Rhythmiar:
Syncing Hearts, Saving Lives

Presentation Scientific Project Design in Drug Discovery 2023

CardioSync Pharmaceuticals

Virtual screening

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In-Vitro studies

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Preclinical Assays

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Claire Damery

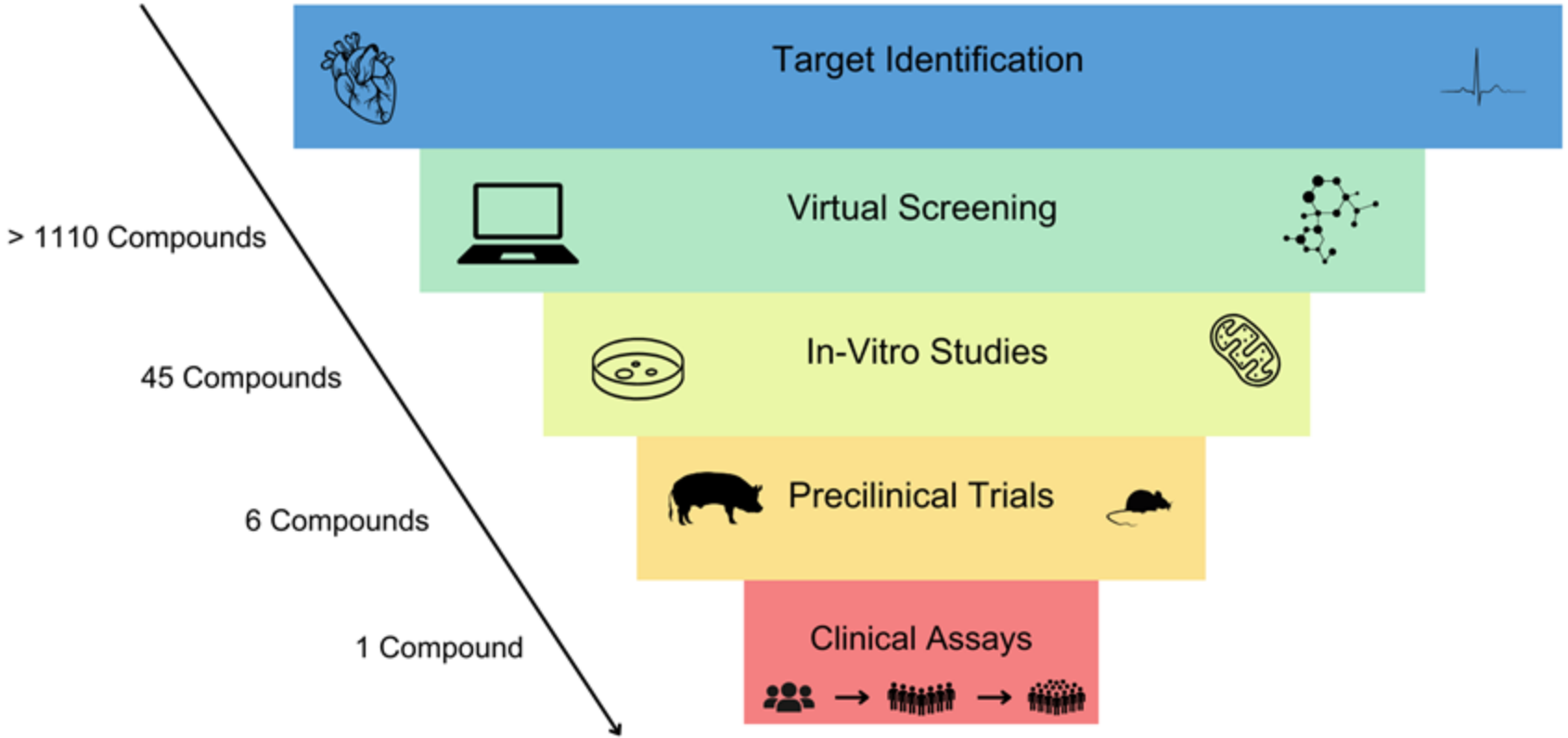
Clinical Trials

Bettina Weber

Danja Zengaffinen

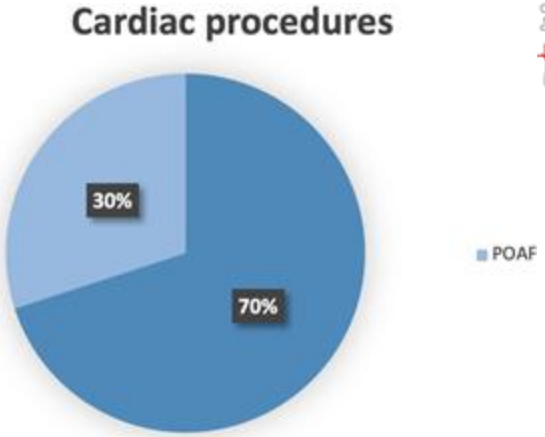
Alexandra Preda

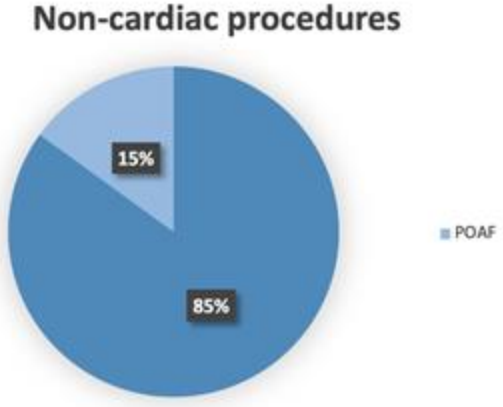
Rhythmiar's development



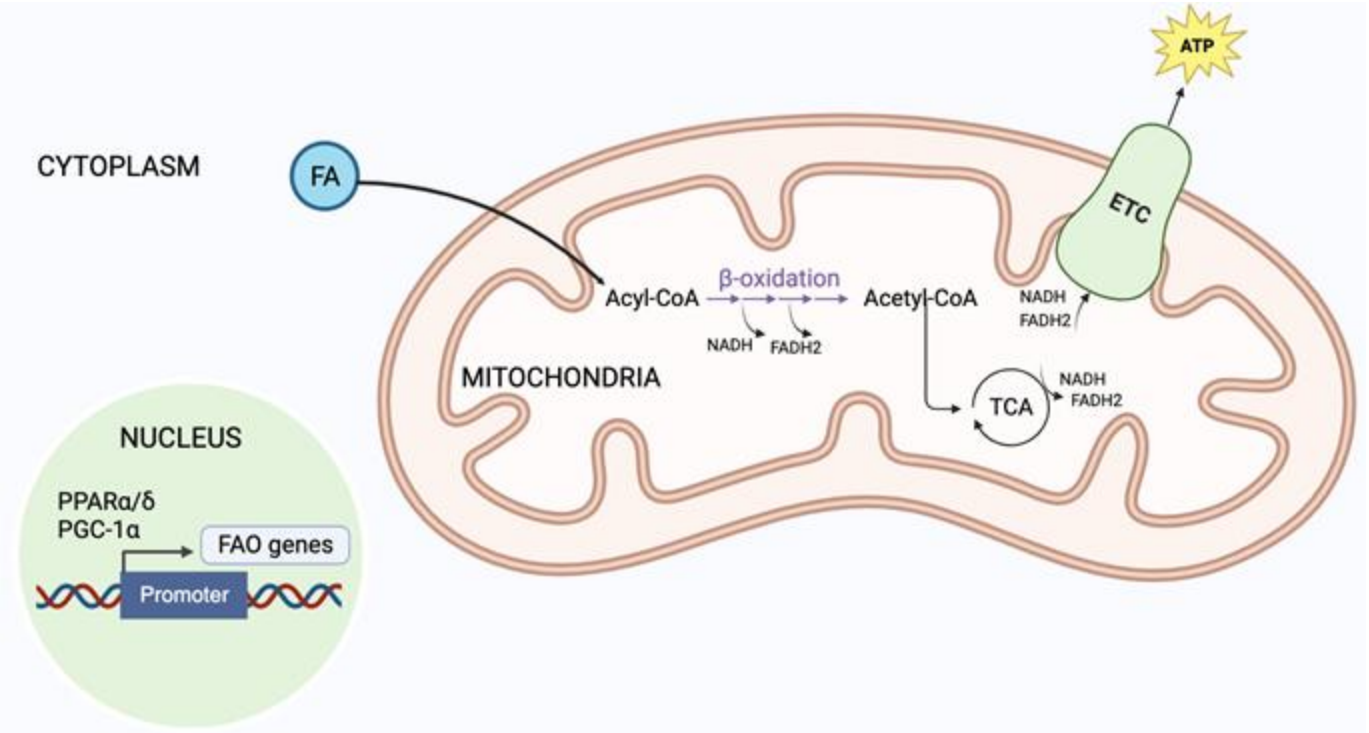
Atrial Fibrillation

- Increased costs, hemodynamic instability stroke risk, morbidity, mortality
