MicroRNAs: A new Class of Substances for the Therapy of Diseases

Thomas Thum, MD, PhD

Disclosures: TT holds and licensed several miRNA/lncRNA patents in CV disease. TT is founder and holds shares of Cardior Pharmaceuticals GmbH. TT received support/holds advisory seats of Sanofi/Genzyme, Novo Nordisk, Takeda, Amicus Therapeutics, Boehringer Ingelheim.

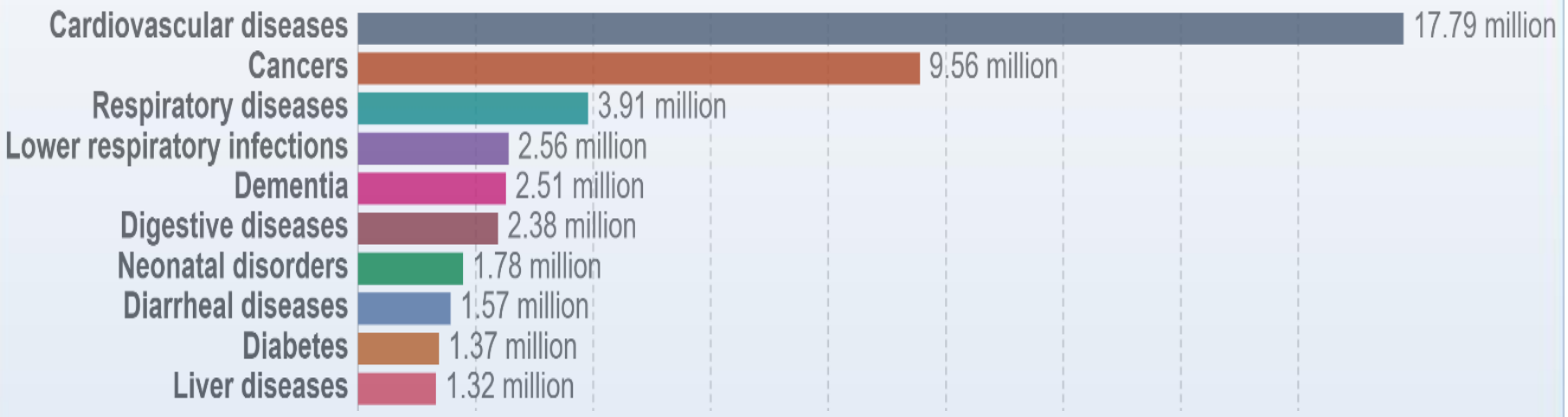
MHH Hannover Medical School

Different Hats

CARDIOR

- Director, Institute of Molecular & Translational Therapeutic Strategies (IMTTS)
  Hannover Medical School
- Director, **Fraunhofer Institute of Toxicology & Experimental Medicine (ITEM)**
- Founder & CSO,
  Cardior Pharmaceuticals GmbH

