EPIGENETIC EDITING: NEW FRONTIER FOR GENETIC MEDICINES

TUNE THERAPEUTICS

29th June 2023

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The new frontier

Epigenomic therapies will transform the treatment of disease

protein-coding genes

epigenomic control

- Only a fraction of the genetics associated with disease involves changes in protein-coding genes.
- The vast majority of human disease is the result of changes in gene expression, driven by epigenomic regulators that control cell state and fate.

Epi-editing will unlock our ability to

change cell states at will, without damage to DNA sequence –

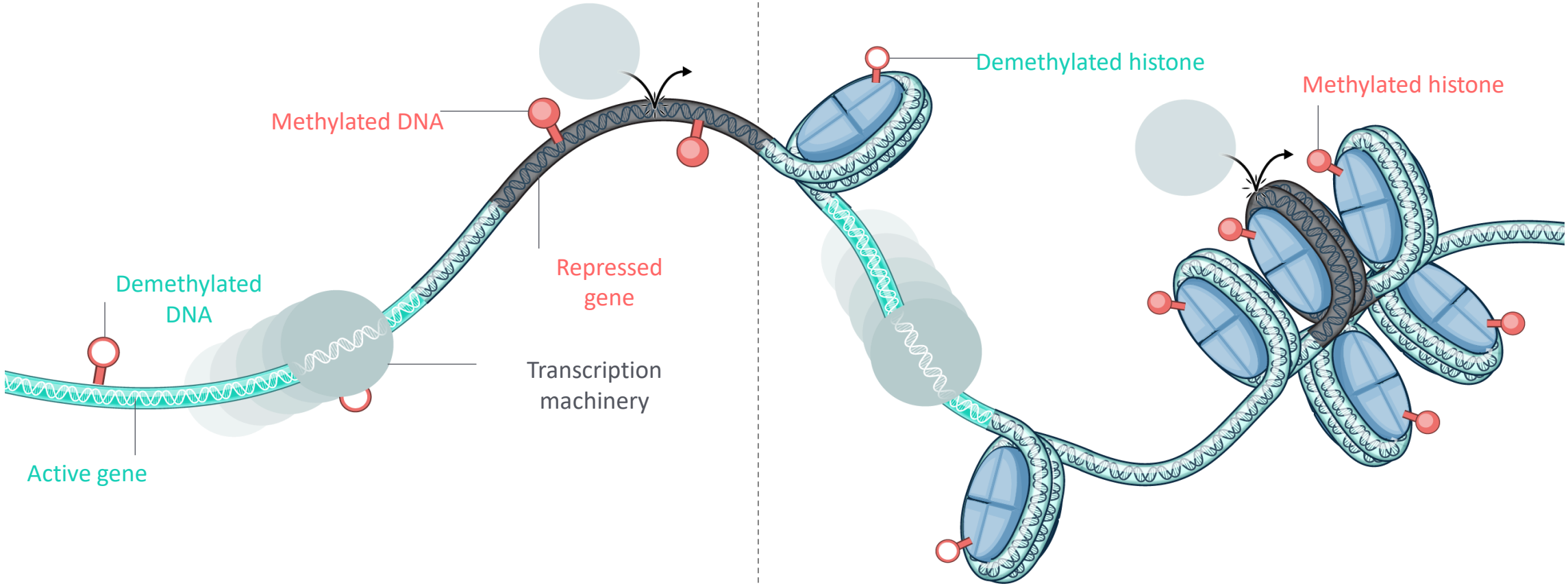
transforming the treatment of common and complex diseases

The epigenome controls levels of gene expression

Mechanisms either increase or restrict the ability of transcription factors to read DNA

DNA modification (methylation or demethylation) alters binding site recognition by transcription factors

Histone modification alters DNA shape, and can act synergistically with DNA modification to regulate expression

