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Endoarterial Biopsy in Pulmonary Hypertension

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Outline

- Pulmonary endoarterial biopsy
- Precision therapy
- Creation of large animal models
- Selective therapy

Pulmonary Arterial Hypertension

- Occlusive disease of the pulmonary arteries leading to severe hemodynamic abnormalities, right heart failure, and premature death
- Despite recent advances and new clinically approved drugs, yearly mortality continues to be about 15%, and the 5-year survival for PAH remains close to 60%

A limitation

- PAH is associated with disordered signaling and growth in both pulmonary endothelial and smooth muscle cells
- The molecular mechanisms of PAH are incompletely understood
- The inaccessibility of pulmonary vascular tissue has limited studies to better define the mechanisms of PAH

A potential solution

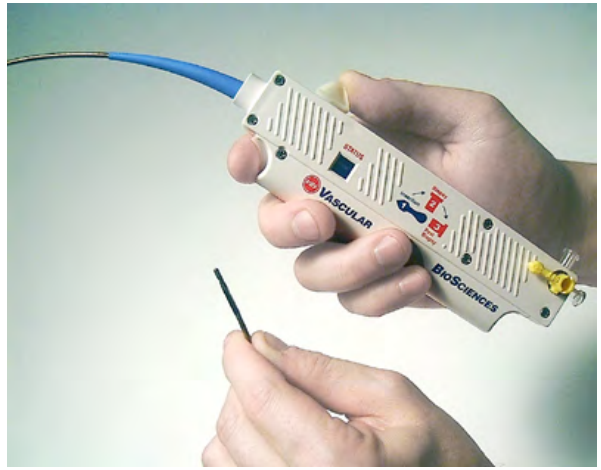
- A minimally invasive method to obtain endovascular samples coupled with recently developed genetic expression analyses could enhance our understanding of the histomolecular processes associated with PAH and lead to improved therapies

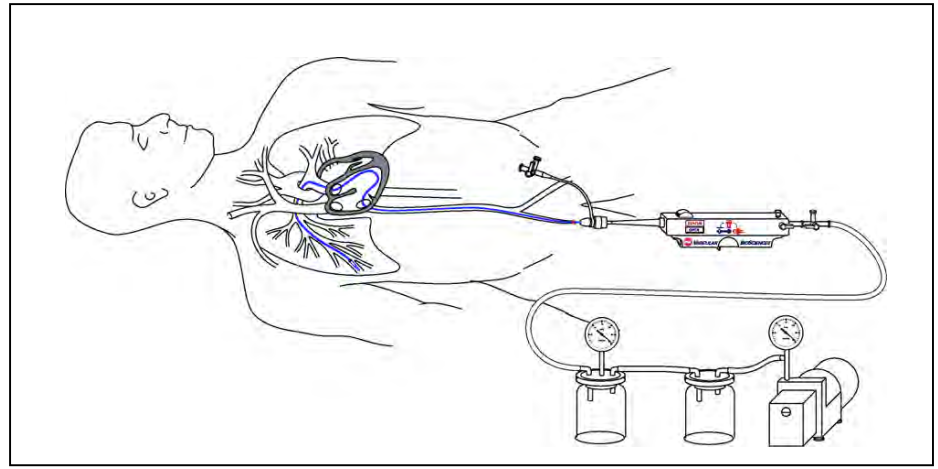
Experimental studies – endoarterial biopsy

- OBJECTIVE: Evaluate the performance of a new arterial biopsy catheter in obtaining endovascular samples in normotensive and hypertensive animals, in a lung transplant model of rejection and in “shunt” models of pulmonary hypertension

The Endoarterial Biopsy Catheter

- 7.9F device (8F - long sheath)
- Percutaneous (jugular or femoral venous access)

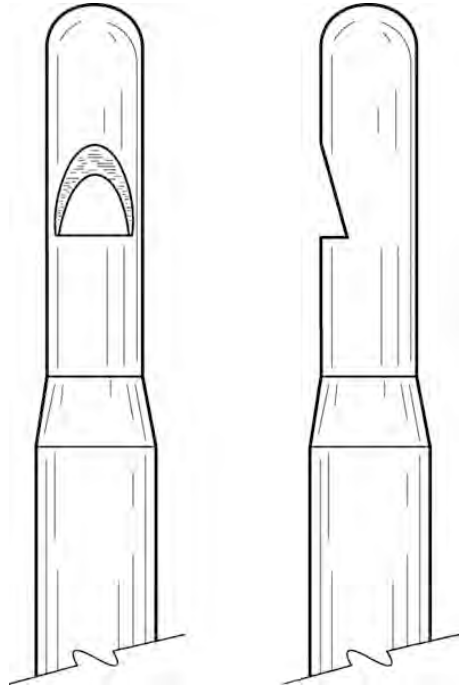




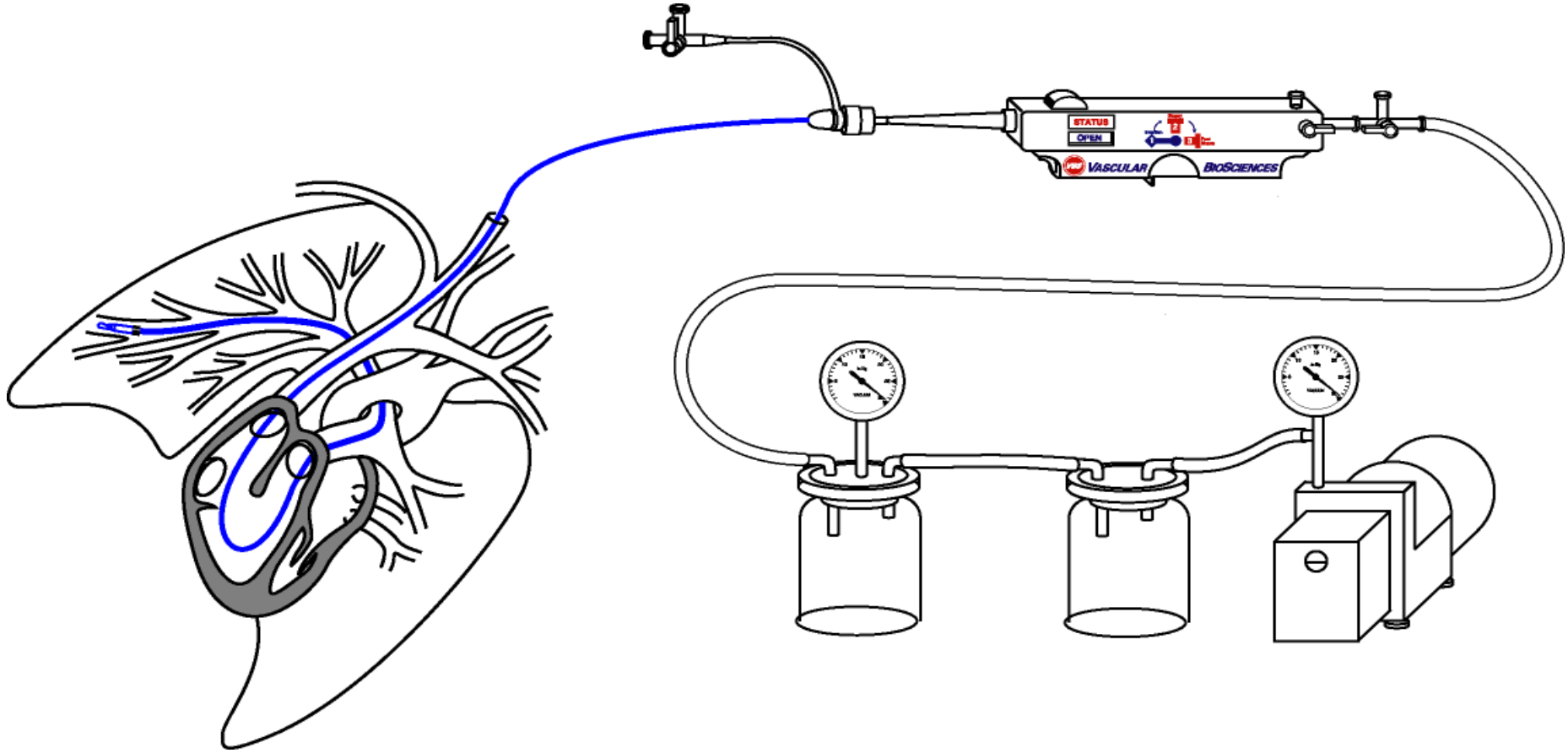
The Endoarterial Biopsy Catheter

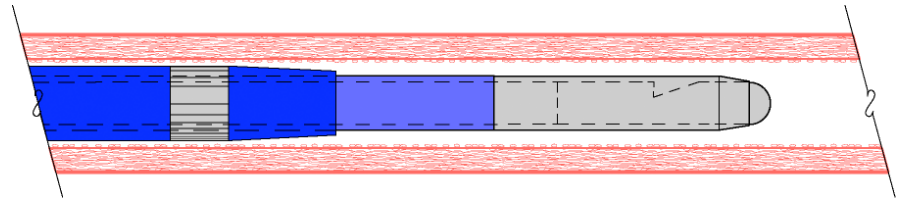
- Two wire-reinforced flexible tubes
- Inner tube has a stainless steel distal end with a beveled opening
- A vacuum is coupled to the inner tube to draw in arterial tissue into the beveled opening
- Outer tube stainless steel cutting distal end cuts arterial tissue when advanced

