circio

Disruptive circRNA technology for genetic medicine

Company presentation
September 2024

Circio executive summary

The challenge

- **Gene therapy market** is expected to **grow sharply** during the next decade
- However, **suboptimal vectors, cost and safety issues** hold back progress
- **Urgent need** for strategies that can increase potency, improve safety and reduce cost → **effective and affordable gene therapy for more patients**

Circio´s Solution

- Unique, proprietary approach to **circRNA, a next generation RNA format**
- **circVec** technology can **enhance** current gold-standard **gene therapy**
- Differentiated ´**remove & replace**´ **dual functionality** gene therapy concept

Milestones

- ✓ **In vivo proof-of-concept demonstrated for circVec vs. mRNA expression**
- ✓ **In vivo technical PoC for circVec-AAV** protein expression → mid´24
- **Gene therapy disease model data for circVec-AAV** → 6-9 months
- **Enter first strategic partnership,** technology or target deal → 1H 2025

AAV: Adeno-Associated Virus **TA**: Therapeutic Area **AATD**: Alpha-1-AntiTrypsin Deficiency **PoC**: Proof of Concept

1

circRNA introduction

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

The circRNA field was established by Circio scientists

Dr Thomas B Hansen

Dr Erik D Wiklund

nature — 6,373 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 — 922 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 — 2,291 citations

Review Article | Published: 08 August 2019

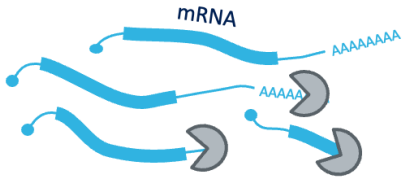
The biogenesis, biology and characterization of circular RNAs

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

circRNA increases durability and expression level, thereby enhancing the potency of gene therapy