- Cardiac ischemia, inflammation and comorbidities linked to POAF
- Current treatments: prophylaxis and rate control, i.e. β-blockers



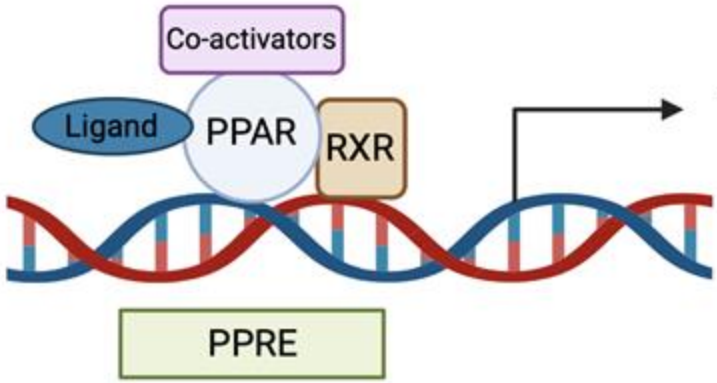


Mitochondria and Fatty acid oxidation

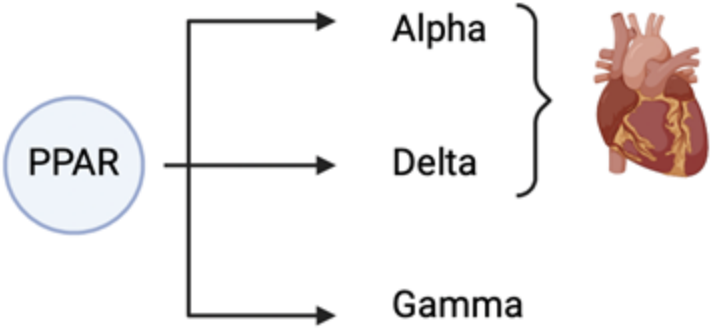


- Primary source of energy for the myocardium
- ATP production
- Anti-inflammatory effects

PPARs as Potential Targets



Transcription FAO genes



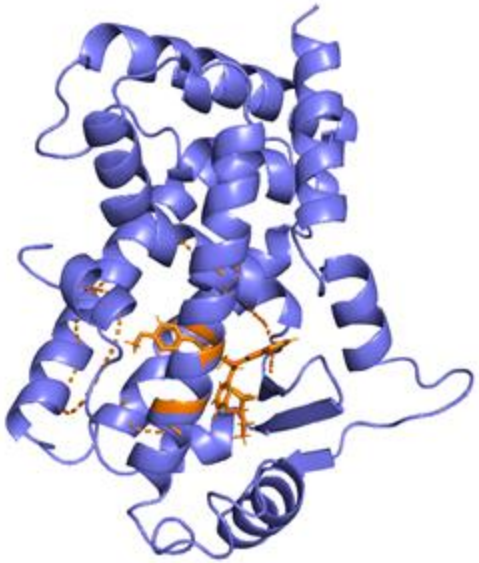
PPAR: Peroxisome proliferator-activated receptor.
PPRE: Peroxisome Proliferator Response Element.
RXR: retinoid X receptor is a type of nuclear receptor that is activated by 9-cis retinoic acid.

