Signaling β-adrenergico nell'insufficienza cardiaca

dagli studi pre-clinici ai clinical trials

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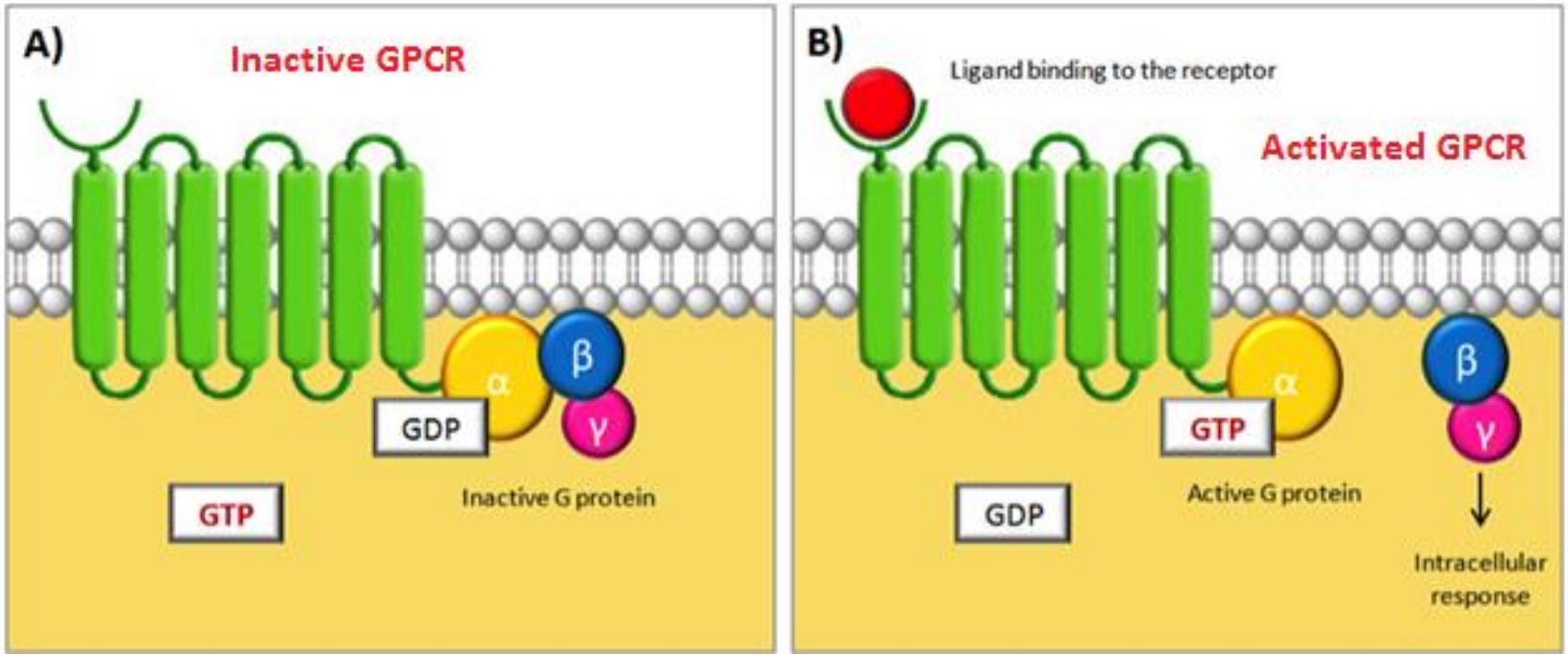
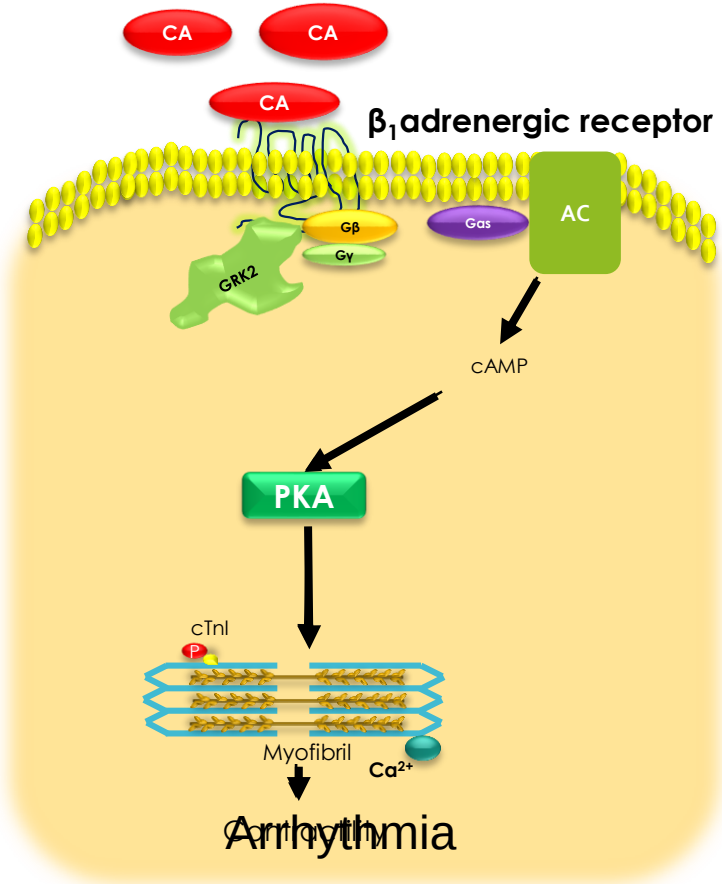
Recettori accoppiati alle protein G (GPCR)

