Chameleon Biosciences
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CHAMELEON BIOSCIENCES INC.

EVADER—stealth mode, next-generation AAV-based gene therapy vectors

Chameleon Biosciences is developing next-generation adeno-associated virus vectors that elicit minimal immune responses, allowing repeat dosing and resulting in superior efficacies. These EVADER vectors have universal application for existing adeno-associated virus vectors, and Chameleon is seeking partners to advance its lead program in severe hemophilia B.

Chameleon Biosciences, a gene therapy company based in Berkeley, CA, is developing next generation, adeno-associated virus (AAV)–based vectors designed to evade the body's anti-AAV immune response. The company's proprietary EVADER vectors, which consist of enveloped AAV particles engineered with potent, checkpoint immunosuppressive molecules, overcome some of the key immunological challenges faced by AAV therapies, including low efficacy due to pre-existing immunity against AAV and adverse post-dosing responses that can trigger neutralizing immune responses and cause acute toxicities (Fig. 1).

Chameleon's technology can be applied to any AAV, providing a potential boost in efficacy and number of patients that can be treated with existing AAV-based therapies and with new ones in development. The company's lead program is in severe hemophilia B.

Over the past decade, gene therapies, and in particular AAV-based approaches, have seen stunning clinical successes including saving the lives of infants with spinal muscular atrophy (SMA), restoring sight in children with certain inherited retinal diseases, such as Leber congenital amaurosis (LCA) or retinitis pigmentosa (RP), and reversing hemophilia disease symptoms in adults. Yet, despite these successes, the wider application of AAV-based gene therapies remains limited due to the complex interaction of the AAV vectors with a patient's immune system.

Chameleon's EVADER vectors are surrounded by a lipid bi-layer membrane that makes the vectors more resistant to neutralizing antibodies than current AAV technologies and helps increase overall gene delivery efficiency. Beyond its lead program, the company has several other programs in exploratory or early preclinical stages.

"At Chameleon every day is Rare Disease Day. We are committed to advancing gene therapy tools so that any child with a genetic disorder can be treated with a safer, more efficacious gene therapy. My goal is to improve the lives of children and adults suffering from any disease that a titratable, redosable gene therapy could alleviate," said Genine Winslow, CEO and co-founder of Chameleon. "EVADER technology brings hope to children and families for which AAV-based gene therapy was previously not a viable option."

Chameleon's partnering model is flexible, and the company is seeking strategic partners to advance its existing programs, as well as to develop novel programs for additional genetic disorders.

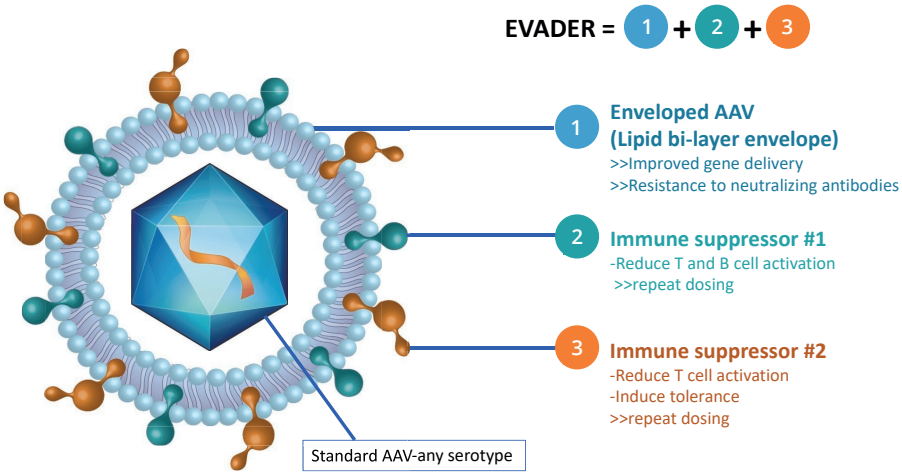

Fig. 1 | EVADER platform technology. Chameleon Biosciences' EVADER vectors consist of enveloped AAV particles decorated with potent, checkpoint immunosuppressive molecules to help overcome some of the key immunological challenges faced by standard AAV therapies. AAV, adeno-associated virus.

The limited promise of AAV-based gene therapy

As of June 2021, three AAV-based gene therapies had been approved for clinical use and many others were in clinical trials. Although these approvals and the growing number of clinical trials underscores the promise of AAV-based gene therapies, a major challenge for their broad implementation is safety concerns related to immunogenicity triggered by the AAV particles. As a result, eligibility for treatment either with marketed therapies or with those in clinical development, remains low.