Rythmiar as a Drug Choice

- PPARα and PPARδ dual agonist
- Activates PPAR to promote FAO in cardiomyocytes

Delivery Method:

- Intravenous administration
  - Precise dosing and rapid onset
- Continuous infusion pre- and post-surgery
  - Optimal drug levels



Target product profile

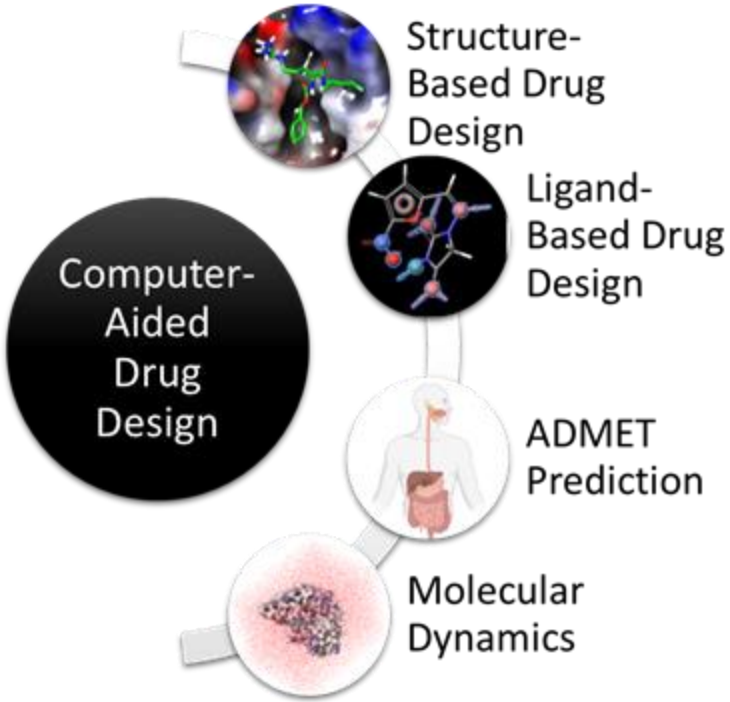
Parameter	Essential Profile	Extended Profile
Indications	Post-operative, acute phase AF	Expansion to AKI and CAF
Patient Population	40+ years old, undergoing bypass surgery	Expansion to other cardiac surgeries, AKI and CAF
Therapeutic modality	Nuclear Receptor Ligand	
Efficacy	≥ 70% decrease in AF occurrence	TBD
Safety	≤ 20% incidence of side effects	
Dosing and Administration	Continuous infusion Start of treatment: 1 day pre-operatively Duration of treatment: 6 days post-surgery	Oral, one-daily administration
Mechanism of Action	Activation of PPARα / δ resulting in increased mitochondrial FA metabolism	

AKI : acute kidney injury
be defined

CAF : chronic atrial fibrillation

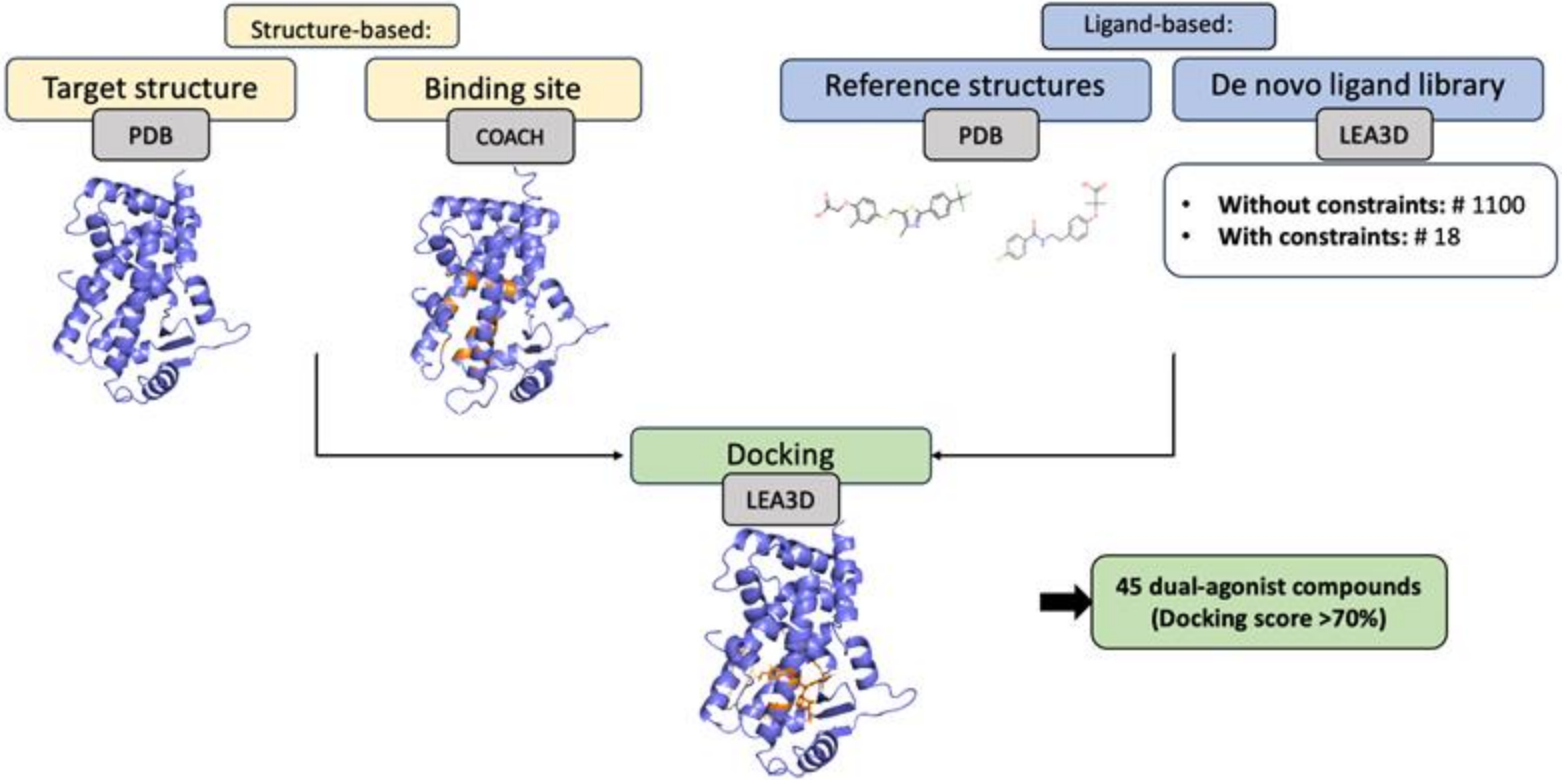
TBD : to

Streamlining Drug Discovery with In Silico Screening – Reducing Compounds, Saving Resources