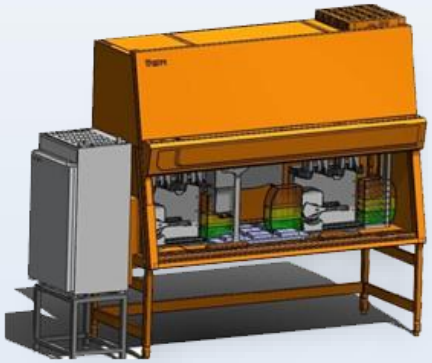
Unmet Medical Needs



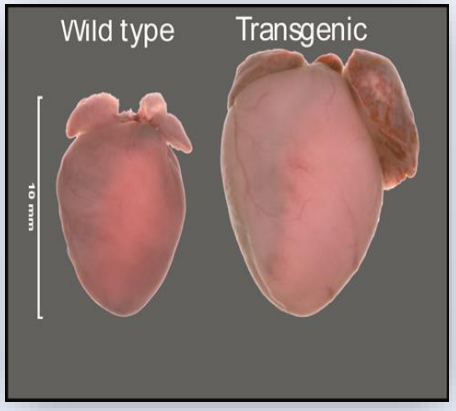
From Discovery Research to Translation

Established RNA drug development pipelines

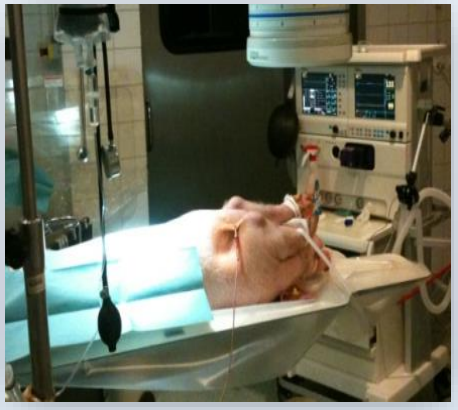
High Throughput Screenings



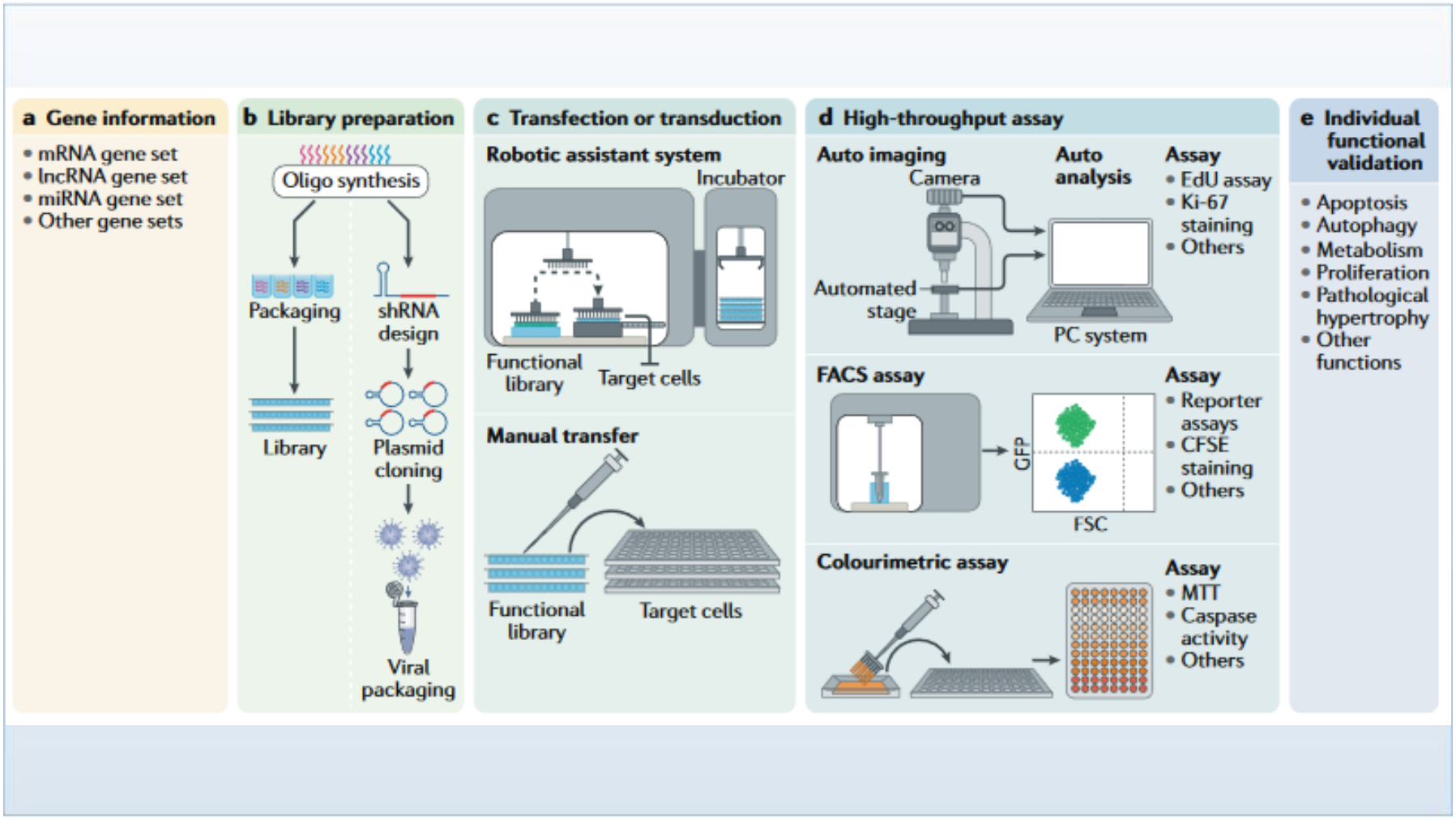
Target Validation & Indicative Safety



Efficacy in small & large animals

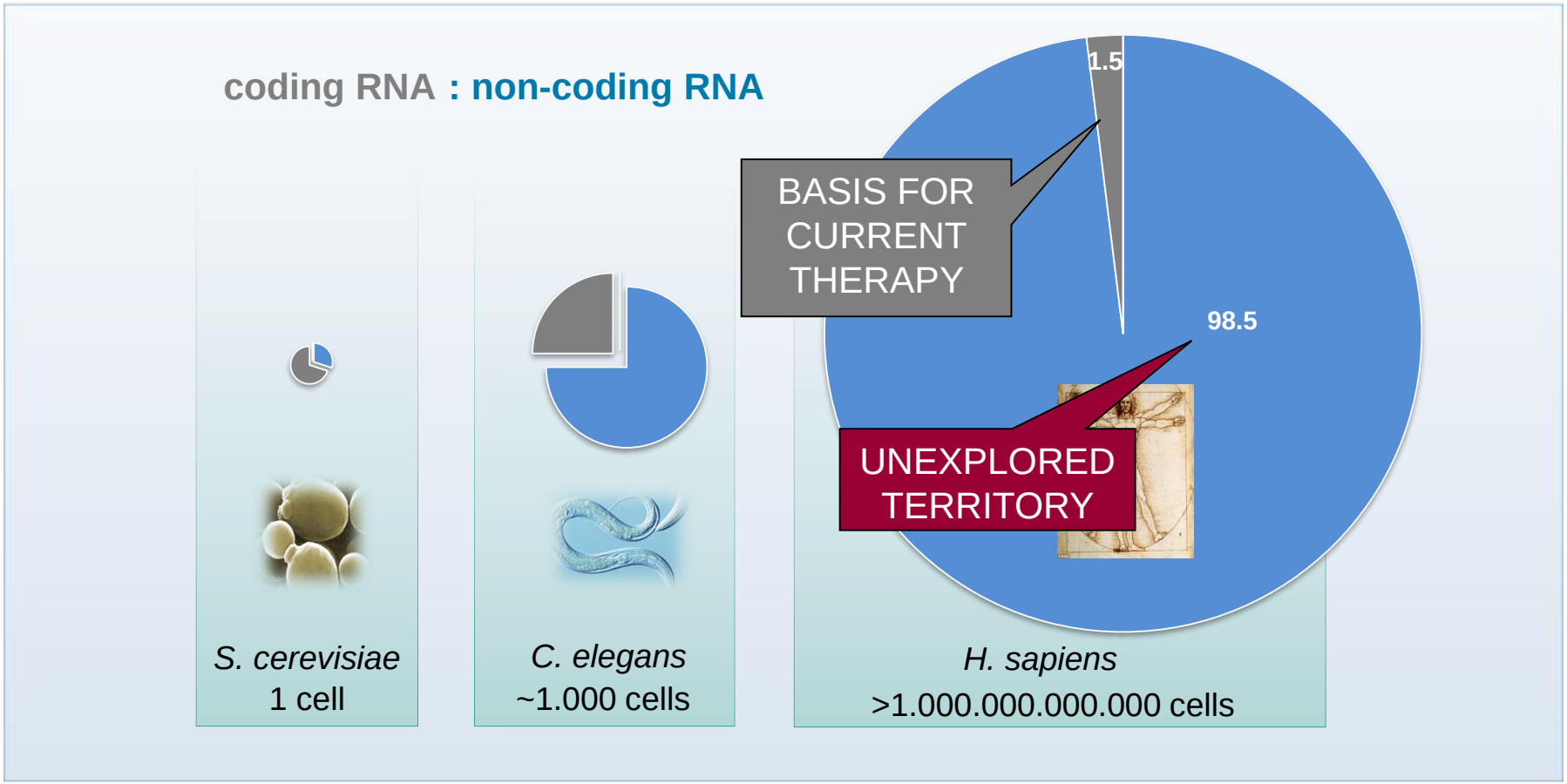


Functional high throughput screening of RNAs



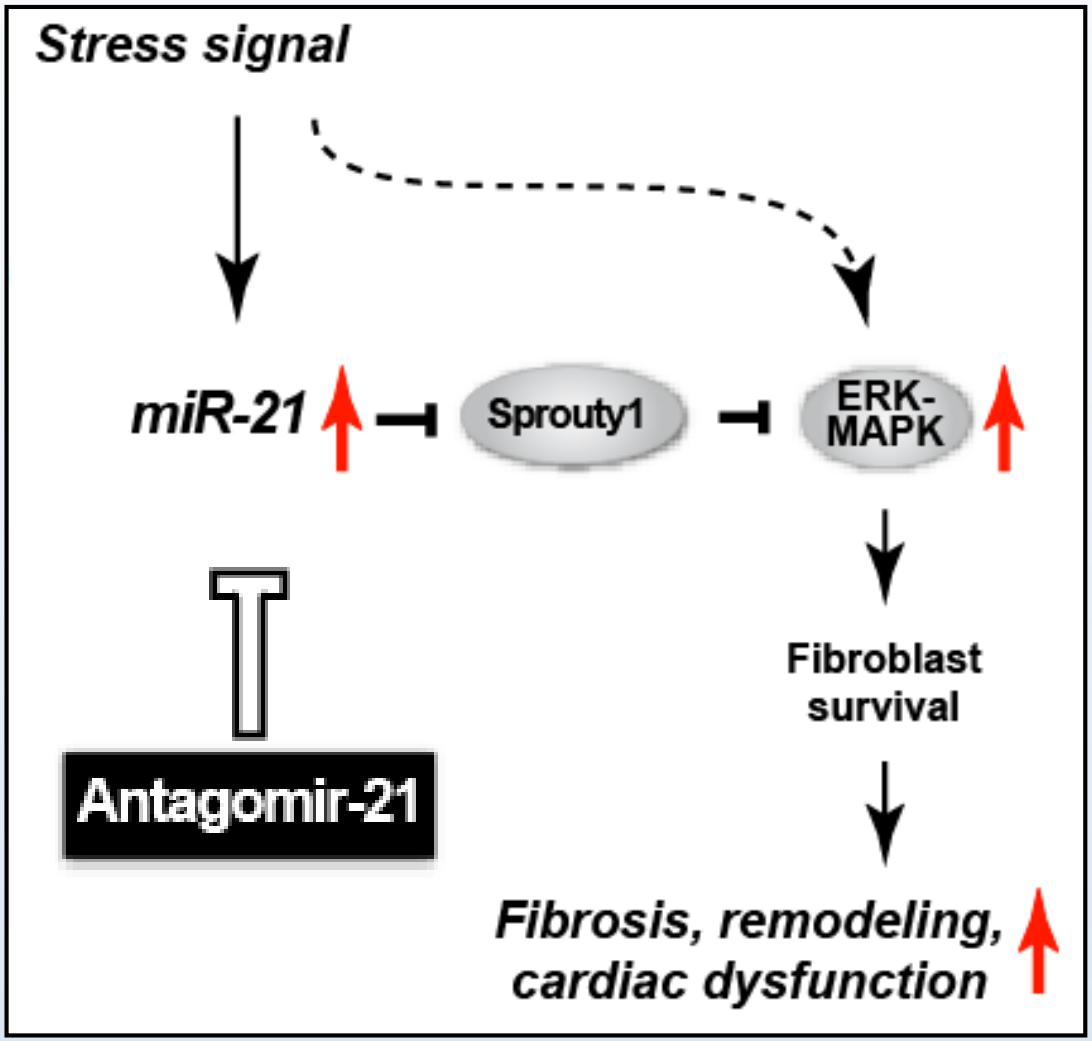
Lu & Thum, **Nature Reviews Cardiology**, 2020

Noncoding RNAs in evolution



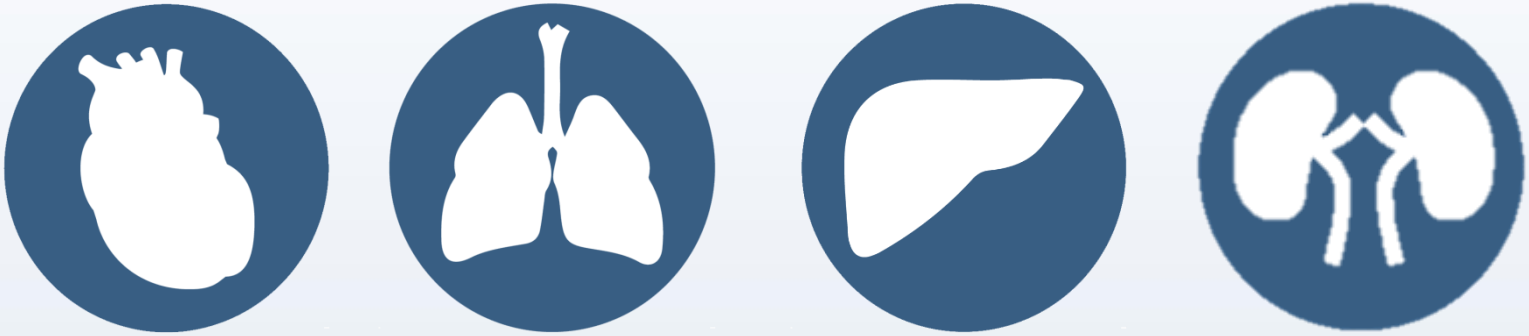
Druggable targets: Silencing through inhibitory synthetic small oligonucleotides