Distal End of Biopsy Catheter

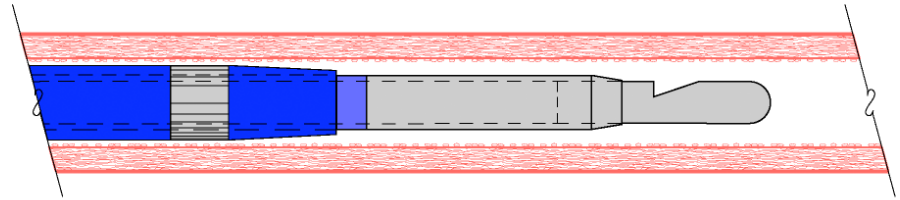


Procedural Configuration

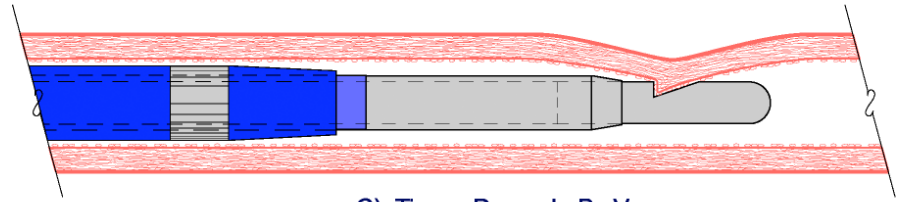




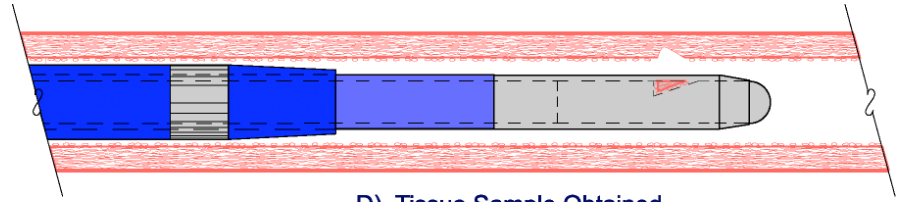
A) Closed Catheter



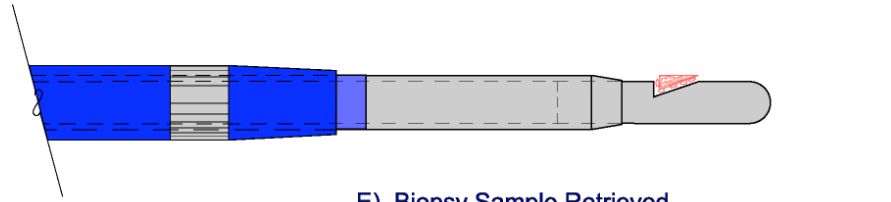
B) Open Catheter



C) Tissue Drawn In By Vacuum

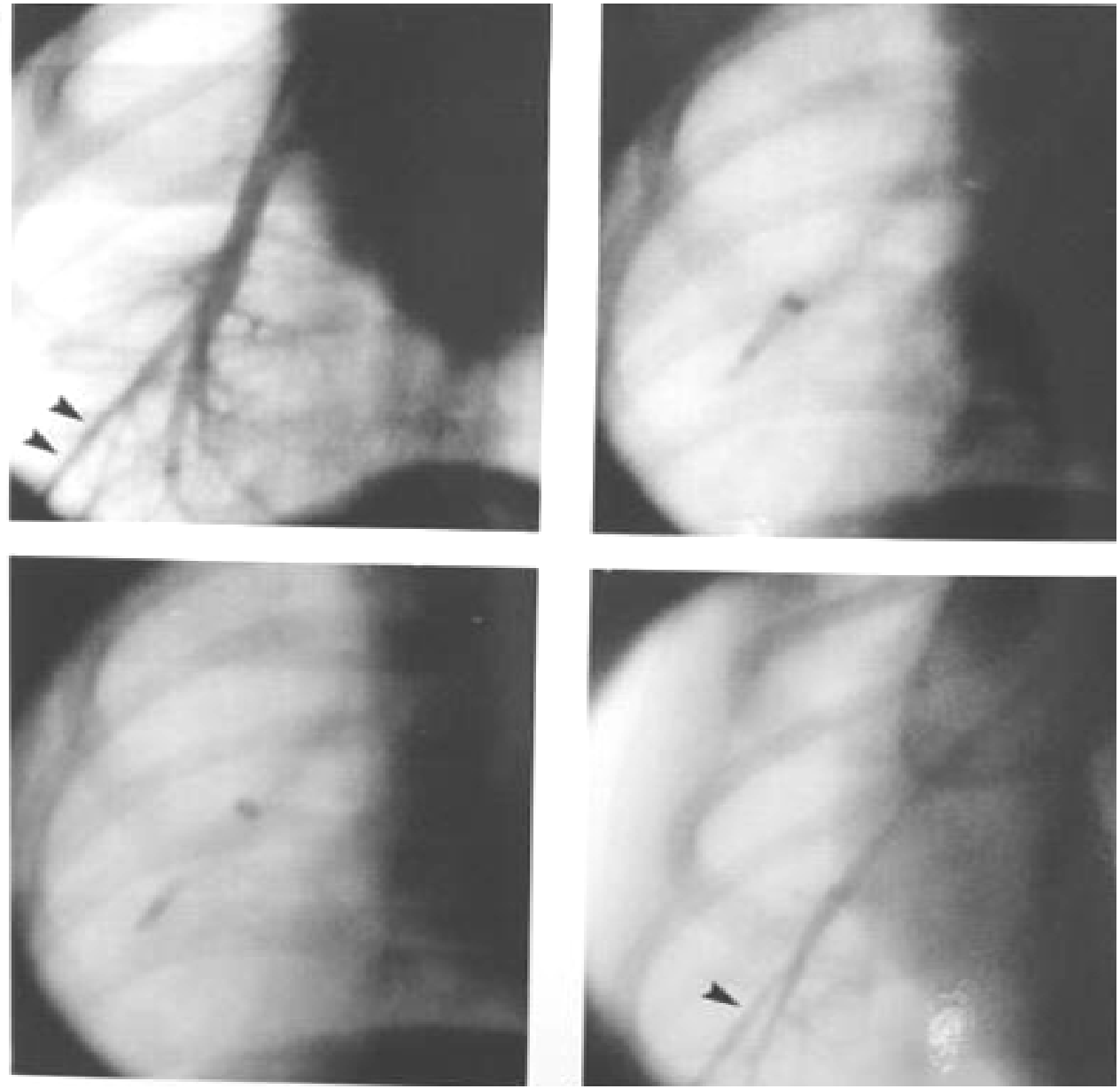


D) Tissue Sample Obtained



E) Biopsy Sample Retrieved

Biopsy Sample



Methods

- Normotensive and hypertensive animals
- External jugular vein catheterization
- 2-3 mm distal pulmonary artery biopsy
- Multiple biopsy samples obtained from targeted vessels
- Angiograms obtained pre and post biopsy

Hypertensive Model

- Monthly infusion of 0.3-0.9 mm ceramic microspheres into superior vena cava
- PA pressure increased acutely after each microsphere infusion, partially recovered between infusions, and steadily increased over time
- Biopsy at systolic PA pressures 10-100 mmHg

Endoarterial biopsy results

- Sample retrieval success rate > 75%
- Minimal clinical effect of the procedure: no deaths, uncomplicated recovery from anesthesia
- Biopsy samples: average size = 1.0 x 1.0 x 0.3 mm, presence of endothelium = 68%, increased intimal thickness with more hypertension

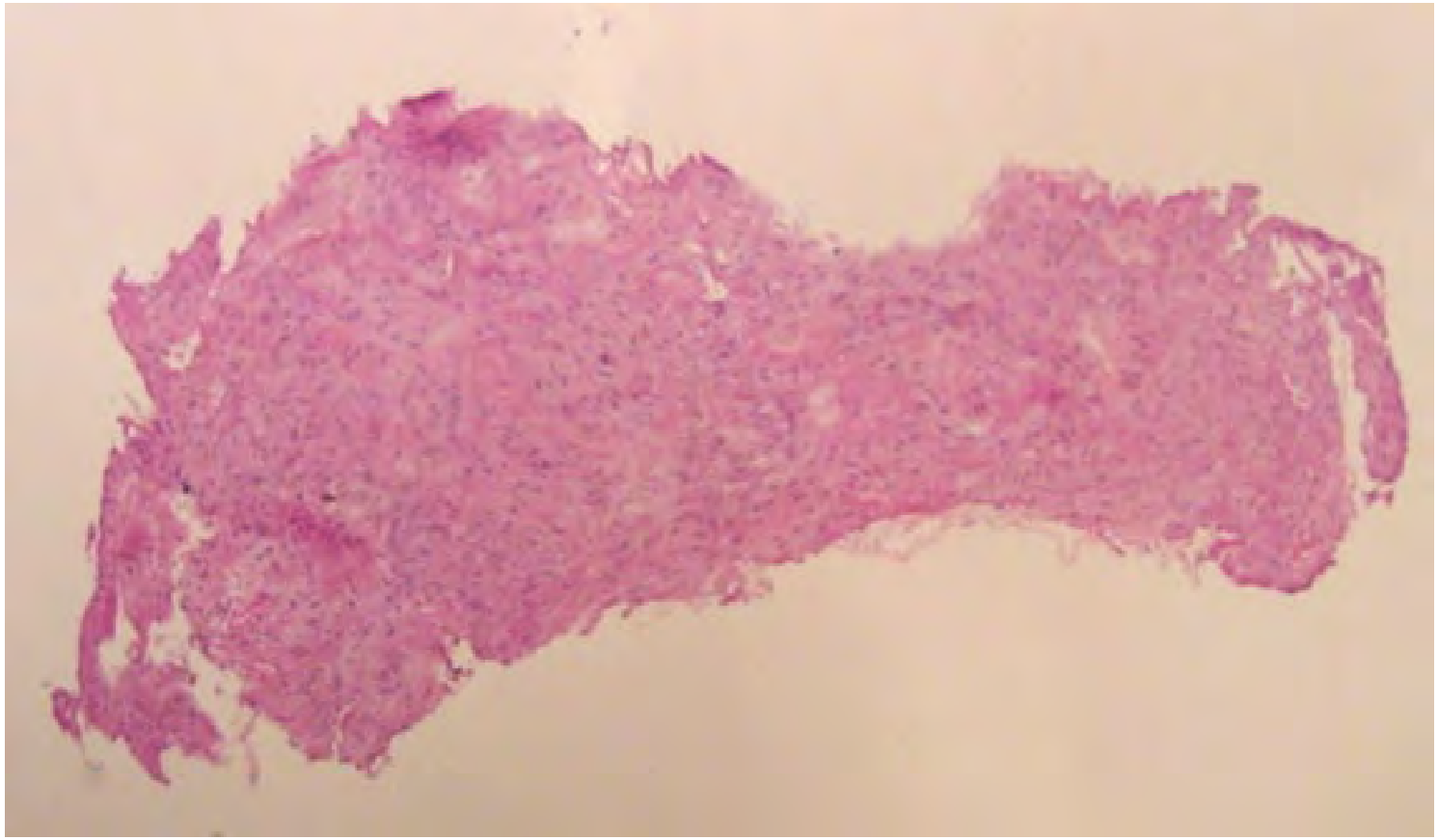
Angiography

- normotensive: 15% vascular irregularities
- hypertensive: rare vascular irregularities, 16% transiently occluded vessels
- Follow-up angiography 1 month later – occlusions resolved, no aneurysms, thrombi or hemorrhage

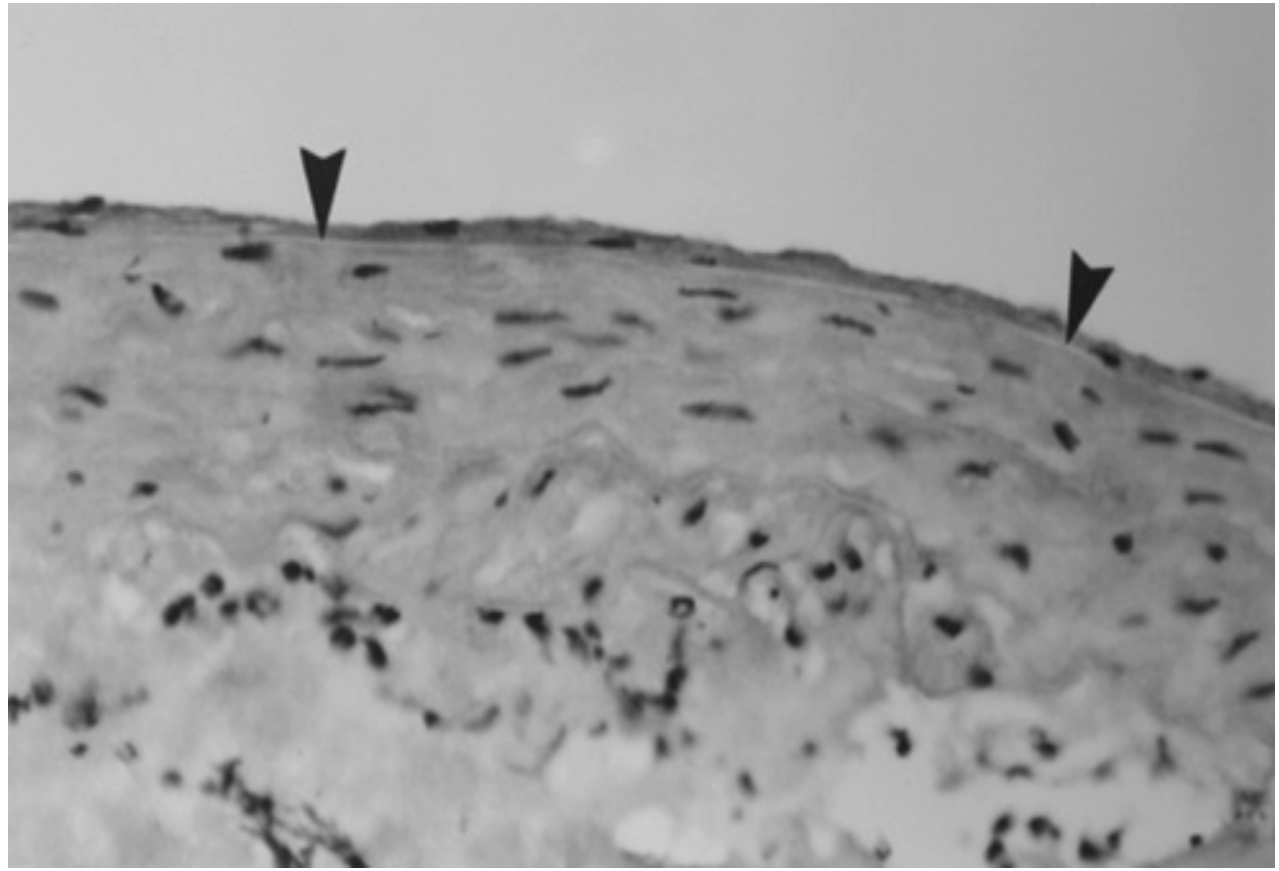
Cell Culture

- Smooth muscle cells and endothelial cells successfully cultured from biopsy samples

