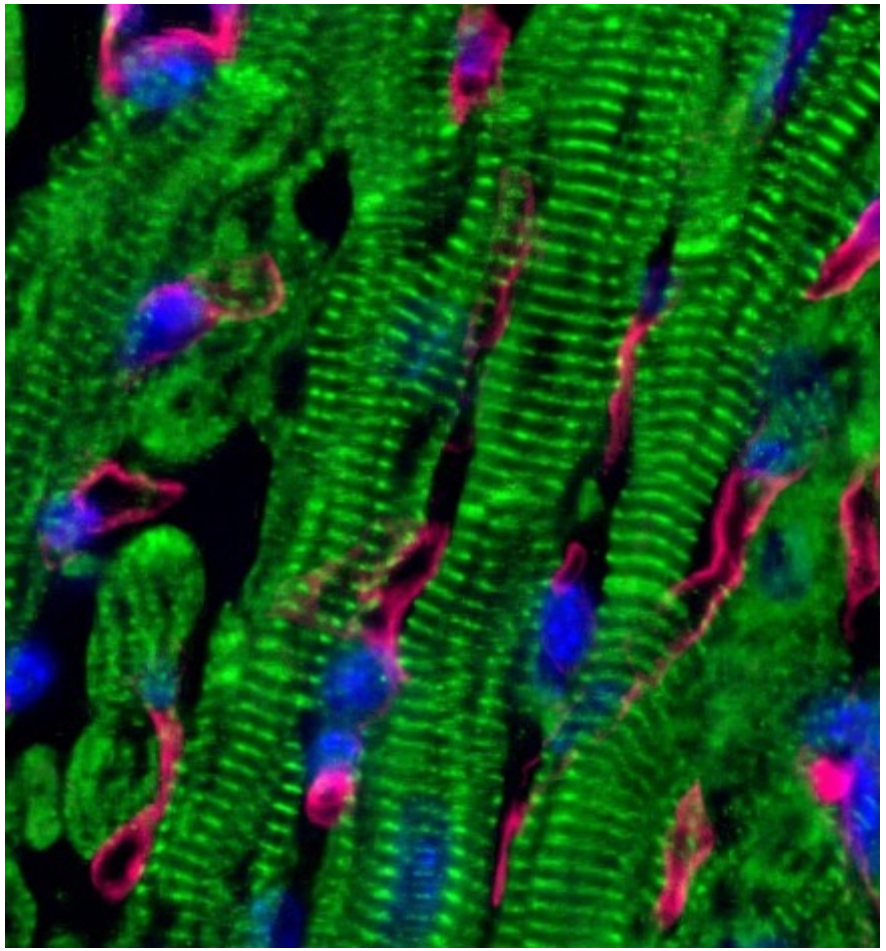
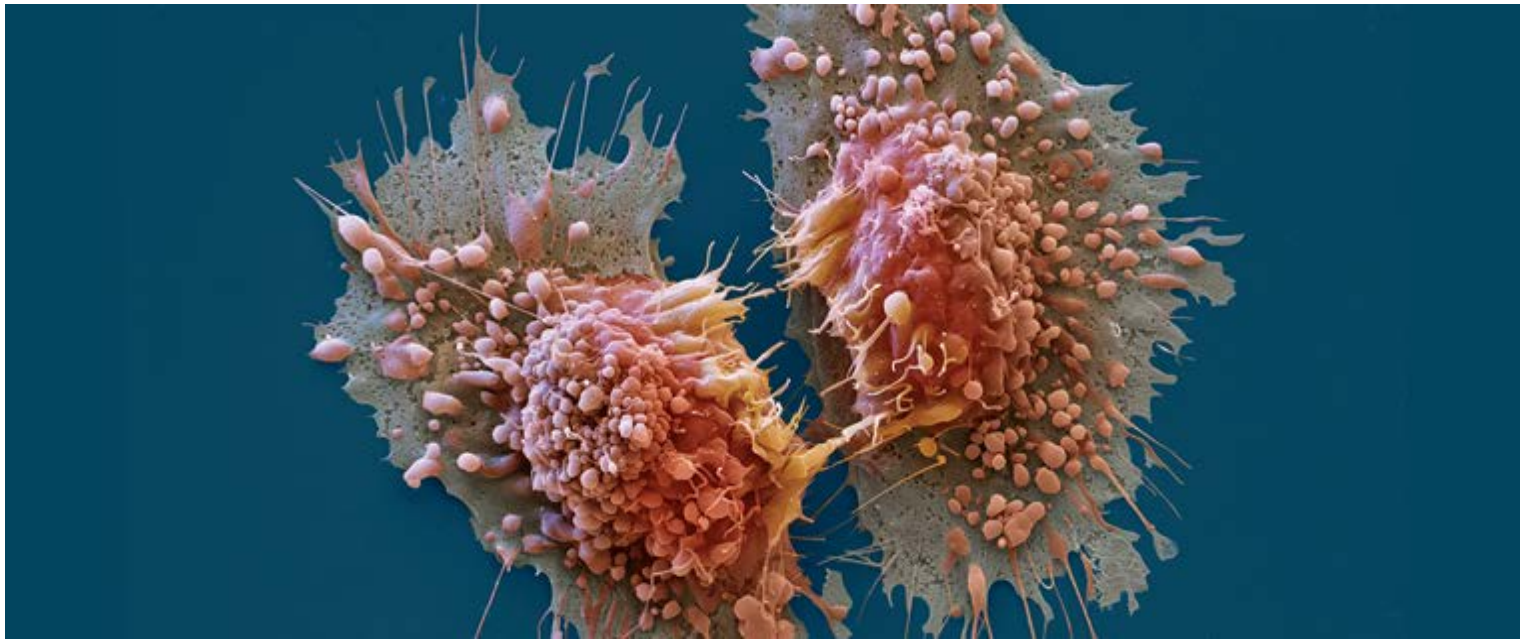




Myocardial biology of kinase inhibitor cardiotoxicity: Predictable on-target and surprising off-target effects

Brian Jensen MD

Associate Professor of Medicine and Pharmacology

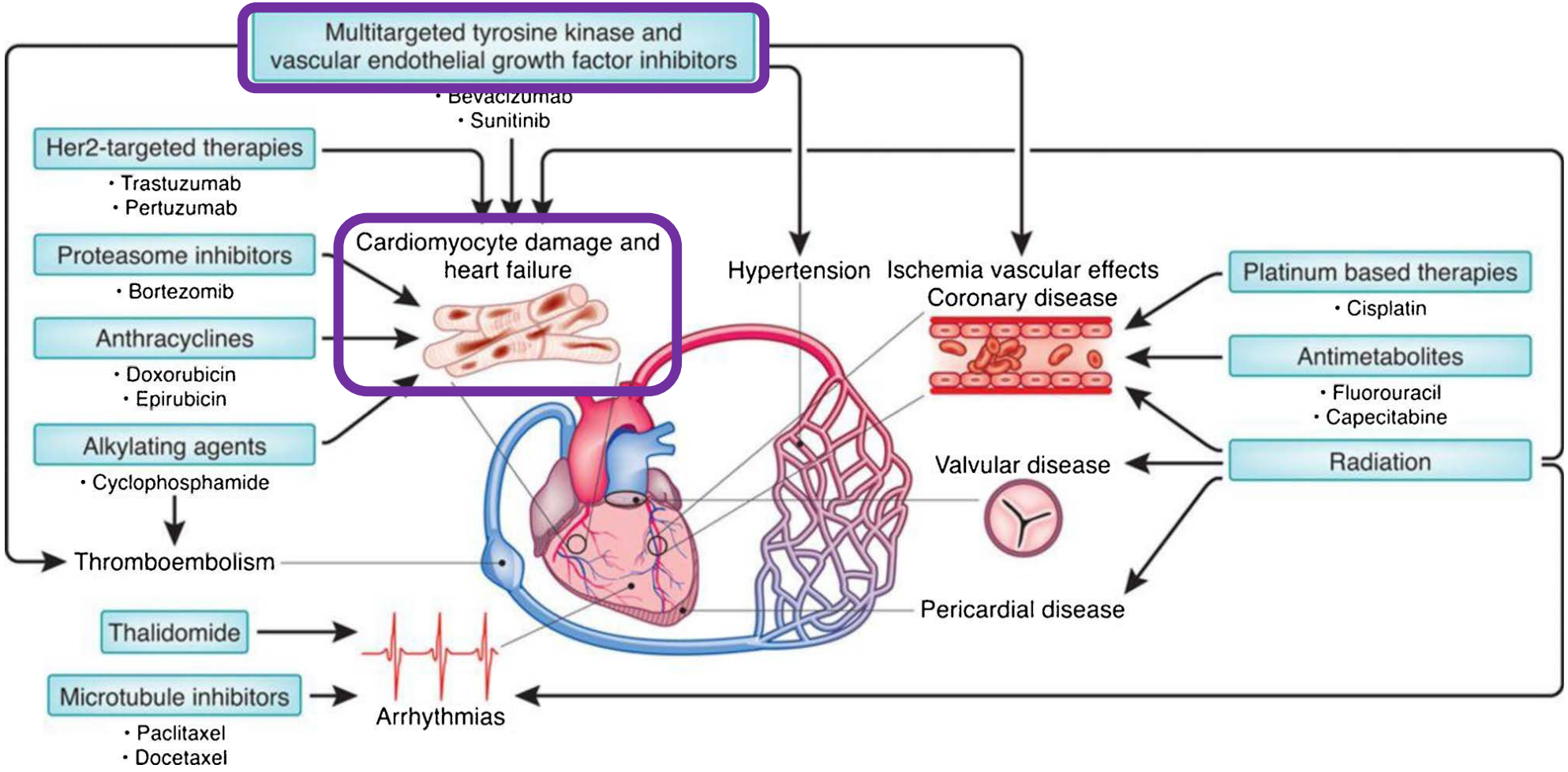
UNC School of Medicine

Conclusions

- Heart failure is a relatively rare but important adverse effect of some targeted cancer therapies
- Current preclinical testing strategies do not adequately predict cardiomyocyte injury
- Though uninjured cardiomyocytes are very different from cancer cells, there is overlap between the biology of a failing cardiomyocyte and a cancer cell.
- Cardiomyocytes are metabolically vulnerable. Expanded preclinical testing for kinase inhibitor cardiotoxicity might include assays of metabolism and mitochondrial function.

