circio

Disruptive circRNA technology for genetic medicine

Dr. Erik Digman Wiklund, CEO

LSX RNA Leaders USA
4 September 2024

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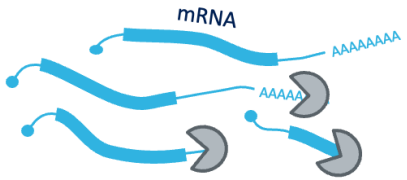
circRNA introduction

2. circVec technical development
3. circVec therapeutic application
4. Summary

The circular mRNA format improves durability and protein expression level

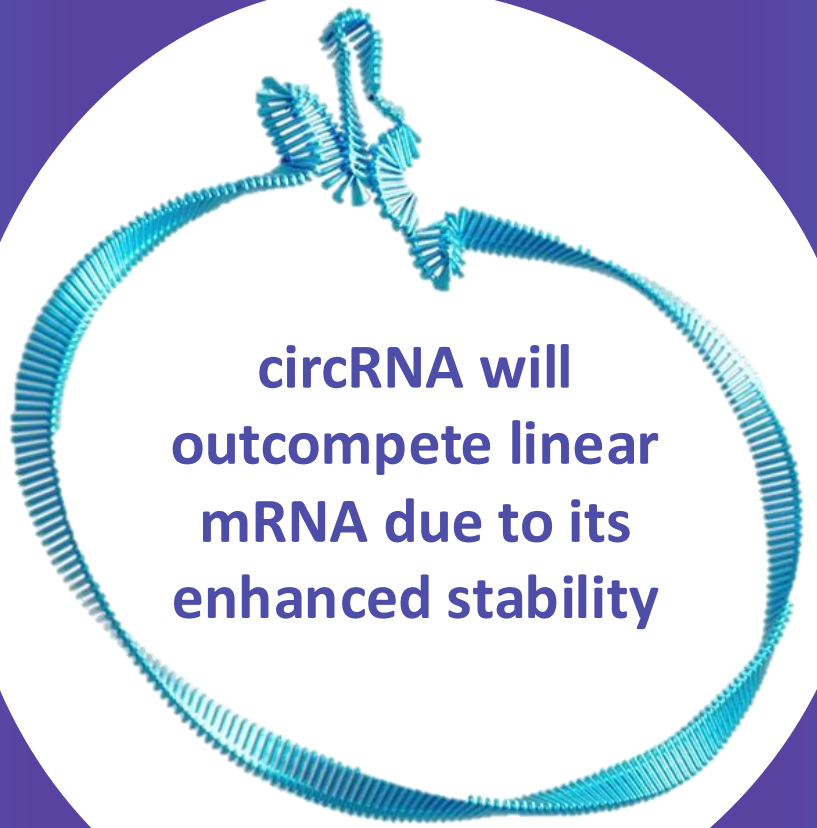
Extended RNA durability

15x half-life vs. mRNA



microRNA sponging

mRNA is destabilized by microRNAs



circRNA will outcompete linear mRNA due to its enhanced stability

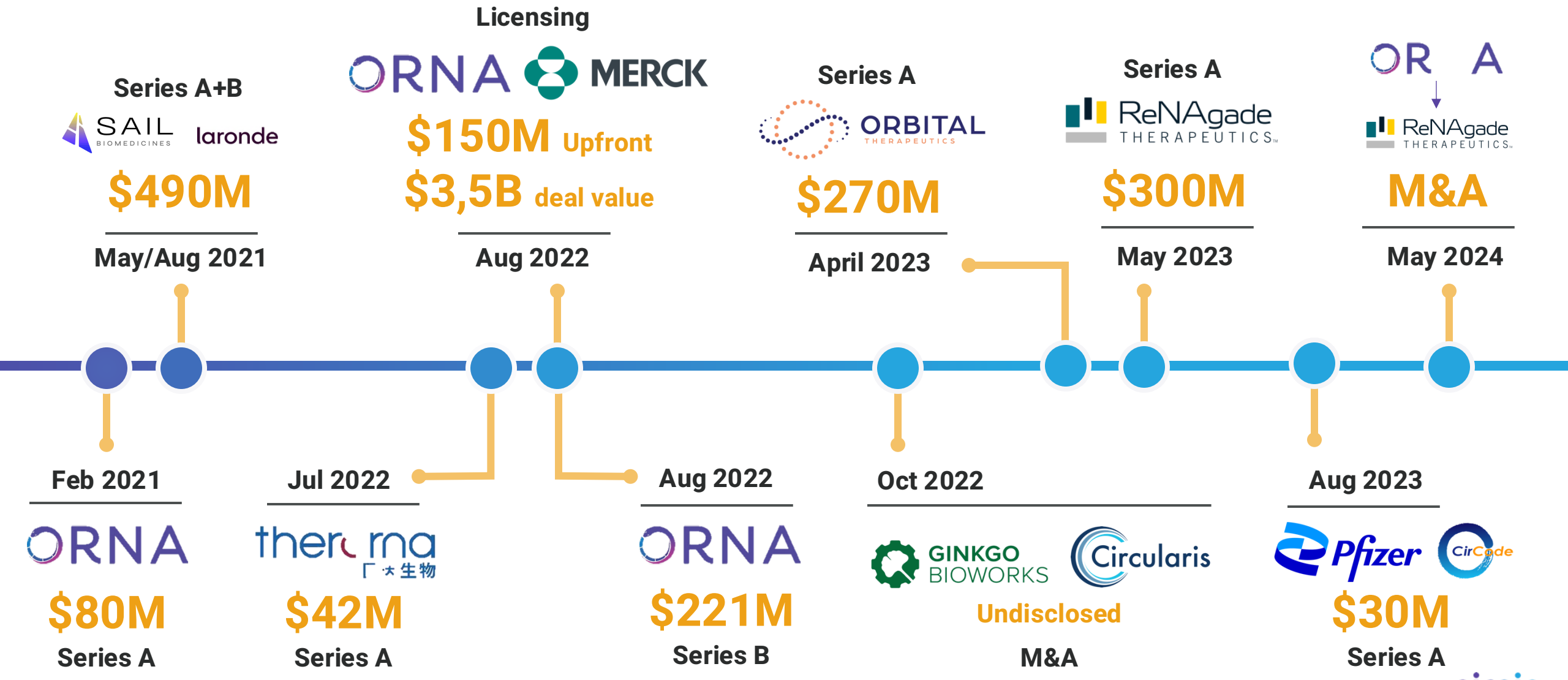
Higher protein expression

5x translation rate vs. mRNA

Modular & multi-functional

Enables ´remove & replace´ strategy

Substantial deal activity in the circular RNA space



The circRNA field was established by Circio scientists

Dr Thomas B Hansen

Dr Erik D Wiklund

nature — 7,400 citations

Published: 27 February 2013

Natural RNA circles function as efficient microRNA sponges

Thomas B. Hansen, Trine I. Jensen, Bettina H. Clausen, Jesper B. Bramsen, Bente Finsen, Christian K. Damgaard & Jørgen Kjems

THE EMBO JOURNAL — EMBOpress — 30 September 2011 — 1,000 citations

CURRENT ISSUE ABOUT INFORMATION ARCHIVE ALERTS SUBMIT

miRNA-dependent gene silencing involving Ago2-mediated cleavage of a circular antisense RNA

Thomas B Hansen, Erik D Wiklund, Jesper B Bramsen, Sune B Villadsen, Aaron L Statham, Susan J Clark, Jørgen Kjems

