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Recent Developments in Gene Therapy for Homozygous Familial Hypercholesterolemia

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Abstract

Homozygous familial hypercholesterolemia (HoFH) is a life-threatening Mendelian disorder with a mean life expectancy of 33 years despite maximally tolerated standard lipid-lowering therapies. This disease is an ideal candidate for gene therapy and in the last few years, a number of exciting developments have brought this approach closer to the clinic than ever before. In this review, we discuss in detail the most advanced of these developments, a recombinant AAV vector carrying a low-density lipoprotein receptor (*LDLR*) transgene which has recently entered phase 1/2a testing. We also review ongoing development of approaches to enhance transgene expression, improve the efficiency of hepatocyte transduction and minimise the AAV capsid-specific adaptive immune response. We include a summary of key gene therapy approaches for HoFH in preclinical development, including RNA silencing of *HMG-CoA reductase* and induced pluripotent stem cell transplant therapy.

Introduction

Homozygous familial hypercholesterolemia (HoFH) is a life-threatening autosomal co-dominant disorder with an estimated prevalence of up to 1 in 160 000–300 000 [1,2]. It is estimated that more than 90% of HoFH patients harbor loss-of-function mutations in both alleles of the low-density lipoprotein receptor gene (*LDLR*). These mutations substantially or fully impair LDLR function, resulting in low-density lipoprotein cholesterol (LDL-C) levels frequently in excess of 500mg/dL and development of cardiovascular disease (CVD), principally coronary artery and aortic valve disease, during adolescence and death in the 20s, if left untreated [2]. Interestingly, significant phenotypic variability has recently been reported in a cohort of molecularly defined HoFH subjects, with LDL-C levels that extensively overlap with levels clinically associated with heterozygous status [1]. A major determinant of the severity of the HoFH phenotype is the residual LDLR activity associated with a given mutation. Based on *in vitro* assays in cultured fibroblasts, HoFH subjects can be classified as receptor-negative (<2% residual LDLR activity) or receptor-defective (2–25% residual LDLR activity) [3]. Receptor-defective FH subjects have significantly lower mean LDL-C levels and develop symptomatic coronary artery disease almost a decade later than their receptor-negative counterparts [3,4]. Furthermore, they respond better to conventional

and novel therapies. In the TESLA B study with the proprotein convertase subtilisin/kexin type 9 (PCSK9) monoclonal antibody evolocumab, receptor-defective HoFH subjects experienced a ~25% LDL-C reduction, in contrast with the lack of effect seen in receptor-negative subjects [5]. Taken together, these findings suggest that even a small amount of LDLR activity is beneficial compared to no receptor activity at all.

The treatment of HoFH has historically been very challenging due to the extremely elevated baseline LDL-C levels and poor responsiveness to standard lipid-lowering therapies (LLT) [2]. Statin treatment is associated with a 10–25% reduction in LDL-C in this population [6] and the addition of ezetimibe results in a further reduction of 10–15% [7]. Despite a 60% reduction in mortality due to these treatments [8], mean life expectancy in HoFH remains low at 33 years [8]. Substantial reduction in LDL-C is obtained with LDL apheresis. However its effect is transitory and availability is limited [9]. The recently approved lipid-lowering agents mipomersen [10], lomitapide [11] and evolocumab, [5] produce additional LDL-C reduction on top of standard therapy in HoFH patients; however, issues of tolerability or lack of efficacy in receptor-negative patients will limit the potential of these agents to fully address the serious unmet medical need in this population. Thus, despite significant therapeutic progress over the last few years, HoFH remains a challenging condition to treat for many of these patients.

