



circio

Disruptive circRNA technology for genetic medicine

circVec R&D update
17 June 2024

Meet today's Circio executive team presenters



Dr Erik D Wiklund
CEO

Circular RNA
co-discoverer &
entrepreneur

CV:

- *PhD Molecular Biology*
- *Biotech CFO & CBO*
- *McKinsey & Company*



Dr Thomas B Hansen
CTO

Circular RNA
co-discoverer &
field pioneer

CV:

- *PhD Molecular Biology*
- *Academic group leader in RNA and bioinformatics*



Dr Victor Levitsky
CSO

Leading immunology &
virology expert, deep
translational experience

CV:

- *PhD Virology*
- *Big pharma R&D*
- *Biotech executive*

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The opportunity

2. The circVec approach
3. Therapeutic application of circVec
4. Summary

Gene therapy is one of the fastest growing therapeutic areas, with increasing priority from industry and regulators

POLICY

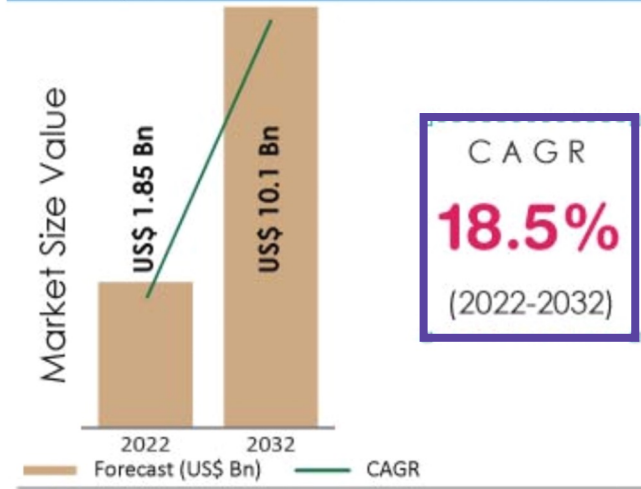
FDA adopts Operation Warp Speed lessons for rare disease pilot program

The FDA announced the launch of a pilot program, dubbed START, to address challenges associated with rare disease development and speed up the regulatory process.

Lecia Bushak | November 22, 2023 | 10:51 AM



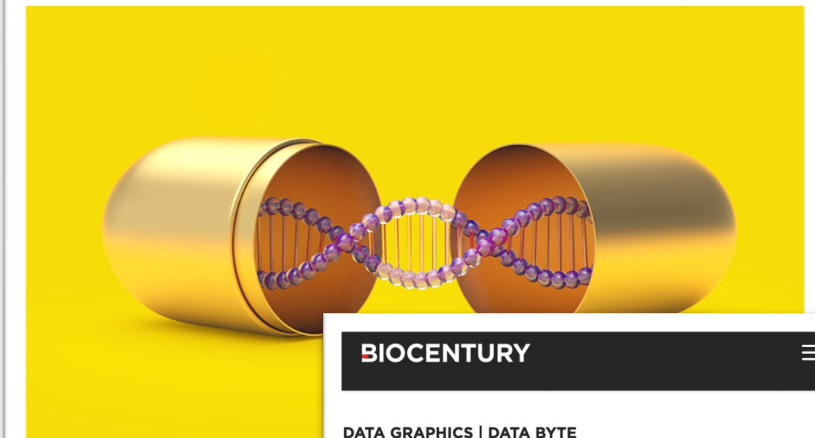
Gene Therapy Market Forecast Analysis, 2022-2032



Source: Fact.MR

TECHNOLOGY

Have Million-Dollar Gene Therapies Finally Reached An Inflection Point?



BIOCENTURY

DATA GRAPHICS | DATA BYTE

Novartis' Zolgensma first gene therapy to top \$1B

The era of effective, but pricey, gene therapies is at hand

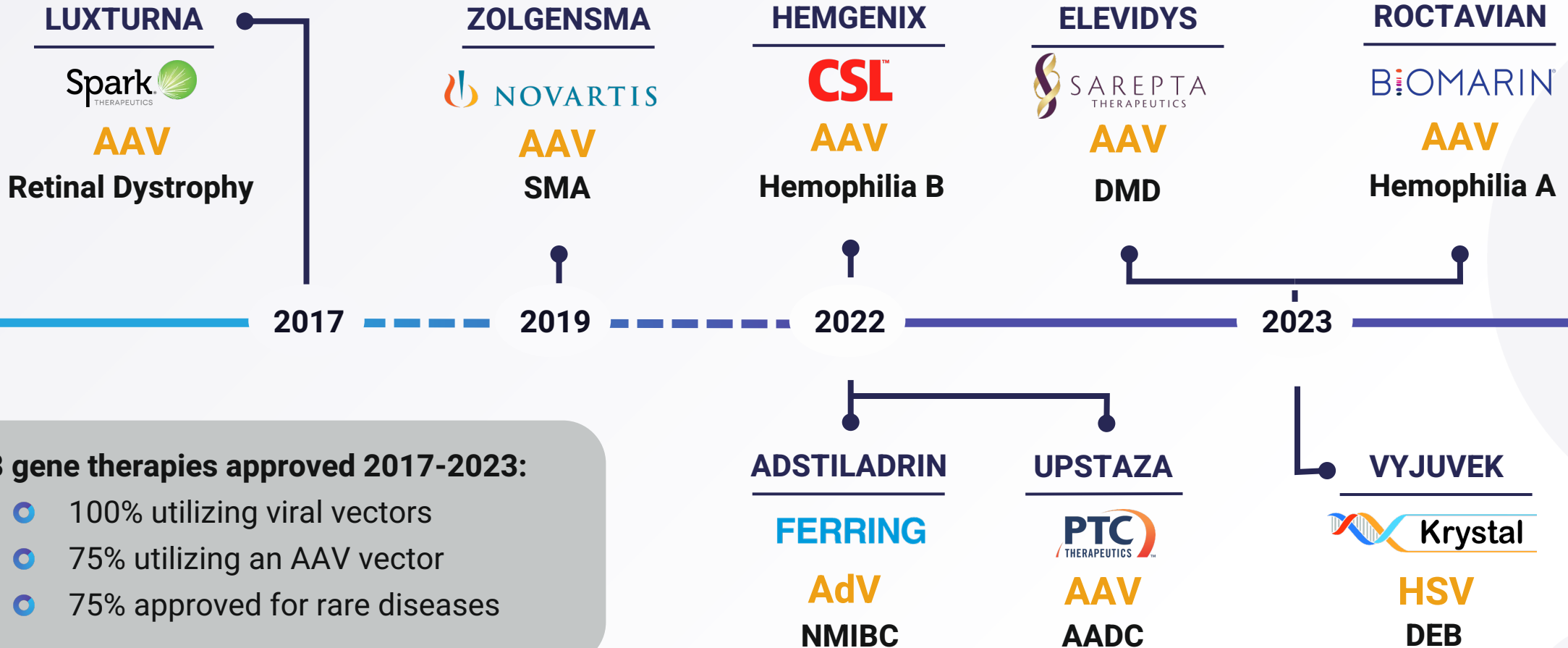
f t in Licensing

ALLISON GATLIN | 12:50 PM ET 09/15/2023

Get ready for a world of million-dollar drugs. Pricey gene therapies that could cure devastating genetic disorders in one fell swoop are gaining momentum, brightening the horizon for biotech stocks like Sarepta Therapeutics (SRPT) and BioMarin Pharmaceutical (BMRN).