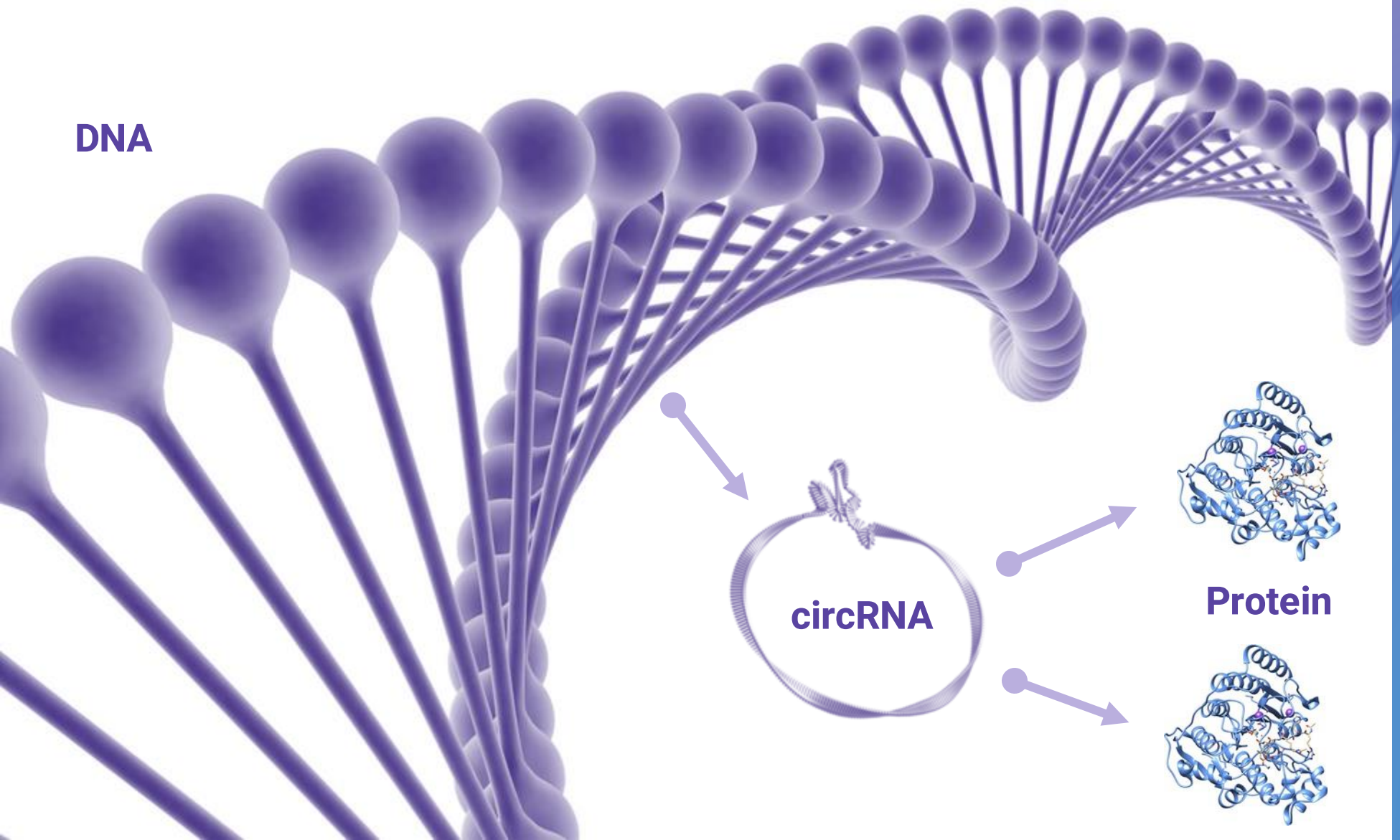
nature reviews genetics — 3,400 citations

Review Article | Published: 08 August 2019

The biogenesis, biology and characterization of circular RNAs

Lasse S. Kristensen, Maria S. Andersen, Lotte V. W. Stagsted, Karoline K. Ebbesen, Thomas B. Hansen & Jørgen Kjems

The unique circVec expression system:
Turning the patient´s cells into circRNA factories



circVec
DNA or viral vector

Inject

circRNA biogenesis

Potent and durable protein expression

Why express protein from circular mRNA?

Growth-decay model:

$$X(t) = Ce^{-Dt} + \frac{P}{D}$$

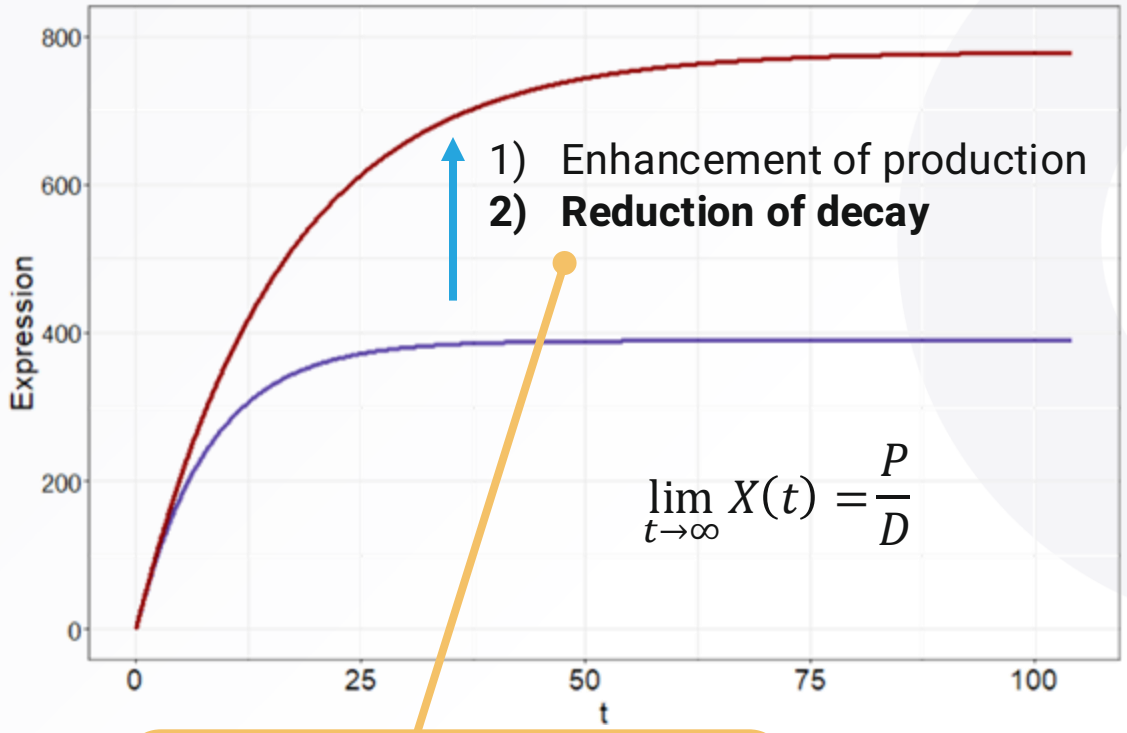
$Expression$

$P = production\ rate$

$D = decay\ rate = \frac{\log_e 2}{T½}$

Gene expression is determined by production rate and **decay rate**.

Two ways to increase expression



circRNAs are **significantly more stable** than linear mRNA

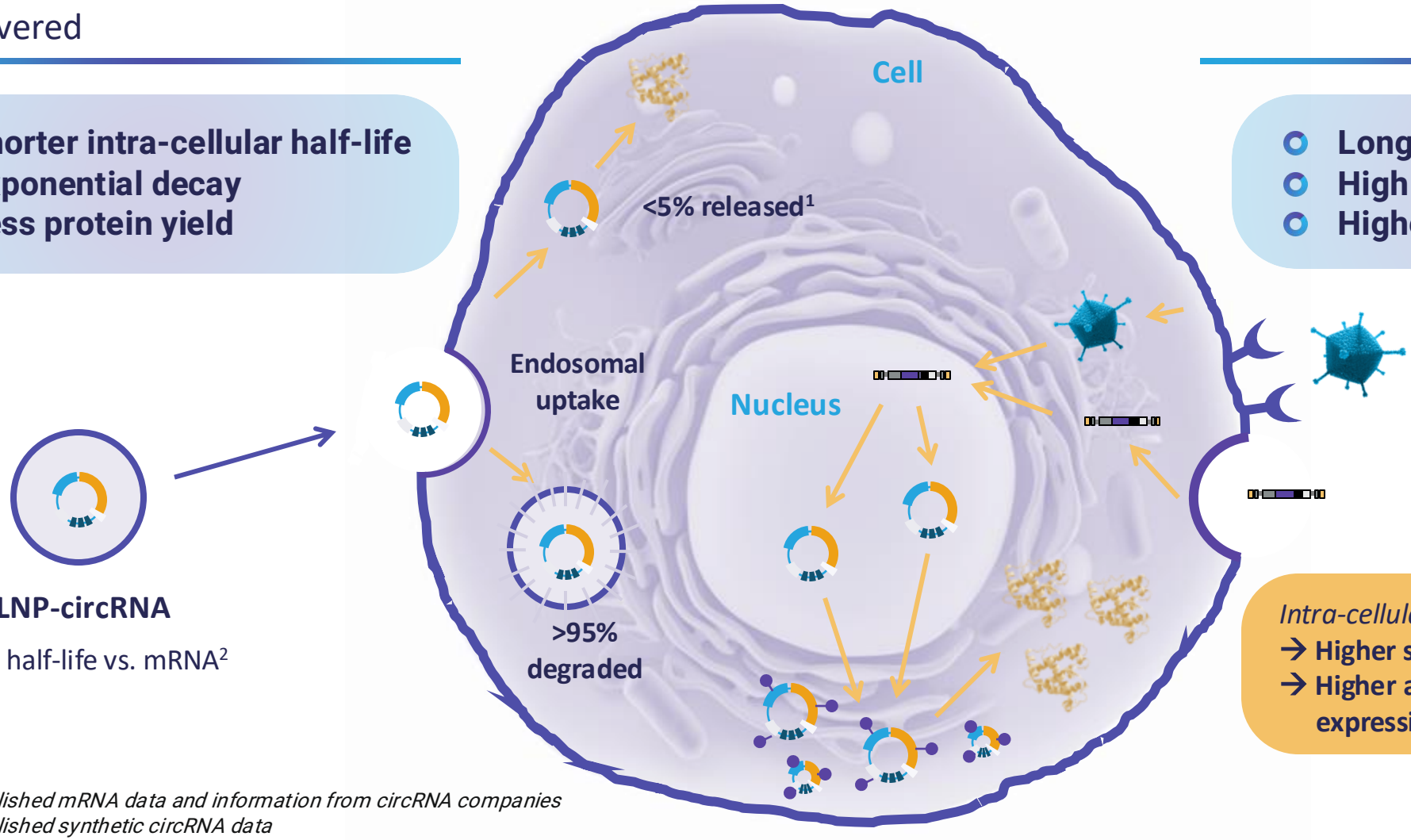
Why use vector-based circRNA delivery?