HoFH is an ideal candidate for liver-directed gene therapy. Approximately 75% of the body's LDL receptors are expressed within the liver making it the most important organ in LDL metabolism [12]. This role is underscored by the dramatic correction and even resolution of the HoFH phenotype with orthotopic liver transplantation [12–14]. The disadvantages and risks of transplantation and long-term immunosuppression limit the viability of this approach in this disease. However, the delivery of a functional *LDLR* transgene to the liver has been of great interest. In the early nineties, Grossman et al. conducted the first ever gene therapy clinical trial in HoFH subjects, using an ex vivo approach to transfer replication-deficient human *LDLR*-expressing retroviruses into the livers of five HoFH patients [15]. To achieve this, the patients underwent resection of the hepatic left-lateral segment, hepatocytes were harvested, transduced ex vivo using the retrovirus vector, and autologous transduced cells were then infused back into their donors via the portal vein. In contrast with its effects in a natural rabbit model of HoFH [16], disappointing results were observed in human subjects with few transduced hepatocytes and variable (6–25% LDL-C reduction in three subjects) but mostly clinically unremarkable transient metabolic effects. These findings precluded further clinical development of the *ex vivo* approach but confirmed the feasibility and safety of *LDLR* gene therapy in humans. A number of different vectors and transgene constructs have since been tested in the pre-clinical setting, however none have achieved stable gene expression with durable metabolic effects [17,18]. In the last few years, however, several novel approaches have yielded promising results, bringing us to an exciting time in this field. In this review we discuss the most advanced of these developments, a recombinant adeno-associated virus (AAV) vector which has recently entered clinical testing. We also review approaches in pre-clinical development including gain-of-function *LDLR* transgene variants, RNA silencing of *HMG CoA reductase* and induced pluripotent stem cell transplant therapy.

Approaches in clinical development: recombinant AAV vectors

Development of AAV vectors for gene therapy—AAV is a replication-defective, non-pathogenic parvovirus with a linear 4.7kB single-stranded DNA [19]. In its latent phase, AAV DNA is retained in the host cell nucleus in a circular episomal form with negligible rates of genomic integration allowing for stable gene expression in post-mitotic tissues [20]. The first AAV vector to be developed, AAV2, achieved efficient gene transfer and stable hepatocyte transduction with low immunogenicity in pre-clinical models [20,21]; in human subjects, however, AAV2 had poor liver tropism and was associated with a limiting adaptive immune response [20,22]. A number of different AAV vectors have since been developed from other naturally occurring AAV serotypes. These differ from AAV2 in tropism and immunogenicity allowing for broader use of the AAV vector class as gene therapy platforms [23–25]. In addition, bioengineering approaches have generated novel variants that, in pre-clinical testing, evaded cellular and humoral immune responses more effectively, and transduced a wide range of cell types more efficiently than their naturally occurring parent serotypes [26]. In 2006, the first bioengineered AAV vector, AAV2.5 (a chimeric serotype derived from AAV1 and AAV2), entered clinical development in a Duchenne's muscular dystrophy cohort with mixed results [27].

AAV vectors are now widely used in gene therapy agents and have performed well clinically [26,25]. Most notable among these successes is that of the lipoprotein lipase (LPL) expressing AAV1 vector, AAV1-*LPL*^{S447X}, which became the first gene therapy agent to gain regulatory approval in the Western world in 2012 [28]. In LPL deficient subjects, AAV1-*LPL*^{S447X} improved post-prandial triglyceride metabolism with clinically significant reductions in the incidence and severity of pancreatic episodes for up to 2 years after administration of the vector with follow-up ongoing [28,29]. The factor IX (*FIX*) expressing liver-targeted AAV8 vector, scAAV2/8-LP1-h*FIX*co, also performed well in a phase 1/2 hemophilia B trial [30] achieving serum FIX levels of 1–7% of normal from baseline levels of <1% with clinically significant phenotypic improvement. This has persisted for up to 4 years in all of the subjects treated [31]. The results from this trial hold particular significance for the prospects of a liver-targeted recombinant AAV8 agent for HoFH.

