Novel methodologies in siRNA delivery for familial hypercholesterolaemia patients unsuitable for statin treatment

Introduction

High cholesterol is one of the leading causes of poor cardiovascular disease (CVD) outcomes worldwide, with CVD providing the NHS with a yearly cost burden of £7.4 billion and globally responsible for 2.6 million deaths in the same period. As such, combatting abnormal cholesterol levels is a key pathway to improving not only the lifespan of individuals in society but also the length of healthy years of life that patients experience.

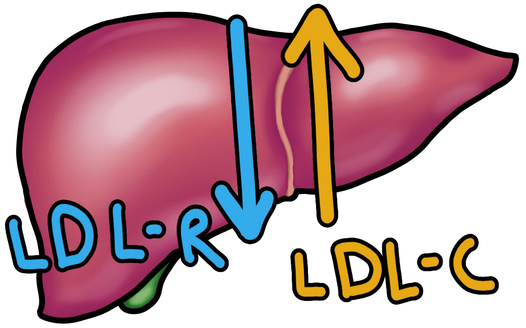
In this poster we will present a novel administration pathway for a cholesterol reducing small interfering RNA (siRNA) treatment known as inclisiran which targets the PCSK9 enzyme. We will take advantage of both traditional delivery molecules in the form of GalNAc conjugates, and new developments in siRNA delivery in the form of hydrogel delivery matrixes.

Familial Hypercholesterolemia

The genetic disorder of familial hypercholesterolemia (FH) can occur due to mutations in some gene resulting in LDL cholesterol (LDL-C) greater than 190 mg/dl in heterozygotes and greater than 450 mg/dl in homozygotes for that gene. One example, is a defect in the LDL receptor (LDL-R) leading to it malfunctioning or missing entirely, thus LDL-C uptake into the cell - where it is broken down - is not possible. Hundreds of mutations of the LDL receptor have been identified, which express themselves as hypercholesterolemia and those that can be passed down are known as 'familial'.

Clinical Significance of FH

Familial hypercholesterolemia in the UK population is believed to affect approximately 1 in 250 people. It is estimated that 270,000 of people in the UK have it [12]



A person afflicted with FH leading to higher LDL cholesterol levels can experience a greater increase in risk of:

- Heart attacks
- Strokes
- Premature and accelerated coronary artery disease
- Sudden cardiac arrest
- Peripheral artery disease
- Microvascular disease
- Pulmonary embolisms (Blood clotting)

Current Treatments and Management

Statins are currently considered the gold standard in the treatment of patients afflicted with FH. However, factors need to be considered before the prescription of statins. For example:

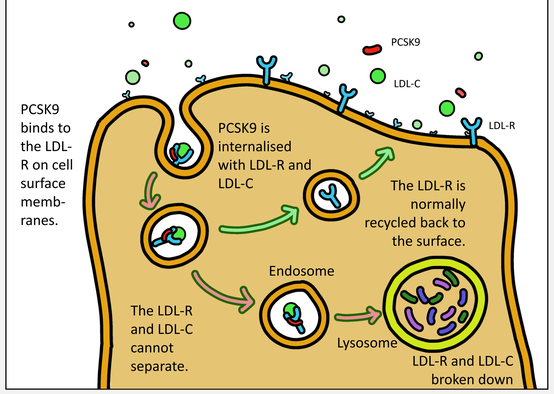
- Age (>70 years)
- Family history of liver disease
- Alcohol intake
- Hyperactive thyroid
- History of adverse effects when taking statins
- Family history of myopathy

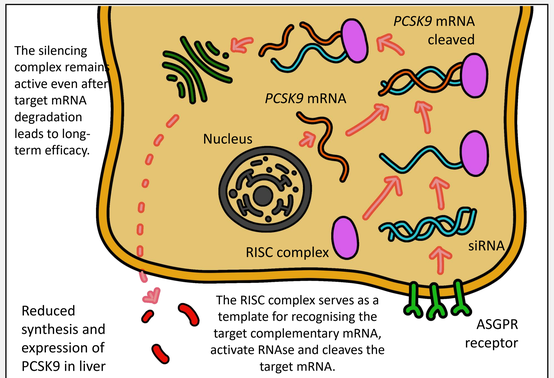
As such, many patients (approximately 5%), are unable to take statins. Therefore, other forms of treatment must be able to be provided for this selection of people.

What is PCSK9?

What is PCSK9?
Proprotein convertase subtilisin kexin type 9 (PCSK9) is a protease enzyme present and synthesised in hepatocyte cells, the major functional cells in the liver. PCSK9 plays a pivotal role in the regulation of cholesterol levels in the bloodstream. Specific mutations in the *PCSK9* gene can lead to familial hypercholesterolemia.