Cardiotoxicity of kinase inhibitors

…the most common class of novel targeted cancer therapies

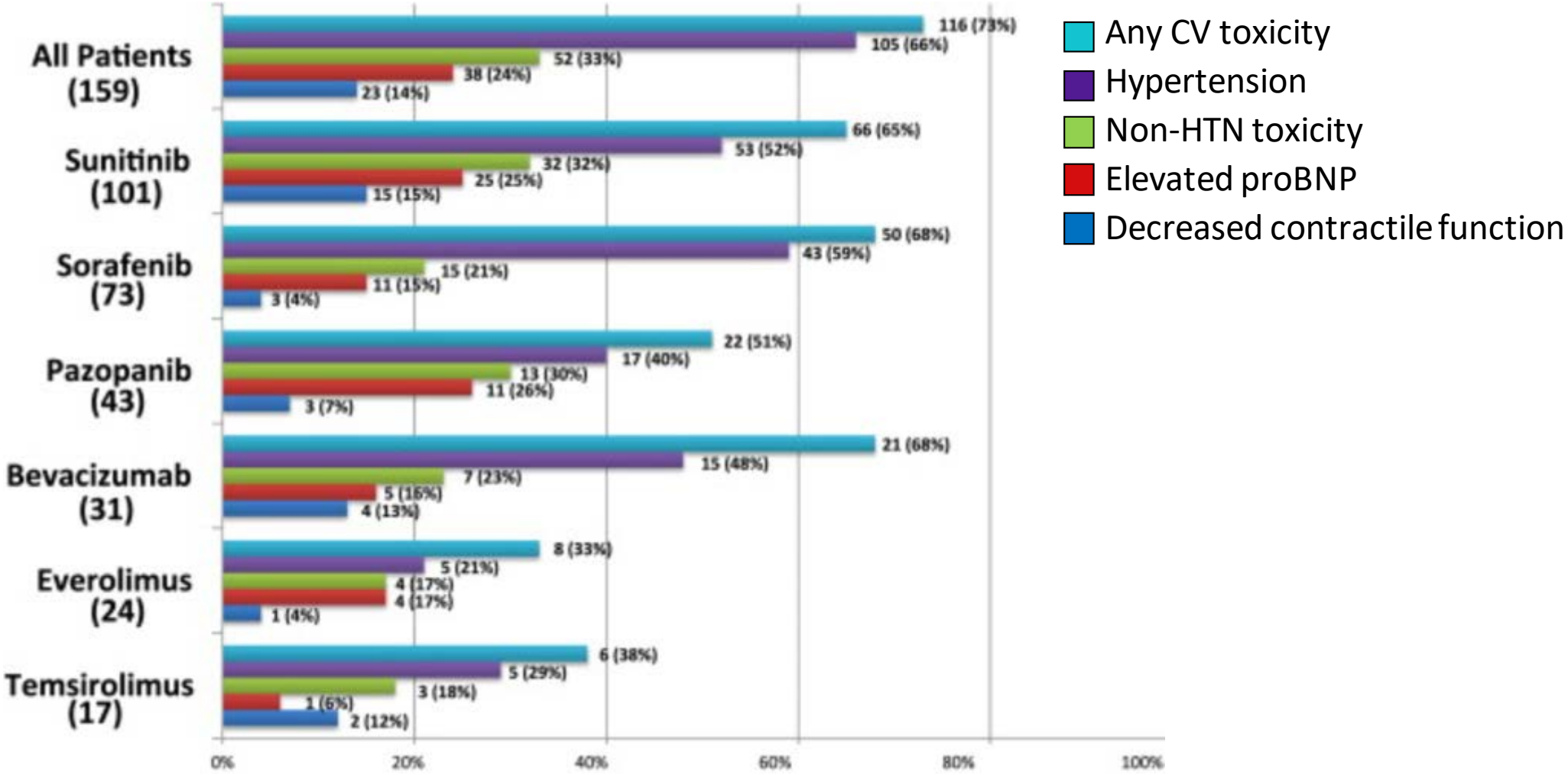


Kinase inhibitors generally do not kill cardiomyocytes, so how do they lead to heart failure?

Babiker HM. Critical Reviews in Oncology / Hematology 126 (2018) 186–200

Toxicity from targeted therapies: scope of the problem

Kinase Inhibitors in the treatment of Renal Cell CA (and others)

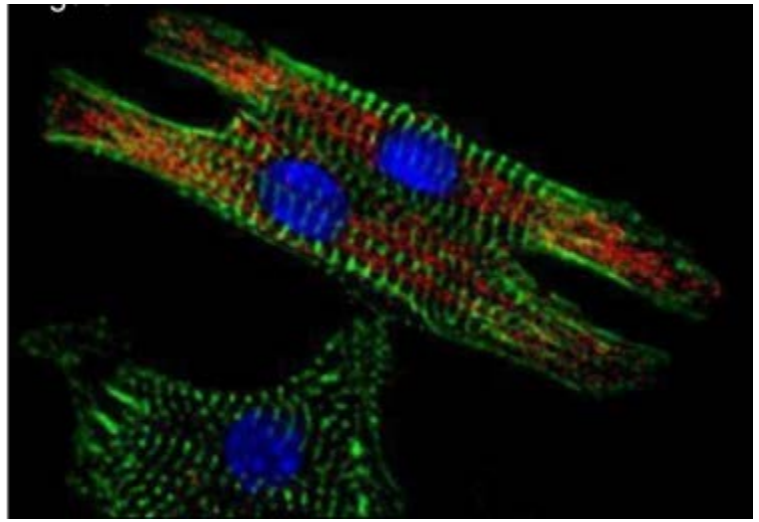


Hypertension is most common, but cardiomyopathy/heart failure occurs in 4-15%

JCHF. 2013;1(1):72-78

Contrasting cardiomyocytes and cancer

Cardiomyocytes

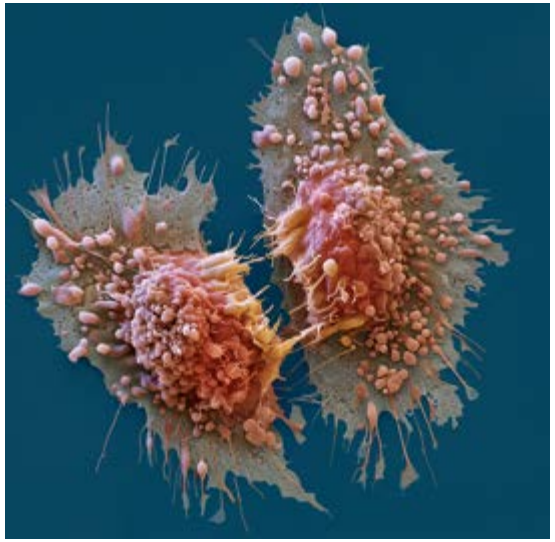


Terminally differentiated

Very limited regeneration

Energy derived from fatty acids

Cancer cells



Undifferentiated

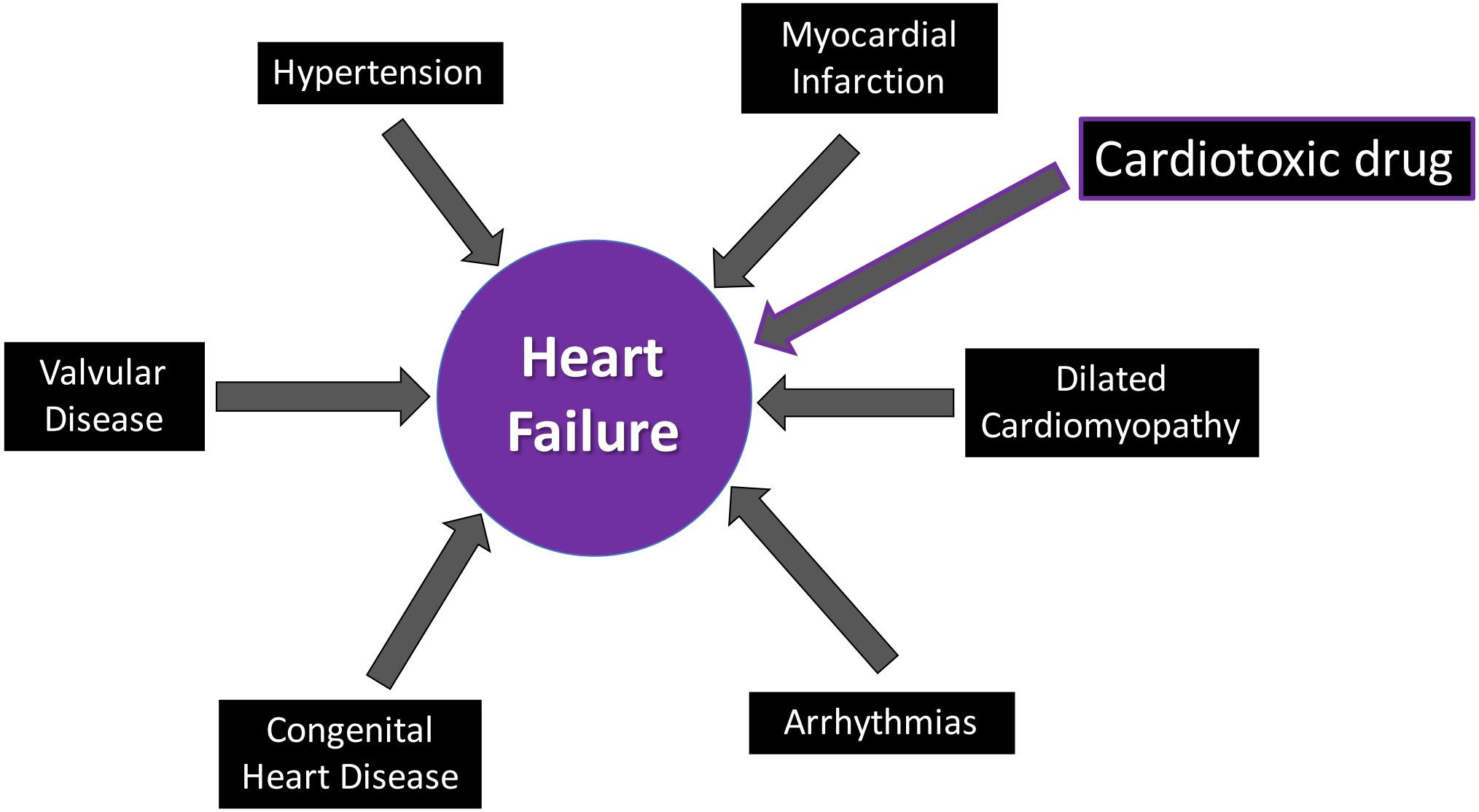
Nearly limitless replication

Energy derived from glucose and glutamine

The differences between cardiomyocytes and cancer cells suggest the possibility that we could develop truly targeted and “cardiosafe” cancer drugs.