Synthetic circRNA,
LNP delivered

- **Shorter intra-cellular half-life**
- **Exponential decay**
- **Less protein yield**

Intra-cellularly generated circRNA,
vector delivered

- **Longer intracellular half-life**
- **High steady-state expression**
- **Higher protein yield**



LNP-circRNA

3-5x half-life vs. mRNA[2]

circVec
Virus or DNA vector
15x half-life vs. mRNA[3]

Intra-cellular circRNA biogenesis:
→ **Higher steady-state circRNA levels**
→ **Higher and longer-lasting protein expression**

(1) Based on published mRNA data and information from circRNA companies
(2) Based on published synthetic circRNA data
(3) Based on Circio's in vitro results

The circVec technology brings the advantages of circRNA into the field of genetic medicine

		Expression durability	*Main opportunity in vaccines*	*Suitable for gene therapy*	*Delivery system*	*Existing CDMO manu-facturing*
circio	**circVec vector approach**	**months to years**	✗	✓	Viral or DNA-LNP	✓
ORNA, SAIL BIOMEDICINES	**Synthetic circRNA**	7-10 days	✓	✗	circRNA-LNP	✗
moderna, BIONTECH	**Synthetic mRNA**	2-3 days	✓	✗	mRNA-LNP	✓

Source: Circio analysis

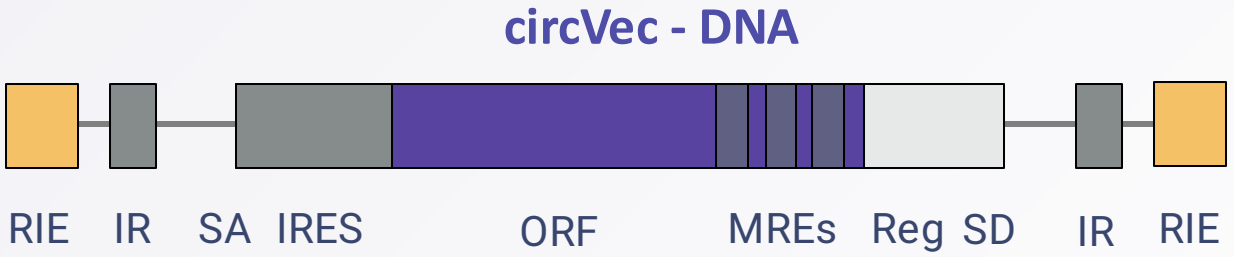
2

circVec technical development

3. circVec therapeutic application
4. Summary

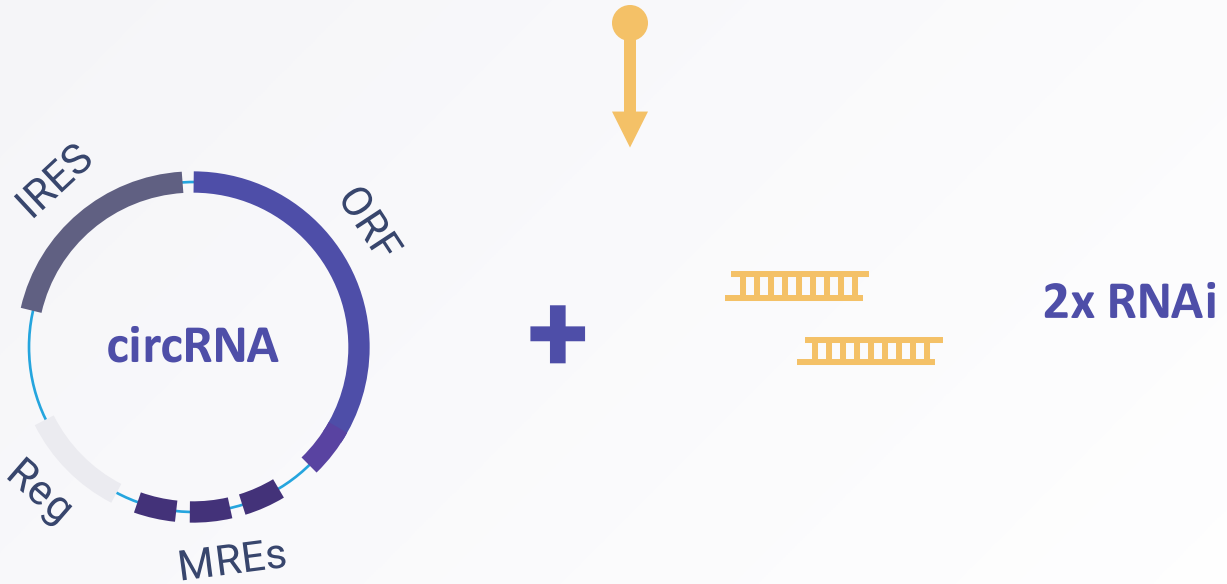
circVec is a genetic cassette optimized for intra-cellular circRNA biogenesis

Genetic cassette design



- **Highest published circRNA biogenesis rate**
- **Intra-cellular production of coding circular mRNA**
- **Vector agnostic** – applicable to a variety of DNA and viral systems

Multi-functional circRNA design



- **15x extended half-life vs. mRNA**
- **Up to 5x enhanced translation rate vs mRNA**
- **Modular, multi-functional design** - protein expression, RNAi, miRNA

RIE: RNAi element
IR: Inverted repeat element
SA: Splice acceptor
SD: Splice donor
IRES: Internal ribosome entry site
ORF: Open reading frame
MRE: microRNA response element
Reg: Regulatory element

circVec substantially outperforms the expression level and durability of mRNA-based systems