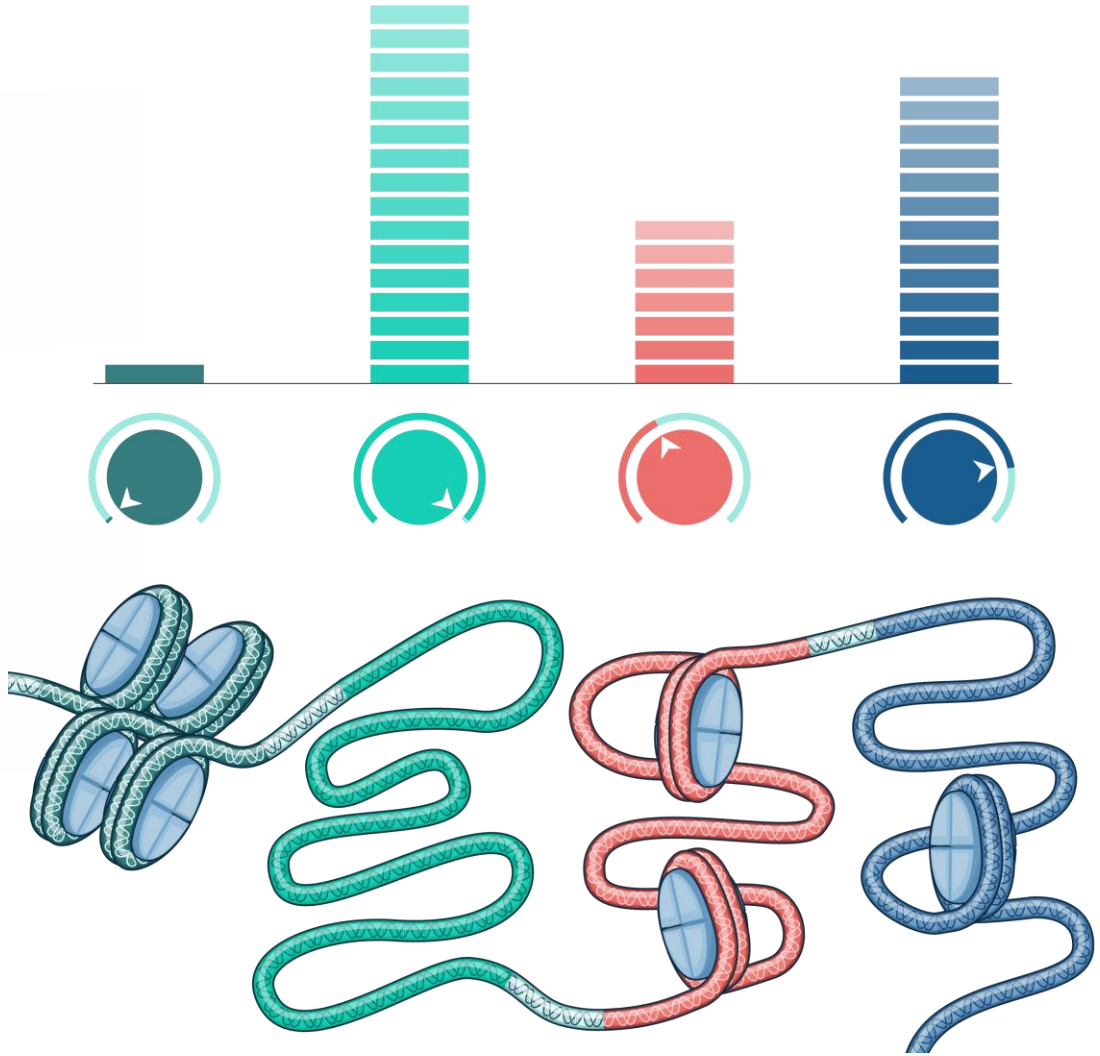


TEMPO tuning platform enables deterministic control

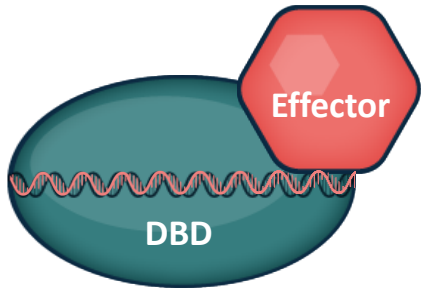
Epi-editing platform dials volume of gene expression up or down without breaking DNA

Genetic tuning with TEMPO can control which genes are expressed, and how much, by affecting the packing and accessibility of cellular DNA

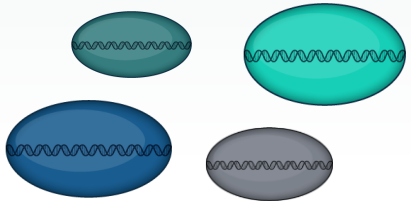
TEMPO enables extensive multiplexing – unlocking the potential to reverse pathways of complex and common disease

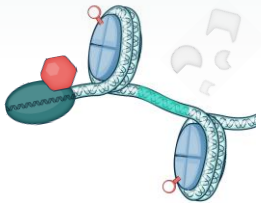
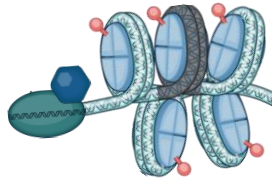
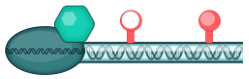
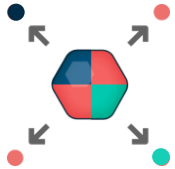


Anatomy of the modular TEMPO tuning platform- Tune's drug product



Choice of **DNA-binding and targeting domain** (DBD) mediates overall **size and potency** of construct