(e.g. ***Nature***, 2008; ***Nature Genetics***, 2010; ***Nature Comm***, 2012; ***Science Trans Med***, 2016; **Nature Comm**, 2017, 2019, 2020; **Eur Heart J**, 2020, **Eur Heart J**, 2021 a,b, **JACC**, 2021)



Nature, 2008:456:980-4

miR-21 inhibitors as general anti-fibrotic innovative drugs



Translational Success / Tech Transfer

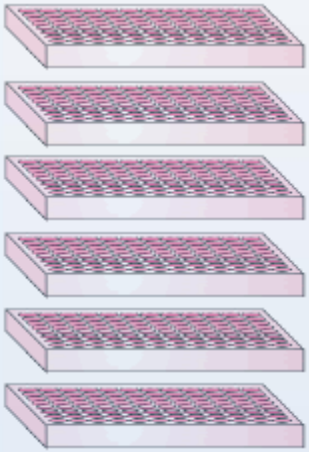
- 2010: **IP licensed** to Regulus Therapeutics; sublicensed to Sanofi
- 2017–18: **Phase I** Clinical Study; NCT03373786
- 2019: HERA, a **Phase II** randomized (1:1), double-blinded, placebo-controlled study evaluating the safety and efficacy of RG-012 in Alport syndrome patients (NCT02855268)

J Exp Med. 2010;207:1589-97; **J Clin Invest**. 2011 Feb;121(2):461-2;
Cancer Res. 2015 May 1;75(9):1859-67; **Clin Sci (Lond).** 2016 Aug 1;130(16):1469-80

High-throughput functional microRNA profiling

Noncoding RNA libraries
(300 – 26.000 inhibitors/shRNAs)

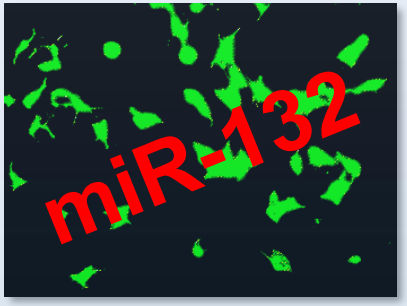
Transfection/
Transduction



fibroblasts/
cardiomyocytes

Quality checks

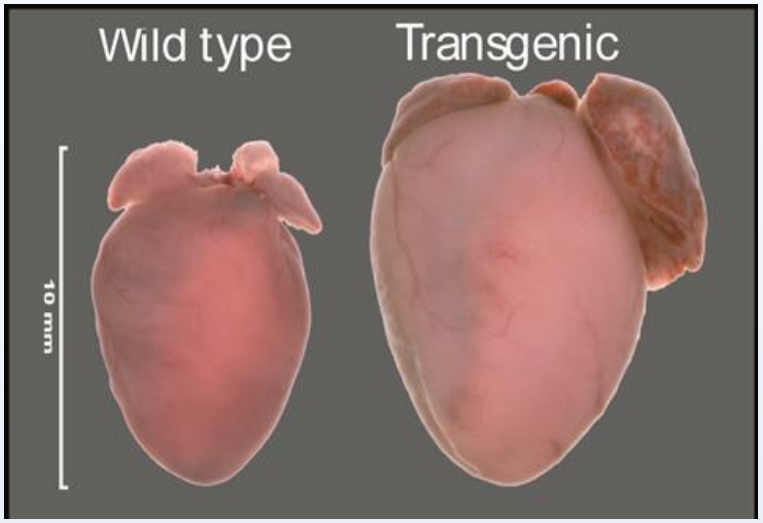
Endpoint analyses

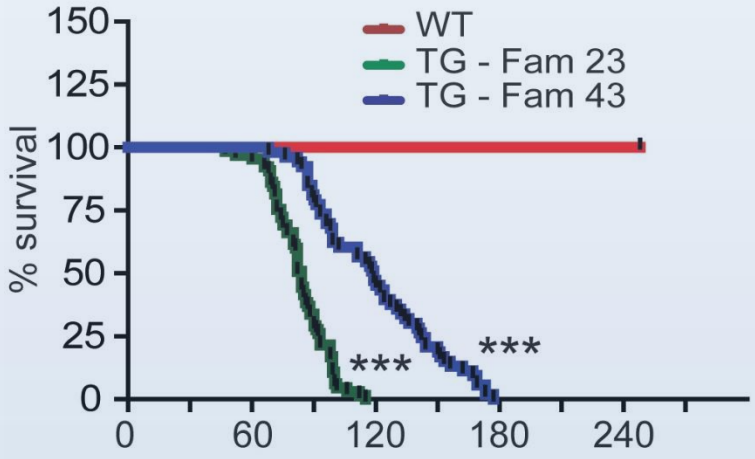


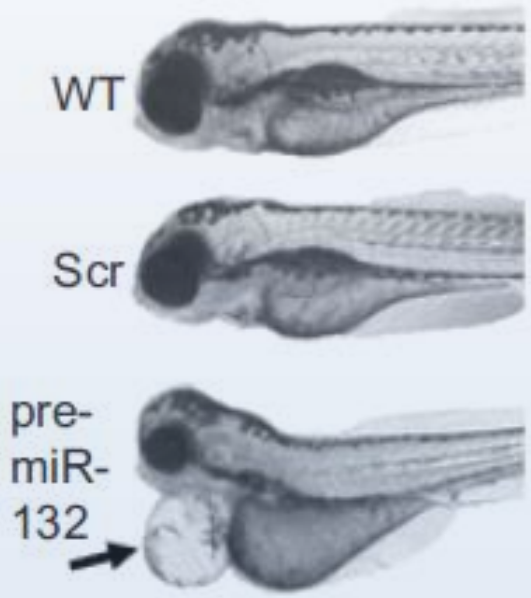
Ucar, Gupta et al., ***Nature Comm***, 2012;

Hit to Lead studies:

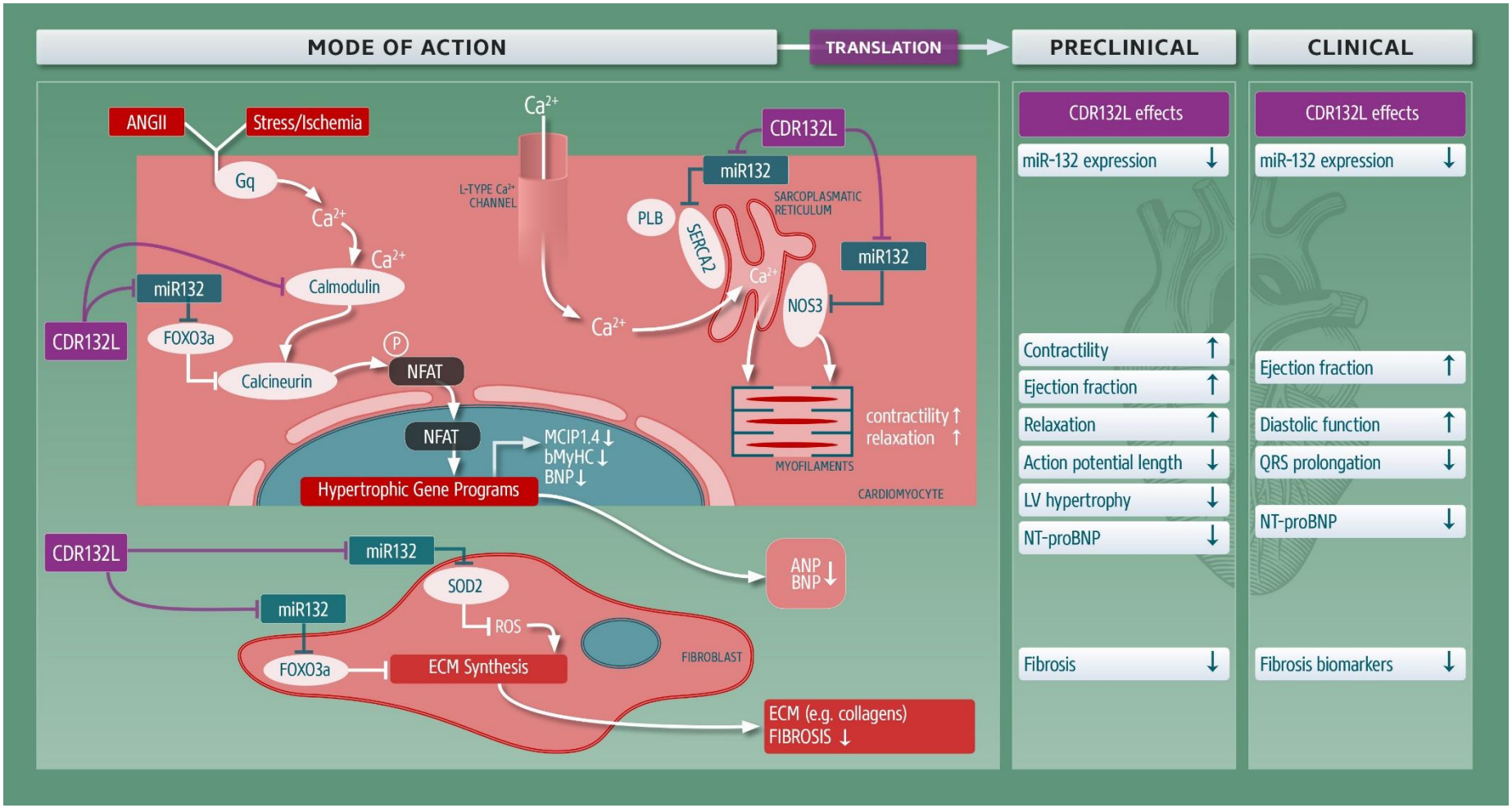
Generation of a cardiomyocyte-specific miR132 transgenic mouse model