Focus area for regulators → Fastest growing class of new approvals → Commercial success

Circio aims to improve current gold-standard gene therapy: 6 out of 8 approved gene therapies are AAV-based



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

circular RNA can:

→ boost potency

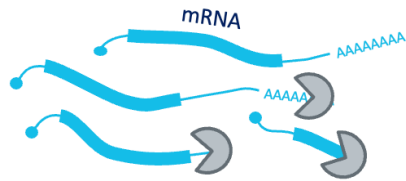
→ lower toxicity

→ reduce cost

circRNA can increase durability and expression level, thus enhancing the potency of gene therapy

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

The circRNA field was established by Circio scientists



Dr Thomas B Hansen

Dr Erik D Wiklund





nature

7,263 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 | 1,031 citations

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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

3,224 citations

Review Article | Published: 08 August 2019

The biogenesis, biology and characterization of circular RNAs

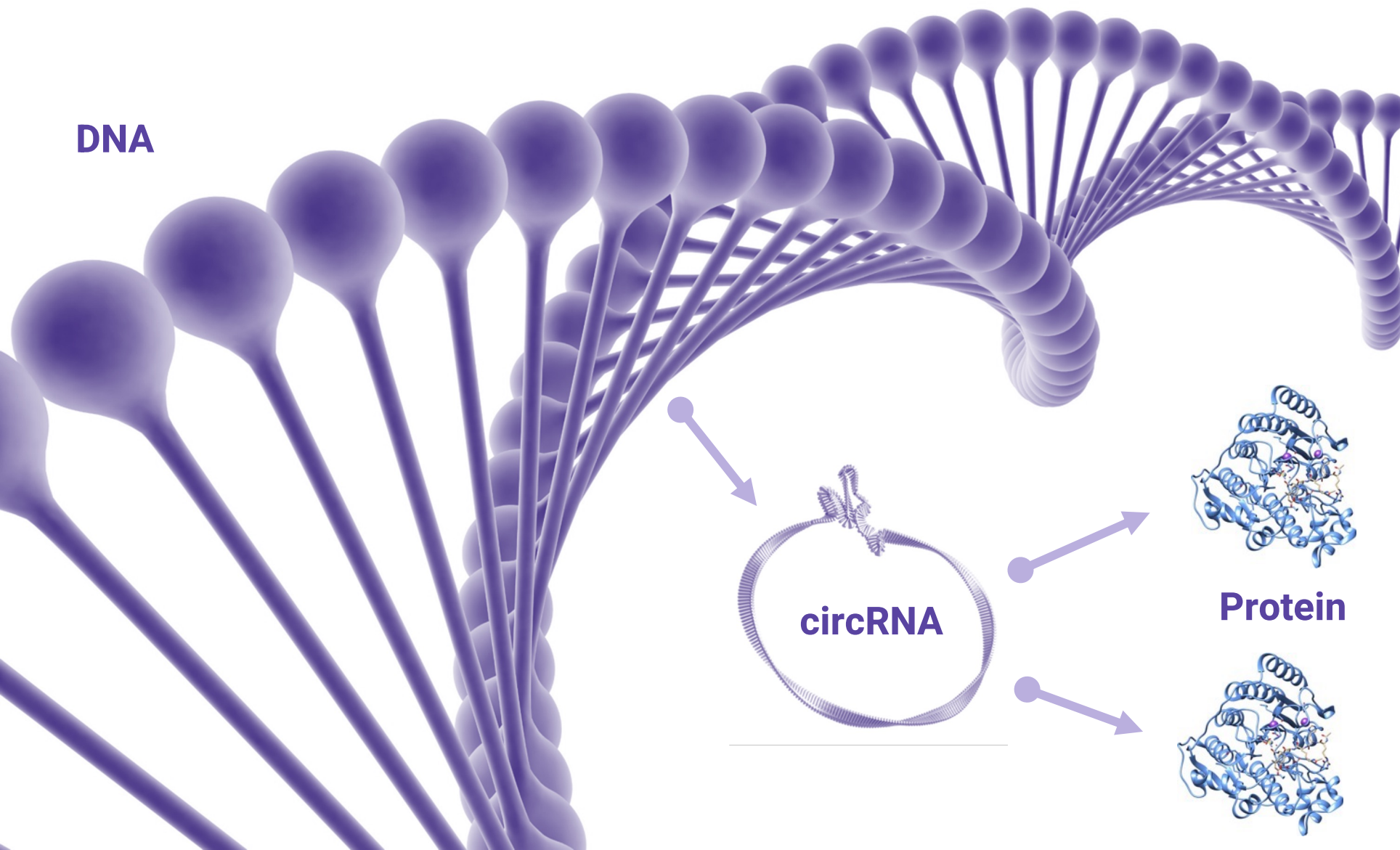
[Lasse S. Kristensen](#) , [Maria S. Andersen](#), [Lotte V. W. Stagsted](#), [Karoline K. Ebbesen](#), [Thomas B. Hansen](#) & [Jørgen Kjems](#)

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The circVec approach

3. Therapeutic application of circVec
4. Summary

The circVec expression system: making circRNA from a DNA starting point








circVec
DNA or viral
vector

Inject

circRNA
biogenesis

Enhanced and
durable protein
expression

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

		<i>Expression durability</i>	<i>Main opportunity in vaccines</i>	<i>Suitable for gene therapy</i>	<i>Delivery system</i>	<i>Existing CDMO manufacturing</i>
	circVec vector approach	months to years	✗	✓	Viral or DNA-LNP	✓
 	Synthetic circRNA	7-10 days	✓	✗	circRNA-LNP	✗
 	Synthetic mRNA	2-3 days	✓	✗	mRNA-LNP	✓

