Precision Medicine and PAH

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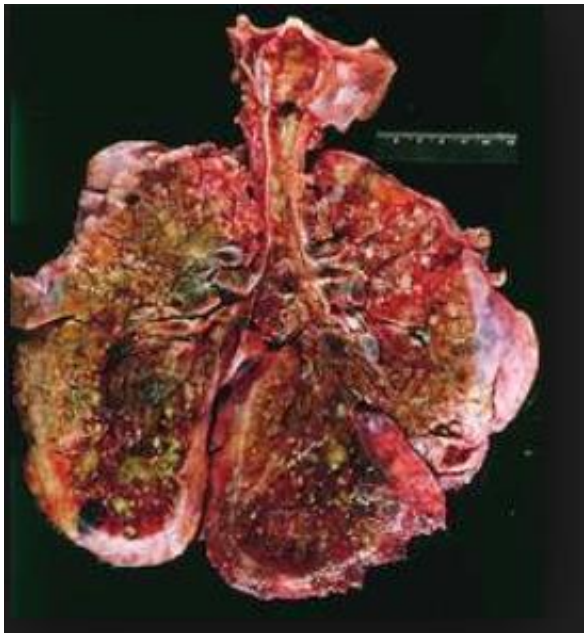
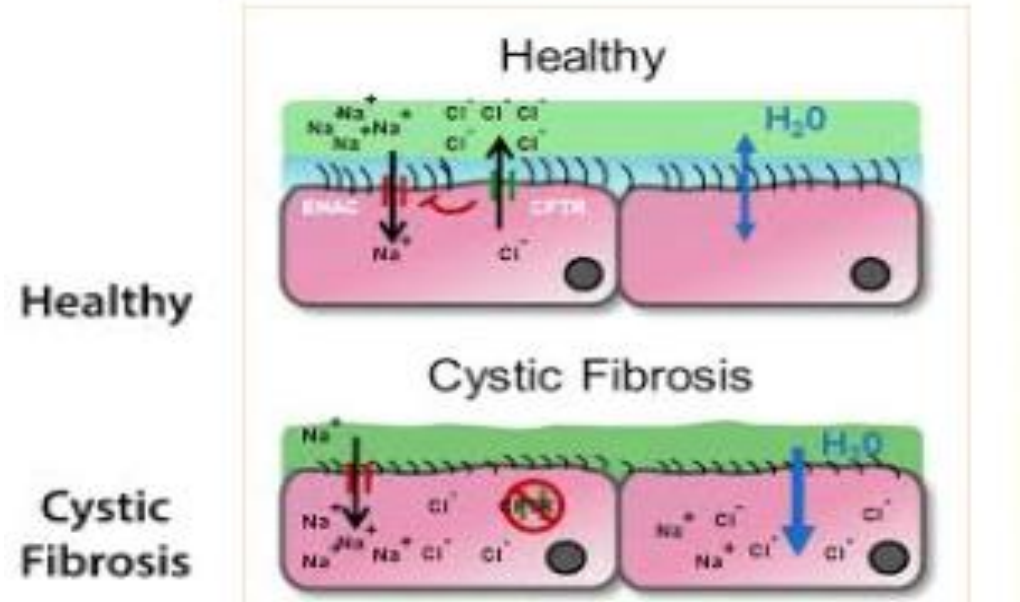
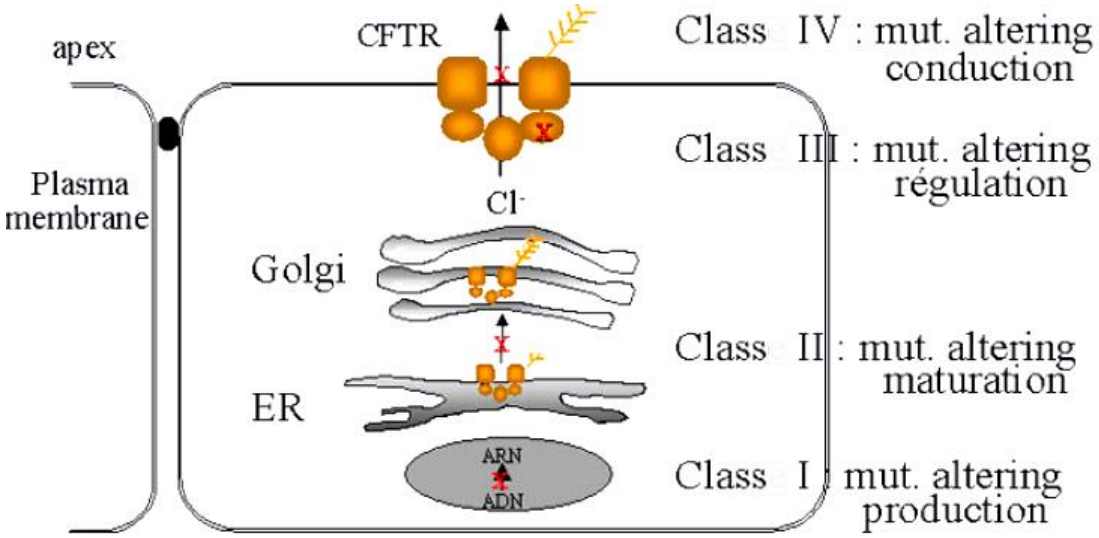
Eva Markvoort, 25: 65_RedRoses
COURTESY MARKVOORT

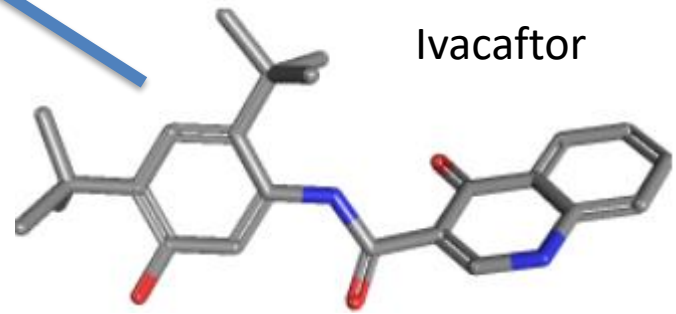



Healthy
Cystic Fibrosis
Healthy
Cystic Fibrosis
H_2O
ENaC
CFTR
Na+
Cl-



Approved in 2012

Ivacaftor

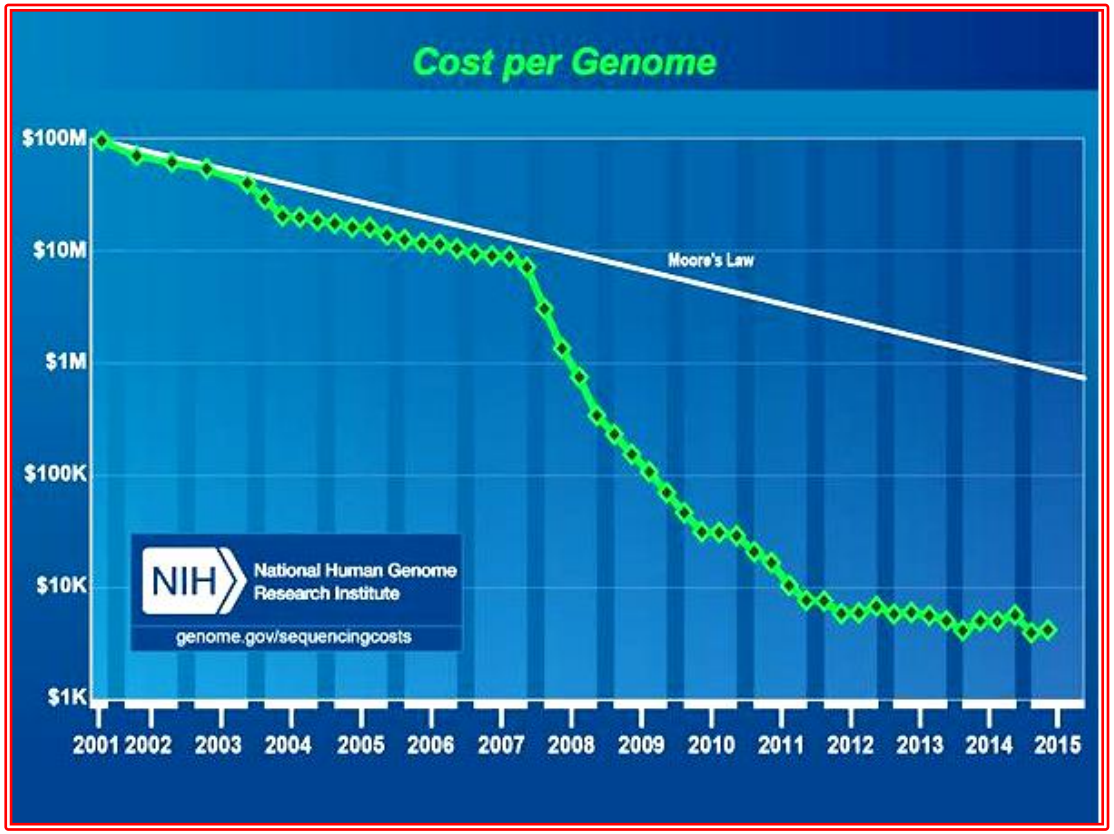


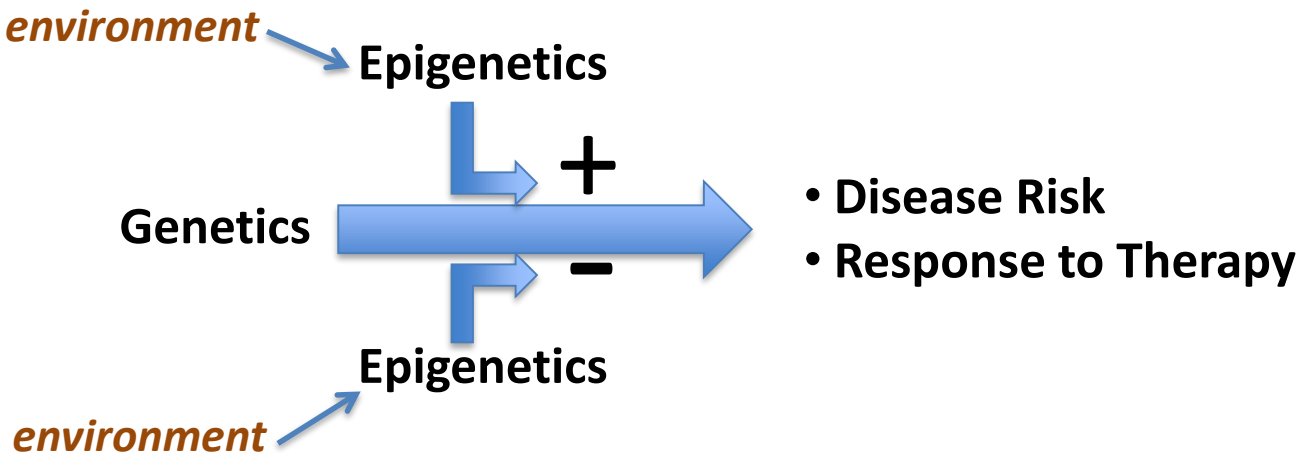
$311,000/pt/year

~ 5% of CF patients

What is early success?

- "… a hypothetical 50 year woman with suboptimal diabetes control… Within 2 Years: enrolls, WGS, implant chip, smartphone, upload data- Using these data she changes her diet and medication dose…Within 5 Years: new drug based on molecular understanding of T2D, adjust dose to genotype"
- Francis Collins, NIH Director


Cost per Genome
$100M
$10M
$1M
$100K
$10K
$1K
Moore's Law
NIH
National Human Genome Research Institute
genome.gov/sequencingcosts
2001 2002 2003 2004 2005 2006 2007 2008 2009 2010 2011 2012 2013 2014 2015


environment
Epigenetics
+
Genetics
−
Epigenetics
environment
• Disease Risk
• Response to Therapy

blood

lungs

cells

Table 1. Select list of proteomic studies in PH and pulmonary vascular remodeling, methodologies used, and the proteins identified

Study/year	PH classification and tissue source	Method	Major protein(s) of interest
Yano et al. [86]; 2011	CTEPH; human serum	SELDI-TOF MS	Fibrinogen A alpha
Wong et al. [87]; 2012	IPAH; human plasma	2D gel MS	Carbonylated proteins
Min et al. [85]; 2007	IPAH; human serum	2D gel MS	Alpha-1-antitrypsin; vitronectin
Yuditskaya et al. [83]; 2009	PAH with and without sickle-cell anemia; human plasma	SELDI-TOF MS	Apolipoprotein A-1
Zhang et al. [82]; 2009	IPAH; human serum	2D gel MS	Leucine-rich alpha-2 glycoprotein; haptoglobin; albunin; transferrin; C3 complementhydroxypyruvate reductase-1; RAF1
Salam et al. [81]; 2006	IPAH; human plasma	SELDI-TOF MS	C4a complement
Salam et al. [78]; 2010	IPAH; human lung	SDS-PAGE LC-MS	CLIC-4; periostin; annexin A3; ager; GD11; FHL1
Veith et al. [76]; 2013	Hypoxia PH; mouse lung	2D gel MS	Cofilin; ager
Kwapiszewska et al. [77]; 2008	Hypoxia PH; mouse lung	2D gel MS	FHL-1
Wang et al. [73]; 2013	Hypoxia PH; rat distal pulmonary artery	2D gel MS	Proteasome subunit beta 6
Ohata et al. [72]; 2013	Hypoxia PH; rat lung	2D gel MS	HSP70; PPI
Schott et al. [75]; 2005	MCT PH; rat RV	2D gel MS	Troponin; tropomyosin; NADH dehydrogenase; p97; BRCA1-assoc. protein; HSP27
Ostergaard et al. [74]; 2011	Hypoxia PH; rat lung	2D gel MS	Guanine nucleotide-binding protein beta; GST-omega-1; cathepsin D; CLIC5; annexin A4; F-actin capping protein
Laudi et al. [66]; 2007	Hypoxia PH; MCT PH; rat lung	2D gel MS	F-actin capping protein; septin2; HSc70; ERp57; CLIC1; annexin 3; HSP27
Watts et al. [94]; 2012	Microparticle emobolization; rat MP	SDS-PAGE LC-MS	vWF; haptoglobin
Meyrick et al. [59]; 2008	Familial PAH; human transformed lymphocytes	2D gel MS	14–3–3; Caspase-3 precursor; Grb2; vimentin; GS1
Lavoie et al. [58]; 2014	Hereditary pulmonary hypertension with BMPR2 mutation; human blood-outgrowth endothelium cells	2D gel MS	Translationally controlled tumor protein
Terrier et al. [95]; 2008	IPAH, FPAH, Dex-PAH, SSc-PAH; human serum	2D immunoblotting LC-MS	G6PD; HSP27;Pi3-kinase; HSP70; DAP kinase; BRDT
Fessel et al. [49]; 2012	Human PAH; BMPR2 mutant pulmonary microvascular endothelial cells	HPLC-MS; GC-MS	Metabolic reprogramming
Zhang et al. [96]; 2009	Hypoxia; human pulmonary artery smooth muscle cells	2D gel MS	Gelsolin-like actin-capping protein; transgelin
Lame et al. [47]; 2000	Human lung endothelial cells treated with MCT pyrrole	2D gel MS	Galectin-1; protein disulfide isomerase (PDI); ER60; actin; tropomyosin
Lame et al. [48]; 2005	Human lung endothelial cells treated with MCT pyrrole	2D gel MS	ERp57; PDI; thioredoxin; galectin-1; annexin A2; cofilin-1
Hassel et al. [46]; 2004	GST-BMPR2 pull-down assays; mouse myoblast C2C12 cells	2D gel MS	Crystallin; tubulin; PKC beta; MAPKKK8; TRP1; tubulin 5 homolog; C4 binding protein