The binding of PCSK9 to LDL-R on the surface of liver cells prevents the separation of the LDL-R and the LDL within the endosome. Thus, PCSK9 appears to divert internalised LDL receptors to degradation in lysosomes and preventing them from recycling to the cell surface. Alternatively, the PCSK9-LDL receptor complex could be actively recognised and directed towards lysosomes. As a result, LDL-R expression decreases, and uptake of LDL-C by hepatocytes decreases.





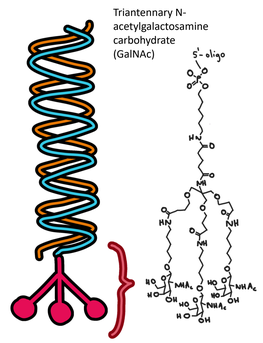
How do we inhibit PCSK9?

One method of treatment is through the use of small interfering RNAs. These are short nucleic acids that silence the expression of PCSK9 in liver cells. As a result, they inhibit the secretion of PCSK9 into the bloodstream. A recently developed siRNA treatment is inclisiran.

Inclisiran is the first siRNA drug approved by the US FDA and the European EMA for treatment of hypercholesterolemia, mixed dyslipidemia or heterozygous familial hypercholesterolemia (HeFH). It provides patients unsuitable for statin treatment with an alternative pathway to returning to regular health. We aim to improve the efficacy of the drug through utilisation of modern siRNA delivery systems, which help to maximise siRNA delivery efficacy, thereby improving patient outcomes.

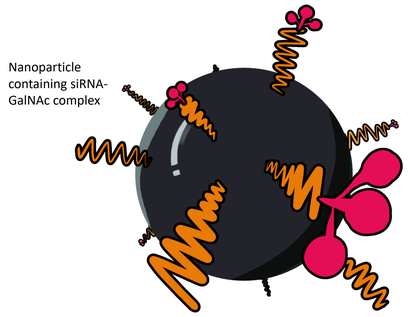
Delivery Mechanisms of Inclisiran

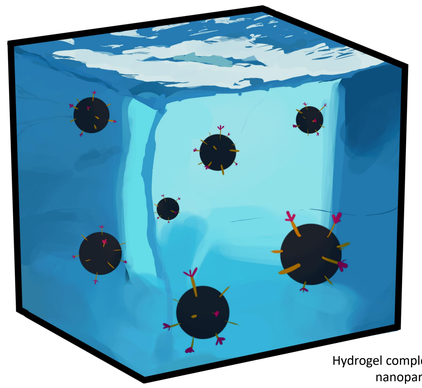
SiRNA is a highly unstable molecule when placed in the human body, with a range of mechanisms designed for clearance of free RNA from cells and the blood stream. This provides many issues in the creation of effective siRNA therapeutics such as inclisiran. Delivery mechanisms therefore are a highly important area to optimize in order to reduce the clearance of siRNA and maximise expression. Our proposal is a novel solution for improving efficacy of inclisiran treatment, incorporating traditional GalNAc conjugate delivery of siRNA with a hydrogel system to allow for extended release post-injection, with higher siRNA stability, and aims of decreasing both siRNA clearance and residual *PCSK9* function [13].



GalNAc conjugates enable precision targeting to liver cells, exploiting a natural receptor interaction with the GalNAc ligands, through the highly expressed asialogylcoprotein receptor (ASGPR) in the liver. They have very high affinity - exhibiting specific and efficient uptake into hepatocytes as well as reduced immune response that enables their suitability for repeated administration [14]. This enables high specificity liver delivery and ensures siRNA-mediated *PCSK9* inhibition in specifically hepatocytes, which make up 80% of the liver's mass and are responsible for PCSK9 protein production [15]

The nanoparticle mechanism carrying the GalNAc conjugates was proposed in previous research into delivery mechanisms and tested with the goal of combatting liver cancers. This has identified the use of cholesterol-modified antimicrobial peptide DP7 (DP7-C) acting as a carrier - a nanoparticle that enables the inclusion of GalNAc conjugated siRNA into the hydrogel network [16].

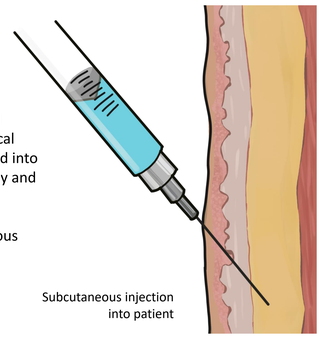




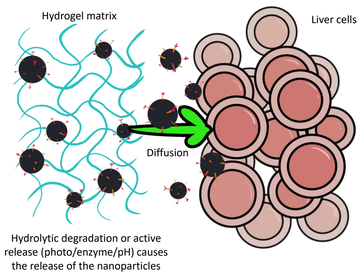
Hydrogel complex containing nanoparticles

A hydrogel delivery system provides a substance that allows for long-term suspension of siRNA-containing carrier molecules, allowing for sustained release [17] which ensures maximal RISC complex binding.
Hydrogel systems also allow for pulsatile release, where groups of siRNA are released at a time, allowing for latent siRNA to remain preserved within the body and released on demand. This can allow for dynamic response to changes in PCSK9 levels, increasing siRNA release to ensure the siRNA treatment produces lasting efficacy [18].

