Translational Studies in Heart Failure

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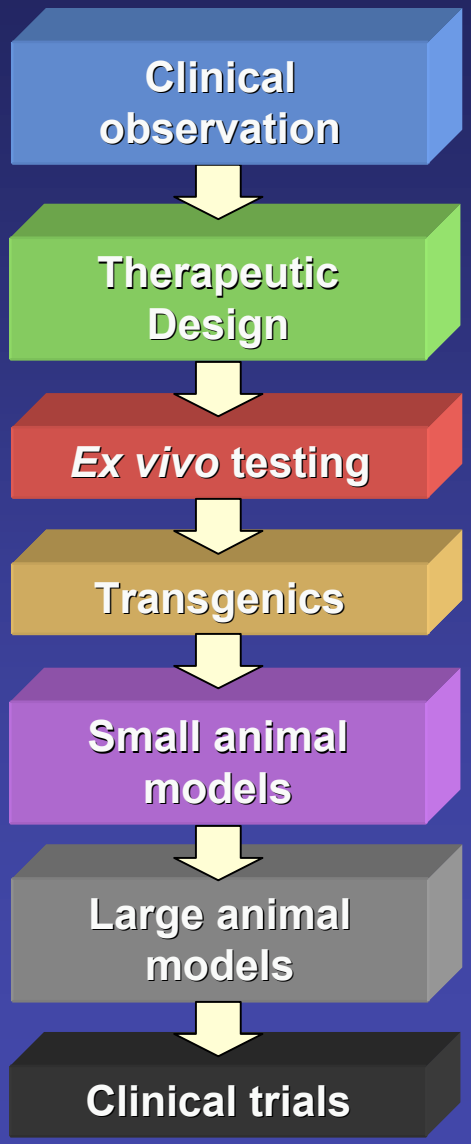
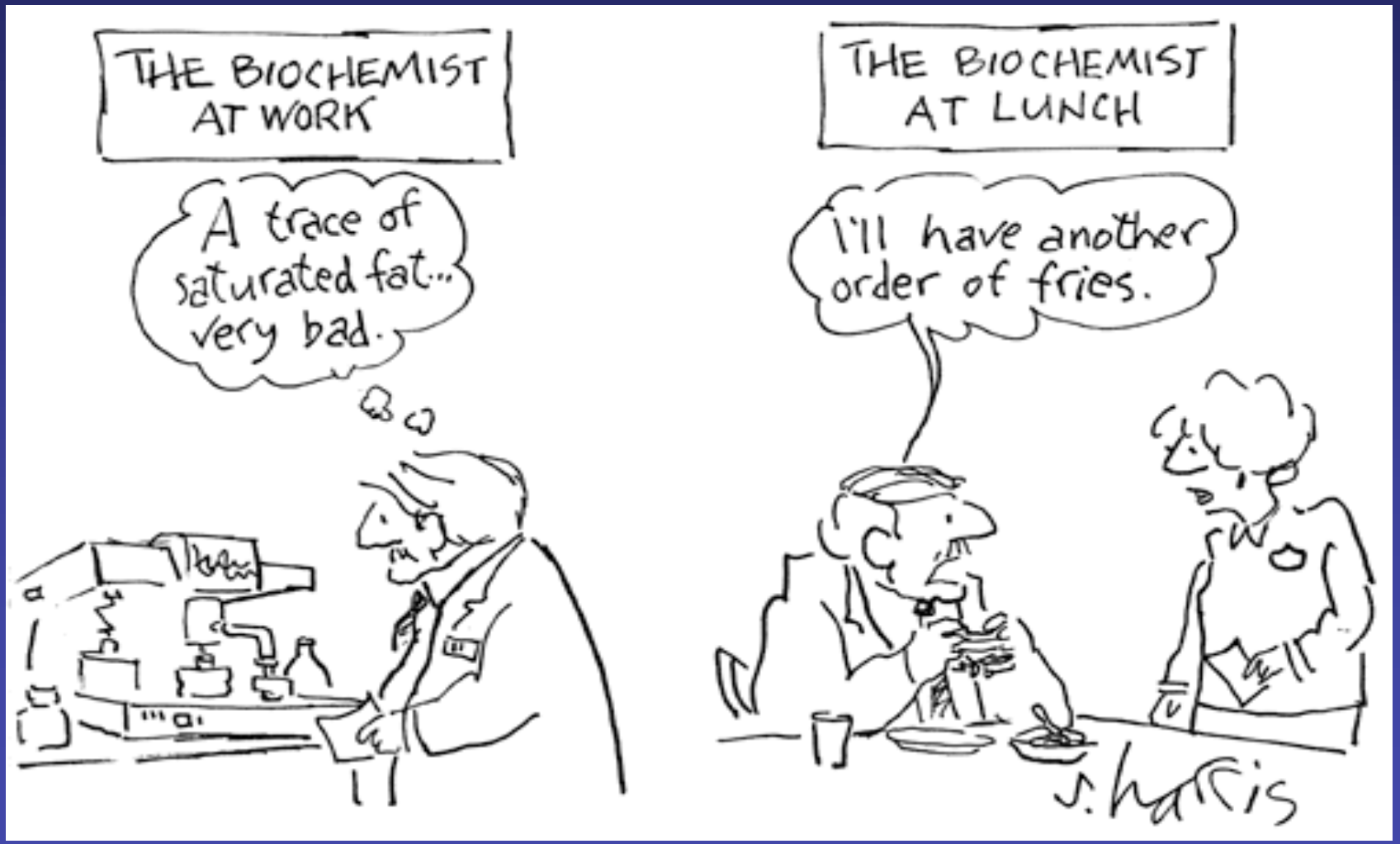
CENTER FOR TRANSLATIONAL MEDICINE

George Zallie and Family Laboratory for Cardiovascular Gene Therapy

Effective Model of Translational Research



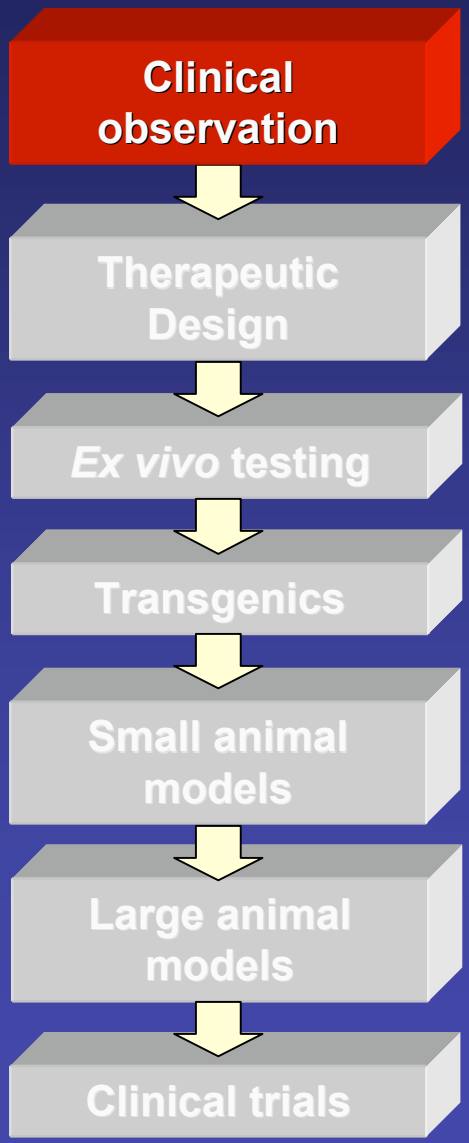
Non-Effective Translational Research Model