The immune challenge presented by AAV-based gene therapies is complex because it involves three different components of the immune response: pre-existing immunity due to exposure to wild-type AAV, an adaptive immune response that results in the de novo generation of neutralizing antibodies against the AAV vector used, and an innate immune response to both the capsid and the nucleotide cargo contained in the AAV vector, that can result in acute toxicities.

Infection in humans with wild-type AAVs is prevalent, with prior exposure to AAV, shown in 30–80% of individuals, based on AAV serotype, patient age, geographical location and several other parameters. While asymptomatic, these infections trigger the generation of neutralizing anti-AAV antibodies that can, even at low titers, substantially impair the therapeutic effect of an AAV vector. This phenomenon of pre-existing immunity is universal across the dozen or so AAV serotypes that have been identified to date and that determine tropism to different organs and tissues in humans, making it equally challenging whether an AAV-based therapy targets the kidney, the liver, the lung, the retinal pigment epithelium or the heart.

The clinical challenges associated with the anti-AAV adaptive immune response are multipronged. First, exposure to the AAV vector triggers the development of neutralizing antibodies, which will act as pre-existing antibodies in the context of potential follow-up vector doses and severely limit, if not eliminate, the possibility of multi-dosing. Secondly, any pre-existing T cell immunity to AAV vectors can result in acute cytotoxic T cell responses that can substantially reduce the effectiveness of a therapy.

Finally, acute toxicities triggered by the innate immune response to the presence of the AAV vector capsid or its cargo can also hamper therapeutic effectiveness and create serious safety issues.

"The immune challenge for AAV-based gene therapies is formidable, but we are committed to developing a solution that will allow the more widespread use of AAV-based therapies to treat greater numbers of patients suffering from rare diseases,"

said Monica Miller, SVP for program development and operations at Chameleon. "Our EVADER platform delivers therapies that significantly reduce the immunogenic burden typically seen with AAV-based therapies and thus open the door to being administered as many times as needed, a critical need for many treatment regimes."

Toward an AAV-based gene therapy for severe hemophilia

Hemophilia A and B are X chromosome–linked coagulation disorders that affect more than 50,000 people in the US and the EU alone, and are associated with uncontrolled bleeding that in severe cases can lead to premature death. Standard care consists mostly of prophylactic administration of two coagulation factors lacking in these patients: factor VIII (FVIII) in the case of hemophilia A, and factor IX (FIX) in the case of hemophilia B. These treatments, however, are short-lived, and over time, recurring dosing can trigger immunological resistance as the body develops neutralizing antibodies against the recombinant coagulation factor proteins. In the case of hemophilia B, alternative but less effective treatments include drugs that promote coagulation directly, bypassing the need for FIX.

Recently, several clinical trials have shown the potential of using AAV-based gene therapy to reverse hemophilia B. Because AAV vectors have particular tropisms and FIX is expressed in hepatocytes, the development of an AAV-based therapy that would target the liver specifically was a key advantage of using the AAV platform. A number of AAV vectors have been engineered using different viral capsids and containing vectors that express FIX at high levels or so called 'hyperactive' variants of FIX. In all cases, single treatments with vectors have resulted in sustained production of the clotting factor and eliminated the need for infusion of recombinant FIX in treated patients.

> **Chameleon's EVADER technology has three key advantages over current AAV therapies: it is more resistant to pre-existing immunity, less immunogenic, and more efficient at gene delivery**
>
> Jeff Vick, Chief Business Officer, Chameleon

These successes, however, have been accompanied by liver toxicities associated with high vector doses that can result in a T cell response directed against the viral capsid. Dose-related T cell responses are thought to be the cause of observed liver cell death and require the use of combined immunosuppression treatments. Importantly, some patients have not qualified for treatment in the first place due to preexisting immunity to viral capsids. In particular, existing hemophilia B gene therapies cannot be administered to children due to the risks associated with multiple dosing. In growing children, the effectiveness of gene therapies is predicted to wane over time because new liver tissue does not express the corrected gene, requiring re-dosing to maintain disease correction.