Colvin and Yeager
Proteomics Clin Appl, 2015

Personal Omics Profiling Reveals Dynamic Molecular and Medical Phenotypes

Rui Chen,[1,11] George I. Mias,[1,11] Jennifer Li-Pook-Than,[1,11] Lihua Jiang,[1,11] Hugo Y.K. Lam,[1,12] Rong Chen,[2,12] Elana Miriami,[1] Konrad J. Karczewski,[1] Manoj Hariharan,[1] Frederick E. Dewey,[3] Yong Cheng,[1] Michael J. Clark,[1] Hogune Im,[1] Lukas Habegger,[6,7] Suganthi Balasubramanian,[6,7] Maeve O'Huallachain,[1] Joel T. Dudley,[2] Sara Hillenmeyer,[1] Rajini Haraksingh,[1] Donald Sharon,[1] Ghia Euskirchen,[1] Phil Lacroute,[1] Keith Bettinger,[1] Alan P. Boyle,[1] Maya Kasowski,[1] Fabian Grubert,[1] Scott Seki,[2] Marco Garcia,[2] Michelle Whirl-Carrillo,[1] Mercedes Gallardo,[9,10] Maria A. Blasco,[9] Peter L. Greenberg,[4] Phyllis Snyder,[1] Teri E. Klein,[1] Russ B. Altman,[1,5] Atul J. Butte,[2] Euan A. Ashley,[3] Mark Gerstein,[6,7,8] Kari C. Nadeau,[2] Hua Tang,[1] and Michael Snyder[1,*]

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Cell *148*, 1293–1307, March 16, 2012

Michael Snyder, PhD
Head, Medical Genetics, Stanford
A healthy and fit 54 year old male

- Studied 20 serial samples of his blood during a 14-month period and performed deep WGS, transcriptomic, proteomic and metabolomic analysis, developing the first "**integrative personal omics profile" (iPOP**).

- During the 14-month period he suffered two minor viral infections (with a rhinovirus and RSV).

- His team tracked ~20,000 transcripts coding 12,000 genes and measured more than 6,000 proteins and 1,000 metabolites in Snyder's blood.

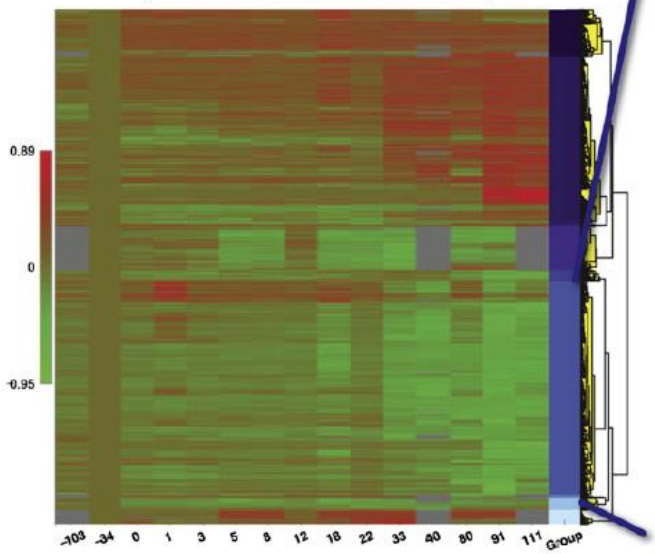
- They identified ~2,000 genes that were expressed at higher levels during the viral infections, and ~2,200 genes expressed at lower levels, including some involved in insulin signaling.

Integrated Omics clustering

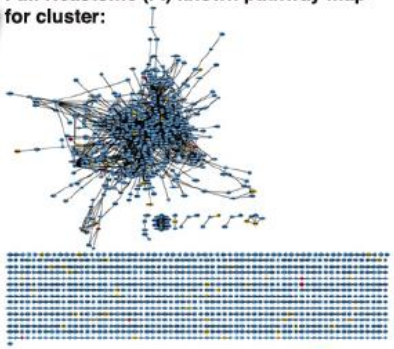
Transcriptome
Proteome
Metabolome

→ RSV Infection → Healthy: Integrated omics

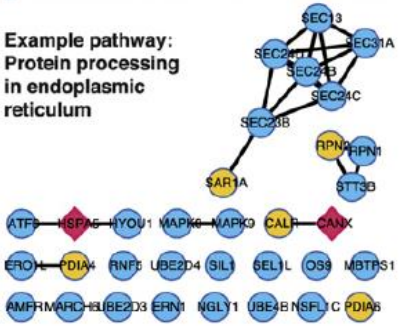
(I) Autocorrelated data clusters



Full Reactome (FI) known pathway map for cluster:

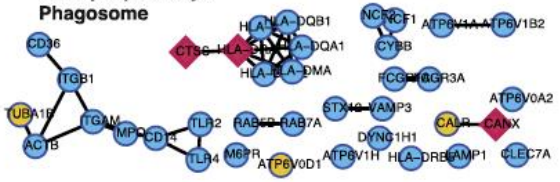


Example pathway: Protein processing in endoplasmic reticulum

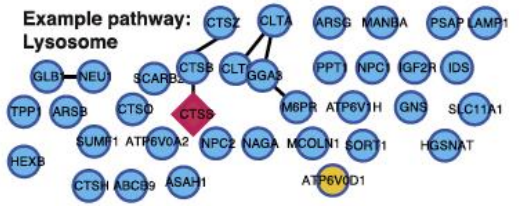


Dynamic expression pattern observed in:
RNA Protein Both RNA + Protein

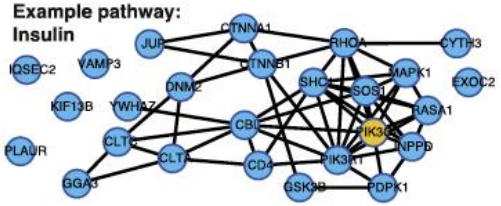
Example pathway: Phagosome



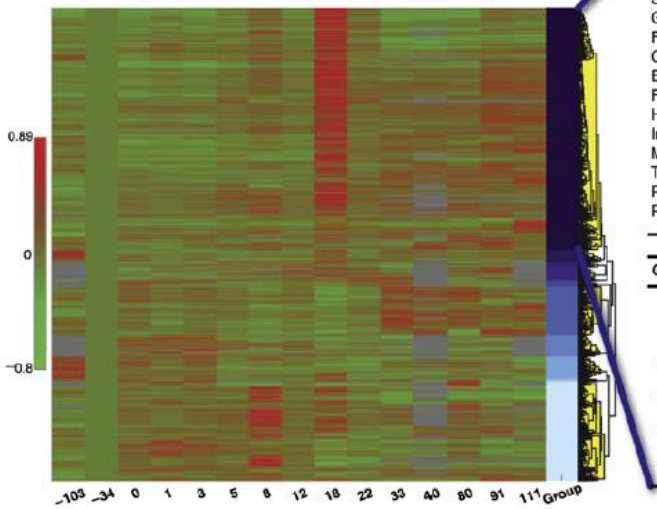
Example pathway: Lysosome



Example pathway: Insulin



(II) Spike maxima data clusters



Eg. Pathway Analysis Results - FDR < 5e-02:

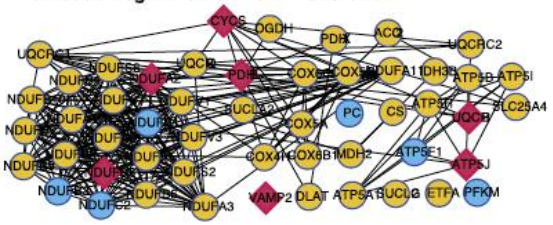
Spliceosome(K)
Glucose Regulation of Insulin Secretion(R)
Formation and Maturation of mRNA Transcript(R)
Oxidative phosphorylation(K)
Electron Transport Chain(R)
Parkinson's disease(K)
Huntington's disease(K)
Influenza Life Cycle(R)
Metabolism of non-coding RNA(R)
Transport of Mature Transcript to Cytoplasm(R)
Protein export(K)
Pyruvate metabolism and TCA cycle(R)

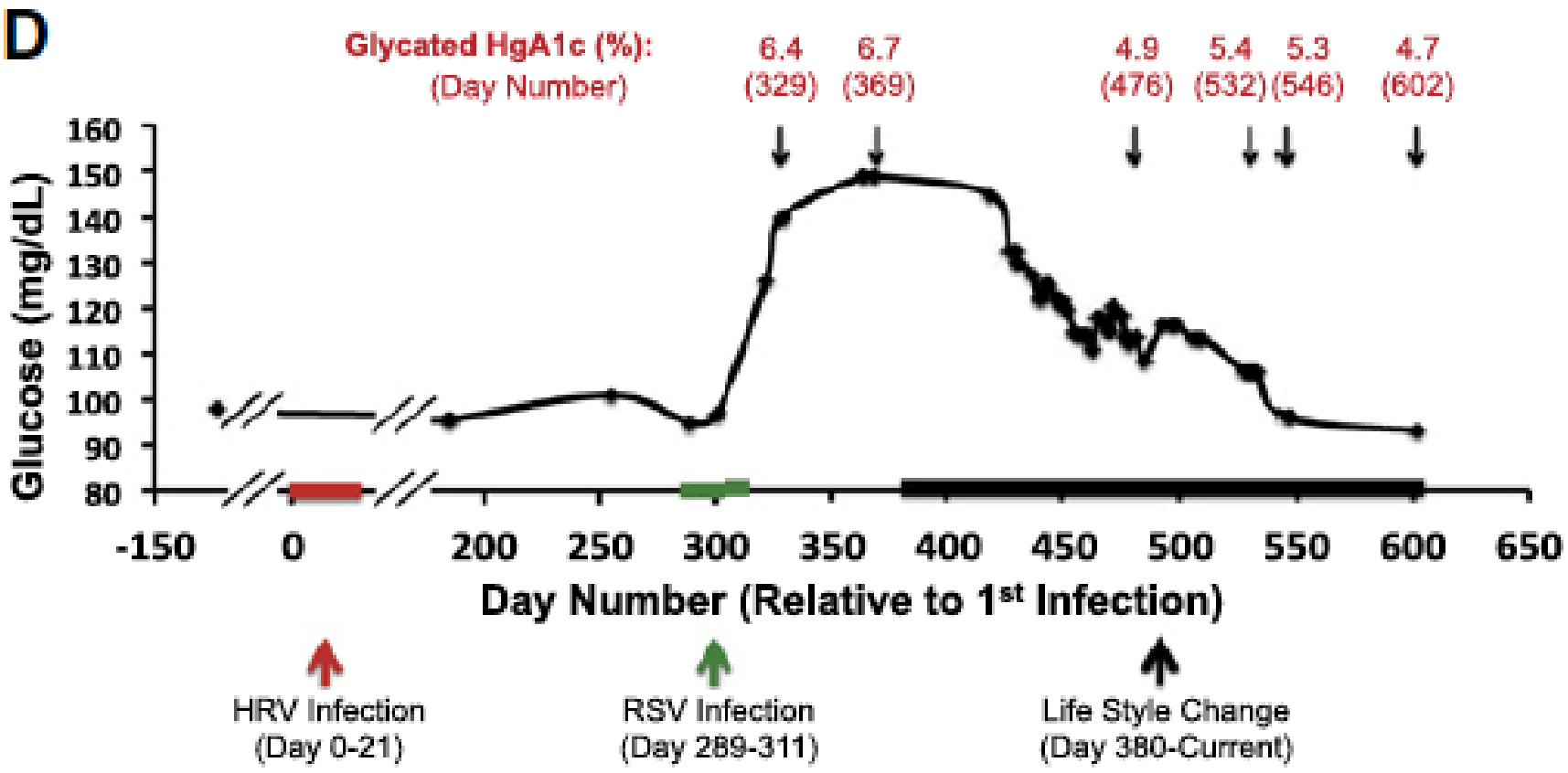
GO-ID	corr p-value	Description
8380	3.59E-71	RNA splicing
6396	2.53E-57	RNA processing
16070	5.93E-54	RNA metabolic process
16071	6.10E-50	mRNA metabolic process
10467	1.74E-48	gene expression
90304	1.78E-45	nucleic acid metabolic process

Eg. Metabolites in Cluster

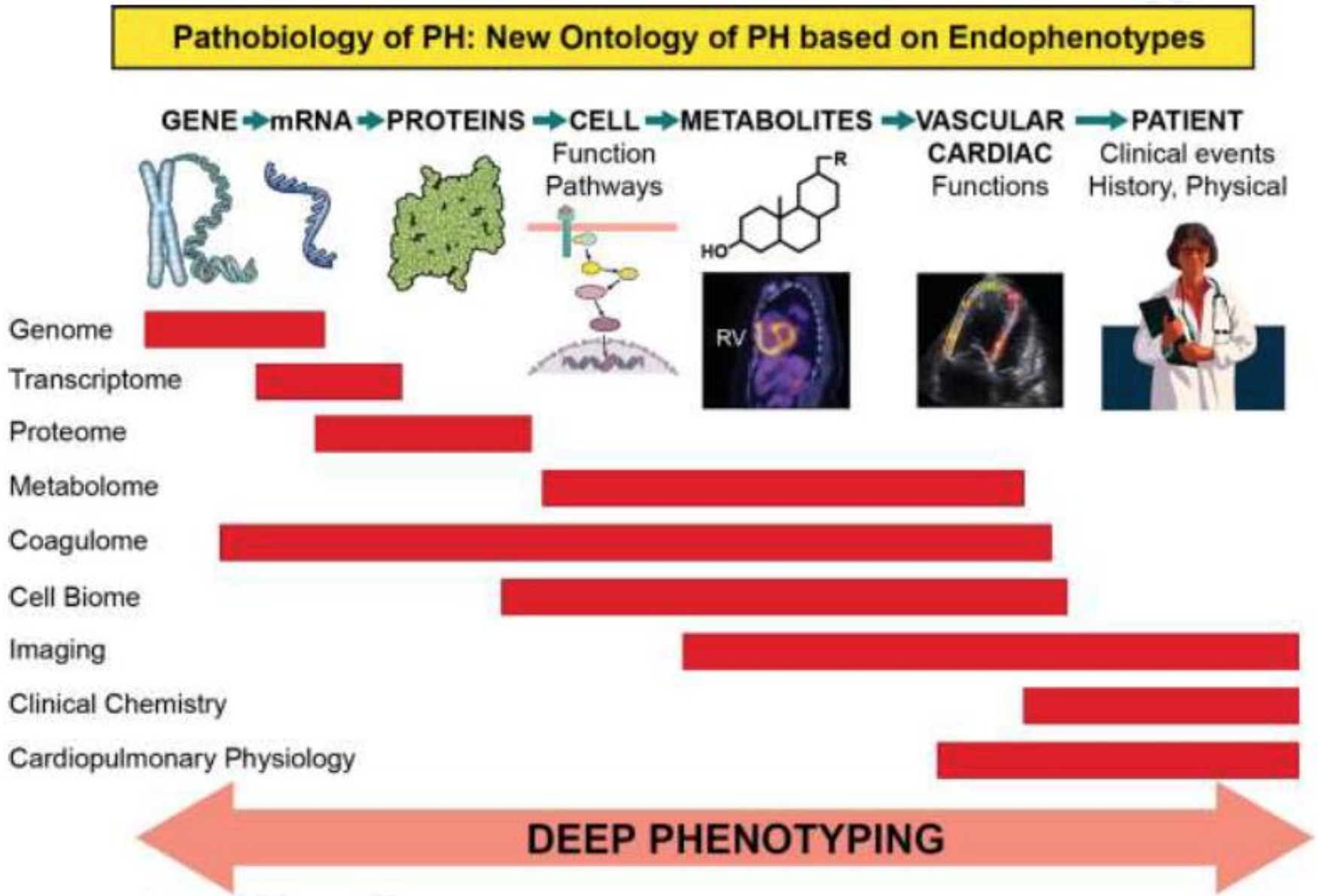
3R-hydroxy-5Z-dodecenoic acid
5,6-DiHETrE-EA
7-Ethoxycoumarin
Lauric acid
1-O-(1Z-tetradecenyl)-2-(9Z-octadecenoyl)-sn-glycerol
(23R)-1alpha,23,25-trihydroxy-24-oxovitamin D3 / (23R)-1alpha,23,25-trihydroxy-24-oxocholecalciferol
1alpha-hydroxy-26,27-dinorvitamin D3 25-carboxylic acid / 1alpha-hydroxy-26,27-dinorcholecalciferol
12-oxo-9-octadecynoic acid
GPCho(O-16:0/O-4:0[U])
19-hydroxy-17-oxoandrost-5-en-3-beta-yl sulfate - 11.538899