Extended RNA durability

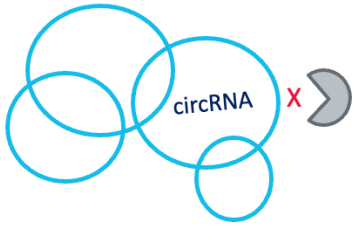
15x half-life vs. mRNA



circRNA will outcompete linear mRNA due to its enhanced stability

Higher protein expression

5x translation rate vs. mRNA



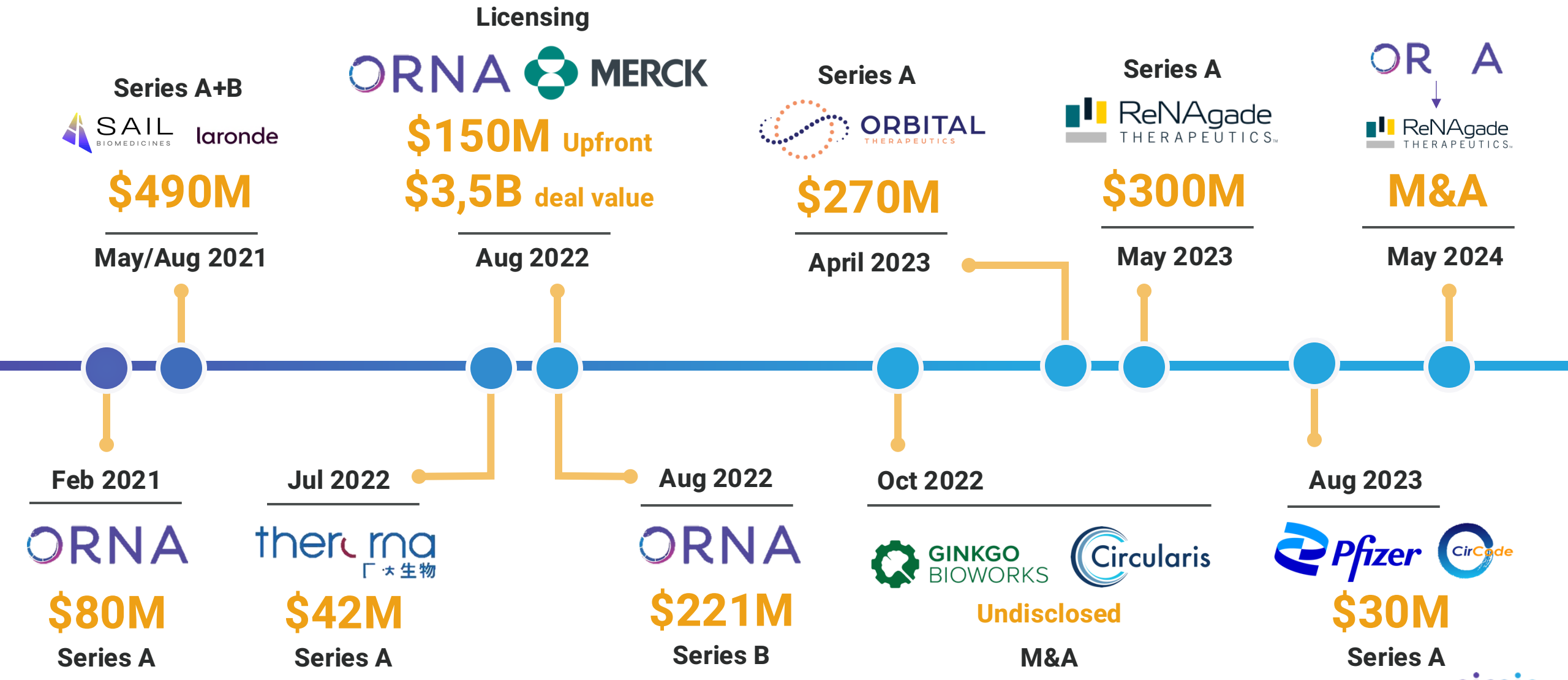
microRNA sponging

mRNA is destabilized by microRNAs

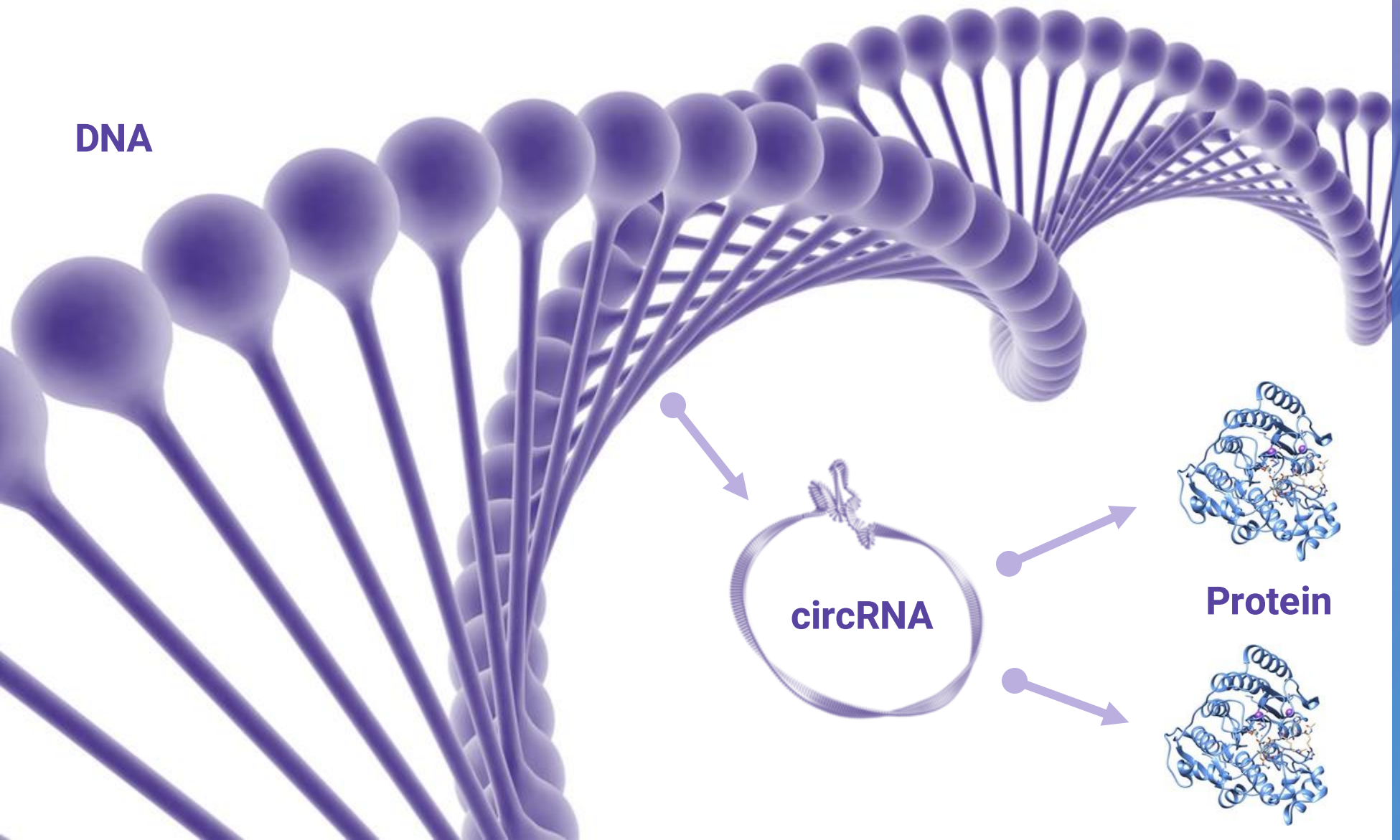
Modular & multi-functional

Enables ´remove & replace´ strategy

Substantial deal activity in the circular RNA space



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

The circVec platform is technologically differentiated and creates novel opportunities for circRNA

		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

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circVec technical development

3. circVec therapeutic application
4. Summary

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

Increased expression level

Prolonged durability

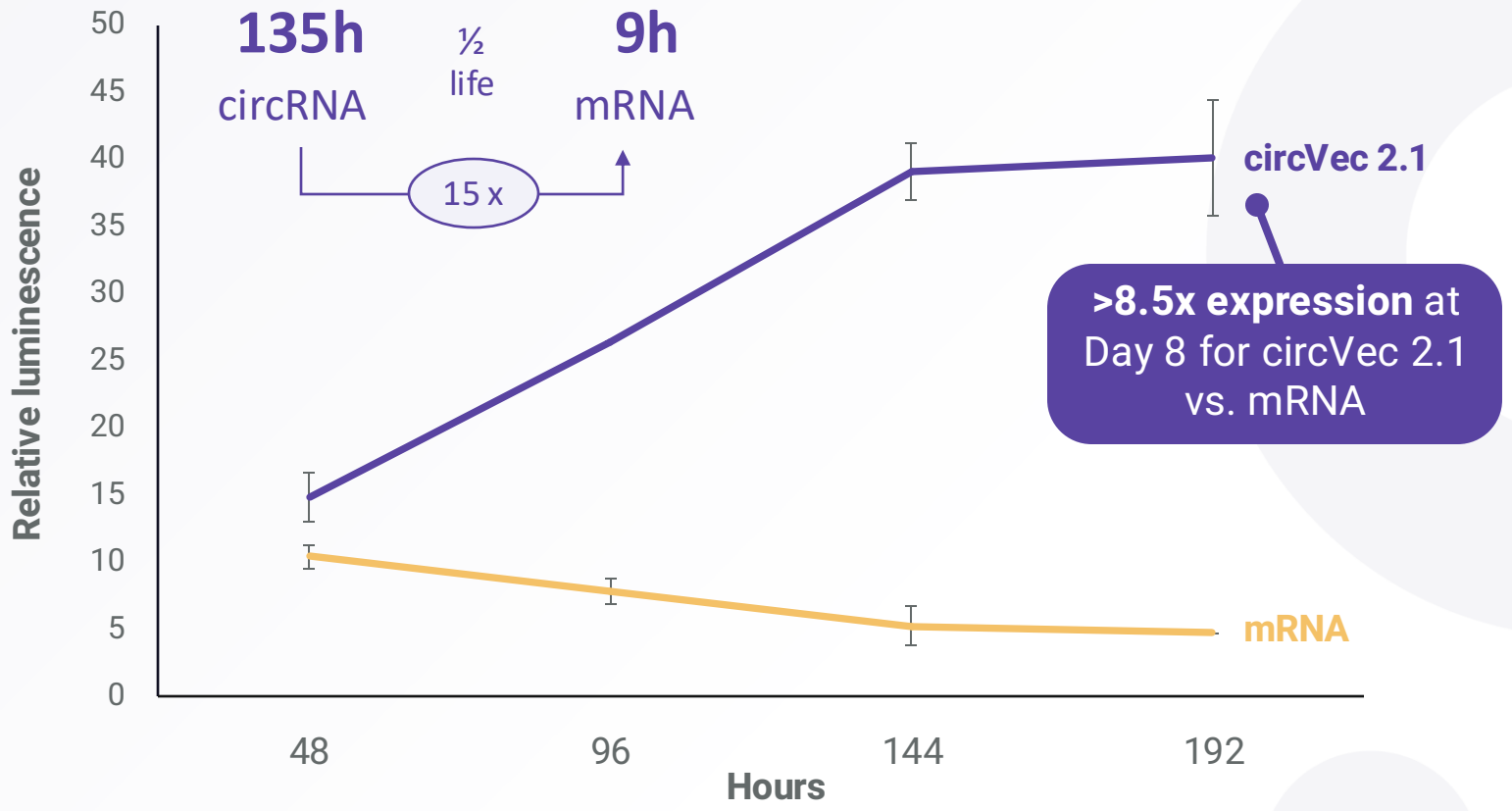
Enhanced therapeutic potency

"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"