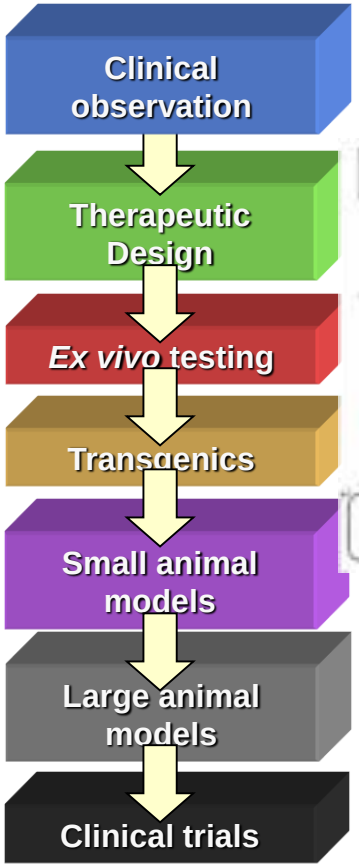


Ruolo cardiaco di GRK2

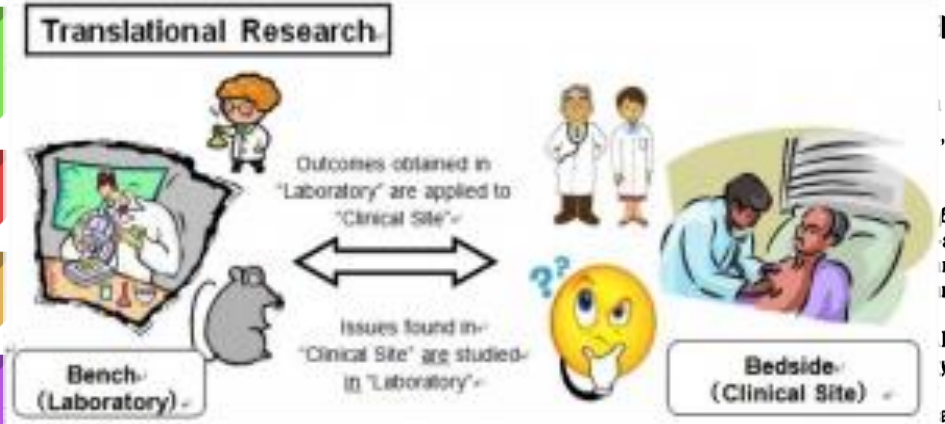


Modificato da Cannavo et al. *Front Physiol.* 2013

A Paradigm for Translational Research



Altered Expression of β-Adrenergic Receptor Kinase and β_1-Adrenergic Receptors in the ırt



S. Elce, PhD;
, MD

β-adrenergic receptor agonists are adrenergic receptors: loss of their regulation). In vitro studies have ıpling involves phosphorylation of

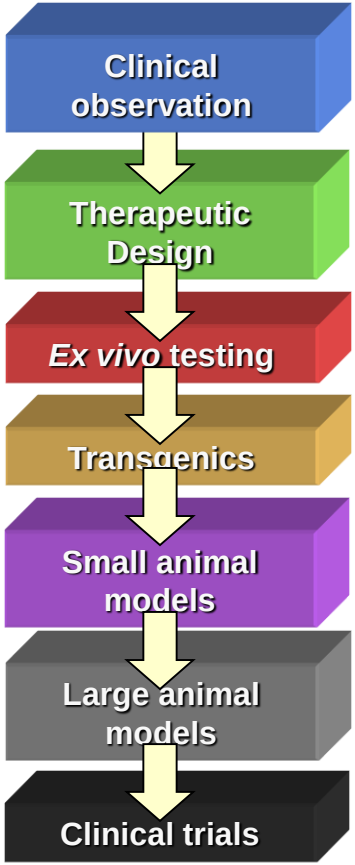
RK and β-adrenergic receptors in y or ischemic cardiomyopathy and stimulation were decreased in the and calcium remained unchanged.

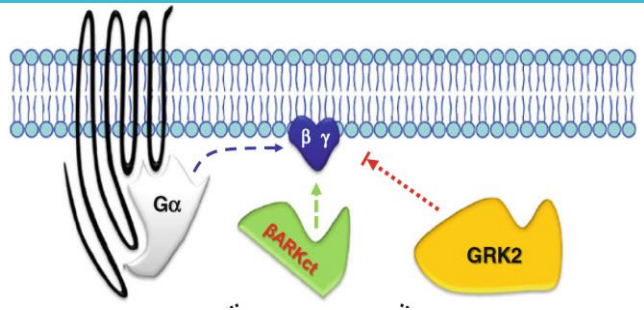
The messenger RNA (mRNA) levels of βARK, β_1- and β_2-receptors, and of glyceraldehyde phosphate dehydrogenase and β-actin as controls were measured by quantitative polymerase chain reactions. In addition, βARK enzyme activity assays were performed, and the levels of β_1- and β_2-receptors were determined by radioligand binding. βARK mRNA levels were increased almost threefold in both forms of heart failure, and βARK activity was enhanced. β_1-Receptor mRNA levels and β_1-receptor numbers were decreased by ~50% in both failing groups, whereas these levels were unaltered for β_2-receptors. There were no differences between dilated and ischemic cardiomyopathy for any of these parameters.

Conclusions. **In addition to other alterations found in failing hearts, the diminished response to β-receptor agonists appears to involve the combined effects of enhanced expression of βARK and reduced expression of β_1-receptors. (*Circulation* 1993;87:454–463)**

KEY WORDS • heart failure • receptors, β-adrenergic • kinase, β-adrenergic receptor • RNA, messenger

Theraputic Design





Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 3637–3641, April 1994
Biochemistry

Functionally active targeting domain of the β-adrenergic receptor kinase: An inhibitor of $G_{\beta\gamma}$-mediated stimulation of type II adenylyl cyclase

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Contributed by R. J. Lefkowitz, January 7, 1994

ABSTRACT The β-adrenergic receptor kinase (βARK) phosphorylates its membrane-associated receptor substrates, such as the β-adrenergic receptor, triggering events leading to receptor desensitization. βARK activity is markedly stimulated by the isoprenylated βγ subunit complex of heterotrimeric guanine nucleotide-binding proteins ($G_{\beta\gamma}$), which translocates the kinase to the plasma membrane and thereby targets it to its receptor substrate. The amino-terminal two-thirds of βARK1 composes the receptor recognition and catalytic domains, while the carboxyl third contains the $G_{\beta\gamma}$ binding sequences, the targeting domain. We prepared this domain as a recombinant His_6 fusion protein from *Escherichia coli* and found that it had both independent secondary structure and functional activity. We demonstrated the inhibitory properties of this domain against $G_{\beta\gamma}$ activation of type II adenylyl cyclase both in a reconstituted system utilizing Sf9 insect cell membranes and in a permeabilized 293 human embryonic kidney cell system. $G_{i\alpha}$-mediated inhibition of adenylyl cyclase was not affected. These data suggest that this His_6 fusion protein derived from the carboxyl terminus of βARK1 provides a specific probe for defining $G_{\beta\gamma}$-mediated processes and for studying the structural features of a $G_{\beta\gamma}$-binding domain.

the γ subunit of $G_{\beta\gamma}$ (15), allowing the kinase to be translocated to the rhodopsin-rich membranes of the retina in a light-dependent fashion (16).