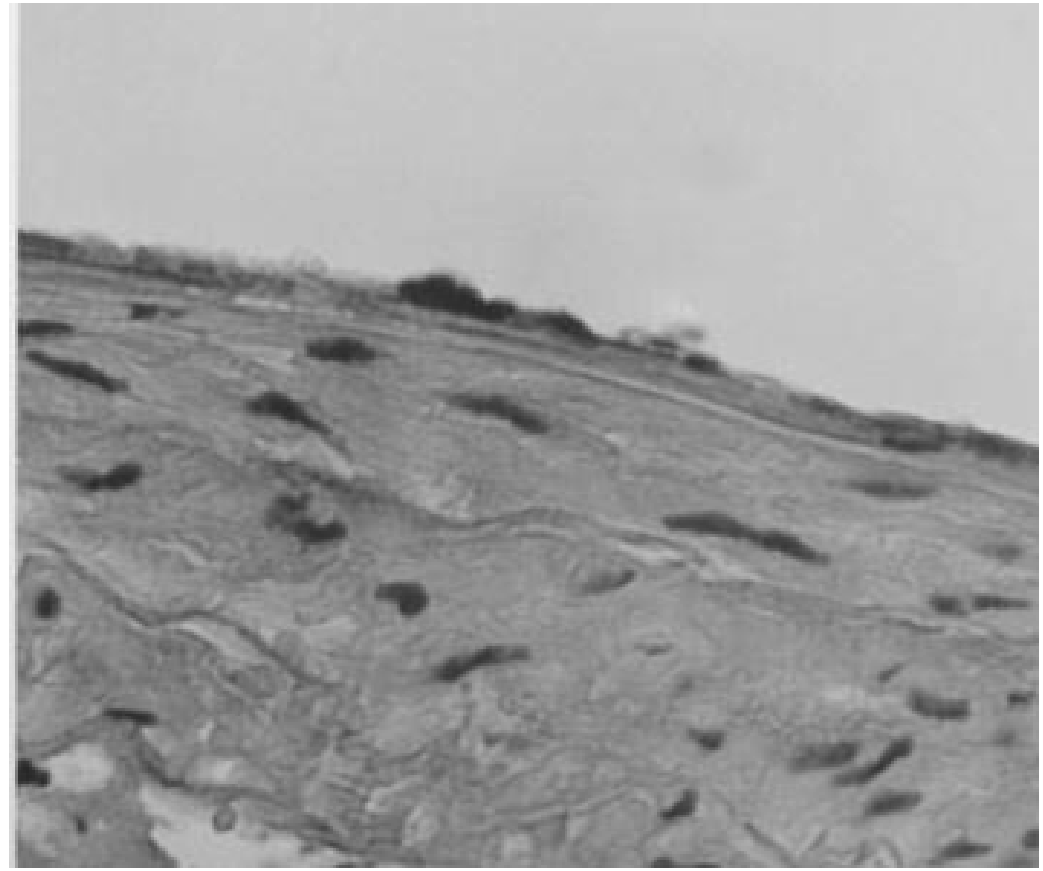
Biopsy Sample



Elastic Lamina



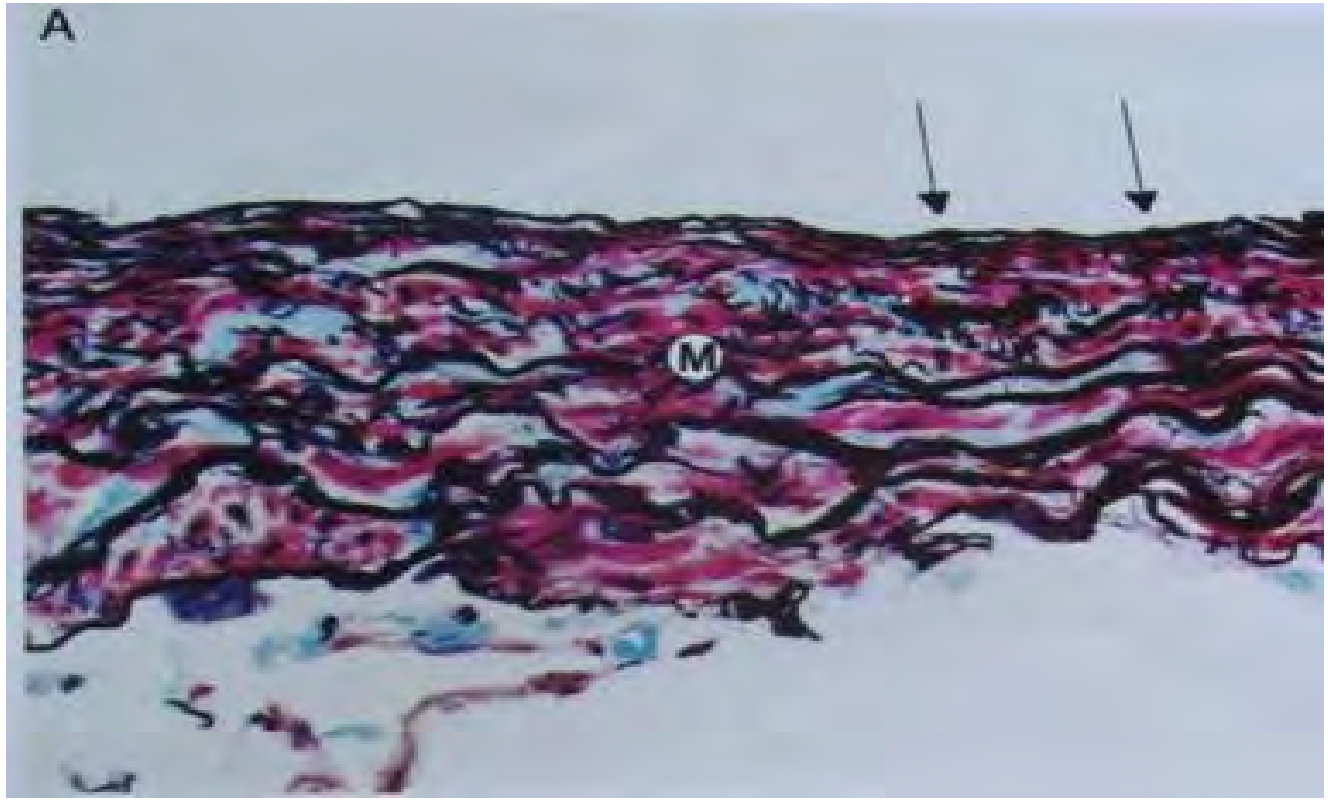
Endothelial Layer



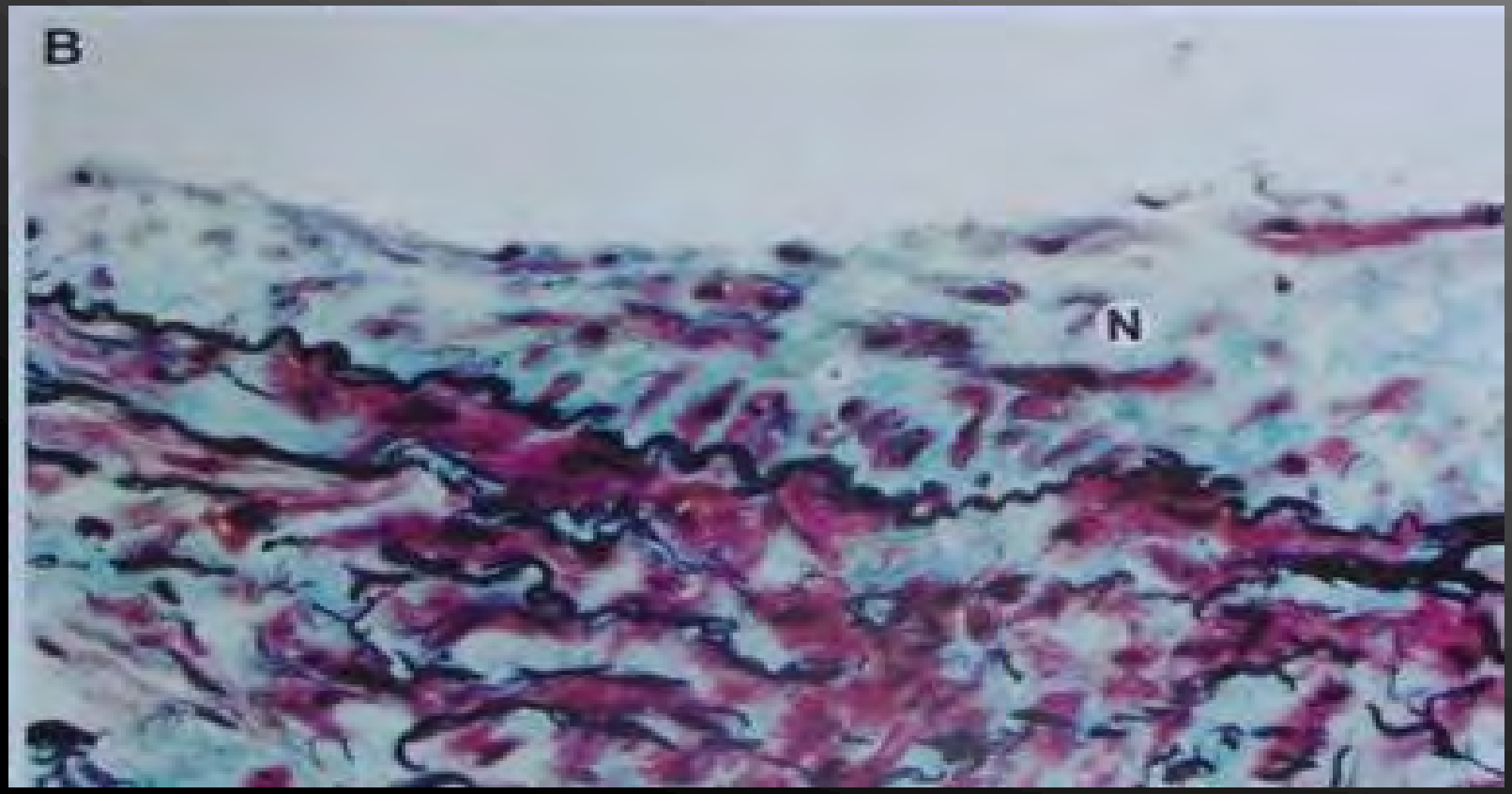
Chronic Study

- Follow-up angiography at 2 weeks and 2 months
  All had smooth vascular contours
  At necropsy, biopsy sites were difficult to identify
  Histology: biopsy sites showed mild localized neointimal hyperplasia, no perivascular hemorrhage