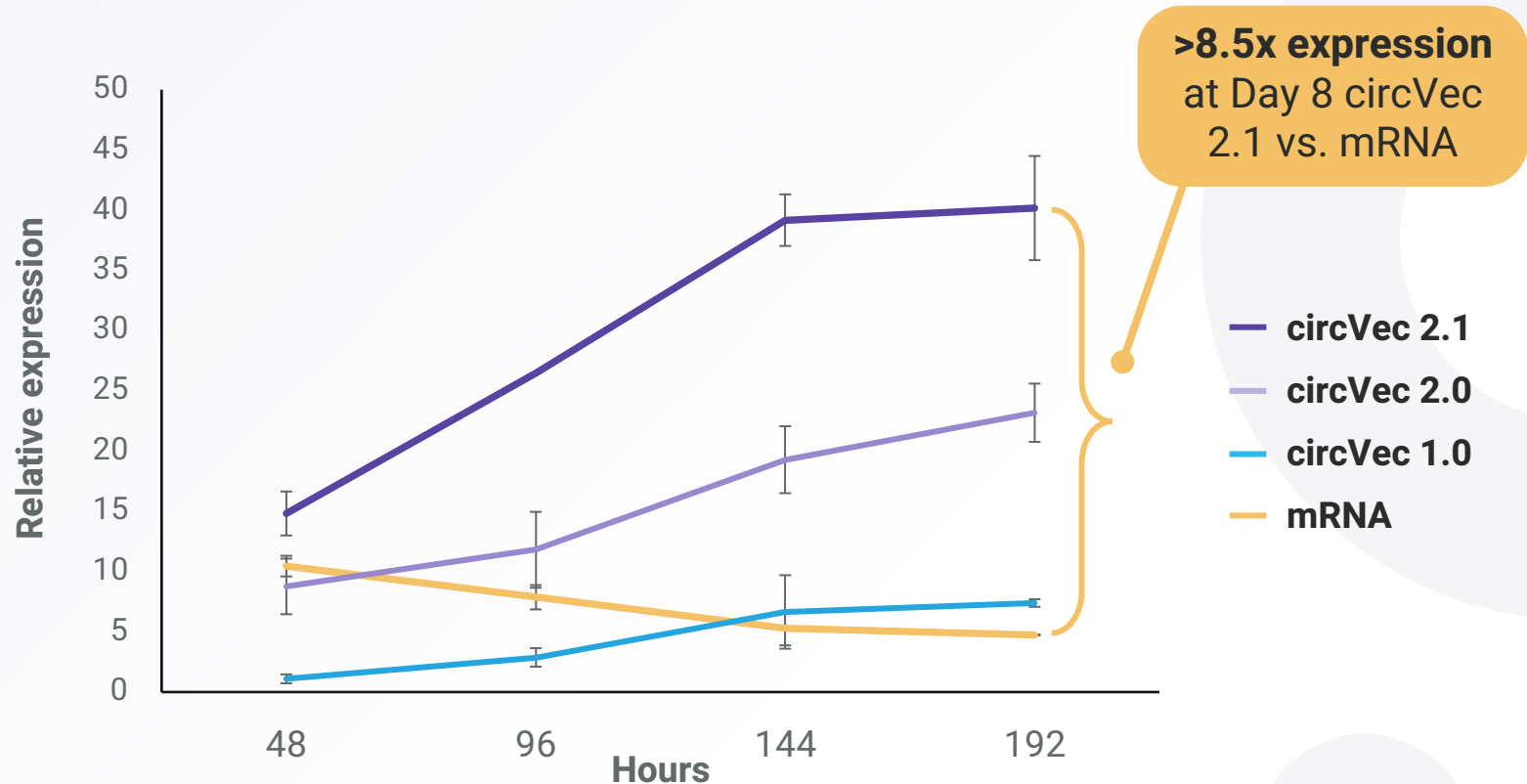
circVec substantially outperforms the expression level and durability of mRNA-based systems in vitro

circVec RNA stability

135h vs. **9h**
circRNA vs. mRNA

15x

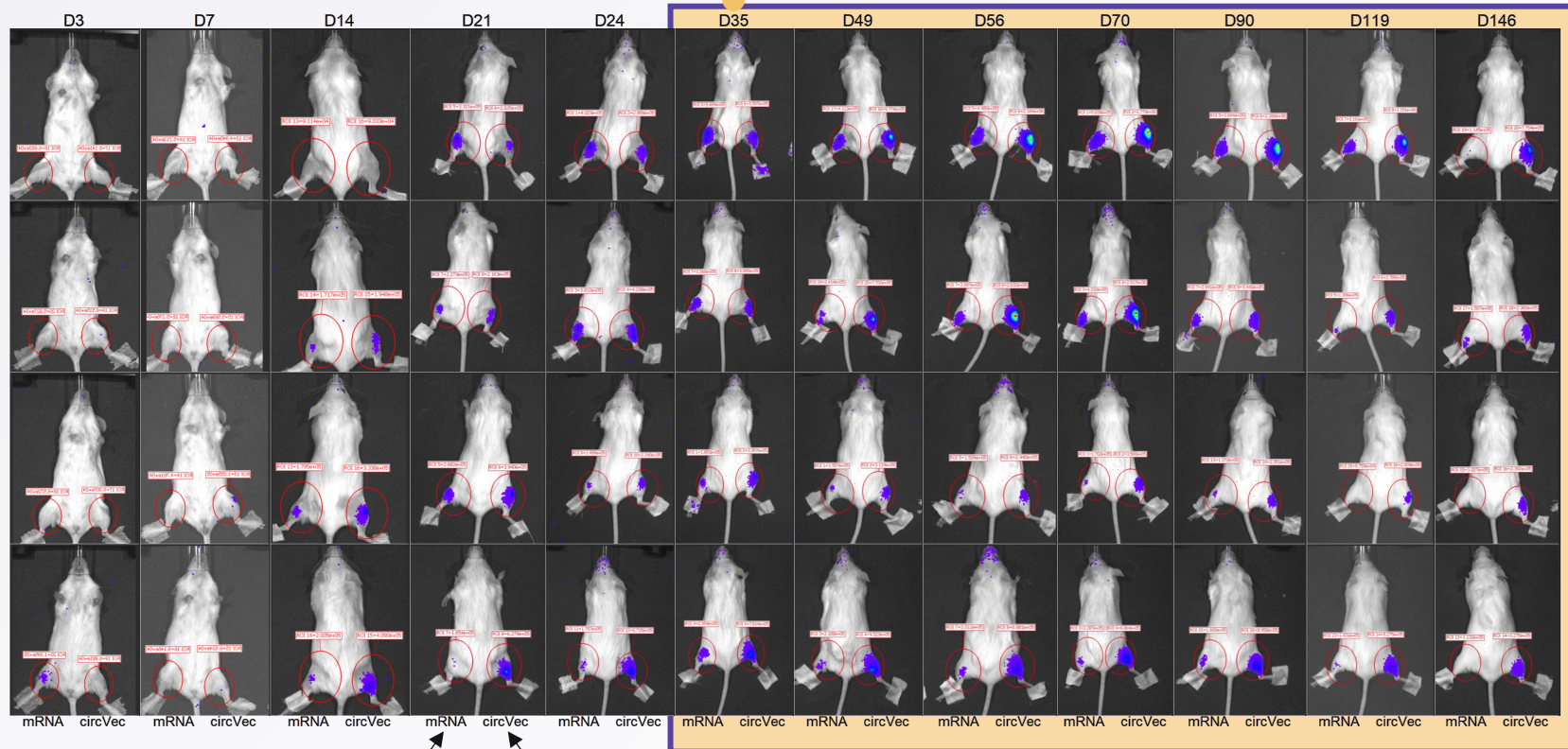
circVec vs. mRNA protein expression assay; time course