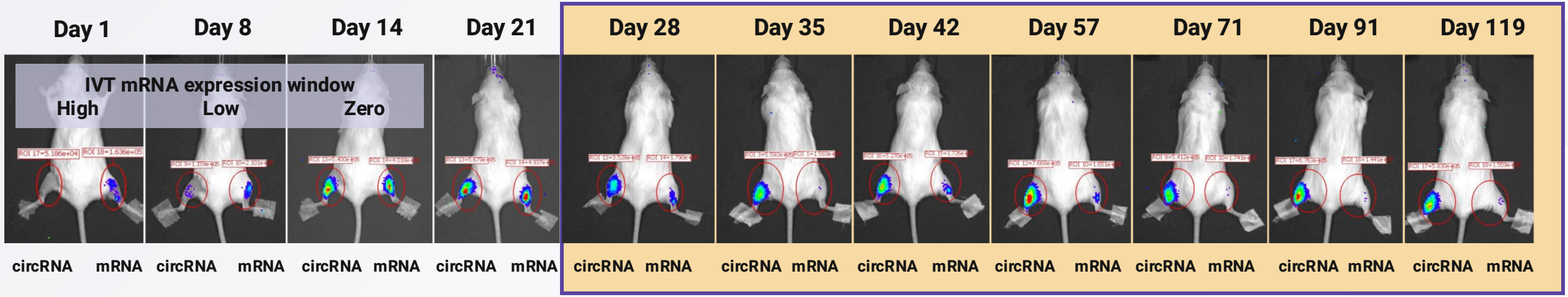
Dr. Alex Wesselhoeft

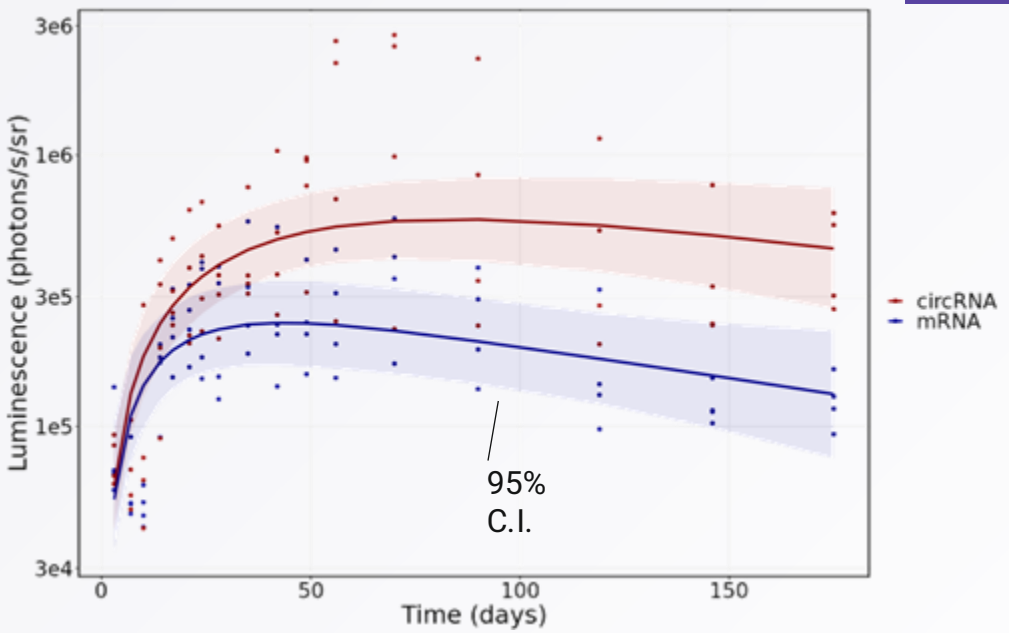
Scientific founder
oRNA Therapeutics

circVec vs. mRNA luciferase reporter expression; time course



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

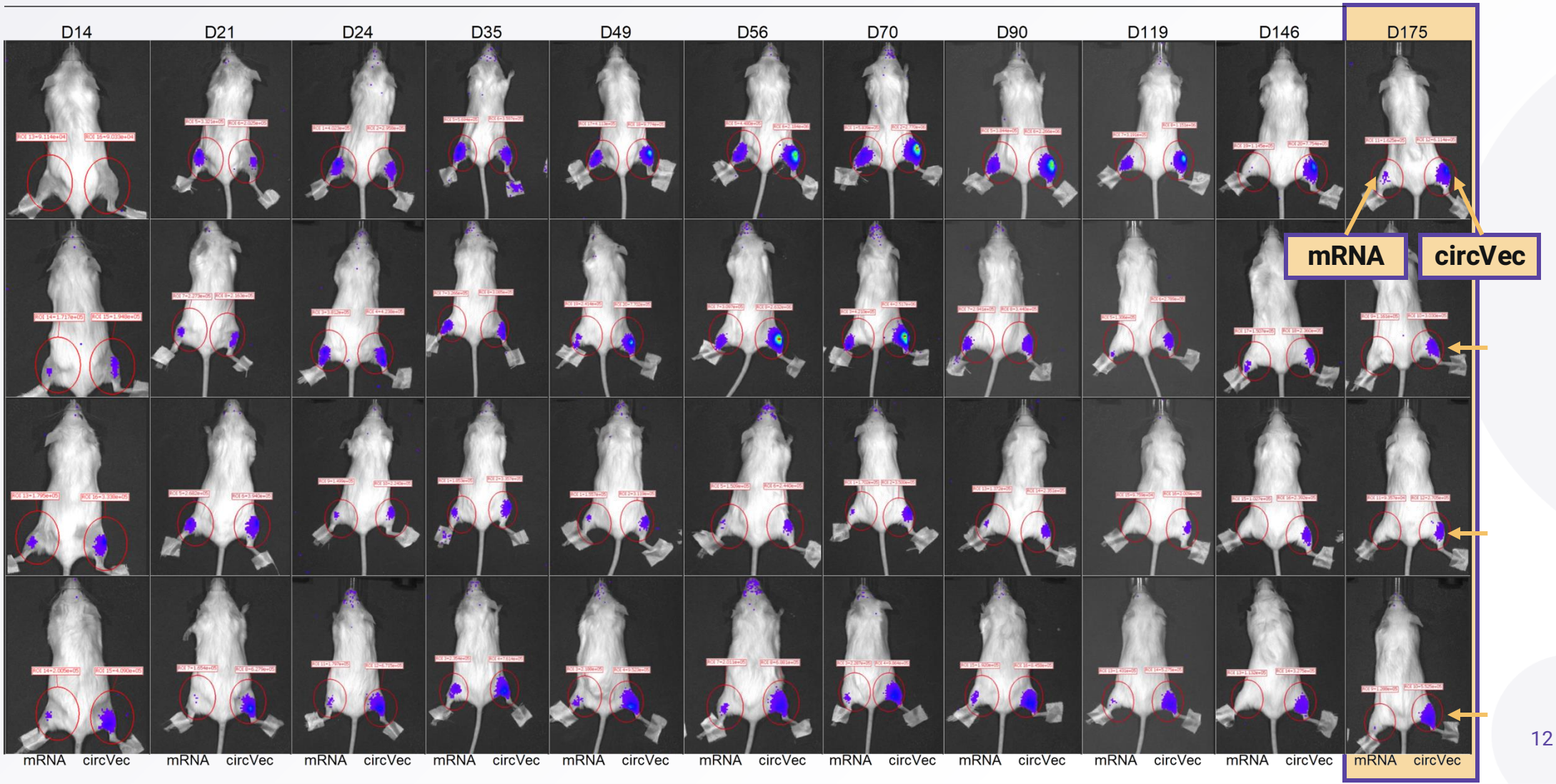




- **circVec 2.1 pDNA constructs show statistically significant enhanced durability vs. mRNA-based expression in vivo**
- **Major advantage and opportunity in gene therapy**

