune response

Cells from the adaptive immune system recognize **antigenic epitopes** on the surface of cells that have taken up the foreign particle, resulting in destruction of antigen-containing cells[4,10,11]

Long-lasting protection following an immune response is conferred by the production of memory B and T cells that respond rapidly to a repeated attack by the same pathogen[1,4]

Possible rAAV vector-related factors triggering an immune response



dsRNA: Double-stranded RNA; MDA5: Melanoma differentiation-associated protein 5; rAAV: Recombinant adeno-associated virus; ssDNA: Single-stranded DNA; TLR: Toll-like receptor.
Figure based on Shirley JL, et al. 2020.[8]

The liver's unique immune environment

- While the liver generally exhibits a strong innate immune response to "foreign" particles, this has been shown to be low toward rAAV vectors[12,13]
- Under basal conditions, liver-resident cells functionally suppress the adaptive immune response, resulting in a state of relative immune unresponsiveness[14]
  - The presentation of foreign particles by hepatic antigen-presenting cells can increase the activity of regulatory T cells and reduce the activity of effector T cells, which promote immune tolerance[15]
- The cell types that make up the liver most likely act synergistically to skew the immune responses toward tolerance,[13] a state that can be utilized for liver-directed hemophilia gene therapy[15]

Find out more at www.genetherapyscience.com

Explore how capsid engineering has been used to optimize transduction in gene therapy — see the brochure *"Optimizing transgene expression for hemophilia gene therapy"*

Understand the mechanisms and impact of pre-existing immunity to rAAV on transduction — see the brochure *"Understanding pre-existing immunity against AAV: implications for hemophilia gene therapy"*

Find out more about the role of the liver in gene therapy for hemophilia — see the brochure on *"The liver takes a leading role in the hemophilia gene therapy story"*

Gene therapies for hemophilia are currently being studied to determine their safety and efficacy.
Approved gene therapies for hemophilia may have different labeling in different countries.

Pre-existing immunity to AAV

- The highly specific B and T memory-cell population that develops during first exposure to AAV (and remains after initial immune response) can be reactivated upon exposure to the same antigen[1,4,8]
- An individual with pre-existing exposure to the same or closely related AAV serotype (e.g. natural exposure to wild-type AAV) can therefore experience a rapid, highly specific adaptive immune response toward antigens on the surface of the rAAV vector following administration of gene therapy[2,4,10,11]
  - **Pre-existing anti-AAV antibodies** can bind and neutralize the rAAV vector, and the **memory B cell** population expands, resulting in the release of more antigen-specific NAbs[4,11]
  - **Memory T cells** will also be activated on recognition of antigens presented on the surface of transduced cells or antigen-presenting cells, resulting in expansion of the cytotoxic T cell population, and the attack and subsequent destruction of transduced cells presenting capsid-derived antigens[11,16]

For more information, see the brochure *"Understanding pre-existing immunity against AAV: implications for hemophilia gene therapy"* or visit www.genetherapyscience.com