Example Pathway: Glucose Regulation of Insulin Secretion




D
Glycated HgA1c (%):
(Day Number)
6.4 (329)
6.7 (369)
4.9 (476)
5.4 (532)
5.3 (546)
4.7 (602)
Glucose (mg/dL)
160
150
140
130
120
110
100
90
80
-150
0
200
250
300
350
400
450
500
550
600
650
Day Number (Relative to 1st Infection)
HRV Infection
(Day 0-21)
RSV Infection
(Day 289-311)
Life Style Change
(Day 380-Current)

PVDOMICS: A prospective (n=1500 patients) collaborative project of 6 academic US centers and one coordinating center

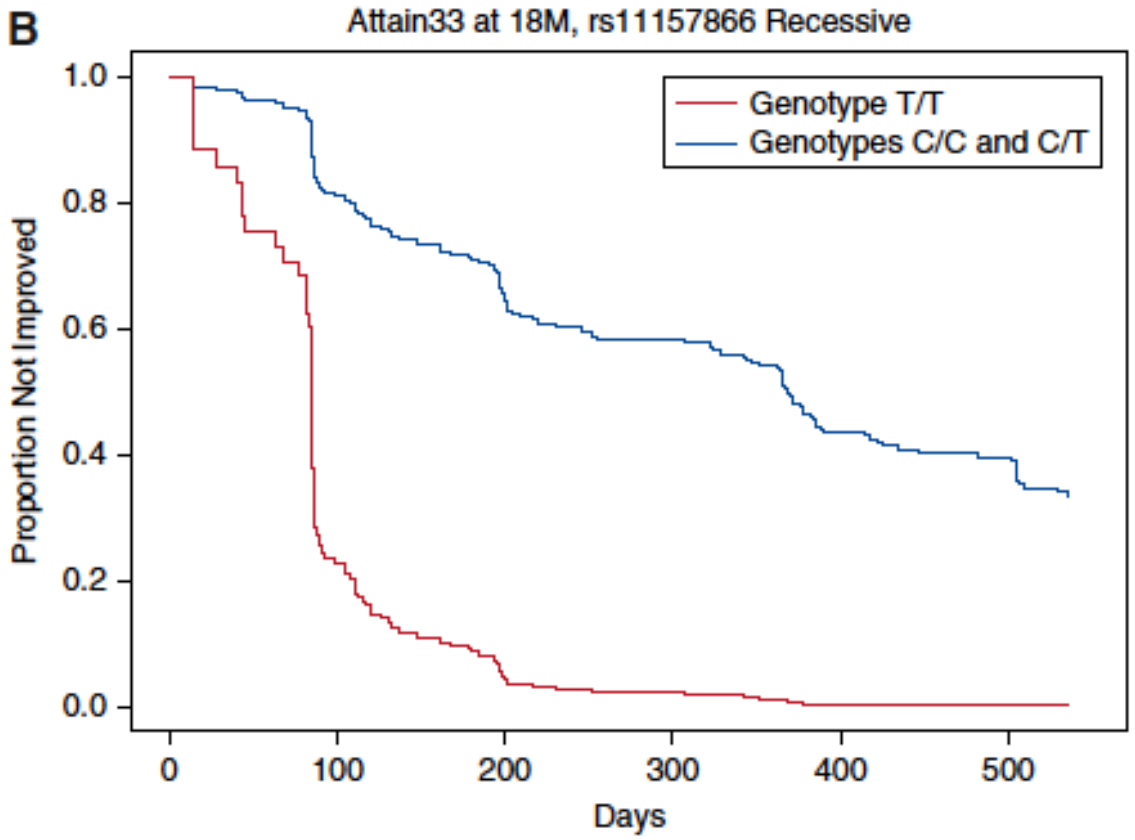


Endothelin-1 Pathway Polymorphisms and Outcomes in Pulmonary Arterial Hypertension

AJRCCM, 2015

Raymond L. Benza[1], Mardi Gomberg-Maitland[2], Teresa Demarco[3], Adaani E. Frost[4], Adam Torbicki[5], David Langleben[6], Tomas Pulido[7], Priscilla Correa-Jaque[1], Michael J. Passineau[1], Howard W. Wiener[8], Mayumi Tamari[9], Tomomitsu Hirota[9], Michiaki Kubo[9], and Hemant K. Tiwari[10]

" A single-nucleotide polymorphism (rs11157866) in the G-protein alpha and gamma subunits gene was significantly associated, accounting for multiple testing, with a combined improvement in functional class and 6-minute-walk distance …"



Hypothesis-driven Precision Medicine Trials that also address *causation* of the gene variant

I. Preclinical: animal work

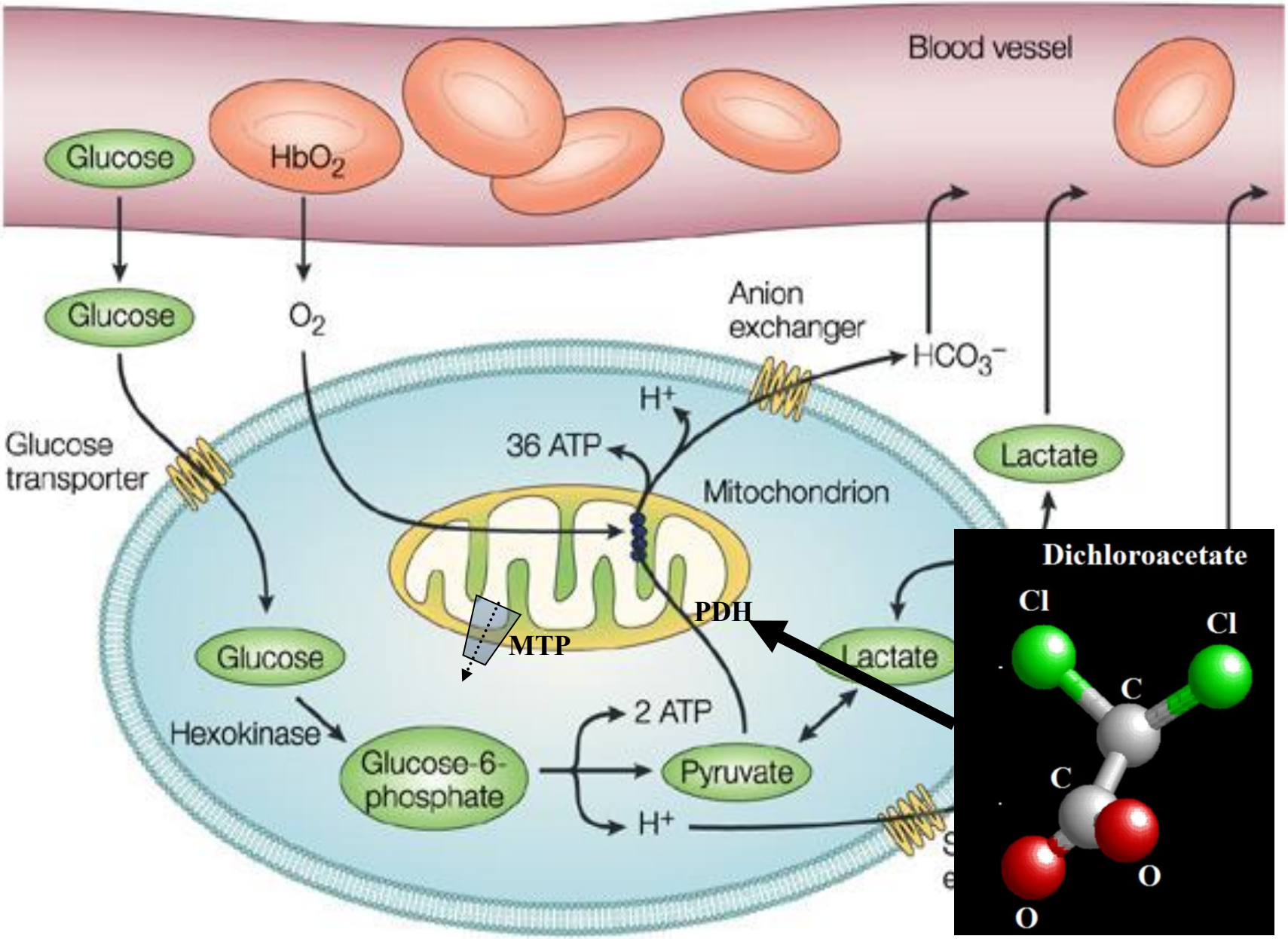
II. Preclinical: human tissues - therapeutic target

III. Preclinical: human tissues – drug effects

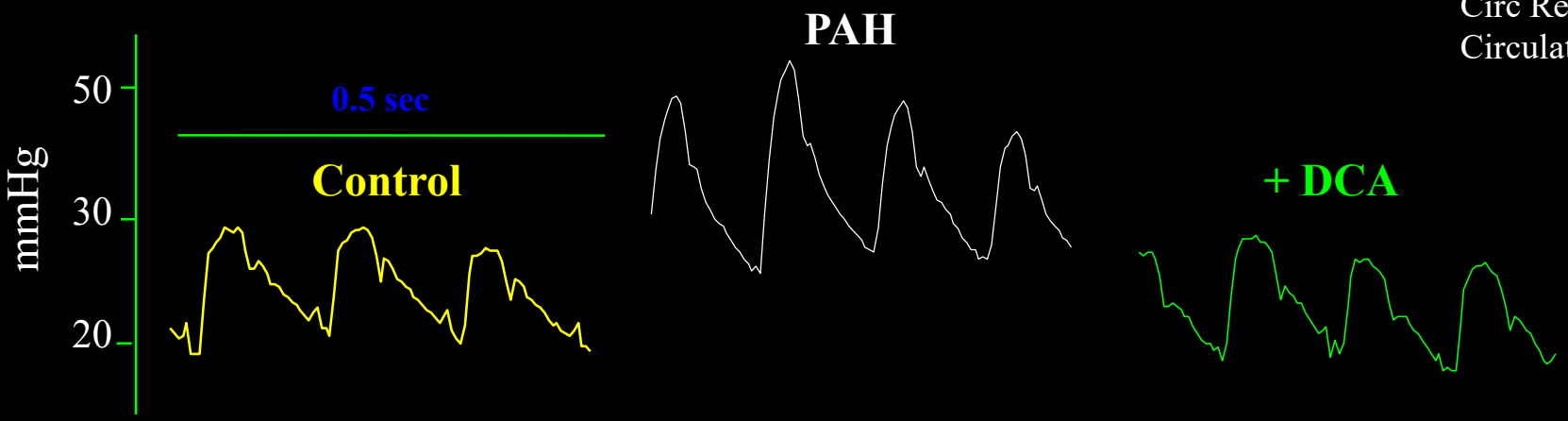
IV. Clinical: phase I-II trials - safety

V. Clinical: phase I-II trials – possible efficacy

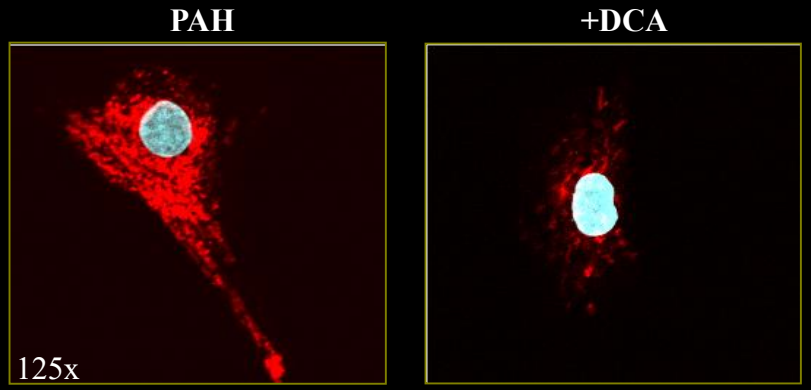
VI. Clinical: phase I-II trials – exploratory biomarkers

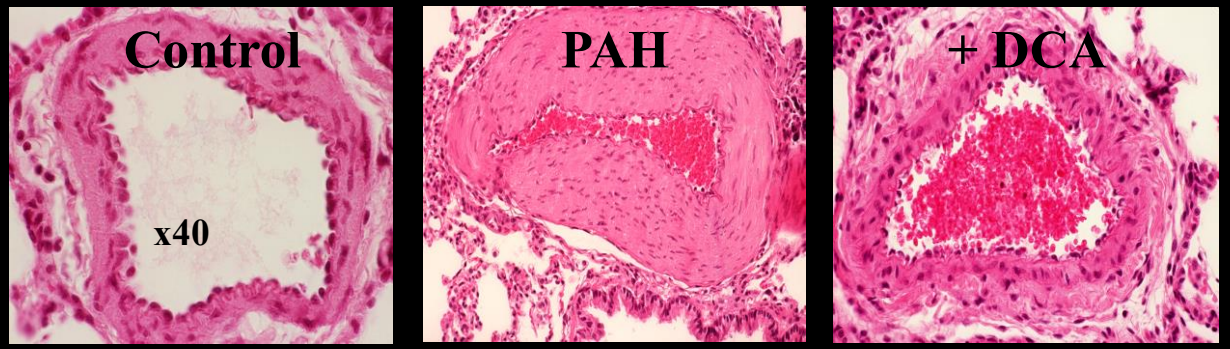

Blood vessel
Glucose
HbO_2
Glucose
O_2
Anion exchanger
HCO_3^-
H^+
36 ATP
Glucose transporter
Mitochondrion
Lactate
PDH
MTP
Glucose
Lactate
Hexokinase
2 ATP
Glucose-6-phosphate
Pyruvate
H^+
Dichloroacetate
Cl
Cl
C
C
O
O