Trichrome Stain Normotensive



Trichrome Stain Hypertensive



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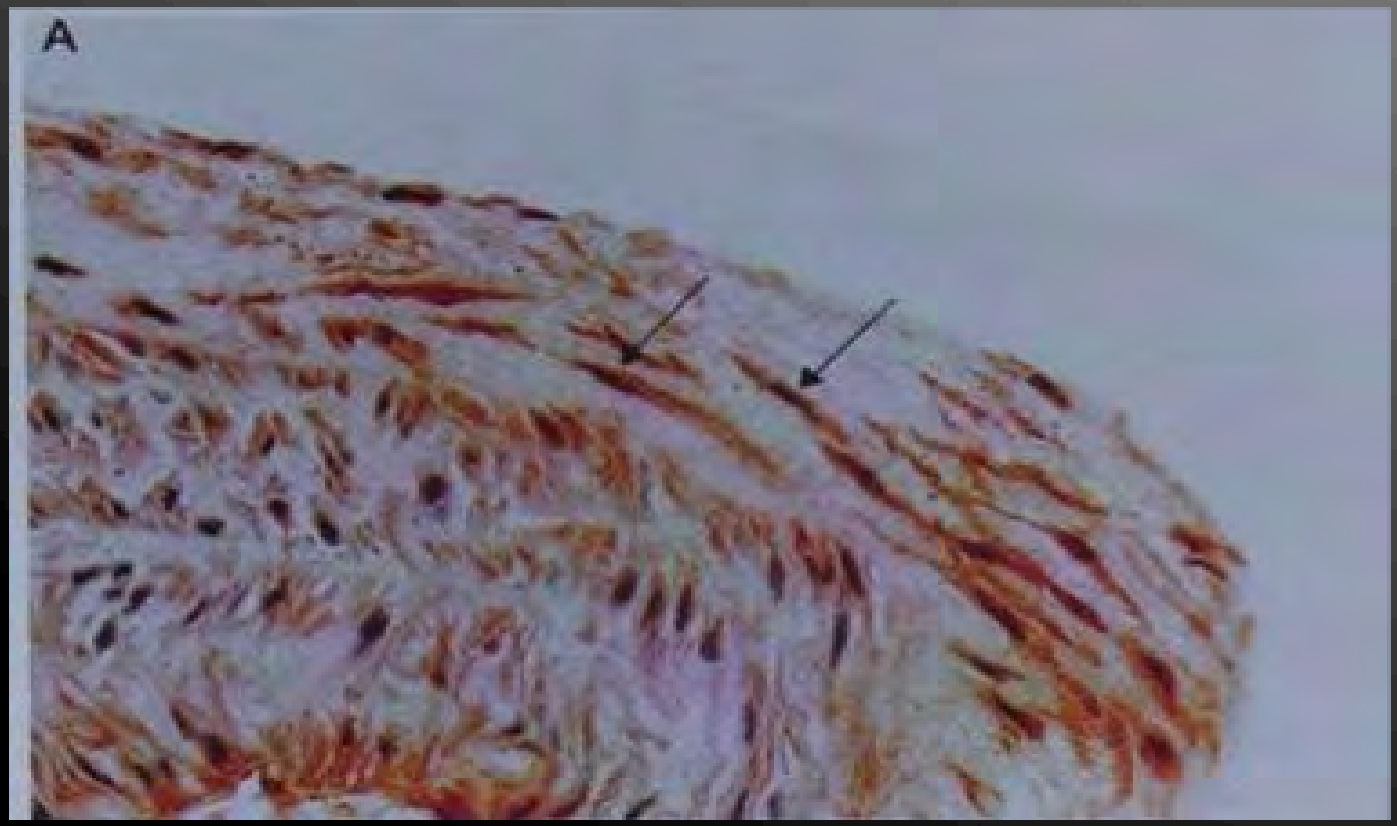
Smooth Muscle Cell Stain



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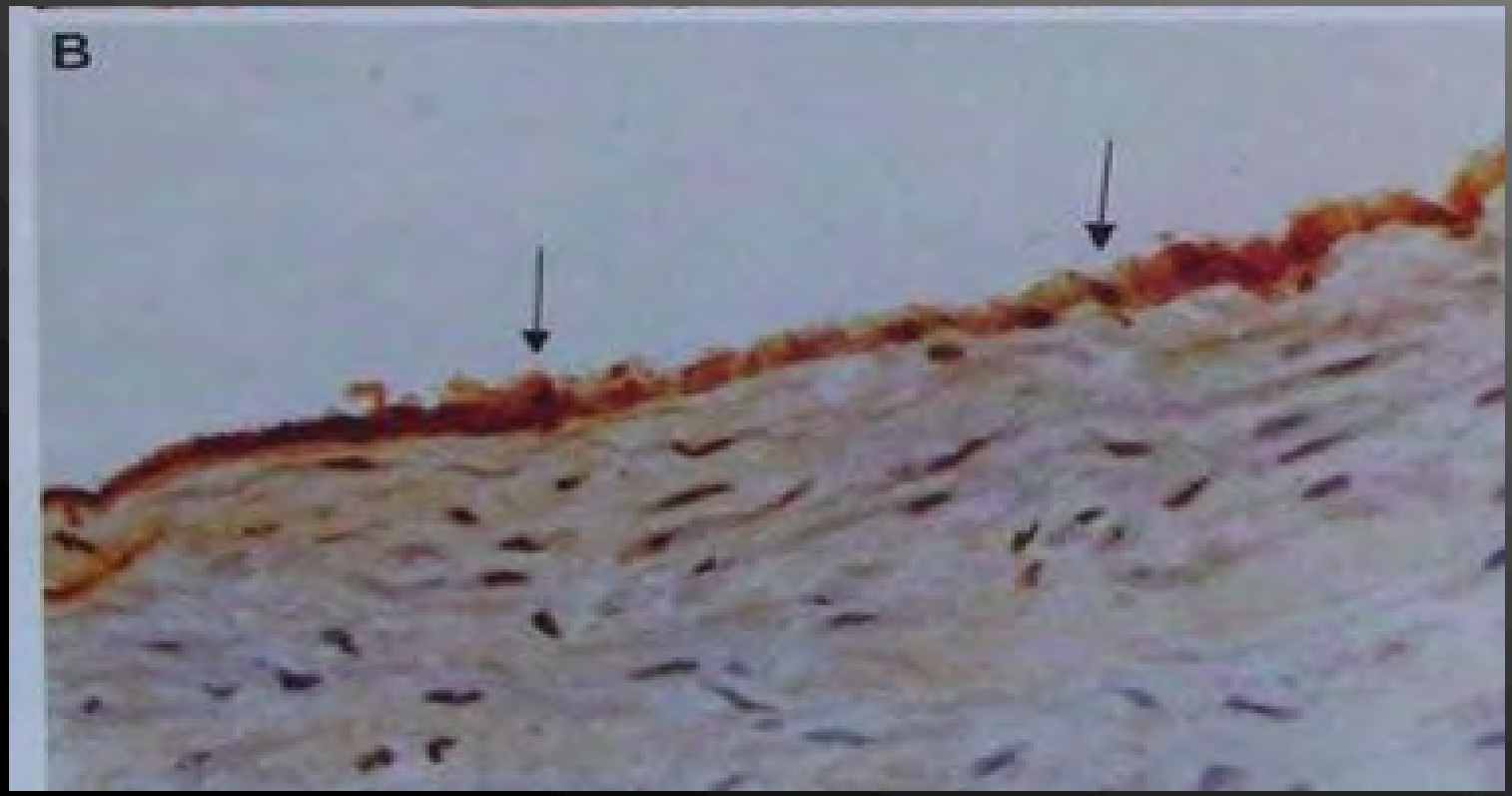
Endothelial Cell Stain



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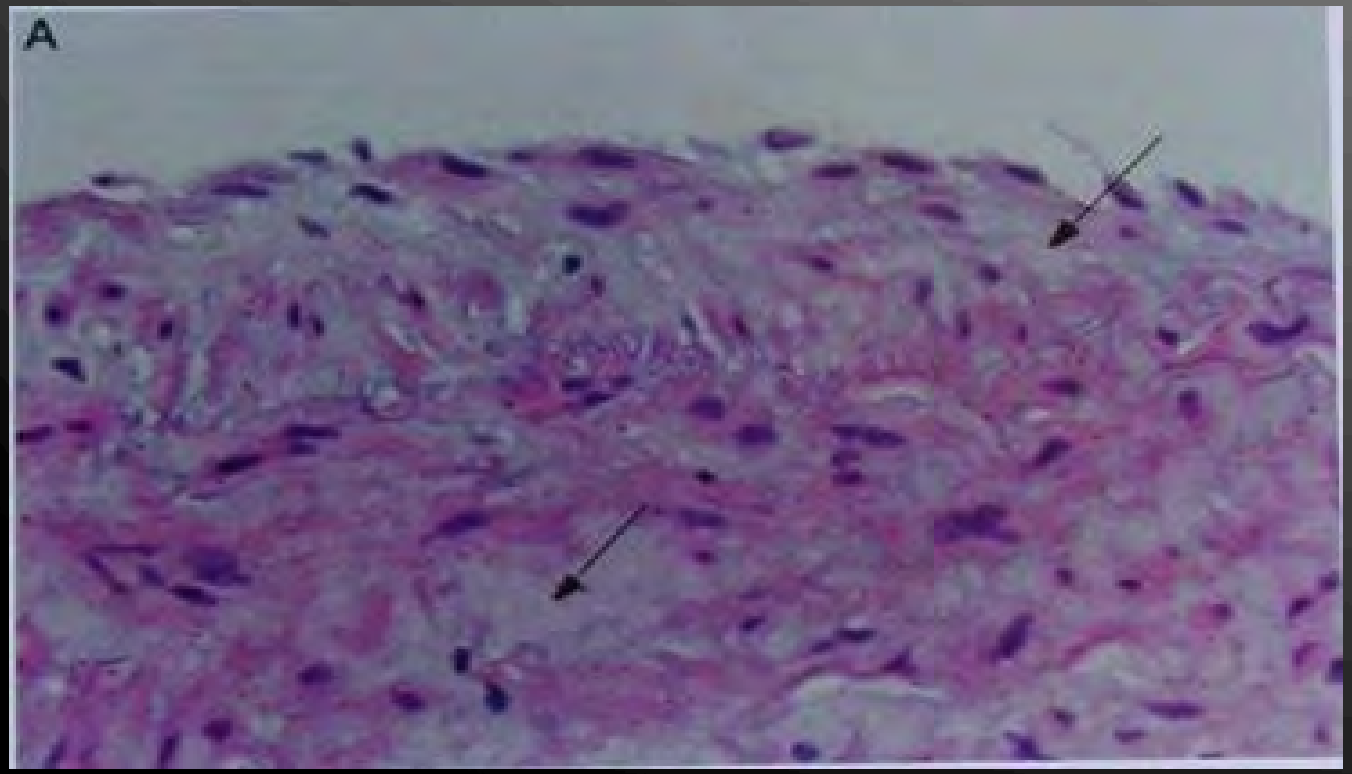
Myxoid Degeneration