In an effort to develop a functionally active $G_{\beta\gamma}$-binding domain, we expressed the carboxyl-terminal domain of βARK1 in *Escherichia coli* as a His_6-tagged fusion protein. Here we describe the properties of this stable 27-kDa domain which retains the ability to form a complex with $G_{\beta\gamma}$ and inhibits the $G_{\beta\gamma}$ stimulation of type II adenylyl cyclase both in a reconstituted membrane system and in intact cells.

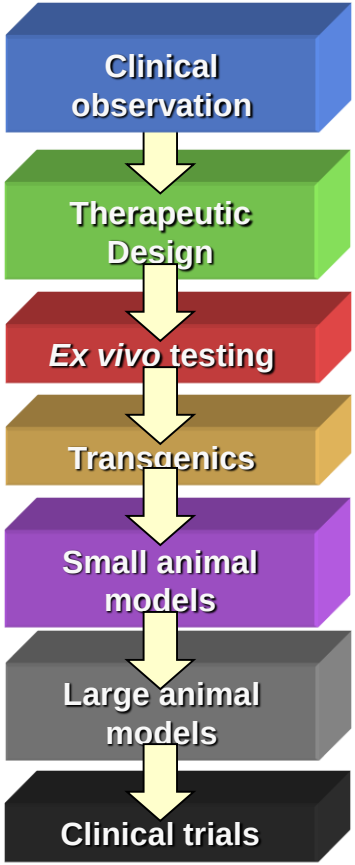
MATERIALS AND METHODS

Materials. The full-length cDNA encoding the human dopamine 1A receptor ($D_{1A}R$) was provided by Marc Caron (Howard Hughes Medical Institute, Duke University) and that for the rat type II adenylyl cyclase (17) by Randall Reed (Johns Hopkins University). Human embryonic kidney 293 (HEK-293) cells (ATCC CRL-1573) were from the American Type Culture Collection. Eagle's minimum essential medium, fetal bovine serum, and gentamicin were from GIBCO. Reduced streptolysin O was from Wellcome. Radioligands, including [^{3}H]cAMP, [^{3}H]SCH23390, [^{3}H]yohimbine, and

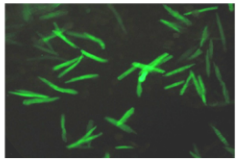
Walter J. Koch, PhD

CM CENTER FOR TRANSLATIONAL MEDICINE

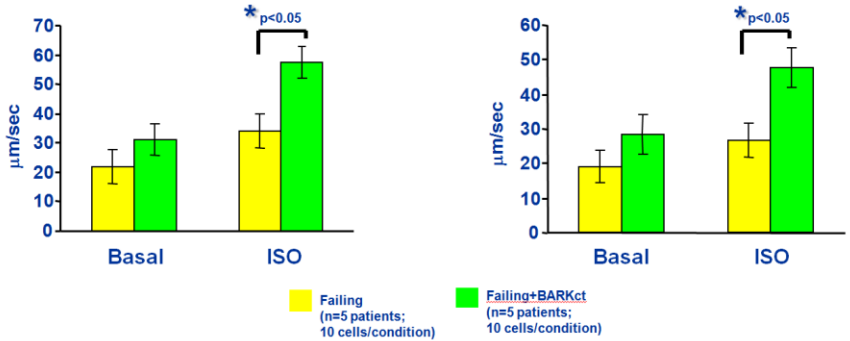
Ex-vivo testing



Restoration of Contractility and βAR Function by βARKct in Failing Human Myocytes