Circulation 2002
Circ Res 2004
Circulation 2006

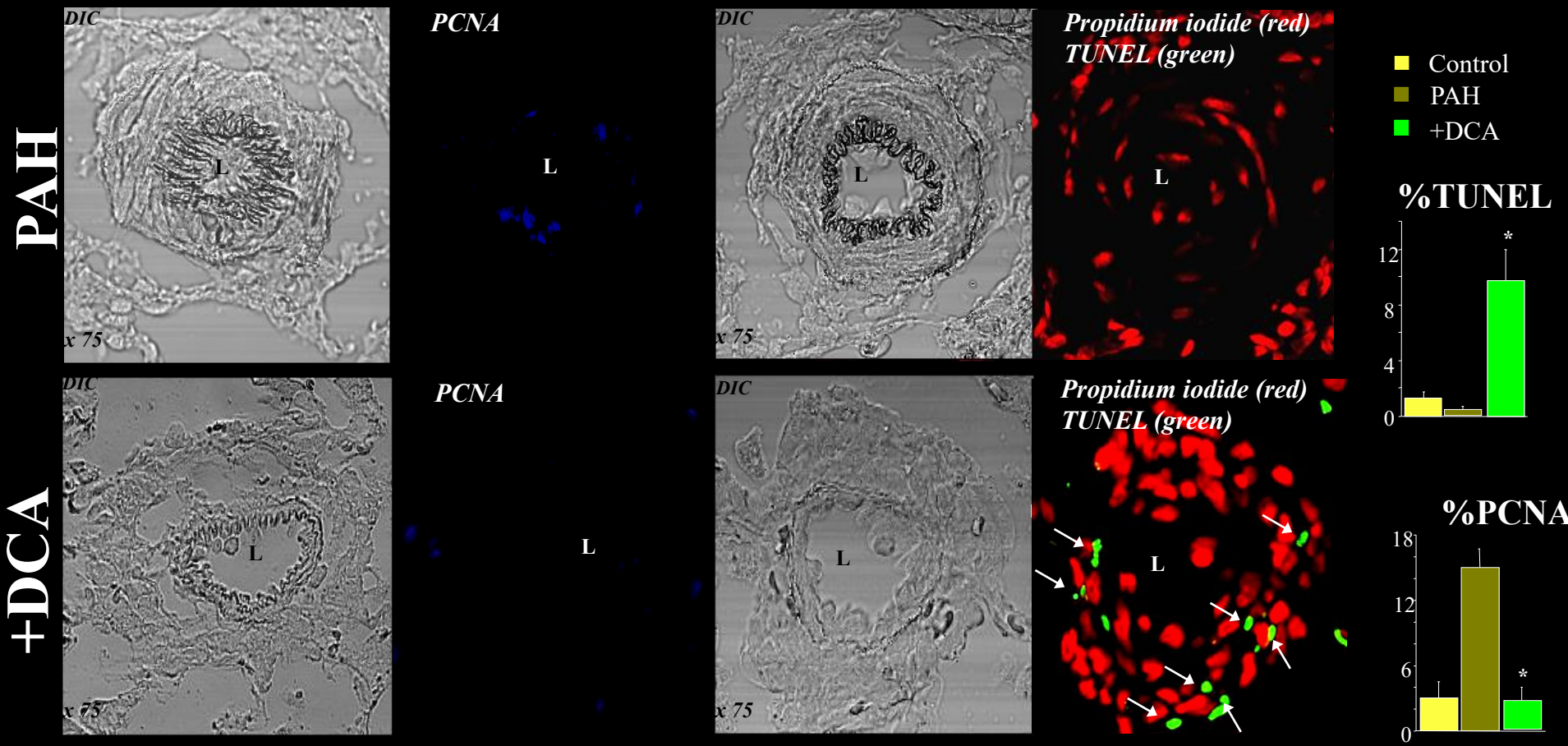


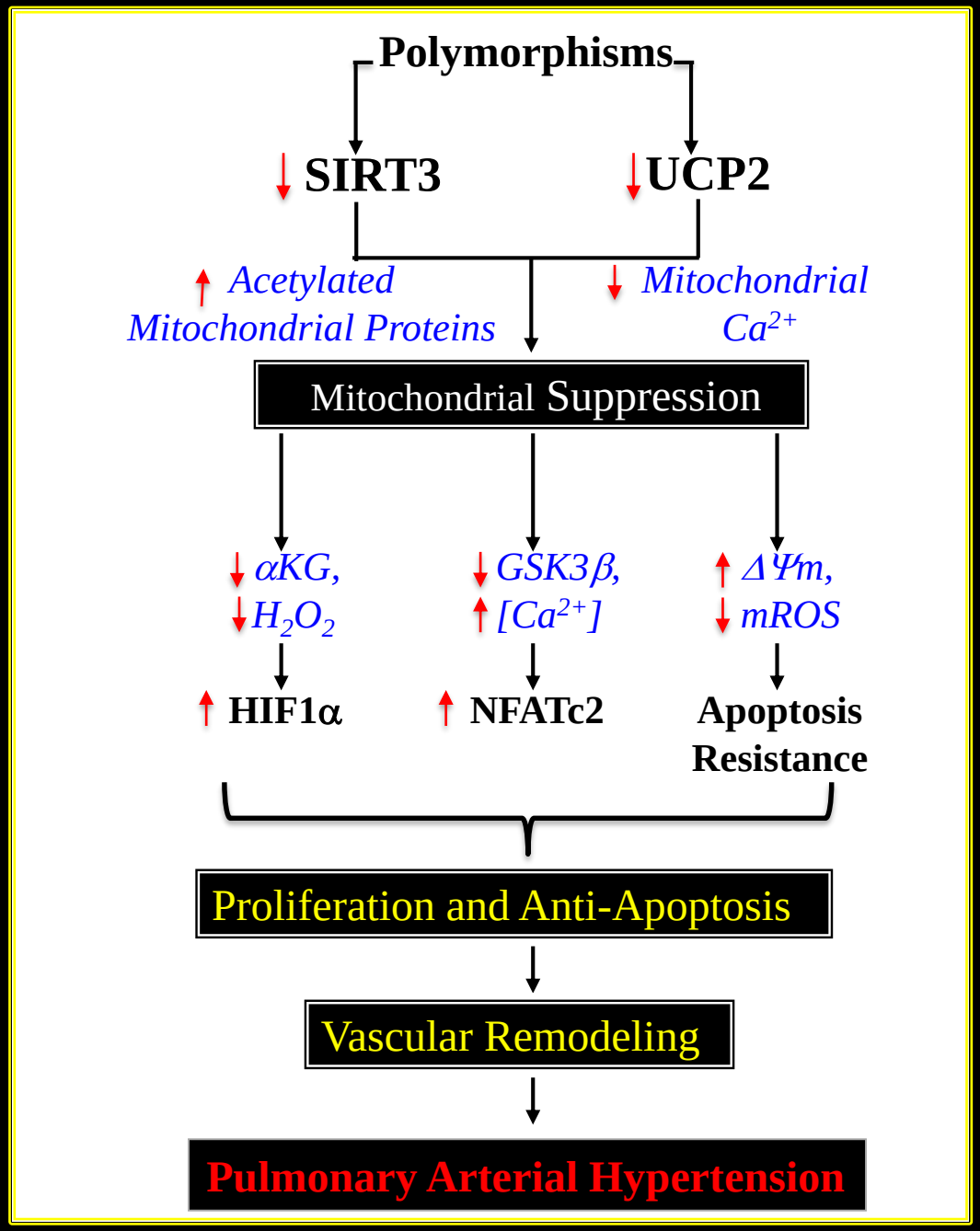
PASMC MITOCHONDRIA



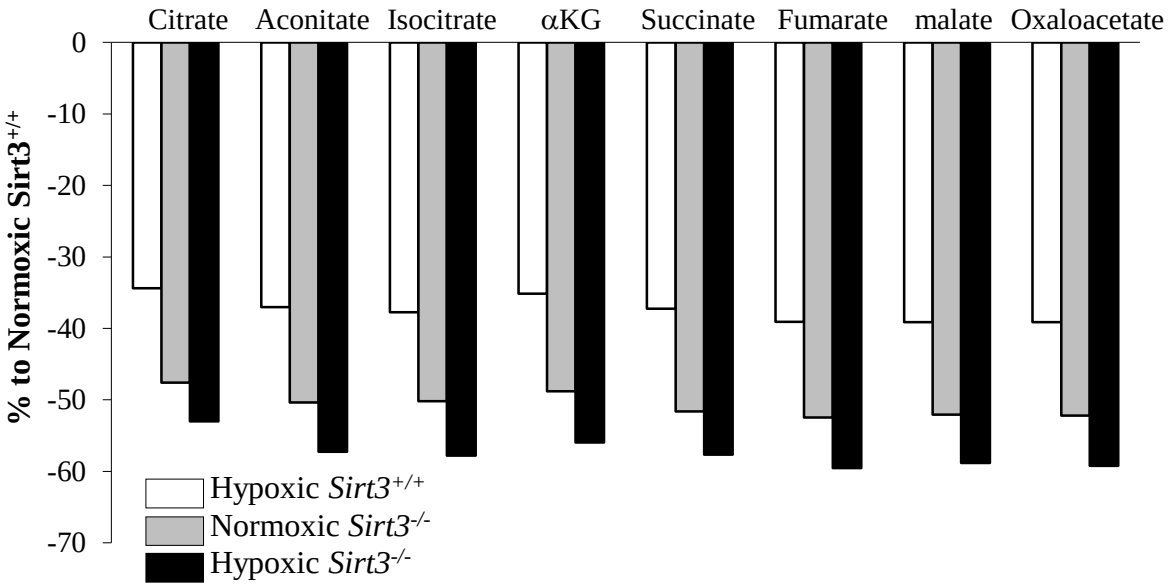


DCA therapy induces apoptosis in the PA wall and reverses vascular remodeling

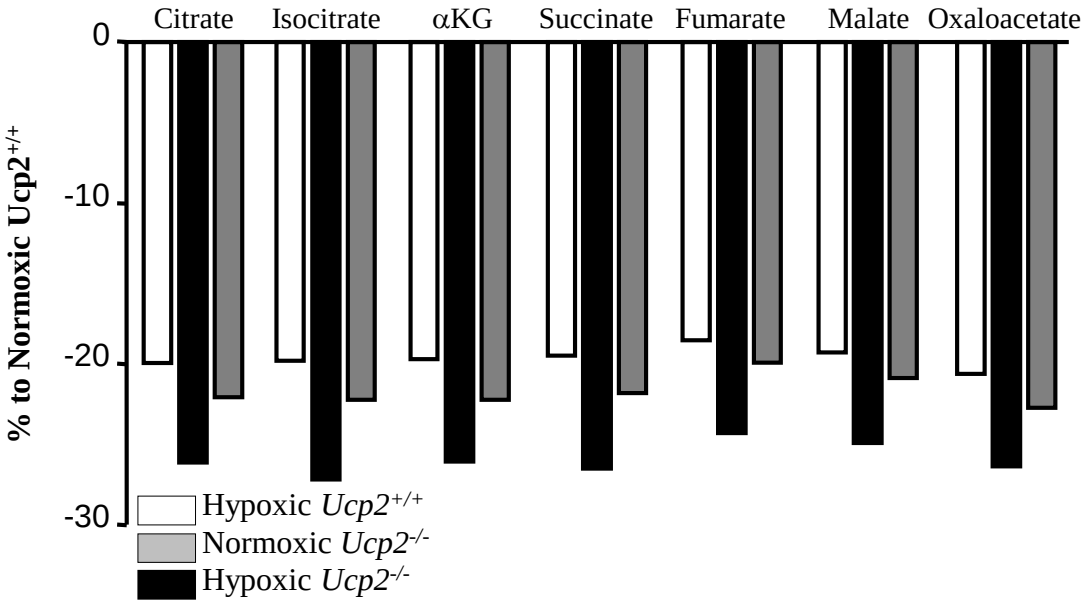



Polymorphisms
↓ SIRT3
↓ UCP2
↑ Acetylated Mitochondrial Proteins
↓ Mitochondrial Ca^{2+}
Mitochondrial Suppression
↓ αKG, ↓ H_2O_2
↓ GSK3β, ↑ $[Ca^{2+}]$
↑ ΔΨm, ↓ mROS
↑ HIF1α
↑ NFATc2
Apoptosis Resistance
Proliferation and Anti-Apoptosis
Vascular Remodeling
Pulmonary Arterial Hypertension

All Krebs' cycle intermediates are decreased in Sirt3$^{-/-}$ and Ucp2$^{-/-}$ PASMCs