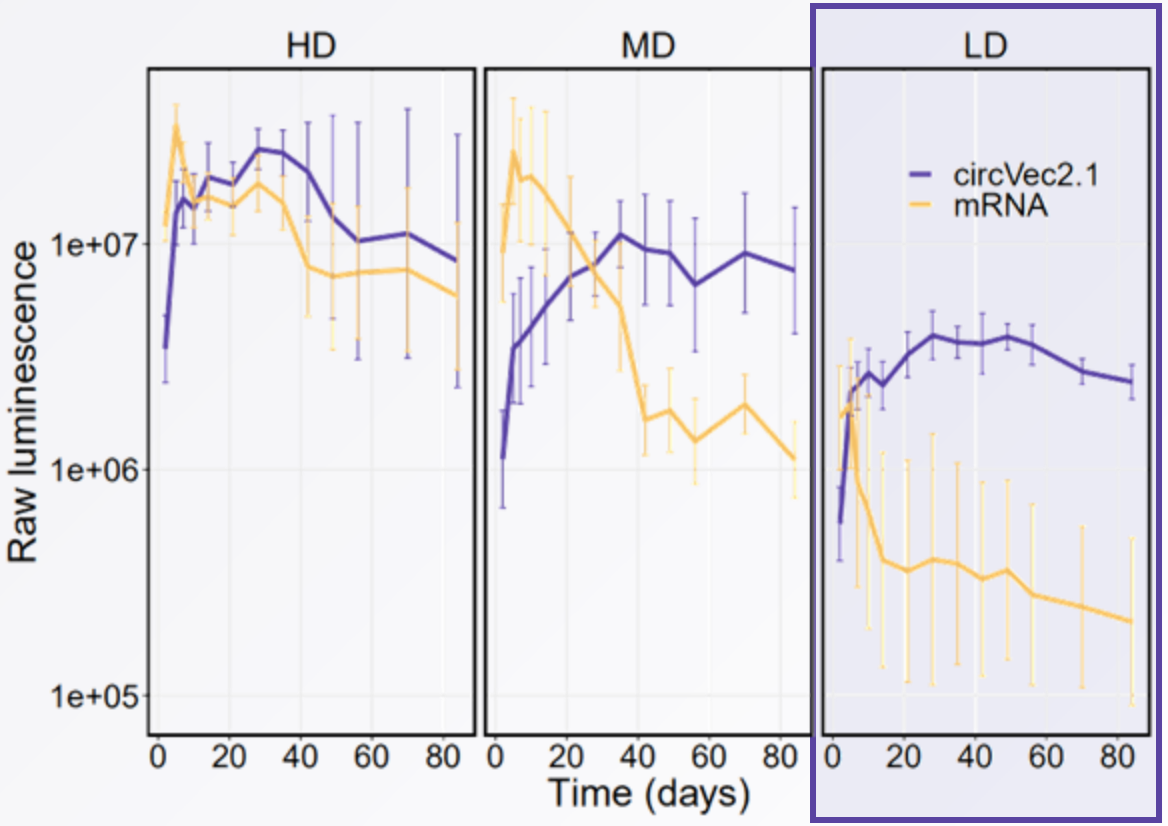
circVec 2.1 advantage vs. mRNA expression has been validated for up to six months

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

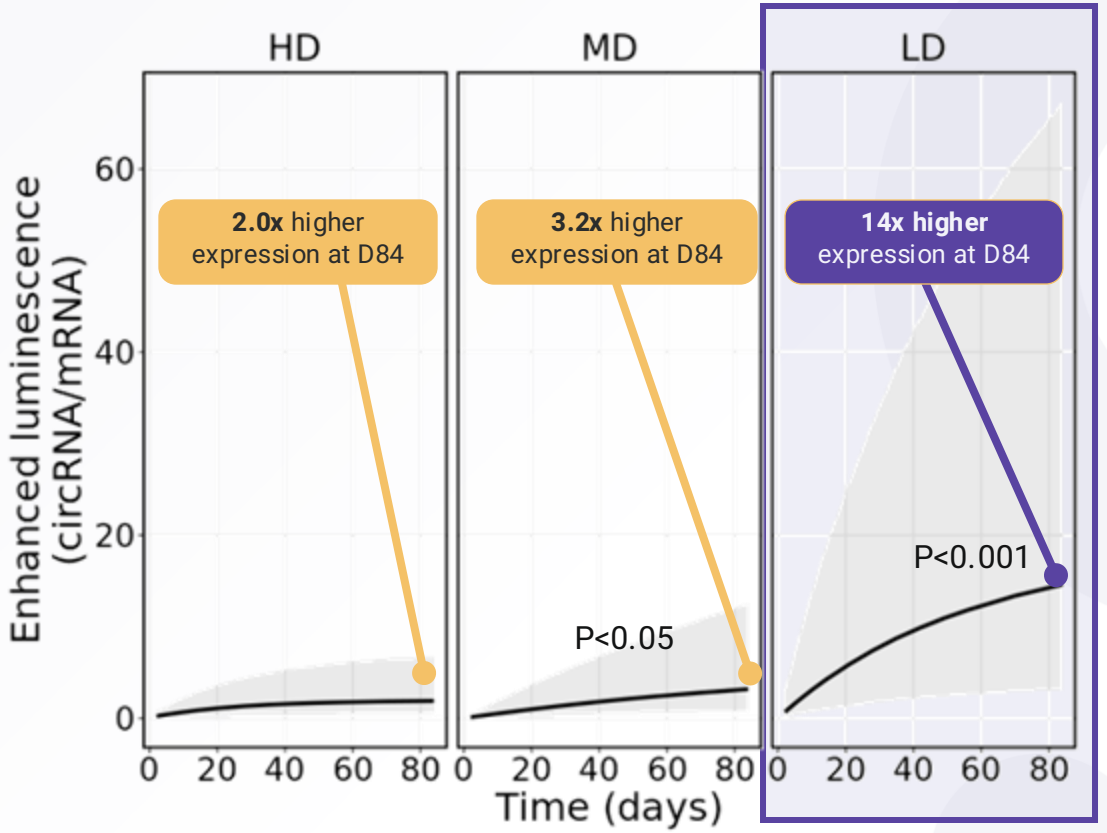


circVec in vivo advantage is enhanced at lower dose levels, up to 14x higher expression than mRNA

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



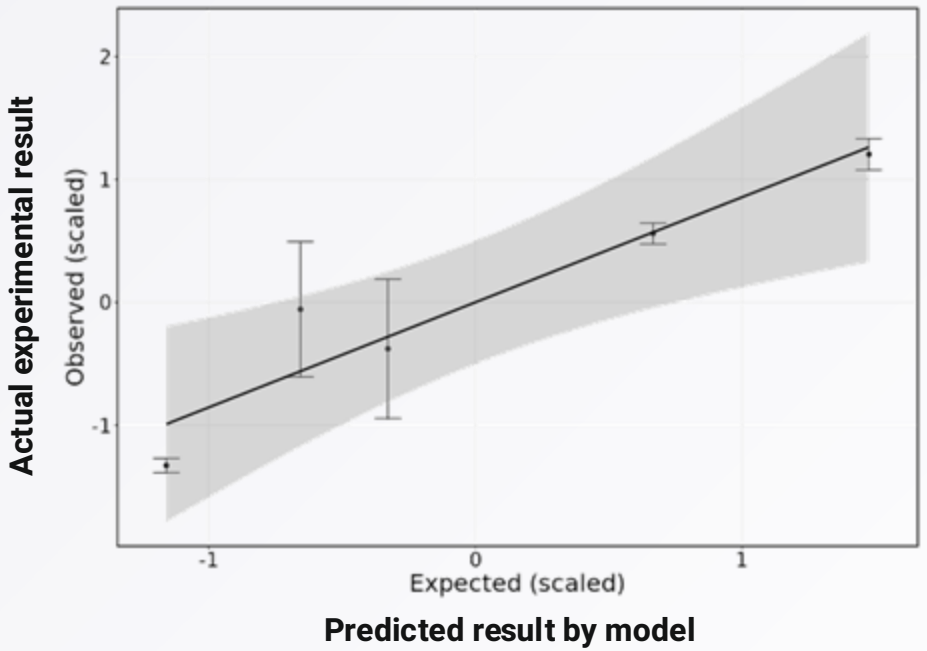
Relative expression (luminescence)
-fold change circVec 2.1 vs. mRNA expression