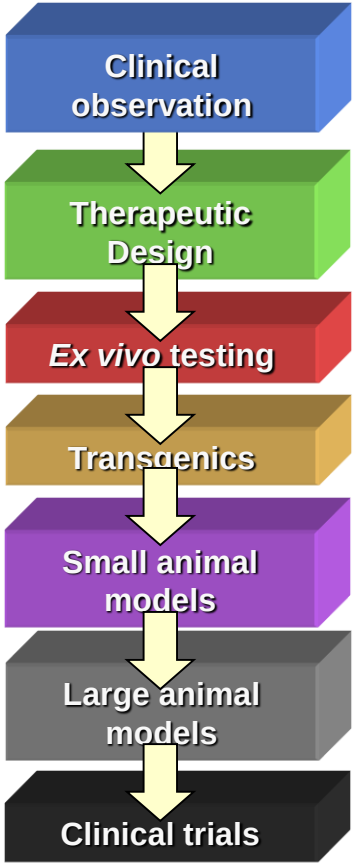


Human myocytes infected with Adeno-GFP/BARKct

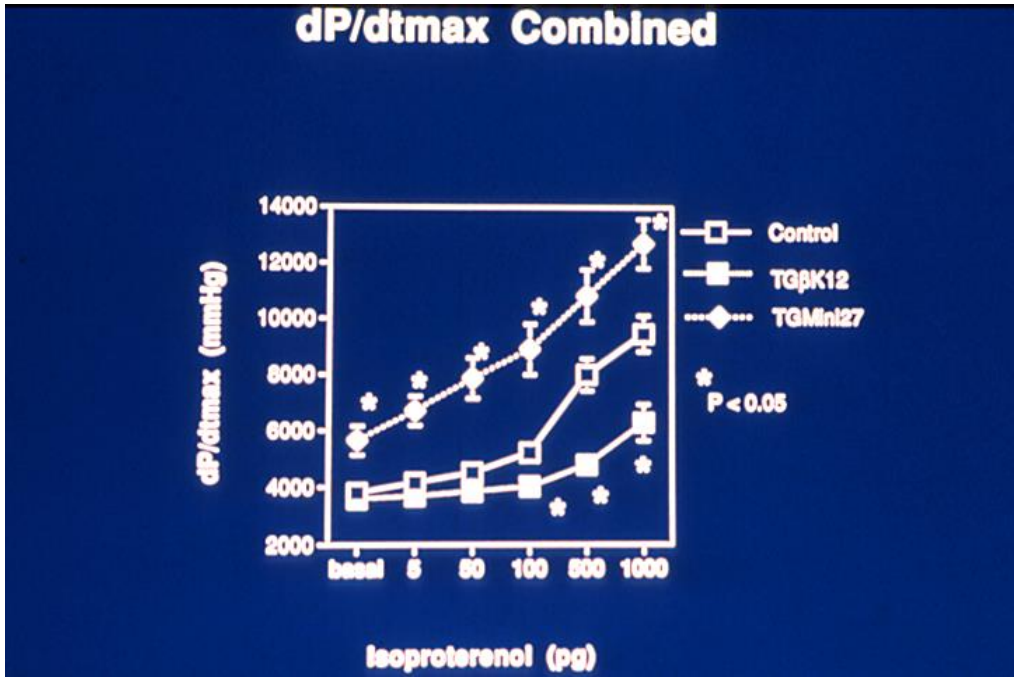


Williams et al Circulation 2004.

Transgenic models



αMHC | GRK2 or βARKct | SV40



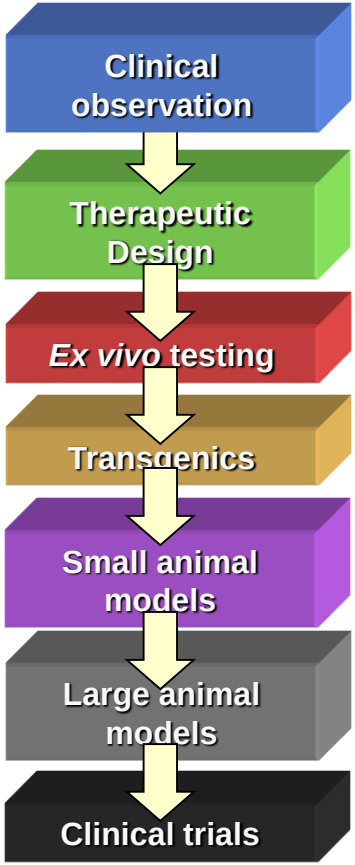
Koch et al., Science, 1995.

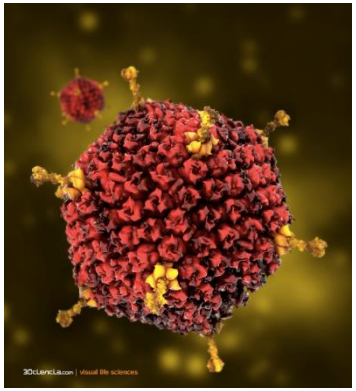
Transgenic models

Murine model	Result of βARKct cross	Reference
MLP$^{-/-}$ Knockout	Complete functional rescue with restored βAR responsiveness	1
Transgenic Cardiac CSQ Overexpression	Rescue of cardiac function with smaller cardiac dimension and also improved survival	2
Transgenic Cardiac Expression of a Mutant Myosin Heavy Chain (HCM)	Rescue of function, prevention of hypertrophy and dimensions and improved exercise tolerance	3
Transgenic Cardiac Overexpression of MCP-1	Hypertrophy prevented	4
Transgenic Cardiac Overexpression of dominant-Negative mutant of CREB ($CREB_{A133}$)	Only βAR signaling improved with no functional or mortality rescue	5

1. **Rockman et al.** 1998 PNAS 95:7000-7005.
2. **Harding et al.** 2001 PNAS 98:5809-5814.
3. **Freeman et al.** 2001 J Clin Invest 107:967-974.
4. **Khouri et al.** 2002 J Amer Coll Cardiol 39:I-164.
5. **Eckhart et al.** 2002 J Mol Cell Cardiol 34:669-677.