Confirmatory in vivo study validates circVec 2.1 expression advantage vs. mRNA up to Day 146

circVec expression superior from week 4 and onwards

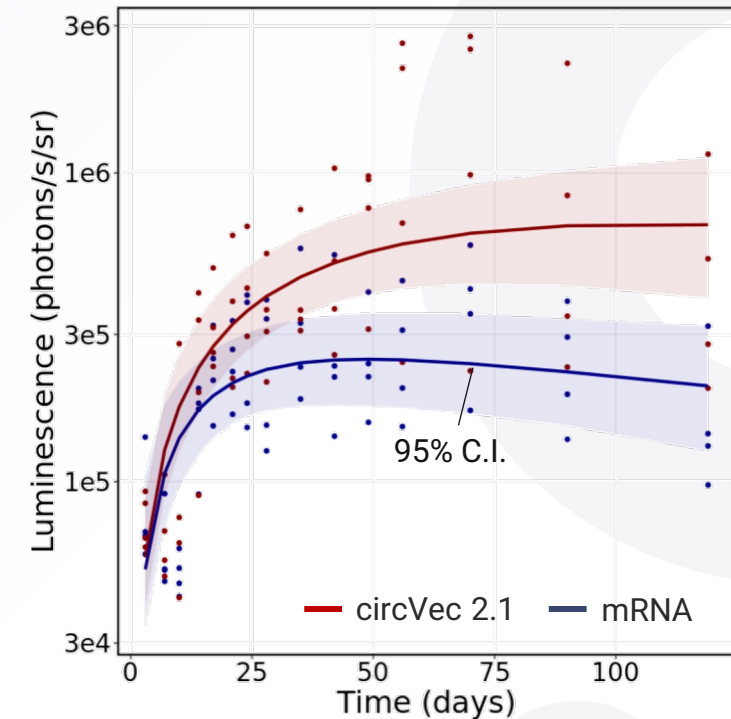
Day 146



mRNA-vector in left hindleg
circVec in right hindleg

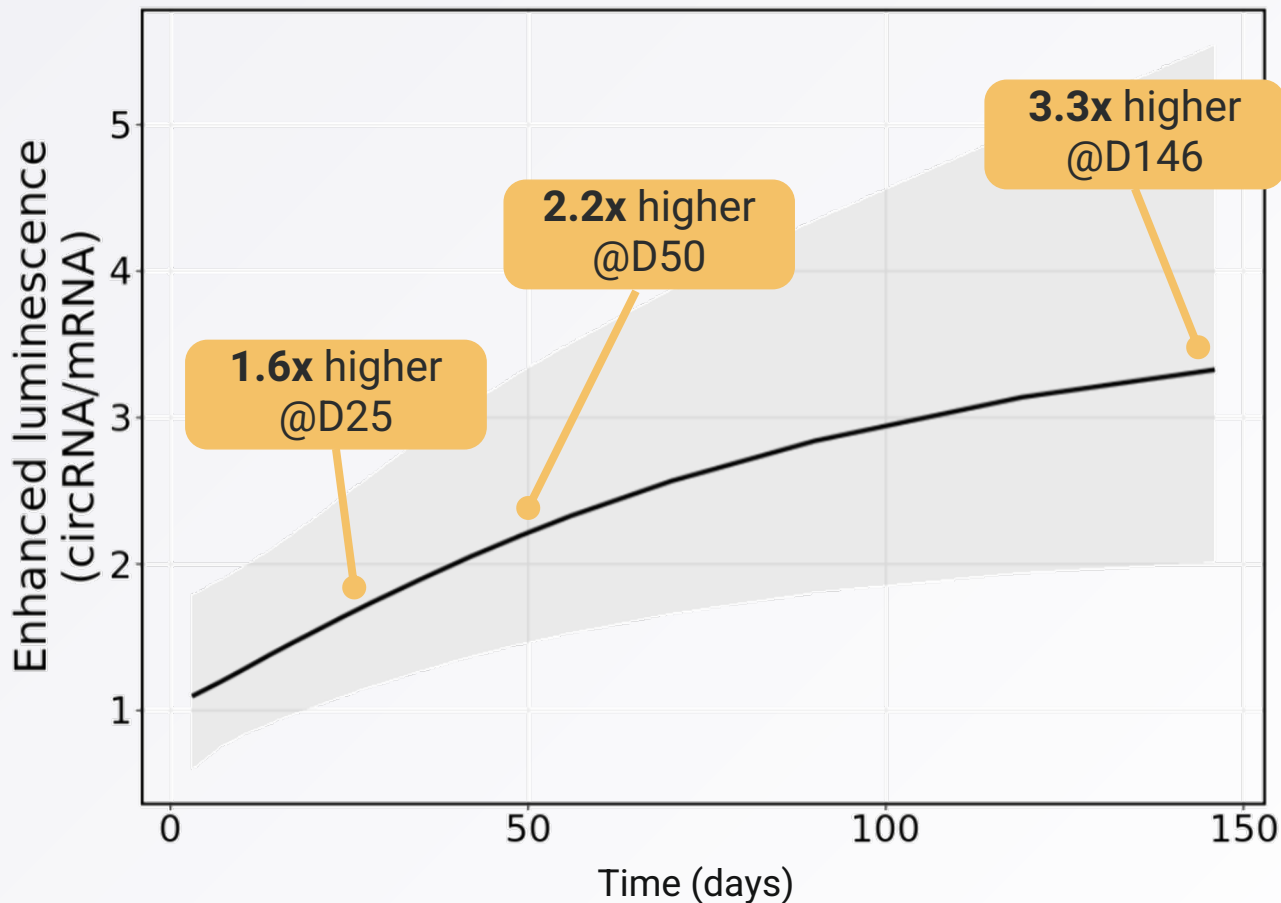
Statistical modelling based on growth-decay equation:

$$E(t) = e^{-D_{VEC}t} (1 - e^{-D_{RNA}t}) \frac{P_{RNA}}{D_{RNA}}$$

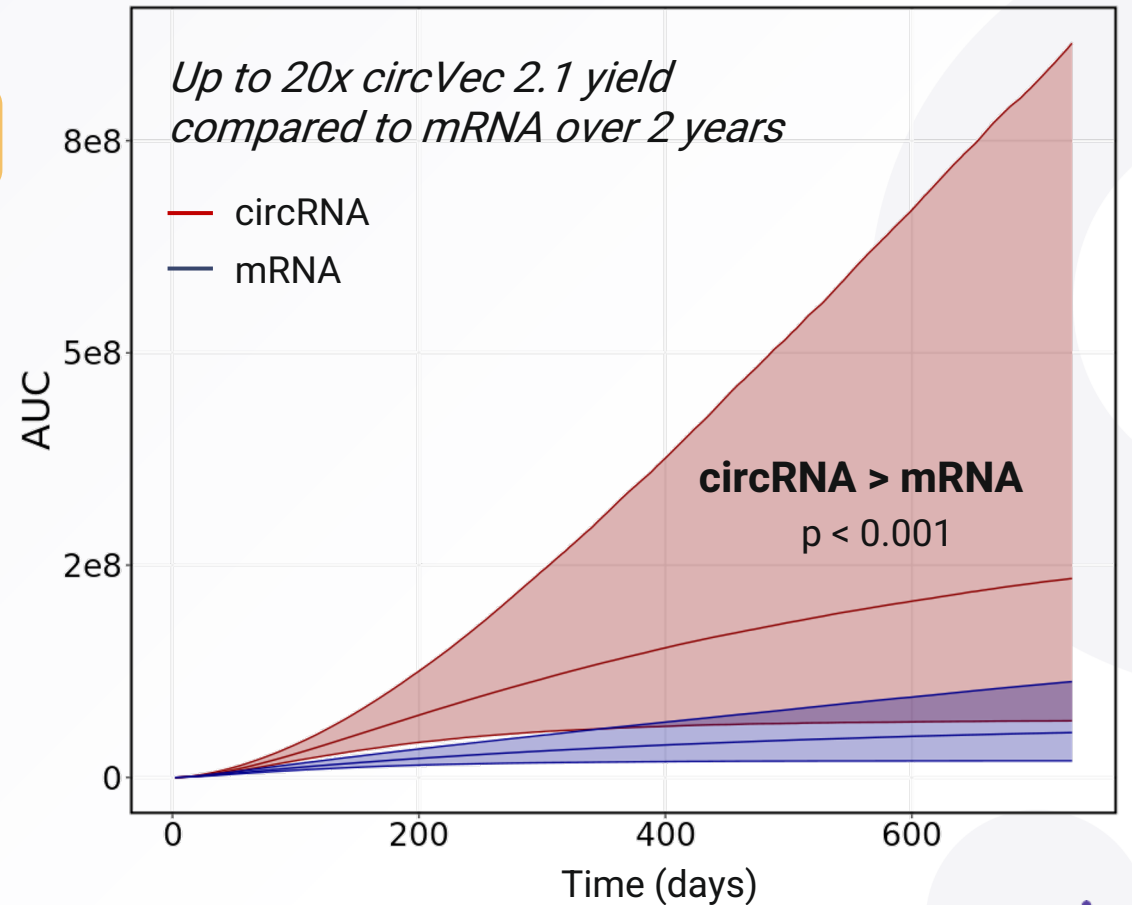


Statistical analysis of in vivo data demonstrates significant advantage vs. mRNA increasing over time

Luciferase signal in vivo, -fold change
circVec 2.1 vs. mRNA pDNA vector expression



Statistical modelling of long-term expression
circVec 2.1 vs. mRNA expression dynamics, 2 years

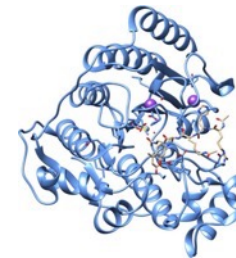
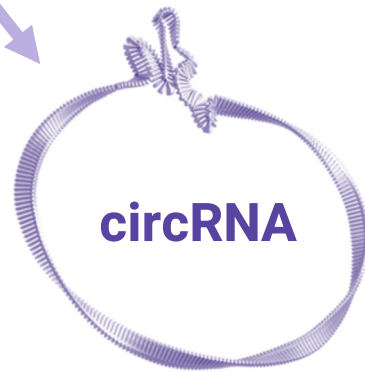


Improving the circVec expression system: Towards circVec 3.0

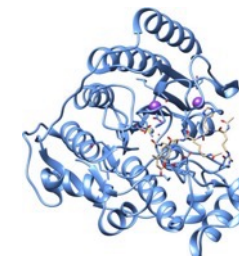
circVec

Improve biogenesis

Improve translation



Protein



circVec
DNA or viral
vector

Inject

circRNA
biogenesis

Enhanced and
durable protein
expression

Using machine learning to establish circVec 2.2 design

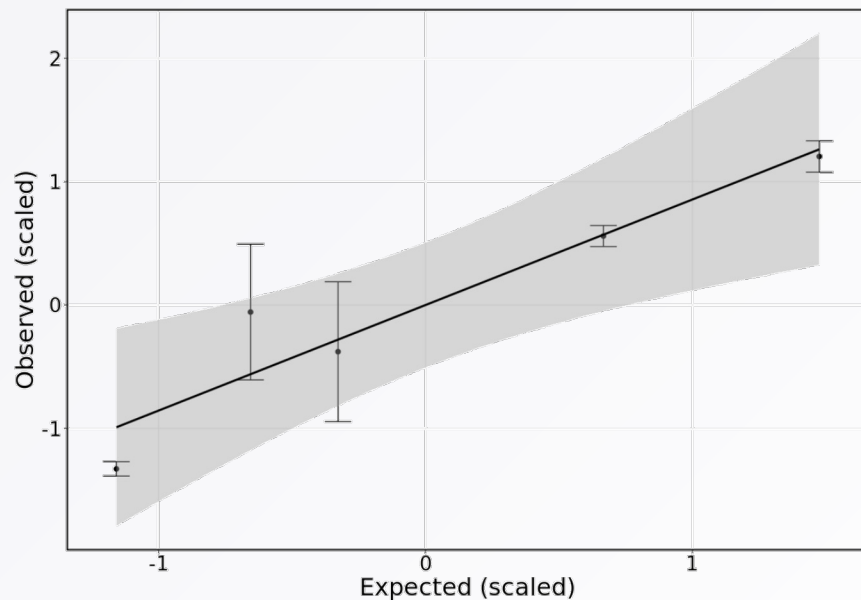
Codon composition sampling

Select most informative variants

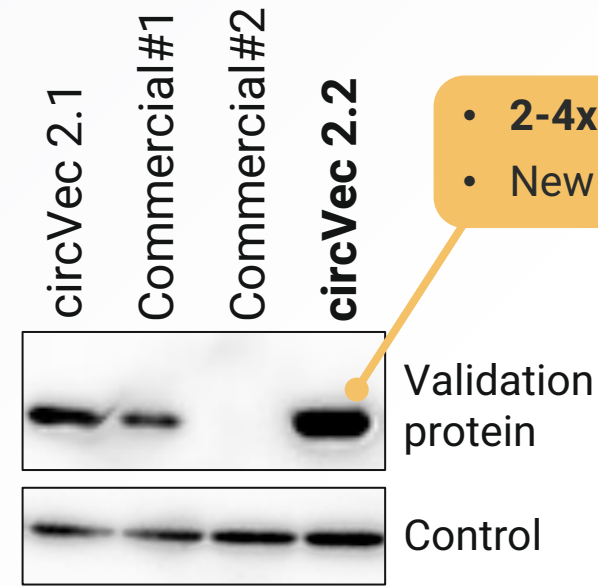
Generate experimental data

Build machine learning model

Model validation



circVec expression



- 2-4x protein yield
- New **circVec 2.2** feature

Validation protein

Control

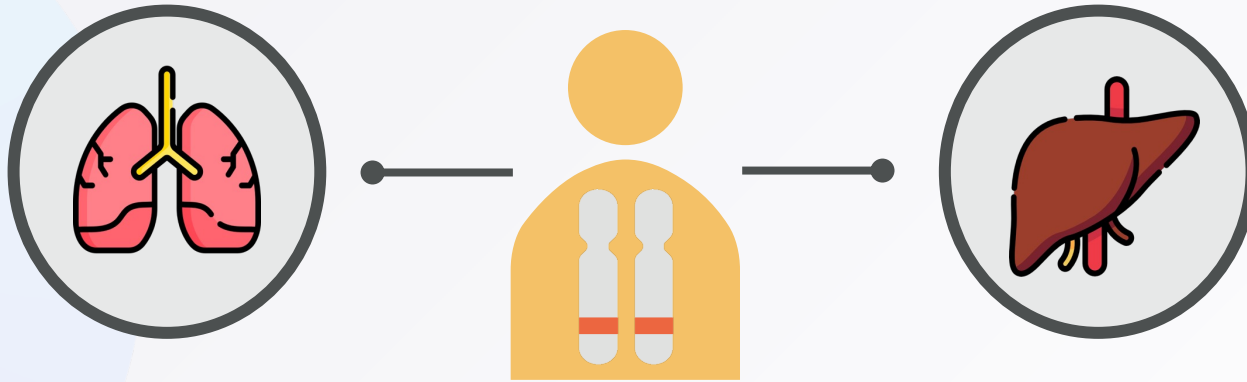
3

Therapeutic application of circVec

4. Summary

Lead indication: Alpha-1 antitrypsin deficiency (AATD)