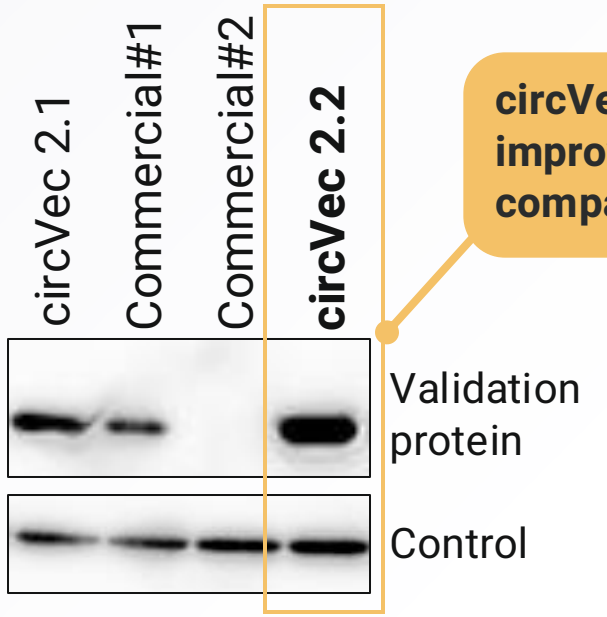
HD: High dose MD: Mid dose LD: Low dose

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

Circio is being recognized by industry media as an emerging leader in the circRNA space

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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News > Drug Development

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

Share

Clinical Trials Arena

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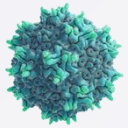
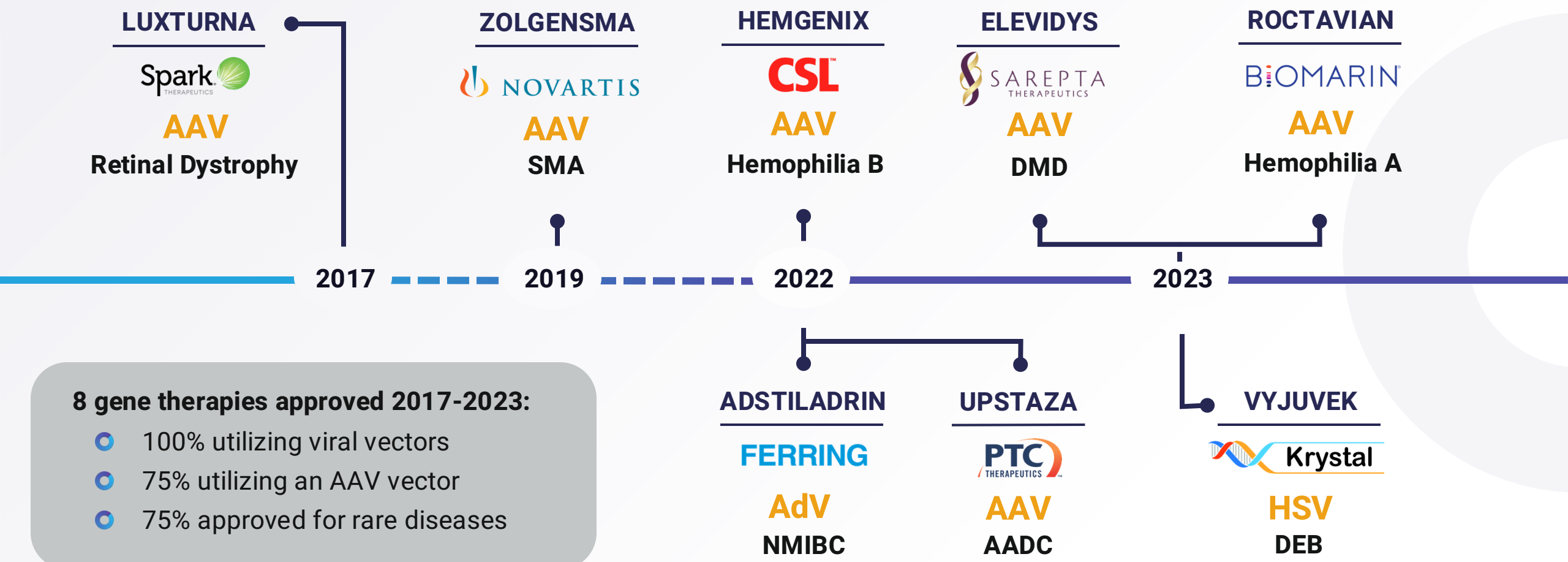
circVec therapeutic application

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

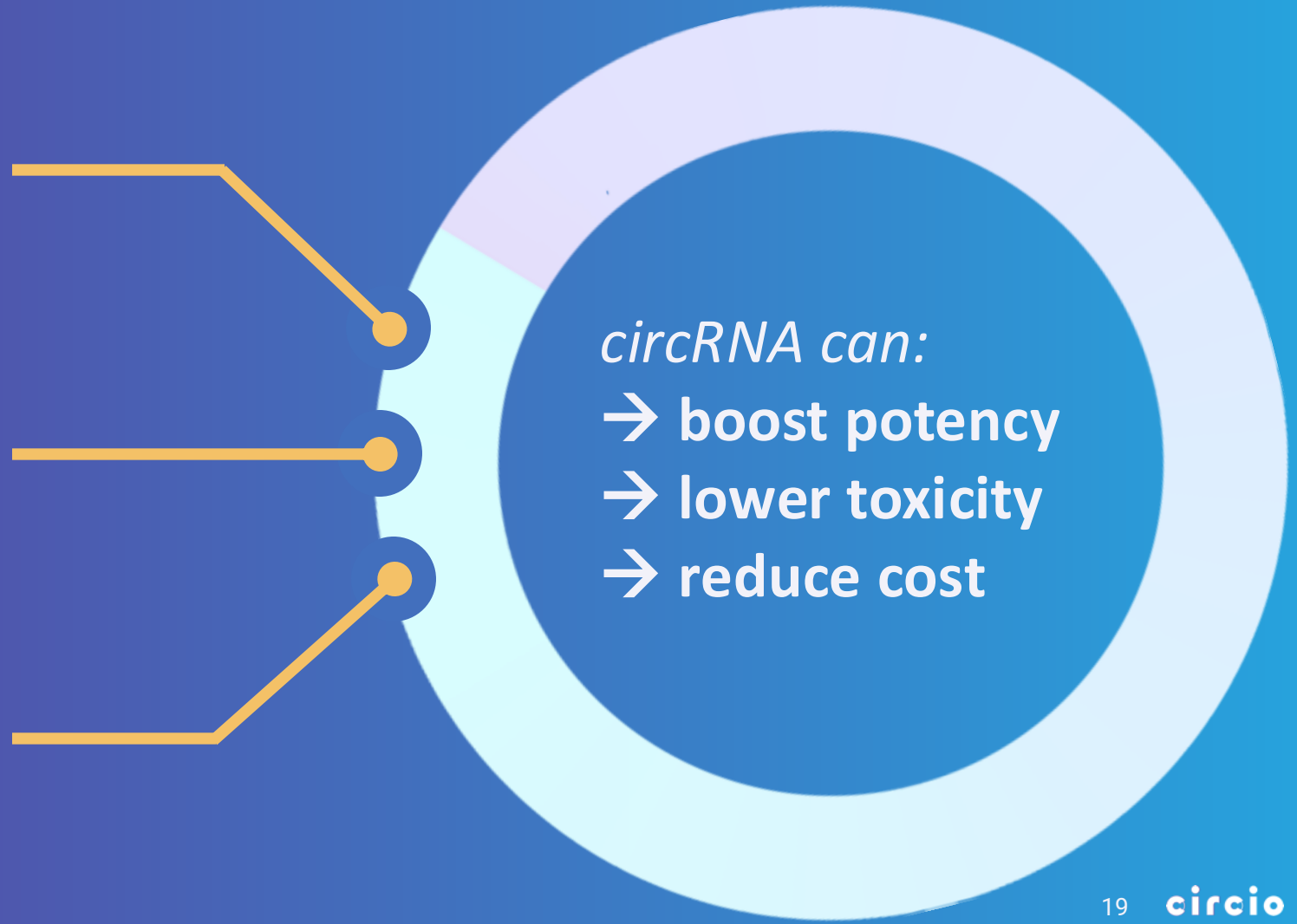
AAV: Adeno-Associated Virus, currently best known vector for long-term protein expression in humans