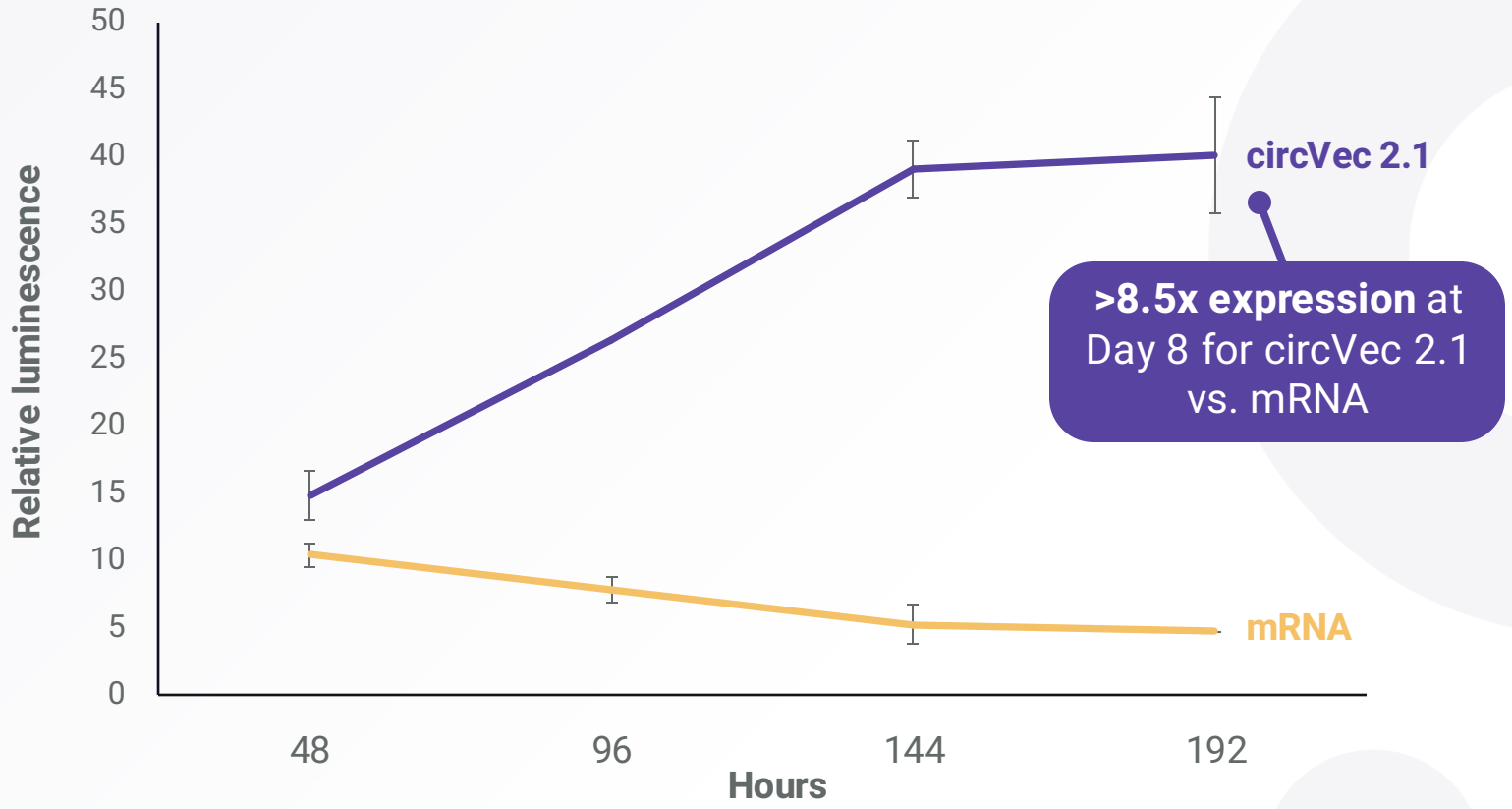
circVec RNA stability

135h vs. **9h**

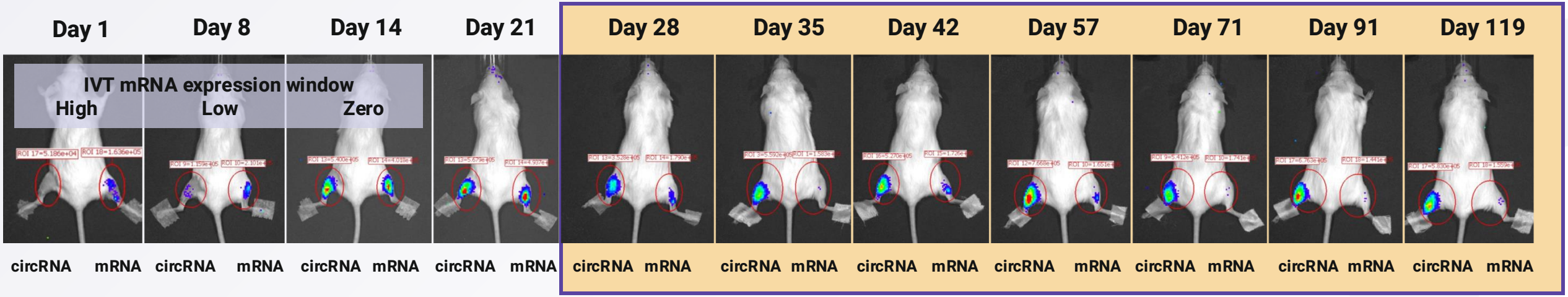
circRNA mRNA

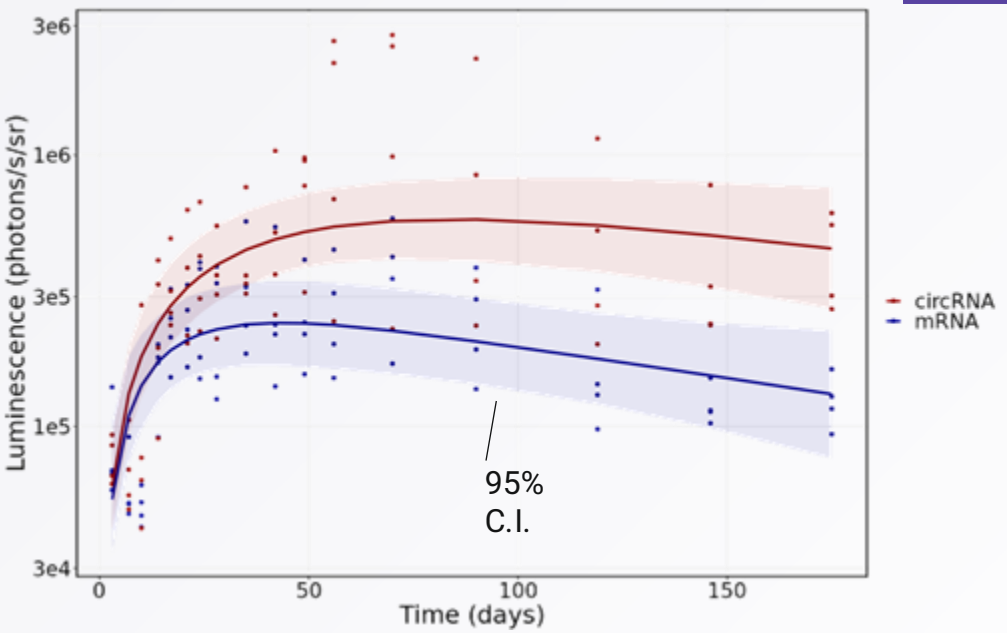
15 x

circVec vs. mRNA in vitro luciferase reporter expression; time course



circVec 2.1 significantly outperforms conventional mRNA-based expression in mouse models



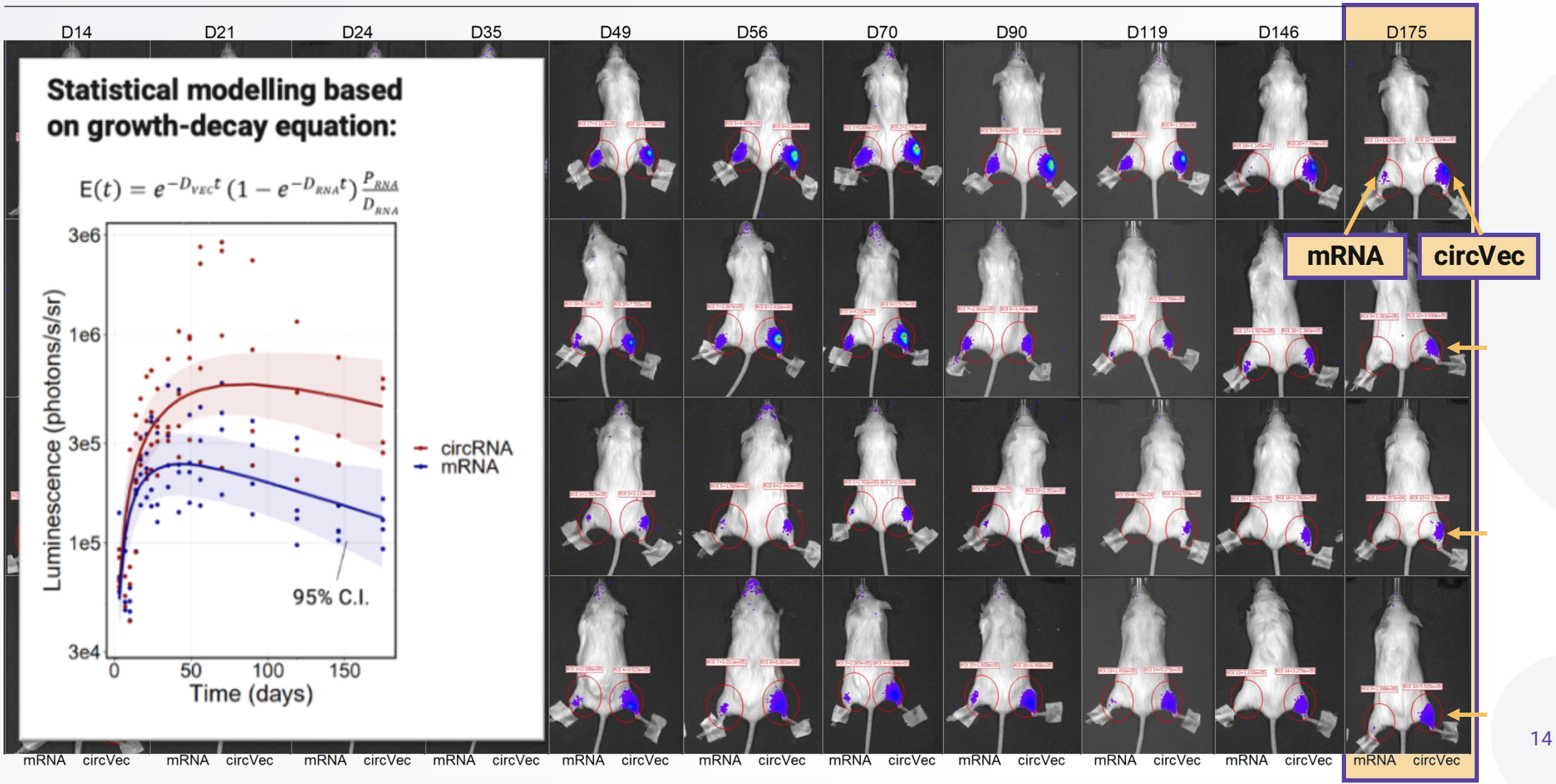


- **circVec 2.1 pDNA constructs show statistically significant enhanced durability vs. mRNA-based expression in vivo**
- **Major advantage and opportunity in gene therapy**

circVec 2.1 advantage vs. mRNA expression has been validated for up to six months