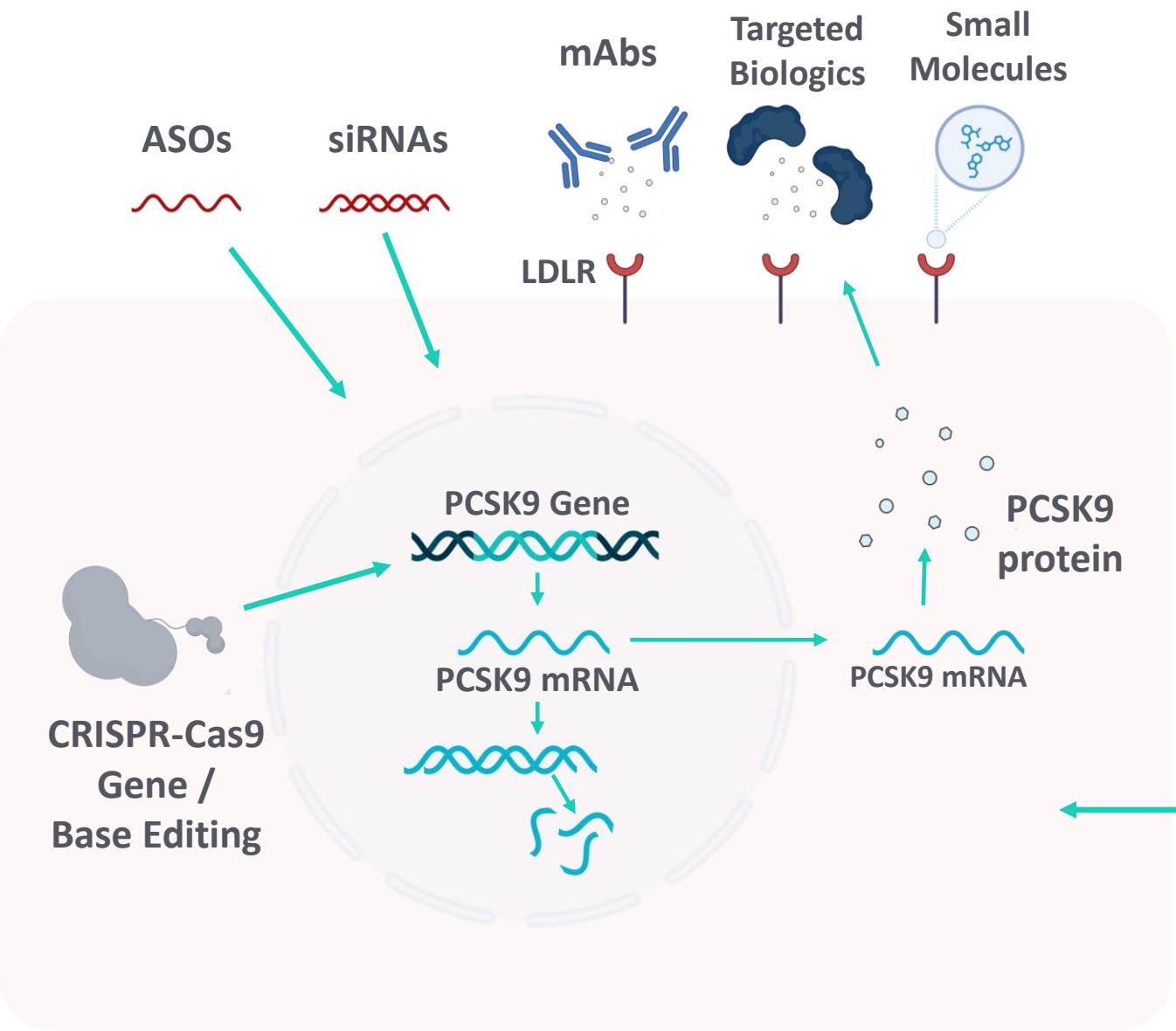


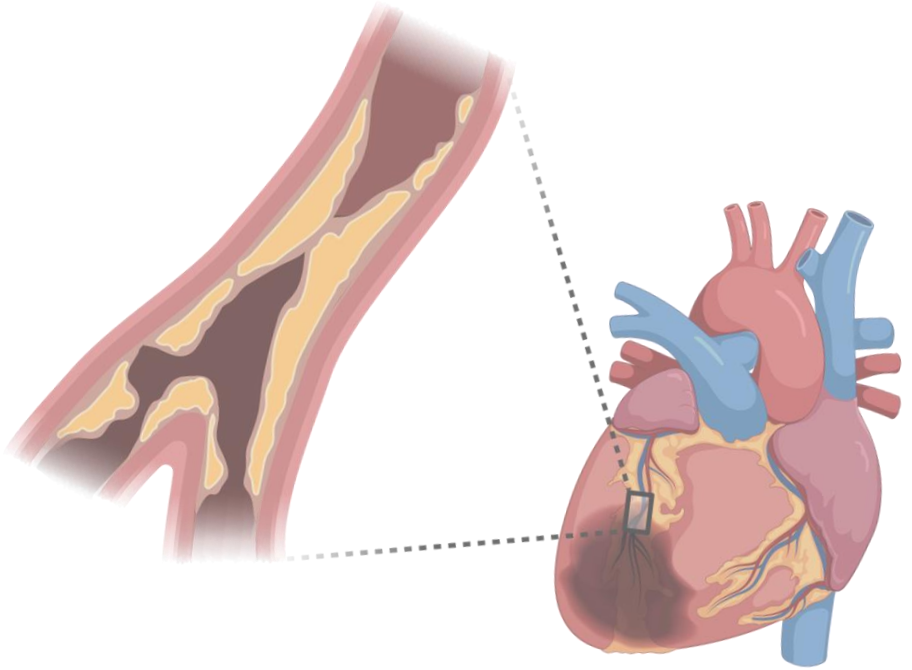
Effector controls…	native, epigenomic mechanisms		duration of effect	
VP64 / P65		Recruits transcription machinery		Transient, reversible activation
KRAB		Hetero-chromatin formation		Transient, reversible repression
TET / DNMT		DNA methylation / demethylation		Stable, durable repression or activation
Novel		Combine to create unique properties & effects		Transient or durable

Liver-Directed Disease

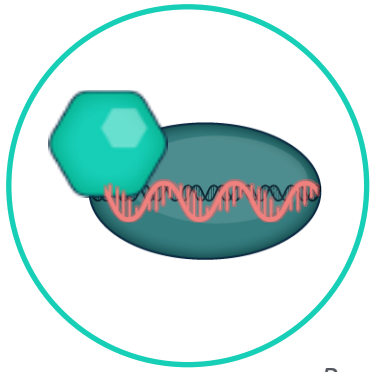
Transient Delivery, Durable Repression

PCSK9: a well-defined target for therapeutic regulation of LDL-C



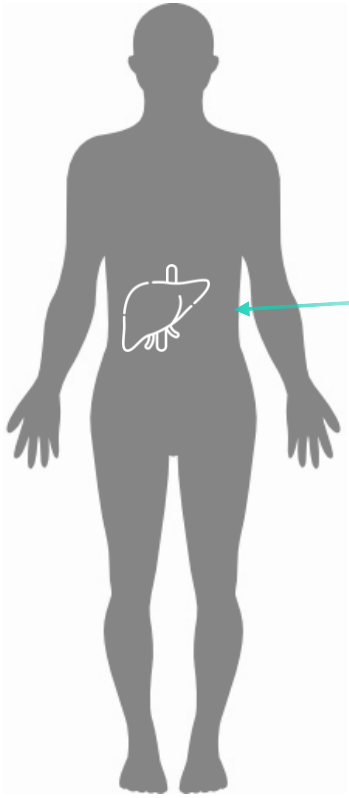


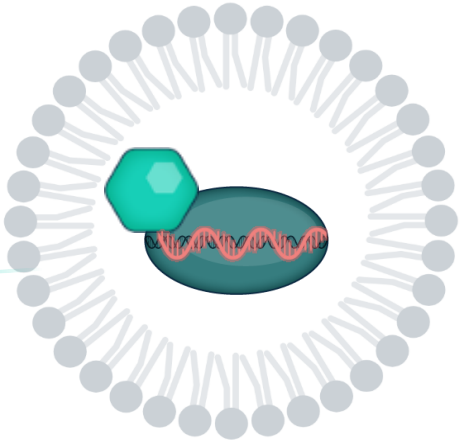
Epigenome Editing
Single dose, durable effect