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

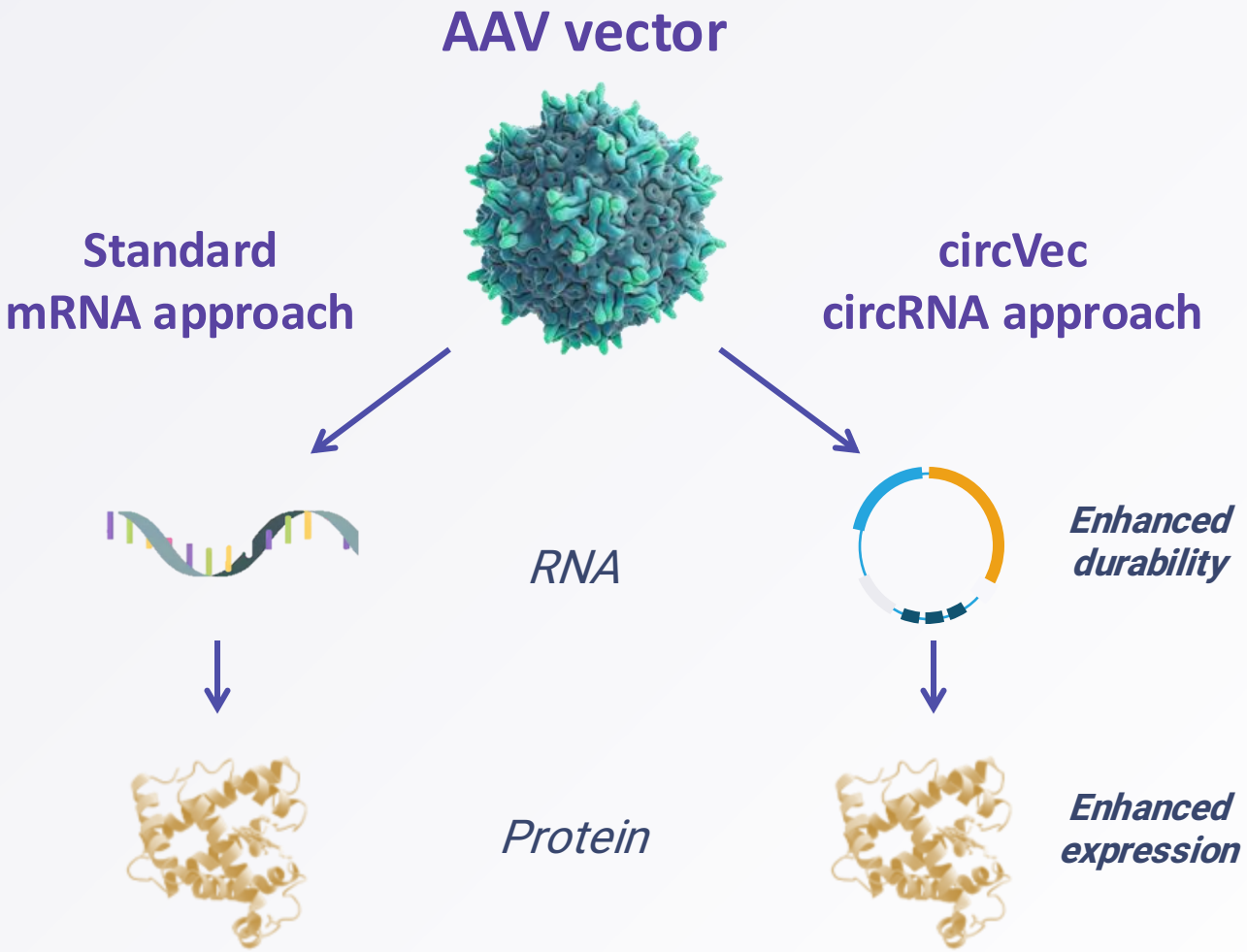
Limited applicability
Low expression level not sufficient for many genetic diseases

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

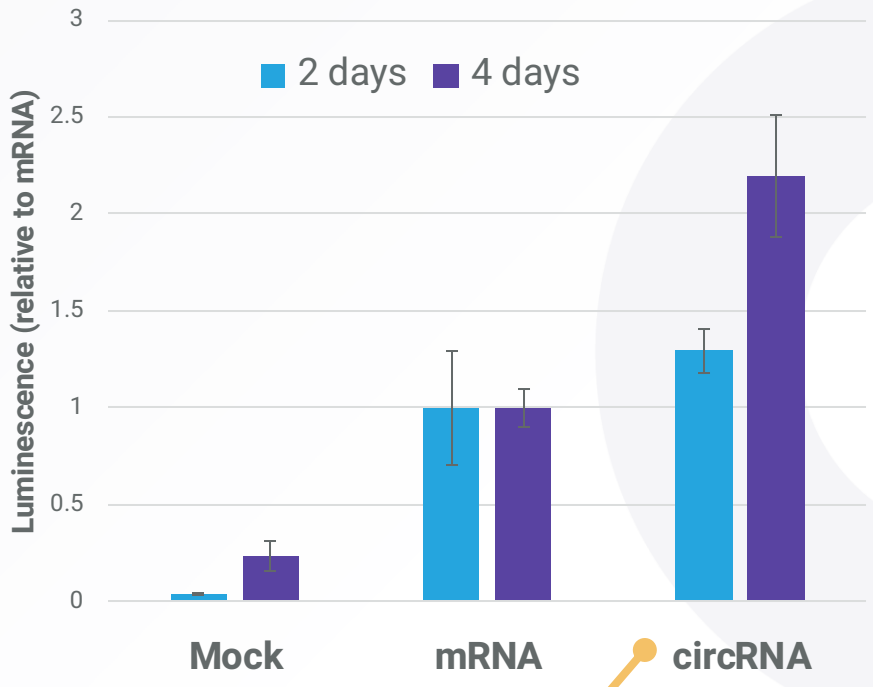
High dosing → High cost
High dose requirement drives high manufacturing cost



Can circVec be deployed to enhance AAV gene therapy?