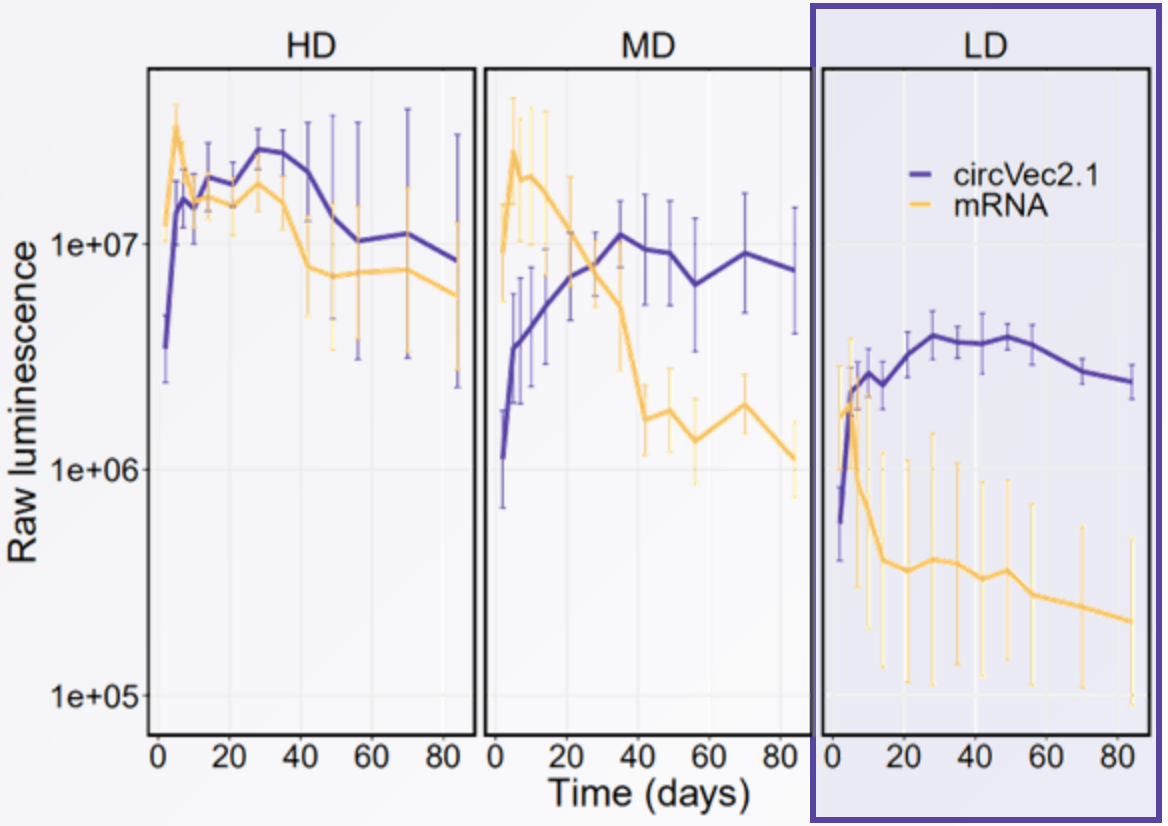
circVec vs. mRNA Luciferase expression, single intra-muscular injection of pDNA @ day 0

Day 175

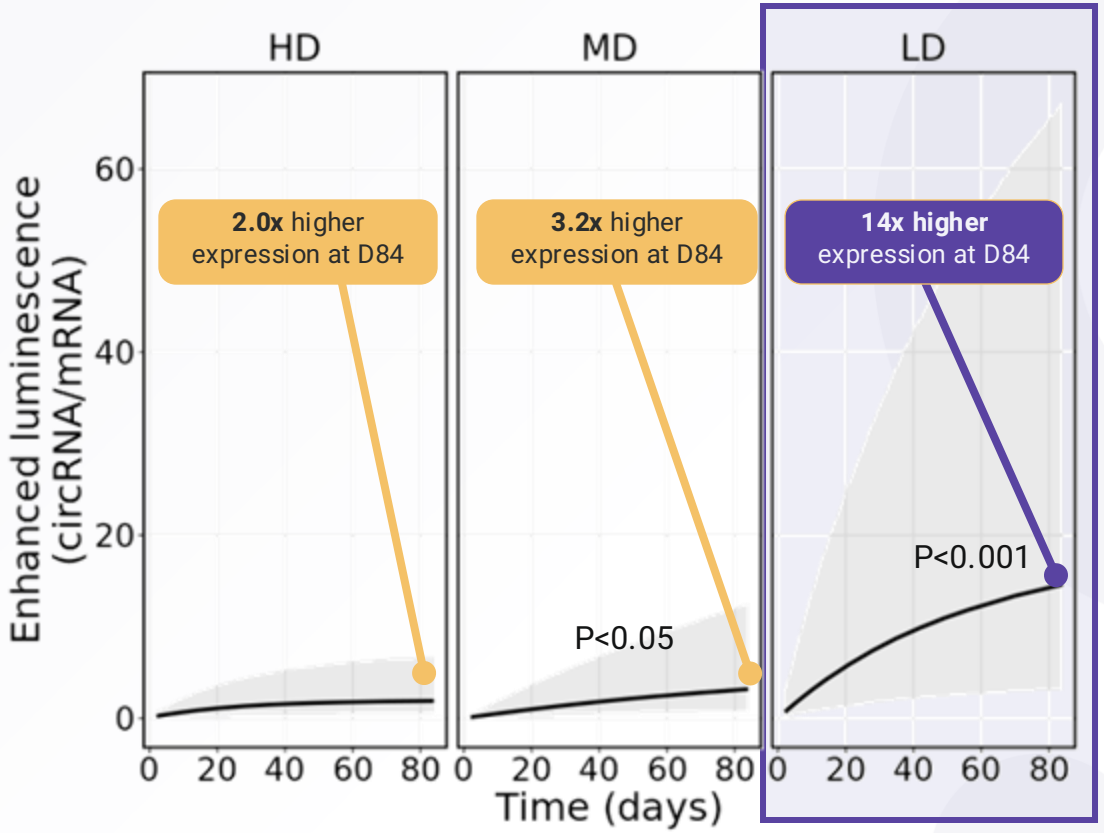


circVec in vivo advantage is enhanced at lower dose levels, up to 14x higher expression than mRNA

Absolute expression (luminescence)
circVec 2.1 vs. mRNA pDNA vector expression



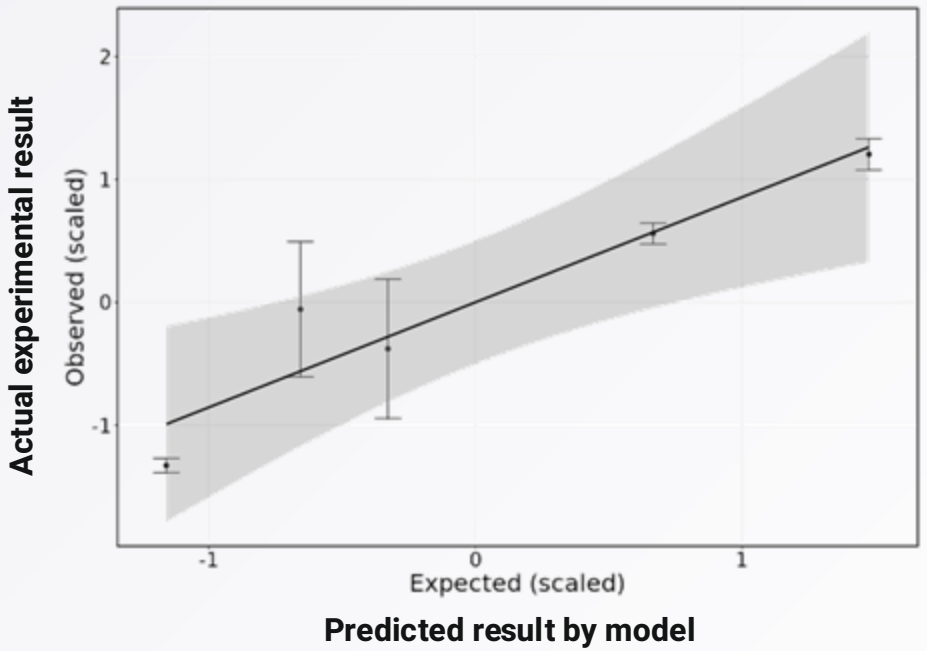
Relative expression (luminescence)
-fold change circVec 2.1 vs. mRNA expression



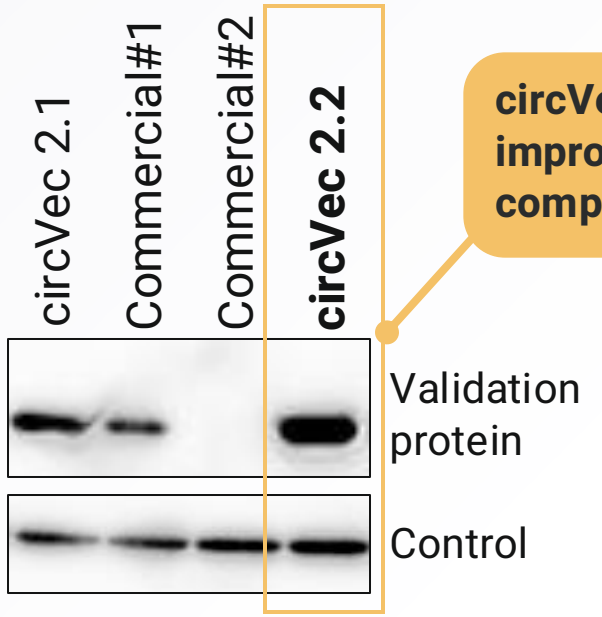
HD: High dose MD: Mid dose LD: Low dose

Machine learning has been deployed to further optimize circVec design – generation 2.2 and beyond

Machine learning model validation



circVec 2.x performance



circVec 2.2 shows 2-4x improved expression compared 2.1 design

Interest in the therapeutic potential of circRNA is growing rapidly – new opportunity in gene therapy

BIOCENTURY

ARTICLE | PRODUCT DEVELOPMENT

Emerging circular RNA field split on what to deliver and how to deliver it

The rising therapeutic modality is more durable than linear mRNA, promising efficacy and manufacturing advantages

BY DANIELLE GOLOVIN, BIOPHARMA ANALYST

August 17, 2023 11:34 PM UTC

Drug TARGET REVIEW

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ARTICLE

Enhancing gene therapy with Circio

In this Q&A, Erik Wiklund, CEO of Circio, explains the key findings of their circVec circular RNA platform technology, why they chose AAV-based gene therapy for AATD as the lead programme, and their plans for the future to enhance the potency and reduce the cost of current gold-standard gene therapy.