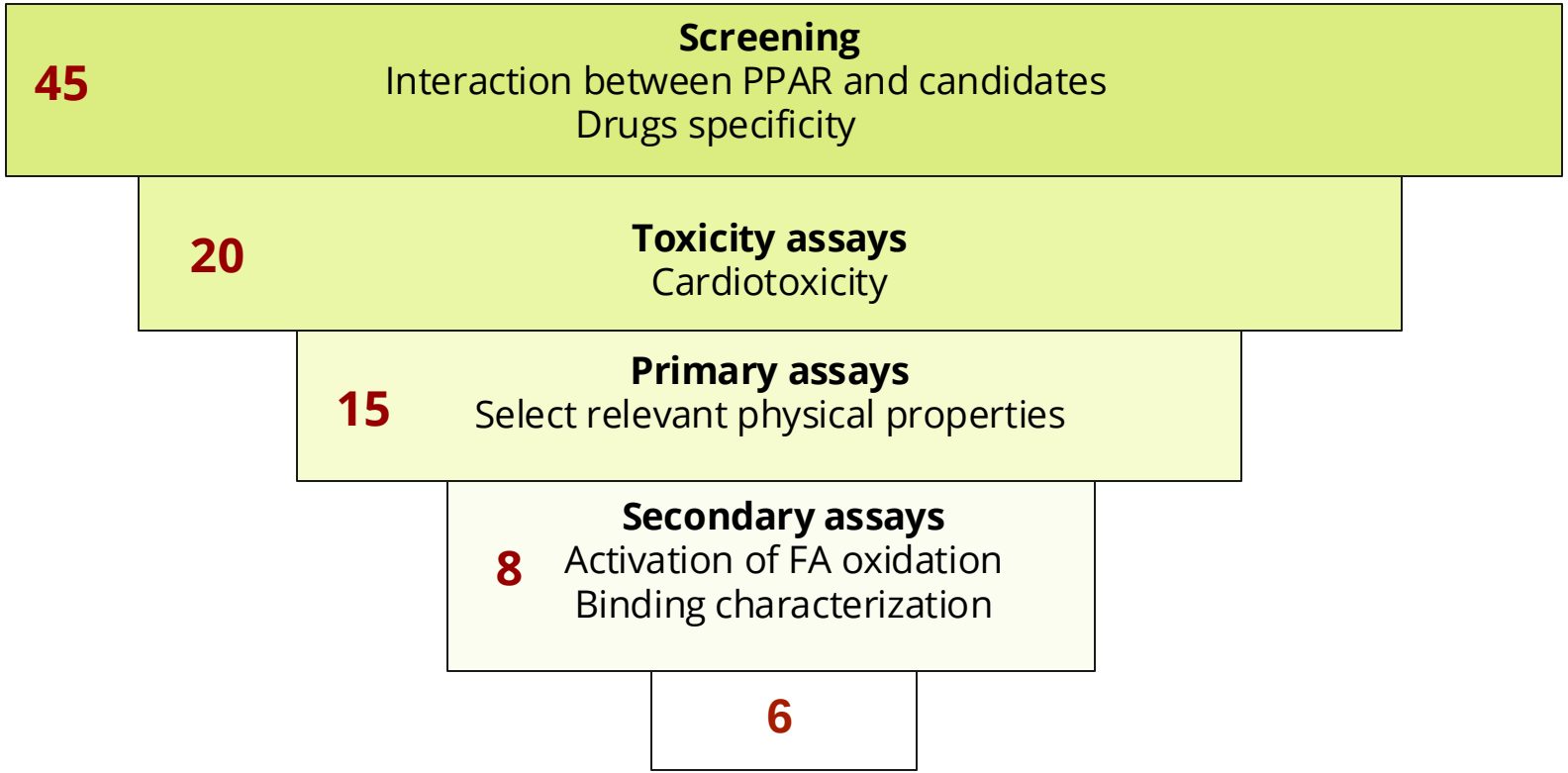


Chang, Y. et al. A guide to in silico drug design. *Pharmaceutics*, **2022**, *15*(1), 49.

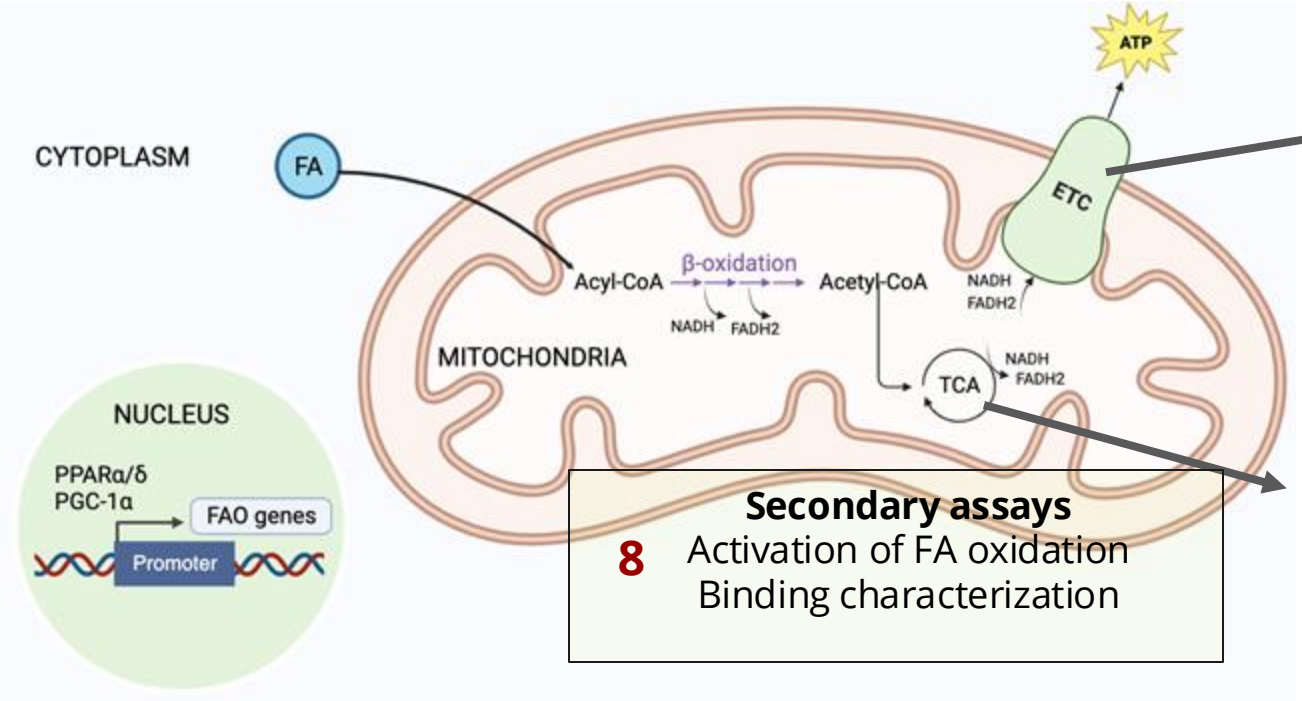
In silico


Structure-based:
Target structure
PDB
Binding site
COACH
Ligand-based:
Reference structures
PDB
De novo ligand library
LEA3D
• Without constraints: # 1100
• With constraints: # 18
Docking
LEA3D
45 dual-agonist compounds
(Docking score >70%)