Single dose of epi-editor targeting PCSK9 to durably reduce LDL-C

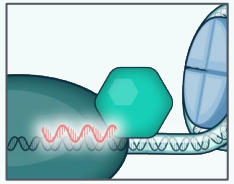
In vivo delivery with LNP





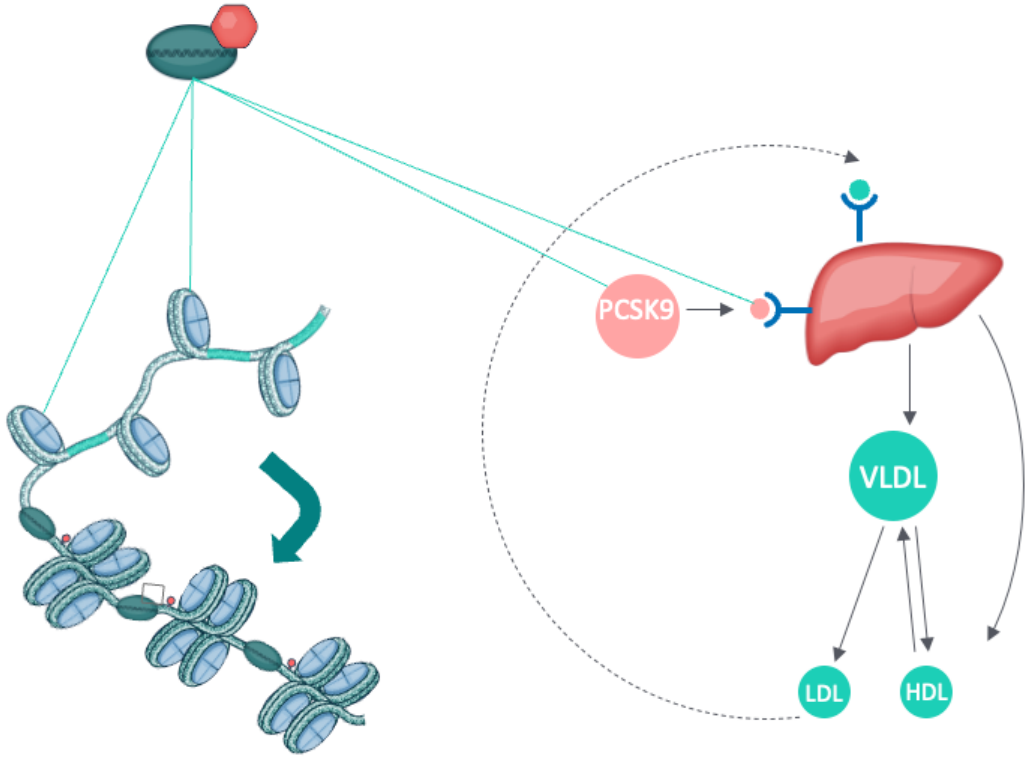
Lipid nanoparticles (LNP) make ideal transient delivery vehicles for liver targets with lipid accumulation