How does circVec technology compare to conventional mRNA?

3 July 2024

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Posted in News | Tagged Circio Holding, circular RNAs, Gene therapy, Genetic diseases, In vivo, mRNA

Circio has announced updated *in vivo* data that demonstrates a substantial durability advantage of Circio's circVec technology over conventional mRNA expression. In addition, Circio has undertaken sequence optimisation resulting in a new circVec 2.2 design.

BioSpace

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Opinion: Circular RNA Will Soon Replace mRNA in Biopharma

July 31, 2024 | 5 min read | Erik Digman Wiklund

Features

Circular RNA: Vaccines, therapeutics and biomarkers could be revolutionised

CircRNA is still in very early days of development, but it is expected to trialled in vaccines, therapeutics and biomarkers trials in the next few years.

Abigail Beaney | May 15, 2024

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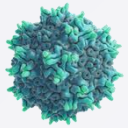
Clinical Trials Arena

3

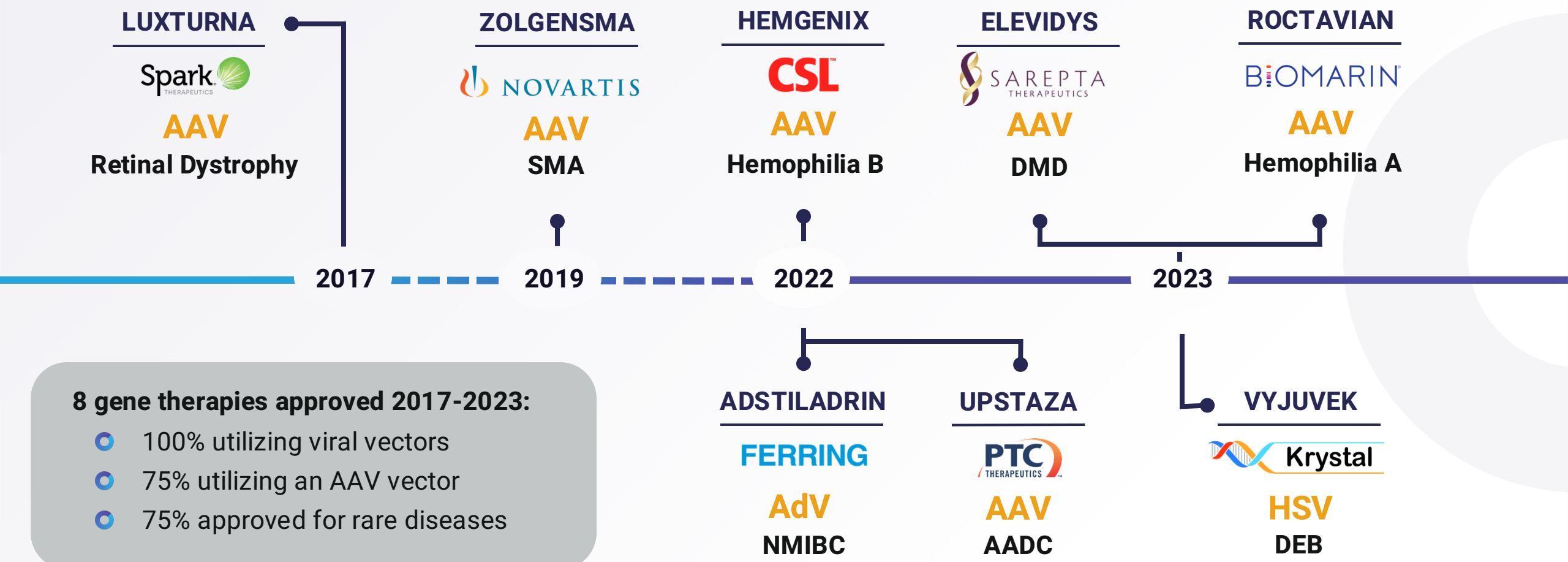
circVec therapeutic application

4. Summary

circVec is being explored in both viral and synthetic DNA vector formats for therapeutic applications

	Viral		Synthetic DNA	
	 AAV	 Adenovirus	DNA format 1 	DNA format 2
Application	• **Gene therapy**	• **Vaccines** • **Oncology**	• **Gene therapy** • **Vaccines**	• **Gene therapy** • **Cell therapy**
Aim	• **Improved expression and reduced dosing** vs. mRNA AAV	• **Single-dose** vaccine • **Therapeutic protein delivery** to tumors	• **Enable repeat-dosing** for gene therapy • Enhanced **nuclear uptake**	• **Improved uptake** • Reduced **immunogenicity**
	Advantage: *Efficient delivery of genetic material* ***Challenge***: *Repeat dosing and immune response*		***Advantage***: *Repeat dosing and manufacturing* ***Challenge***: *Nuclear delivery and innate immunity*	

AAV virus is the main gene therapy format today



8 gene therapies approved 2017-2023:

- 100% utilizing viral vectors
- 75% utilizing an AAV vector
- 75% approved for rare diseases

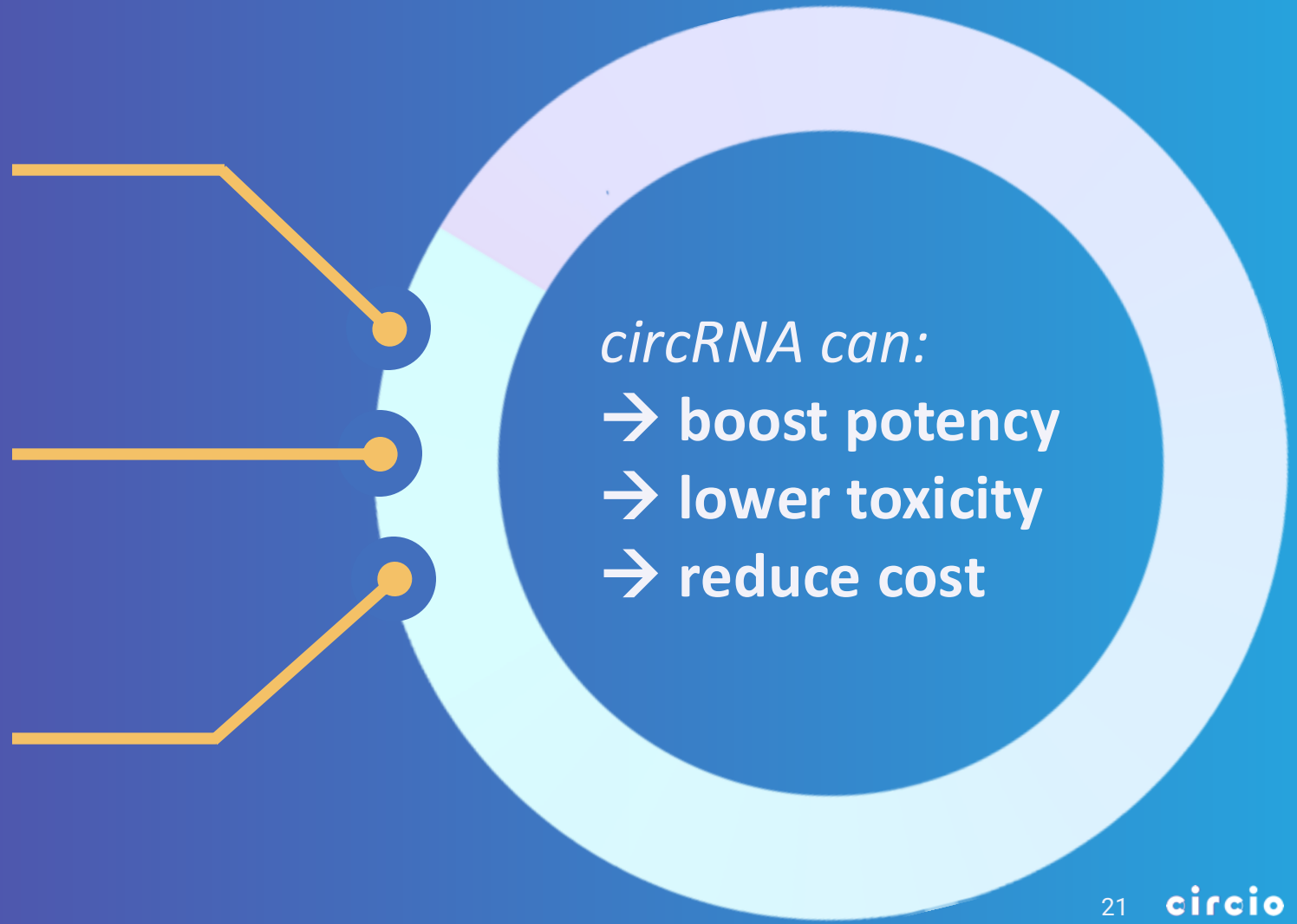
AAV: Adeno-Associated Virus, currently best known vector for long-term protein expression in humans

The need for high dosing is a major limitation for current gold-standard AAV gene therapy