In Vitro assays

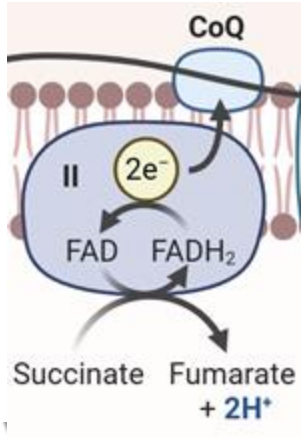


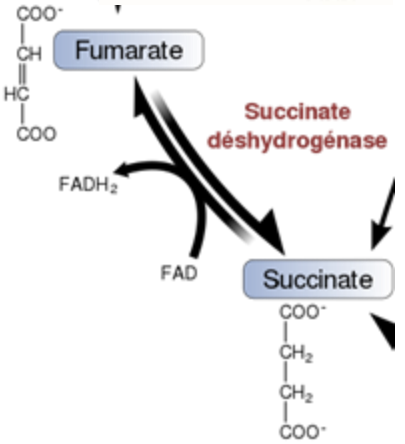
Verification of the activation of the fatty acid oxidation pathway



Secondary assays

8 Activation of FA oxidation
Binding characterization





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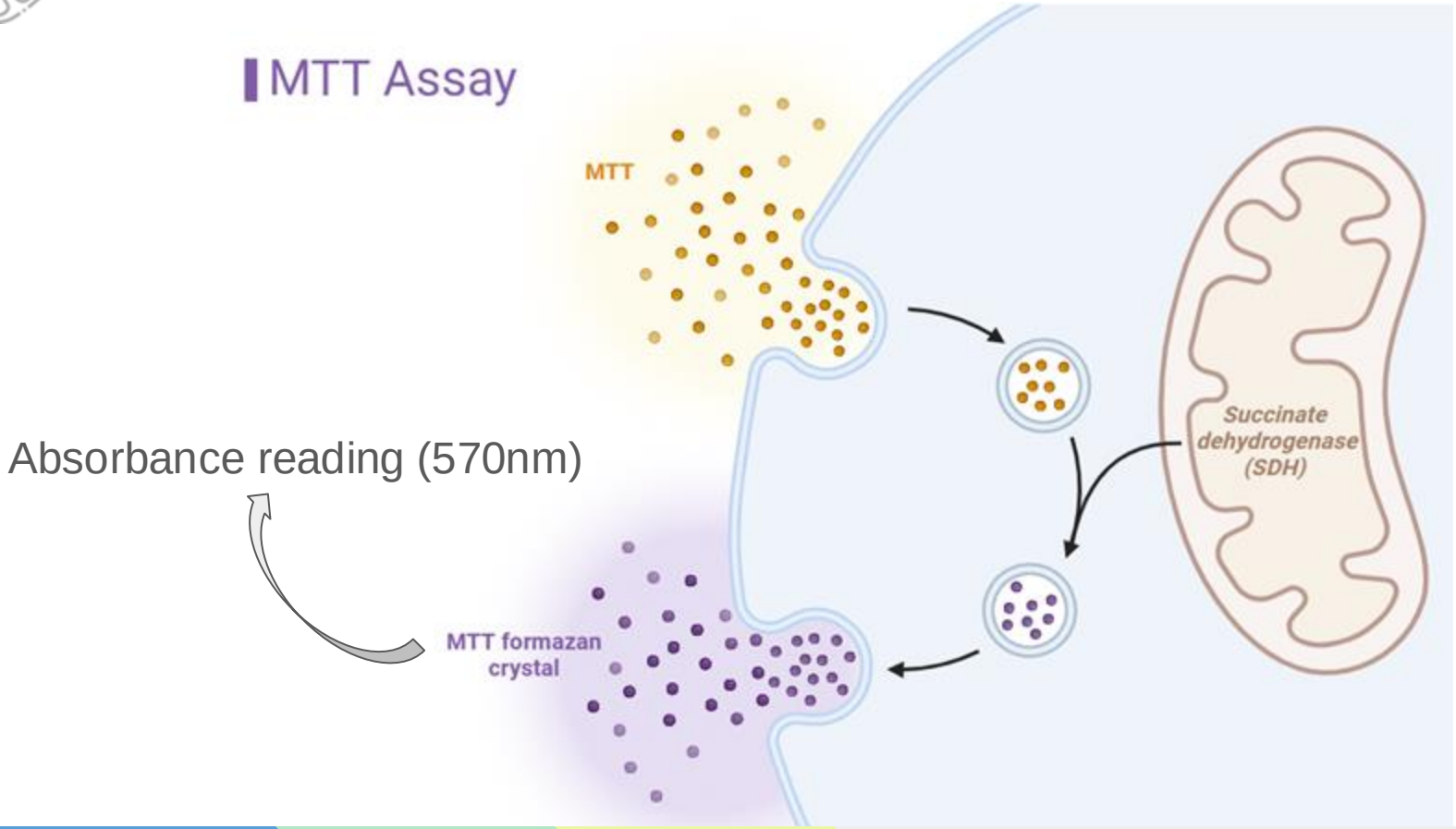
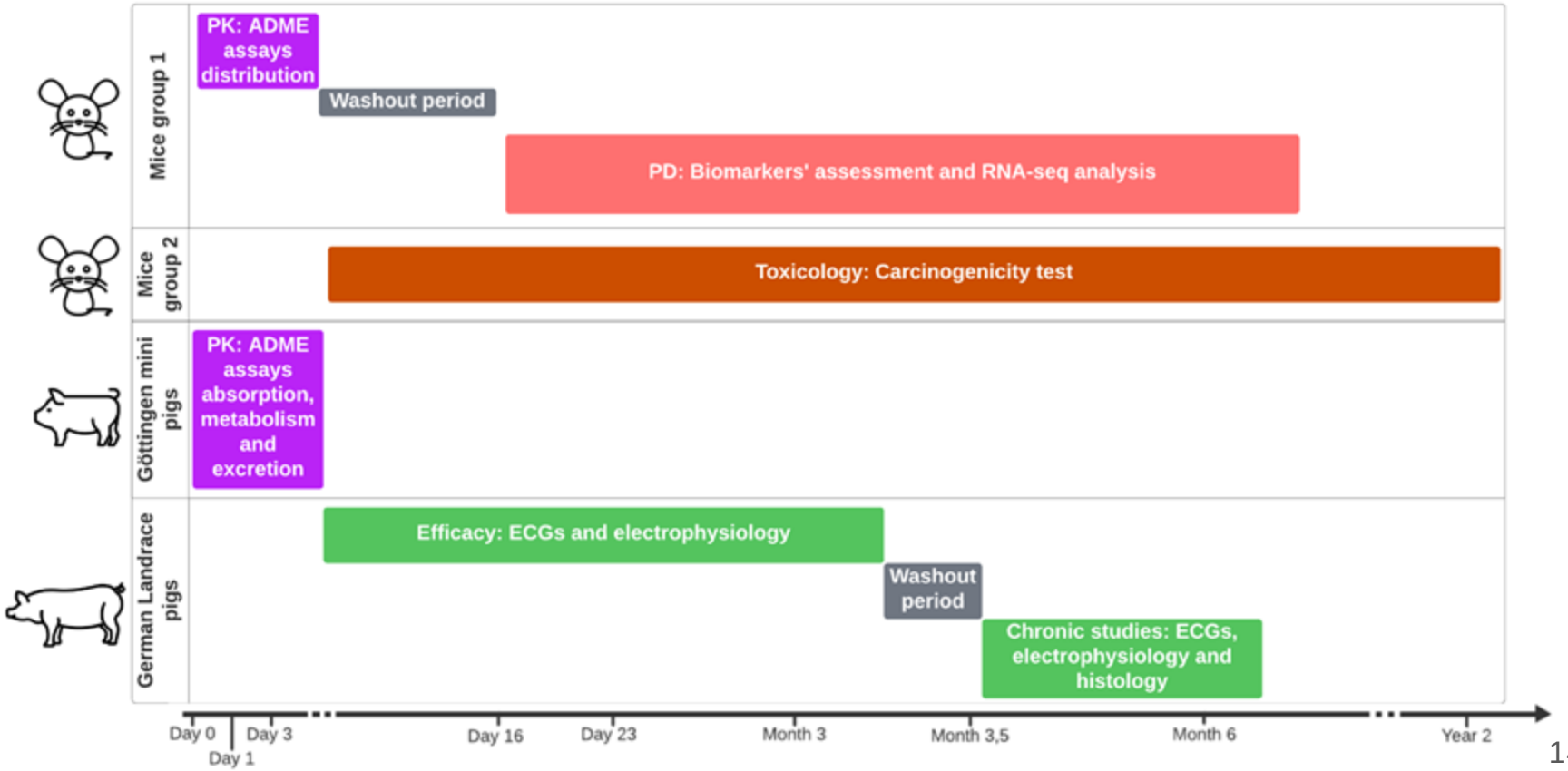