Efficacy of recombinant AAV8 in mouse models of HoFH—AAV8 was identified for liver-directed gene therapy in HoFH due to its strong liver tropism and relatively low (38%) seroprevalence in Western populations [24,32]. The first AAV8-based HoFH gene therapy study compared the efficacy of a human *LDLR*-expressing recombinant AAV8 vector to AAV2 and AAV7 in *Ldl*^{-/-} mice on a high-fat diet. Compared to AAV2, AAV8 was associated with more efficient gene transfer (2.1 gc/cell vs. 52 gc/cell), hepatocyte transduction (4.2% vs. 81.2% of all hepatocytes), and more complete lipid correction (total cholesterol –600mg/dL vs. –1300mg/dL; p<0.001) [33]. AAV8 was also superior to AAV7 in these endpoints. Moreover, crucially, in AAV8-treated mice metabolic effects were maintained for up to 20 weeks and the development of atherosclerosis was almost completely attenuated.

The differences in the preclinical and clinical results observed with the HoFH ex vivo gene therapy approach highlight important limitations with the use of animal models. This has led

to substantial efforts in developing an animal model for HoFH that more accurately reflects the human pathophysiology. By deleting the apolipoprotein B mRNA editing catalytic polypeptide-1 (*Apobec1*) and *LDLR* genes (*Ldlr*^{-/-}/*Apobec1*^{-/-}), Kassim et al. designed a murine model of HoFH that, on a low-fat chow diet, develops a lipid and atherosclerotic phenotype comparable to that seen in human HoFH subjects [34,35]. In *Ldlr*^{-/-}/*Apobec1*^{-/-} mice maintained on a chow diet, increasing doses of a recombinant AAV8 vector containing a mouse *LDLR* transgene driven from a liver-specific thyroxine-binding globulin (TBG) promoter (AAV8.TBG.m*LDLR*) produced a significant dose-dependent reduction in total cholesterol and non-HDL-C within seven days of treatment [35]. Interestingly, the lowest dose that produced a significant lipid improvement (total cholesterol: -141mg/dL; non-HDL -130mg/dL at day 35) was associated with transduction of only 5–10% of hepatocytes. Complete metabolic correction was achieved with doses at least an order of magnitude higher than this dose and maintained for up to six months. Gene transfer and hepatocyte transduction were also dose-dependent and, at the highest dose tested, achieved 60–70% hepatocyte transduction at 35 days post vector treatment.

In a further study, a more advanced humanized model was designed using *Ldlr*^{-/-}/*Apobec1*^{-/-} mice engineered to express the human apoB100 transgene (*Ldlr*^{-/-}/*Apobec1*^{-/-}/*human ApoB* transgenic) [36]. In these mice, an AAV8 vector expressing the human *LDLR* transgene (AAV8.TBG.h*LDLR*) produced comparable metabolic effects to that seen in the previous study [36]. Of interest, experiments performed in heterozygous FH (HeFH) mice in this study showed that, although AAV8.TBG.h*LDLR* also produced a dose-dependent reduction in serum lipid levels in HeFH mice, the minimum effective dose in this cohort was an order of magnitude higher than that recorded in the setting of the *LDLR* knockout mice.

Atherosclerotic regression is an important therapeutic endpoint for lipid-lowering therapies used in HoFH patients. Atherosclerotic progression was assessed in a cohort of *Ldlr*^{-/-}/*Apobec1*^{-/-} mice maintained on a high-fat for two months [36]. At this point, these mice received a tail-vein injection of AAV8.TBG.m*LDLR* and were followed for an additional two months while remaining on the high-fat diet. Within seven days of receiving the vector, a significant reduction in mean total cholesterol was observed in these mice (1555mg/dL to 266mg/dL), and that normalized by the end of the follow-up period (67mg/dL). This was associated with an 87% reduction in the aortic plaque burden. Histological analysis of these lesions showed smaller lesions, fewer inflammatory cells and favorable plaque remodeling [36].