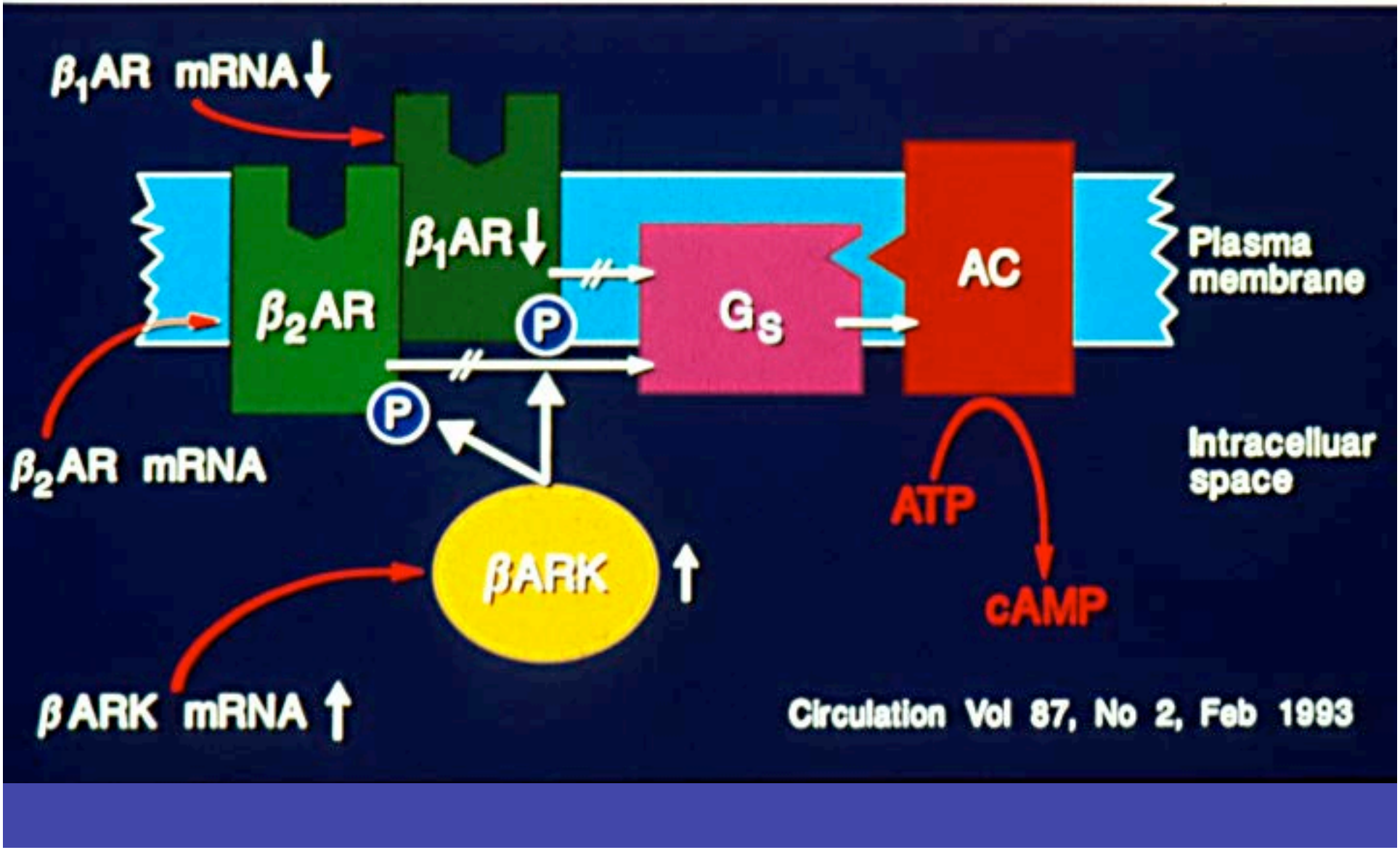
Heart Failure (HF) Statistics

- **Epidemic Proportions**
  - >400,000 new cases per year in U.S. (5 million total)
- **Death-rate still Increasing**
  - CAD down 49% - CHF up 64% in last 20 years
- **Morbidity and Costs also High**
  - #1 cause of all hospitalizations – >$300 Billion per year
- **Therapies not Ideal**
  - Improvements but no truly effective therapy

Translational Research

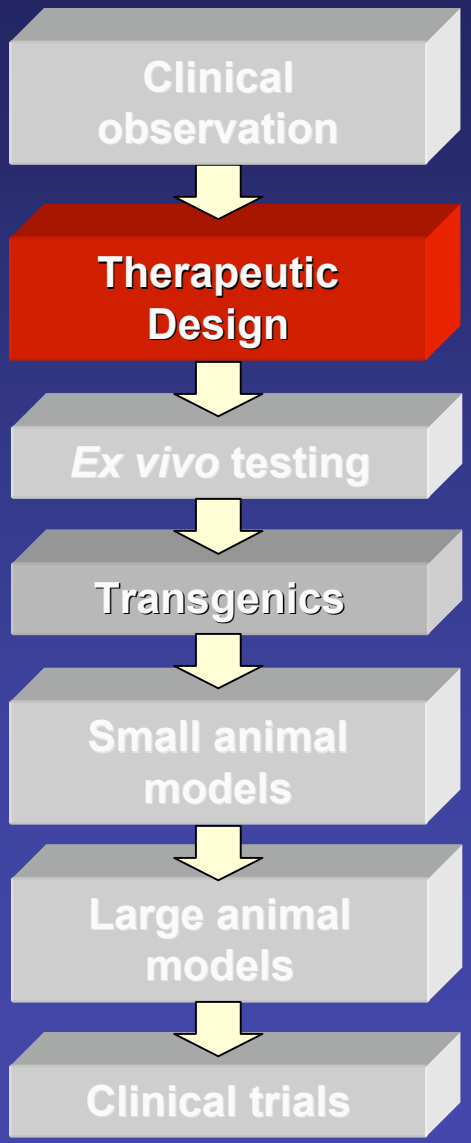


β-Adrenergic Receptor System in Heart Failure



Circulation Vol 87, No 2, Feb 1993

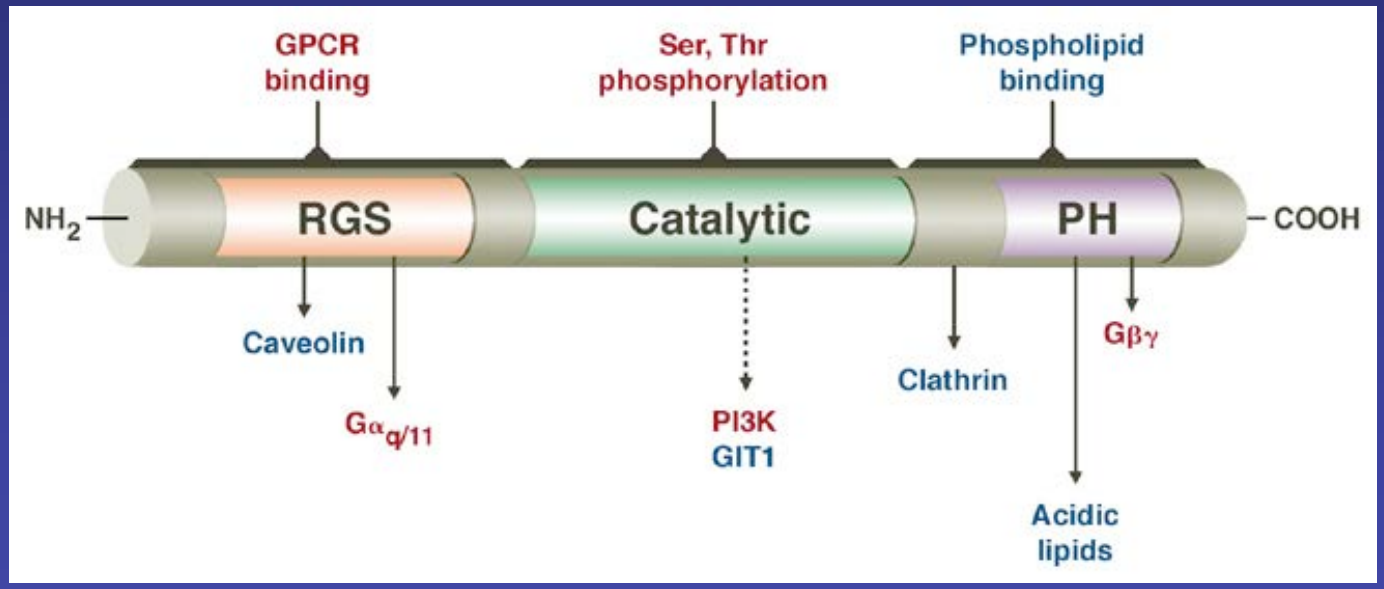
Translational Research



The G Protein-Coupled Receptor Kinases (GRKs)