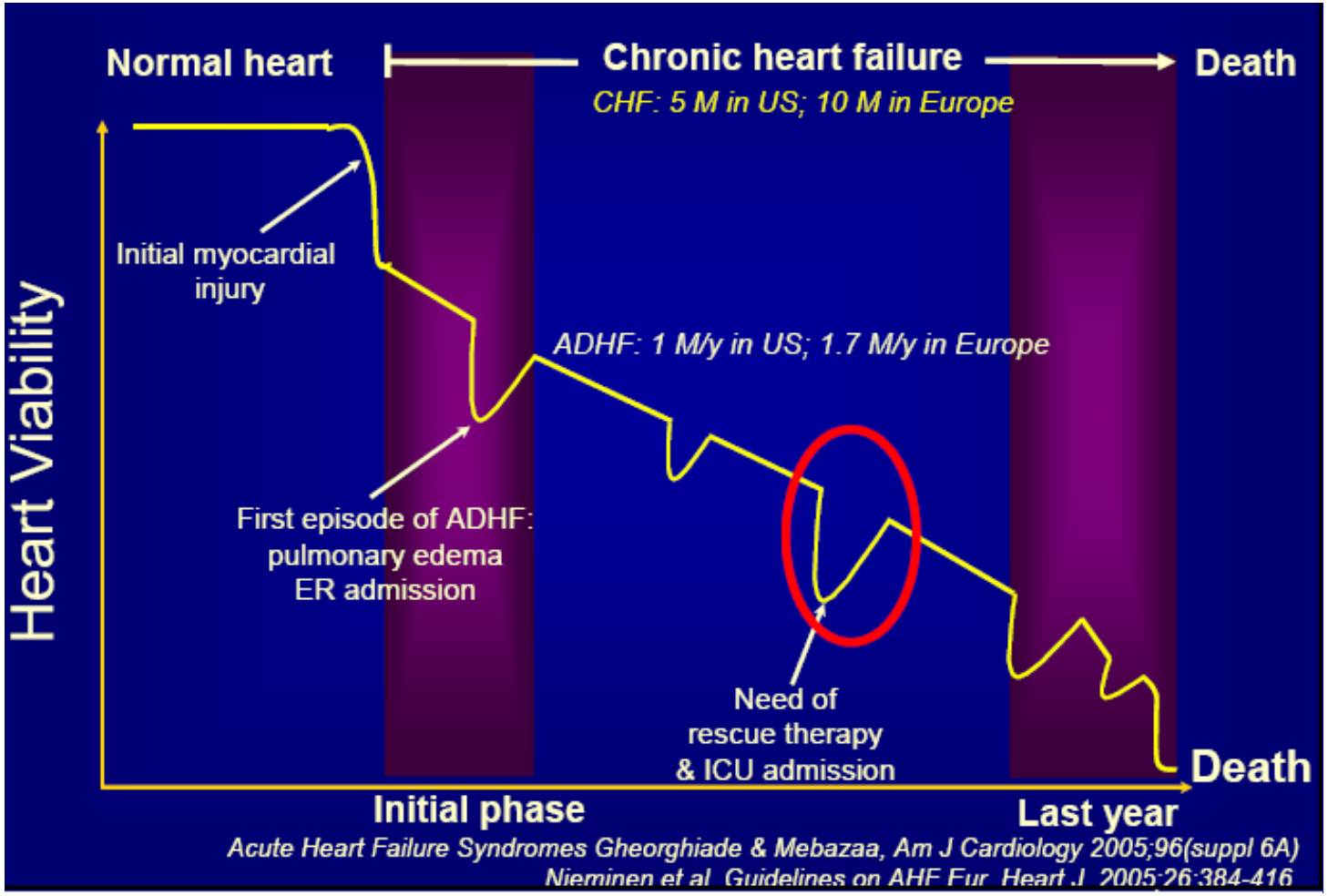
Causes of Heart Failure

Heart Failure as a final common pathway



Heart failure is progressive and has a poor prognosis

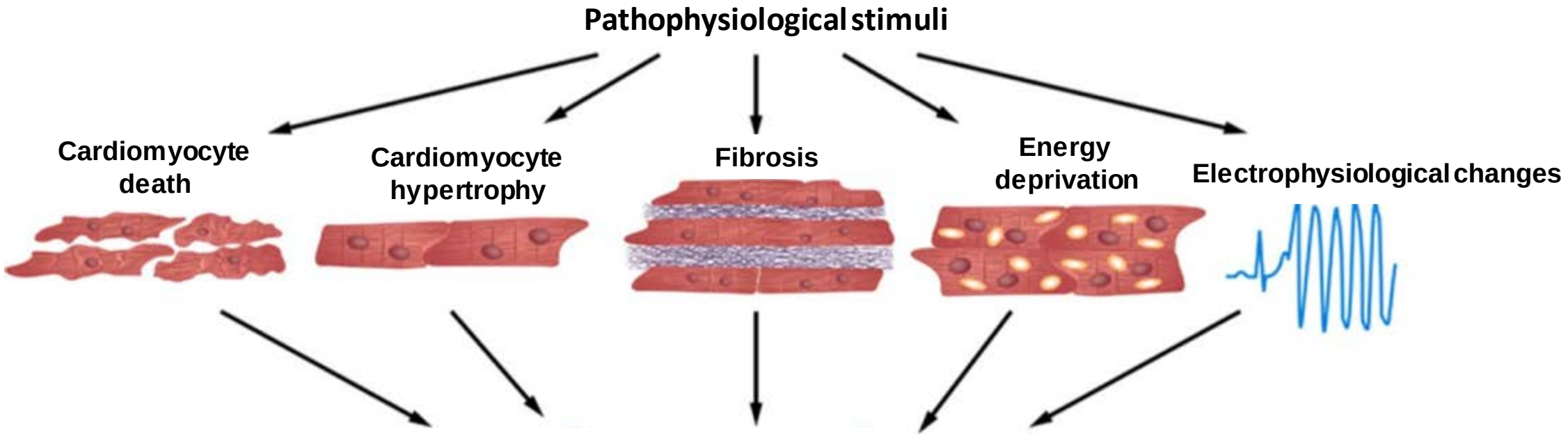
…trading one bad disease for another…

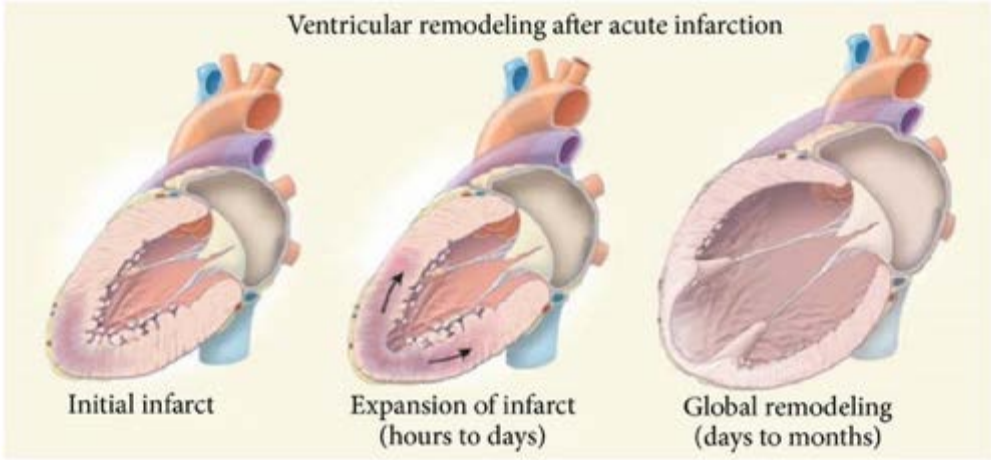


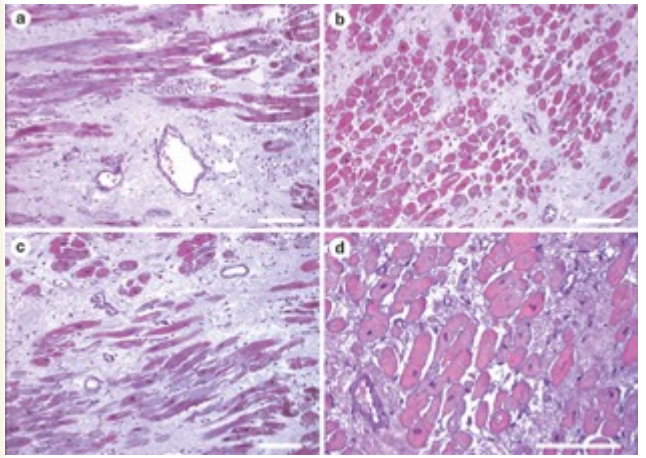
Mean survival after diagnosis of heart failure is ~5 years
Mean survival after first hospitalization for heart failure = ~2.5 years