Disorganized Elastic Layers

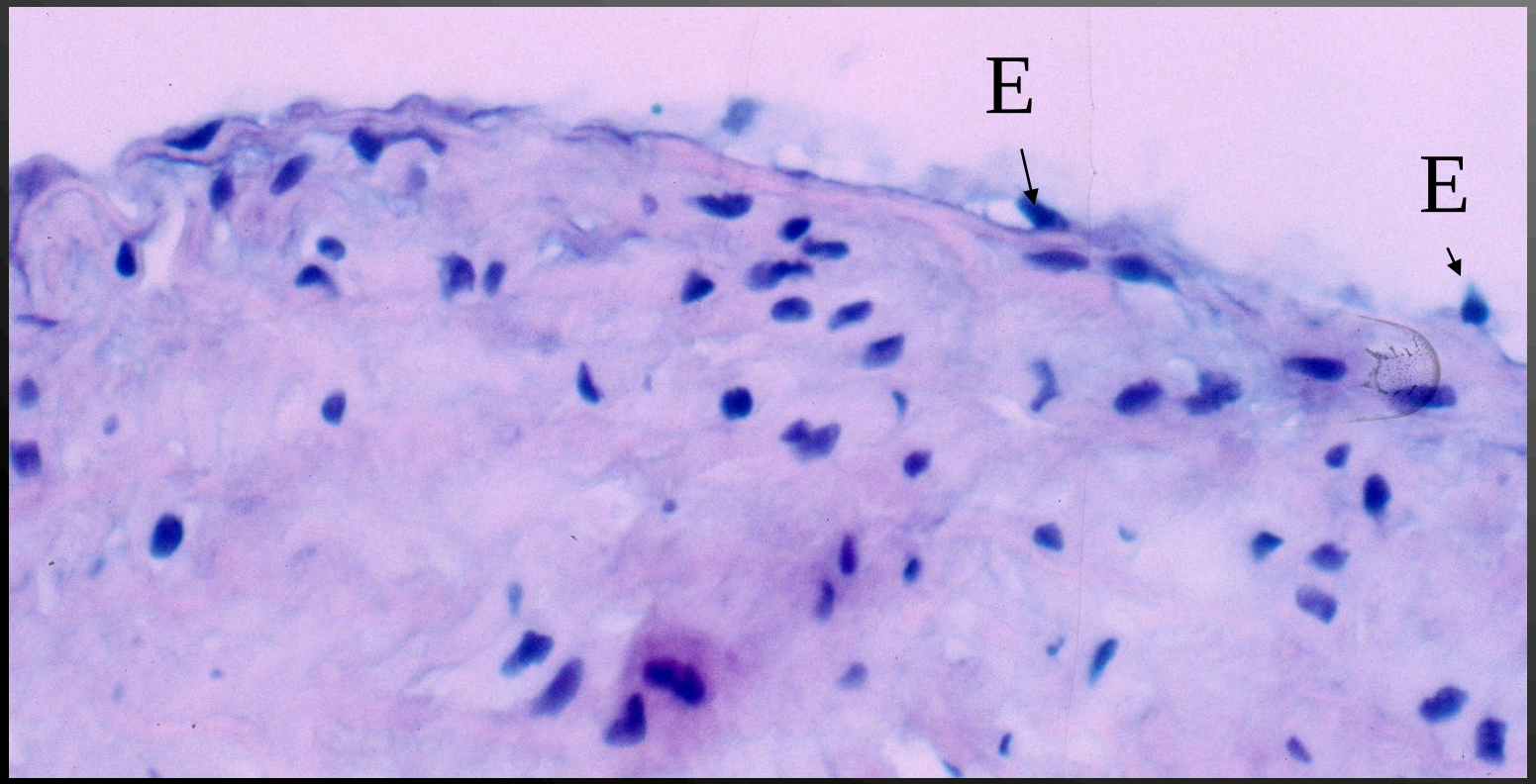
Lung Transplantation

- For patients with severe pulmonary hypertension
- Survival is limited by rejection, infection and bronchiolitis obliterans
- A method to improve our understanding and diagnosis of transplant rejection is needed
- Pulmonary endovasculature may be revealing

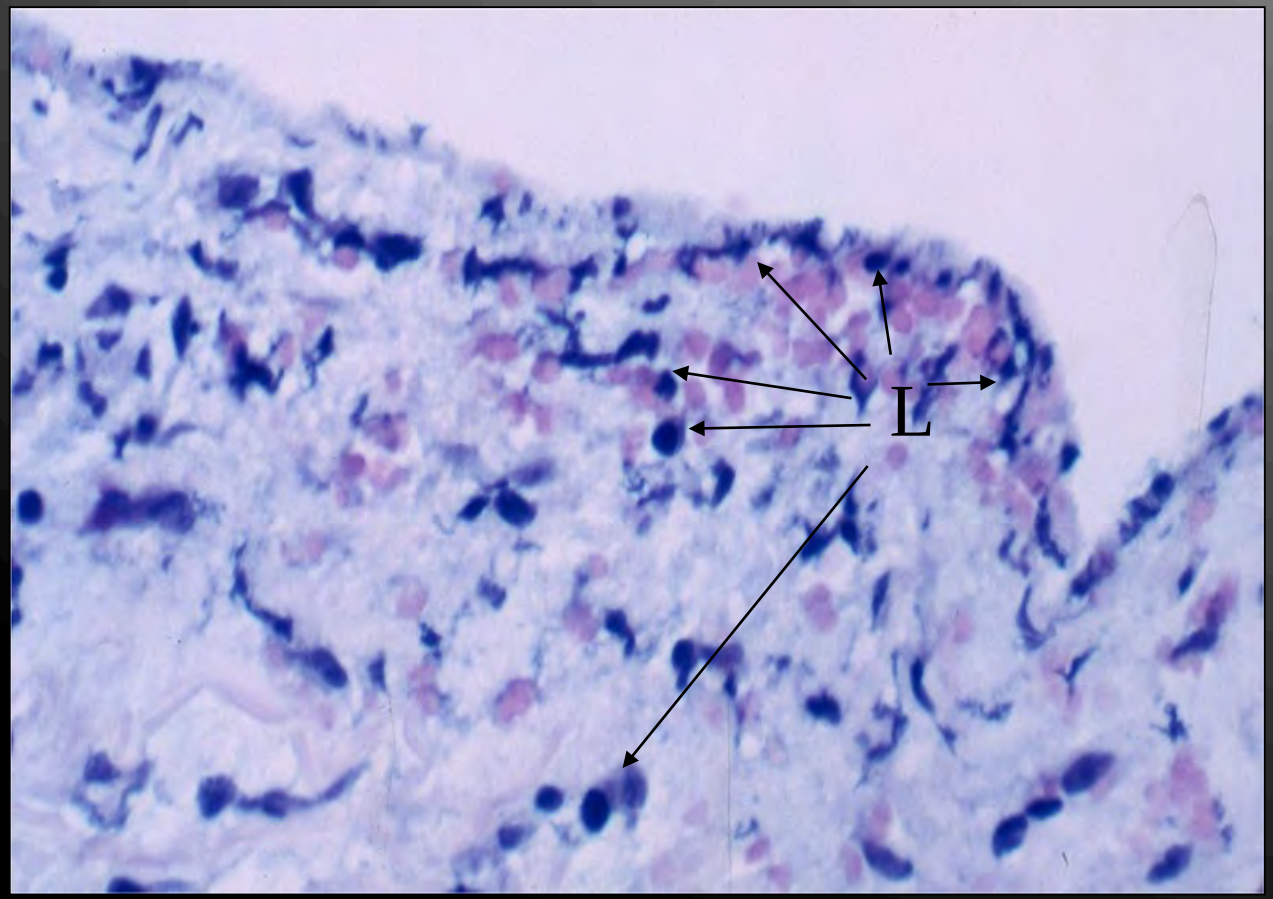
Lung Transplant Rejection Model

- Single left lung transplant
- Immunosuppresion for 2 weeks, then stop
- Biopsy every 2-3 days until full rejection and euthanasia
- Test biopsy samples for ICAM-1, VCAM-1 and E-selectin mRNA levels

Endothelial Cell Changes



Lymphocytes



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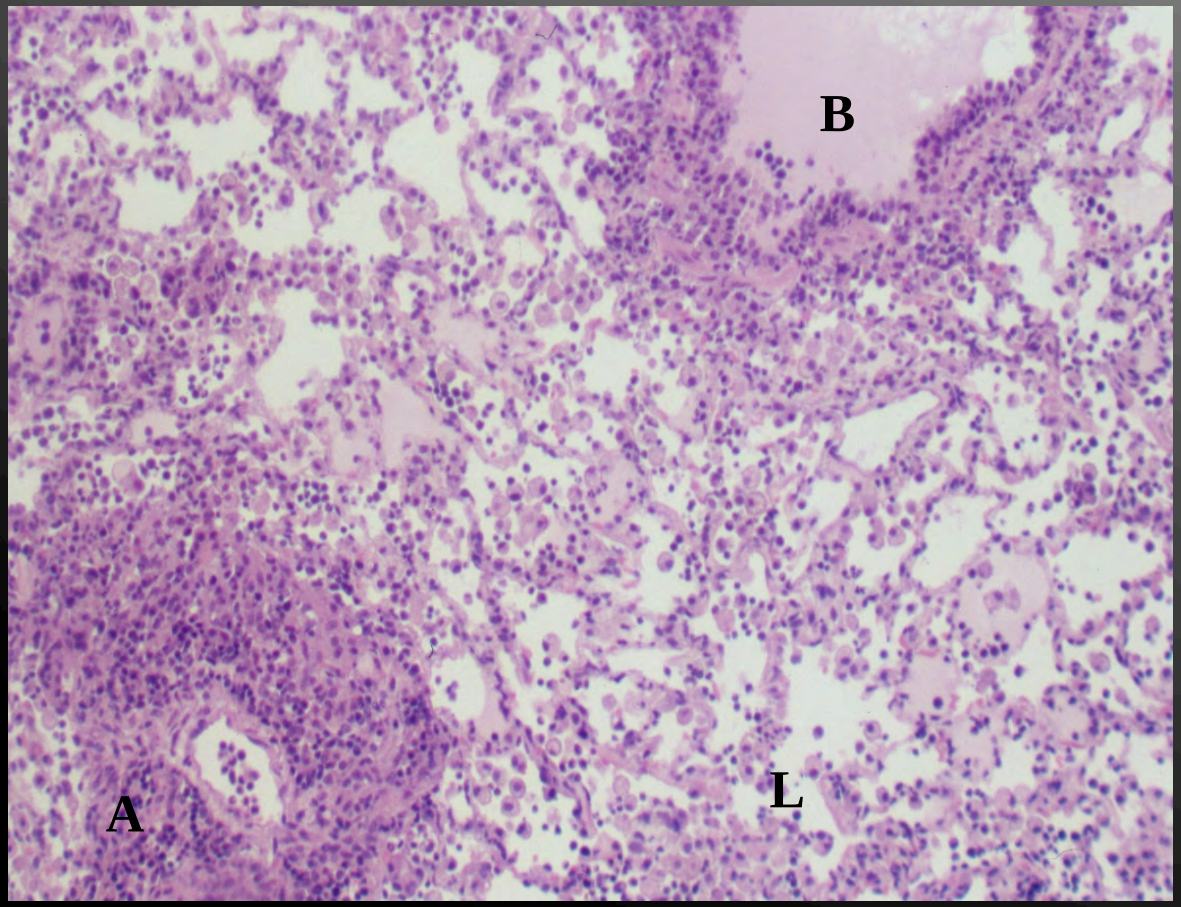
Advanced Rejection - Necropsy



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Results

- No increase in ICAM-1 or E-selectin mRNA levels
- Increase in VCAM-1 mRNA from biopsies of transplanted lung vs native lung
- Progressive increase in VCAM-1 with longer rejection times
- VCAM-1 mRNA changes detected prior to histologic changes

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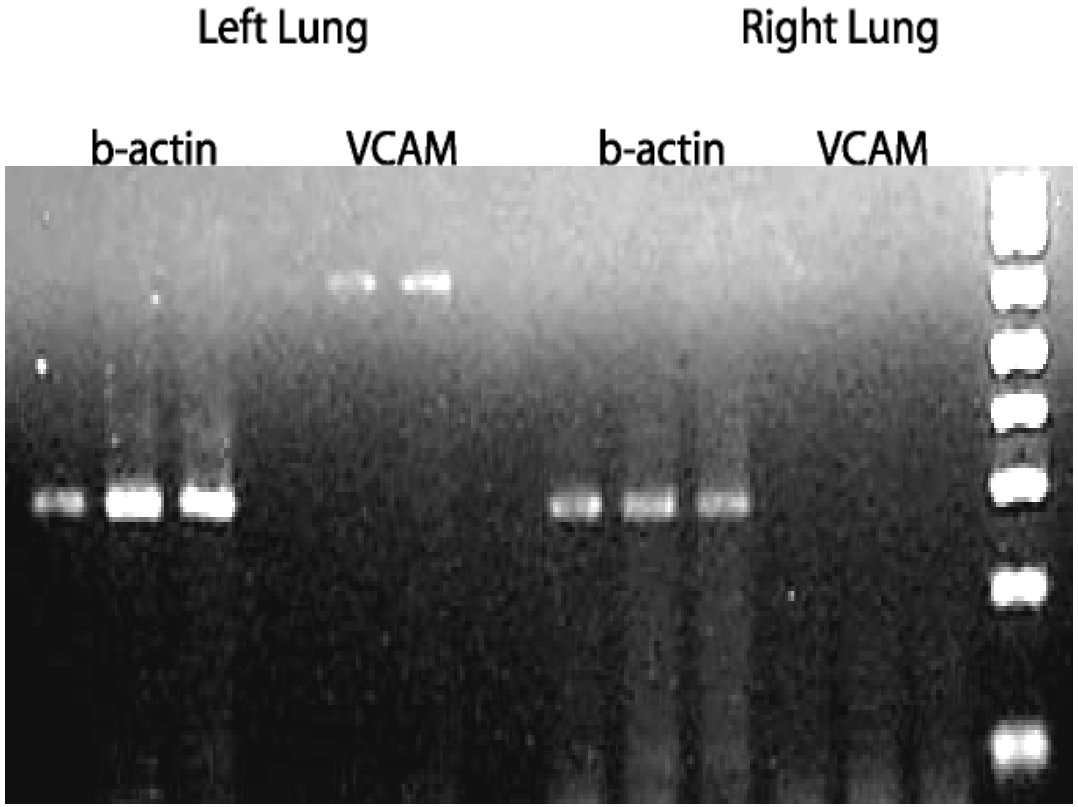
PCR Analysis on Biopsy Samples