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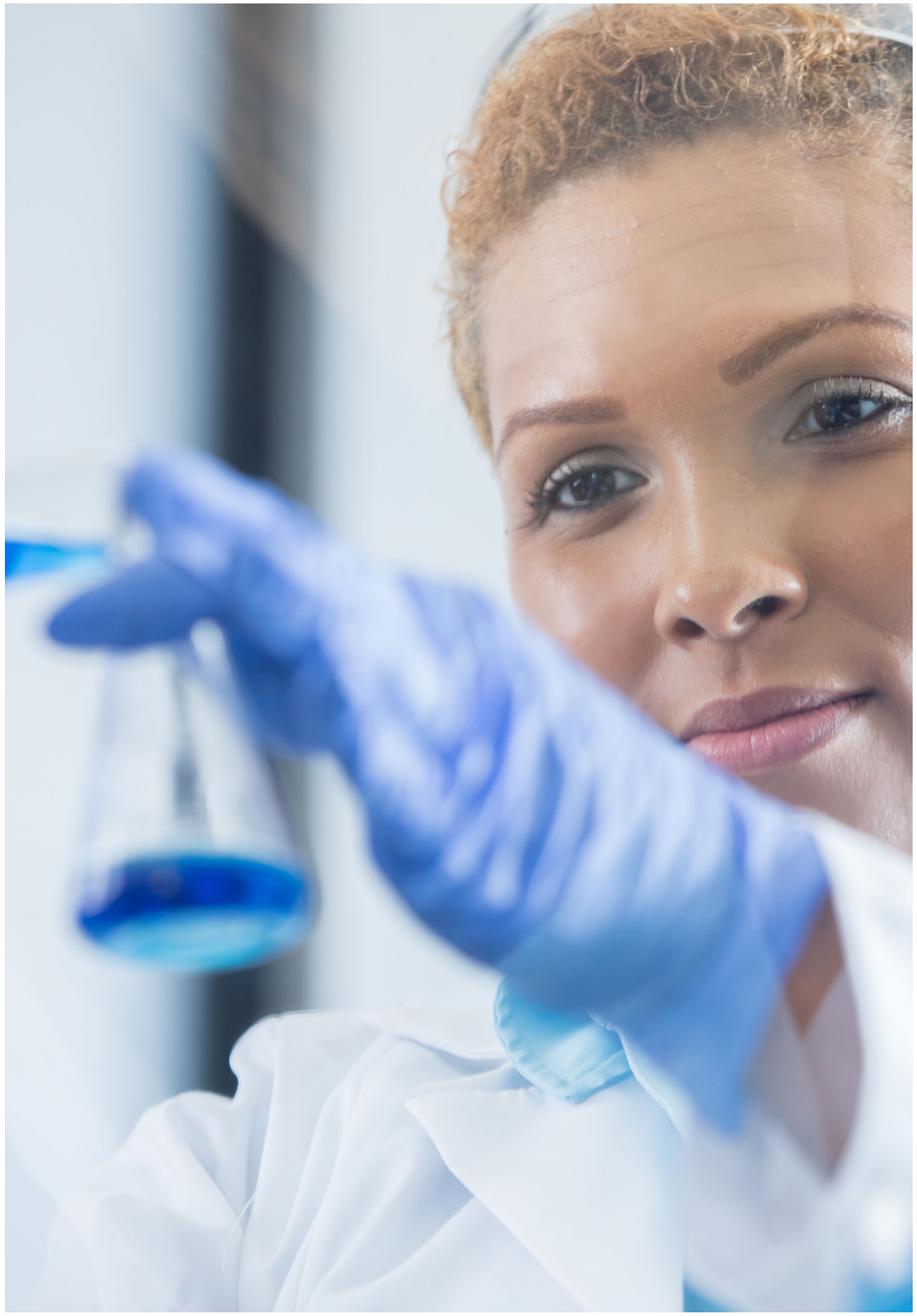

Innate immunity to rAAV vectors

- In the absence of prior exposure to a pathogen, the non-specific innate immune system is responsible for initial detection of the foreign rAAV vector[7,8,11]
- Although the innate immune response against rAAV gene therapy is considered to be mild and transient,[8,9] it is understood to have a key role in determining the extent of any subsequent adaptive immune response[2,4]
- Activation of the innate immune system relies on pattern recognition receptors (PRRs), such as membrane-bound Toll-like receptors (TLRs), which allow innate immune cells to recognize pathogen-associated molecular patterns on foreign particles[4,9,11]

- Downstream signaling results in the expression of type I interferons and other pro-inflammatory cytokines,[4] leading to the recruitment of cells of the innate immune system.[7] This also provides activation signals for an adaptive immune response[7,8]
- Following successful transduction, dsRNA may form (due to the presence of 5' and 3' inverted terminal repeats (ITRs) in the rAAV transgene) and be detected by cytosolic RNA sensors, leading to the expression of interferon-β, potentially resulting in immune-mediated, delayed loss of transgene expression[8,9,17]

What is the role of the complement system?

- The complement system is the part of the innate immune system responsible for amplifying the antibody response as well as recognizing pathogen-associated immunostimulants via inactive proenzymes in serum[9,18]
- It may serve as a costimulatory signal to increase the magnitude of an antibody response, as well as maintaining T-cell viability, proliferation and differentiation[9,18]