Mechanisms of remodeling

The multifactorial pathobiology of heart failure

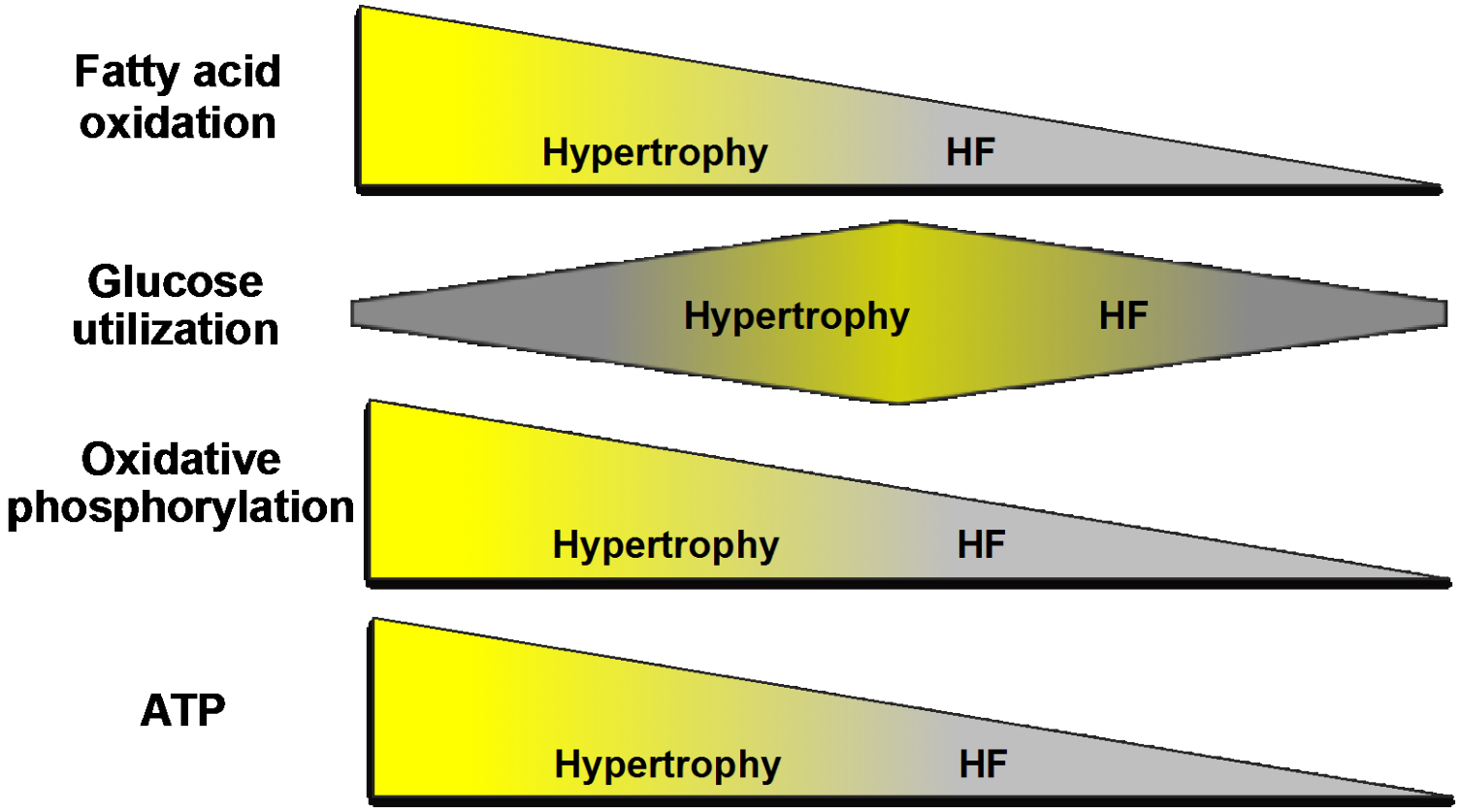


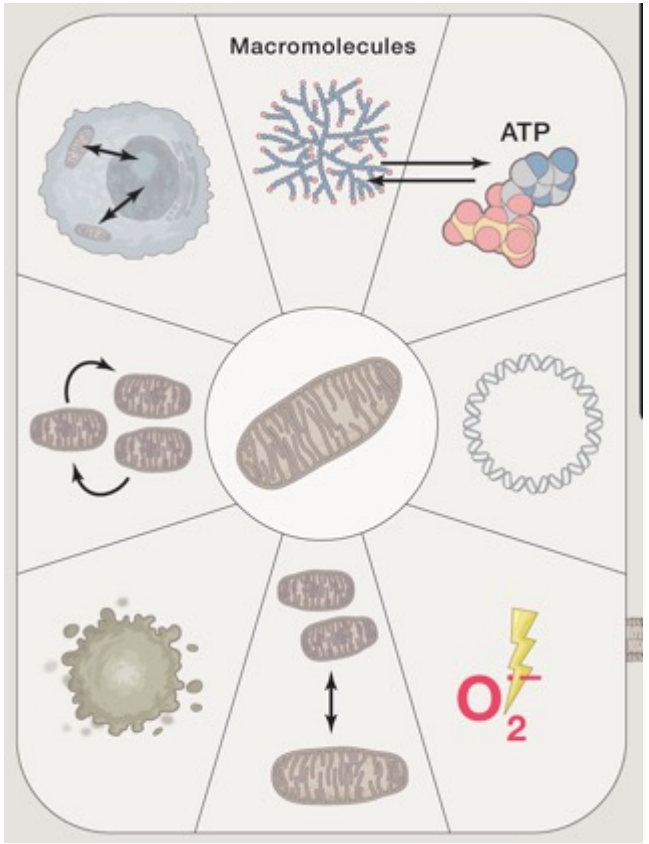




Energy deprivation is central to HF pathobiology

Substrate switching and mitochondrial dysfunction





Cell 2016 166, 555-566DOI: (10.1016/j.cell.2016.07.002)

N.B. The heart has the highest ATP requirement of any organ

Could drug-induced energy deprivation cause heart failure?

Neubauer, *NEJM* 2007

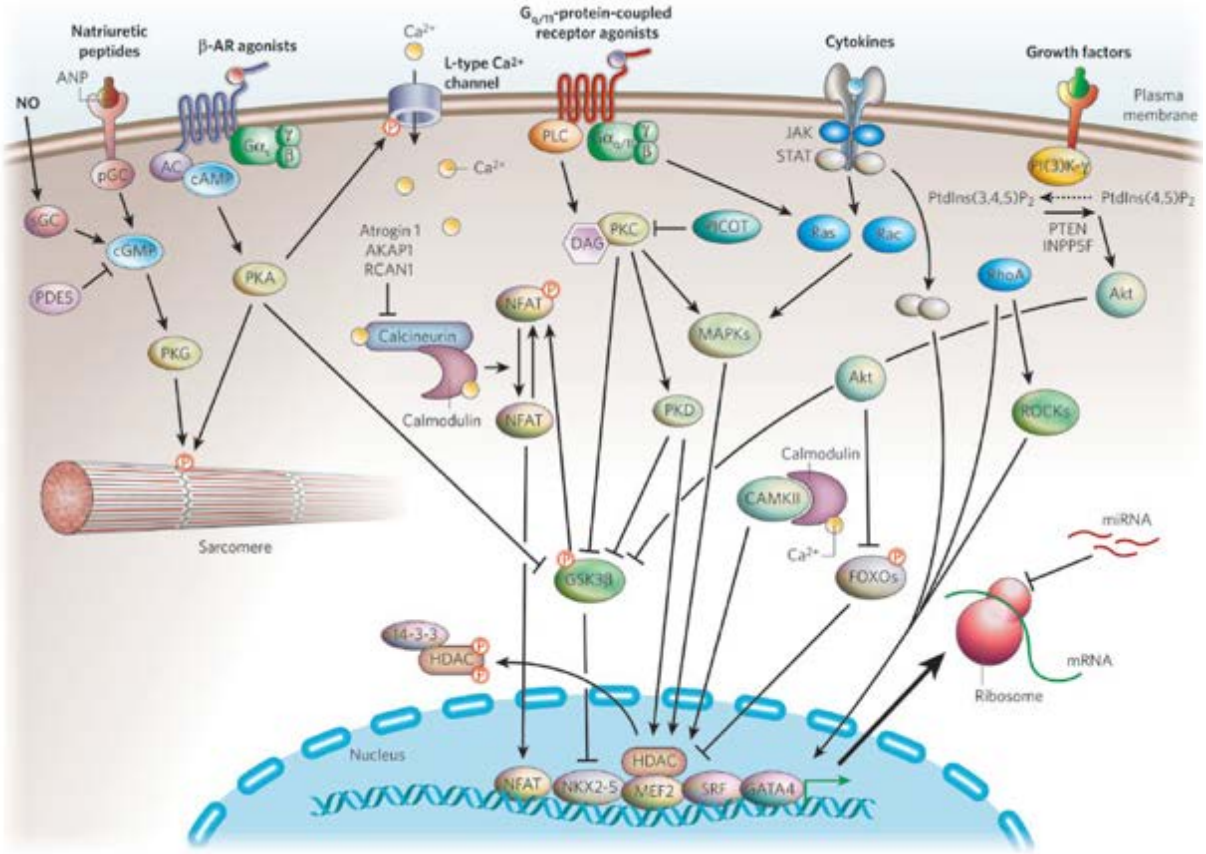
Heart failure vs. cancer…

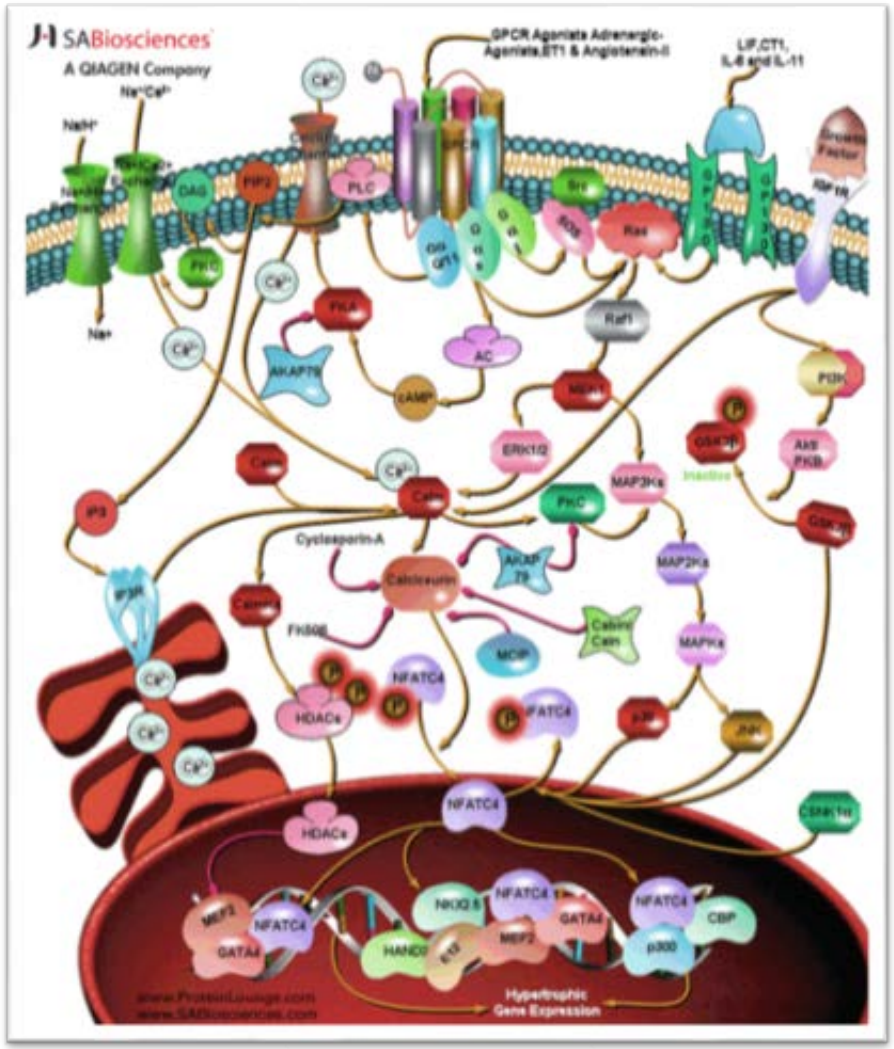
Compare and contrast

Heart failure		Cancer
Cellular hypertrophy	✗	Cellular hyperplasia
Vascular rarefaction	✗	Angiogenesis
Enhanced glucose metabolism Impaired oxidative phosphorylation	✓	Enhanced glucose metabolism Impaired oxidative phosphorylation Warburg effect: aerobic glycolysis
Inflammation	✓	Inflammation
Oxidative stress	✓	Oxidative stress

Signaling in the failing heart

Complex…like cancer





Some oncogenic pathways are also cardioprotective

Targeted cancer therapies

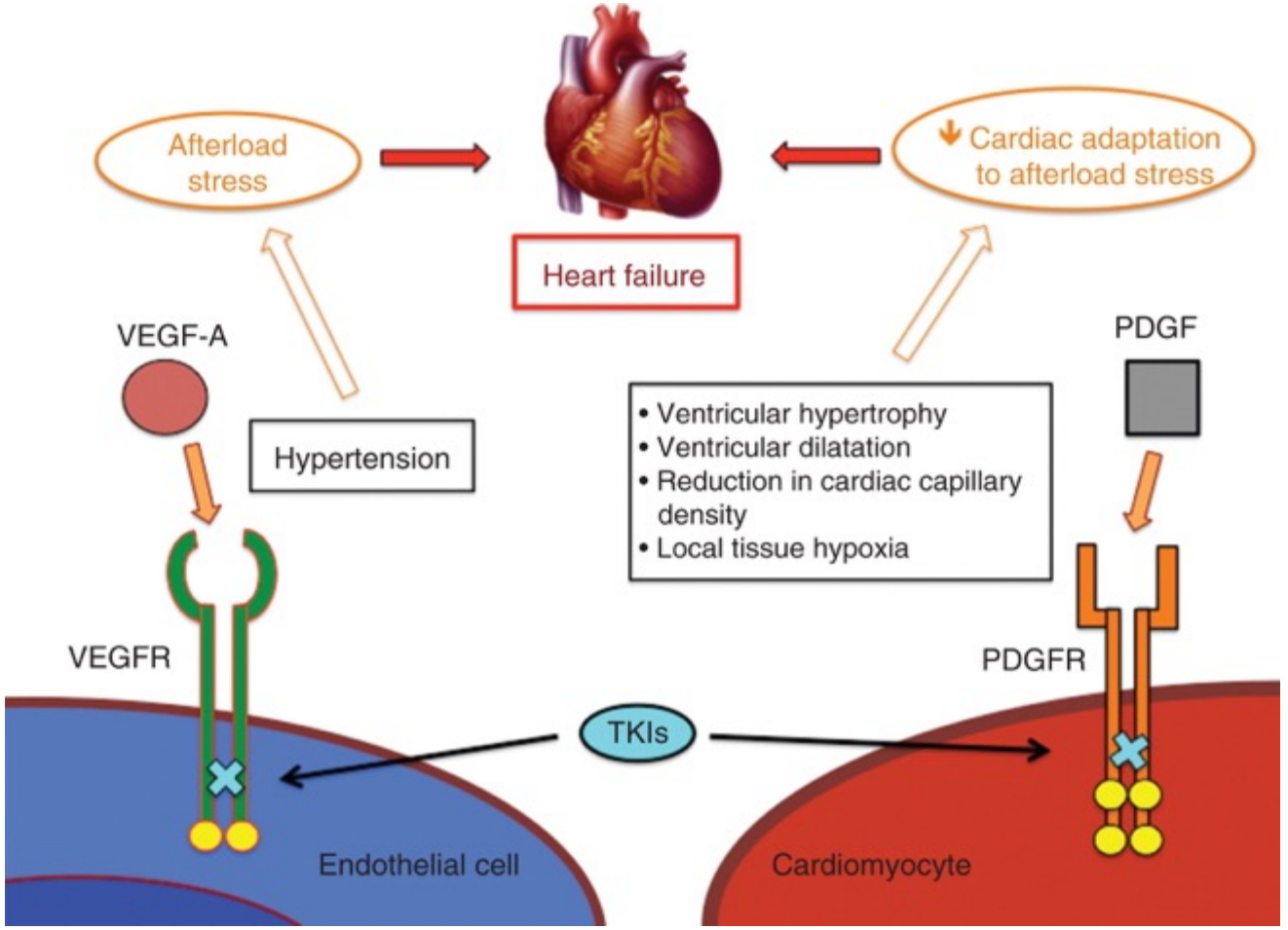
VEGF signaling pathway: on-target CV toxicity (?)