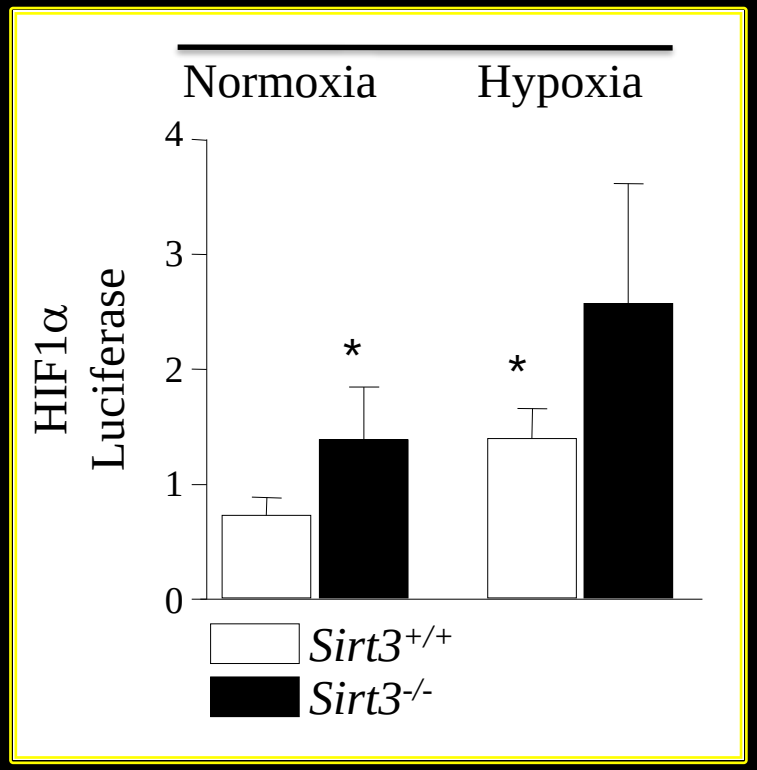
Paulin et al,
Cell Metabolism, 2014



Dromparis et al,
Circulation Research 2013

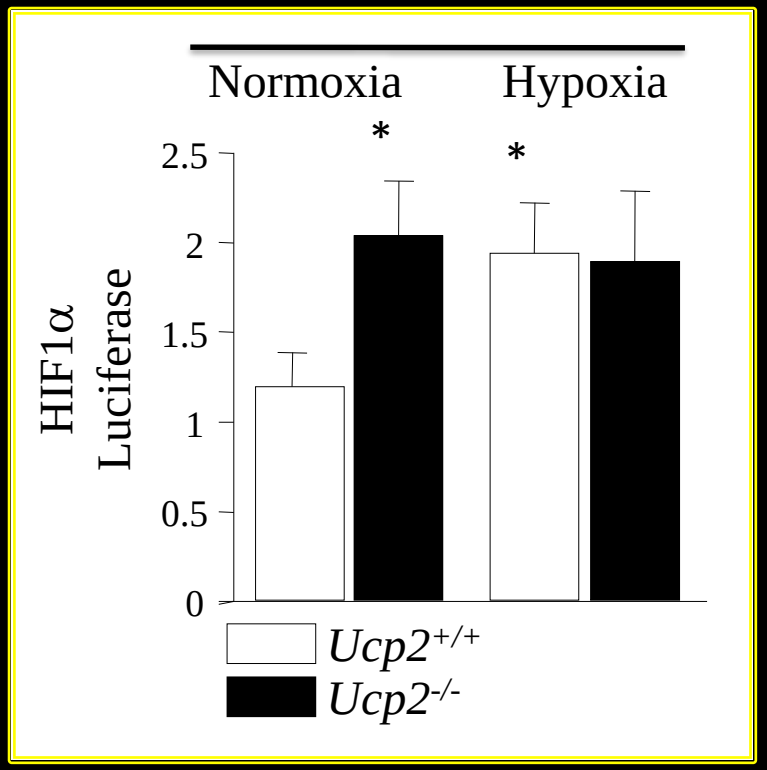
HIF1a is activated in both Sirt3$^{-/-}$ and Ucp2$^{-/-}$ PASMCs even under normoxia

SIRT3



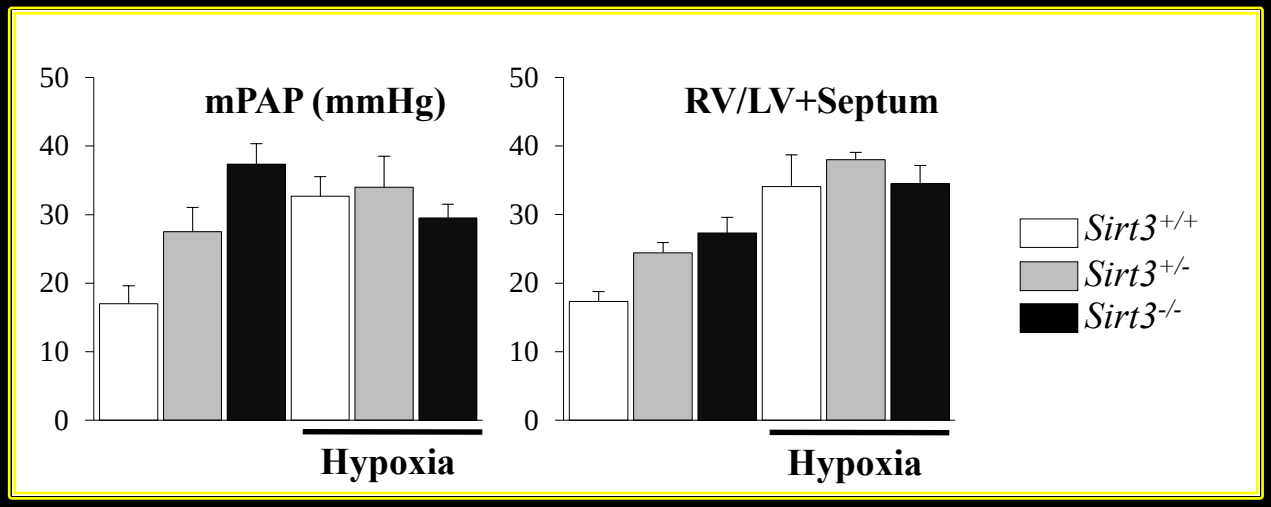
Paulin et al, Cell Metabolism, 2014

UCP2



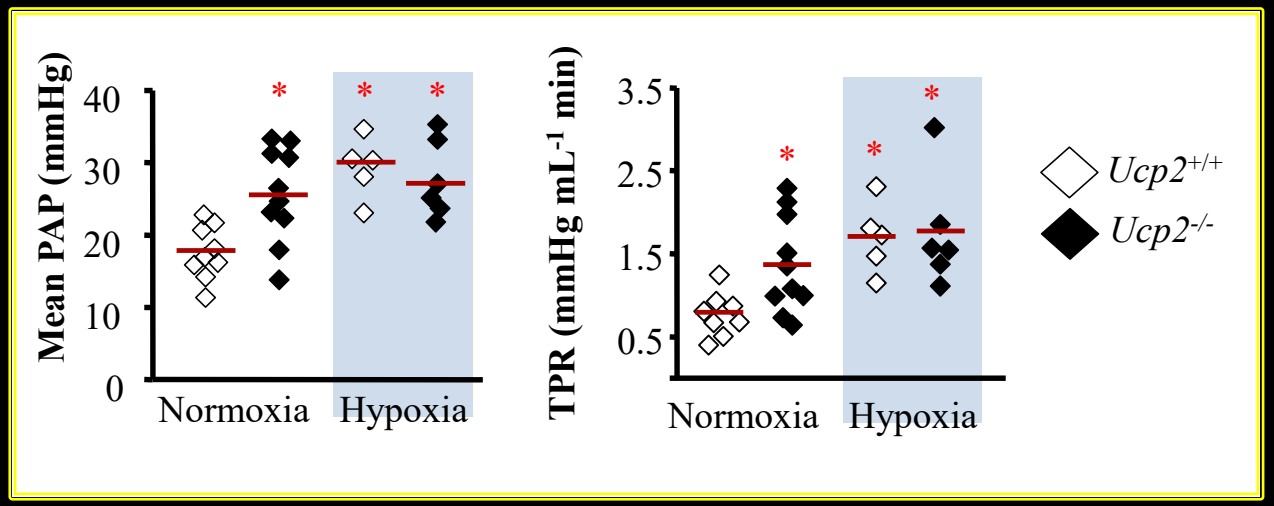
Dromparis et al, Circulation Research 2013

Sirt3$^{-/-}$ mice develop spontaneous PAH in a dose-dependent manner



Paulin et al,
Cell Metabolism, 2014

Ucp2$^{-/-}$ mice develop spontaneous PAH



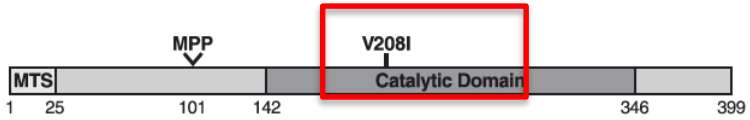
Dromparis et al,
Circulation Research 2013

Human SIRT3 Polymorphism

Chromosome 11: NCBI NC_000011.9

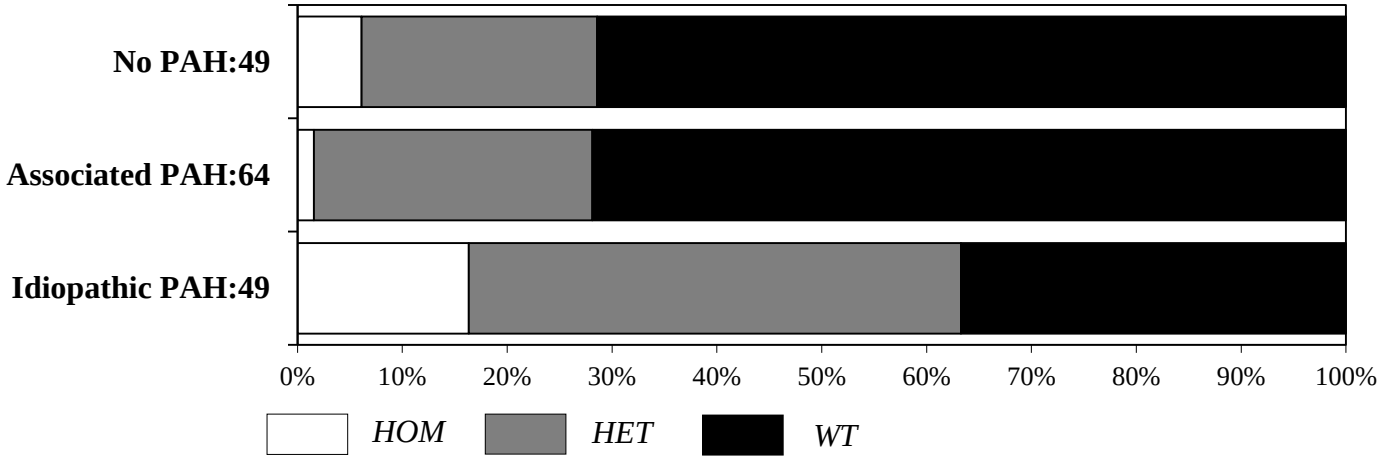
Mol Cell. 2011 Oct 21;44(2):177-90.

Allele change: [C>T] → 208 [Val>Ile]

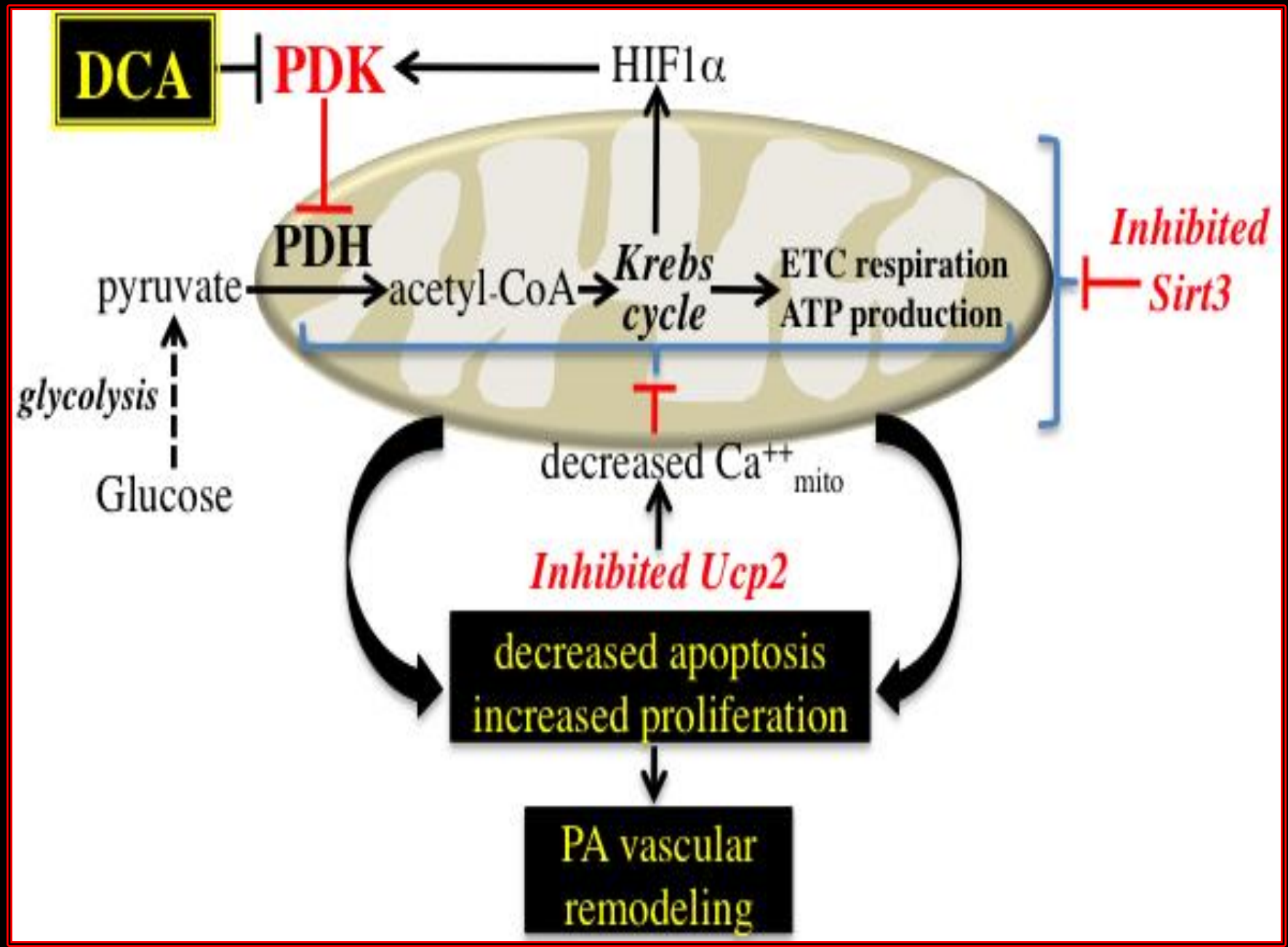


34% decrease in enzyme activity

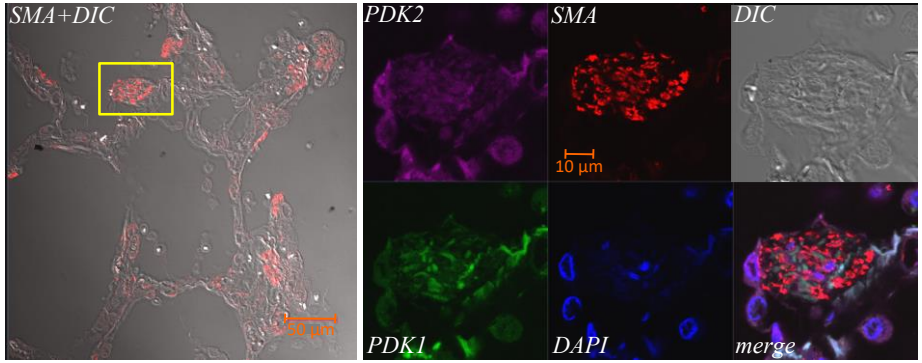
	HOM (AA)	HET (AG)	WT (GG)
No PAH n=49	3 (6.12%)	11 (22.45%)	35 (71.42%)
Associated PAH n=64	1 (1.56%)	17 (26.56%)	46 (71.88%)
Idiopathic PAH n=49	8 (16.32%)	23 (46.94%)	18 (36.73%)