miR132: Mode of Action

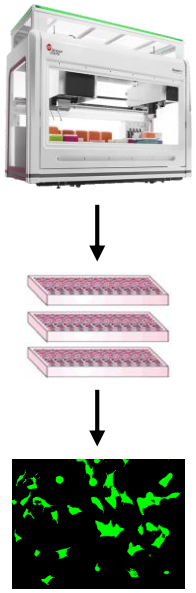


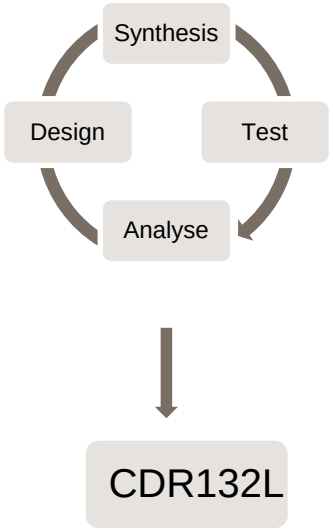
Thum et al., ***Circulation***, 2007
Ucar et al., ***Nature Genet***, 2010
Ucar, Gupta, et al., ***Nature Comm***, 2012
Kumarswamy et al., ***Eur Heart J***, 2014
Foinquinos et al., ***Nature Comm***, 2020
Batkai et al., ***Eur Heart J***, 2021
Hinkel et al., ***JACC***, 2021

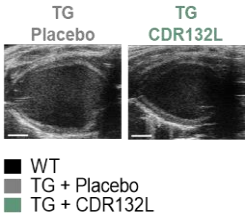
Drug Development: From University to Spin-off

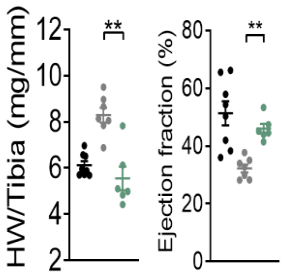
University → **Spin-off** → **Spin-off** → **Spin-off** → **Spin-off**

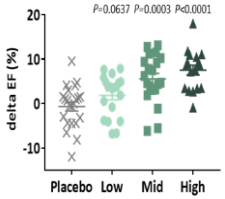
Target identification	Lead optimization	In vivo small animal studies	In vivo large animal studies	Clinical Phase 1b study
In vitro high-throughput miRNA functional profiling	In vitro toxicological and efficacy screening of ASOs	miR-132 TG during subacute post-MI HF	Subacute post-MI HF	



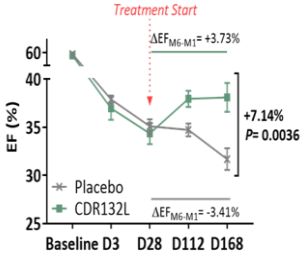


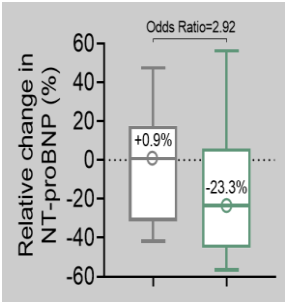






Chronic post-MI HF





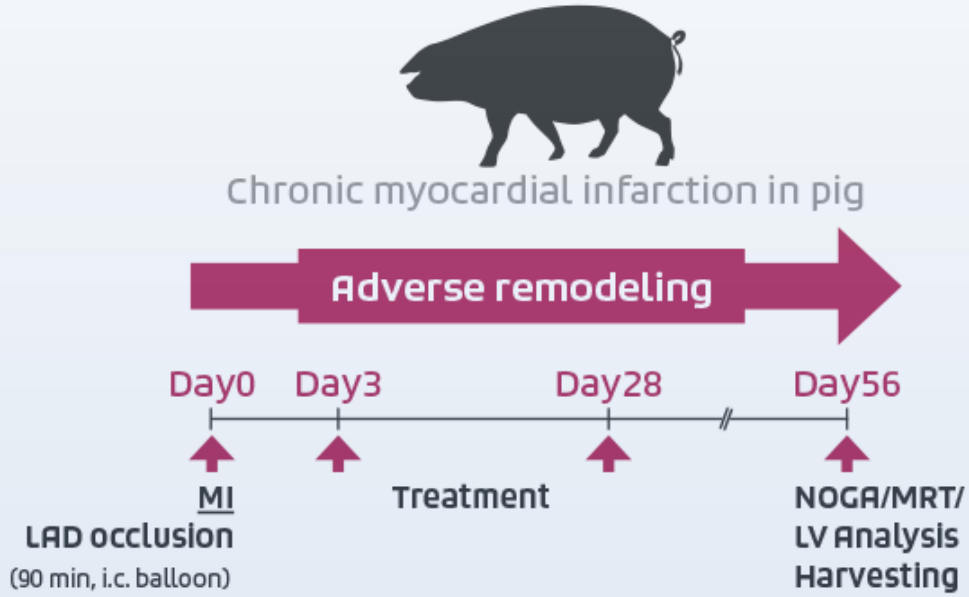
(Eur Heart J. 2021)

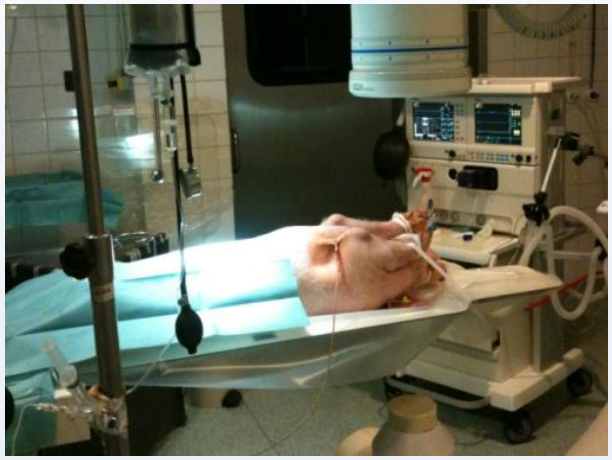
(Nat Commun. 2012)

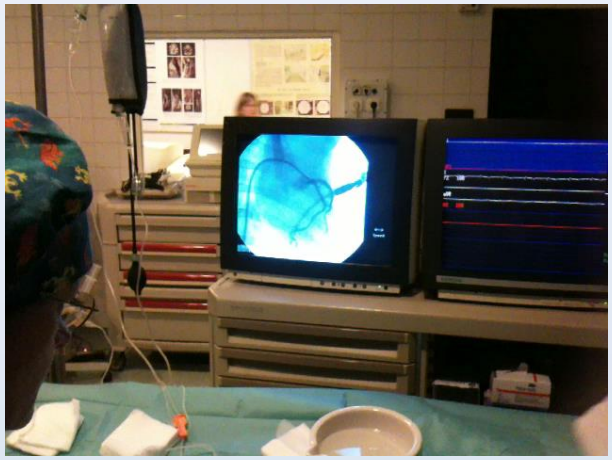
(Nat Commun. 2020)

(Nat Commun. 2020; Eur Heart J. 2020a)