Serine/ Threonine Kinases

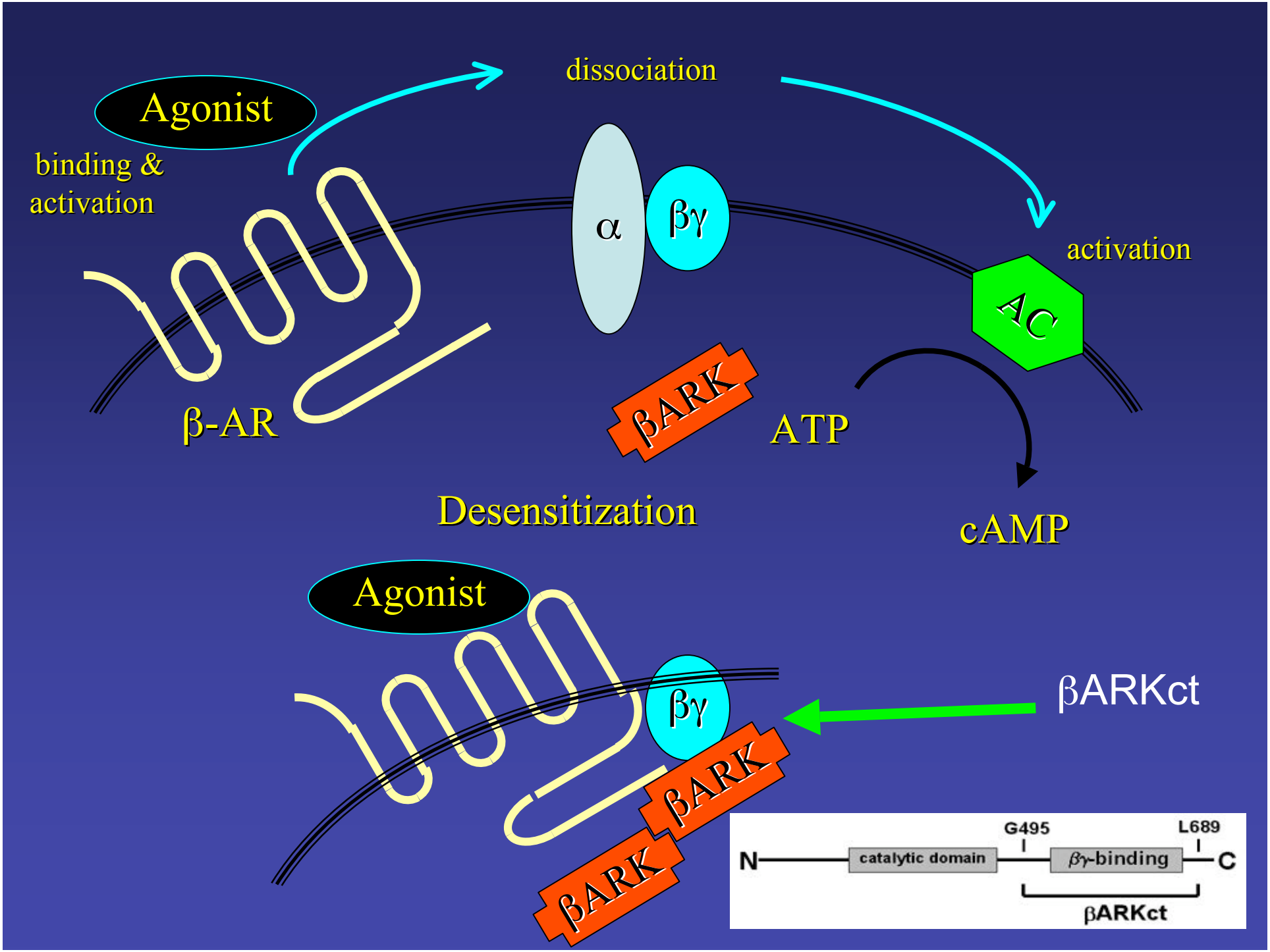
3 classes: GRK1 (Rhodopsin Kinase), GRK7
GRK2 (βARK1), GRK3 (βARK2)
GRK4, GRK5, GRK6



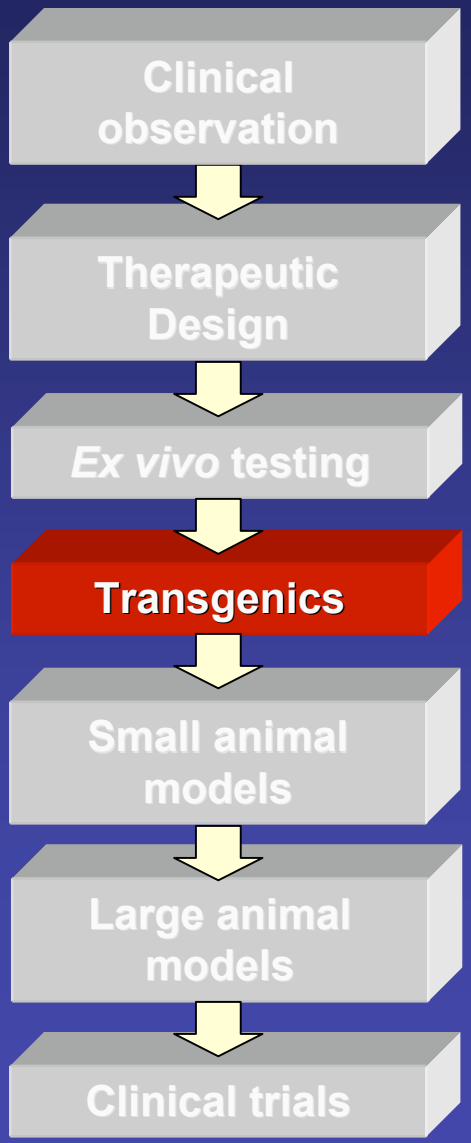
Additional Interacting Partners:

Tubulin, Actin, α-actinin

Adapted from Pao CS and Benovic JL, *Science's STKE* 8 October 2002, pp. pe42

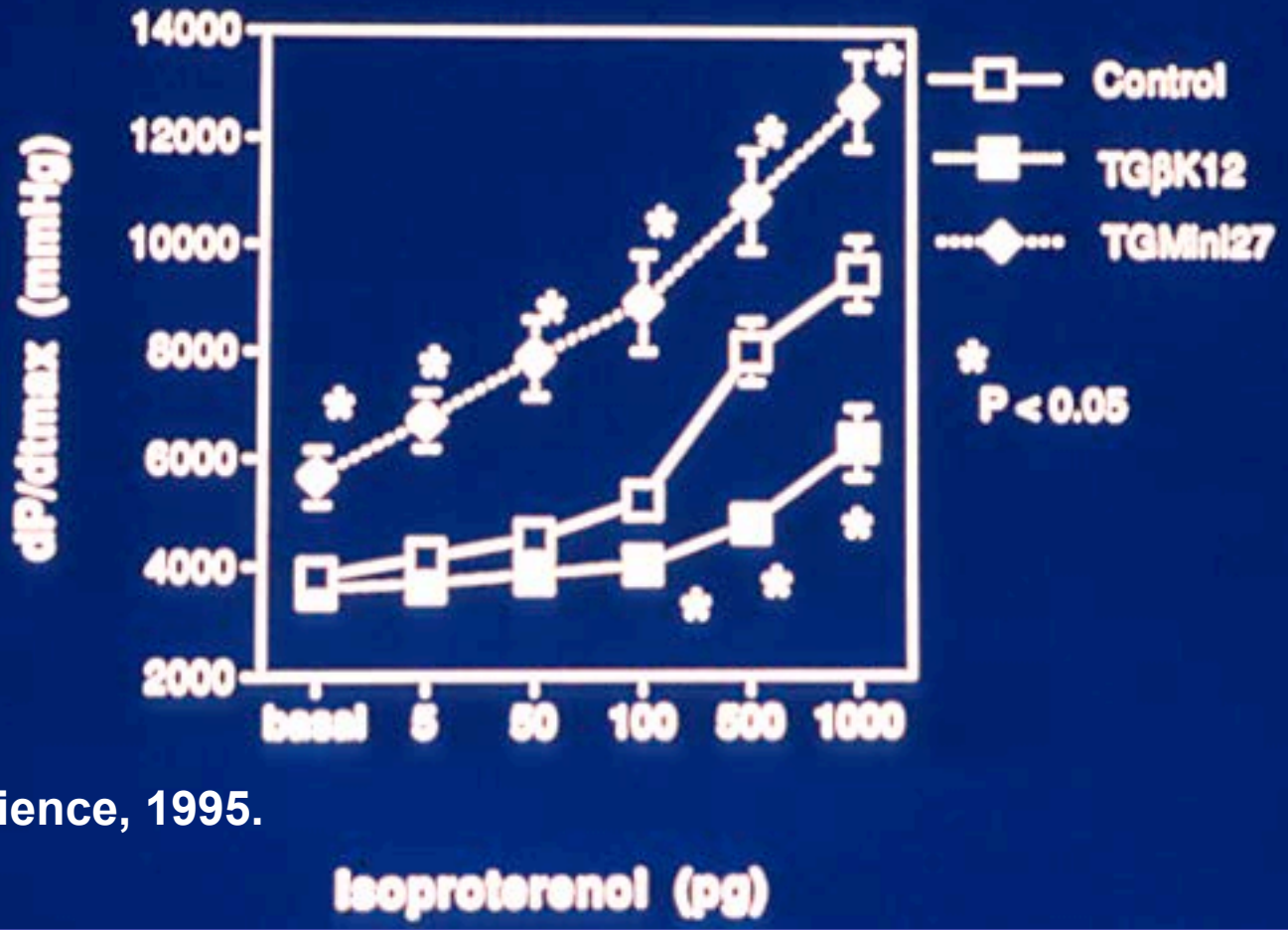

Agonist
binding &
activation
dissociation
α
βγ
activation
AC
β-AR
βARK
ATP
Desensitization
cAMP
Agonist
βγ
βARKct
βARK
βARK
βARK
G495
L689
N
catalytic domain
βγ-binding
C
βARKct