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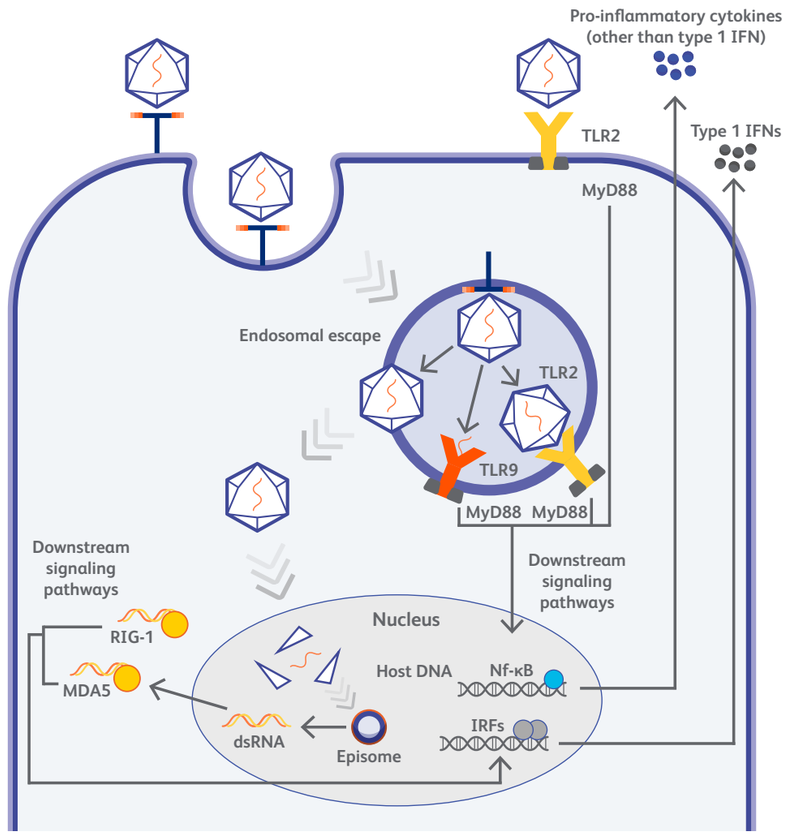
- TLR2 and TLR9, with the downstream mediator, MyD88, are believed to be the key PRRs responsible for initiating an innate immune response against rAAV-mediated gene therapy[2,9]



- **TLR2:** expressed by dendritic cells and macrophages, recognizes microbial protein and glycolipid structures. It has been shown to detect the rAAV capsid in endosomes and in circulation[2,9]
- **TLR9** (endosomal DNA receptor): can detect the rAAV genome, particularly unmethylated CpG motifs, on the endosomal membranes of plasmacytoid dendritic cells[2,8-10]
- **MyD88** is a downstream mediator of TLR2 and TLR9. It functions as a signaling adaptor in pathways that induce host defense mechanisms such as proinflammatory cytokines[9]

dsRNA: Double-stranded RNA; IFN: Interferon; IRF: Interferon regulatory factor; MDA5: Melanoma differentiation-associated protein 5; MyD88: Myeloid differentiation primary response 88; NF-κB: Nuclear factor-kappa B; RIG-1: retinoic acid–inducible gene 1; TLR: Toll-like receptor.
Figure developed from Mingozzi F and High KA 2013,[2] Vandamme C, et al. 2017,[4] Muhuri M, et al. 2021[9] and Colella P, et al. 2018[11]

What is a CpG motif?

A site in the DNA sequence where a guanine (G) nucleotide is positioned after a cytosine (C) nucleotide, connected by a phosphodiester bond (p).[19] Methylation of DNA can occur at a CpG site, which typically represses DNA transcription[20]

The impact of CpG motifs in the rAAV genome in clinical trials

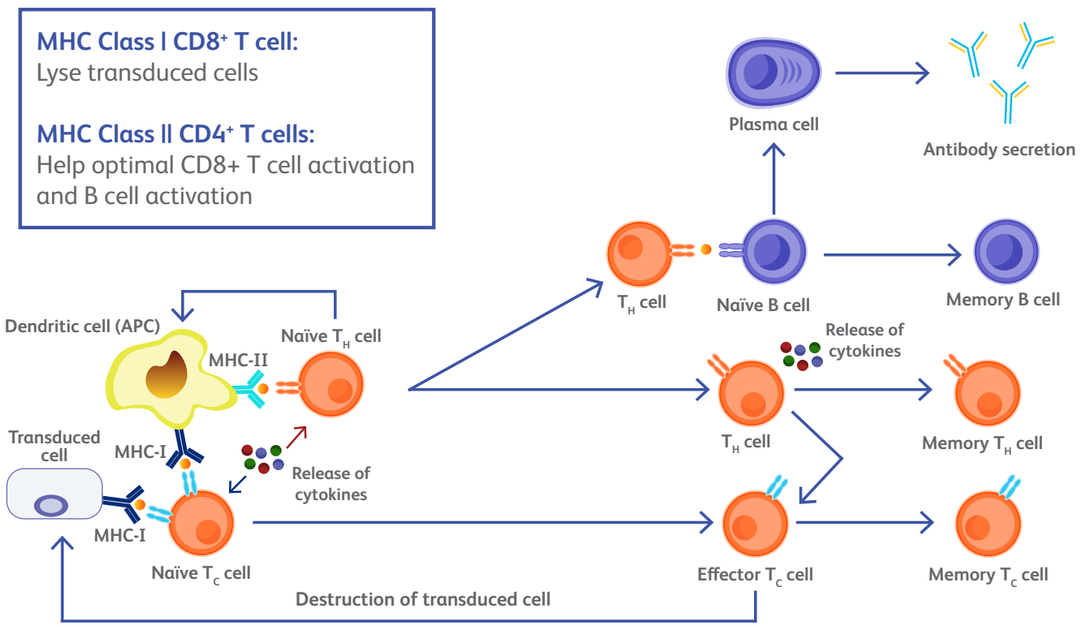
- In clinical trials for hemophilia B gene therapy, codon optimization of the transgene resulted in the introduction of CpG motifs into the open-reading frame of the transgene[21,22]
- FIX activity declined within 3 months in trials, suggesting that loss of transgene expression may have been due to recognition of CpG oligodeoxynucleotides by TLR9 and subsequent destruction of transduced cells[21,22]