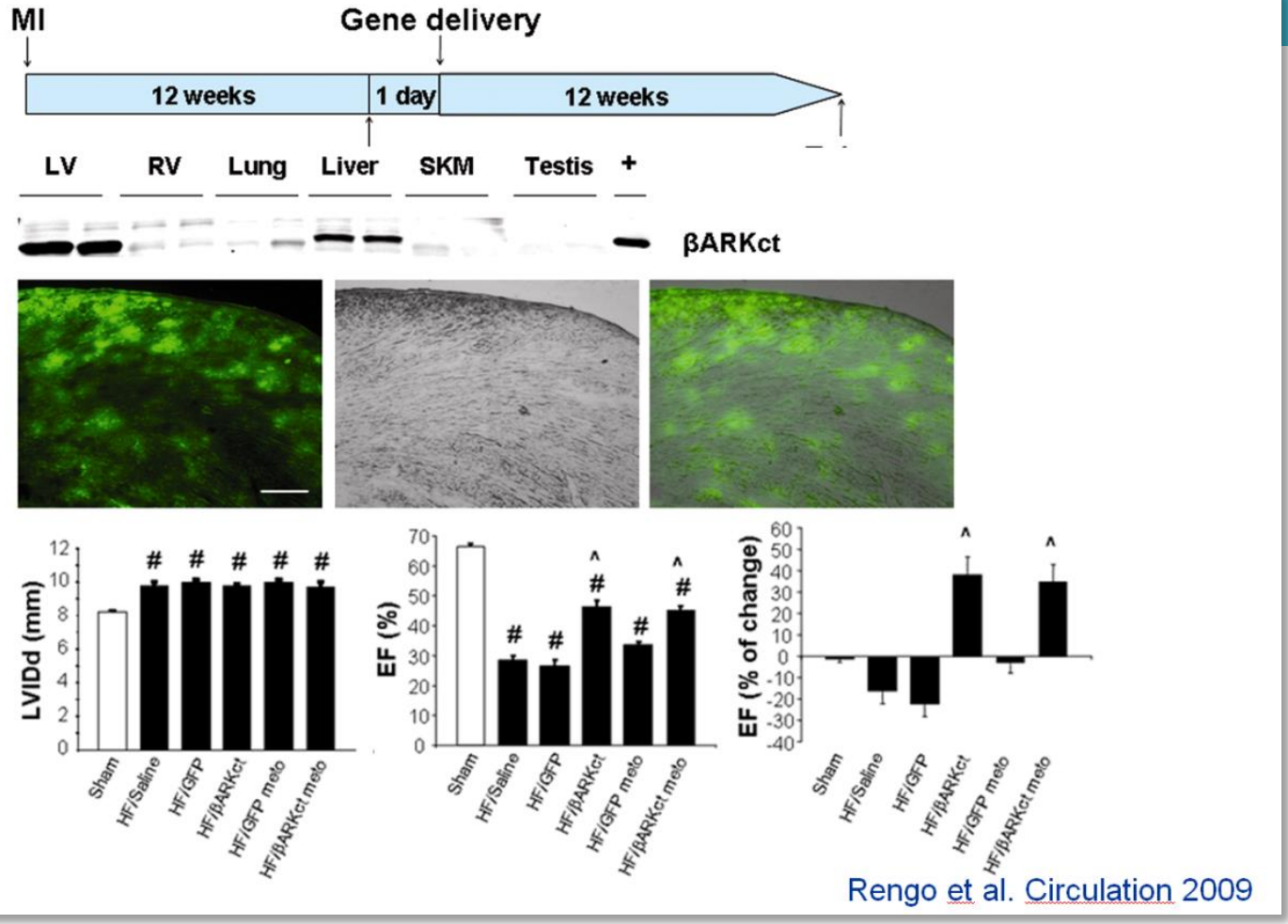
Small animal models





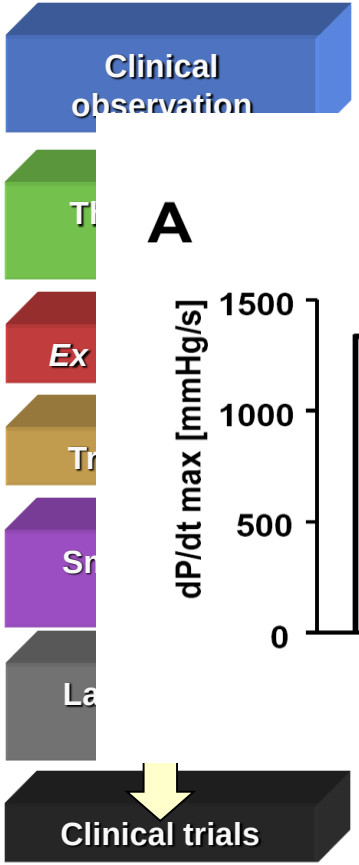
- The βARKct transgene was cloned into replication-deficient adenoviral vectors
- Intracoronary gene transfer to the hearts of larger animal models has rescued LV dysfunction (White PNAS, 1999, Shah, Circulation, 2001).
- Now using AAV Vectors -

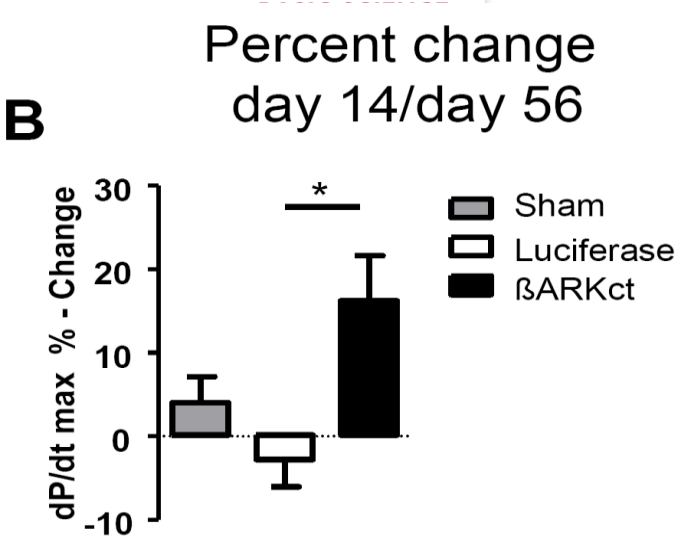
Small animal models



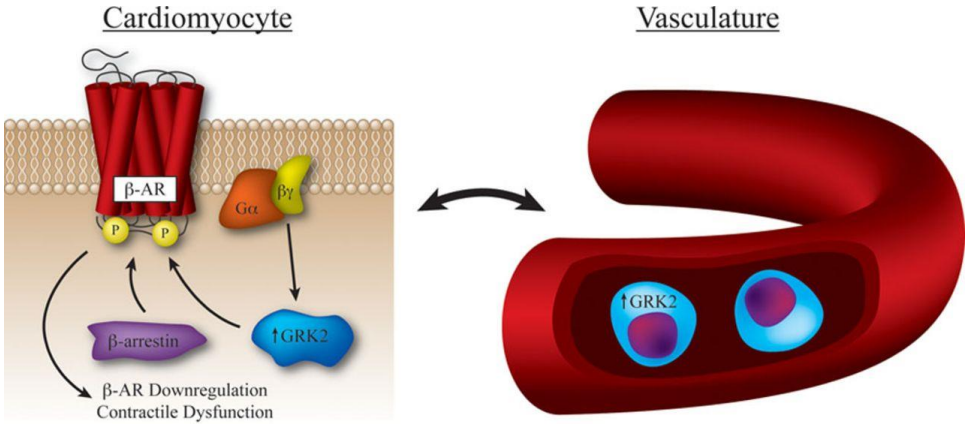
Rengo et al. Circulation 2009

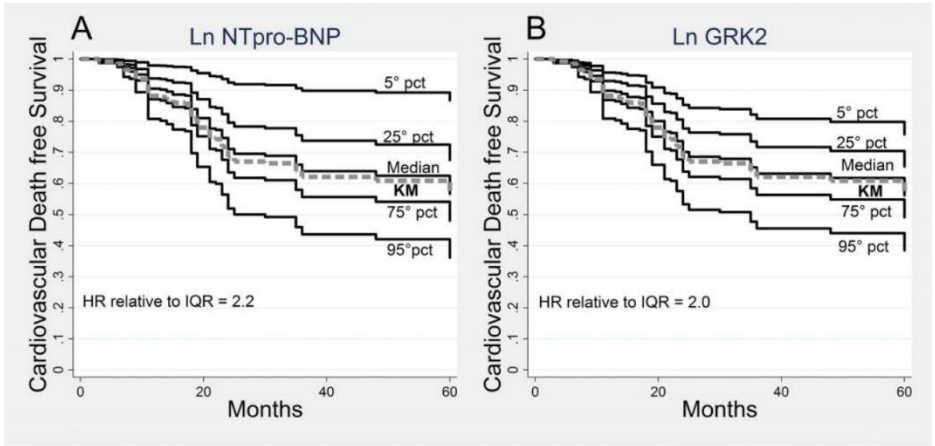
Large animal models





Prognostic Value of Lymphocyte G Protein-Coupled Receptor Kinase-2 Protein Levels in Patients With Heart Failure.