Tumors require angiogenesis to proliferate. Targeted therapies block angiogenesis by blocking the effects of VEGF, which decreases NO bioavailability. Hypertension is a frequent response, due to the importance of NO to endothelial function.

Moslehi JJ N Engl J Med 2016;375:1457-67

Multiple mechanisms of KI cardiotoxicity

Direct myocardial effects and indirect effects from vasculature (?)



Multitargeted kinase inhibitors (e.g. sunitinib and sorafenib) target both PDGFR and VEGFR
PDGFRs and VEGFRs both are protective in cardiomyocytes

Expert Opinion on Drug Safety **2015,** 14, 253-267

Can we predict cardiotoxicity of targeted therapy?

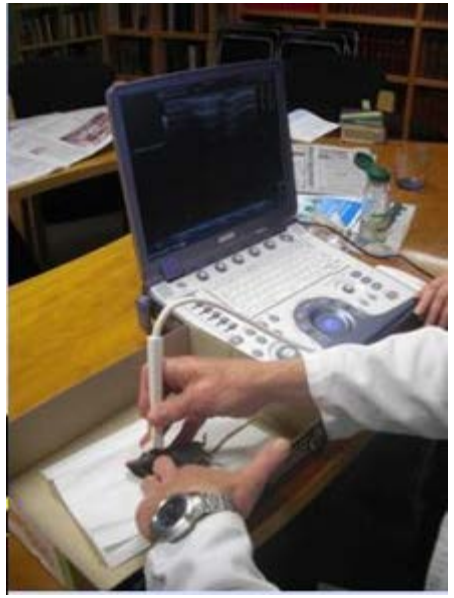
…not very well

Target	Cardioprotective?	Drug example	Heart failure?
HER2 (ErbB2)	Yes	Herceptin	Yes
MEK-ERK	Yes	Trametinib	Yes
PDGFR	Yes	Sunitinib	Yes
EGFR	Yes	Erlotinib	No
PI3 Kinase/Akt	Yes	Idelalisib	No
VEGFR	No	Bevacizumab	Yes
CDK4/6	No	Palbociclib	No *
BTK	No	Ibrutinib	No**
ALK	?	Crizotinib	No***

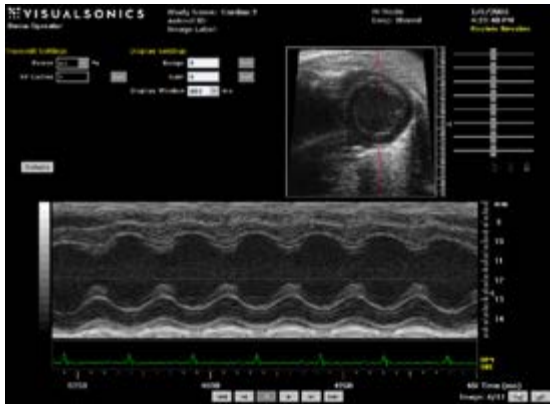
* Ribociclib causes QT prolongation
** Ibrutinib causes arrhythmias
*** Crizotinib causes bradycardia

Do mice accurately model human KI cardiotoxicity?

Echocardiography measures cardiac contractile function

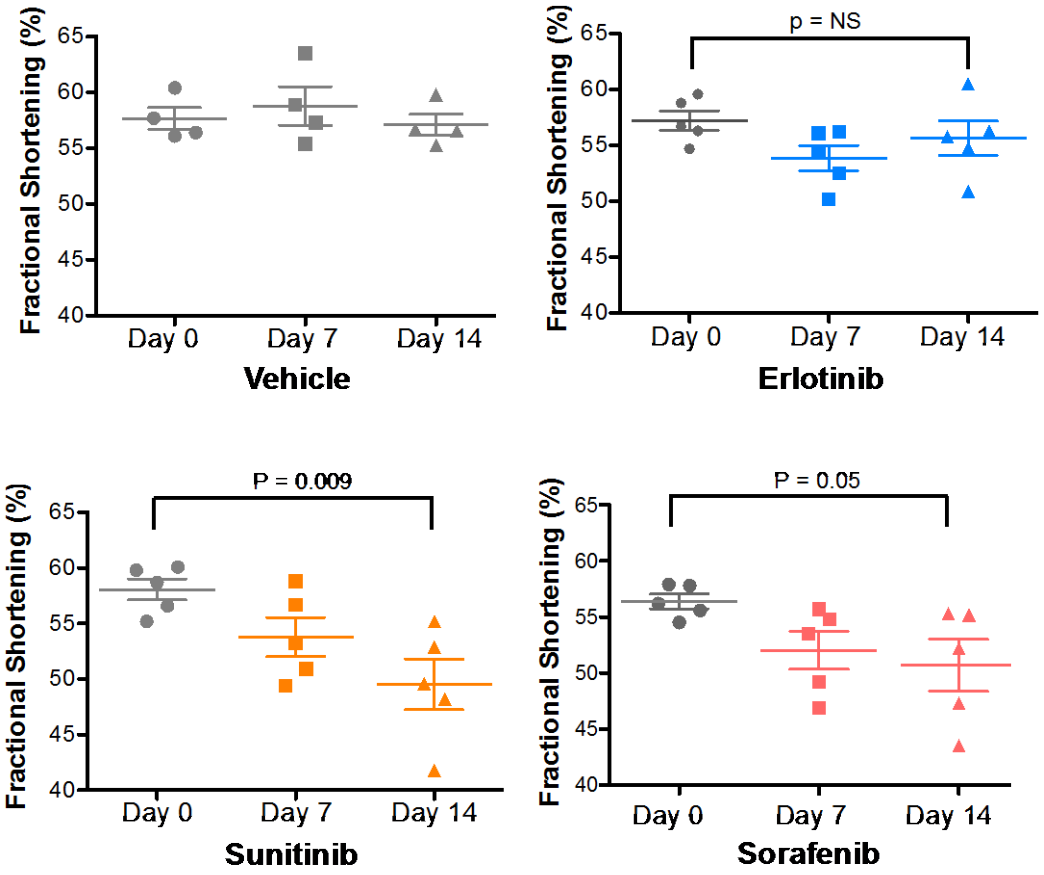


Conscious transthoracic echocardiography



Calculating fractional shortening

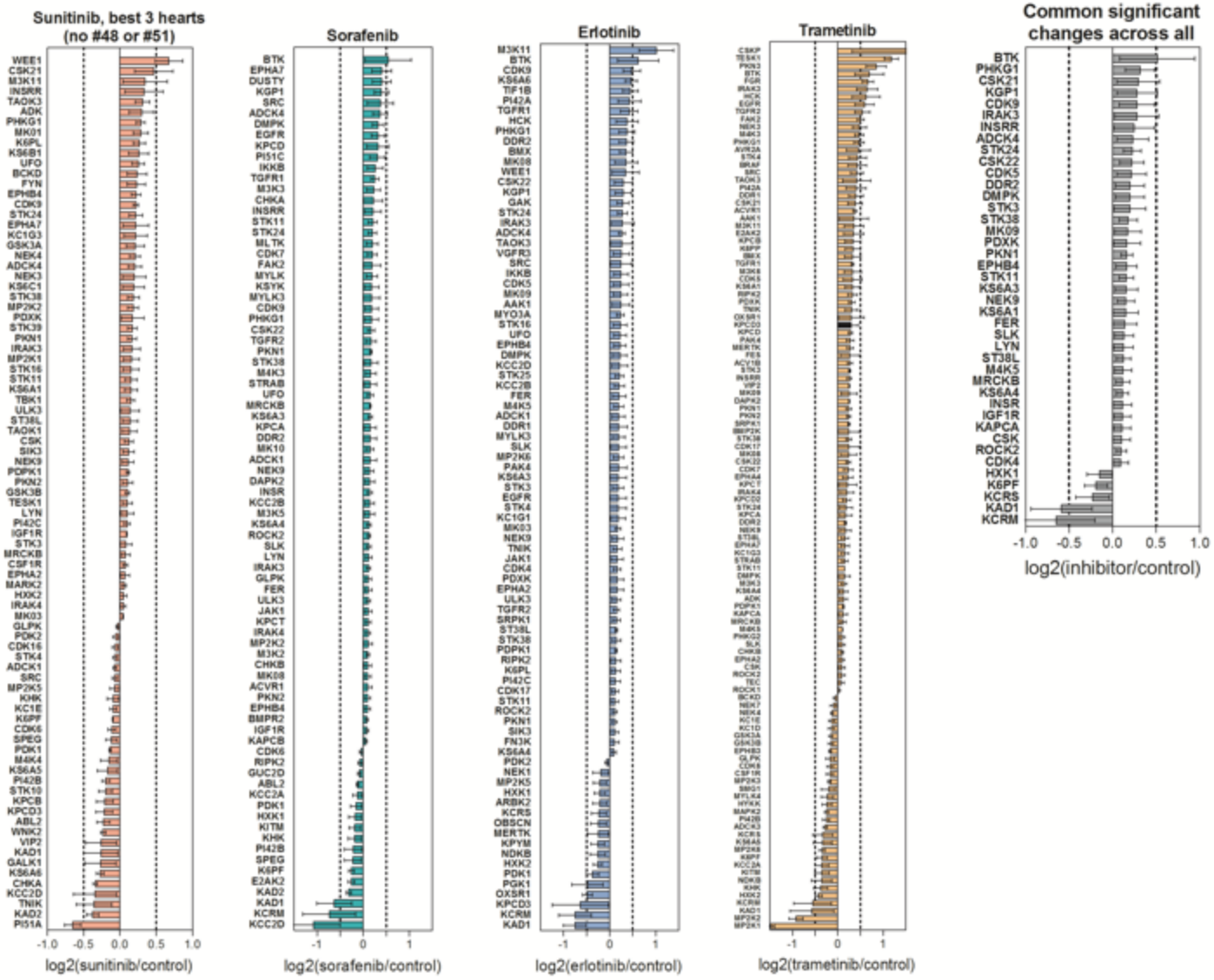
Once daily oral gavage with kinase inhibitors or vehicle
Echocardiogram at Day 7 and 14
Sacrifice at Day 14



Fractional shortening is an index of contractile function

Can we identify the molecular basis of KI cardiotoxicity?