Adaptive immunity to rAAV vectors

- Adaptive immune responses are **highly specific** responses mediated by the inflammatory environment created by the innate immune response following recognition of antigens[4,8]
- Adaptive immunity can be subdivided into the **cellular and humoral immune responses**, which both involve the activation of antigen-specific effector cells, elimination of foreign antigens (e.g. pathogens), and the generation of immunological memory[4,8]

MHC Class I CD8⁺ T cell:
Lyse transduced cells

MHC Class II CD4⁺ T cells:
Help optimal CD8+ T cell activation and B cell activation



APC: Antigen-presenting cell; MHC: Major histocompatibility complex; T_C cell: CD8⁺ cytotoxic T cell; T_H cell: CD4⁺ T helper cell. Figure developed from Vandamme C, et al. 2017,[4] Ronzitti G, et al. 2020,[6] Shirley JD et al. 2020,[8] Muhuri M, et al. 2021,[9] and Pennock ND, et al. 2013[23]

- Upon target cell binding and endocytosis, some rAAV vectors can be targeted for degradation by the proteasome[4,10]
- Following degradation by the proteasome, antigenic epitopes of the rAAV capsid can be presented by major histocompatibility complex Class 1 (MHC Class I) on the surface of transduced cells[4,10]
- Antigens can also be presented – via MHC Class I or MHC Class II – on the surface of dendritic cells (antigen-presenting cells [APCs]), which act as a bridge between the innate and adaptive immune systems[8,9]

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Humoral immune response to rAAV vector gene therapy

- Presentation of capsid-derived antigens via MHC Class II on APCs, along with the release of pro-inflammatory cytokines, leads to the activation of CD4⁺ T helper cells, which in turn activate naïve B cells, resulting in differentiation into antibody-secreting B cells (plasma cells) and memory B cells[8,9]
- Neutralizing antibodies (NAbs) bind to the rAAV capsid, rendering the rAAV vector unable to bind to the receptor on the target cell, thereby preventing transduction[10]

Can an immune response to the transgenic protein occur?

- Confirmed immune responses to the transgene product have not been observed to date in clinical trials, although subjects with a history of inhibitors to FIX or FVIII are typically excluded[2,24]
- No anti-FIX neutralizing antibodies (inhibitors) have been observed in any of the clinical studies of liver-directed hemophilia B gene therapy, even though some subjects had null mutations and were thereby at a higher risk of inhibitor development[2,16,25,26]
- To date, one case of inhibitor development to FVIII has been reported in an early hemophilia A gene therapy trial using a non-viral engineered cell-based therapy. Investigations are ongoing to understand if there is a causal relationship between this adverse event and the treatment used in the study[27]
- Some hemophilia gene therapy trials are investigating gene therapy in individuals with inhibitors[28,29]

Cellular immune response to rAAV vector gene therapy

- CD8⁺ cytotoxic T cells recognize antigens on MHC Class I of transduced cells and APCs, resulting in the cytotoxic T-cell-mediated destruction of transduced cells[4,9,10]
- CD4⁺ T helper cells interact with CD8+ cytotoxic T cells to facilitate the response[6]

How does the adaptive immune response develop immunological memory?

