A novel EPAC1 inhibitor with cardioprotective effects
Role of cyclic AMP in cardiac remodeling

- **Acute cAMP/PKA signaling is beneficial to regulate cardiac function:**
  - Intracellular second messenger produced from ATP upon **β-adrenergic receptor** stimulation;
  - Involved in physiological processes such as cardiac contractility and relaxation through modulation of calcium cytosolic concentration;
  - Exerts its effects via the Protein Kinase A (PKA).

- **Chronic activation of cAMP/EPAC signaling leads to cardiac dysfunction:**
  - Induces **pathologic cardiac remodeling**, notably ventricular **hypertrophy and fibrosis**;
  - Promotes cardiac arrhythmia and heart failure development;
  - Molecular mechanism: switch from PKA to **EPAC deleterious signaling**.
Target validation: EPAC1

- Exchange proteins directly activated by cAMP -1 ;
- Promotes activation of the small G protein Rap ;
- Highly expressed in the heart and upregulated in cardiac diseases ;
- **Target validation:**
  - EPAC1 gene or pharmacological inhibition does not alter basal cardiac function ;
  - EPAC1 gene or pharmacological inhibition **prevents pathological remodeling** *(e.g fibrosis, cell death, inflammation)* and **improves cardiac function** in experimental models of ischemic and non-ischemic heart failure ;
  - EPAC1 gene deletion is protective against **atrial arrhythmia**.

Target drugability: AM, a novel family of EPAC1 inhibitors

- Small molecules identified by chemical bank screening;
- **Non competitive inhibitors**;
- **Very specific of EPAC1 isoform** : no effect on EPAC2 and PKA activity;
- Half maximal inhibitory concentration IC50 ~ 50 μM;
- **In vitro and in vivo** pharmacologic efficacy;
- **No apparent toxicity** on cultured cell lines, nor on mice liver and kidney after **in vivo** treatment.
AM-001 prevents EPAC1 detrimental effect in vitro

- **Protective effect against EPAC1-induced cardiomyocyte hypertrophy:**

  A. Fluorescent microscopic analysis of neonatal rat ventricular myocytes surface (NRVMs) treated or not with Epac1-specific agonist 8-CPT-AM, in the presence or absence of AM-001. B. AM-001 decreases the expression of pathological hypertrophic markers.

- **Protective effect against hypoxia-reoxygenation-induced cardiomyocyte death:**

  C. Cell viability, assayed by LDH release, of isolated adult cardiomyocytes subjected to normoxia (NX) or hypoxia-reoxygenation (HX+R) treatment.

→ **AM-001 prevents EPAC1-induced cardiomyocyte hypertrophy and cell death.**
Protective effects of AM-001 in a mouse model of ischemia-reperfusion:

- AM-001 reduces the infarct size in mice hearts and has a therapeutic potential in cardiac ischemia.

A. Schematic time scale and experimental strategy to evaluate the effect of AM-001 on I/R injury.

B. Representative cross-sections stained with Evans blue and TTC of mice pretreated or not with AM-001. C. Quantification of the area at risk expressed as percentage of left ventricle size and infarct size.
Protective effects of AM-001 against chronic β-adrenergic receptor activation-induced cardiac dysfunction:

A. Schematic time scale and experimental strategy. (B) Mouse heart sections were stained with sirius red (left panel) to detect fibrosis. (C) Fibrosis quantification

- ISO - ISO
- + ISO + ISO

D. Comparison of the ratio of left ventricular weight (LVW) to tibia length (LVW/TL), and E. fractional shortening (FS), in ISO treated animals in the presence or absence of AM-001.

⇒ AM-001 attenuates cardiac fibrosis and hypertrophy, and improves cardiac function during chronic activation of β-adrenergic receptors.
AM inhibitors, new drugs for cardiac disease therapy

- **AM FAMILY INHIBITORS BENEFITS:**
  - Novel molecular therapeutics for acute and chronic heart failure treatment;
  - First EPAC1 specific inhibitors, with no impact on PKA;
  - *In vitro* and *in vivo* efficiency, reproducing EPAC1 knock-down effects;
  - No evidence of toxicity *in vitro* and *in vivo*;
  - No reduction of cardiac contractility, contrary to β-blockers;
  - Potential protection against cardiotoxic side effects of anticancer therapies.

- **ONGOING WORK FOR AM INHIBITORS VALIDATION:**
  - Rational design to improve AM-001 efficacy dose;
  - ADMET studies;
  - Comparison of AM-001 treatment to current standard-of-care β-blocker therapy;
  - Co-administration of AM-001 and β-blocker therapy in animal models of HF.
- **Patent** EP18305685 *(June 2018)*
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