Kinome profiling (MIB/MS)



Dynamic Reprogramming of the Kinome in Response to Targeted MEK Inhibition in Triple-Negative Breast Cancer

Cell

James S. Duncan,[1,8] Martin C. Whittle,[1,8] Kazuhiro Nakamura,[1] Amy N. Abell,[1] Alicia A. Midland,[2] Jon S. Zawistowski,[1] Nancy L. Johnson,[1] Deborah A. Granger,[1] Nicole Vincent Jordan,[1] David B. Darr,[3] Jerry Usary,[3] Pei-Fen Kuan,[4] David M. Smalley,[1] Ben Major,[5] Xiaping He,[3] Katherine A. Hoadley,[3] Bing Zhou,[3,6] Norman E. Sharpless,[3,6] Charles M. Perou,[3] William Y. Kim,[3,6] Shawn M. Gomez,[2] Xin Chen,[7] Jian Jin,[7] Stephen V. Frye,[7] H. Shelton Earp,[1,6] Lee M. Graves,[1] and Gary L. Johnson[1,*]

Multiplexed inhibitor beads (MIBs) bind activated kinases from hearts of mice treated by KIs

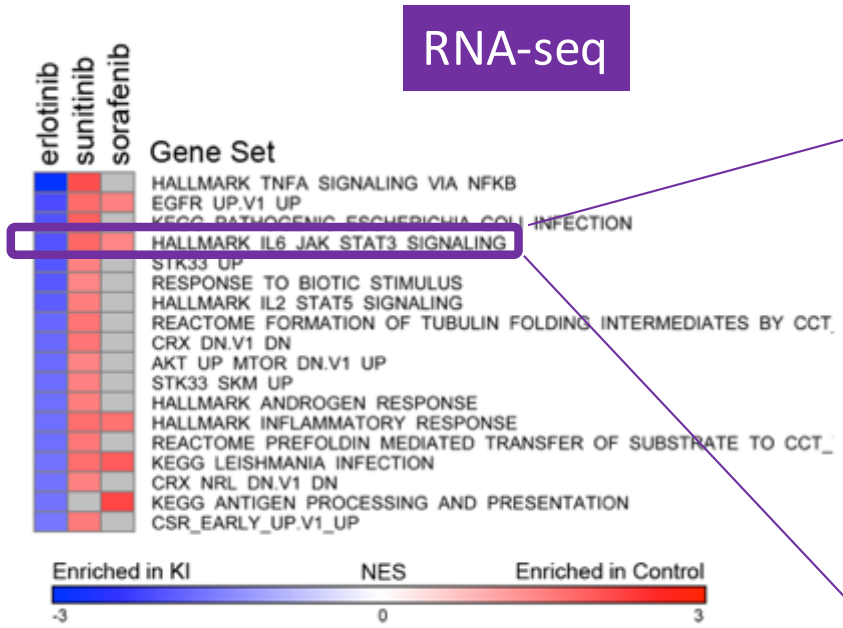
Mass spectrometry quantitates the bound kinases

Treatment	Kinases assayed	Upregulated	Downregulated
Erlotinib	200	70	18
Sunitinib	214	54	30
Sorafenib	215	62	18

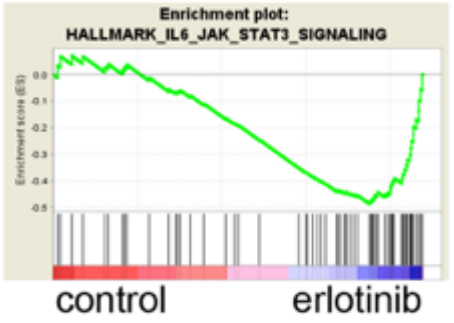
Why isn't erlotinib cardiotoxic?

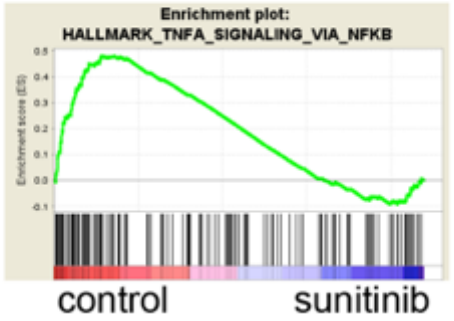
Its target, EGFR, is cardioprotective…

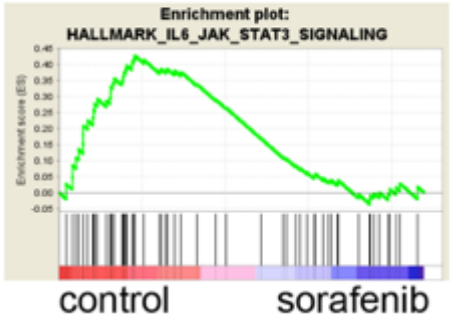
RNA-seq



HALLMARK_IL6_ JAK_STAT3 SIGNALING

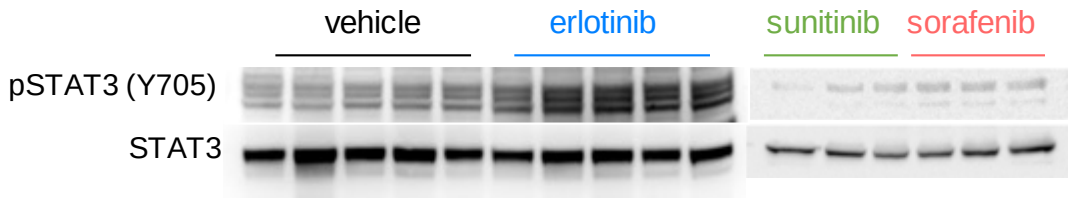


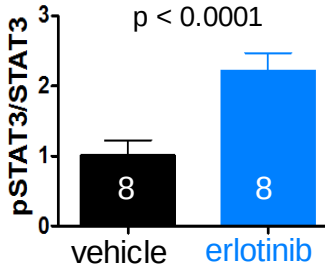




RNA-seq suggested that the JAK-STAT pathway was upregulated by erlotinib, but downregulated by sunitinib and sorafenib.

Western blot





Immunoblotting confirmed activation of STAT3 by erlotinib

STAT3 upregulation facilitates tumor "escape" from EGFR inhibition

Similarities between heart and tumor?

www.impactjournals.com/oncotarget/ Oncotarget, Vol. 7, No. 16

Overcoming resistance of targeted EGFR monotherapy by inhibition of STAT3 escape pathway in soft tissue sarcoma

Xiaochun Wang[1,3], David Goldstein[2], Philip J. Crowe[1,3], Mark Yang[3], Kerryn Garrett[4,5], Nikolajs Zeps[4,5] and Jia-Lin Yang[1,3]

INTERNATIONAL JOURNAL OF ONCOLOGY 46: 2083-2095, 2015

Continuous exposure of non-small cell lung cancer cells with wild-type EGFR to an inhibitor of EGFR tyrosine kinase induces chemoresistance by activating STAT3

JIE TANG[1], FUCHUN GUO[2], YANG DU[1], XIAOLING LIU[2], QING QIN[2], XIAOKE LIU[2], TAO YIN[3], LI JIANG[1] and YONGSHENG WANG[2]

SCIENTIFIC REPORTS

OncoImmunology 2:12, e27091; December 2013; © 2013 Landes Bioscience

EGFR-mediated tumor immunoescape

The imbalance between phosphorylated STAT1 and phosphorylated STAT3

Fernando Concha-Benavente[1], Raghvendra M Srivastava[2], Soldano Ferrone[3], and Robert L Ferris[1,2,4,*]

RESEARCH ARTICLE

CANCER www.SCIENCESIGNALING.org 29 March 2016 Vol 9 Issue 421 ra33

JAK2 inhibition sensitizes resistant EGFR-mutant lung adenocarcinoma to tyrosine kinase inhibitors

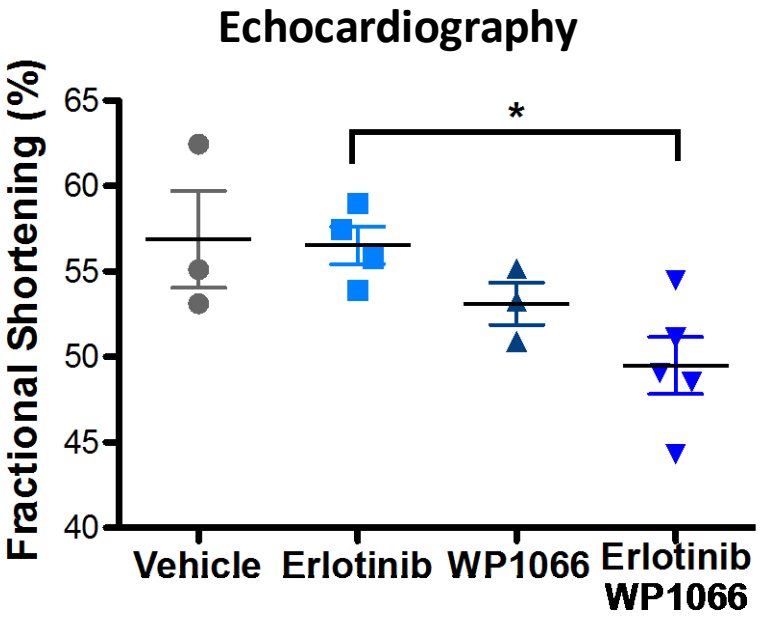
Sizhi P. Gao,[1] Qing Chang,[1] Ninghui Mao,[1] Laura A. Daly,[1] Robert Vogel,[2] Tyler Chan,[1] Shu Hui Liu,[1] Eirini Bournazou,[1] Erez Schori,[1] Haiying Zhang,[3] Monica Red Brewer,[4,5] William Pao,[4,5] Luc Morris,[6] Marc Ladanyi,[7,8] Maria Arcila,[7] Katia Manova-Todorova,[9] Elisa de Stanchina,[10] Larry Norton,[1,11] Ross L. Levine,[1,8,11] Gregoire Altan-Bonnet,[2] David Solit,[1,8,11,12] Michael Zinda,[13] Dennis Huszar,[13*] David Lyden,[3,14,15†] Jacqueline F. Bromberg[1,11†]

STAT3 activation is cardioprotective

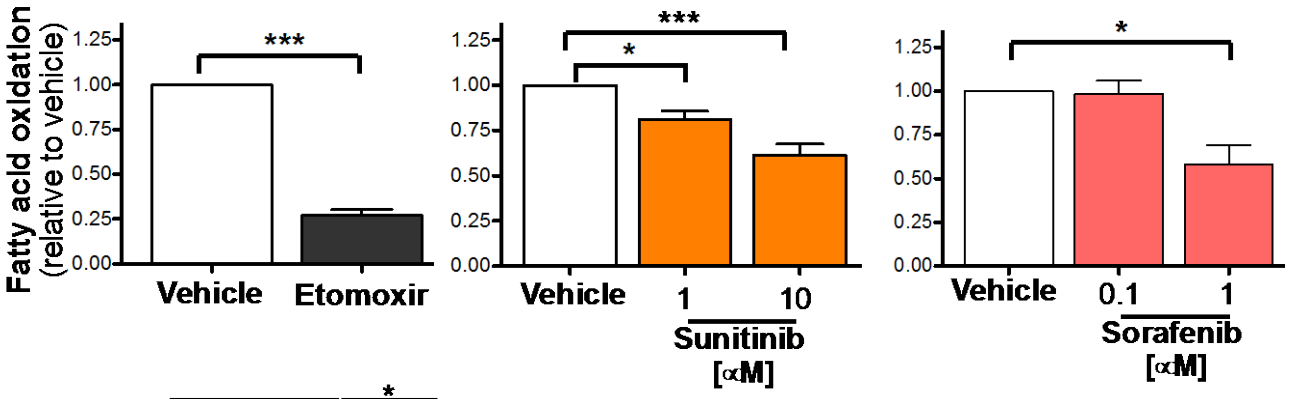
Potentially mitigating effects of EGFR inhibition (?)