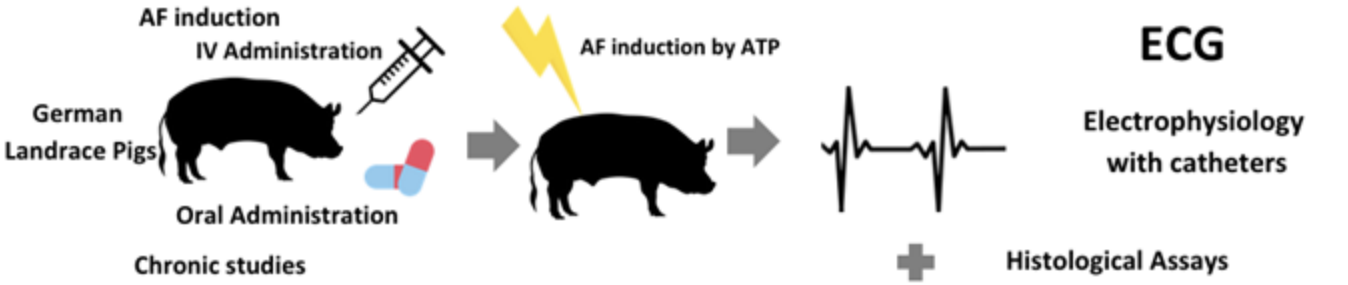
In Vivo Validation: Investing in CardioSync Pharma's bright future



In vivo testing

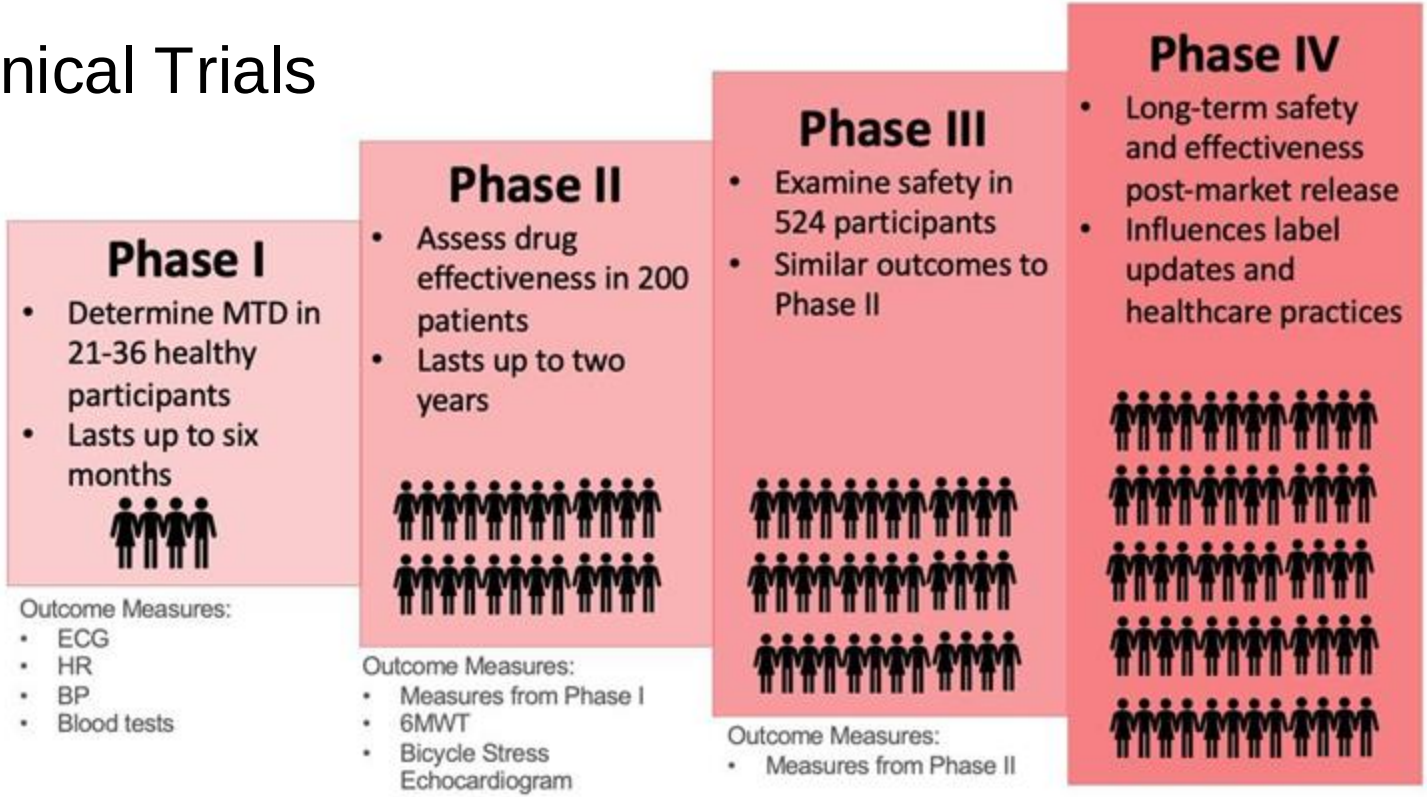
Pharmacokinetics in-vivo → Pharmacodynamics in-vivo