Rengo et al. Circ Res. 2016

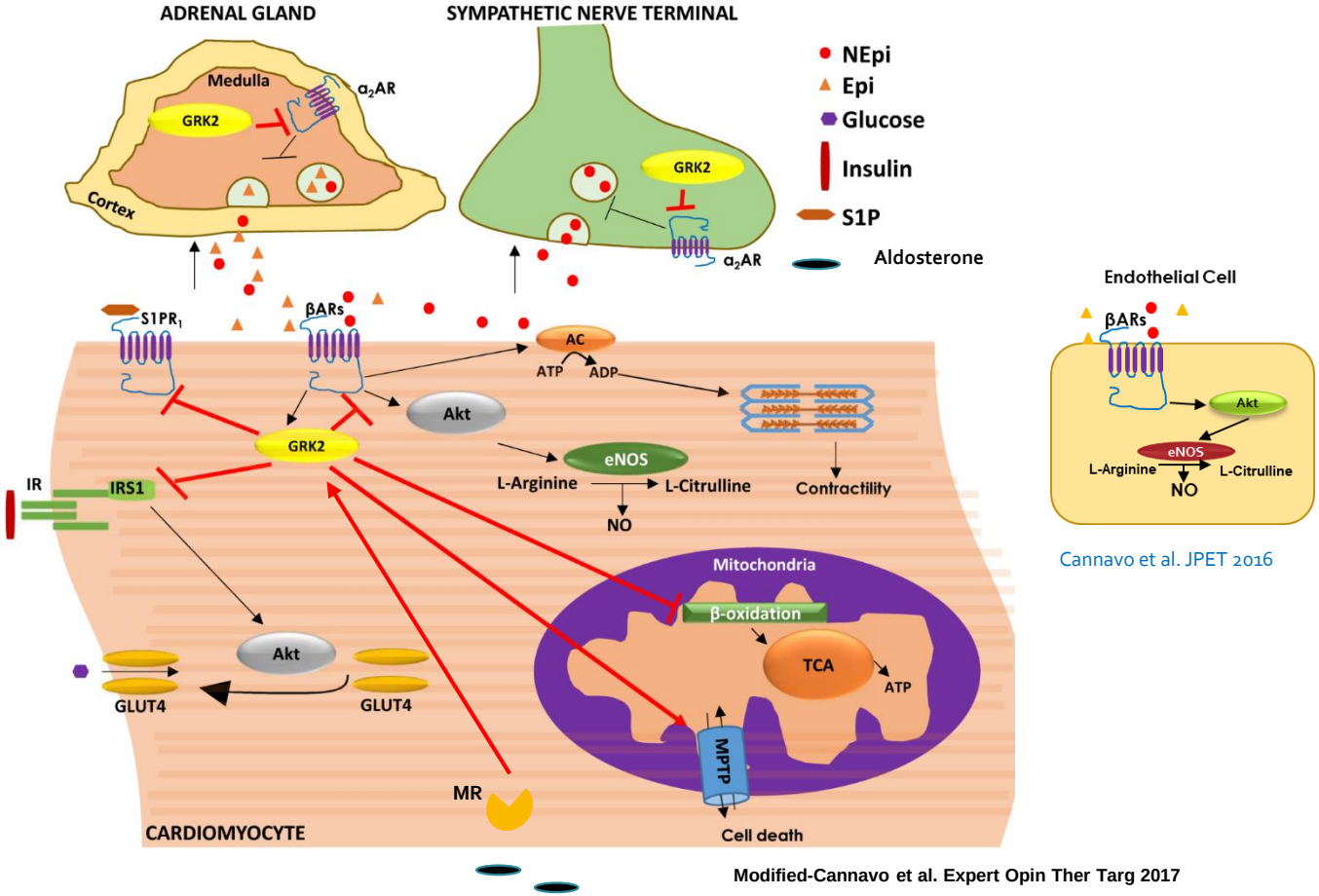
GRK2: This is not just a question of HEART

Lymperopoulos et al. Nat Med 2010
Cannavo et al. J Am Coll Cardiol. 2017

Cannavo et al. Circulation 2013

Ciccarelli et al. Circulation 2011

Cannavo et al. Nat Communications 2016



Cannavo et al. JPET 2016

Modified-Cannavo et al. Expert Opin Ther Targ 2017

Translational Impact

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
1401 Rockville Pike
Rockville, MD 20852-1448

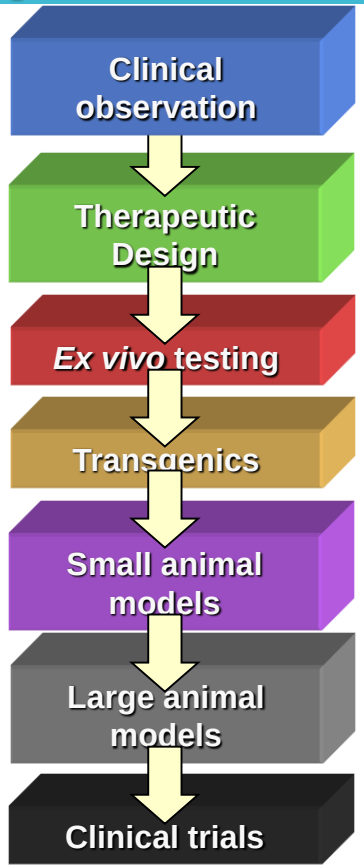
Our Reference: PTS PS 002251
Meeting ID: 9336

April 29, 2014

Walter J. Koch, PhD, FAHA
Director, Center for Translational Medicine
Temple University School of Medicine
3500 N. Broad Street, Suite 941
Philadelphia, PA 19140

Dear Dr Koch:

Pre-IND (Investigational New Drug) application



Pre-IND Feedback

Clinical trials

Phone Call from FDA to discuss their recommendations
then written report follows

Sample recommendations:

Systemic Toxicology study in rats – higher dose than planned (vg per Kg)
Full dose-response (3 doses) efficacy study in pigs with full toxicology
Then come back in with amended Pre-IND application with clinical trial

Gene Therapy with βARKct at Pre-IND stage
Clinical Trial being planned

Lancet. 2016 Mar 19;387(10024):1178-86. doi: 10.1016/S0140-6736(16)00082-9. Epub 2016 Jan 21.