Translational validation in a large animal model







Translational validation in a large animal model

Study Overview

Initial set of pigs **(135)**

IC/IV **(62)**

Randomization

Dropout **(7)**

Excluded D3 EF >40% **(16)**

Included in analysis **(39)**

IC/IV High dose **(10)**

IC/IV Mid dose **(10)**

IC/IV Low dose **(9)**

IC/IV Placebo **(10)**

IV/IV **(73)**

Randomization

RoA part

Dropout **(13)**

Excluded D3 EF >40% **(20)**

Included in analysis **(40)**

DRF part

IV/IV High dose **(7)**

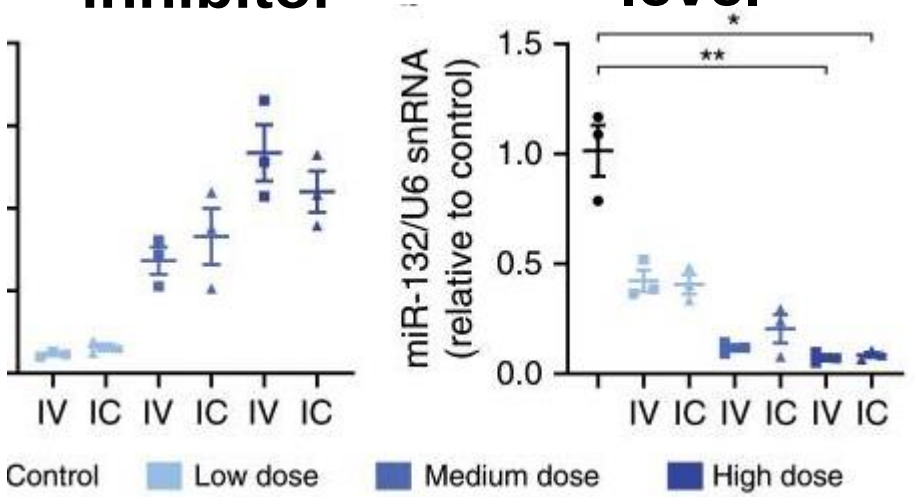
IV/IV Mid dose **(10)**

IV/IV Low dose **(11)**

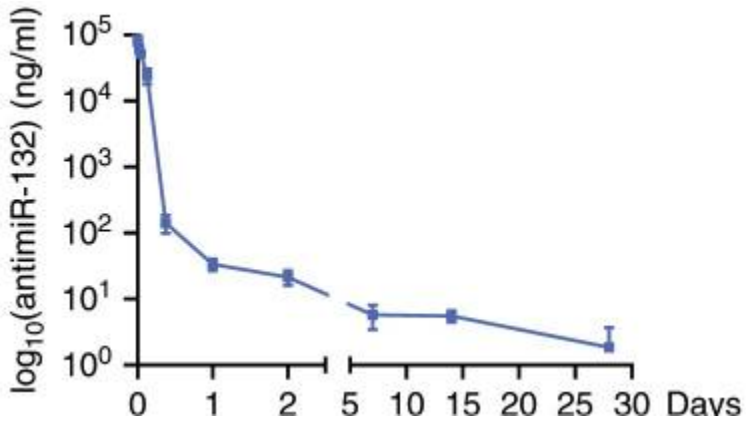
IV/IV Placebo **(12)**

Succesful target engangement

Cardiac tissue



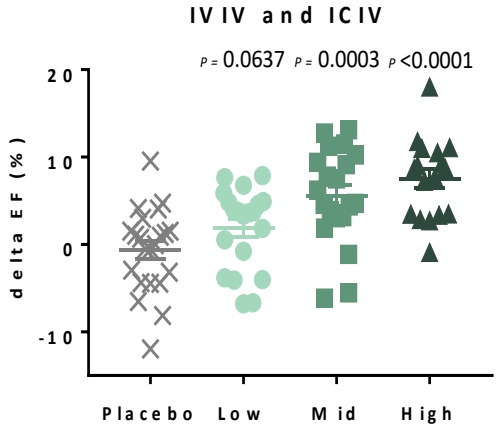
Plasma



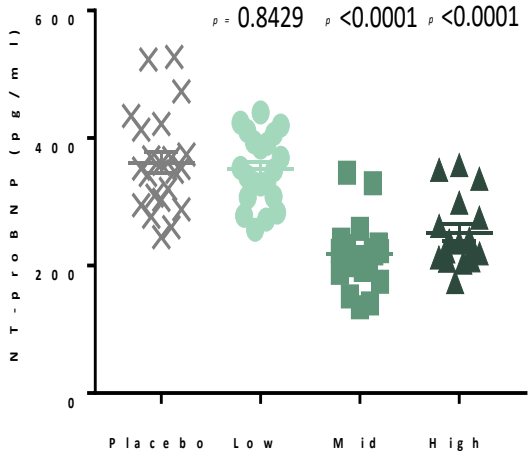
Nature Commun. 2020 Jan 31;11(1):633.

Translational validation in a large animal model of MI
Ejection Fraction

Ejection Fraction



Plasma NT-proBNP



- **Significant beneficial changes in EF** and NT-proBNP from Day 3 to Day 56 (delta EF) post MI in the **mid and high dose** groups indicating functional improvement.

CDR132L reverts early post-MI heart failure

Clinically relevant data confirmed in GLP-like studies of early post-MI (subacute) HF animal models


nature COMMUNICATIONS

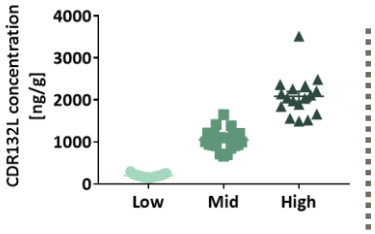
ARTICLE

https://doi.org/10.1038/s41467-020-14349-2 OPEN

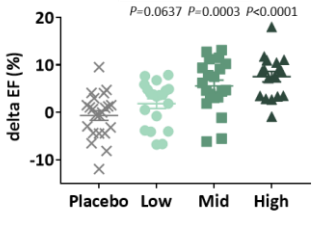
Preclinical development of a miR-132 inhibitor for heart failure treatment

Ariana Foinquinos[1,10], Sandor Batkai[1,2,10], Celina Genschel[1,2], Janika Viereck[1,2], Steffen Rump[2], Mariann Gyöngyösi[3], Denise Traxler[3], Martin Riesenhuber[3], Andreas Spannbauer[3], Dominika Lukovic[3], Natalie Weber[4], Katrin Zlabinger[3], Ena Hašimbegović[3], Johannes Winkler[3], Jan Fiedler[1], Seema Dangwal[1], Martin Fischer[5], Jeanne de la Roche[5], Daniel Wojciechowski[5], Theresia Kraft[4], Rita Garamvölgyi[6], Sonja Neitzel[7], Shambhabi Chatterjee[1], Xiaoke Yin[8], Christian Bär[1], Manuel Mayr[8], Ke Xiao[1] & Thomas Thum[1,2,9*]