TEMPO drives epigenetic change

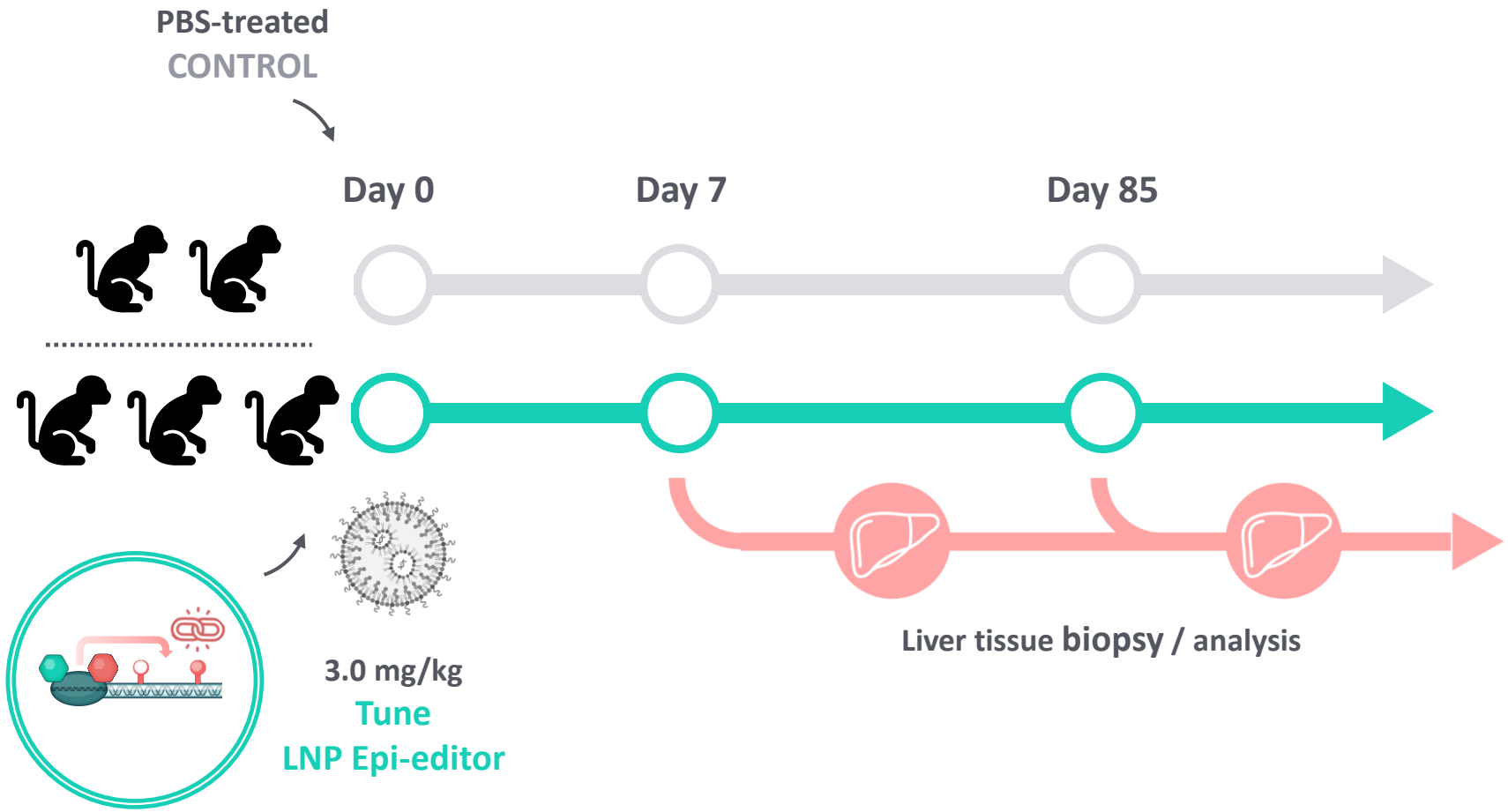


Tuned cell

Epigenetic repression of PCSK9 as a therapeutic strategy for LDL-C reduction



NHP study to demonstrate repression, durability, and safety



Primary Endpoints

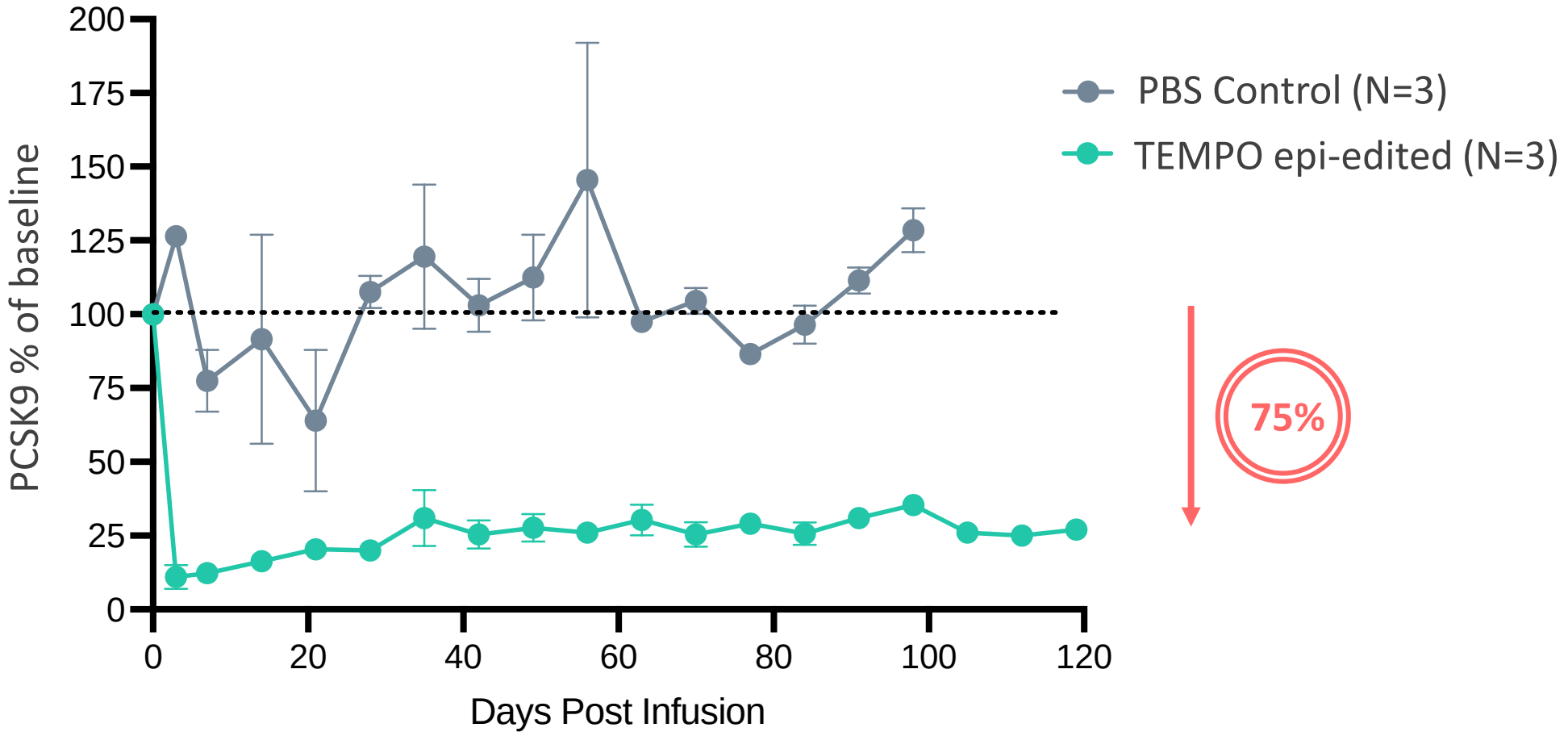
Blood

- PCSK9 protein
- LDL-C level
- Liver enzymes