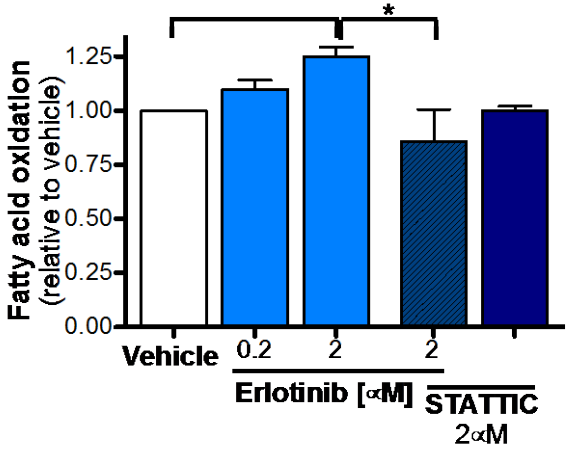
Combined EGFR and STAT3 inhibition is cardiotoxic

Caution for combination targeted therapy?



Neither erlotinib (EGFR inhibitor) nor WP1066 (STAT3 inhibitor) affects cardiac contractile function independently. In combination they cause cardiomyopathy.



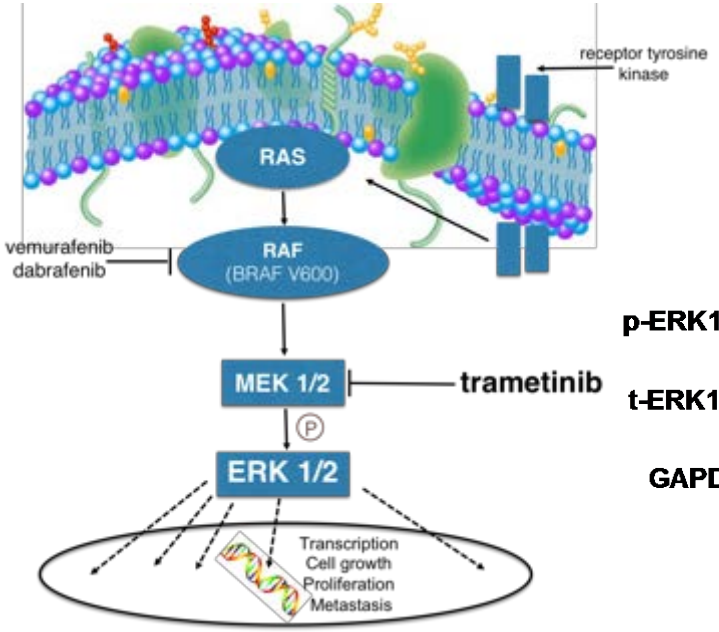


Cardiotoxic KIs, sunitinib and sorafenib, decrease cardiomyocyte fatty acid oxidation (FAO). Erlotinib alone enhances FAO, but erlotinib + the STAT3 inhibitor, STATTIC, decreases FAO.

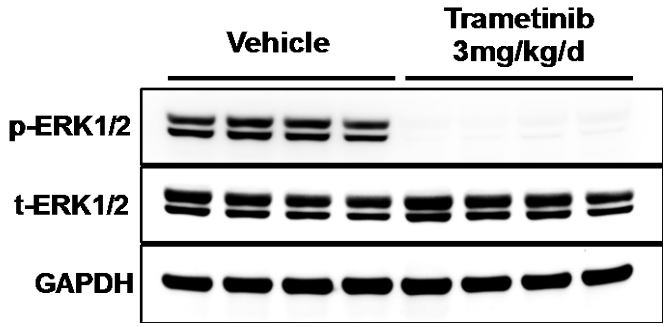
Stuhlmiller et al. J Am Heart Assoc. 2017 Oct 19

Trametinib causes reversible cardiomyopathy and heart failure

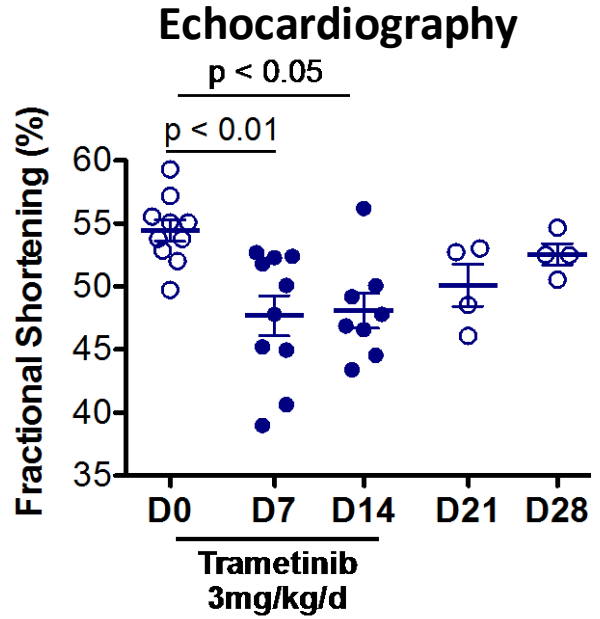
…in mice like in (some) humans



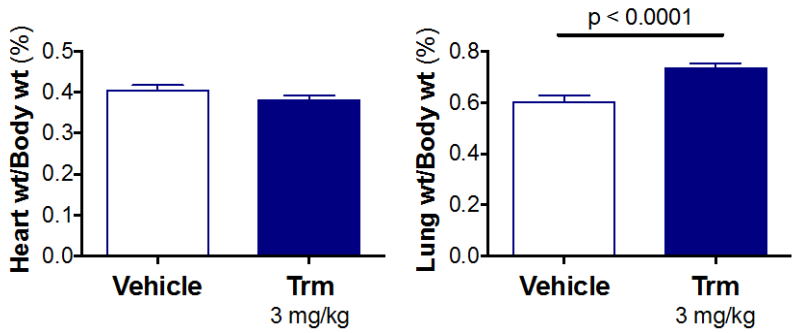
Ras-Raf-MEK-ERK oncogenic signaling pathway



Mouse heart lysates



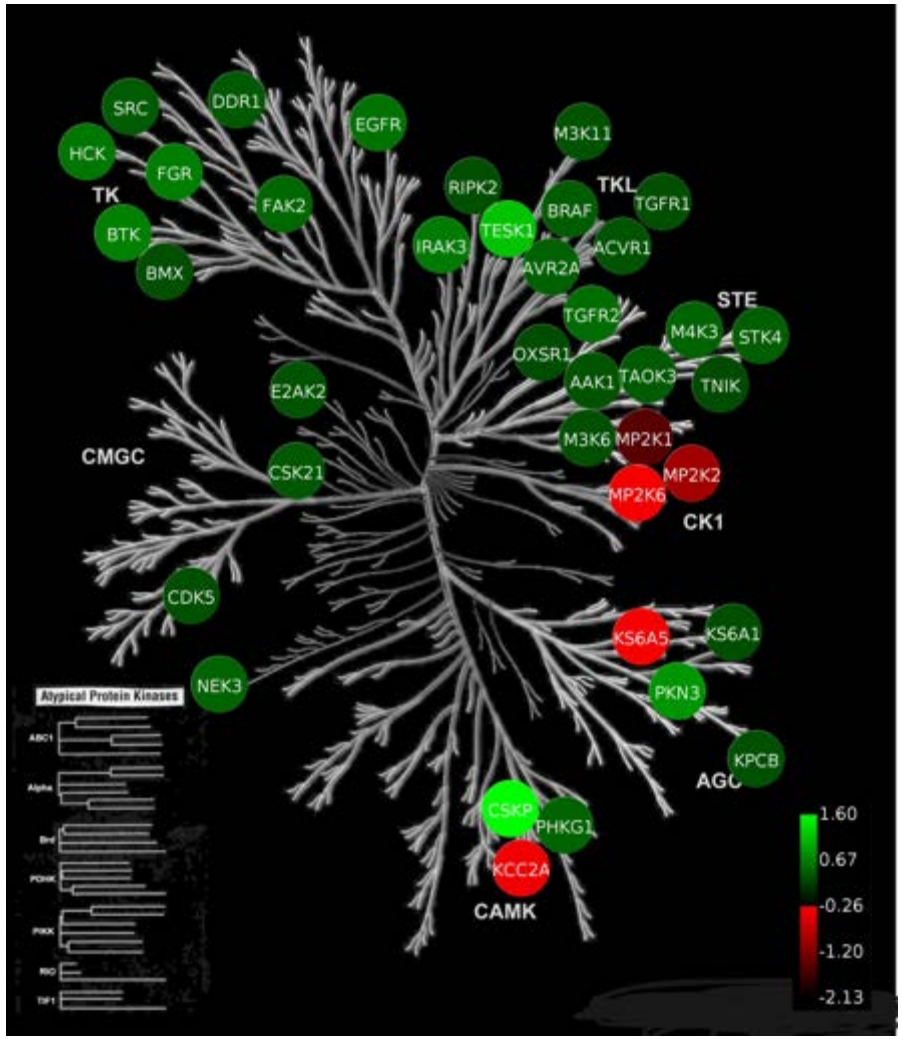
Reversible decrease in contractile function