CDR132L concentration in heart tissue

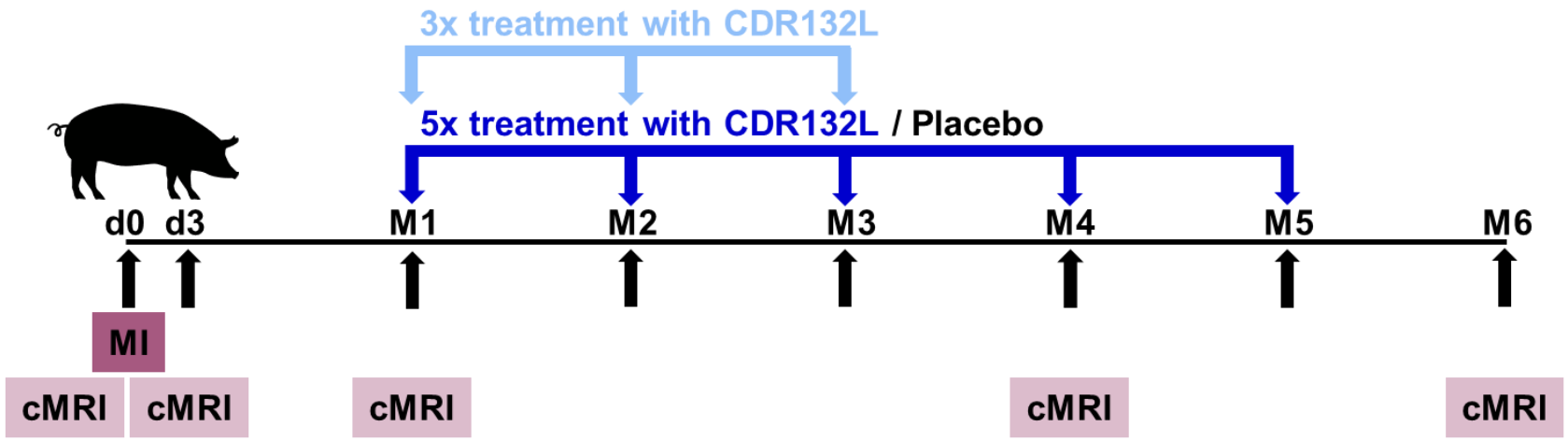


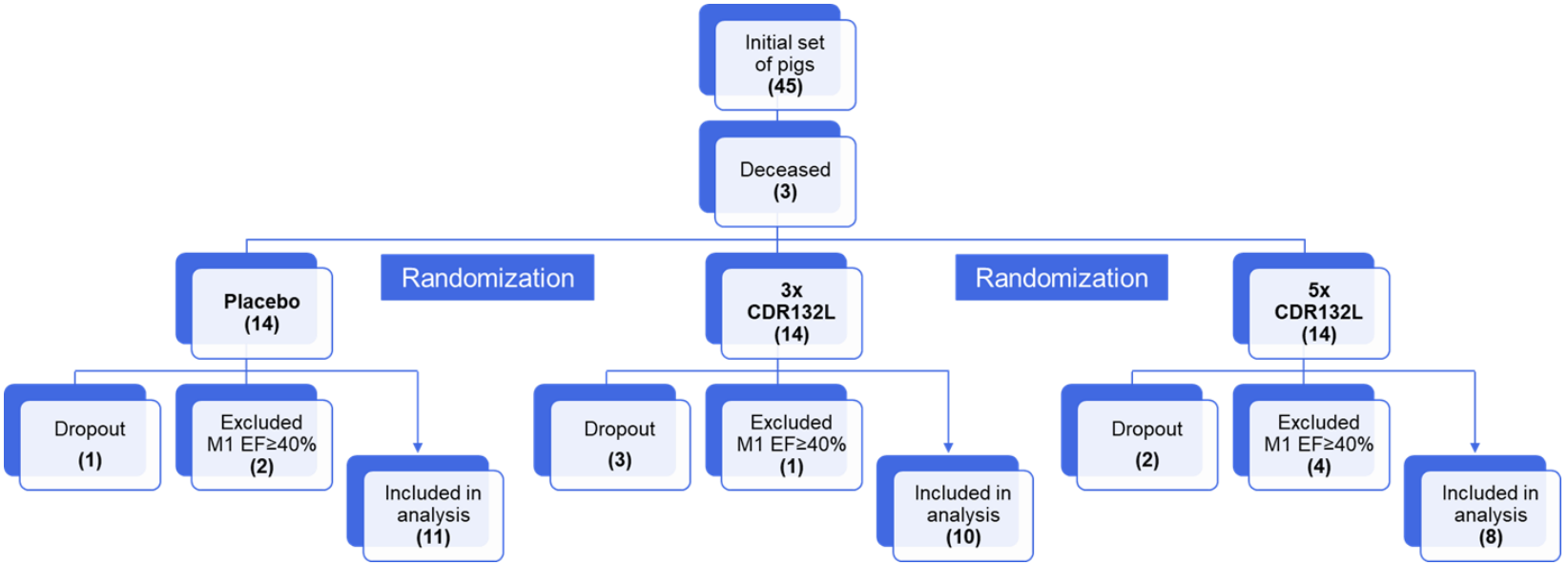
delta EF ($EF_{day56} - EF_{day3}$)



- Dose-dependent **inhibition of** functional levels of **the target miR-132**
- Dose-dependent **improvement of cardiac function**
- **Reduction of NT-proBNP as** highly relevant clinical HF biomarker
- **Inhibition of cardiac fibrosis** and **pathological heart growth** post-MI
- Very broad **safety margin** and very good **tolerability** (no adverse drug reactions)
- **Reduction of circulating miR-132 levels** allowing future use of plasma miR-132 measurements to determine target engagement

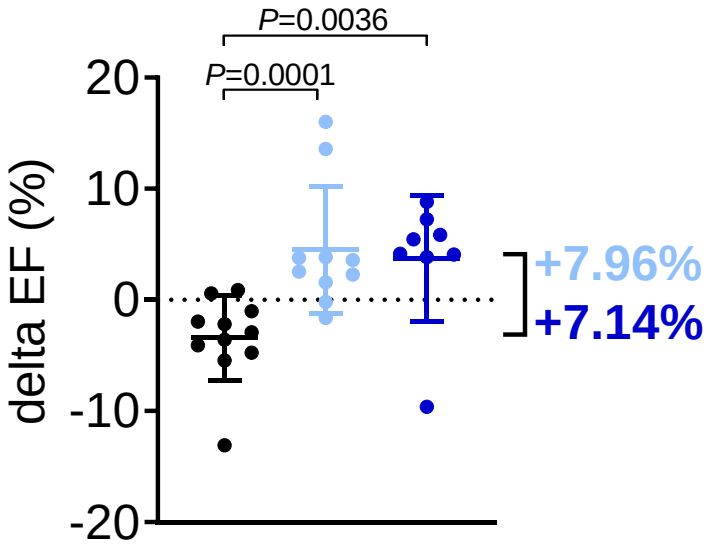
Translational validation in chronic heart failure

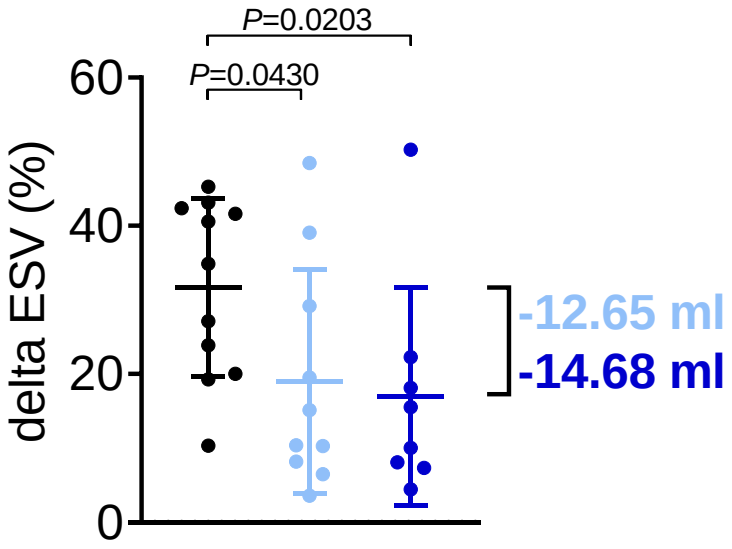




Batkai et al., **Eur Heart J**. 2020 Oct 22:ehaa791.

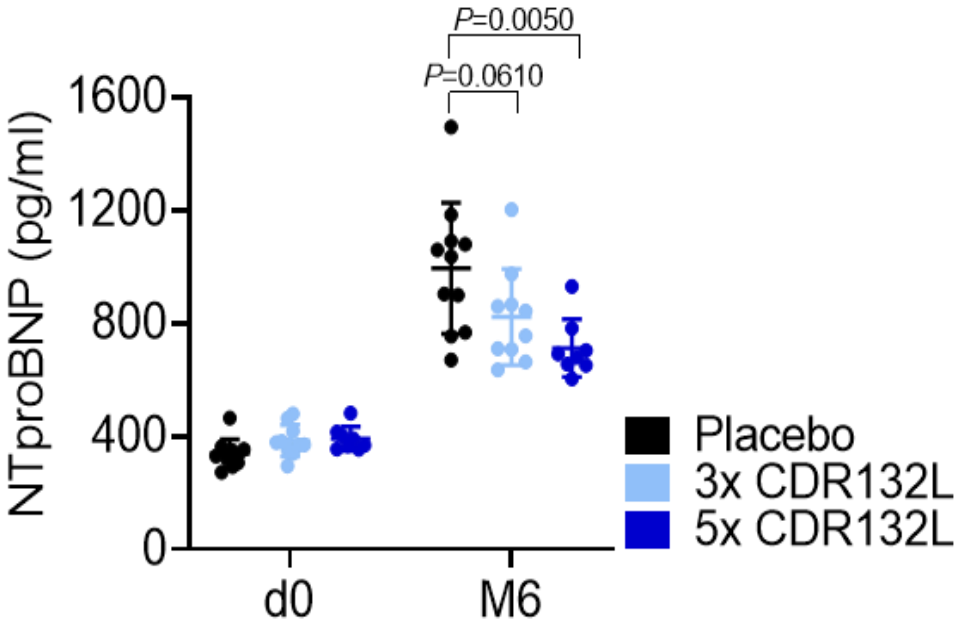
CDR132L leads to substantial improvements in chronic heart failure





Batkai et al., **Eur Heart J**. 2020 Oct 22:ehaa791.

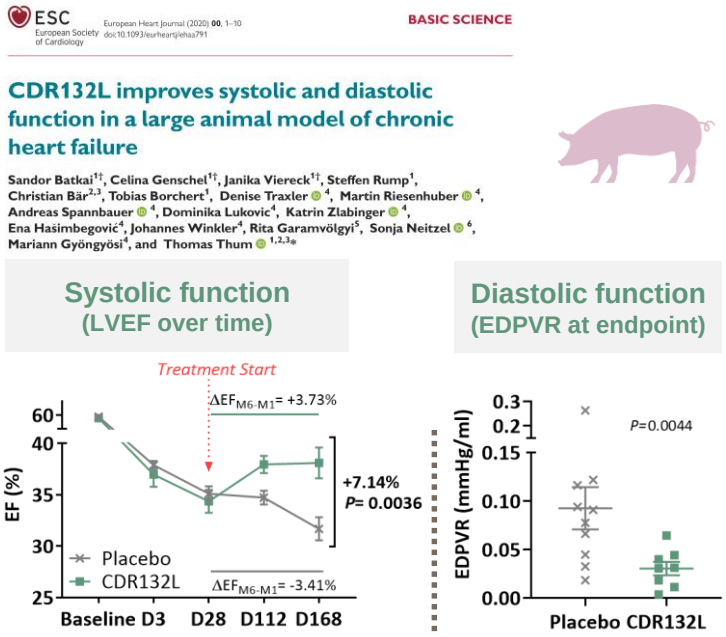
CDR132L leads to significant reductions in circulating NTproBNP in chronic heart failure



Batkai et al., **Eur Heart J**. 2020 Oct 22:ehaa791.