Surgical Shunt Model

- Create a model of high pressure and high flow in the pulmonary arterial system

- Assess the histologic and mRNA changes sequentially

Surgical Shunt Model

- Baseline Biopsy
- Left thoracotomy
- Disconnect LPA from MPA
- Connect LPA to descending aorta
- Biopsy hypertensive LPA at 7, 21, 60, 90 and 180 days after surgery

Aims

- Identify gene expression at different time points
- Analyze upregulated genes, compare to currently available FDA-approved drugs
- Novel genes, novel drug list

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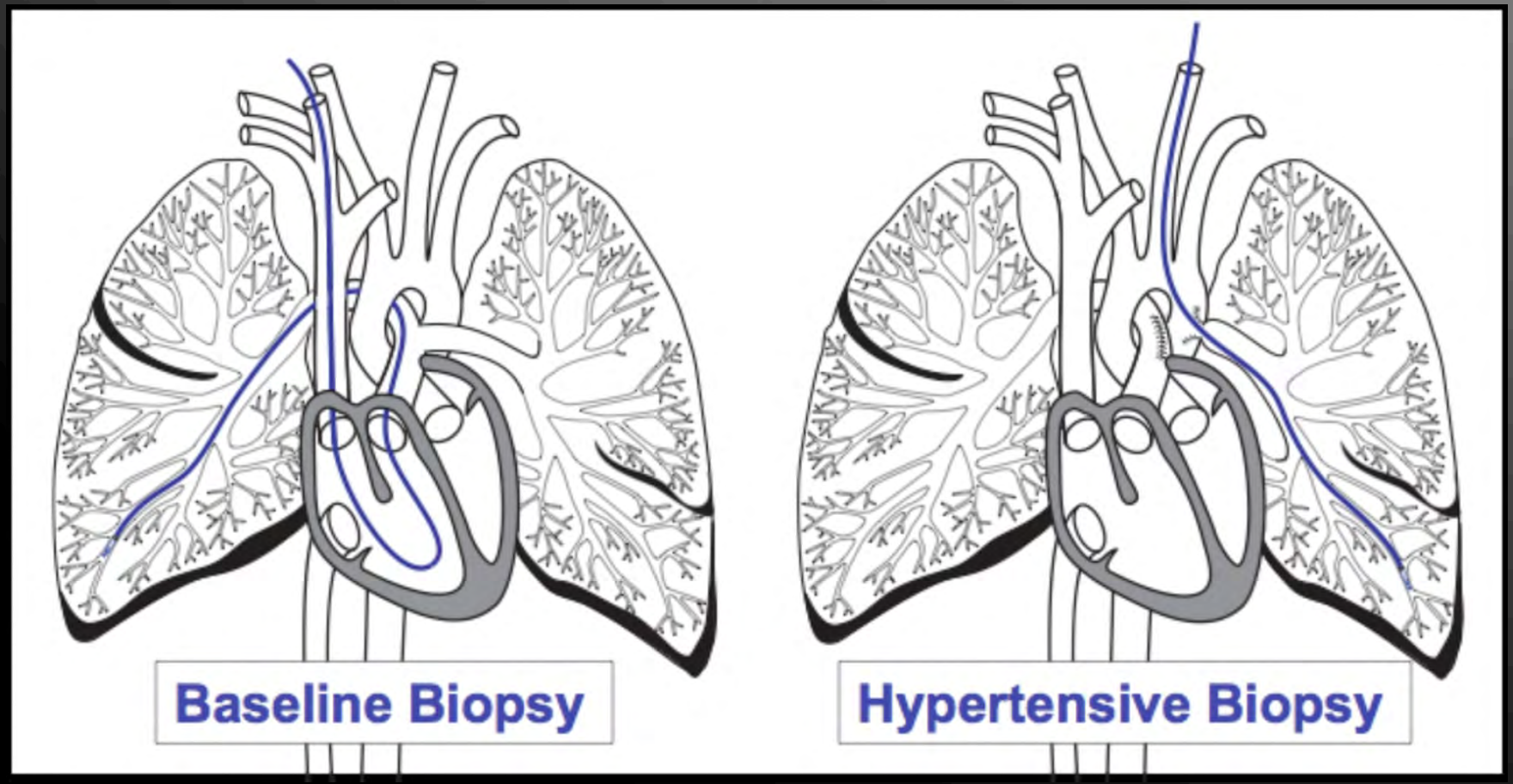
Surgical Shunt Model



Selected Markers

Symbol	Name	7 days	60 days	180 days
EDN	endothelin-1	-1.6	5.2	60.0
5-HT2B	serotonin 2B receptor	-2.6	2.9	9.8
PDE5A	phosphodiesterase 5A	4.3	2.3	4.1
KCNJ2	inwardly-rectifying K+ channel KIR6.1	-4.7	-1.8	-4.6
NOS2A	nitric oxide synthase inducible	-4.4	-4.4	-5.4
VEGFB	vascular endothelial growth factor beta	-21.0	-45.0	-8.3

Known markers

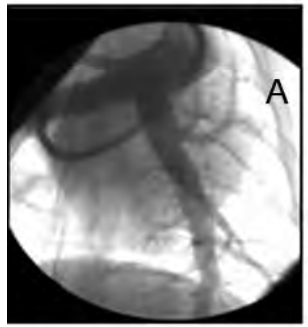
Symbol	Name	7 days	60 days	180 days
C5	complement 5	-17.0	619.0	9.3
TIE2	protein-kinase Tie2	-8.1	34.0	10.4
EDNRB	endothelin receptor B	-5.3	16.0	1.2
FYN	fyn proto-oncogene	4.2	12.0	3.8
ANGPT2	angiopoietin 2	1.7	11.6	6.0
RASA 1	RAS p21 protein activator 1	5.3	9.6	4.9
PDGFRA	PDGF receptor alpha	2.2	8.8	-3.7
CXCR4	chemokine receptor type 4	3.7	8.2	1.7
MAOB	monoamine oxidase B	1.2	7.0	-1.1
5-HT1D	serotonin 1D receptor	3.0	4.3	-2.9
PRKCB1	protein-kinase C, beta	3.4	3.5	-5.0
5-HTT	sodium dependent serotonin transporter	-37.0	3.3	-6.2
GUCY1B3	guanylate cyclase soluble beta 1	-4.3	3.0	2.1
ITGAV	integrin alpha-V	-6.1	2.9	3.1
ROCK1	rho associated protein kinase 1	2.4	2.8	3.0
VDAC1	voltage dependent anion channel 1	2.0	2.7	1.9
CASP3	caspase 3	-3.0	2.3	2.3
HMGCR	hydroxy methylglutaryl coenzyme A reductase	3.3	1.8	-2.6
EDNRA	endothelin receptor A	1.1	1.5	-2.9
PDGFRB	PDGF receptor beta	3.5	1.4	1.6
MAOA	monoamine oxidase A	-1.9	1.1	1.2
PPARG	peroxisome prolif. activ. receptor gamma 2	-14.0	1.1	-2.9
CALM1	calmodulin	-1.3	-2.2	-1.4
APOE	apolipoprotein E	-1.7	-2.7	-1.3
VDAC3	voltage dependent anion channel 3	-5.5	-4.3	-2.0
NOS3	nitric oxide synthase endothelial	-9.0	-5.0	1.2
BIRC5	apoptosis inhibitor survivin	4.4	-5.4	-3.8
KCNB1	voltage-gated K+ channel subunit kv 2.1	1.1	-5.7	2.0

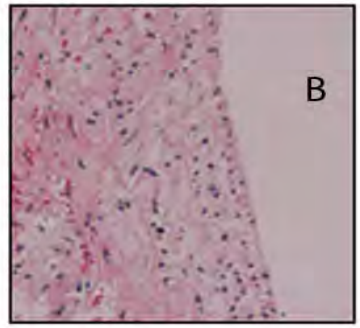
Novel markers

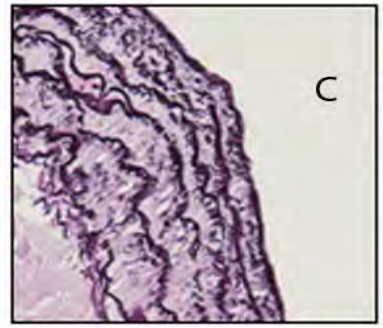
Symbol	Name	7 days	60 days	180 days
TACSTD1	tumor associated calcium signal transducer 1	1.8	177.0	2.1
CD2	T-cell surface antigen CD2	-3.2	93.0	5.2
CD3G	CD3g molecule, gamma	-2.1	11.0	3.6
HDAC2	histone deacetylase 2	-3.2	5.0	4.7
CDK6	cyclin-dependent kinase 6	-6.6	4.8	5.1
CDK7	cyclin-dependent kinase 7	-1.1	4.3	1.2
NP	purine nucleoside phosphorylase	-3.3	3.5	-1.1
CCR5	chemokine (C-C motif) receptor 5	-2.5	3.3	1.3
TLR8	Toll-like receptor 8	1.2	3.1	-1.5
HPSE	heparanase	4.8	3.0	-1.8
PLA2G7	platelet activating factor acetylhydrolase	1.6	3.0	1.5
IFNAR1	interferon alpha/beta receptor alpha chain	10.0	2.6	-1.3
PSEN1	presenilin 1	-14.0	2.5	3.8
RRM1	ribonucleotide reductase M1	-1.9	2.0	-1.0
F5	coagulation factor 5	1.9	1.9	-1.7
PSMB5	proteasome beta 5	-1.8	1.4	5.9
RRM2	ribonucleotide reductase M2	-13.1	1.4	1.7
POLB	DNA polymerase beta	1.1	1.1	5.3
IFNAR2	interferon alpha/beta receptor beta chain	2.3	1.1	-1.6
IL2RB	interleukin-2 receptor beta chain	1.4	-1.1	-5.2
HDAC6	histone deacetylase 6	-1.9	-3.6	21.0
CD19	B lymphocyte antigen CD19	9.5	-10.0	3.0

BASELINE

Mean LPA Pressure: 17 mm Hg



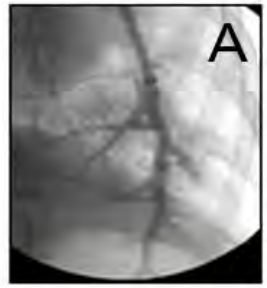


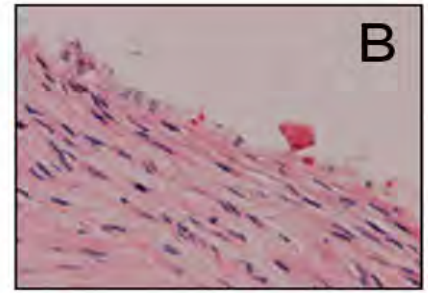


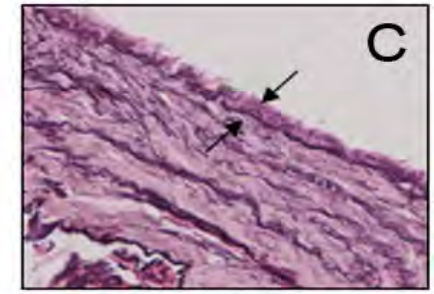
Symbol	Name	Baseline
EDNRB	endothelin receptor B	1.0
EDN	endothelin-1	1.0
5-HT1D	serotonin 1D receptor	1.0
5-HTT	sodium dependent serotonin transporter	1.0
5-HT2B	serotonin 2B receptor	1.0
PDE5A	phosphodiesterase 5A	1.0
EDNRA	endothelin receptor A	1.0
KCNJ2	inwardly-rectifying K+ channel KIR6.1	1.0
NOS2A	nitric oxide synthase inducible	1.0
NOS3	nitric oxide synthase endothelial	1.0
KCNB1	voltage-gated K+ channel subunit kv 2.1	1.0
VEGFB	vascular endothelial growth factor beta	1.0