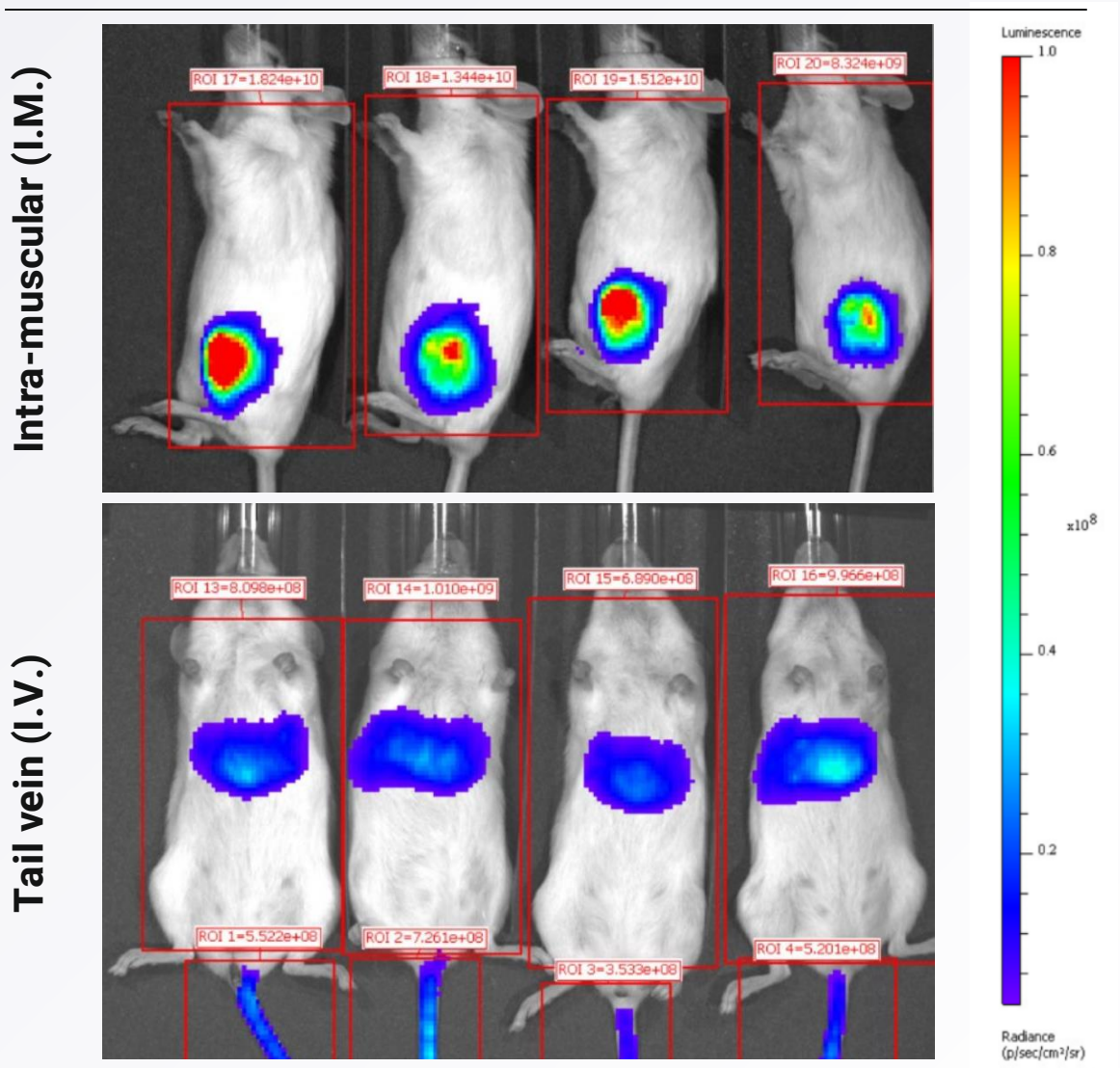
AAV protein expression, luminescence



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

circVec 2.0 AAV vector expression validated in vivo

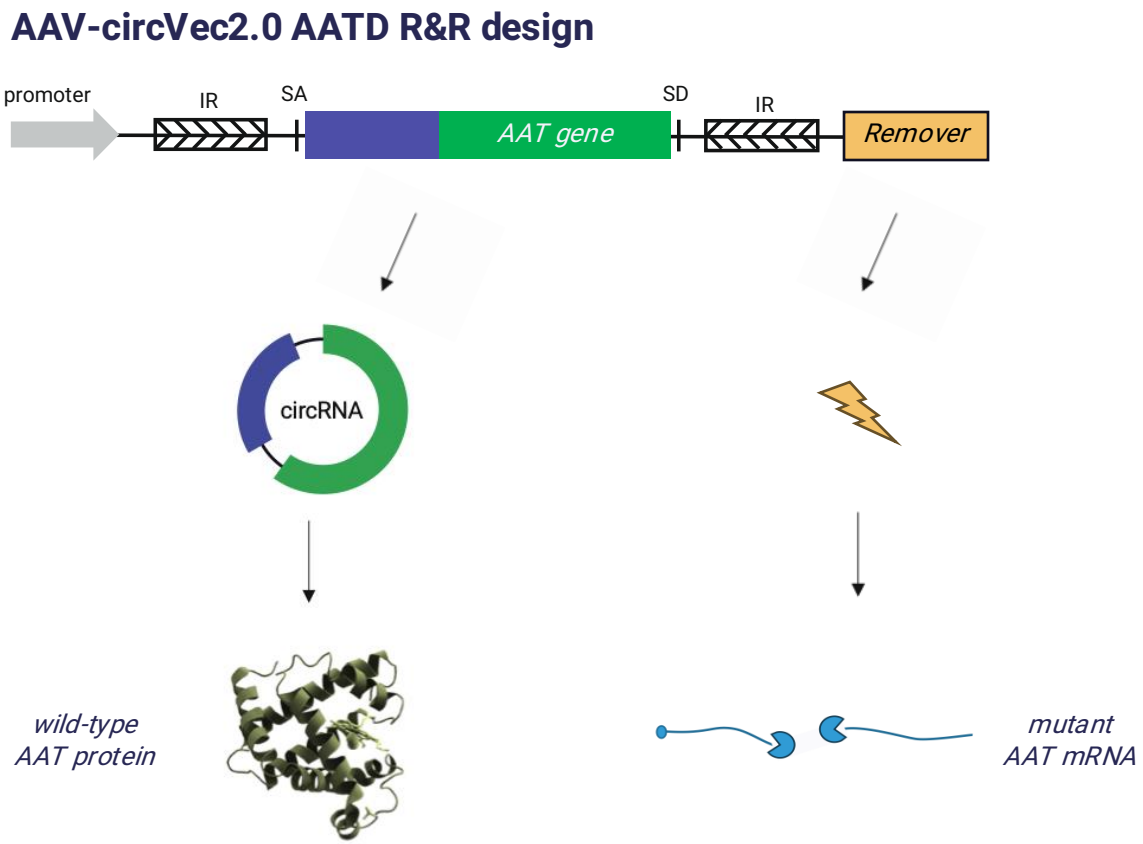
circVec-AAV luminescence, F-luc at Day 20