AATD is a genetic disease manifested in liver and lung



- Lack of functional AAT protein
- Emphysema and/or chronic bronchitis

- Toxic accumulation of mutant form of protein
- Cirrhosis

Number of patients:

120K in EU

75K in US

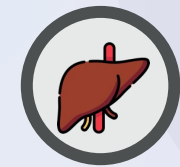
No satisfactory treatment options → Major unmet medical need
Significant commercial opportunity

Current treatment options



Lung-associated AATD

- Replacement therapy with an alpha-1 proteinase inhibitors
- Weekly IV infusions
- Bronchodilators and inhaled steroids used for mild symptoms

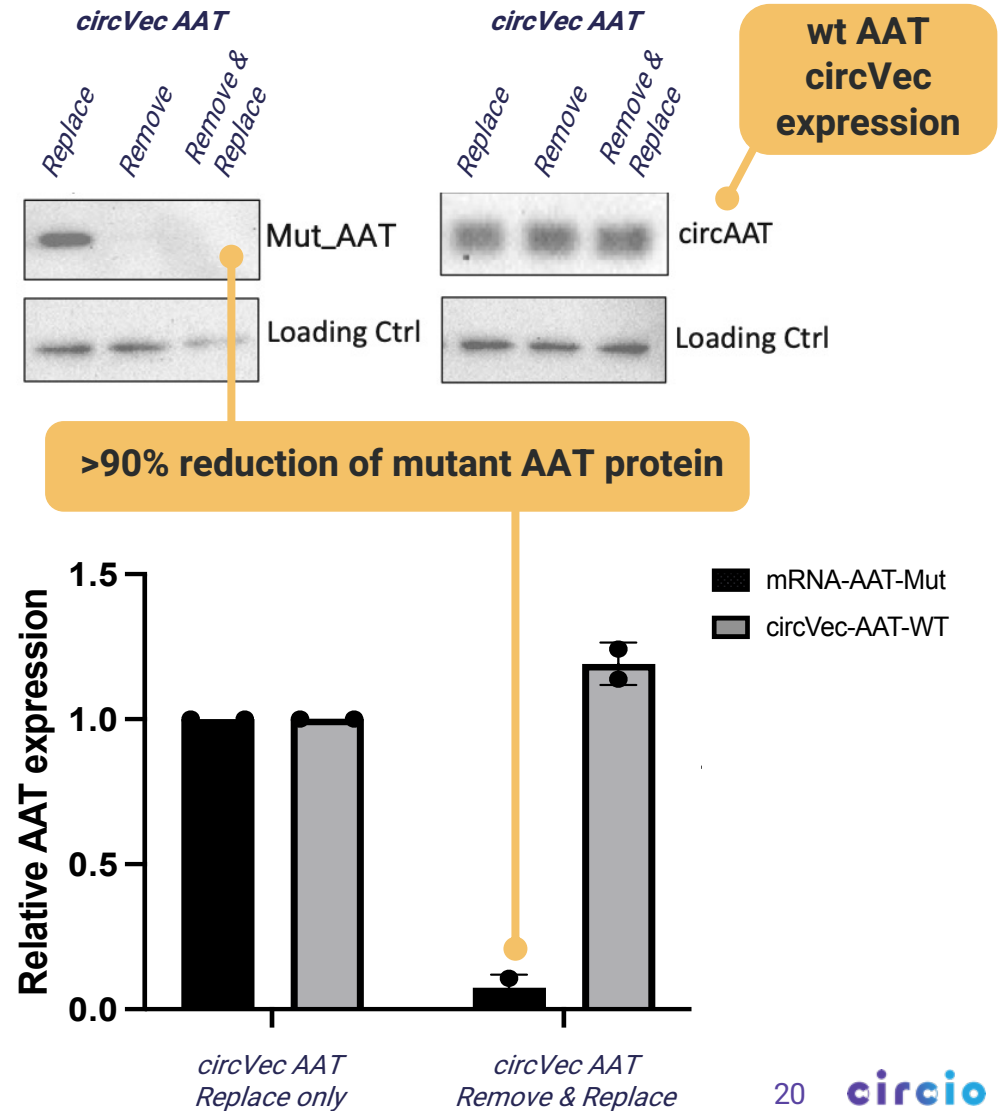
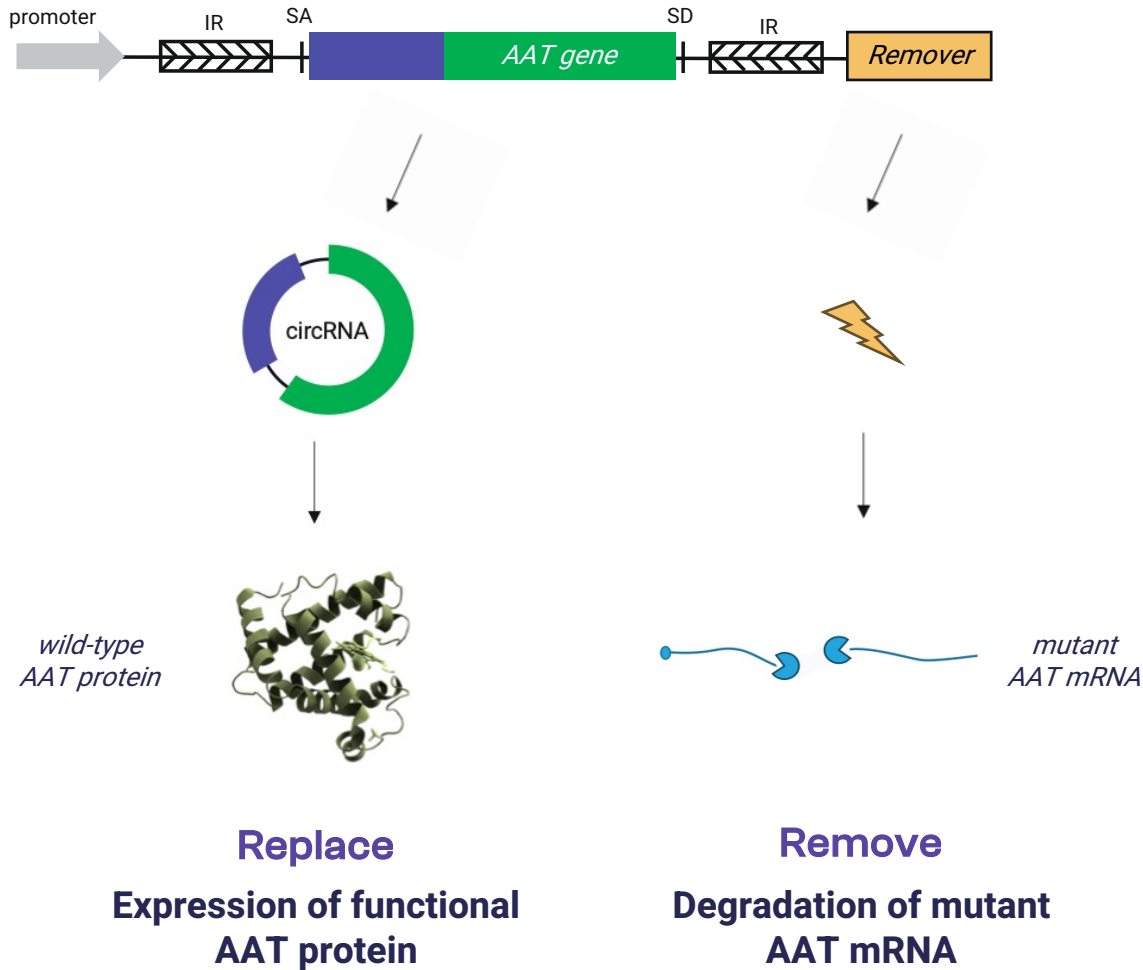