Liver Biopsies

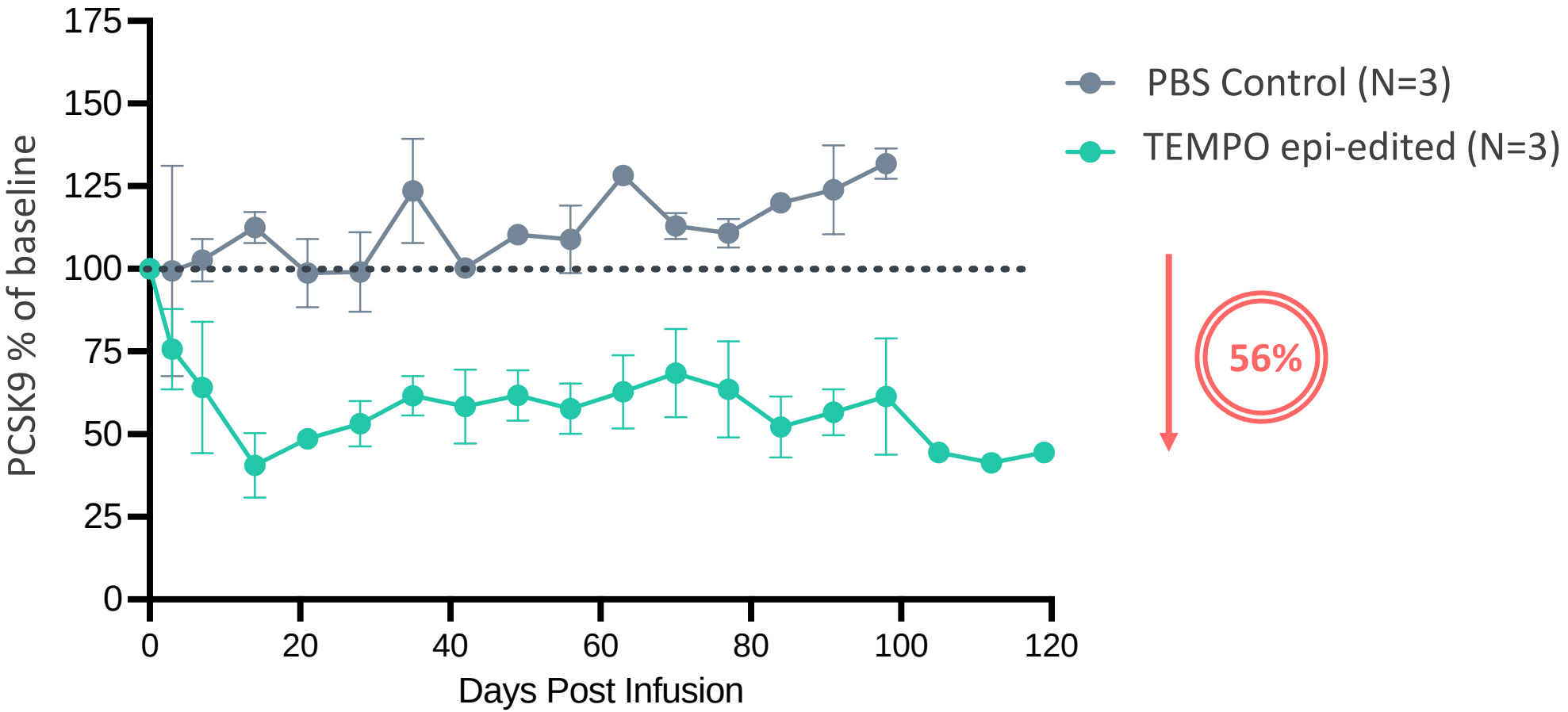
- Methylation analysis

Blood **PCSK9** protein: 75% reduction 120 days after single LNP epi-editor administration



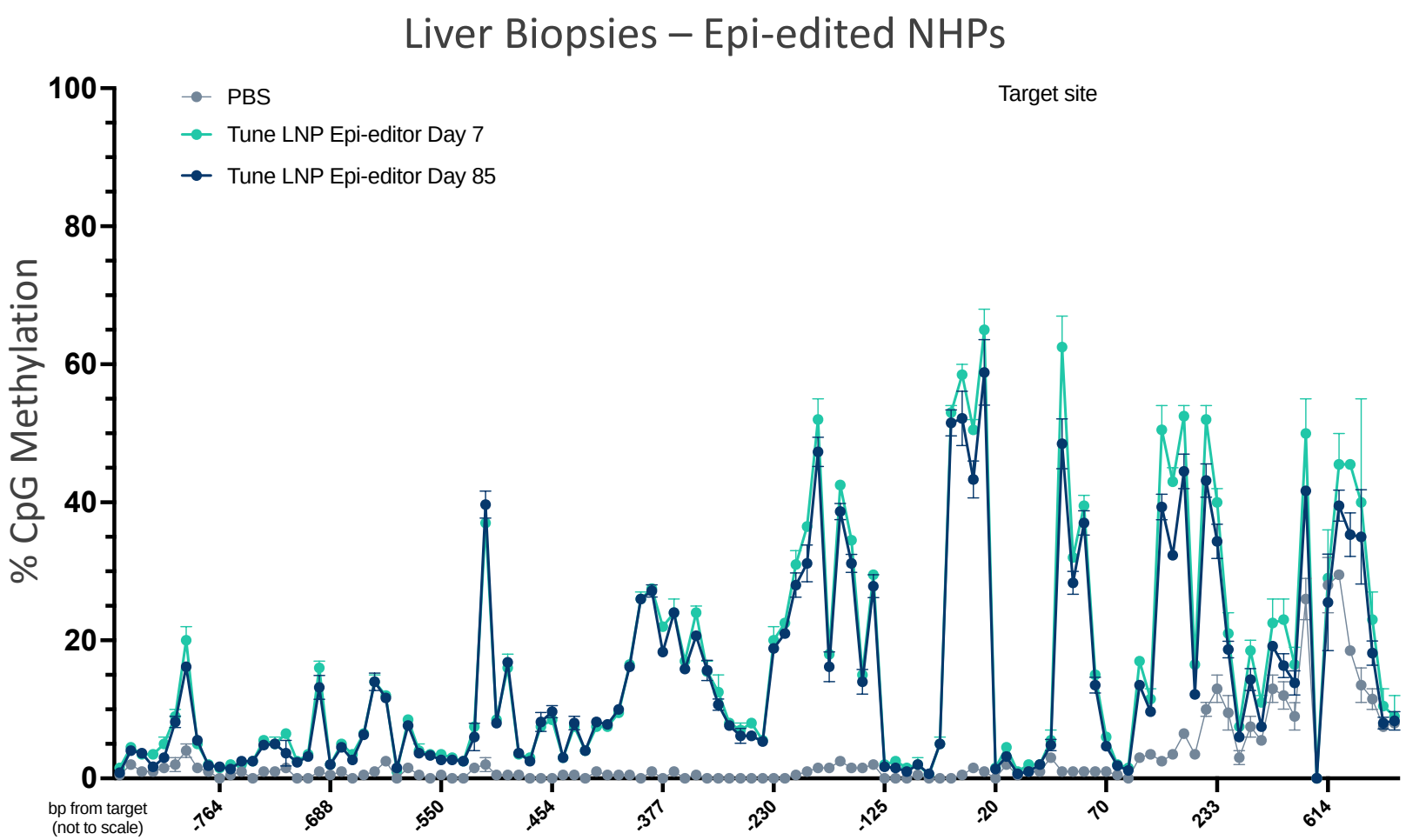
Presented at ASGCT 2023

Blood **LDL-C** protein: 56% reduction 120 days after single LNP epi-editor administration