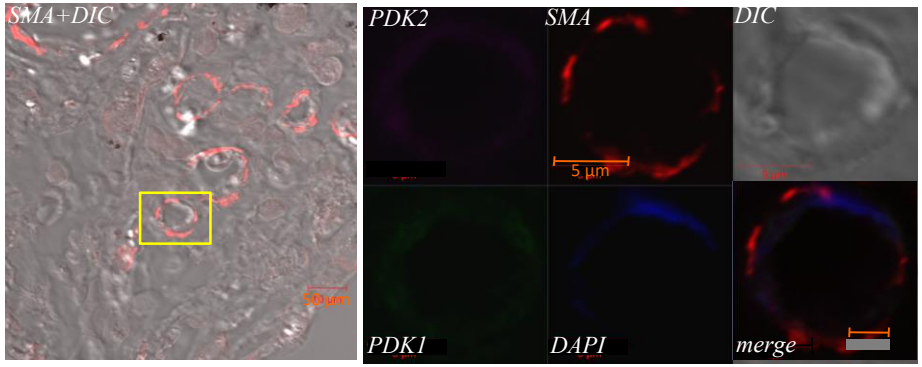
Paulin et al, Cell Metabolism, 2014

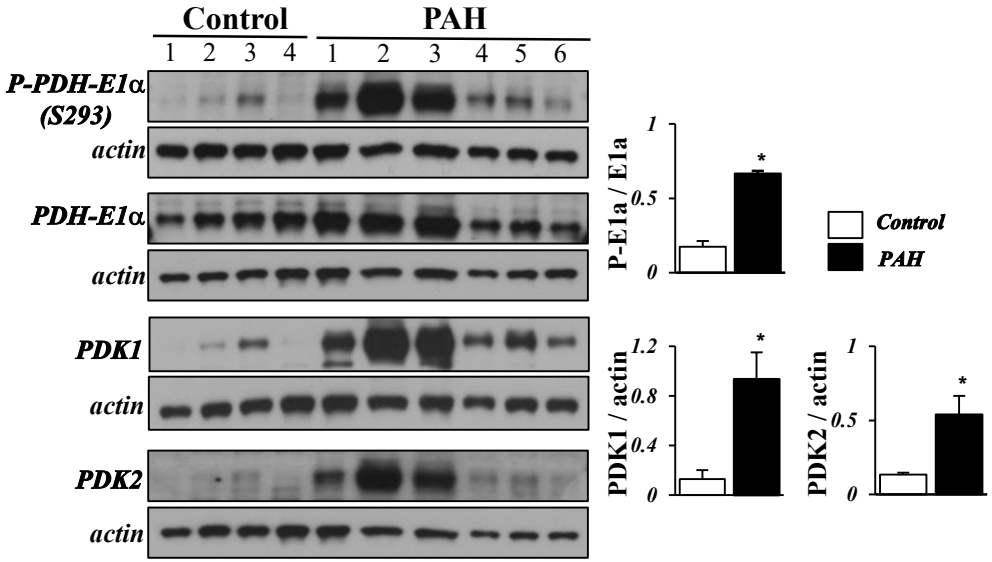

DCA
PDK
HIF1α
PDH
pyruvate
acetyl-CoA
Krebs cycle
ETC respiration
ATP production
Inhibited
Sirt3
glycolysis
Glucose
decreased Ca++ mito
Inhibited Ucp2
decreased apoptosis
increased proliferation
PA vascular remodeling

PAH *patient 1*



Control (no PAH) *patient 2*





“Loss-of-function” gene variants (SNPs) in the Sirt3 and Ucp2 genes

SNP score interpretation

SNP score	Interpretation
0	Homozygous wild-type
1	1 affected allele of either gene (heterozygous)
2	2 affected alleles of the same gene (homozygous) or 1 affected allele for each gene (heterozygous)
3	2 affected alleles for 1 gene (homozygous) plus 1 affected allele for the other (heterozygous)
4	Homozygous for both variants

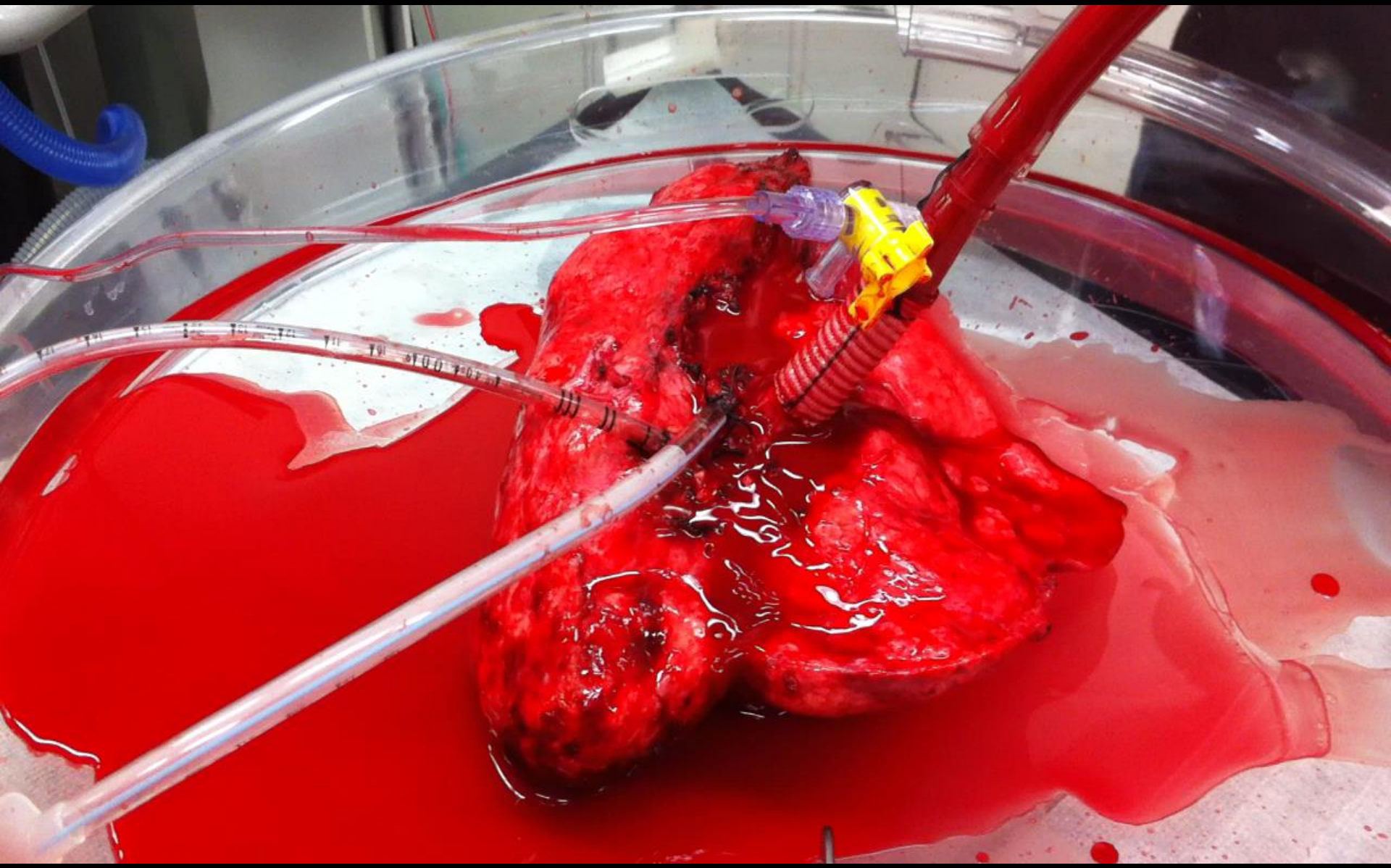
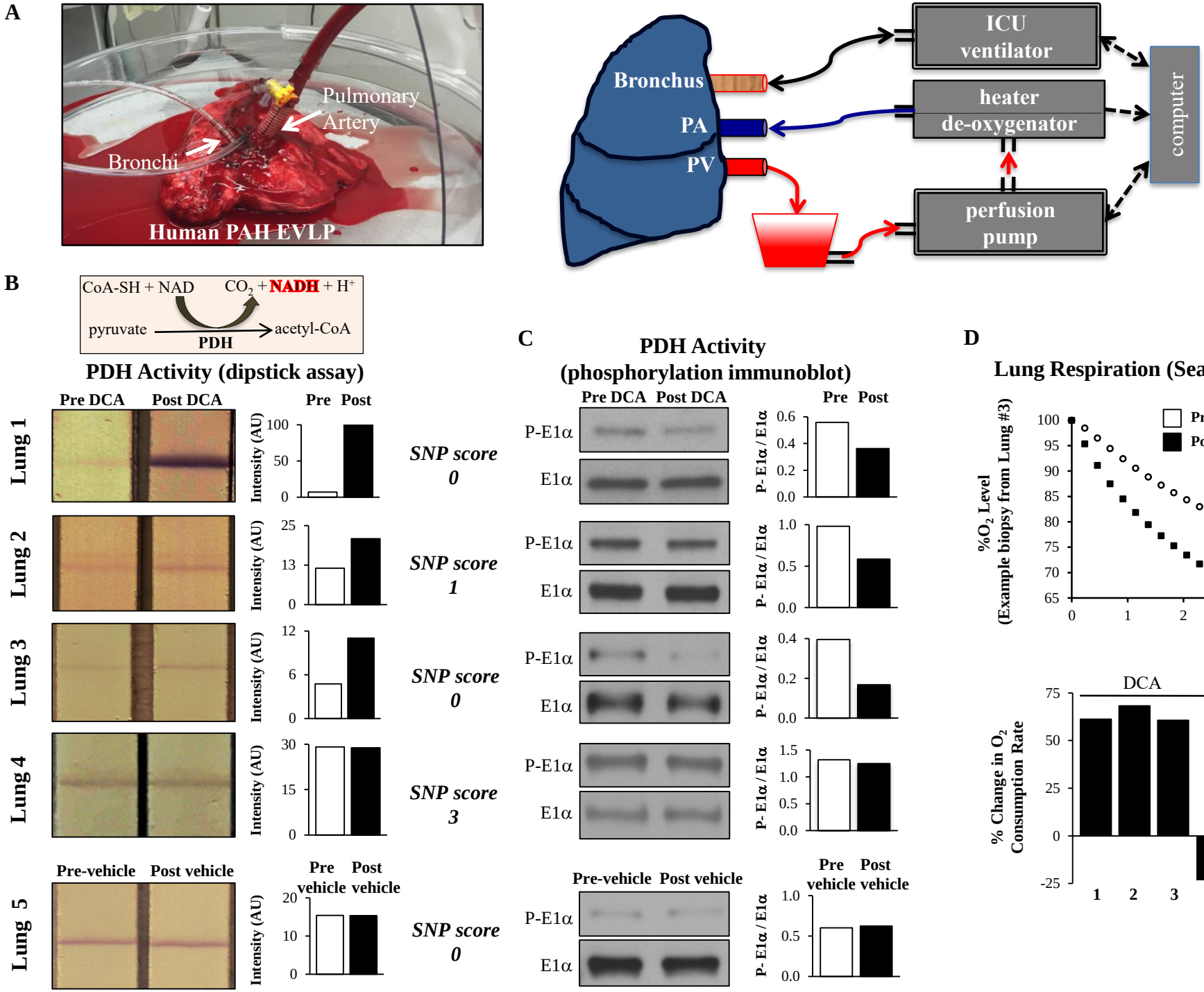
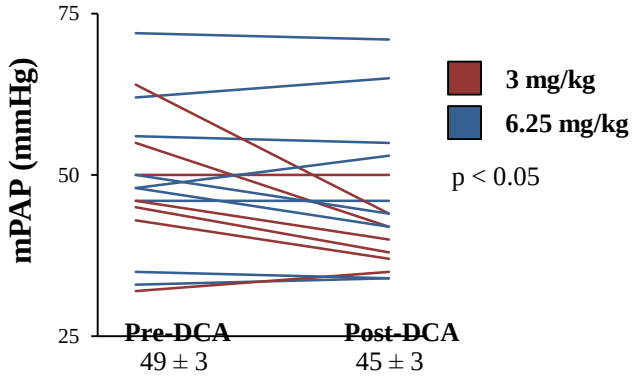
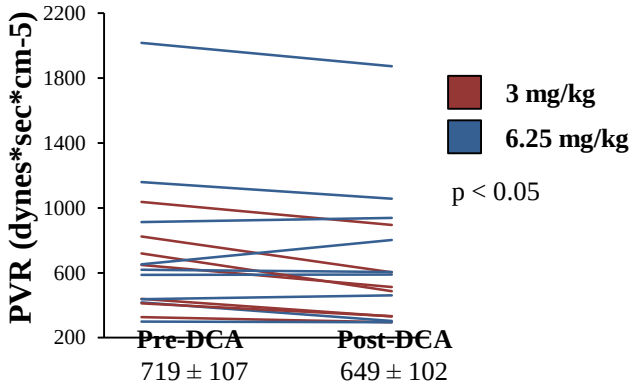
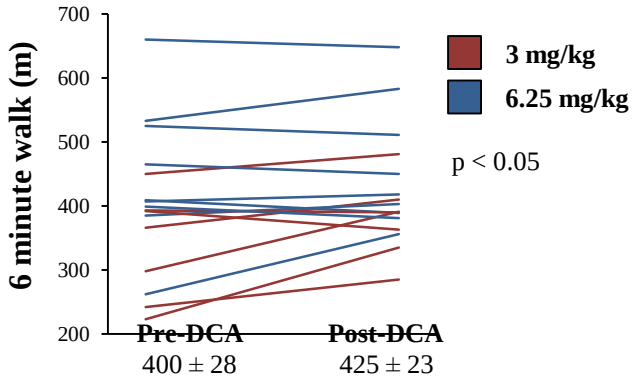


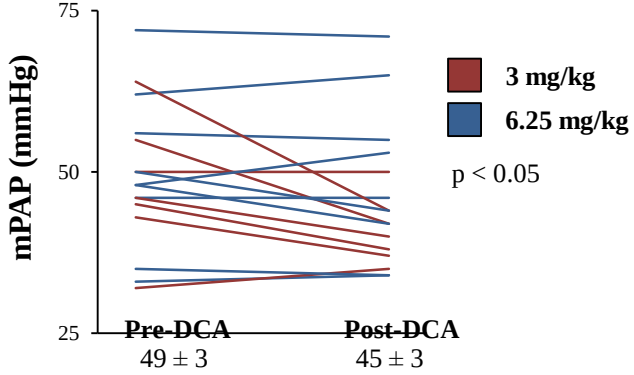
Fig. 2

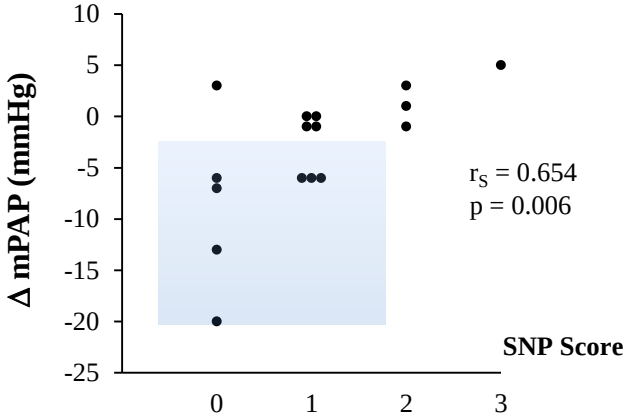

A
Pulmonary Artery
Bronchi
Human PAH EVLP
Bronchus
PA
PV
ICU ventilator
heater de-oxygenator
perfusion pump
computer
B
CoA-SH + NAD
CO2 + NADH + H+
pyruvate
acetyl-CoA
PDH
PDH Activity (dipstick assay)
Pre DCA
Post DCA
Pre
Post
Lung 1
Lung 2
Lung 3
Lung 4
Lung 5
Intensity (AU)
SNP score 0
SNP score 1
SNP score 0
SNP score 3
Pre-vehicle
Post vehicle
SNP score 0
C
PDH Activity (phosphorylation immunoblot)
P-E1α
E1α
P- E1α / E1α
D
Lung Respiration (Seahorse)
%O2 Level (Example biopsy from Lung #3)
Pre-DCA
Post-DCA
Time(s)
% Change in O2 Consumption Rate
DCA
vehicle
Lung #