DAY 7

Mean LPA Pressure: 34 mm Hg



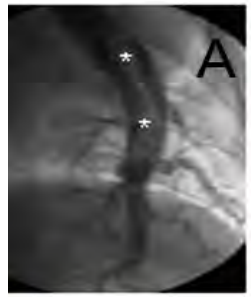


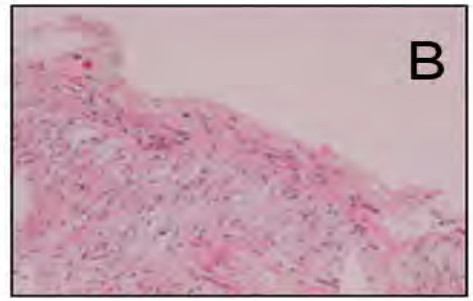


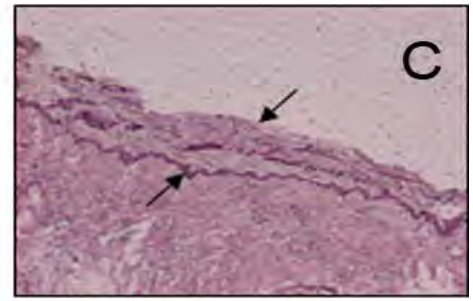
Symbol	Name	7 days
PDE5A	phosphodiesterase 5A	4.3
5-HT1D	serotonin 1D receptor	3.0
EDNRA	endothelin receptor A	1.1
KCNB1	voltage-gated K+ channel subunit kv 2.1	1.1
EDN	endothelin-1	-1.6
5-HT2B	serotonin 2B receptor	-2.6
NOS2A	nitric oxide synthase inducible	-4.4
KCNJ2	inwardly-rectifying K+ channel KIR6.1	-4.7
EDNRB	endothelin receptor B	-5.3
NOS3	nitric oxide synthase endothelial	-9.0
VEGFB	vascular endothelial growth factor beta	-21.0
5-HTT	sodium dependent serotonin transporter	-37.0

DAY 60

LPA Pressure: Systemic



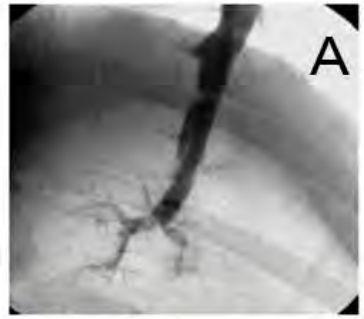


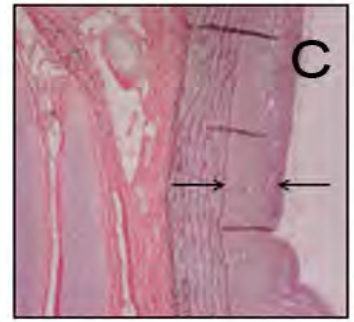


Symbol	Name	60 days
EDNRB	endothelin receptor B	16.0
EDN	endothelin-1	5.2
5-HT1D	serotonin 1D receptor	4.3
5-HTT	sodium dependent serotonin transporter	3.3
5-HT2B	serotonin 2B receptor	2.9
PDE5A	phosphodiesterase 5A	2.3
EDNRA	endothelin receptor A	1.5
KCNJ2	inwardly-rectifying K+ channel KIR6.1	-1.8
NOS2A	nitric oxide synthase inducible	-4.4
NOS3	nitric oxide synthase endothelial	-5.0
KCNB1	voltage-gated K+ channel subunit kv 2.1	-5.7
VEGFB	vascular endothelial growth factor beta	-45.0

DAY 180

LPA Pressure: Systemic



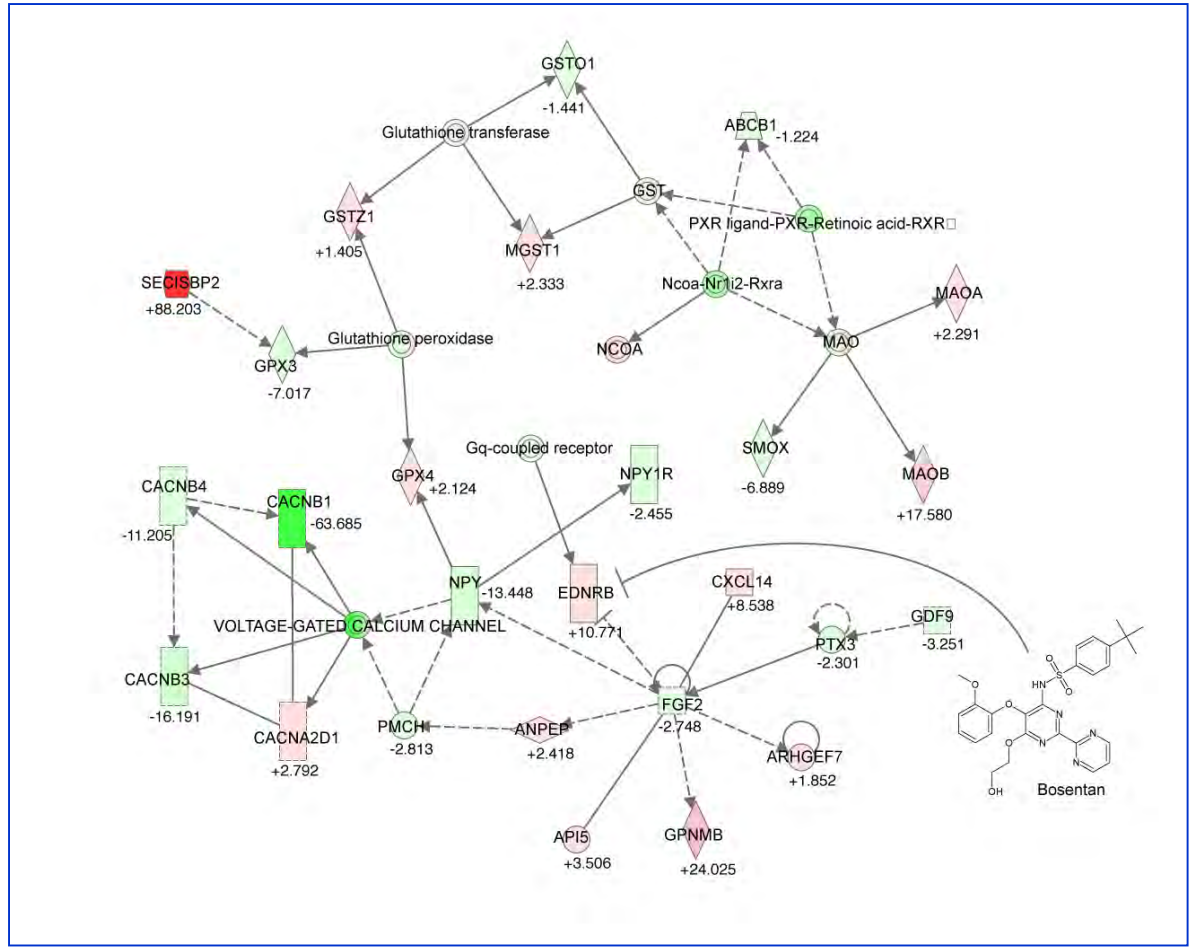


Symbol	Name	180 days
EDN	endothelin-1	60.0
5-HT2B	serotonin 2B receptor	9.8
PDE5A	phosphodiesterase 5A	4.1
KCNB1	voltage-gated K+ channel subunit kv 2.1	2.0
NOS3	nitric oxide synthase endothelial	1.2
EDNRB	endothelin receptor B	1.2
EDNRA	endothelin receptor A	-2.9
5-HT1D	serotonin 1D receptor	-2.9
KCNJ2	inwardly-rectifying K+ channel KIR6.1	-4.6
NOS2A	nitric oxide synthase inducible	-5.4
5-HTT	sodium dependent serotonin transporter	-6.2
VEGFB	vascular endothelial growth factor beta	-8.3

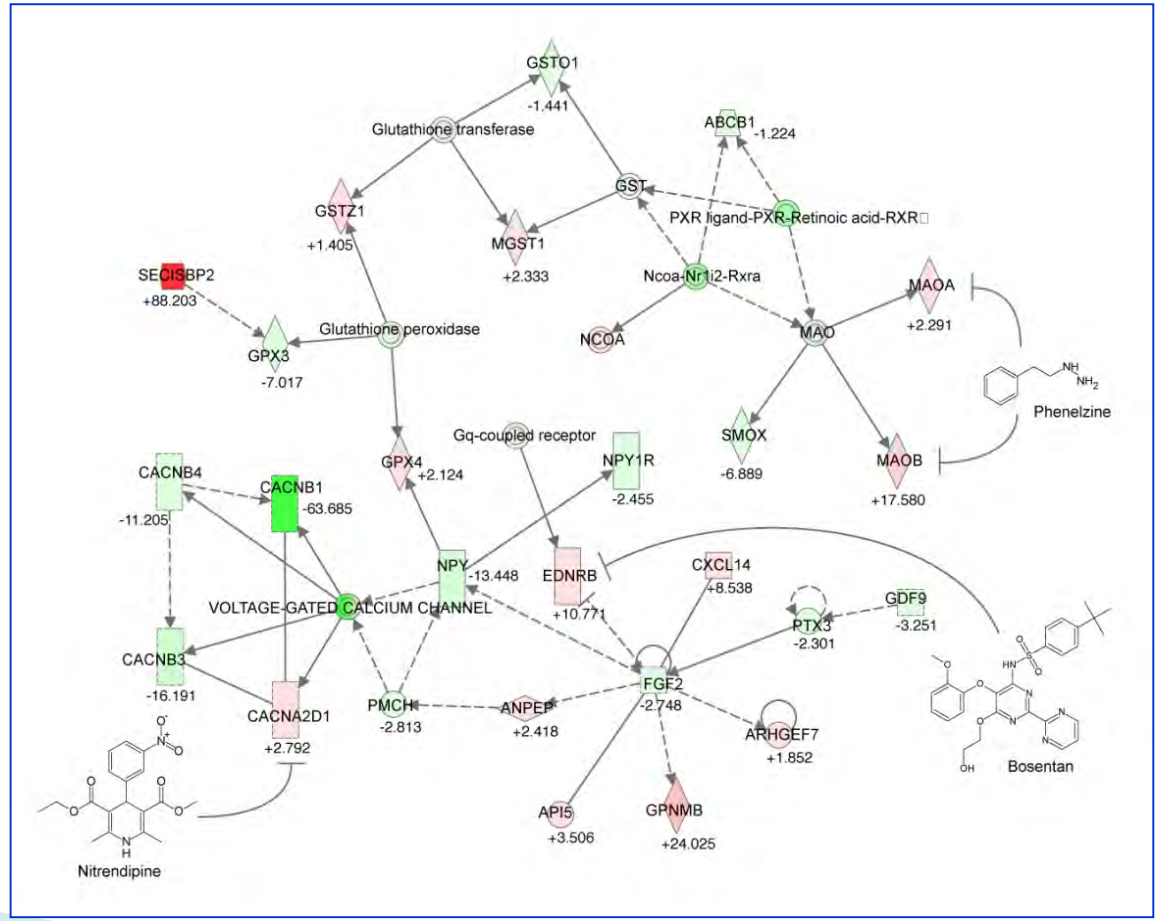
Upregulated genes and drugs

- Generate a list of available drugs that inhibit upregulated PAH genes
- Novel drug list: drugs used for other diseases (cancer, atherosclerosis, inflammation, immunological conditions), not previously used in PAH
- Drug list may be stage-specific
- Novel upregulated genes need to be analyzed

