Identification of a potent, orally bioavailable small molecule PCSK9 inhibitor for lowering LDL-cholesterol

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1 Background

- Cardiovascular disease (CVD) is the leading cause of mortality worldwide, and lowering low-density lipoprotein cholesterol (LDL-C) is an established therapeutic approach for reducing cardiovascular risk. Statins are widely regarded as the front-line therapy for lowering LDL-C but approximately 25% of patients fail to reach the desired LDL-C levels with the maximal statin dose or are intolerant to statins.
- Proprotein convertase subtilisin/kexin 9 (PCSK9) plays an important role in regulating lipoprotein metabolism by binding to low density lipoprotein receptors, leading to their degradation.
- It has been shown that naturally-occurring gain-of-function mutations in PCSK9 significantly increases the risk of heart attacks, whereas naturally-occurring loss-of-function mutations in PCSK9 significantly reduces LDL-C in humans and reduces the risk of heart attacks.
- LDL-C-lowering drugs that operate through inhibition of PCSK9 are being pursued for the management of hypercholesterolemia and reducing its associated CVD risk. In 2015, the PCSK9-blocking monoclonal antibodies alirocumab and evolocumab were approved for hypercholesterolemia. However, the identification of a small molecule, orally bioavailable PCSK9 inhibitor has proven to be a significant challenge for drug discovery, which has been explained by the expansive, 'undruggable' flat binding interface targeted by the antibodies.
- There are no oral PCSK9 inhibitors available to patients or in late-stage development.

2 Hit compound identification

- A screening campaign identified a fragment-like compound that bound to a novel, cryptic site within the PCSK9 protein with micromolar affinity.
- Building out from this fragment produced a higher affinity PCSK9 inhibitor albeit with no appreciable microsomal stability:

Hit compound	
K_D hPCSK9 (nM)	188
ACD LogP	3.2
CL_{int} (h/m; µL/min/kg)	277/234

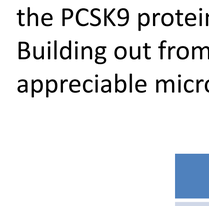
3 Medicinal chemistry

Challenges:

- Significantly improve PCSK9 affinity.
- Reduce LogP and instil microsomal and hepatocyte stability to afford low *in vivo* clearance and a good pharmacokinetic profile.

Achievements:

- Generated numerous highly resolved X-ray crystal structures to design PCSK9 inhibitors that had optimised binding interactions with the protein.
- Multiple picomolar and low nanomolar PCSK9 inhibitors were identified following this structure-based approach.



Novel, potent PCSK9 inhibitor bound into X-ray quality PCSK9 crystals

- Compound designs focussed on reducing the overall lipophilicity of the molecule, optimising cell permeability and identifying metabolic hot spots.
- This strategy provided numerous potent PCSK9 inhibitors with low plasma clearance and high oral bioavailability across pre-clinical species.

Compound 1	
K_D hPCSK9 (nM)	0.1
ACD LogP	1.7
Microsomes CL_{int} (h/m/r/cyno; µL/min/kg)	<14/<14/<14/<14
Plasma clearance (m/r/d/cyno; L/h/kg)	0.69/0.16/0.08/0.27
F (m/r/d/cyno; %)	86/84/77/100

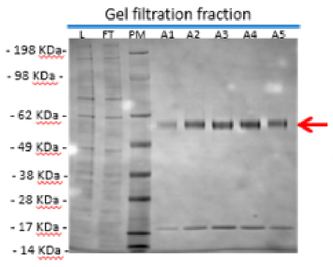
4 Biology

Challenges:

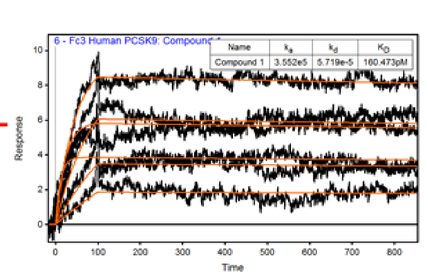
- Initial Hit Finding campaign used isothermal titration calorimetry (ITC) to measure PCSK9 affinity – ITC was not a viable primary assay for the Lead Optimisation program.
- No cell assay in the literature that we could use in our program to measure functional effect.

Achievements:

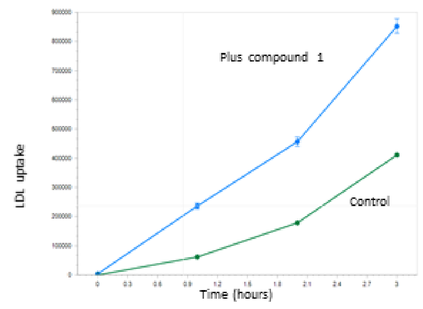
- Surface plasmon resonance (SPR) assay was developed to measure K_D for our PCSK9 inhibitors and used as the front-line assay.
- Cell assay developed with no literature precedent to measure the functional effect on LDL expression – a key decision-maker for compound progression.



Protein production of multiple constructs and species to support SPR and crystallography



SPR assay developed



Functional cell assay developed measuring exogenous LDL uptake

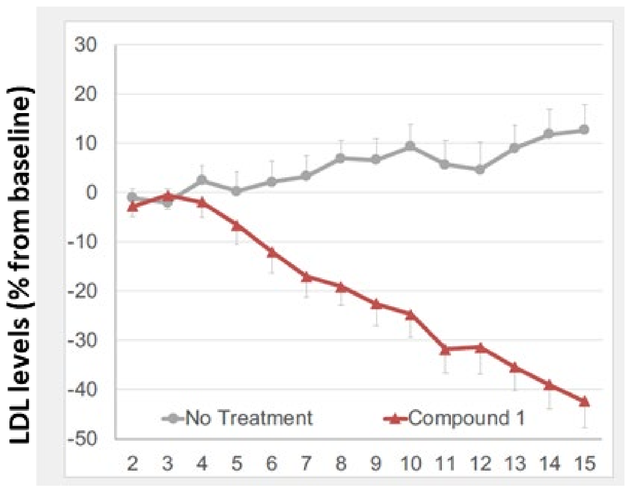
5 PD study

Challenges:

- No monkey PD studies in the literature for small molecule PCSK9 inhibitors.

Achievements:

- PK study designed to provide >85% theoretical receptor occupancy of PCSK9.



Compound 1 orally administered to non-human primates

When orally administered to dyslipidemic non-human primates, multiple PCSK9 inhibitors elicit significant and robust lowering of LDL-C following multiple days of administration

6 Acknowledgements

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7 More information

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