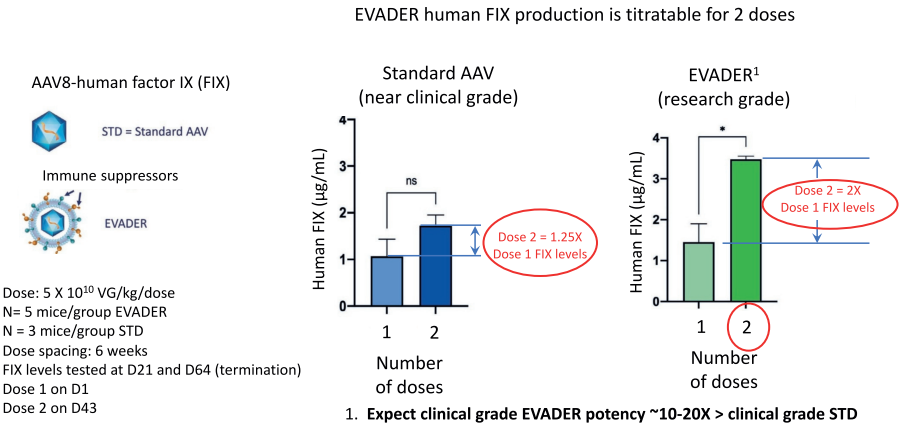

Fig. 2 | EVADER human FIX production is titratable for two doses. Studies in mice have shown that treatment with an AAV8 Evader/FIX particle not only results in a reduced production of neutralizing antibodies, but most remarkably, results in twice the levels of FIX with two doses of vector as compared to one, which stand in contrast with what has been observed with a standard AAV8 FIX vector. AAV, adeno-associated virus.

Chameleon is now conducting preclinical studies on an AAV8 EVADER particle containing a human FIX transgene payload. In in vitro experiments, these particles have shown reduced T cell responses compared to wild-type AAV8, and studies in mice have demonstrated a significantly reduced adaptive immune response resulting in markedly lower levels of B cell activation, which translates to fewer neutralizing antibodies being generated. Most remarkable, however, was the ability of the AAV8 EVADER/FIX particle to produce twice the levels of FIX with two doses of vector compared to one. In contrast, a second dose of standard AAV8 FIX vector did not result in a statistically significant increase in a FIX expression (Fig. 2).

"The early preclinical results with our AAV8 EVADER/FIX particle give us reason to be optimistic about the prospect of developing improved gene therapies for patients with hemophilia B," said Winslow. "The combination of low immunogenicity and high delivery and expression efficacies we have observed can change the current gene therapy paradigm of 'one and done', opening the possibility of treating at lower initial doses, for significantly reduced immune responses, followed by subsequent doses as needed to safely and effectively reverse disease symptoms."

With initial pilot scale studies having demonstrated the company's ability to scale the manufacture of the EVADER particles, Chameleon is now seeking strategic partners to collaborate on the next stages of preclinical and clinical development of its AAV8 EVADER/FIX technology.

Expanding the promise of gene therapy through collaborations

Chameleon is rapidly expanding its portfolio of gene therapies. Following its lead program in severe hemophilia B, the company has launched programs in three other rare monogenic genetic diseases with potentially rapid paths to the clinic: Niemann-Pick disease type C (NPC), mitochondrial neurogastrointestinal encephalomyopathy (MNGIE), and severe hemophilia A. Just as with hemophilia B, these conditions have been designated as orphan diseases by the US Food and Drug Administration, and their clinical endpoints are clearly defined.

NPC is a rare lysosomal disorder characterized by a progressive loss of the ability to transport cholesterol and other lipids throughout the body, resulting in localized accumulations that can be harmful, and in many cases fatal. NPC is caused by mutations in one of two genes encoding an intracellular cholesterol transporter: NPC1 or NPC2. Chameleon's preclinical program is focused on in vitro and in vivo testing of EVADER particles containing an NPC1 transgene.

MNGIE is a rare multisystem disorder characterized by progressive muscular degeneration, primarily of the gastrointestinal tract, but also of other areas such as the extraocular space, resulting in gastrointestinal dysmotility and limited eye movements. MNGIE is caused by mutations in the TYMP gene, which encodes thymidine phosphorylase. Chameleon's foundational program is focused on optimizing an EVADER particle containing a TYMP transgene.

Chameleon is pursuing a partnering strategy consisting of establishing collaborations with biotech and pharmaceutical companies interested in developing de novo AAV-based gene therapy programs or in applying the company's EVADER technology to their ongoing AAV-based programs.

"Chameleon's EVADER technology has three key advantages over current AAV therapies: it is more resistant to pre-existing immunity, less immunogenic, enabling repeat dosing, and more efficient at gene delivery, allowing lower dosing," noted Jeff Vick, Chief Business Officer at Chameleon. "We think this could be a game changer for expanding potential disease applications of AAV-based gene therapy and potential patient populations that can be addressed. Our hope is that this will make gene therapy more available for treating an increasing number of devastating diseases."

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