Translational Research



dP/dtmax Combined

promoter	coding region	intron -polyA +
αMHC	βARK1 or βARKct	SV40

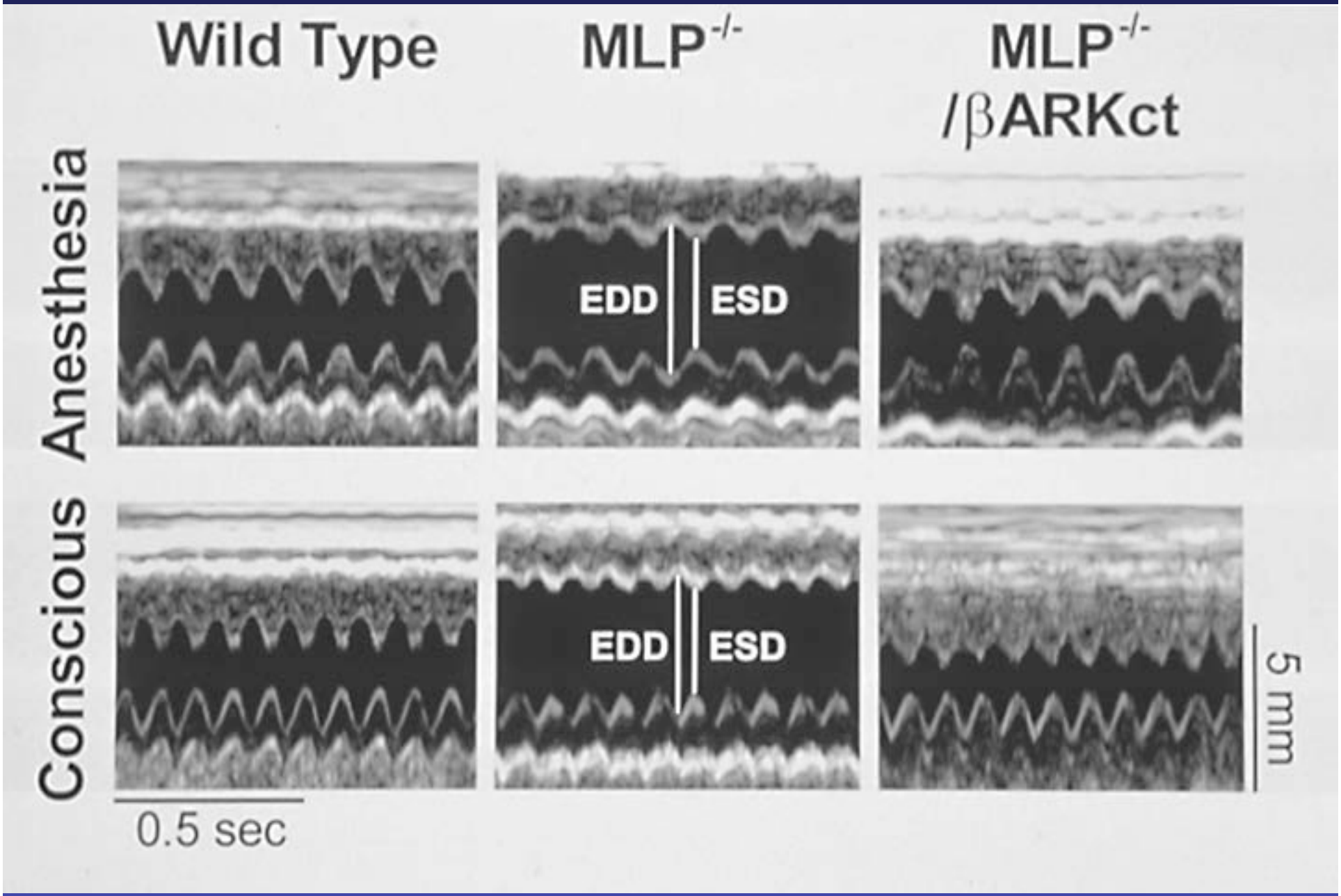


Koch et al., Science, 1995.

βARKct rescues several different murine models of HF

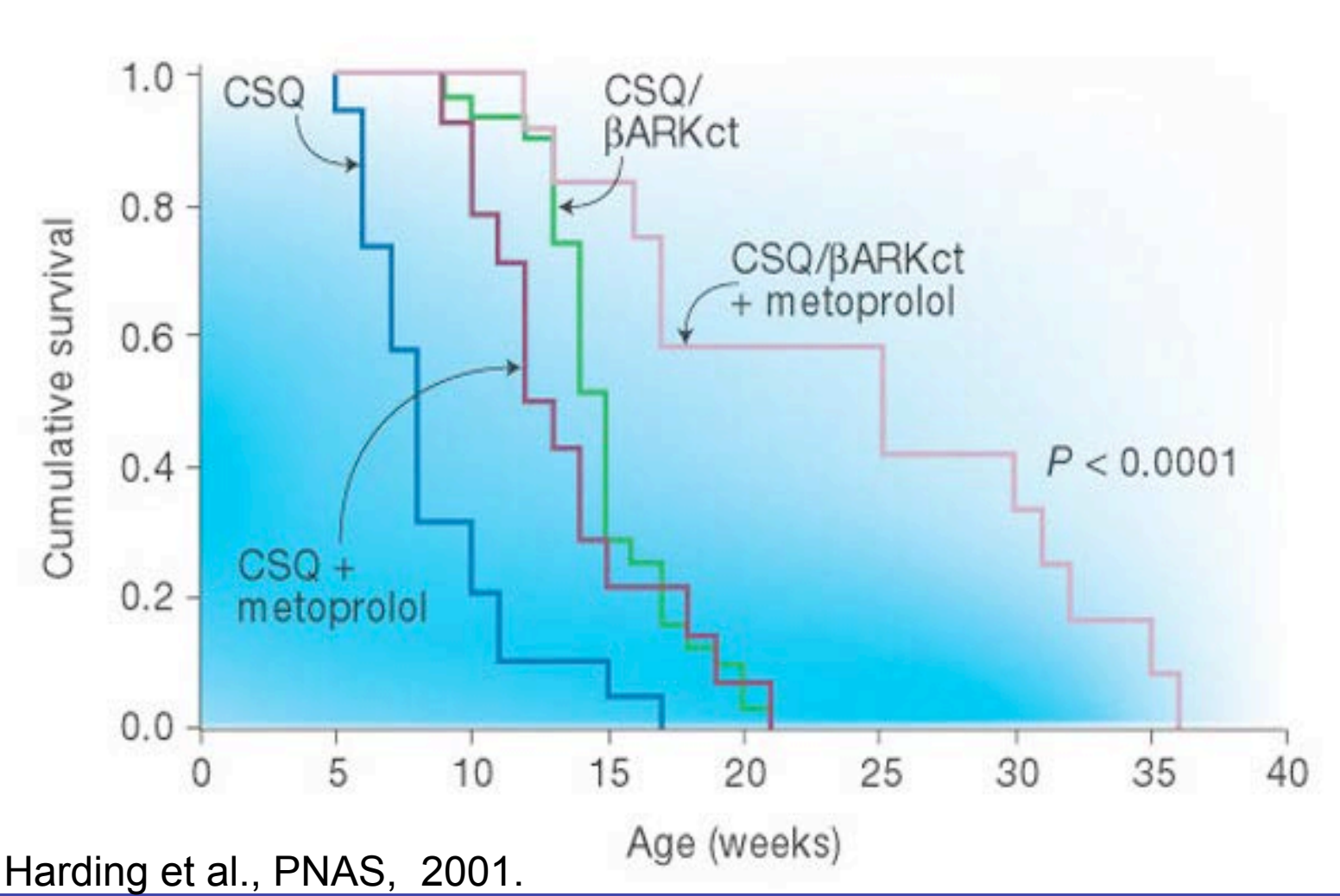
Murine model	Result of βARKct cross	Reference
MLP$^{-/-}$ Knockout	Complete functional rescue with restored βAR responsiveness	1
Transgenic Cardiac CSQ Overexpression	Rescue of cardiac function with smaller cardiac dimension and also improved survival	2
Transgenic Cardiac Expression of a Mutant Myosin 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.



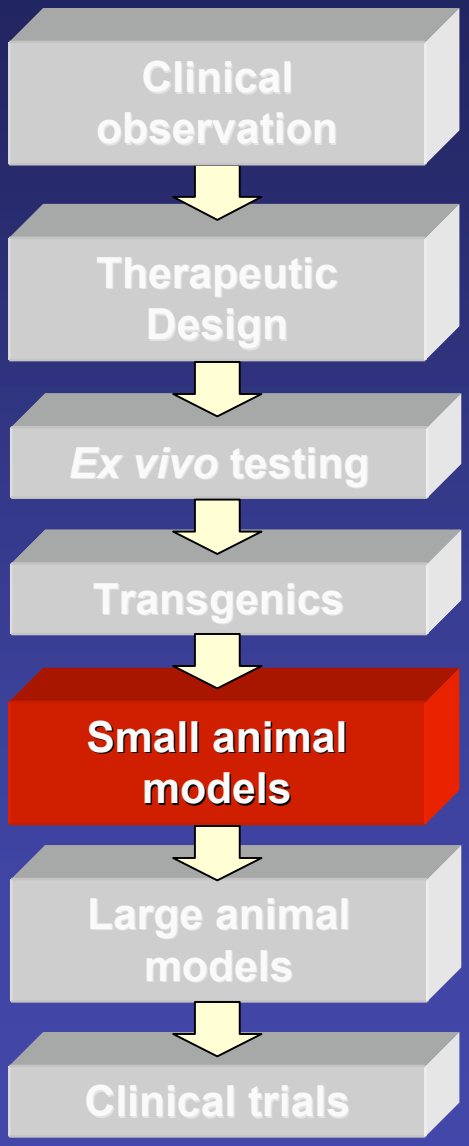
Rockman et al, PNAS 95:7000,1998.