Presented at ASGCT 2023

Methylation of PCSK9 is highly stable over 85 days

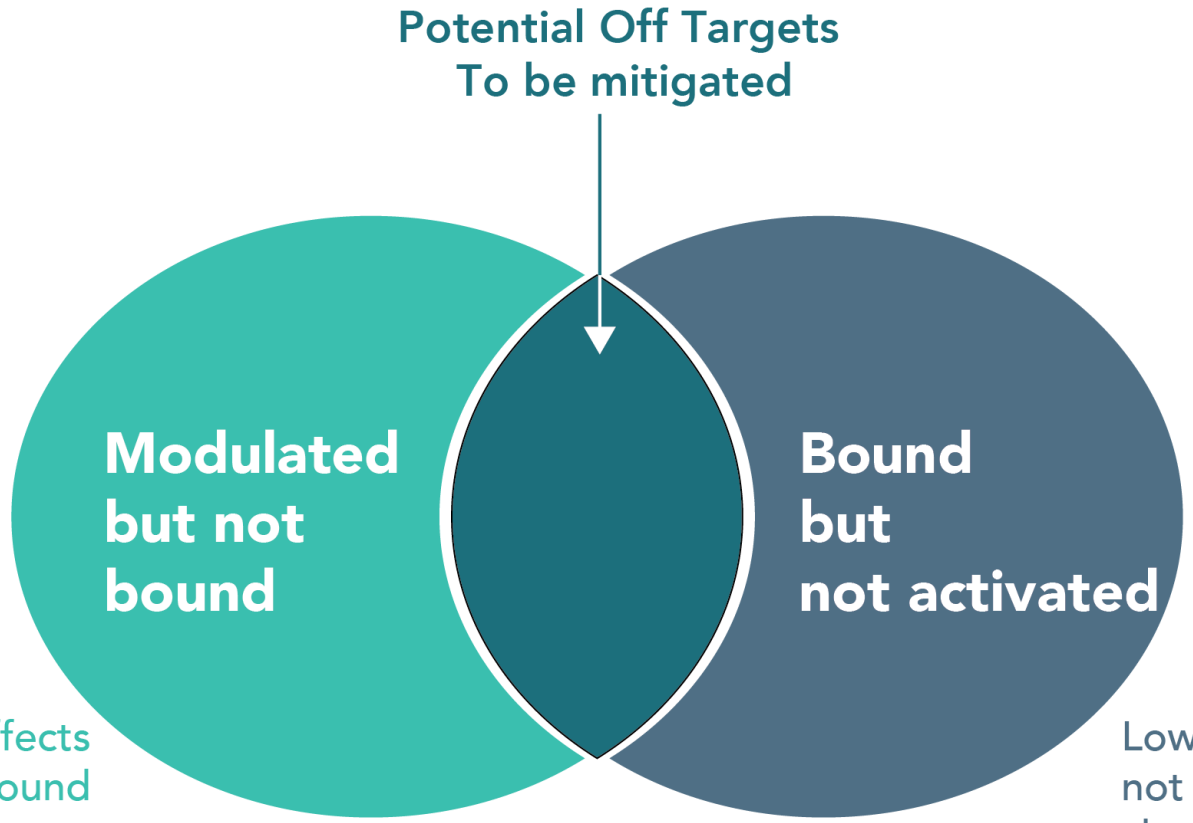


Experimental Layout

- Day 85 FNA biopsies collected
- DNA processed for Methyl-seq with panel designed around PCSK9 locus

Specificity assessment is vital for epi-editing product development

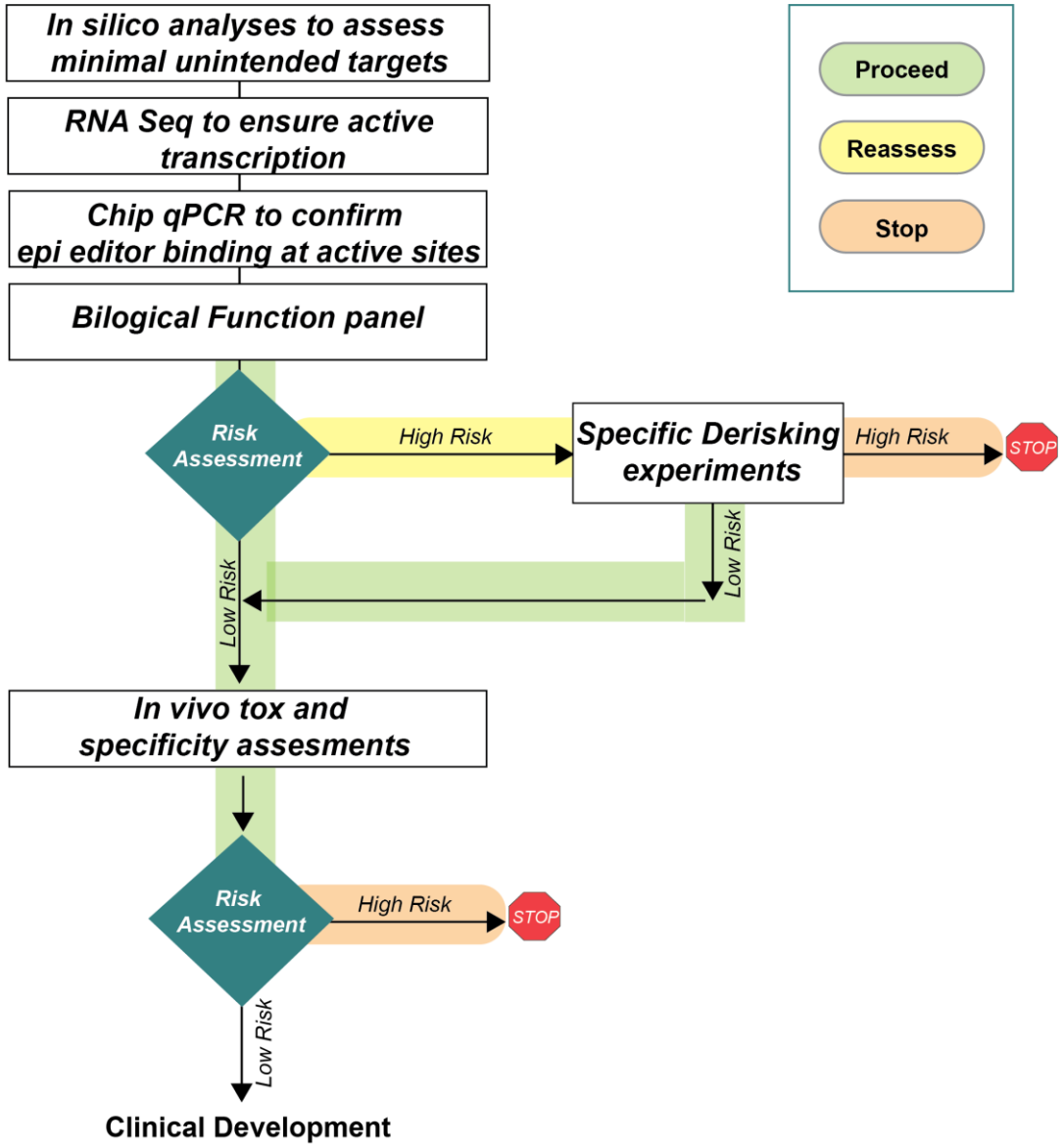
Precise epi-editing requires both binding and modulation of the epi-editor to the target gene