Taken together, these studies demonstrate that a recombinant *LDLR*-expressing AAV8 vector produces efficient gene transfer, stable hepatocyte transduction, significant lipid correction and atherosclerotic regression in humanized mouse models of HoFH. However, it has been shown that AAV vectors transduce human hepatocytes less efficiently than murine hepatocytes [24,37]. Recombinant *FIX*-expressing AAV8 produced substantially greater serum FIX elevations in mouse models of hemophilia B [38] than it did in human hemophilia B subjects [30]. Crucially, however, the levels achieved in the human hemophiliacs produced clinically significant effects that have persisted for more than 3 years [31]. This is of relevance to the use of AAV8 in HoFH because patients carrying *LDLR* mutations associated with >2% residual *LDLR* activity (receptor-defective) have a

significantly better prognosis than patients with *LDLR* mutations associated with <2% residual *LDLR* activity (receptor-negative) [4].

Safety profile of recombinant AAV8 vectors—Published studies in mice have not provided evidence of toxicity associated with the *LDLR*-expressing AAV8 vector [33,35,36]. However, caution must be taken in applying these findings to humans because substantial differences exist between the murine and human immune systems [39,40]. AAVs are naturally present in the environment [41], so it is expected that a percentage of the population have been exposed to them and will have developed anti-AAV8 neutralizing antibodies (nAbs)[32,41] and possibly also AAV memory T cells [42]. Furthermore, as demonstrated by previous gene therapy trials in LPL deficient [28] and hemophilia patients [30,31,43], administration of AAV vectors induces both cellular and humoral adaptive immune responses in human subjects.

Humoral immunity poses a challenge to gene therapy with AAV vectors for a number of reasons. First, approximately 30% of patients from Western populations have pre-existing anti-AAV8 neutralizing antibodies [32], and would be precluded from treatment with these vectors. Pre-existing antibodies against other serotypes such as AAV2 are even more prevalent [32]. In non-Western populations, such as the Chinese where anti-AAV8 seroprevalence is >80% [44], many more could be ineligible for AAV treatment [41]. Second, immunity that develops as a result of AAV8 administration precludes re-administration of the transgene with the same vector should transgene expression falls below therapeutic levels.

The cellular immune response poses the challenge of limiting transgene expression through the destruction of transduced cells [42]. It has been shown that a capsid and, less commonly, a transgene-specific T cell response is elicited by recombinant AAV vectors targeted to skeletal muscle [27,28,45] and the liver [30,43]. Focusing on the liver data, in the first liver-directed hemophilia B trial with AAV2, serum FIX levels of 10–12% normal were achieved at the highest dose tested in one patient, however within 2–4 weeks of vector administration, levels declined and returned to baseline by 10 weeks [43]. This was accompanied by a capsid-specific T cell response and a rise in transaminases which was asymptomatic and reversible. In this study, the T cell response was attributed to expansion of AAV2-specific memory T cells [42,43], however, alternative theories exist [40,46–48]. The capsid-specific T cell response was subsequently shown to be dose-dependent, induced by other AAVs and amenable to immunosuppression in the AAV8 hemophilia B trial [30,31]. In this study, capsid-specific T cells were detected in the intermediate and higher dosing groups and accompanied by a reversible transaminitis in 4 out of the 6 subjects in the high-dose cohort. As in the previous study, FIX levels declined in association with the cellular response however, in the highest dosing group, mean steady-state FIX levels of up to 7% of normal were achieved and maintained [31]. Importantly, a course of prednisolone was administered to subjects that experienced a rise in transaminases. The decline in serum FIX levels was more pronounced when the initiation of prednisolone was delayed for >2 days [31]. Of note, a dramatic 50–70% reduction in serum FIX levels was also observed in association with modest transaminase elevations (peak ALT 36–202 IU/l), highlighting the detrimental effect of the cellular immune response on gene expression, and the importance of early initiation of

immunosuppression [31]. Crucially, the capsid-specific T cell responses in these trials were subclinical and no transgene-specific T cell responses were detected.