βARKct Rescue of Survival in A Transgenic Mouse Model of HF

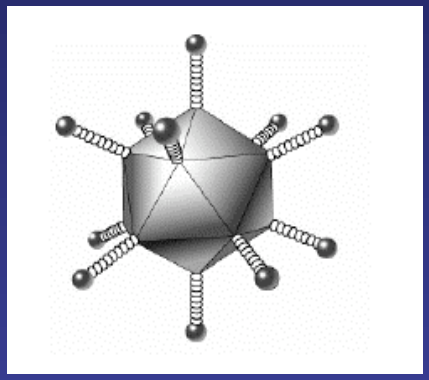


Harding et al., PNAS, 2001.

Translational Research Model



FROM MOUSE to MAN

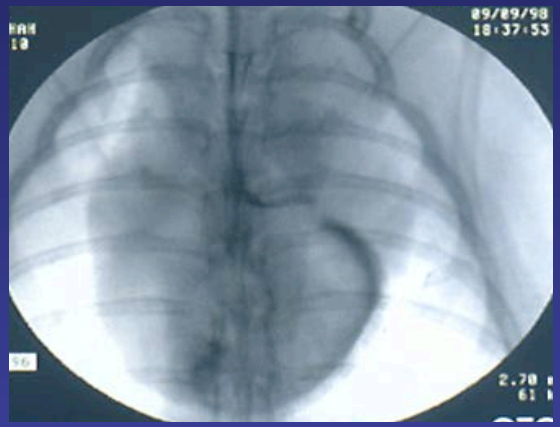


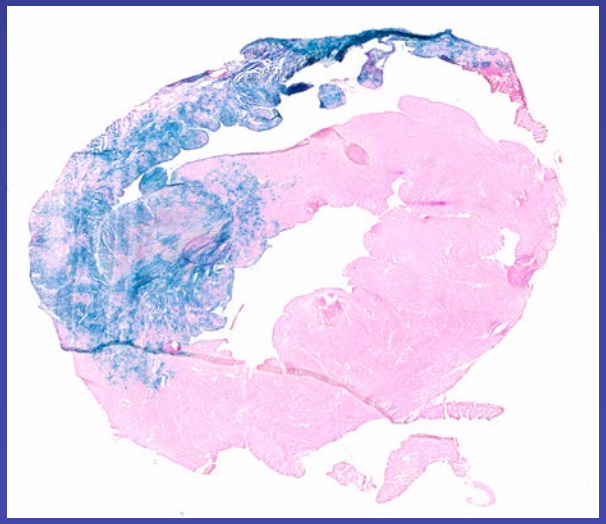
- The βARKct transgene was cloned into replication-deficient adenoviral vectors
- Attempt intracoronary gene transfer to the hearts of larger animal models

Intracoronary Adenoviral-mediated Myocardial Delivery

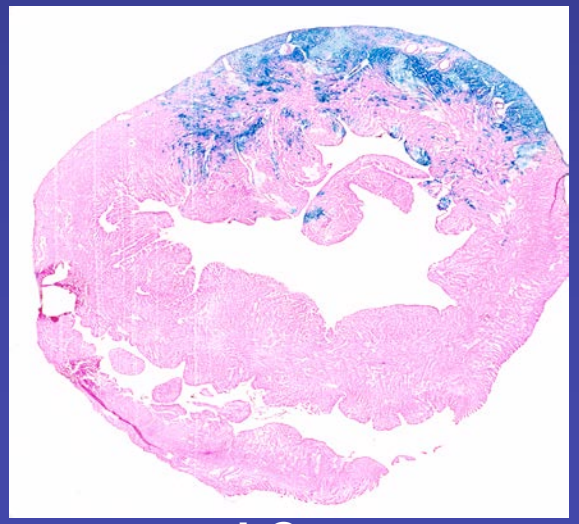
Sub-selective coronary artery catheterization

Shah et al. Circulation 101:408-414, 2000.





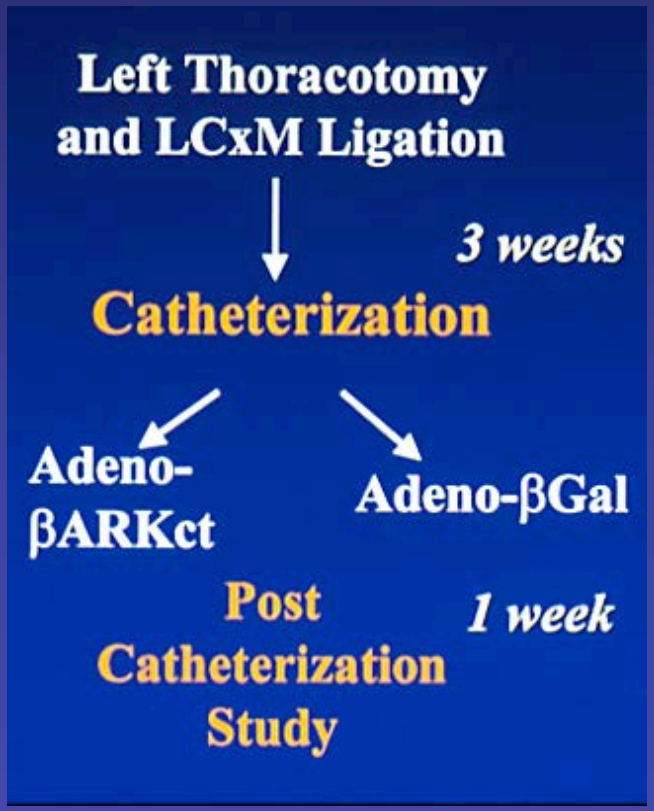
RCA

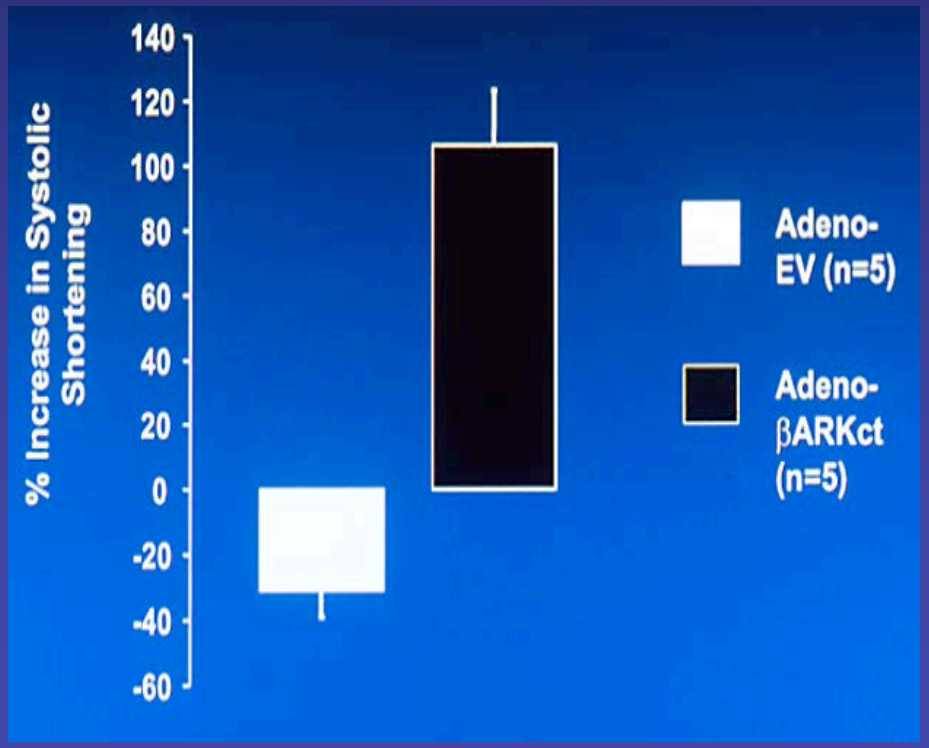


LCx

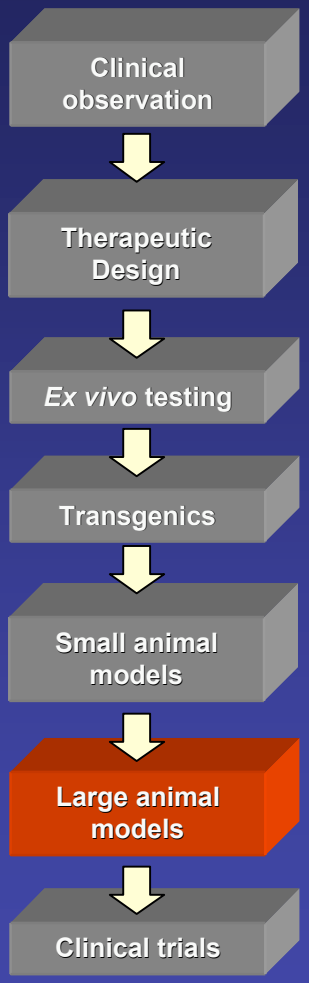
Sub-selective catheter-mediated delivery of βARKct: Chronic HF model