Liver-associated AATD

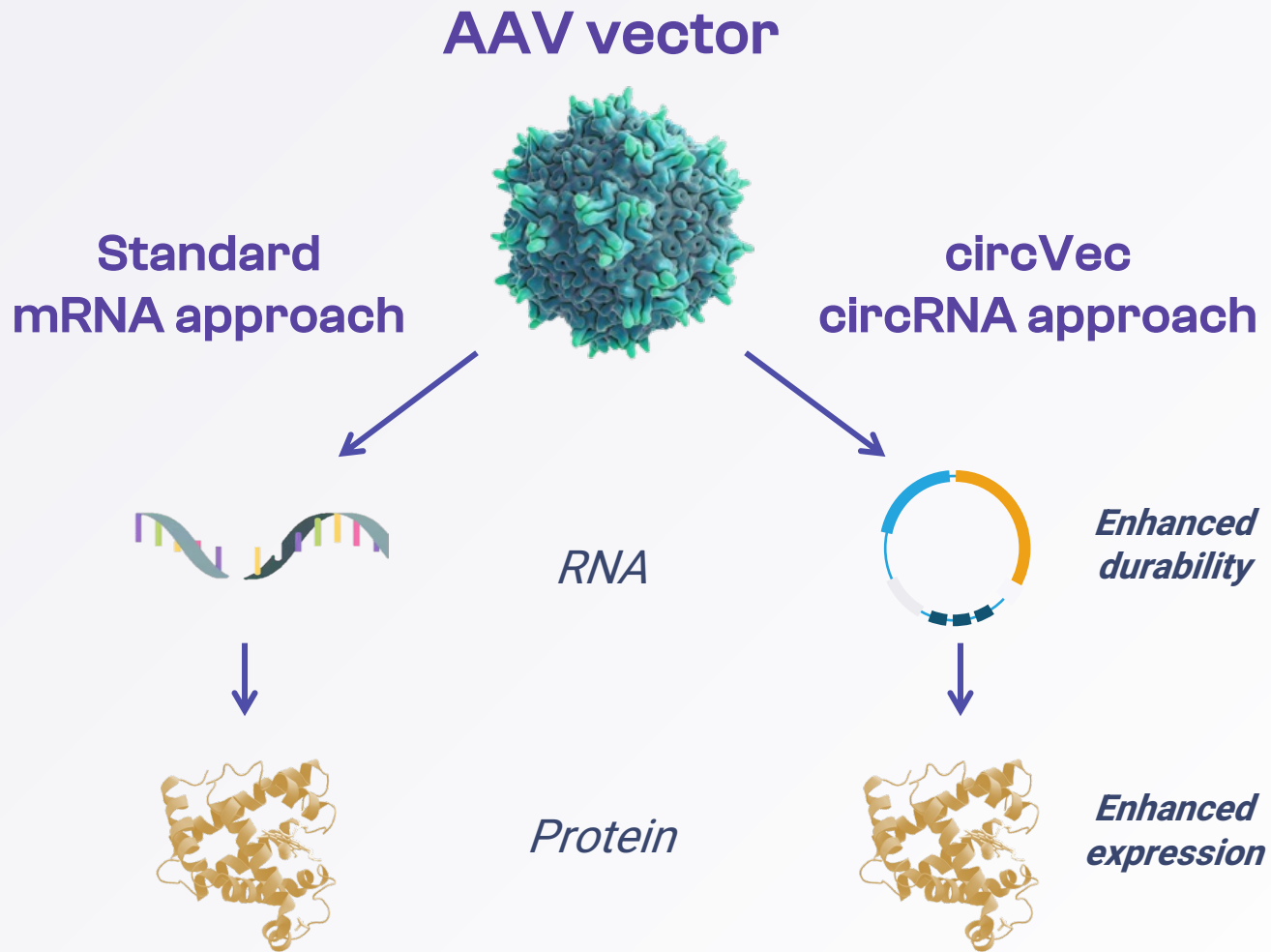
- No approved therapeutics
- Liver transplantation is the only treatment alternative in severe cases

Lead circVec gene therapy program: Differentiated 'Remove-&-Replace' concept for AATD