Based on this preliminary safety and efficacy data, a first-in-human phase 1/2a trial has been announced with the recombinant AAV8 vector AAV8.TBG.h*LDLR* (NCT02651675) [see Table]. Its primary outcome measure is the number of participants that experience vector-related adverse effects by 52 weeks following administration. The total follow-up of subjects in this study is 5 years.

Recombinant AAV8 in HoFH: future directions—The development of strategies to improve transgene expression and minimise the immune response is ongoing. Bioengineering approaches are being used to develop AAV variants with attenuated immunogenicity and improved transgene transfer and hepatocyte transduction efficacies [40,49]. One AAV2 variant developed with this strategy was almost 100-fold more resistant to neutralization by nAbs specific for its parent AAV2 capsid in vitro with consistent findings in vivo [50]. AAV2.5 is thus far the only engineered AAV2 variant to undergo clinical testing. In the first-in-human study with this agent, six boys with Duchenne's muscular dystrophy received an intramuscular injection of one of two doses of a recombinant AAV2.5 vector carrying a *minidystrophin* transgene (AAV2.5-*minidystrophin*) under corticosteroid cover [27]. Vector genome transfer was confirmed in all of the subjects treated however gene expression was poor overall. Both humoral and adaptive immune responses to the AAV capsid were detected in this study although pre-existing seropositivity for AAV2.5 was shown to be ~25% lower than for AAV2. These clinical findings support the safety of engineered AAV serotypes in humans; however, the mixed efficacy and immunological results suggest further work is needed to develop variants with clear superiority to native AAV vectors.

Based on the successes of the *LPL* [28] and *FIX* [51,52] gain-of-function variants, a further approach in development are gain-of-function *LDLR* transgene variants. Using site-directed mutagenesis of h*LDLR* cDNA, Somanathan et al. developed three gain-of-function *LDLR* transgene variants that expressed LDLR proteins resistant to PCSK9 and/or inducible degrader of LDLR (IDOL)-mediated LDLR degradation. These variants (carried by AAV8 vectors) produced significantly greater cholesterol corrections in vivo and in vitro compared to the wild-type *LDLR* transgene in the setting of PCSK9 or IDOL overexpression (see Table) [53]. In vivo studies with physiological levels of PCSK9 and IDOL are needed to verify these findings. Moreover, it is possible that these variants could reduce responsiveness to the PCSK9 inhibitors so this should also be addressed in future studies.

Approaches in pre-clinical development

RNA silencing of 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGCR)—In a 2010 paper, Hibbitt et al. showed that combining statin therapy with a *LDLR* transgene construct containing *LDLR* genomic regulatory elements increased transgene expression up to 5-fold [54]. Building on this work, they piloted an approach that combined RNA silencing of *HMGCR*, which encodes the enzyme target of statins, with a plasmid that contained the *LDLR* transgene driven by *LDLR* genomic regulatory elements (*pLDLR-LDLR*) [55]. Using

a luciferase reporter gene driven by *LDLR* regulatory elements, they showed that small interfering RNA oligonucleotides and microRNA (miRNA) specific for murine *HMGCR* transcripts increased *LDLR* promoter activity by 300-fold and 12-fold, respectively [55]. Furthermore, when they co-transfected four *HMGCR*-specific miRNA plasmids with *pLDLR-LDLR* into *Ldl*^{-/-} mice, they observed significantly lower LDL-C levels (~1.5mmol/L, *p*=0.0036) in these mice as compared with controls that only received the *pLDLR-LDLR* plasmid. Thus, RNA-mediated knockdown of *HMGCR* provides an effective complementary strategy for enhancing *LDLR* transgene expression. How this approach would compare to combining *LDLR* gene transfer with regular statin therapy, which is the standard of care in HoFH, is of importance and remains to be established.