Shah et al. *Circulation* 2001;103:1311-1316



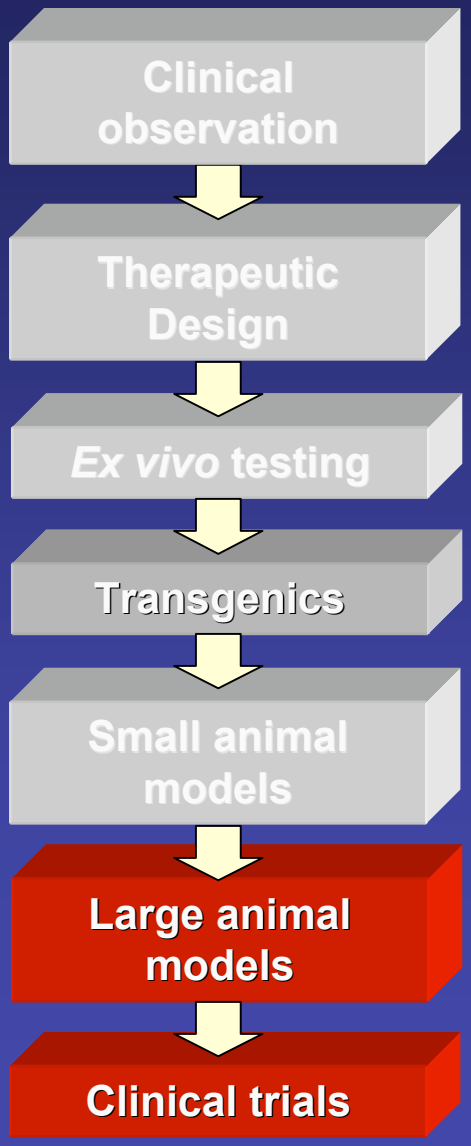


Large Animal Gene Therapy



- Cardiopulmonary bypass with RA cannulation
- Cardioplegic arrest (30 min) → gene delivery
- Global transgene expression at 7 days

Translational Research



Final Proof of Concept for the βARKct

Will βARKct be beneficial in failing human myocytes ?

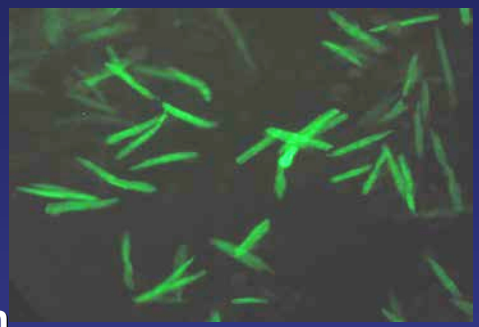
Heart arrested with 1L cardioplegia and explanted.

Coronary artery (LAD or graft) cannulated and perfused with collagenase.

Myocytes incubated on Matrigel®-coated plates.

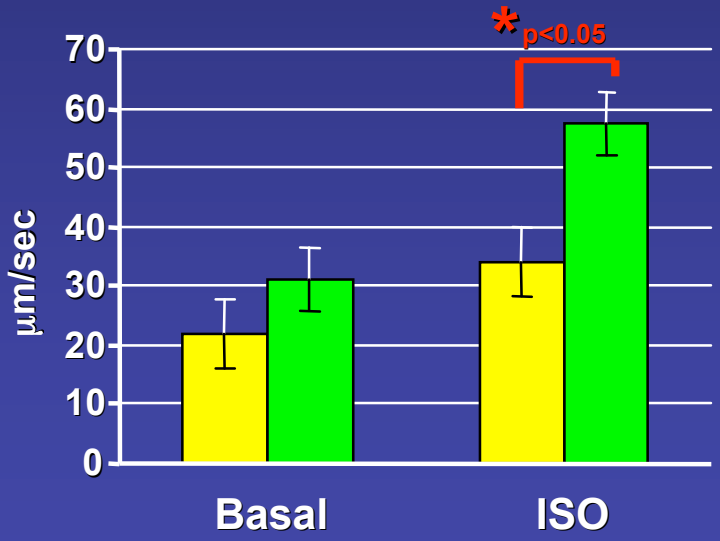
Treated with Adenovirus and single cell contraction measured as well as βAR signaling.

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

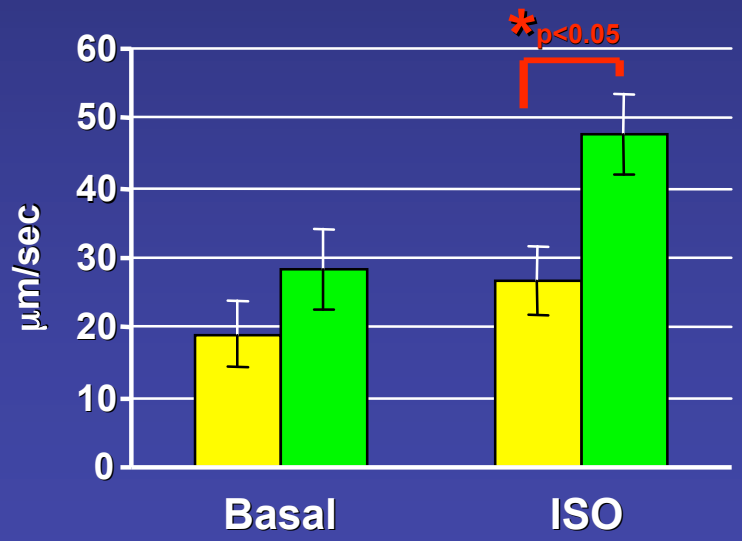


Human myocytes infected with Adeno-GFP/BARKct

dL/dT contraction



dL/dT relaxation



Failing (n=5 patients; 10 cells/condition)

Failing+BARKct (n=5 patients; 10 cells/condition)

Williams et al, Circulation 2004.

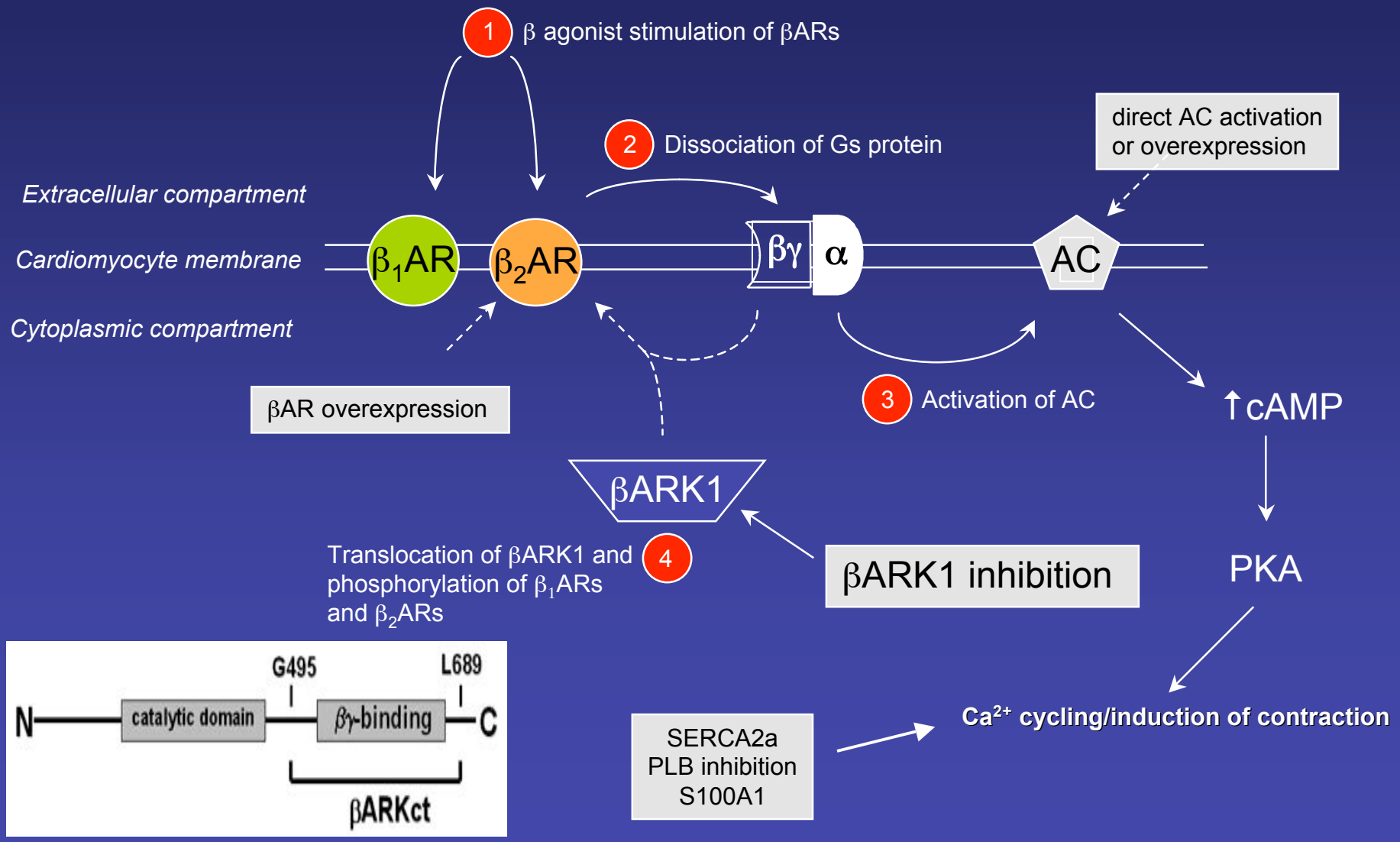
Conclusions

Inhibition of GRK2 (βARK1) represents a potential new drug class, targeting βAR and other GPCR systems from "the inside out"

Molecular Normalization or "Molecular Remodeling" of the βAR System via GRK Inhibition is Beneficial in Heart Failure and a Novel Therapeutic Strategy. Synergistic with current HF therapy with βAR antagonists.