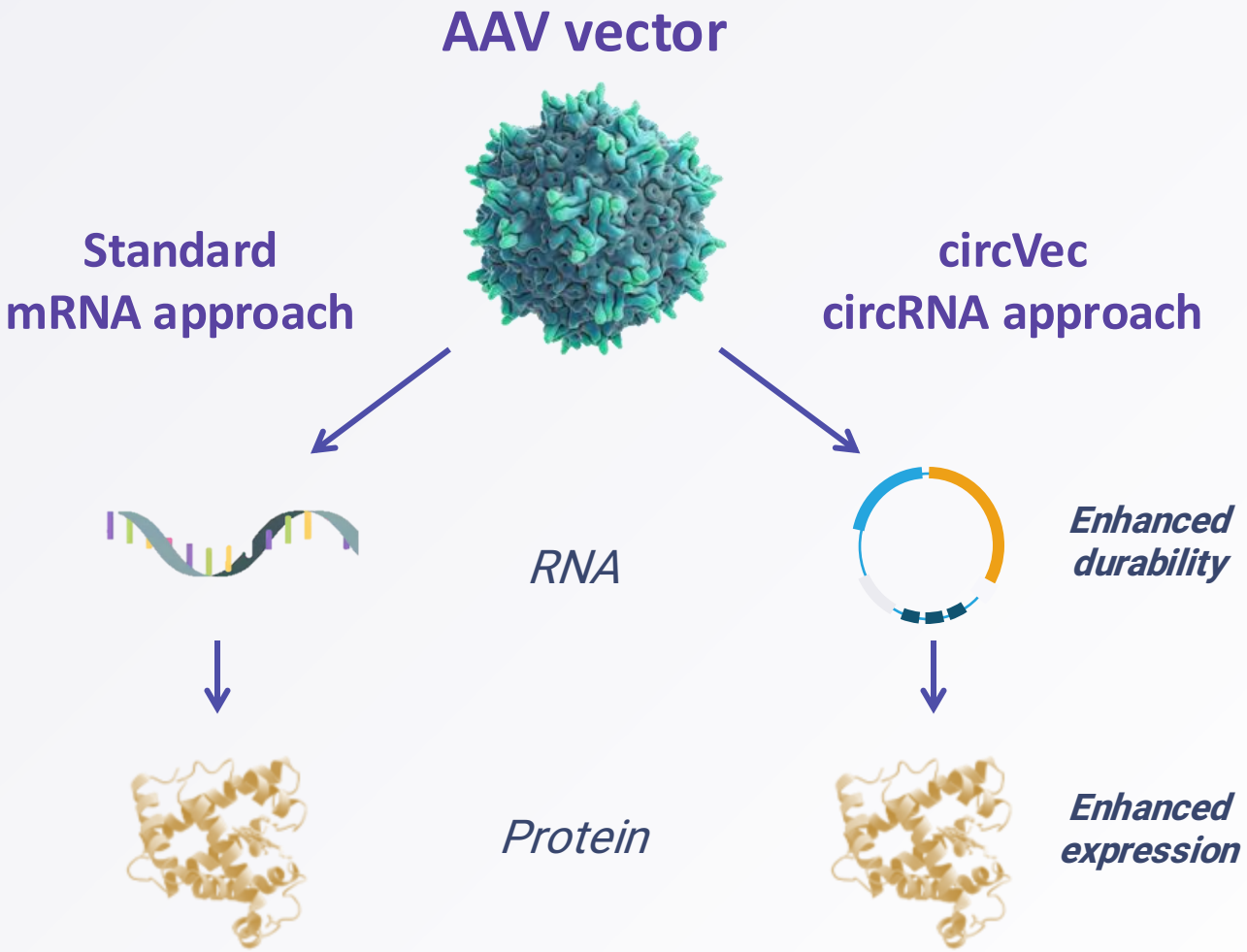
Limited applicability
Low expression level not sufficient for many genetic diseases

Low expression → High dosing
Safety issues, liver and immunological toxicity

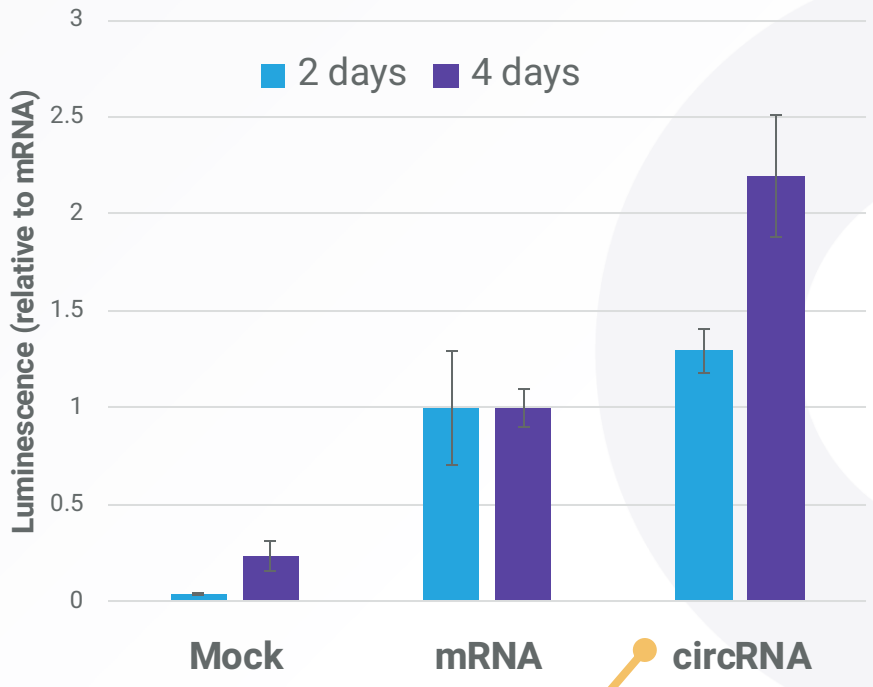
High dosing → High cost
High dose requirement drives high manufacturing cost



Can circVec be deployed to enhance AAV gene therapy?



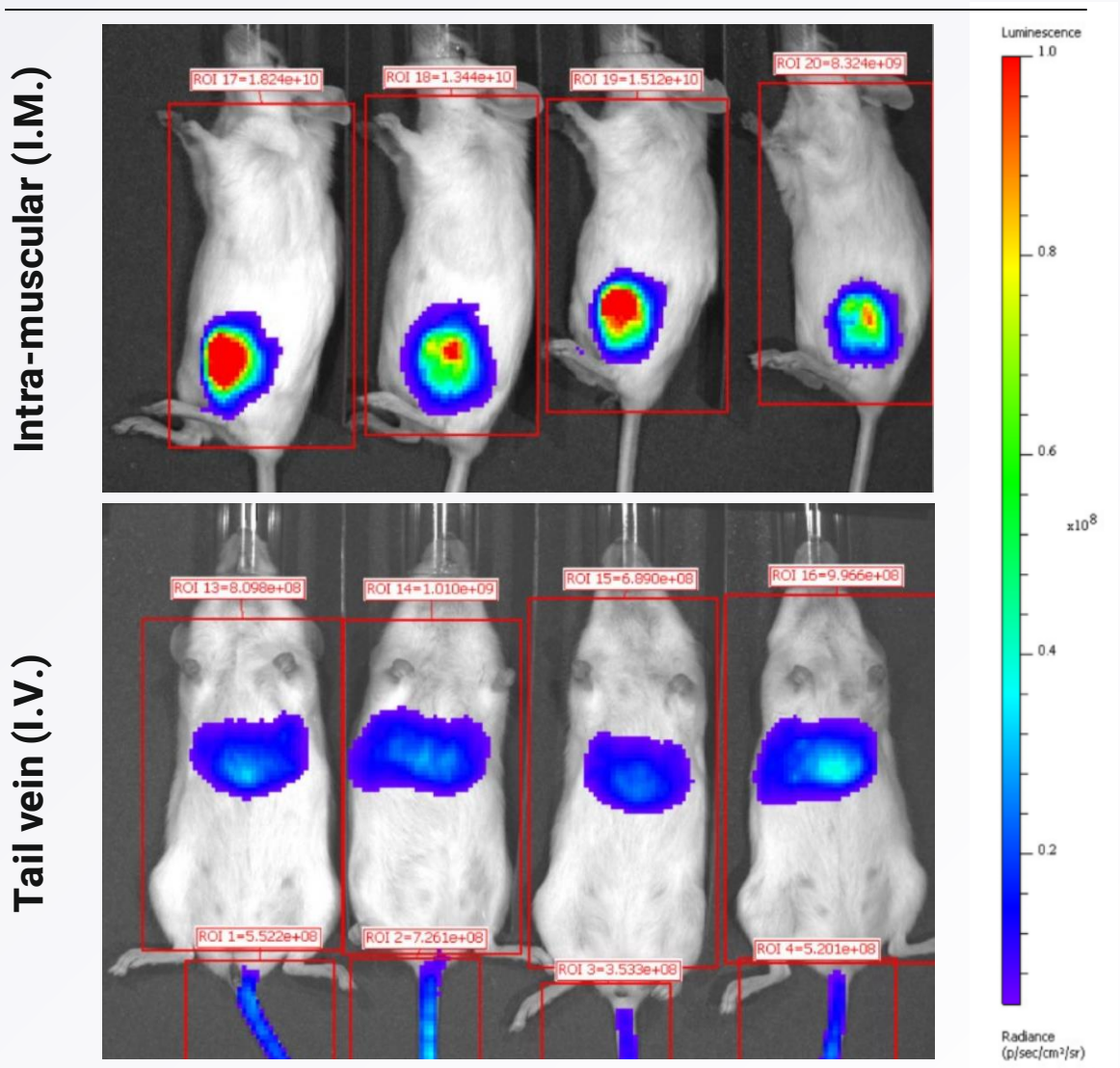
AAV protein expression, luminescence



Enhanced circVec-AAV expression vs. mRNA-AAV, validated by **multiple experimental methods** *in vitro*