Induced pluripotent stem cell-based cell (iPSC) transplant therapy—iPSCs are somatic cells generated by overexpression of specific transcription factors. As orthotopic liver transplantation is curative in HoFH [2], autologous transplantation of genetically corrected cells derived from iPSCs has been of interest as a way of harnessing the benefits of transplantation while avoiding its undesirable effects. It has been shown that iPSCs derived from patients with familial hypercholesterolemia (FH) can be differentiated into hepatocyte-like cells that closely recapitulate the FH phenotype in vitro [56,57]. Building on this, Fattahi et al. showed that transfecting iPSCs derived from a HoFH patient with a plasmid containing the *LDLR* transgene generated hepatocyte-like cells that expressed LDLR mRNA and were able to internalize LDL under feedback regulation from extracellular LDL-C [58]. In a subsequent study, Ramakrishnan et al., showed that LDL internalization into such cells was also responsive to extracellular lovastatin and sterol, consistent with the restoration of physiological LDL metabolism [59].

In vivo data in HoFH preclinical models are not yet available, however, corrected iPSCs that were transplanted into the liver of fumarylacetoacetate hydrolase deficient (FAH) mice, another inherited metabolic disease of the liver, reversed the disease phenotype [60]. Of note, however, genetically corrected hepatocytes in FAH mice possess a unique survival advantage to native hepatocytes and expand robustly to repopulate the liver. Conversely, engraftment and repopulation of the liver is known to be less effective with iPSCs than with mature human hepatocytes [61]. Translation of iPSC technology to the clinic will also require significant refinement of the iPSC technology itself to ensure the genetic stability of derived cells, production of high-quality iPSCs, and efficient and reproducible differentiation to fully functional hepatocyte-like cells [61]. In addition, the risk of tumorigenesis associated with retained stem cells and inadvertently activated oncogenes during reprogramming must be mitigated in cells that are to be used therapeutically [62]. Thus, although appealing, the iPSC technology remains very much experimental and is unlikely to be a viable therapeutic for HoFH in the near future.

Conclusion

After more than two decades, gene therapy for HoFH has returned to clinical testing. The AAV vector class which has been widely successful in clinical trials in other Mendelian diseases is the backbone on which this agent has been developed. The recently announced first-in-human clinical trial is expected to recruit a dozen HoFH subjects, who will receive a

recombinant *LDLR*-expressing AAV8 vector with follow-up of up to five years. It is worth noting that none of the AAV gene therapy agents that have been clinically successful in the past decade have produced complete reversal of the disease phenotype. As such, the recombinant *LDLR*-expressing AAV in testing is unlikely to prove curative but might produce clinically significant LDL-C reduction and improved response to lipid-lowering agents such as PCSK9 inhibitors. Nevertheless, a 'cure' remains the ultimate goal of any gene therapy approach. Achieving this will depend on achieving sufficient and sustained levels of stable *LDLR* expression. Bioengineering strategies have been employed to develop gain-of-function *LDLR* transgenes and AAV vectors adapted to enhance transgene transfer, transduction efficacy and minimize immunogenicity compared to their natural occurring AAV counterparts. Combining *LDLR* gene therapy with RNA silencing of *HMGCR* is a further strategy intended to enhance *LDLR* gene expression. These approaches are still largely in pre-clinical development however, with promising early results, it is hoped that they will advance us further towards a genetic cure for HoFH in the future.

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Table

Gene therapy agents in recent development for Homozygous Familial Hypercholesterolemia