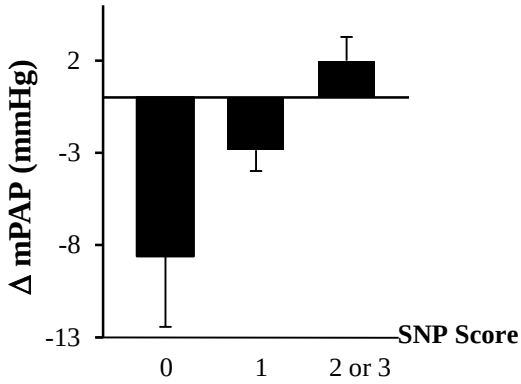

mPAP (mmHg)
75
50
25
3 mg/kg
6.25 mg/kg
p < 0.05
Pre-DCA
49 ± 3
Post-DCA
45 ± 3

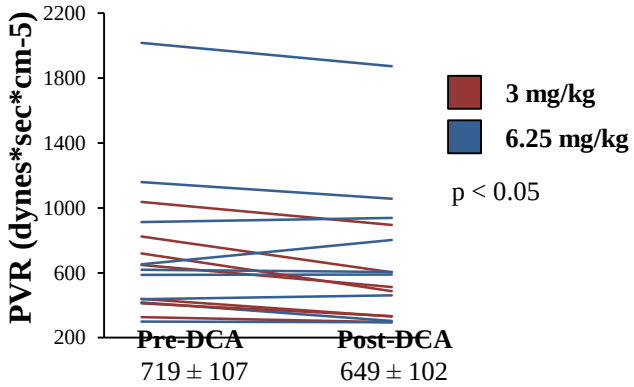

PVR (dynes*sec*cm-5)
2200
1800
1400
1000
600
200
3 mg/kg
6.25 mg/kg
p < 0.05
Pre-DCA
719 ± 107
Post-DCA
649 ± 102

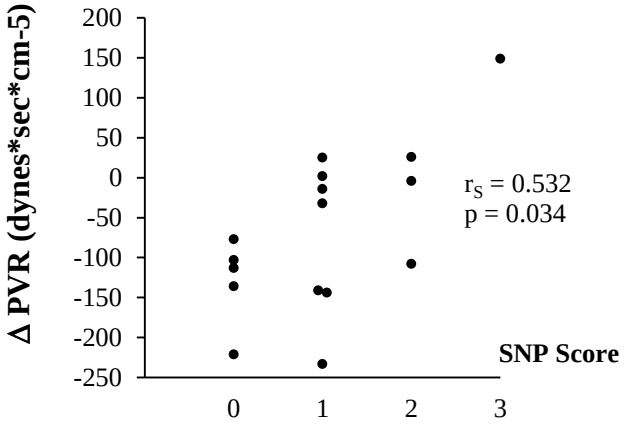

6 minute walk (m)
700
600
500
400
300
200
3 mg/kg
6.25 mg/kg
p < 0.05
Pre-DCA
400 ± 28
Post-DCA
425 ± 23

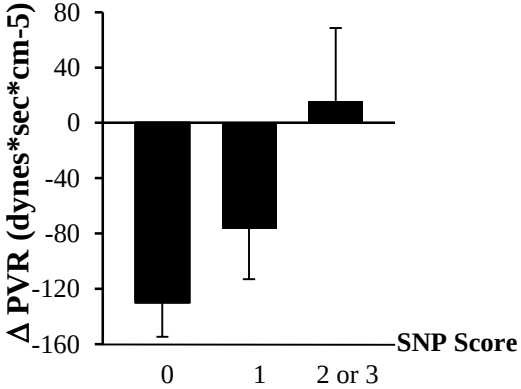

mPAP (mmHg)
75
50
25
3 mg/kg
6.25 mg/kg
p < 0.05
Pre-DCA
49 ± 3
Post-DCA
45 ± 3

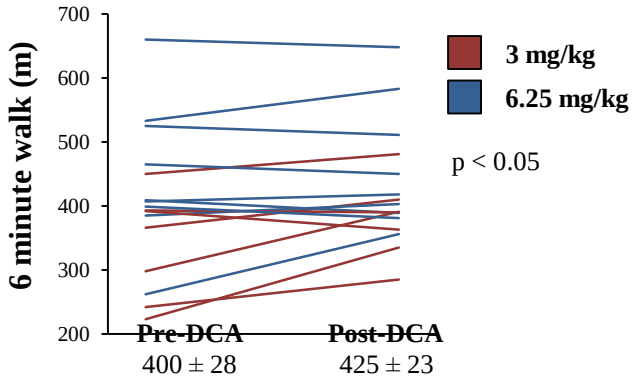

Δ mPAP (mmHg)
10
5
0
-5
-10
-15
-20
-25
rS = 0.654
p = 0.006
SNP Score
0
1
2
3

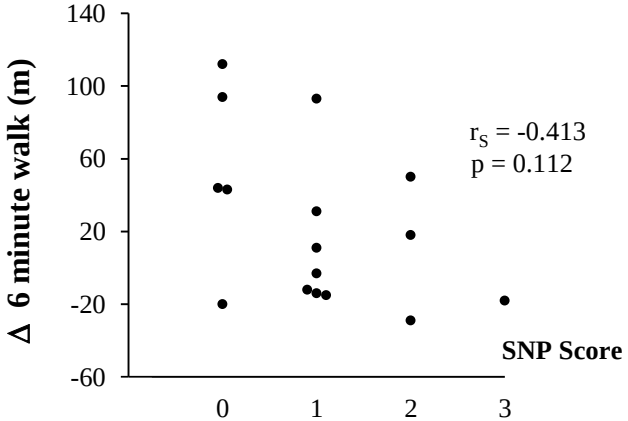

Δ mPAP (mmHg)
2
-3
-8
-13
SNP Score
0
1
2 or 3

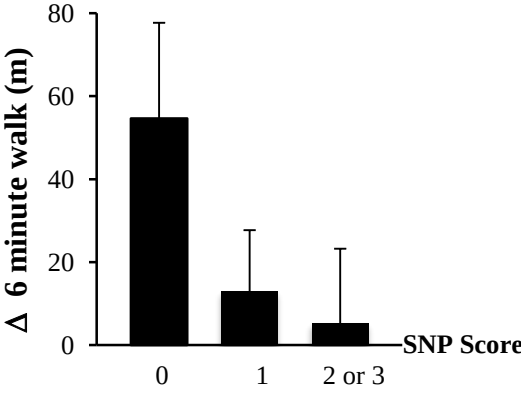

PVR (dynes*sec*cm-5)
2200
1800
1400
1000
600
200
3 mg/kg
6.25 mg/kg
p < 0.05
Pre-DCA
719 ± 107
Post-DCA
649 ± 102

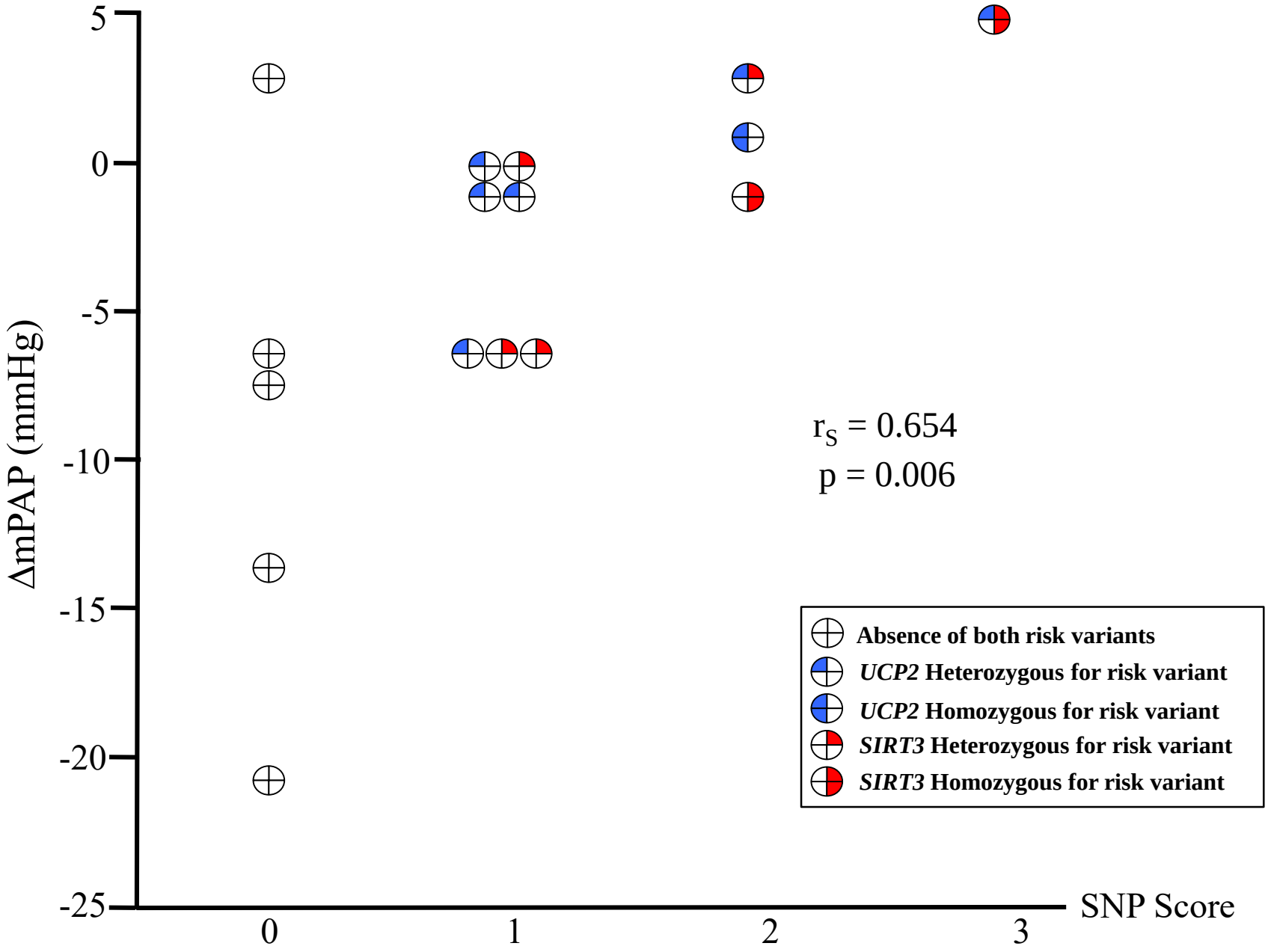

Δ PVR (dynes*sec*cm-5)
200
150
100
50
0
-50
-100
-150
-200
-250
rS = 0.532
p = 0.034
SNP Score
0
1
2
3


Δ PVR (dynes*sec*cm-5)
80
40
0
-40
-80
-120
-160
SNP Score
0
1
2 or 3


6 minute walk (m)
700
600
500
400
300
200
3 mg/kg
6.25 mg/kg
p < 0.05
Pre-DCA
400 ± 28
Post-DCA
425 ± 23

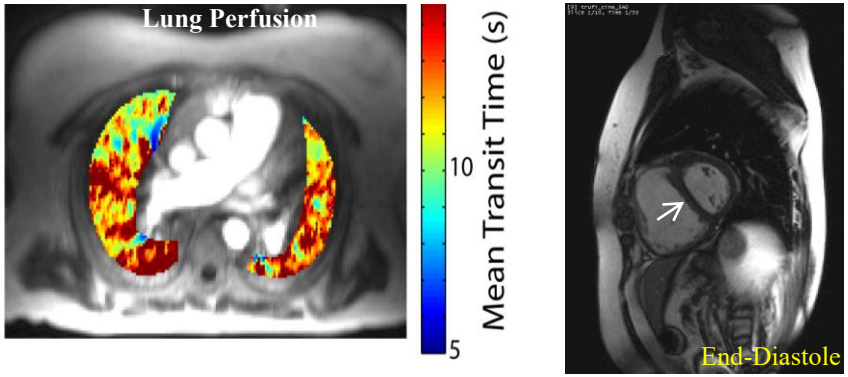

Δ 6 minute walk (m)
140
100
60
20
-20
-60
rS = -0.413
p = 0.112
SNP Score
0
1
2
3


Δ 6 minute walk (m)
80
60
40
20
0
SNP Score
0
1
2 or 3


5
0
-5
-10
-15
-20
-25
ΔmPAP (mmHg)
0
1
2
3
SNP Score
r_S = 0.654
p = 0.006
Absence of both risk variants
UCP2 Heterozygous for risk variant
UCP2 Homozygous for risk variant
SIRT3 Heterozygous for risk variant
SIRT3 Homozygous for risk variant