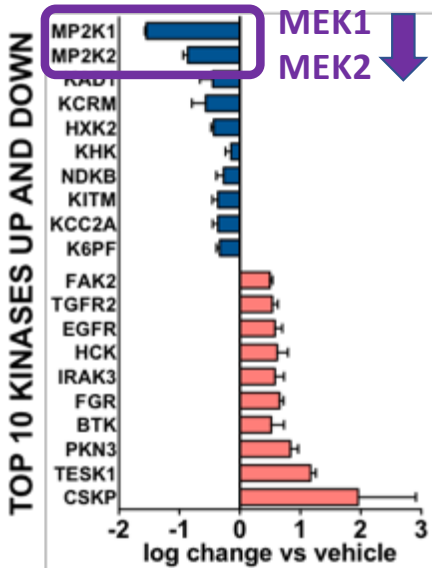


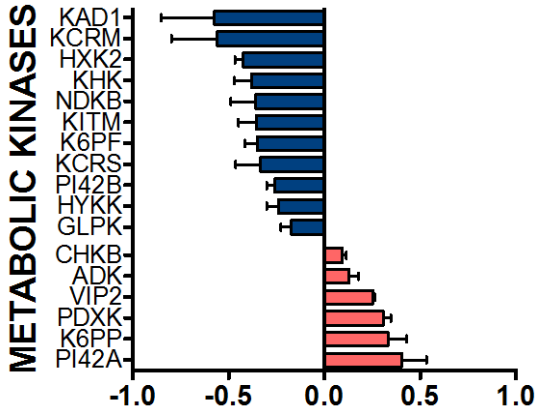
Increased lung weight consistent with pulmonary edema (heart failure)

MIB-MS and RNA-seq suggest metabolic injury

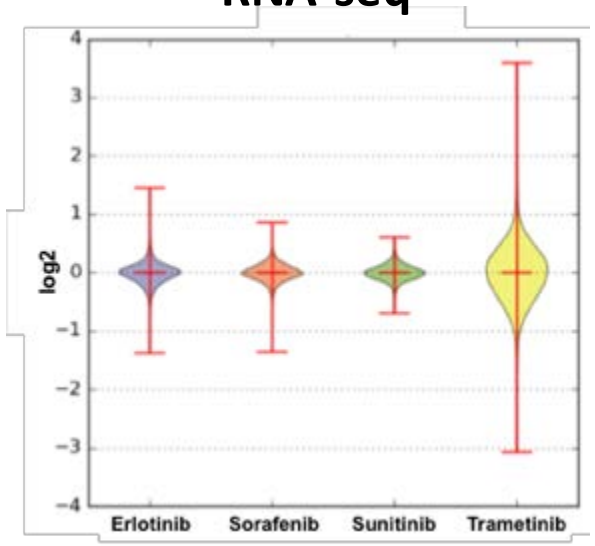
MIB-MS



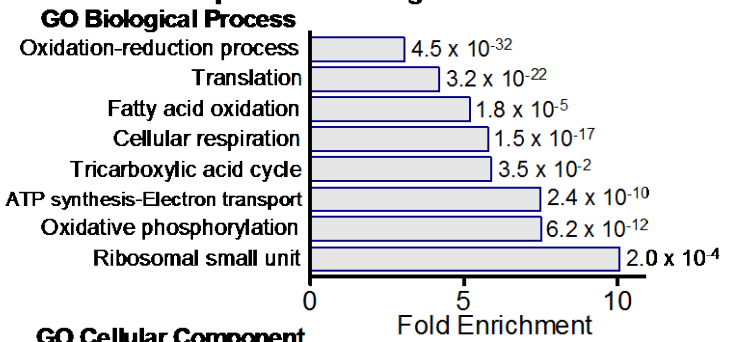


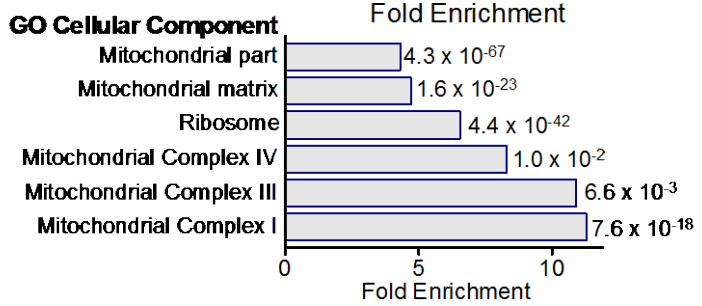


RNA-seq



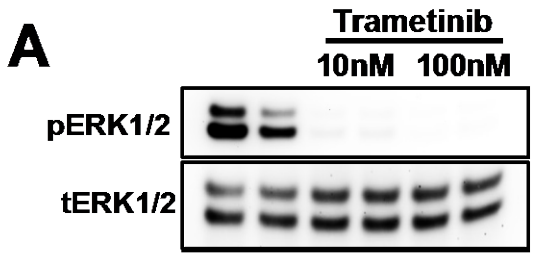
RNAseq 2-fold downregulated

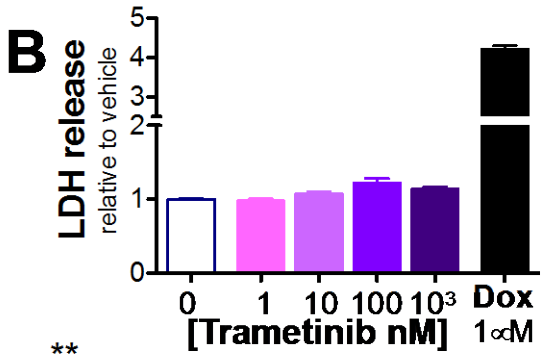


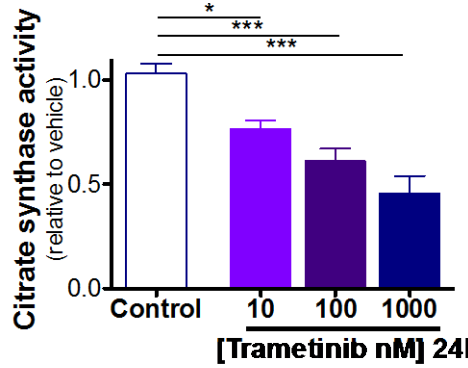


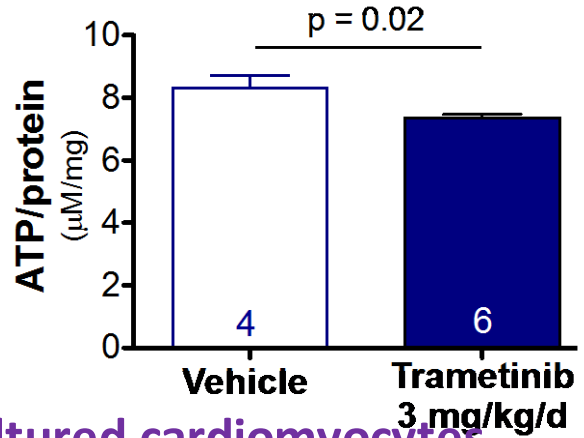
More upregulated than downregulated kinases, though metabolic kinases are disproportionately downregulated

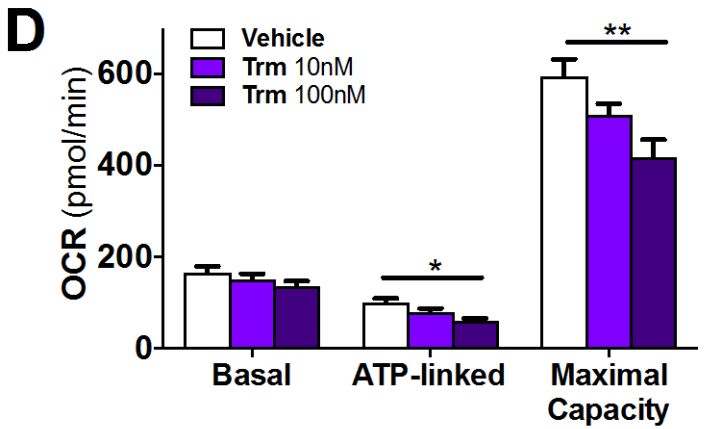
Trametinib impairs mitochondrial number and function *in vivo* and *in vitro*

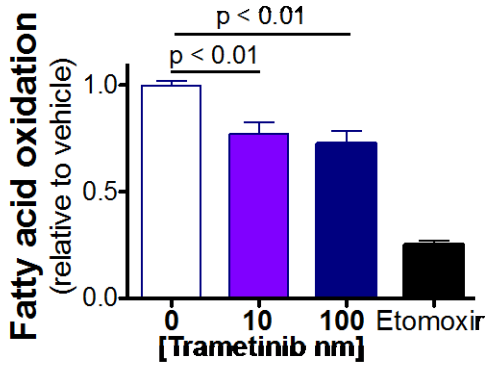


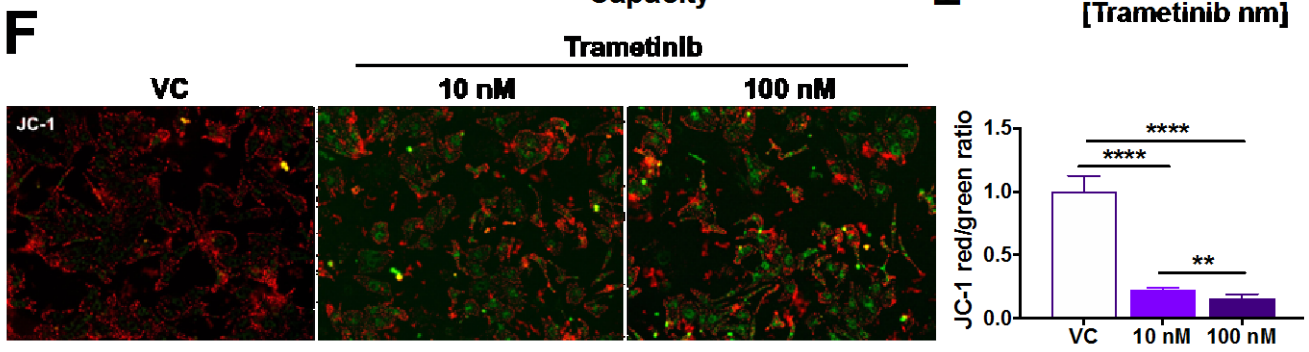












Cultured cardiomyocytes

Decreased ATP *in vivo*

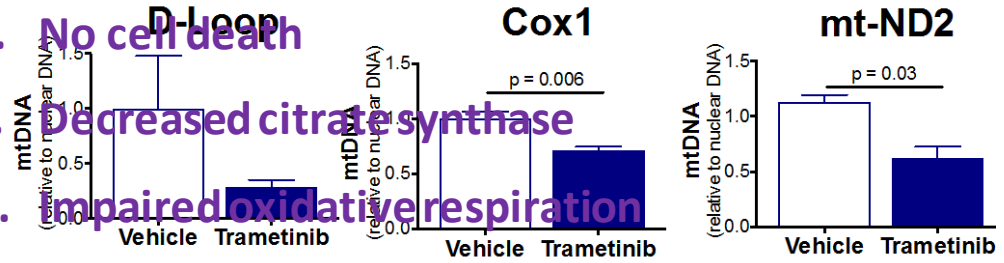
A. ERK inhibition by WB

B. No cell death

C. Decreased citrate synthase

D. Impaired oxidative respiration

(Seahorse bioanalyzer)



Fewer mitochondria *in vivo*

E. Impaired fatty acid oxidation

(radiolabeled oleate uptake)

F. Compromised mitochondrial membrane potential

Conclusions

- Heart failure is a relatively rare but important adverse effect of some targeted cancer therapies
- Current preclinical testing strategies do not adequately predict cardiomyocyte injury
- Though uninjured cardiomyocytes are very different from cancer cells, there is overlap between the biology of a failing cardiomyocyte and a cancer cell.
- Cardiomyocytes are metabolically vulnerable. Expanded preclinical testing for kinase inhibitor cardiotoxicity might include assays of metabolism and mitochondrial function.

Thank you!