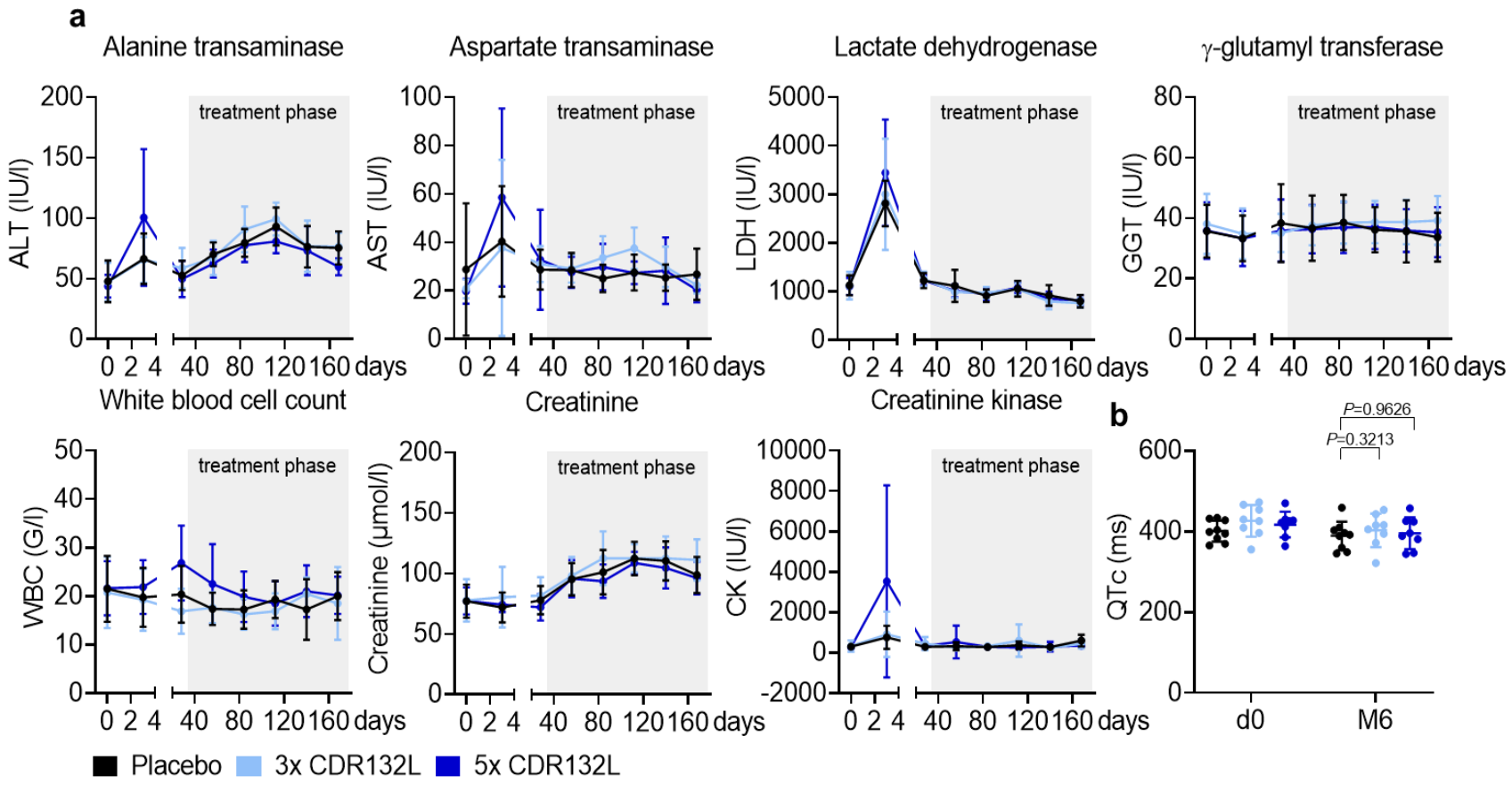
CDR132L reverts chronic heart failure

Clinically relevant data confirmed in GLP-like studies of chronic HF animals



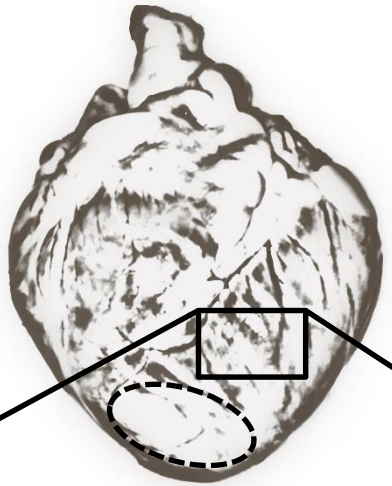
- Significant **improvement of cardiac function in chronic HF**
- **Reversal of adverse remodeling** in chronic HF post-MI
- **Reduction of HF biomarker NT-proBNP**
- **Decrease of fibrosis** and **maladaptive cardiomyocyte hypertrophy (tissue level)**
- **Sufficient tissue exposure** and **successful target engagement**
- Chronic treatment is **safe and well tolerated**

Up to 5x 10mg/kg antimiR-132 did not lead to any changes in lab chemistry (esp. liver, kidney, inflammation, platelets)



Batkai et al., **Eur Heart J**. 2020 Oct 22:ehaa791.

Summary



Myocardial infarction

Cardiac remodelling

Heart Failure

Pathological hypertrophy

Cardiac remodelling

miR-132

Fibrosis

CDR132L

- Improved cardiac function
- Reduced adverse remodelling
- Alleviated fibrosis and cardiomyocte hypertrophy

Development in line with regulatory requirements

- Legal requirements acting as **legal sponsor of clinical trials**:
  - **European level:** council directive 2001/20/EC including its amendments implements good clinical practice (GCP)
  - **German law:** AMG and its subsequent GCP-ordinance (GCP-Verordnung, GCP-V).

- Legal requirements on **quality and manufacture**
  - **European level:** EudraLex Volume 4 GMP
  - **German law:** Arzneimittel- und Wirkstoffherstellungsverordnung – AMWHV a implementing regulation of Arzneimittelgesetzes (AMG)

- Legal requirements on **non-clinical** development
  - **European level:** Organization for Economic Cooperation and Development (OECD)
  - **German law:** Chemical Law (ChemG), § 19a-d

Regulators involved in development planning and performance from the beginning on

- To ensure regulatory **compliance** of the proposed development program (incl. Pharmacology, Toxicology and PK)
- For **alignment** on manufacturing strategy and specifications
- To ensure regulatory **acceptance** of the proposed clinical setting and indication

➢ Central development milestones were presented to regulators in **Scientific Advice** meetings

Regulatory confirmation of development allowed to go straight into patients

Scientific advice from German regulators **(BfArM)** in Nov 2017 and Nov 2018:

Pre-clinical package and CMC sufficient to start FiH studies.

Risk/benefit analysis of efficacy and safety data allow to go straight into HF patients.

UK regulators **(MHRA)** approved in May 2019 the CTA for the FiH - study:

Confirming stable HF patients as volunteers and agree to dose escalation and repeat dose in the same patient.

Advantage**:** PK, safety, and PD data already from target population available.

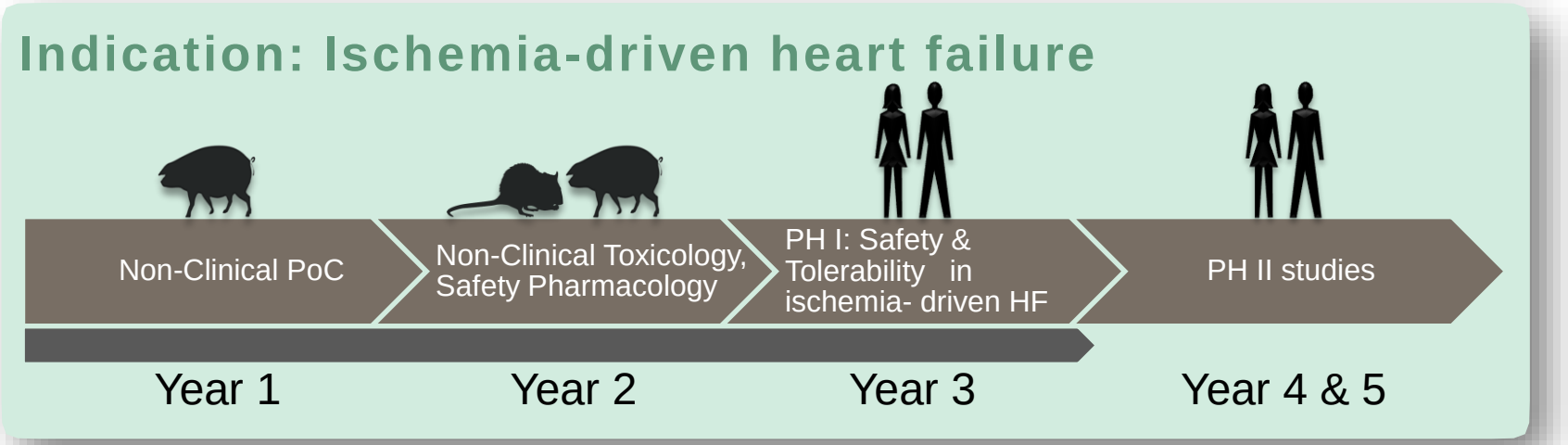
FDA feedback in pre-IND meeting on Oct 31, 2019:

Pre-clinical data-package incl. planned chronic tox studies (rat, minipig) agreed

CMC suitable

Phase 2 development plan and study outlines for PoC studies in early post-MI and chronic HF agreed.