Gene Therapy with βARKct will be first but also a definite need for small molecule.

Potential Targets for Heart Failure Gene Therapy



Hurdles to Human Application

- Target validation present for βARKct as well as other targets (S100A1, SERCA, Adenylyl Cyclase)
- Choice of vector
  - Advanced Adenovirus vs. AAV (or Lentivirus?)
- Route of vector administration
  - Invasive vs. non-invasive (CPB, coronary cath. or intra-ventricular)
- Choice of patient population
  - End-stage, -/+ LVAD ? Or Class III/V, post CPB dysfunction

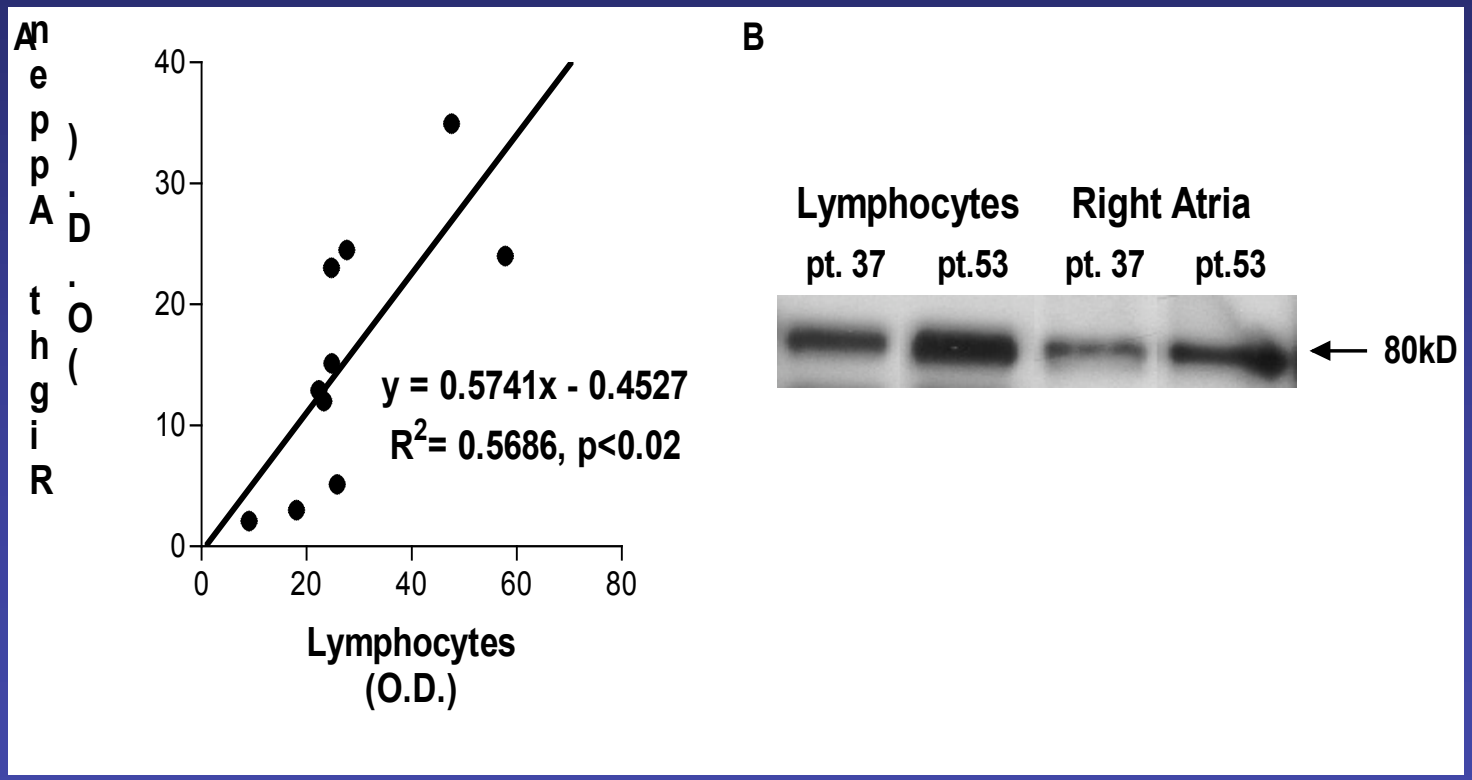
GRK2 as a Novel Biomarker for Heart Failure

Another Translational Approach – Clinical Research

Can GRK2 be a Biomarker for Human HF?

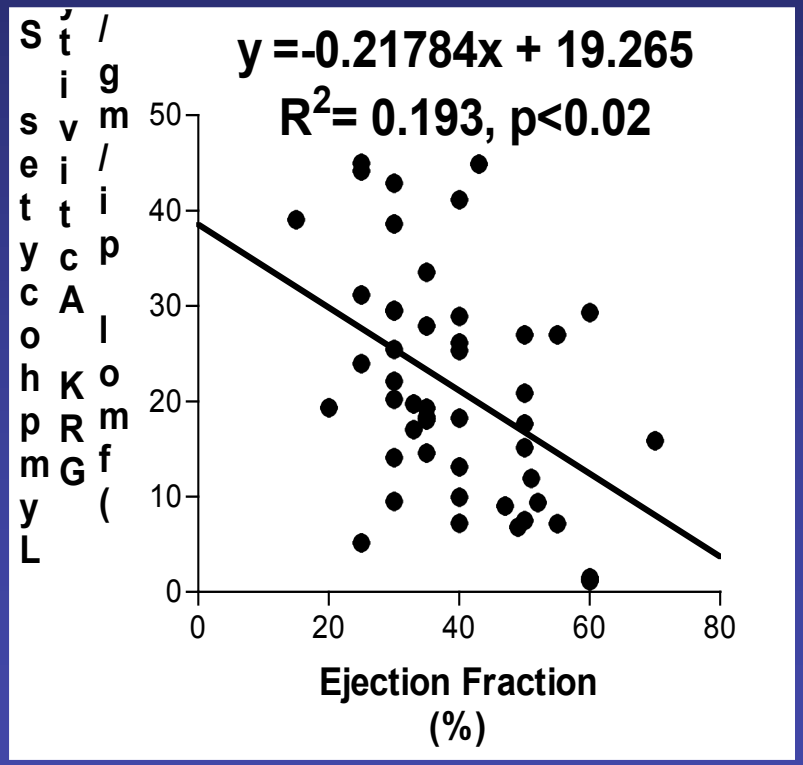
- A biomarker for heart failure is much needed
- More therapeutic tools are needed for the treatment of this condition
- Evidence available in animals indicating this molecule as a key player in experimental HF where its levels are regulated by the activation of the sympathetic nervous system
- To be exported in human settings we need confirmation that in HHF
  1) GRK2 is pathophysiologically relevant
  2) is dysregulated during the disease
  3) is GRK2 important for prognosis in HHF
  4) its reduction can be beneficial
- To answer these and more questions we need a way to monitor cardiac GRK2 repeatedly over the time

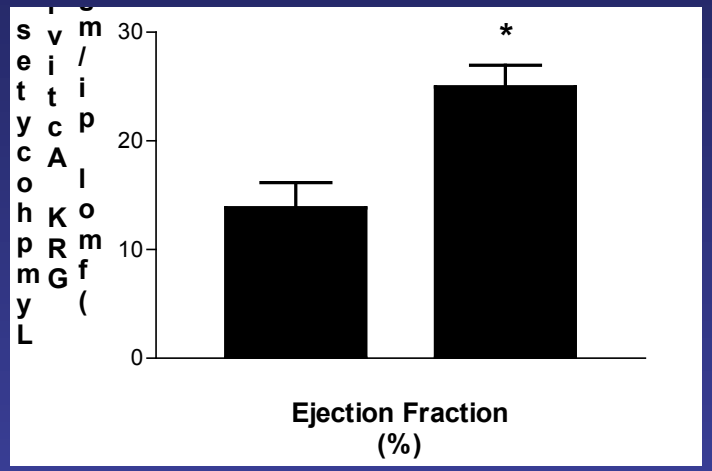
Cardiac GRK2 Tracks with Levels Found in White Blood Cells

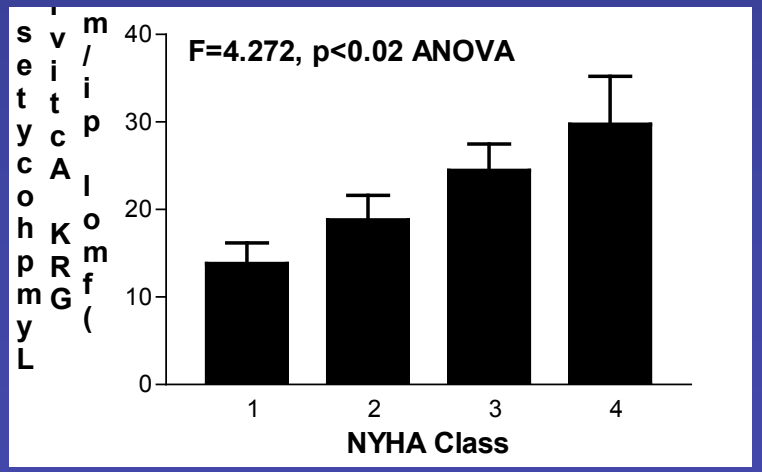


Iaccarino et al., Eur Heart J, 2005

Lymphocyte GRK2 Negatively Associated with Cardiac Function







If High GRK2 is Associated with Worsened Function - is GRK2 Lowered With Treatment ?