Likely intended secondary effects epi editor isn't bound to the gene.
Method: RNA Seq

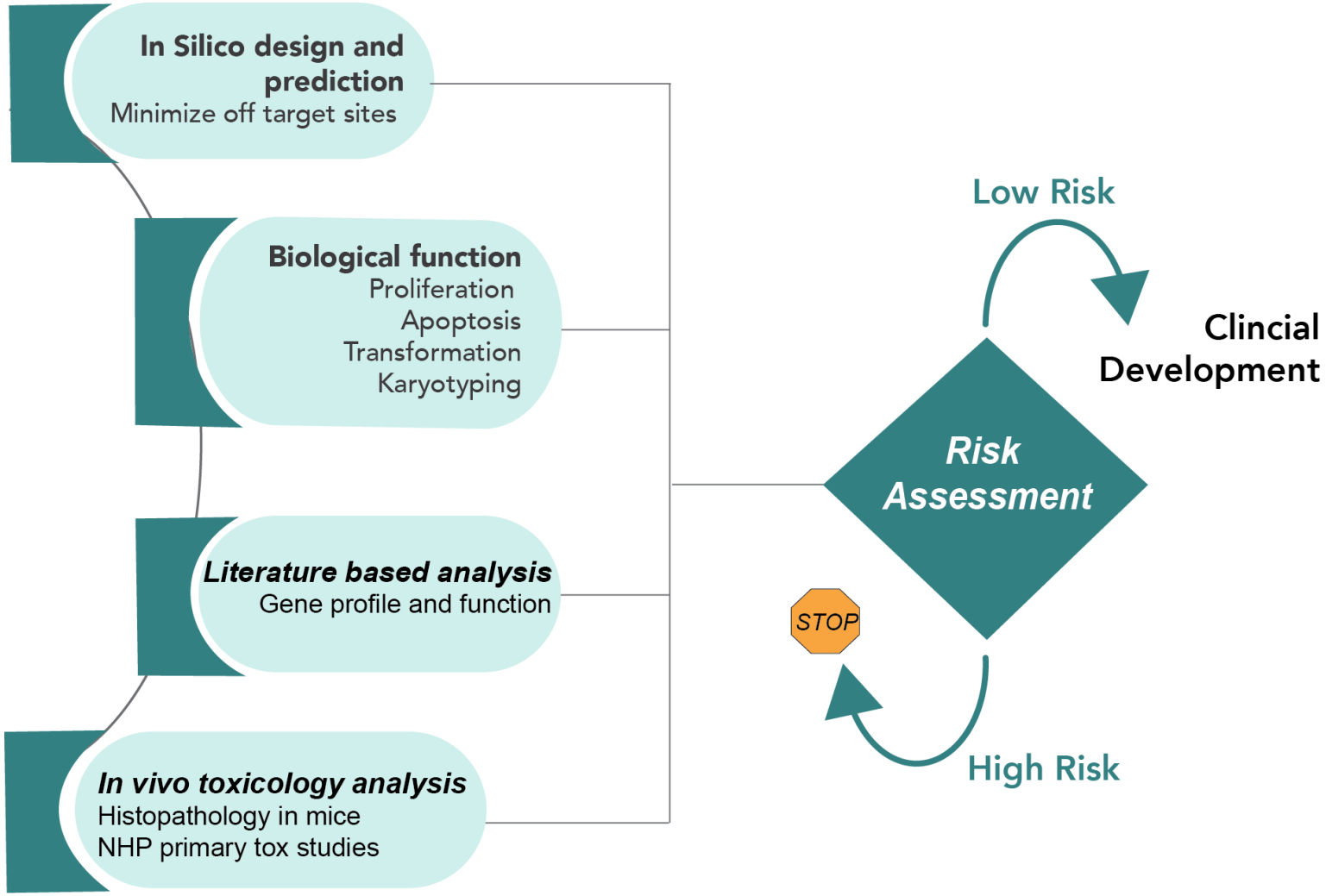
Low risk as binding of epi-editor is not causing gene expression changes.
Method: Genomewide binding assays

Path for comprehensive assessment of specificity



- *Biological function and impact analyses would be weighted the most.*
- *In Silico and predictive analyses will complement biological function.*
- *In vivo tox and specificity assessments, with an eye on functional readout would ultimately determine the path for clinical development.*

Overall risk assessment is based on multipronged input



Inherent safety features of Epi-editing

- **No DNA cuts:** Structural integrity of the chromosome is NOT impacted.
- Leverage naturally occurring epigenomic mechanisms for therapeutic purpose.
- Functional activation of genes can be accurately and precisely measured (RNA seq).

Conclusions

- Epi editing can drive expression and/or repression of genes without ***cutting or nicking the DNA.***
- Transient Tune Epi-editor demonstrated ***efficacy and durability*** of PCSK9 repression in cells lines, mice and NHP.
- The specificity strategy for mitigation of any potential off target is a holistic consideration of binding and modulation, centered around ***functional significance***.

TUNE
THERAPEUTICS

THANK YOU