Calcium upregulation by percutaneous administration of gene therapy in patients with cardiac disease (CUPID 2): a randomised, multinational, double-blind, placebo-controlled, phase 2b trial.

Greenberg B[1], Butler J[2], Felker GM[3], Ponikowski P[4], Voors AA[5], Desai AS[6], Barnard D[7], Bouchard A[8], Jaski B[9], Lyon AR[10], Pogoda JM[11], Rudy JJ[11], Zsebo KM[12].

Author information

Abstract

BACKGROUND: Sarcoplasmic/endoplasmic reticulum Ca(2+)-ATPase (SERCA2a) activity is deficient in the failing heart. Correction of this abnormality by gene transfer might improve cardiac function. We aimed to investigate the clinical benefits and safety of gene therapy through infusion of adeno-associated virus 1 (AAV1)/SERCA2a in patients with heart failure and reduced ejection fraction.

METHODS: We did this randomised, multinational, double-blind, placebo-controlled, phase 2b trial at 67 clinical centres and hospitals in the USA, Europe, and Israel. High-risk ambulatory patients with New York Heart Association class II-IV symptoms of heart failure and a left ventricular ejection fraction of 0·35 or less due to an ischaemic or non-ischaemic cause were randomly assigned (1:1), via an interactive voice and web-response system, to receive a single intracoronary infusion of 1 × 10(13) DNase-resistant particles of AAV1/SERCA2a or placebo. Randomisation was stratified by country and by 6 min walk test distance. All patients, physicians, and outcome assessors were masked to treatment assignment. The primary efficacy endpoint was time to recurrent events, defined as hospital admission because of heart failure or ambulatory treatment for worsening heart failure. Primary efficacy endpoint analyses and safety analyses were done by modified intention to treat. This trial is registered with ClinicalTrials.gov, number NCT01643330.

FINDINGS: Between July 9, 2012, and Feb 5, 2014, we randomly assigned 250 patients to receive either AAV1/SERCA2a (n=123) or placebo (n=127); 243 (97%) patients comprised the modified intention-to-treat population. Patients were followed up for at least 12 months; median follow-up was 17·5 months (range 1·8-29·4 months). AAV1/SERCA2a did not improve time to recurrent events compared with placebo (104 vs 128 events; hazard ratio 0·93, 95% CI 0·53-1·65; p=0·81). No safety signals were noted. 20 (16%) patients died in the placebo group and 25 (21%) patients died in the AAV1/SERCA2a group; 18 and 22 deaths, respectively, were adjudicated as being due to cardiovascular causes.

INTERPRETATION: CUPID 2 is the largest gene transfer study done in patients with heart failure so far. Despite promising results from previous studies, AAV1/SERCA2a at the dose tested did not improve the clinical course of patients with heart failure and reduced ejection fraction. Although we did not find evidence of improved outcomes at the dose of AAV1/SERCA2a studied, our findings should stimulate further research into the use of gene therapy to treat patients with heart failure and help inform the design of future gene therapy trials.

FUNDING: Celladon Corporation.

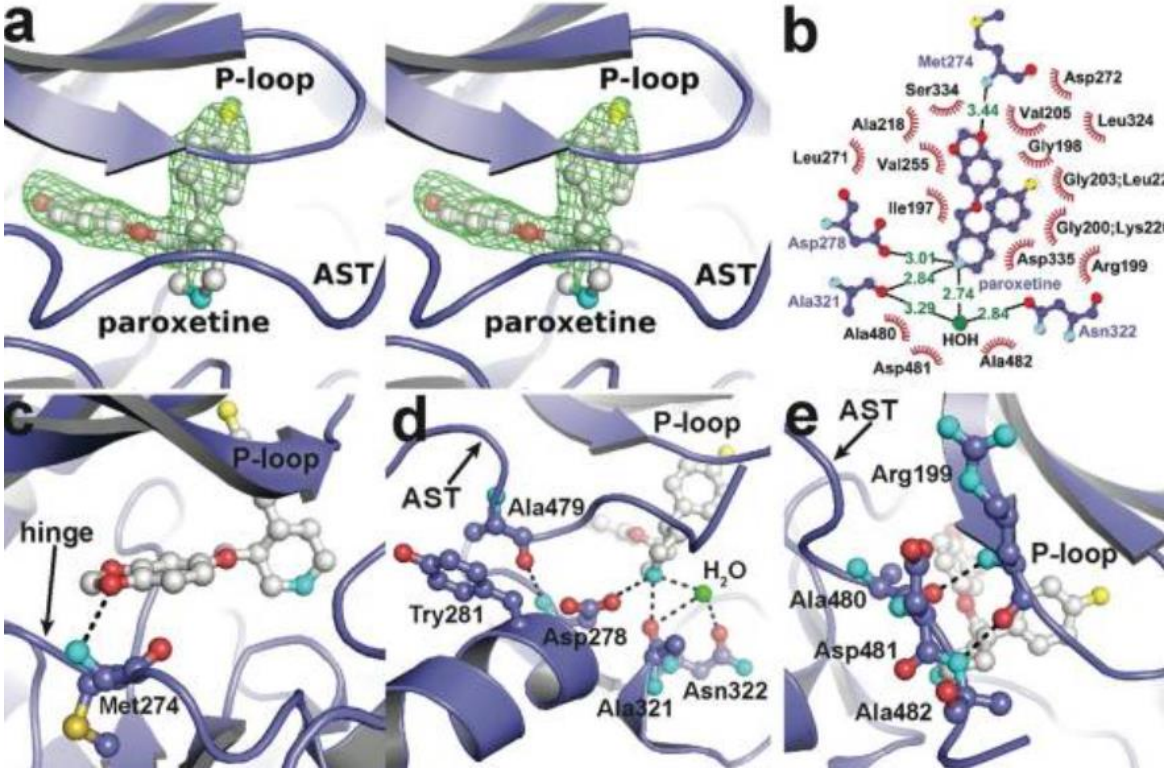
Comment in

Cardiac gene therapy: a call for basic methods development. [Lancet. 2016]