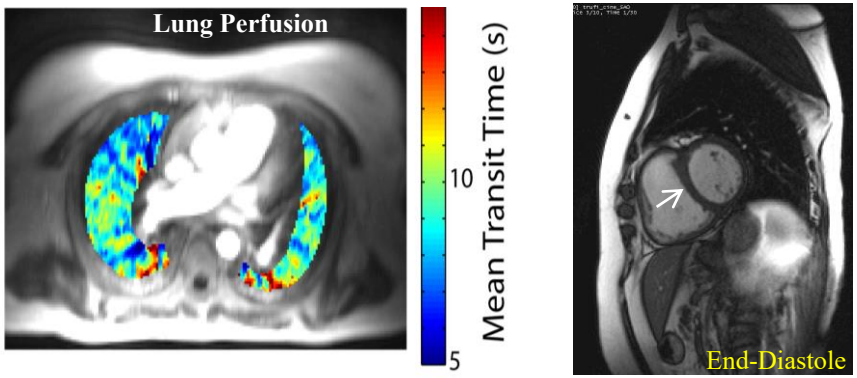
Pre-DCA

mPAP=55

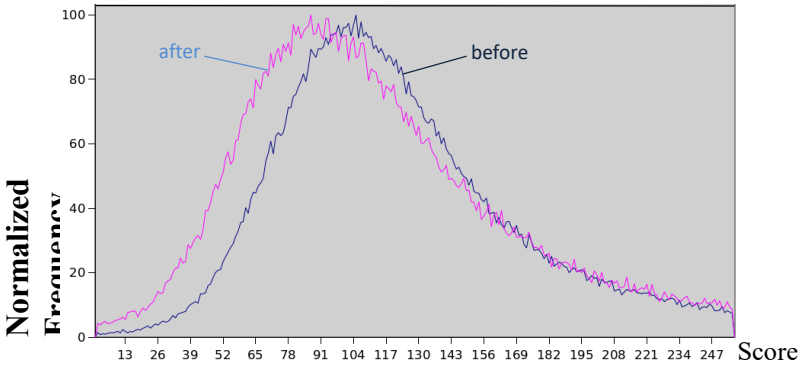


Post-DCA

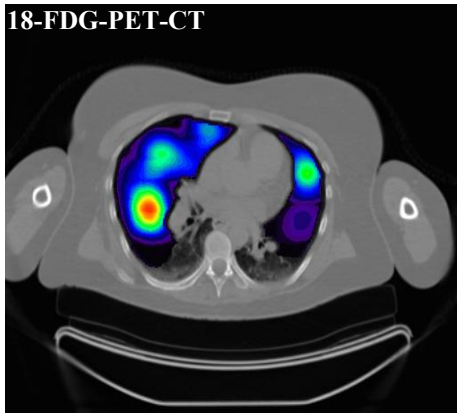
mPAP=42



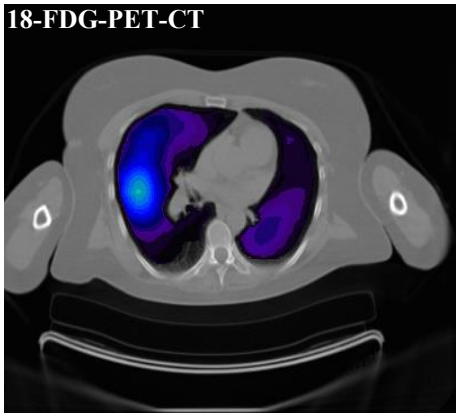
Distribution of 18-FDG uptake before and after DCA

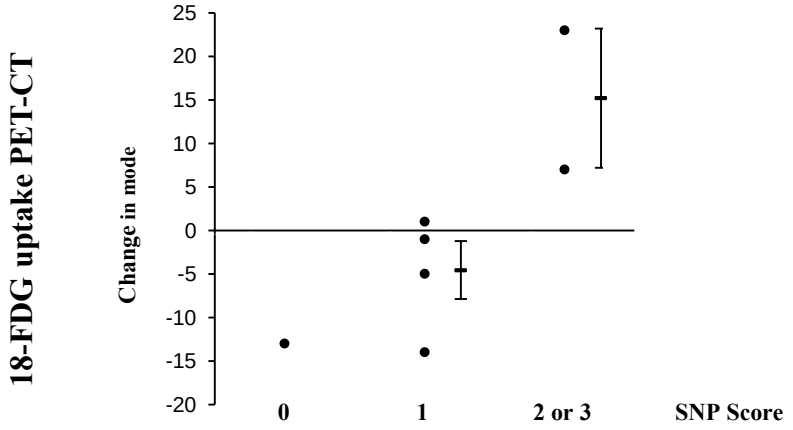


Pre-DCA



Post-DCA

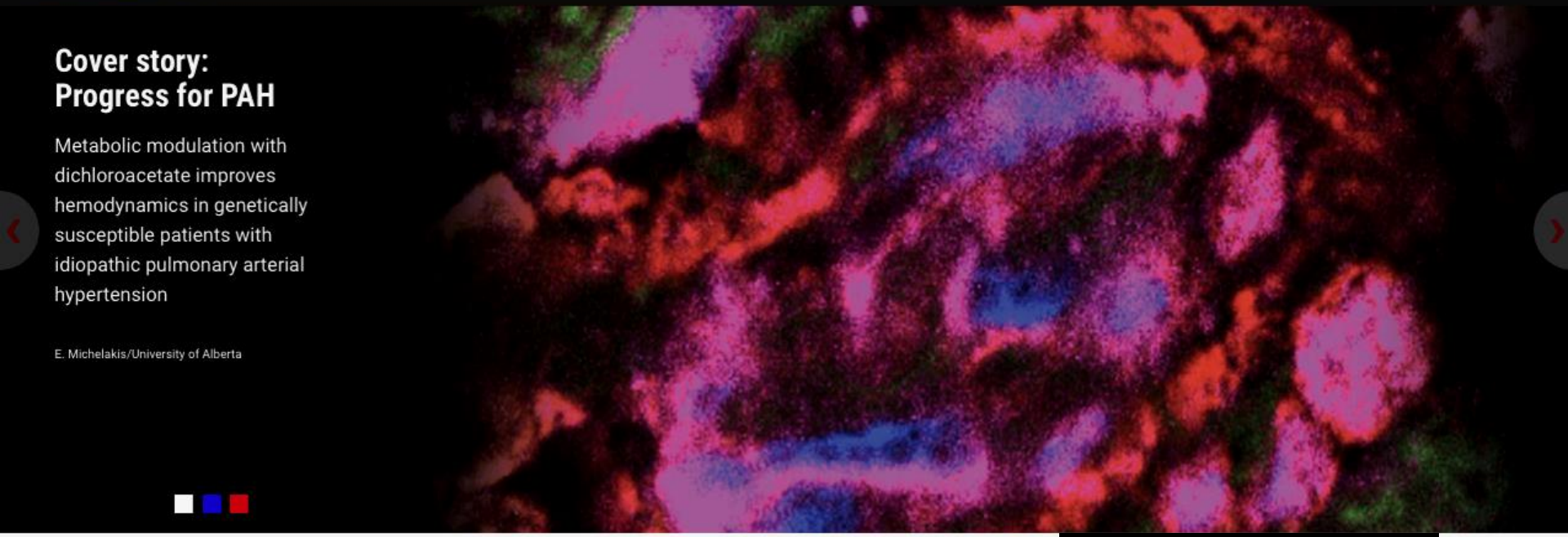




Cover story: Progress for PAH

Metabolic modulation with dichloroacetate improves hemodynamics in genetically susceptible patients with idiopathic pulmonary arterial hypertension

E. Michelakis/University of Alberta



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RESEARCH ARTICLES

Inhibition of pyruvate dehydrogenase kinase improves pulmonary arterial hypertension in genetically susceptible patients

BY EVANGELOS D. MICHELAKIS, VIKRAM GURTU, LINDA WEBSTER, GARETH BARNES, GEOFFREY WATSON, LUKE HOWARD, JOHN CUPITT, IAN PATERSON, RICHARD B. THOMPSON, KELVIN CHOW, DECLAN P. O'REGAN, LAN ZHAO, JOHN WHARTON, DAVID G. KIELY, ADAM KINNAIRD, ARISTEIDIS E. BOUKOURIS, CHRIS WHITE, JAYAN NAGENDRAN, DARREN H. FREED, STEPHEN J. WORT, J. SIMON R. GIBBS, MARTIN R. WILKINS

SCIENCE TRANSLATIONAL MEDICINE | 25 OCT 2017 |

Metabolic modulation with dichloroacetate improves hemodynamics in genetically susceptible patients with idiopathic pulmonary arterial hypertension.

THE CHANGING FACE OF CLINICAL TRIALS

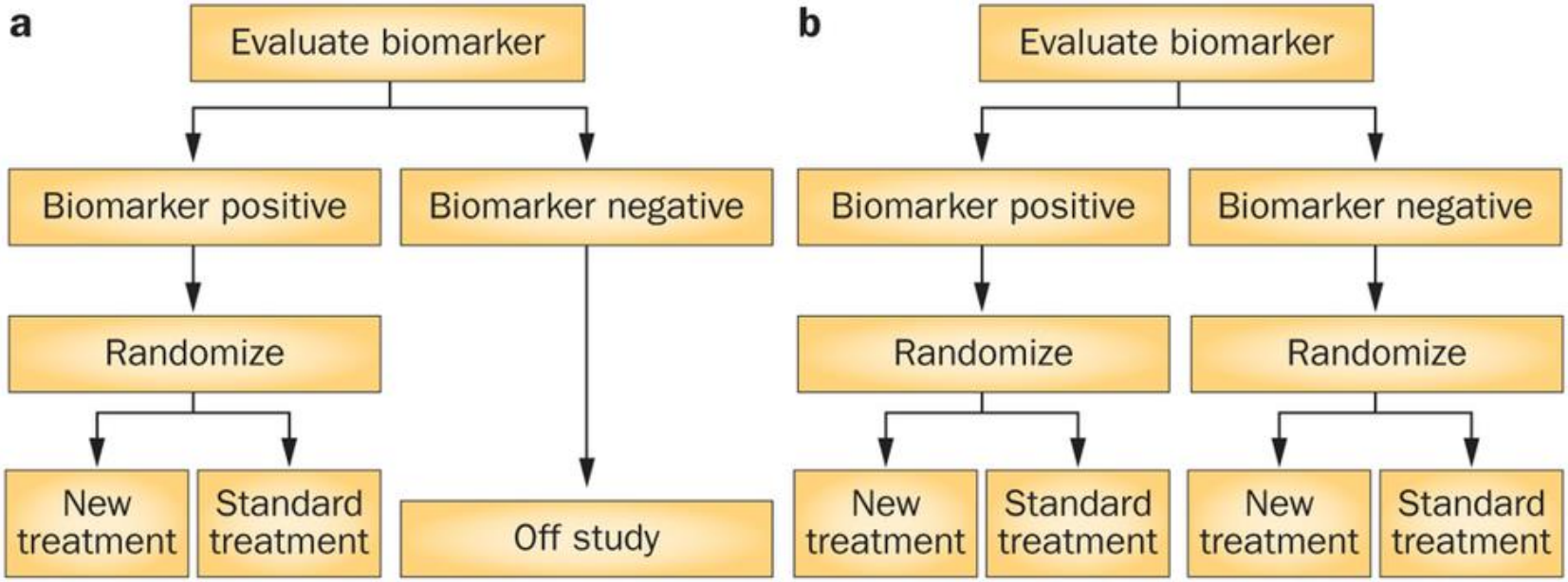
Jeffrey M. Drazen, M.D., David P. Harrington, Ph.D., John J.V. McMurray, M.D., James H. Ware, Ph.D., and Janet Woodcock, M.D., *Editors*

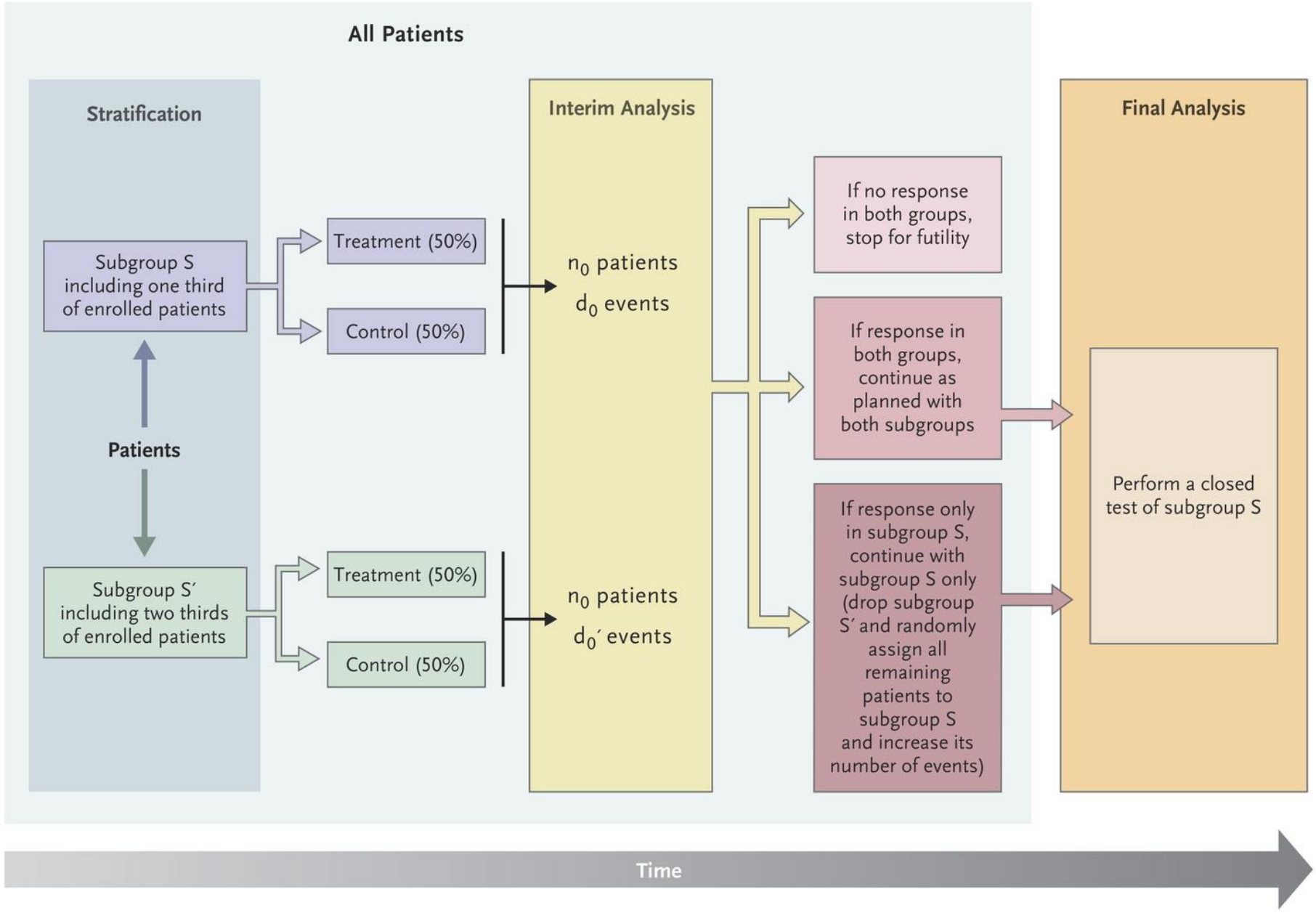
Adaptive Designs for Clinical Trials

Deepak L. Bhatt, M.D., M.P.H., and Cyrus Mehta, Ph.D.

N Engl J Med 2016;375:65-74.

Biomarker-Driven Adaptive Population-Enrichment Designs




All Patients
Stratification
Subgroup S including one third of enrolled patients
Patients
Subgroup S´ including two thirds of enrolled patients
Treatment (50%)
Control (50%)
Treatment (50%)
Control (50%)
Interim Analysis
n_0 patients
d_0 events
n_0 patients
d_0´ events
If no response in both groups, stop for futility
If response in both groups, continue as planned with both subgroups
If response only in subgroup S, continue with subgroup S only (drop subgroup S´ and randomly assign all remaining patients to subgroup S and increase its number of events)
Final Analysis
Perform a closed test of subgroup S
Time

H. Matisse, Icarus (1943)

Thank you

Vikram Gurtu
Sotiris Zervopoulos
Aris Boukouris
Peter Dromparis
Roxane Paulin
Adam Kinaird
Al Haromy

Linda Webster

Gopi Sutendra
Sebastien Bonnet
Daren Freed
Jayan Nagendran
Martin Wilkins