Agent	Stage of development	Efficacy data	Safety data
Recombinant <i>LDLR</i> -expressing AAV8 (AAV8.TBG.h <i>LDLR</i>)	Phase I/2a first-in-human study (NCT02651675). 12 patients. Primary safety endpoint at 1 year. Total follow-up of 5 years.	<ul style="list-style-type: none"> In <i>Ldlr</i>^{-/-}/<i>Apobec1</i>^{-/-} mice maintained on a high-fat diet for two months prior to the administration of AAV8.TBG.m<i>LDLR</i> and then followed and analysed after two further months on this diet, TC was reduced by ~1500mg/dL and atherosclerotic burden by 87% [35]. Lipid lowering effects were maintained for up to 6 months post-vector administration in a cohort of <i>Ldlr</i>^{-/-}/<i>Apobec1</i>^{-/-} chow-fed mice. Comparable findings in <i>Ldlr</i>^{-/-}/<i>Apobec1</i>^{-/-} hApoB-Tg mice treated with AAV8.TBG.h<i>LDLR</i> in [36] 	<ul style="list-style-type: none"> In <i>Ldlr</i>^{-/-}/<i>Apobec1</i>^{-/-} mice that received AAV8.TBG.m<i>LDLR</i>, hepatic lipid accumulation measured at day 35 and transaminases measured at day 60 post-vector administration were within normal limits [35]. In <i>Ldlr</i>^{-/-}/<i>Apobec1</i>^{-/-}/hApoB-Tg given the highest deliverable dose of AAV8.TBG.h<i>LDLR</i> (5×10¹³ GC/kg), no transgene or capsid-specific T cell responses were detected. There was also no significant rise in transaminases and no hepatic inflammation or lipid accumulation in AAV8.TBG.h<i>LDLR</i>-treated mice [36].
Gain-of-function <i>LDLR</i> variants (delivered within recombinant AAV8 vectors): <ul style="list-style-type: none"> AAV8.TBG.h<i>LDLR</i>-L318D (<i>confers PCSK9 resistance</i>)* AAV8.TBG.h<i>LDLR</i>-K809R/C818A (<i>confers IDOL resistance</i>)* AAV8.TBG.h<i>LDLR</i>-L318D/K809R/C818A (<i>confers resistance to PCSK9 and IDOL</i>)* 	Pre-clinical (in vivo)	<ul style="list-style-type: none"> Humanized HoFH mice that received AAV8.TBG.h<i>LDLR</i>-L318D, AAV8.TBG.h<i>LDLR</i>-K809R/C818A or AAV8.TBG.h<i>LDLR</i>-L318D/K809R/C818A had lower TC levels and higher <i>hepatic LDLR</i> expression in the context of IDOL or PCSK9 over-expression compared to those that received the wild-type <i>LDLR</i> transgene [53]. 	N/A
<i>HMGCR</i> -specific miRNA/siRNA	Pre-clinical (in vivo)	<ul style="list-style-type: none"> Co-transfecting a plasmid containing a reporter gene driven by <i>LDLR</i> genomic regulatory elements with <i>HMGCR</i>-specific siRNA or miRNA increased <i>LDLR</i> promoter activity 300-fold and 12-fold, after 7 and 10 days, respectively, in vitro. In vivo, co-transfecting 4 <i>HMGCR</i>-specific miRNA plasmids with 	N/A

Agent	Stage of development	Efficacy data	Safety data
		an <i>LDLR</i> -expressing plasmid with <i>LDLR</i> regulatory elements lowered plasma LDL-C by ~1.5mmol/L (p =0.0036) compared to mice treated with the <i>LDLR</i> plasmid alone [55].	
Genetically corrected iPSC	Pre-clinical (in vitro)	• Transfection of <i>LDLR</i> -expressing plasmids into iPSCs derived from patients with HoFH generated hepatocyte-like cells with restored LDL internalisation under feedback control from extracellular cholesterol, statin and sterols [58,59].	N/A

* All three agents are recombinant AAV8 vectors expressing gain-of-function h*LDLR* variants resistant to PCSK9/IDOL or both. PCSK9 and IDOL are negative regulators of *LDLR*.

Key; Apobec, apolipoprotein B mRNA editing catalytic polypeptide-1; gc, gene copies; hLDLR, human LDLR; HMGCR, HMG CoA reductase.; IDOL, inducible degrader of LDLR; iPSC, induced pluripotent stem cell; miRNA, microRNA; TC, total cholesterol; siRNA, small interfering RNA oligonucleotides