- Highly specific memory B and T cell populations and NAbs to the AAV capsid proteins remain after the infection is resolved and can be reactivated on exposure to the same antigen, thus providing long-lasting immune response to AAV[4]

Impact of immune response

Elevated liver enzymes following gene therapy

- Liver transaminases (alanine aminotransferase and aspartate aminotransferase) can sometimes indicate the occurrence of a T-cell response following rAAV vector gene therapy[24]
- A (generally asymptomatic) rise in alanine aminotransferase and aspartate aminotransferase levels (“transaminitis”) often correlates with a loss of FIX or FVIII expression in clinical trials[16,24,30,31]
- Immunosuppressive therapies can be administered either reactively or prophylactically. When initiated at the first indication of transaminase elevation or on detection of vector-reactive T cells (evidenced in a hemophilia B trial using AAV8 vectors), immunosuppressive therapy can result in the rescue of transgene expression[30,32]

Implications of immune response against the rAAV vector for future administration of gene therapy

- In the same way that pre-existing immunity arising from previous exposure to wild-type AAV can result in an immune response to the rAAV capsid and prevent transduction,[2,11] subsequent rounds of gene therapy using the same rAAV vector may be compromised by an adaptive immune response[11]
- The same consideration would apply if closely related rAAV capsids were used for subsequent rounds of gene therapy due to cross-reactivity[33]
  - Cross-reactivity may arise as a result of the high level of similarity in the amino acid sequence and structural homology across the capsid proteins of AAV serotypes[34]

To find out more, see the brochure *“Understanding pre-existing immunity against AAV: implications for hemophilia gene therapy”* or visit www.genetherapyscience.com

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Strategies to address immunity

What strategies are under investigation to address immunity post-administration of gene therapy?

Several different strategies are currently being utilized or are under investigation across the field of hemophilia gene therapy, with the aim of overcoming or evading immune responses to the rAAV vector.

Humoral immune responses post gene therapy

Strategies under investigation (pre-clinical stage in hemophilia)

Strategy	Rationale
Utilizing high vector doses (for hemophilia B, not A)	Dilute the NAb response, acting as "decoys" for AAV-specific antibodies[2,4,35,36]
Engineering of the rAAV capsid	Evade immune detection and/or enable higher expression of the transgene[37,38]
Plasmapheresis	Reduce the titer of circulating NAbs[39]
Treatment with the cysteine protease IdeS	Enable non-specific cleavage of circulating IgG immunoglobulins[9,40]

Cellular immune responses post gene therapy

Strategies under investigation (clinical stage in hemophilia)

Strategy	Rationale
Use of minimal vector doses	Evade the cellular immune response, or using hyperactive transgenes (e.g. FIX high-activity variant [R338L]) to reduce vector dose[11,41]
Administering immunosuppressive drugs (prophylactically and/or reactively)	Block T-cell responses to AAV[32]
Modifying vector sequence	Reduce immunogenicity (e.g. CpG-depleted transgene)[35]
Reducing ectopic or off-target expression of the transgene (e.g. tissue-specific promoter)	Allow for high transgene expression in the desired tissue or be used to increase expression at lower doses[42]

AAV: Adeno-associated virus; IgG: Immunoglobulin G; NAb: Neutralizing antibody; rAAV: Recombinant AAV.

Explore more of the science of hemophilia gene therapy by visiting www.genetherapyscience.com

Key take-home messages

- Vector immunogenicity (i.e. interactions between the rAAV vector and host immune system) remains a challenge in hemophilia gene therapy[6]
- The type and speed of immune response is dependent on the host's prior exposure to a wild-type AAV serotype that cross-reacts with the rAAV vector:[2,4]
  - In the absence of prior pathogen exposure, the **innate immune response** occurs within hours or days of first exposure and is non-specific[4,7-9]
  - The **adaptive immune response** is highly specific, long-lasting and is mediated by the inflammatory environment created by the innate immune response[4,8]
- Although the innate immune response against rAAV gene therapy is typically mild and transient,[8,9] it plays a key role in determining the extent of any subsequent adaptive immune response[2,4]
- Confirmed immune responses to the transgene product have not been observed to date in hemophilia gene therapy clinical trials[2,24]
- The impact of the immune response may be evident from elevated liver enzymes (alanine aminotransferase and aspartate aminotransferase), which may indicate the occurrence of a T-cell response following rAAV vector gene therapy[24]
- Several strategies have been used, or are under investigation, to overcome or evade immune responses to the rAAV vector in hemophilia gene therapy
  - For humoral immune response these include utilizing high-dose vectors,[2,4,35,36] engineering rAAV capsids,[37,38] and plasmapheresis[39]
  - For cellular immune response these include use of minimal vector doses,[11,41] immunosuppressive regimes (prophylactic or reactive),[32] and modifying the vector sequence[35]

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For more information

The Hemophilia Gene Therapy Webinar Series explores the complex science underpinning hemophilia gene therapy. Hosted by an expert hematologist, joined by a specialist, each webinar focuses on providing a high-science review of key areas of interest in hemophilia gene therapy.

Gene Therapy **Webinar Series**

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