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

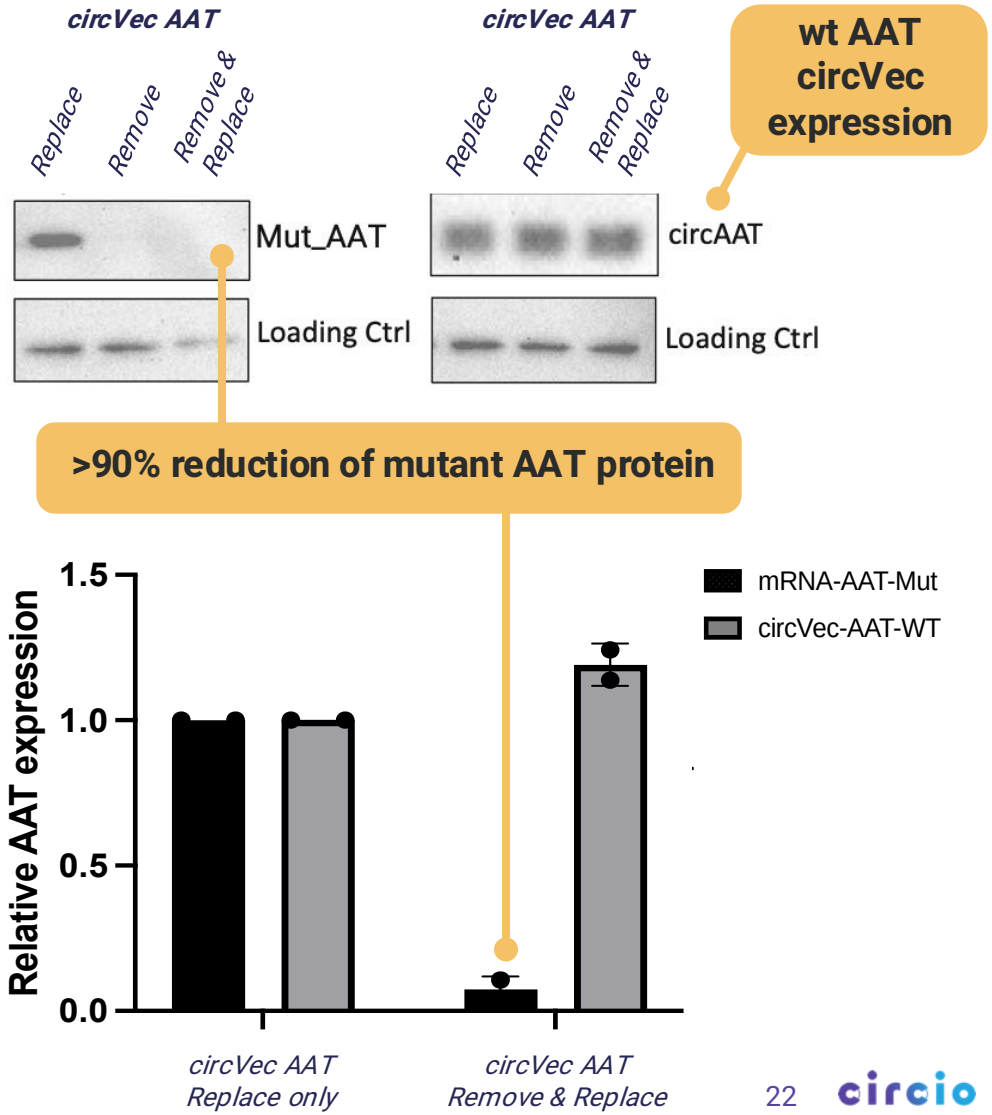


Replace

Expression of functional AAT protein

Remove

Degradation of mutant AAT mRNA

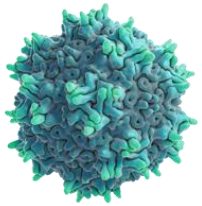


circVec R&D priorities and next steps

circVec platform

- circVec 2.2 generation testing in vivo
- Establish and test circVec 3.0 generation in vitro
- Two new patent filings at drafting / planning stage

Gene therapy applications



- Explore muscle-specific AAV and lower dose levels
- Test delivery and expression in additional tissues
- Implement circVec 2.2 → 3.0 features to AAV

Business Development

- Entered / entering five gene therapy delivery collaborations, data generation during next six months
- Seeking research collaborations with AAV and DNA-delivery companies

4

Summary

Full team in place with strong blend of expertise to build and capitalize on Circio´s platform

Dr Erik D Wiklund

CEO

Overall strategy and execution

CV:

- *PhD Molecular Biology*
- *circRNA co-discoverer*
- *Biotech CFO & CBO*
- *McKinsey & Company*

Dr Lubor Gaal

CFO & CBO

Securing financing and partnering deals

CV:

- *PhD Neuroscience*
- *Big pharma BD*
- *Biotech executive*
- *Investment banking*

Dr Thomas B Hansen

CTO

Building technology platform and IP

CV:

- *PhD Molecular Biology*
- *circRNA co-discoverer and scientific pioneer*
- *Big data analysis*

Dr Victor Levitsky

CSO

Leading immunology and virology expert

CV:

- *PhD Virology*
- *Big pharma R&D*
- *Biotech executive*
- *Top academic centers*

Ola Melin

COO

Operational execution

CV:

- *BSc Chem. Eng*
- *Big pharma and biotech manufacturing, clinical and commercial*

Active strategy to develop shareholder value through revenue-generating partnerships

2023	1H 2024	2H 2024	2025
Initiate partner dialogues	**Tailor R&D strategy**	**Generate requested data**	**Enter revenue-generating deals**
• **Indication of interest** in technology • **Feedback on targets** and applications • **Feedback on data** and R&D plan	• **R&D strategy adapted** to BD feedback • Sharpened focus on **gene therapy** • Experimental plan to **address specific partner requests**	• **Revisit partners when requested data** has been generated • Address any follow-up requests • **Short-list** top priority targets	• **Partner negotiations and closing** • Further supportive data generation • Initiate R&D work on secondary priorities

100+ prospective partners contacted – 30+ requested follow-ups – 10 CDAs entered to date

Approximate numbers

Timing and value of BD deals dependent on strength and timing of experimental data

circVec summary and next steps

In vitro validation

- circVec 2.1 generation outperforms mRNA by 10x
- Validated in various cells, tissues and 20 payloads
- Platform potential, three patent applications filed

In vivo validation

- Statistically significant improvement over mRNA-based expression in multiple in vivo settings
- Multiple vector and delivery strategies in testing
- circVec-AAV functionality confirmed in vivo

Next steps

- Establish circVec 3.0 generation and broaden IP
- circVec-AAV enhancement and in vivo validation
- Enter first strategic partnership in 1H´25