Efficacy testing in-vivo



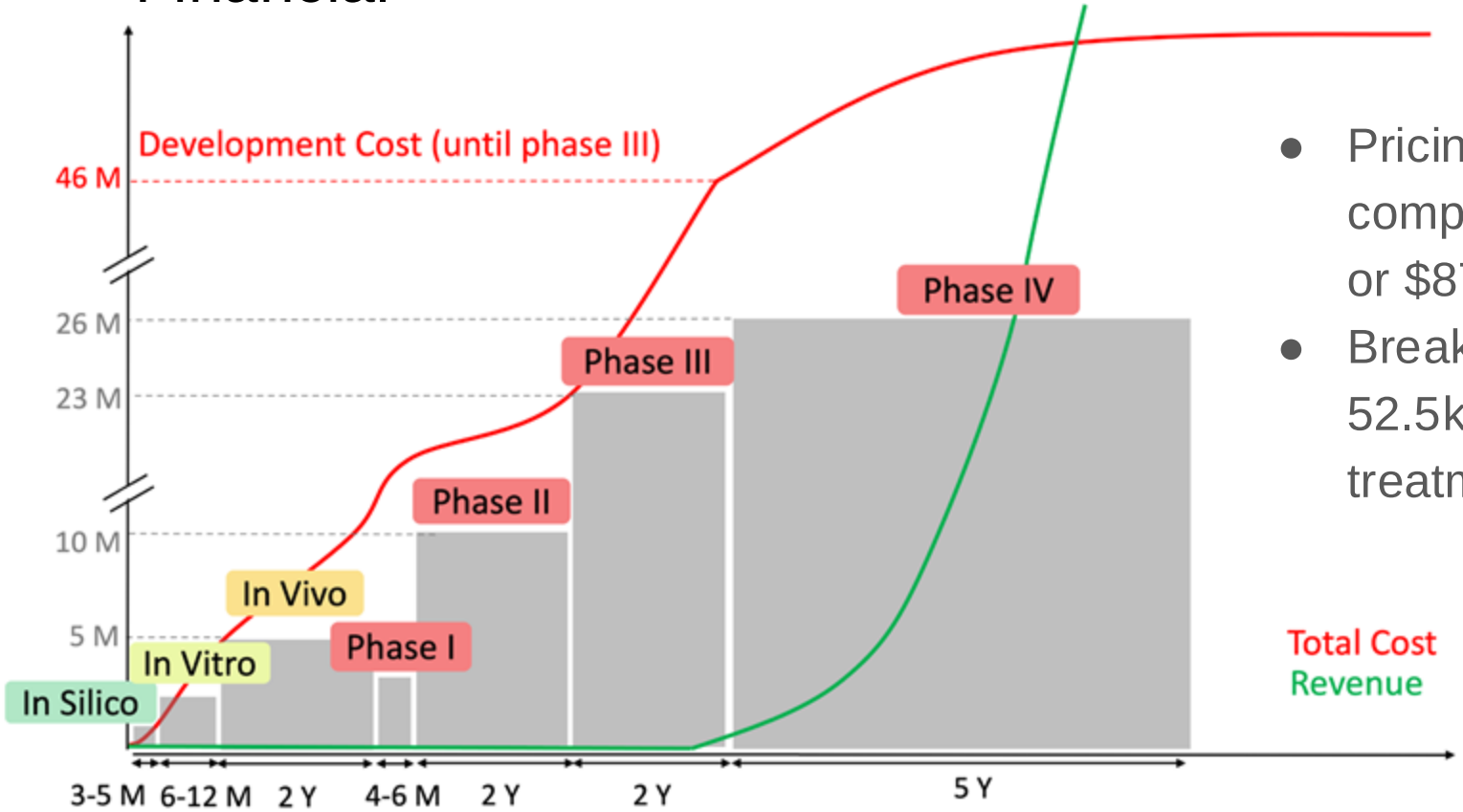
Toxicity in-vivo

Clinical Trials



MTD = Maximum Tolerated Dose, 6MWT = 6 Minute Walking Test

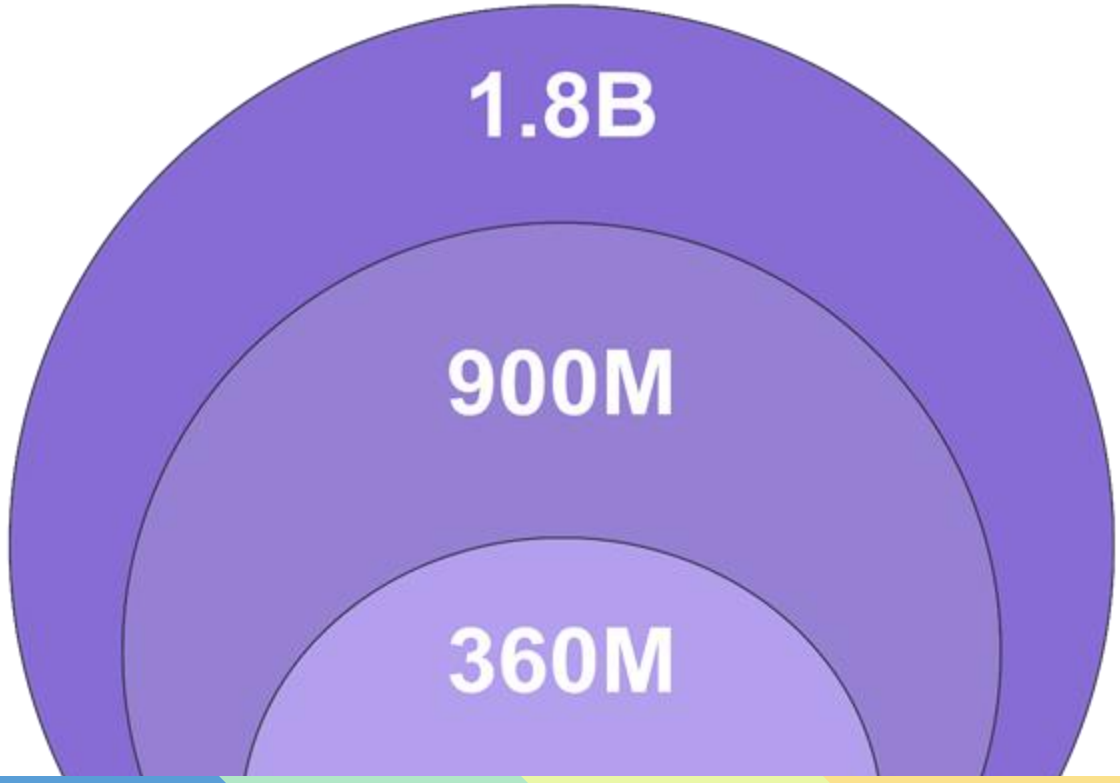
Financial



- Pricing: $7k for the complete treatment, or $875 per dose
- Break-even point: 52.5k doses or 6.5k treatment courses

Target identification | Virtual screening | In-vitro | Preclinical Assays | Clinical Assays | **Business plan**

Size of the Market



TAM = Total Available Market

SAM = Serviceable Adressable Market
Market Penetration = 0.5

SOM = Serviceable Obtainable Market

Target identification | Virtual screening | In-vitro | Preclinical Assays | Clinical Assays | **Business plan**

Why Rhythmiar?

Highly effective & safe drug .
Reduction of hospital stay and treatment costs

High Profit Potential

Low production cost, high profit margin

Big Market

400k annual cardiac surgeries in USA.
No effective alternatives

Potential of Expansion

The applications of the drug can be expanded to a even bigger market

Target identification | Virtual screening | In-vitro | Preclinical Assays | Clinical Assays | **Business plan**

Join the Rhythmiar Revolution

More than just a drug

High impact on patients

Step forward in cardiac healthcare

Commitment to a better future

Target identification | Virtual screening | In-vitro | Preclinical Assays | Clinical Assays | **Business plan**

Thank you

Supplementary Slides

1

Target Identification

Disease

- Post-operative atrial fibrillation's acute phase
- 8-day once-daily in-hospital IV administration
- Possible subsequent expansion to chronic treatment through oral delivery and other diseases (post-operative acute kidney injury)

Drug Target

PPAR-α and PPAR-δ

Intellectual Property

Screening for novel compounds → no existing patent

2

Lead Identification & Optimization

Virtual Screening (1100)

- Identify PPAR-α and PPAR-δ structures via PDB database
- Determine a library of agonist molecules using LEA3D