The hydrogel protects siRNA from degradation by enzymes, preserving functional integrity [16] as the siRNA is not cleared from the body as it would usually be. Local delivery is also enabled through the use of a hydrogel, as the contents are injected into the local cell environment which minimises exposure to other systems of the body and off target delivery to other cells expressing receptors involved in drug uptake [19]. With the inclusion of GalNAcs this is a reduced consideration, but remains a minor benefit of localising a hydrogel treatment to the liver area with subcutaneous injection.



Subcutaneous injection into patient



The synergistic combination of these delivery mechanisms creates a potent delivery system that harnesses the strengths of both methodologies. A hydrogel matrix loaded with GalNAc conjugated siRNA ensures maximally targeted delivery to hepatocytes while providing a protective environment that enhances siRNA stability. This protects siRNA uptake into hepatocytes which ensures gene silencing effects are exerted through binding to the RISC complex and minimises residual expression of *PSCK9*.

Clinical Trials

Inclisiran and GalNAc conjugates are already in use and approved for the treatment of familial hypercholesterolaemia. Questions around their efficacy with current delivery methods have been raised in the ORION clinical trials [17] , and as such our aim will be to identify if our hydrogel delivery mechanism increases drug efficacy.

Finally, trialling will discover whether any contraindications exist due to patient variations, allowing us to better understand the safety profile of the hydrogel delivery mechanism *in vivo*.

Stage 1:
Testing the hydrogel system in vitro on human tissue samples in petri dishes. This will ensure there are no negative impacts that are produced due to the use of the hydrogel. Mice testing in vivo will identify drug functionality in an animal model.

Stage 2:
After checking for initial toxicity, the system will be approved for stage 2 trialling where healthy volunteers will be given the injection to test for safety and the presence of unforeseen side effects. This will help identify if the hydrogel system has contraindications with certain patient profiles.

Stage 3:
This stage involves testing on a larger group of roughly 100 to 200 individuals with familial hypercholesterolemia to allow for comparison with standard inclisiran treatment to evaluate efficacy of the hydrogel delivery system in producing a longer efficacy period.

Stage 4:
Testing on different groups within the population will be carried out to test for any variation between patients particularly in terms of changes in efficacy or side effects.

Long Term:
Once the drug has been approved for use and recommended as cost effective by NICE, it will be slowly introduced as a management technique for those unsuitable for statin treatment. Regular re-evaluation will be performed to ensure the treatment is both safe and effective

Socio-economic Viability

Cost

- Based on research, we approximate that the total cost of this treatment will be ~ £4500, aligning with the average costs of current PSCK9 treatments.
- The treatment will therefore be specifically targeted at those with FH who are intolerant to statins.
- Some estimates suggest less than 5% of statin users experience some form of clinical intolerance. This could equate to as many as 350'000 statin users.
- We believe that this treatment will save money in the long term by limiting costs of apheresis.
- Studies involving current *PSCK9* inhibitor treatments e.g. Alirocumab show that lipoprotein apheresis was discontinued in 63.4% of patients on alirocumab who were previously undergoing regular apheresis, and the rate was at least halved in 92.7% of patients. [11] This can cost up to £1200 per session so use of PSCK9 inhibitors can reduce long term costs.

Feasibility

- SiRNA therapies such as Inclisiran are already approved for use by NICE, however it is still a relatively new treatment option and so long-term effects have not been studied.
- Our proposal of a hydrogel delivery mechanism will aim to maximise efficacy of the drug and improve patient outcomes.

Efficacy

- Compared to placebo, PCSK9 inhibitors ranked first for improving LDL-C (standardised mean difference -50.76, 95% CI), HDL cholesterol (SMD 7.73) and TC levels (-35.81) [6]. Note that statins ranked second for all of these.
- PCSK9 inhibitors were associated with similar decreased risk of CV events as statins.
- [9] Alirocumab trial demonstrated a -62% mean change from baseline in calculated LDL-C compared to a placebo group.
- The efficacy of the hydrogel delivery mechanism will be studied during clinical trials.

Social Acceptability

- The treatment is injected once and then again in 3 months and every 6 months following that. This makes the treatment option very convenient compared to daily medications, especially for those patients who may not be able to keep track of their daily medications.

Meet the Team:

Fadil Adiguna - Art & Design, research into PCSK9 and inclisiran

Michael Domarkas - Research into delivery mechanism and clinical trialling

Toni Perni - Social, economics and feasibility researcher

Nour Mansour - Research into familial hypercholesterolaemia and current treatments

References