Is GRK2 Involved in Reverse Remodeling Associated with LVAD Treatment ?

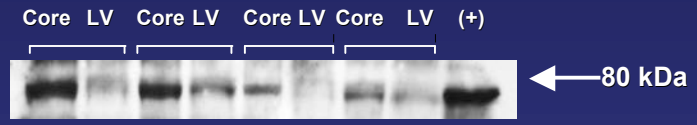
GRKs and LVAD Support in Human Heart Failure

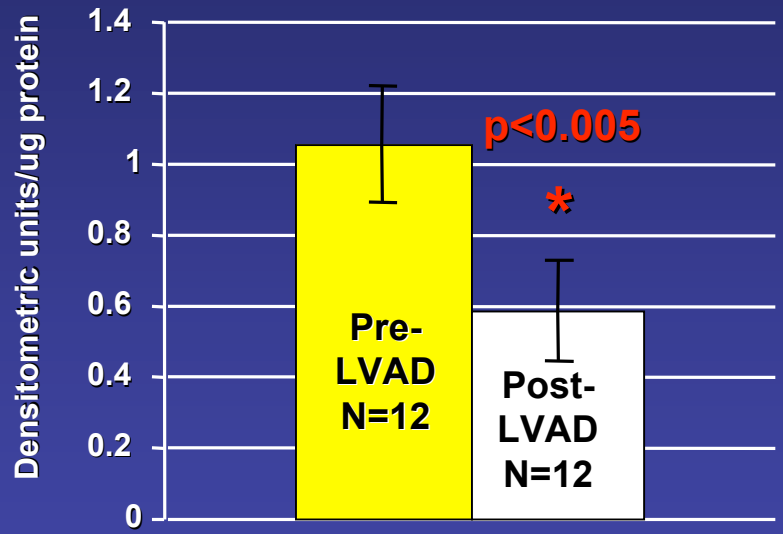
- ~3000 cardiac transplants performed per year, LVADs are commonly used as a “bridge to cardiac transplant”.
- LV unloading by LVAD support leads toward normalization of myocardial structure and function (“reverse remodeling”) including restoration of βAR responsiveness.
- Long-term LVAD support leads to enhanced survival in patients not eligible for transplant compared to optimized medical treatment (REMATCH Trial, Rose, et al, *NEJM*, 2001) …however, 1-year mortality ~50%.

- **Will LVAD support in HF induce significant changes in myocardial GRK2 expression and GRK activity to support improved βAR responsiveness as a positive component to reverse remodeling ?**

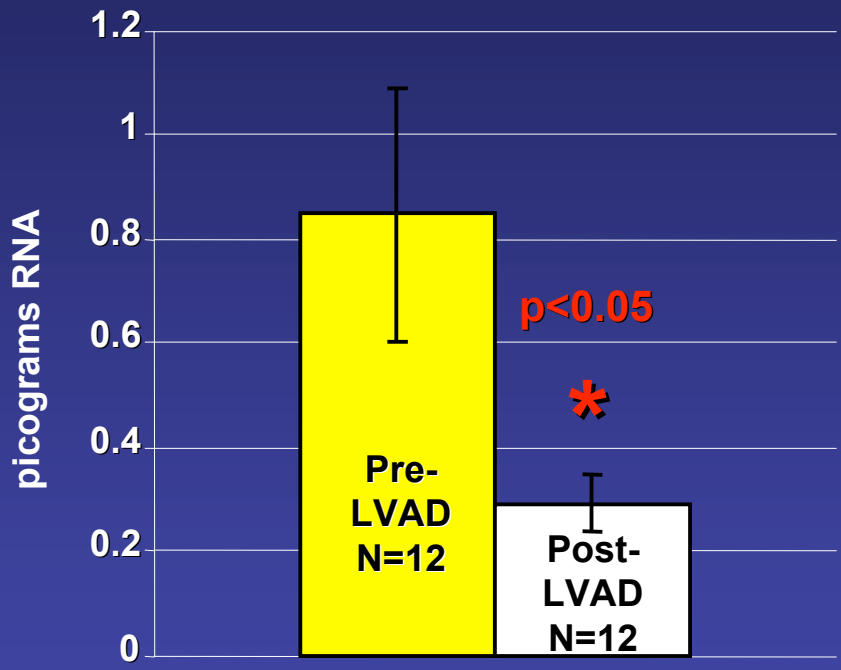
GRK2 Levels After Mechanical Unloading in the Failing Human Heart

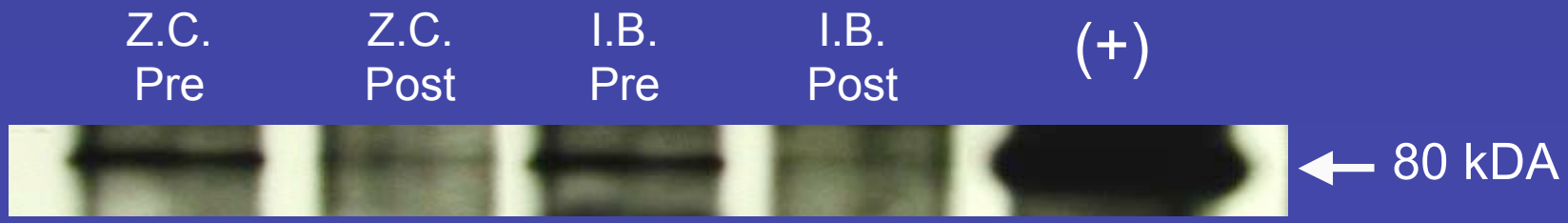
Myocardial βARK1 protein



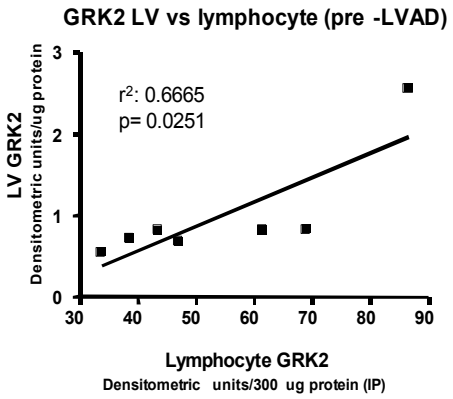


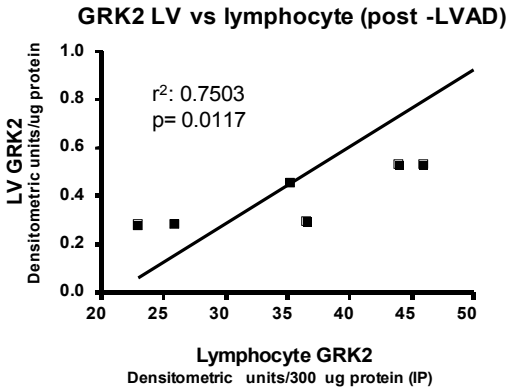
Myocardial βARK1 mRNA





Cardiac (LV) GRK2 Tracks with Levels Found in White Blood Cells





Hata et al., JCF, 2006

Summarizing GRK2 (βARK1) and GRK Activity as a Novel Biomarker in HF

- Alterations in GRK2 expression and GRK activity seen in failing myocardium mirrored in lymphocytes and appears to be associated with severity of disease and decreased GRK2 associated with cardiac functional improvement.
- Potential for GRK2 levels and GRK activity in lymphocytes to be used as a biomarker in HF (surrogate marker for response to therapy currently being tested).

Active Clinical Studies at Jefferson

Does Lymphocyte GRK2 Represent a Novel Biomarker for HF

Measurement of lymphocyte GRK2 in acutely decompensated (hospitalized) HF patients anc comparison to BNP for acute volume reduction.

Measurement of lymphocyte GRK2 and BNP in newly diagnosed HF patients. Temporal assessment after β-blocker therapy. Correlation with treatment response.

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