- Evaluate ligand-receptor docking with LEA3D (PLANTS tool)
- Optimization of the screening by conducting ADMET *in silico* testing (Drug Sniffer pipeline)
- Library narrowed down from **1118** to **45** compounds (Docking score > 70%)

In-Vitro (45)

Screening: 45 compounds
- Specificity screen (SPA)

Toxicity: 20 compounds
- Cytotoxicity (LDH-release assay, micronucleus test)
- Genotoxicity test (Ames test)
- Drug interference (cell-based)
- Cardiotoxicity

Primary Assays: 15 compounds
- Solubility (shake-flask)
- Interaction (HPAC)
- Lipophilicity (shake-flask)
- Permeability (PAMPA)

Secondary Assays: 8 compounds
- Verification of the activation of the FA oxidation pathway (MTT assay)
- Specific characterization of the binding between our drugs and PPAR (ITC)

3

Preclinical Assays

Pharmacokinetics (6)

ADME studies on Göttingen pigs and on mice

Pharmacodynamics (5)

- Investigation of each compound's effects on mice
- RNA-seq analysis

Efficacy (4)

AF induced by sustained ATP, test for:
- ECG
- Electrophysiology

Chronic in vivo studies: Sustain AF for 7 days on pigs, and check for ECG and histological assay

Toxicology (3)

- Tumorigenesis in mice
- Drug interference test

4

Clinical Assays (1)

Phase 1

Assessment of:
- safety
- pharmacokinetics
- maximum tolerated dose

on 21-36 healthy individuals.

Phase 2

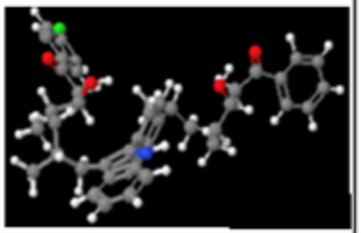
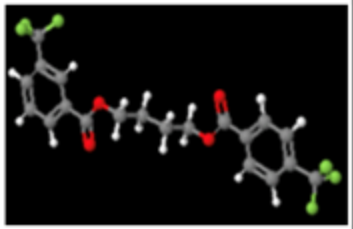
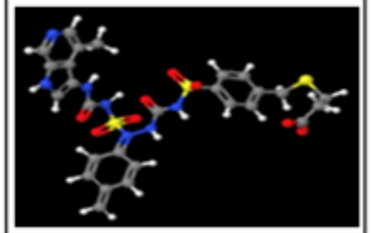
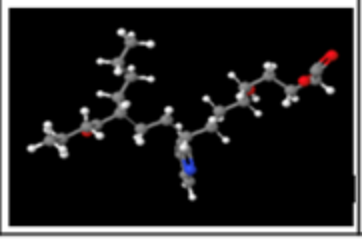
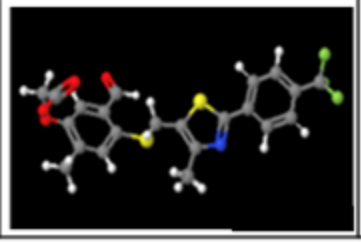
Determine efficacy, optimal dose and safety on 200 patients.
Primary outcome: reduced incidence of AF by 70%

Phase 3

Proof of efficacy and safety in a population of 524 people and longer time period.

Phase 4

Post market release surveillance for rare or long-term adverse effects.

Synthetic Compounds	Fibrates		GW501516		Elafibranor	
	Score	Ball-and-Stick Model	Score	Ball-and-Stick Model	Score	Ball-and-Stick Model
Without Constraints	98.80%		75.62%		82.30%	
With Constraints	76.85%		71.60%		70.32%