Gene therapy: No improvement in outcomes with gene therapy for heart failure. [Nat Rev Cardiol. 2016]

Pharmacologic inhibition of GRK2

John Tesmer, PhD



Pharmacologic inhibition of GRK2

Structural Determinants Influencing the Potency and Selectivity of Indazole-Paroxetine Hybrid G Protein-Coupled Receptor Kinase 2 Inhibitors.
Bouley R, Waldschmidt HV, Cato MC, Cannavo A, Song J, Cheung JY, Yao XQ, Koch WJ, Larsen SD, Tesmer JJG.
Mol Pharmacol. 2017 Dec;92(6):707-717. doi: 10.1124/mol.117.110130. Epub 2017 Oct 25.

Structure-Based Design of Highly Selective and Potent G Protein-Coupled Receptor Kinase 2 Inhibitors Based on Paroxetine.
Waldschmidt HV, Homan KT, Cato MC, Cruz-Rodríguez O, Cannavo A, Wilson MW, Song J, Cheung JY, Koch WJ, Tesmer JJ, Larsen SD.
J Med Chem. 2017 Apr 13;60(7):3052-3069. doi: 10.1021/acs.jmedchem.7b00112. Epub 2017 Mar 29.

Structure-Based Design, Synthesis, and Biological Evaluation of Highly Selective and Potent G Protein-Coupled Receptor Kinase 2 Inhibitors.
Waldschmidt HV, Homan KT, Cruz-Rodríguez O, Cato MC, Waninger-Saroni J, Larimore KM, Cannavo A, Song J, Cheung JY, Kirchhoff PD, Koch WJ, Tesmer JJ, Larsen SD.
J Med Chem. 2016 Apr 28;59(8):3793-807. doi: 10.1021/acs.jmedchem.5b02000. Epub 2016 Apr 13.

Identification and characterization of amlexanox as a G protein-coupled receptor kinase 5 inhibitor.
Homan KT, Wu E, Cannavo A, Koch WJ, Tesmer JJ.
Molecules. 2014 Oct 22;19(10):16937-49. doi: 10.3390/molecules191016937.

Paroxetine is a direct inhibitor of g protein-coupled receptor kinase 2 and increases myocardial contractility.
Thal DM, Homan KT, Chen J, Wu EK, Hinkle PM, Huang ZM, Chuprun JK, Song J, Gao E, Cheung JY, Sklar LA, Koch WJ, Tesmer JJ.
ACS Chem Biol. 2012 Nov 16;7(11):1830-9. doi: 10.1021/cb3003013. Epub 2012 Aug 21.

Pharmacologic inhibition of GRK2: pre-clinical and clinical studies

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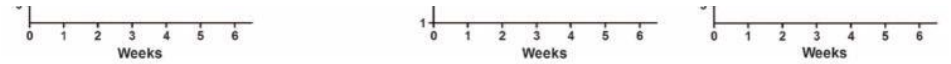
Table 3 Comparison of the changes of the variables in AMID patients before and after treatment with or without paroxetine or fluoxetine

Variables	AMID-N (n=21)		AMID-P (n=23)		AMID-F (n=23)	
	Before	After	Before	After	Before	After
HAMD-17	27.1±4.7	26.9±4.6	27.2±4.5	11.2±4.0***	27.4±4.4	15.9±4.8***
SDS	58.0±4.4	55.4±4.8	57.1±4.3	40.8±5.7***	57.5±4.0	46.9±4.2***
LVEF (%)	38.1±4.1	41.4±4.5	38.0±5.7	49.8±6.9******	38.2±4.0	41.7±5.6***
FS (%)	32.1±3.2	27.1±3.9	31.6±4.0	24.7±3.7*	31.7±3.1	28.9±3.9
LVDd (mm)	51.9±11.3	50.9±8.6	51.5±9.2	44.1±6.3*	51.4±10.7	47.9±8.4
LVPW (mm)	10.97±1.0110	34±1.08	10.08±1.06	9.46±1.04	10.16±1.04	9.46±1.03
HRV						
SDNN (ms)	21±9	44±10*	23±10	59±17***	23±11	58±15***
HF (ms^2)	257±114	379±149*	251±109	660±178******	255±106	430±157***
LF (ms^2)	328±173	406±130*	358±176	729±247******	346±152	519±230***
LF/HF (%)	48±32	97±57*	42±46	328±84******	45±43	189±68***
EPI (pg/mL)	115.6±18.7	76.1±7.7*	117.4±19.6	39.2±5.1******	112.5±17.2	52.4±5.9***
NE (pg/mL)	928±367.1	710±276.3*	972±272.4	467±198.2******	996±280.9	589±253.2***
SBP (mmHg)	131±10.8	124±9.3	138±11.4	115±7.6	137±11.8	119±8.2
DBP (mmHg)	79±6.9	74±6.2	84±7.1	72±6.0	85±6.8	73±6.4
CV after AMI						
PIAP (%)	–	10 (47.6)	–	7 (30.4)**	–	6 (26.1)**
RMI (%)	–	4 (19.0)	–	3 (13.0)**	–	3 (13.0)**

Notes: Data was expressed as mean ± SD. *$P<0.01$ compared with before treatment within groups. **$P<0.01$ compared with after treatments of AMID + N. ***$P<0.01$ compared with after treatments of fluoxetine.



Ringraziamenti

Walter J. Koch

John Tesmer

Giuseppe Rengo
Nicola Ferrara
Dario Leosco

DIPARTIMENTO DI SCIENZE MEDICHE TRASLAZIONALI
Università degli Studi di Napoli - Federico II

Daniela Liccardo, Postdoc
Giuseppina Gambino, Postdoc
Andrea Elia, PhD student
Federica Marzano, PhD student
Claudia Perna, Tech

GRAZIE!!

These studies are supported by:

MINISTERO DELL'ISTRUZIONE, DELL'UNIVERSITÀ E DELLA RICERCA

American Heart Association. life is why™

Compagnia di San Paolo