circVec 2.0 AAV vector expression validated in vivo

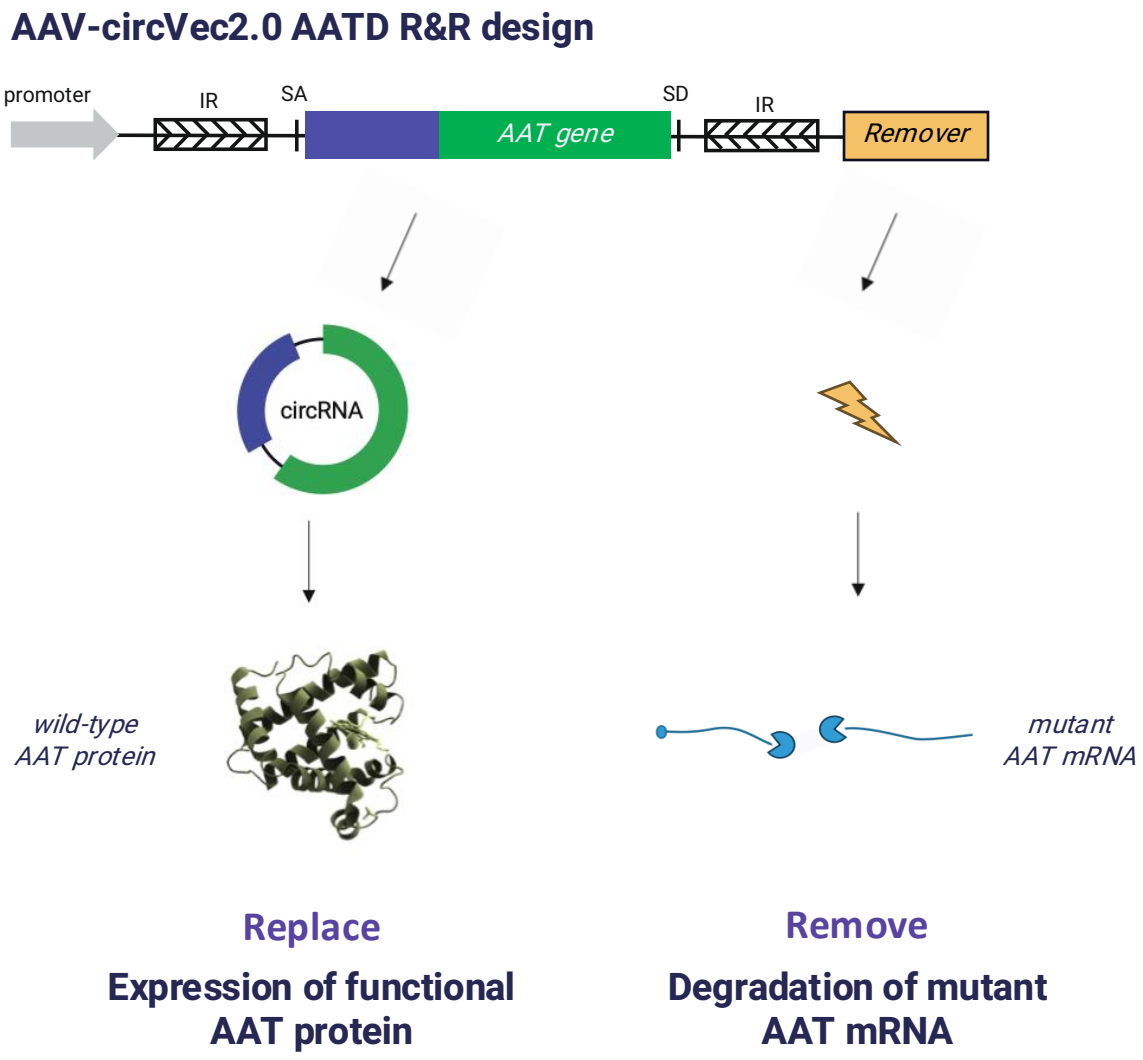
circVec-AAV luminescence, F-luc at Day 20

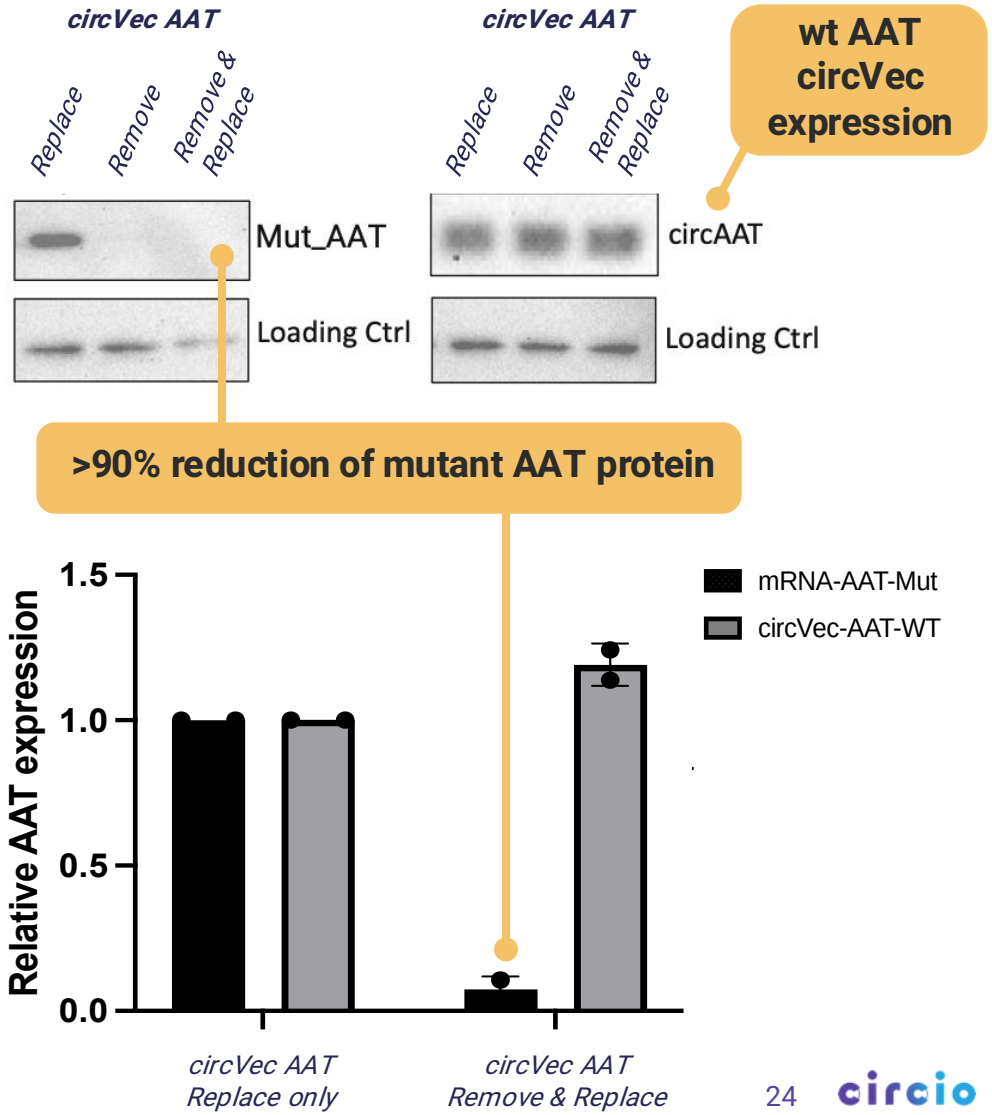


Experimental set-up

Vector:	AAV8
circVec version:	circVec 2.0
Payload:	Firefly luciferase (F-luc)
Mouse strain:	NOD/SCID/IL-2Rγnull immuno-deficient mice
Delivery route:	Tail vein or intra-muscular injection
Single injection, dose:	$1x10^{10}$ or $1x10^{11}$ viral genomes

circVec ´Remove-&-Replace´ gene therapy concept, AATD case example





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Summary

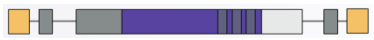
Circio is a leader in the DNA-format circRNA space
Take-home messages:

Durability

The circular mRNA format offers **significant durability and expression level advantages** over conventional linear mRNA

Versatility

The advantages of circular mRNA can be applied to both IVT ”synthetic” mRNA and **vector-based expression systems**



circVec

The circVec platform is a **unique and powerful intra-cellular expression system for circular mRNA**

“Due to its significant advantages, circRNA systems can be expected to replace mRNA-based expression for DNA format therapeutics in the future – just as synthetic circRNA can be expected to replace current mRNA formats”

Dr. Alex Wesselhoeft
Scientific founder
oRNA Therapeutics