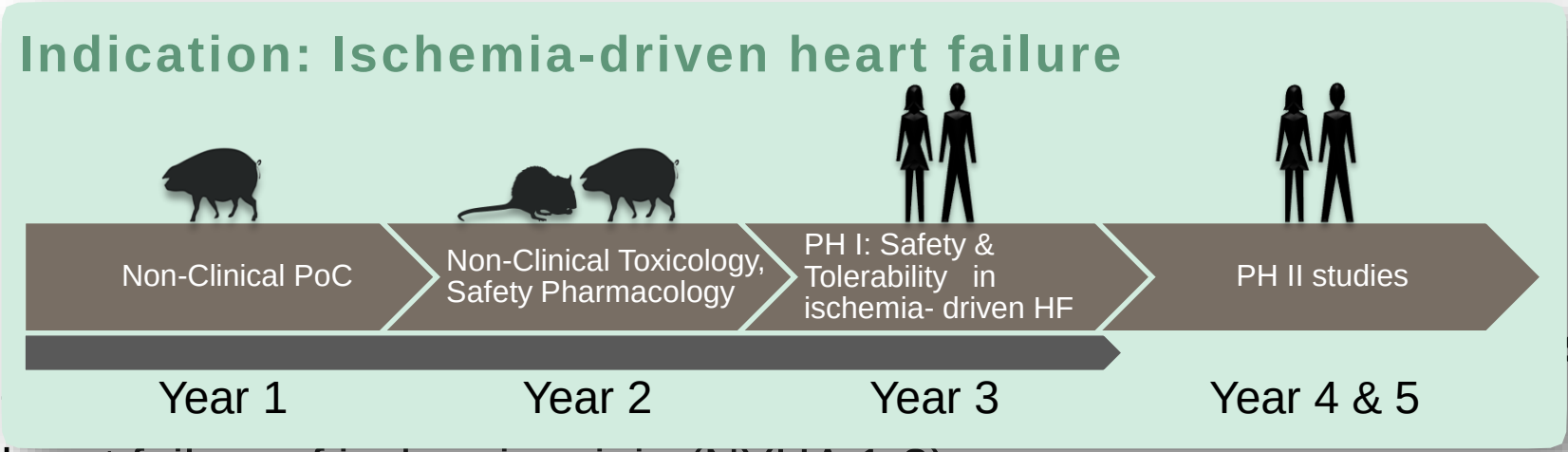
Path to the clinics



<u>Clinical Phase 1b study completed</u>:

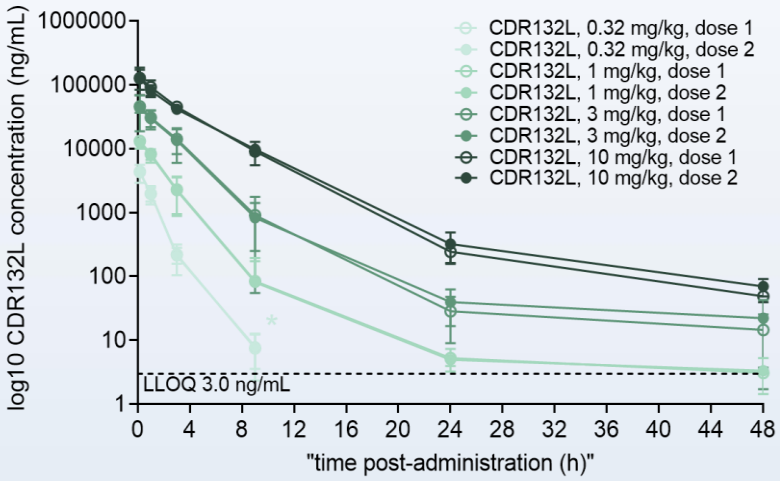
Study to Assess Safety, PK and PD Parameters of CDR132L
ClinicalTrials.gov Identifier: NCT04045405

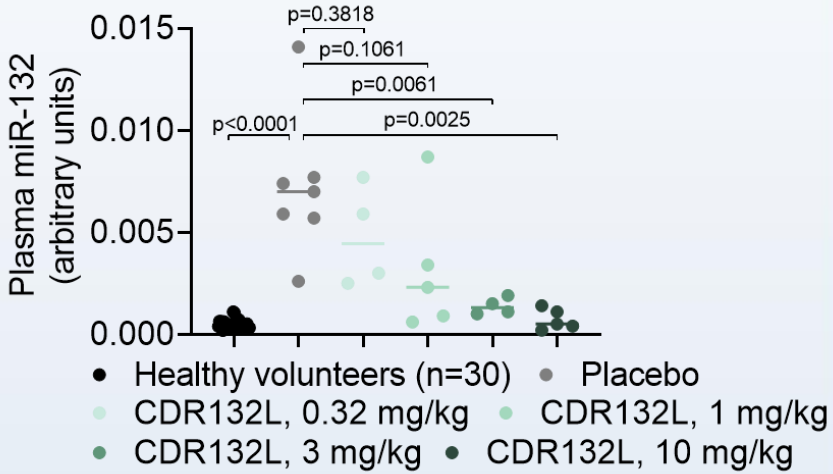
Path to the clinics



- S
  heart failure of ischemic origin (NYHA 1-3).
- CDR132L was administered to 28 patients at dose-escalating single and matching repeat doses (5:2 randomized; at 4 weeks intervals).
- Primary endpoint: safety
- Secondary endpoint: pharmacokinetic (PK) profile of CDR132L

Word-wide first study of an oligonucleotide therapeutics in heart failure patients (phase 1b study; *NCT04045405*)

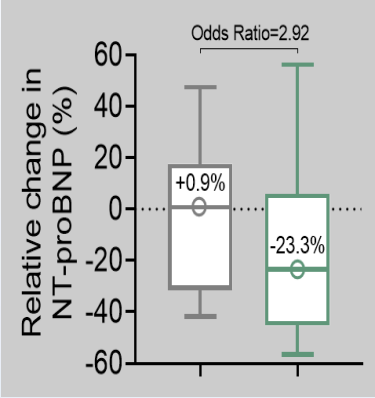




- ✓ **Dose-linear pharmakokinetic profile**
- ✓ **Succesfull target engagement in HF patients**

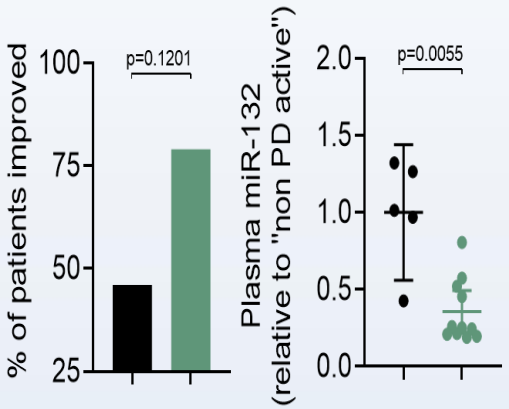
Täubel et al., **Eur Heart J**, *2020*

Word-wide first study of an oligonucleotide therapeutics in heart failure patients (phase 1b study; *NCT04045405*)



NT-proBNP relative change per group

Median (circles) and 25%/75% interquartile ranges (bars) for relative changes of NT-proBNP from baseline to day 112.



LVEF and NT-proBNP (mean±SEM)

% Pat with absolute increase >2% in LVEF and >10% reduction in NT-proBNP

- ✓ **Very good safety and tolerability**
- ✓ **Clinically meaningful** median **reduction** of 23.3 % for **NT-proBNP**
- ✓ **Indicative cardiac functional improvements**

Täubel et al., **Eur Heart J**, 2021

Phase 1b: all endpoints met successfully

- Excellent tolerability and safety of CDR132L in patients with heart failure in single and repeat dose escalating study conducted on top of standard of care.
- PK profile shows high level of dose linearity and no signs of accumulation , as seen in pre-clinical large animal models.
- Unique Mode of Action in heart failure confirmed, target engagement validated, and various exploratory pharmacodynamic (PD) parameters reaffirm best-in-class profile.
- Clinical data are confirming the beneficial effects already observed in pre-clinical studies.
- NT-proBNP decreased in median 23.3% at 4 months.
- Current Phase 1b study results allows further development in HFrEF and HFpEF patients.

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