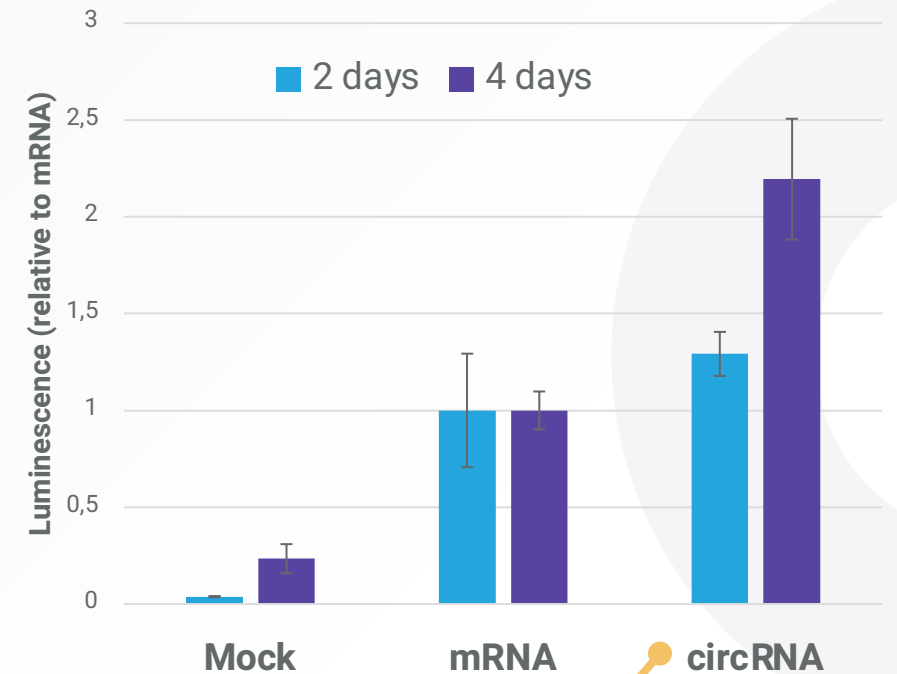
AAV-circVec2.0 AATD R&R design



circVec-AAV gene therapy for AATD



AAV protein expression, luminescence

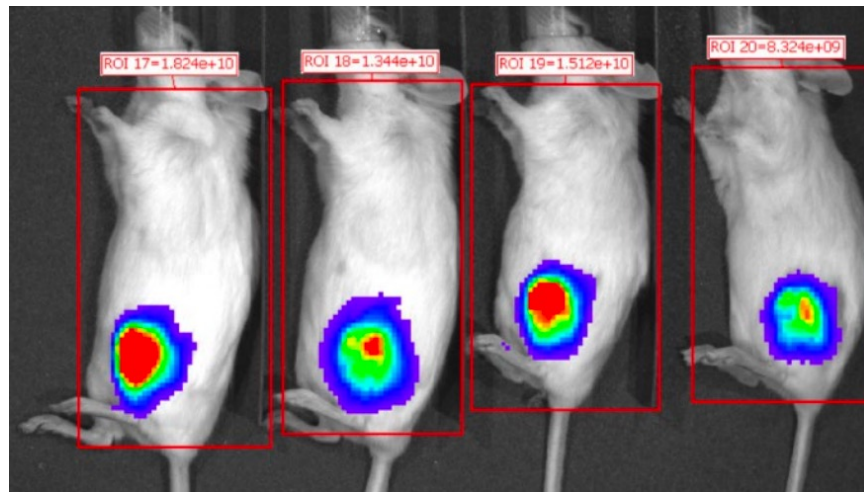


Enhanced circVec-AAV expression vs. mRNA-AAV, validated by multiple experimental methods *in vitro*

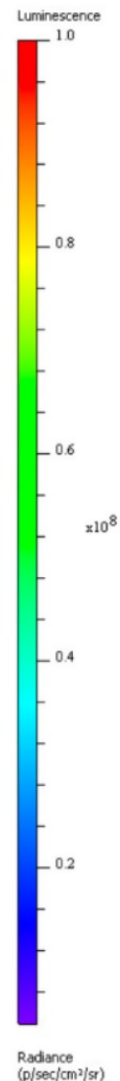
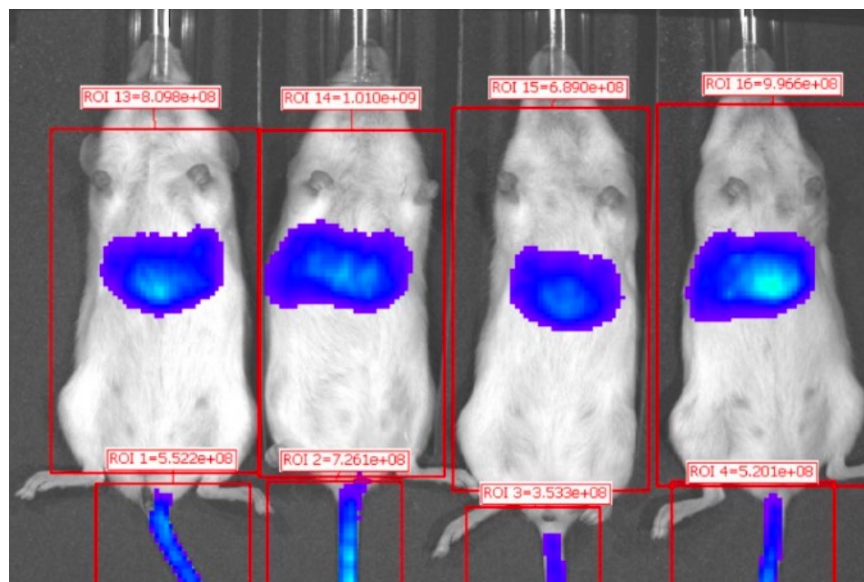
circVec 2.0 AAV vector expression validated in vivo both by I.V. and I.M. delivery – tracking vs. mRNA ongoing

circVec-AAV luminescence, F-luc at Day 20

Intra-muscular (I.M.)



Tail vein (I.V.)



Experimental set-up

Vector:	AAV8
circVec version:	circVec 2.0
Payload:	Firefly luciferase (F-luc)
Mouse strain:	NOD/SCID/IL-2R γ null immunodeficient mice
Delivery route:	Tail vein or intra-muscular injection
Single injection, dose:	1x10 ¹⁰ or 1x10 ¹¹ viral genomes



4

Summary

circVec R&D 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

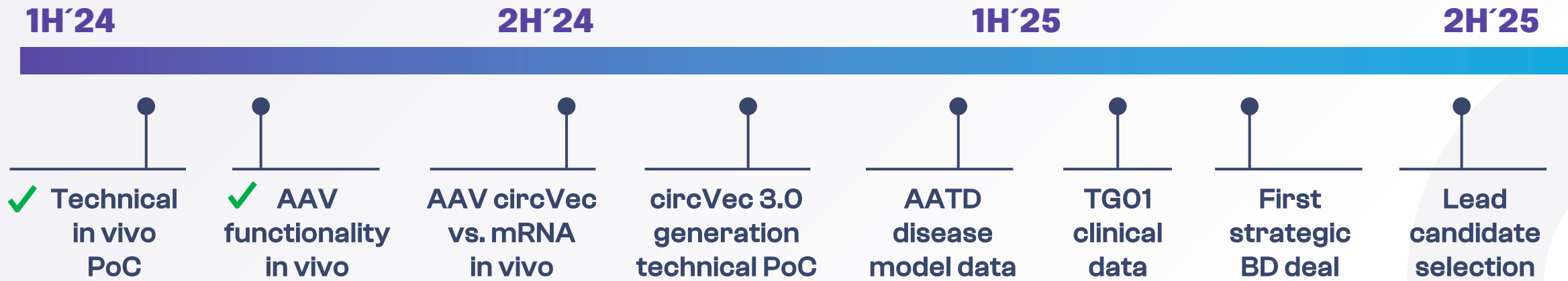
- **Statistically significant improvement over mRNA-based expression**
- **Multiple delivery and dosing strategies confirmed**
- **circVec-AAV functionality confirmed in pilot study**



Next steps

- **circVec-AAV in vivo validation and comparison to mRNA-AAV**
- **circVec disease model data in AATD**
- **Testing of multiple vector and delivery strategies**

R&D & BD value inflection points: Targeting first partnering deal during 1H '25



Financing strategy

NOK 50m financing runway – mid-24 → mid-25

Financing to clinic

BD strategy