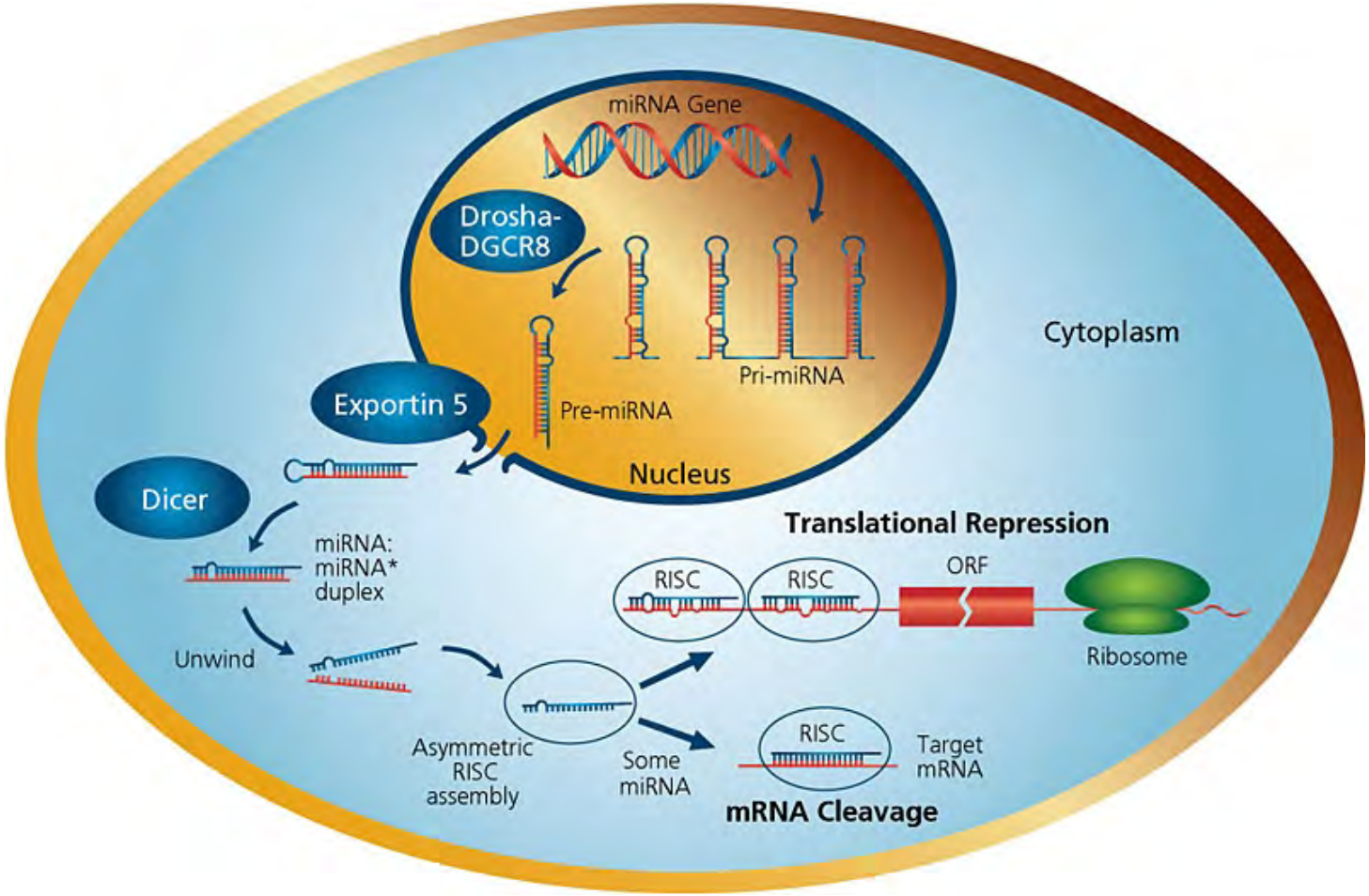
Example - Current PAH therapy with Bosentan



Potential novel uses for Phenelzine & Nitrendipine



Micro RNAs


miRNA Gene
Drosha-
DGCR8
Pri-miRNA
Pre-miRNA
Exportin 5
Nucleus
Cytoplasm
Dicer
miRNA:
miRNA*
duplex
Unwind
Translational Repression
RISC
RISC
ORF
Ribosome
Asymmetric
RISC
assembly
Some
miRNA
RISC
Target
mRNA
mRNA Cleavage

Identification of Dysregulated microRNAs

- Analyze microRNA (miRNA) expression levels during the progression of pulmonary arterial hypertension (PAH) in the surgical shunt model.
- RNA, isolated from biopsy samples, was loaded into illumina miRNA expression microarrays containing ~1200 miRNAs.
- Three groups were defined: (1) Normal (baseline); (2) High Flow Low Pressure ‘HFLP’ and (3) ‘PAH’
- There were pressure sensitive changes in miRNA expression

Table 1A. Animal, days post-shunt surgery, and pulmonary artery pressure (PAP)

Pig # 1			Pig # 2		
Day	**PAP**	**PAP mean**	**Day**	**PAP**	**PAP mean**
Day -7	18/10	16	Day -7	20/11	16
Day 10	22/17	19	Day 6	19/15	16
Day 24	85/62	72	Day 21	20/15	17
Day 59	89/50	58	Day 55	23/15	19
Day 94	100/81	82	Day 83	93/68	80
			Day 104	91/69	80
			Day 140	92/70	81

Table 1B. Animal, days post-shunt surgery, and pulmonary artery pressure (PAP)

BASELINE			HFLP			PH		
Animal, Day	**PAP**	**PAP mean**	**Animal, Day**	**PAP**	**PAP mean**	**Animal, Day**	**PAP**	**PAP mean**
1, Day -7	18/10	16	1, Day 10	22/17	19	1,Day 24	85/62	72
2, Day -7	20/11	16	2, Day 6	19/15	16	1, Day 59	89/50	58
			2, Day 21	20/15	17	1, Day 94	100/81	82
			2, Day 55	23/15	19	2, Day 83	93/68	80
						2, Day 104	91/69	80
						2, Day 140	92/70	81

Downregulated miRNAs

Symbol	Baseline	HFLP	PAH
hsa-miR-586	4183.74	142.81	42.62
hsa-miR-520d	114.97	66.23	14.32
hsa-miR-496	90.38	5.54	5.18
hsa-miR-935	837.14	777.94	14.23
solexa-5620-151	92.30	33.55	5.07
hsa-miR-494	8875.62	546.81	343.70
hsa-miR-1321	31.00	18.23	3.29
hsa-miR-292-2-3p	99.25	43.96	14.28
hsa-miR-95	1310.30	924.85	311.18
hsa-miR-128b	3060.68	1647.50	925.46
hsa-miR-495	370.00	234.11	45.81
hsa-miR-521	493.90	304.45	161.33

Upregulated microRNAs

Symbol	Baseline	HFLP	PAH
hsa-miR-520g	0.01	12.69	2772.39
hsa-miR-331-5p	0.01	8.11	1700.55
hsa-let-7d	0.01	2147.63	1499.40
hsa-miR-187	0.01	558.78	1144.02
hsa-miR-130a	0.01	8.99	912.52
hsa-let-7g	0.01	12.96	216.74
hsa-miR-519e	0.01	15.76	52.02
hsa-miR-192	1.26	430.69	529.41
hsa-miR-568	17.25	883.92	1036.96
hsa-miR-1203	2.62	54.43	148.59
hsa-miR-28-5p	381.67	4105.59	5756.85
hsa-miR-1273	11.78	26.17	80.09

Number of associated PAH genes

miR	Number of genes regulated	miR	Number of genes regulated	miR	Number of genes regulated
miR-548c-3p	113	miR-520g	35	miR-1304	15
miR-520d-3p	106	miR-410	32	miR-219-2-3p	14
miR-130a-5p	103	miR-586	31	miR-154-3p	13
miR-30a-3p	84	miR-29b-1-5p	31	miR-1321	12
let-7g-3p	83	miR-16-2-3p	31	miR-127-5p	12
let-7f-2-3p	78	miR-494	29	miR-331-5p	11
miR-363-5p	49	miR-33a-3p	29	miR-619	11
miR-495	48	miR-548i	29	miR-643	9
miR-519a	48	miR-192-3p	29	miR-1287	8
let-7d-3p	48	miR-185-3p	27	miR-208b	8
miR-548n	46	miR-1205	25	miR-99a-3p	7
miR-519e-5p	46	miR-212	24	miR-28-5p	7
miR-33a-3p	45	miR-187	23	miR-556	7
miR-135a-3p	43	miR-10b-3p	23	miR-610	5
miR-133b	40	miR-371-5p	22	miR-524-3p	4
miR-548c-5p	39	miR-218-1-3p	21	miR-525-3p	3
miR-377	38	miR-1237	18	miR-497	2
miR-548a-5p	37	miR-935	17	miR-664	1

Endoarterial biopsy – useful tool:

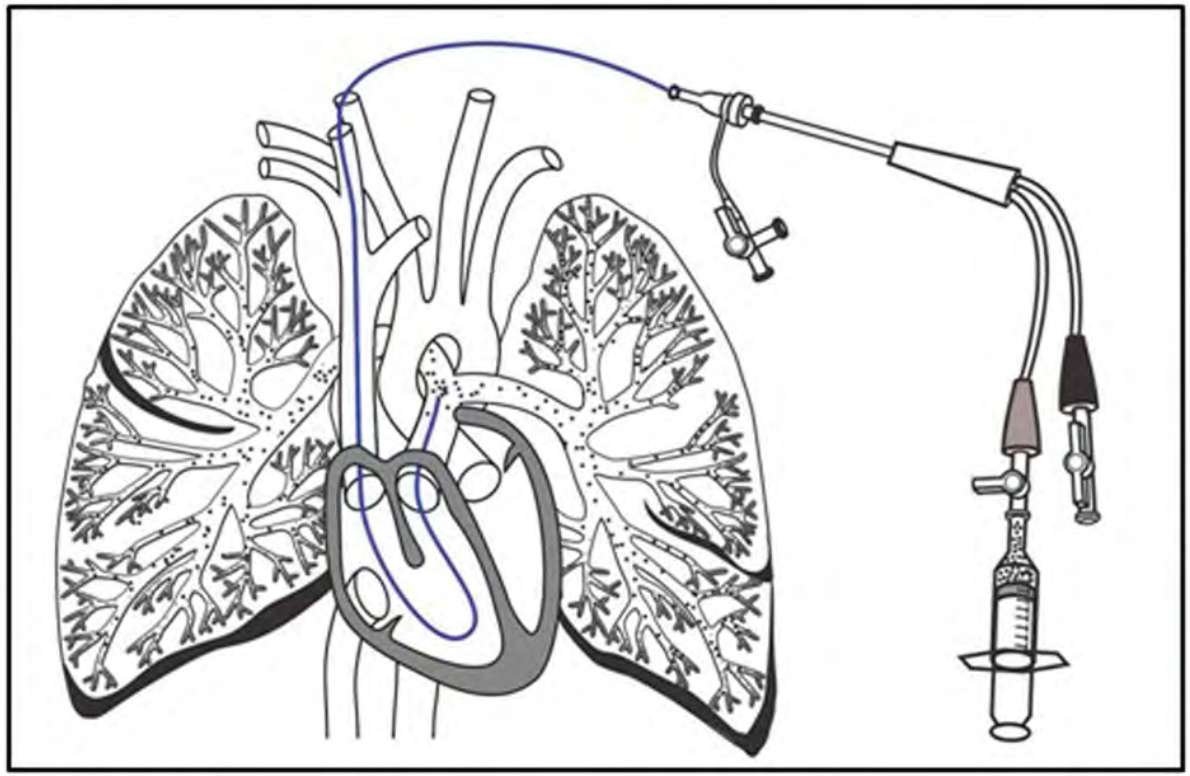
- Sequentially assess vessel biology in PAH, transplant rejection and other pulmonary vascular diseases
- Detect disease- & stage-specific gene/miRNA changes
- Identify novel applications for existing drugs
- Identify new genes, miRNAs and new drugs
- Individualize pharmaco-transcriptomics
- Assess histo-molecular responses to therapy
- Final FDA validation study just concluded (2019).
- HDE designation (2019)

Creation of large animal models

- Microspheres – canine
- DAo-LPA shunt
- Microspheres – porcine
- Carotid-Jugular shunt
- Microspheres + LNAME

Microspheres

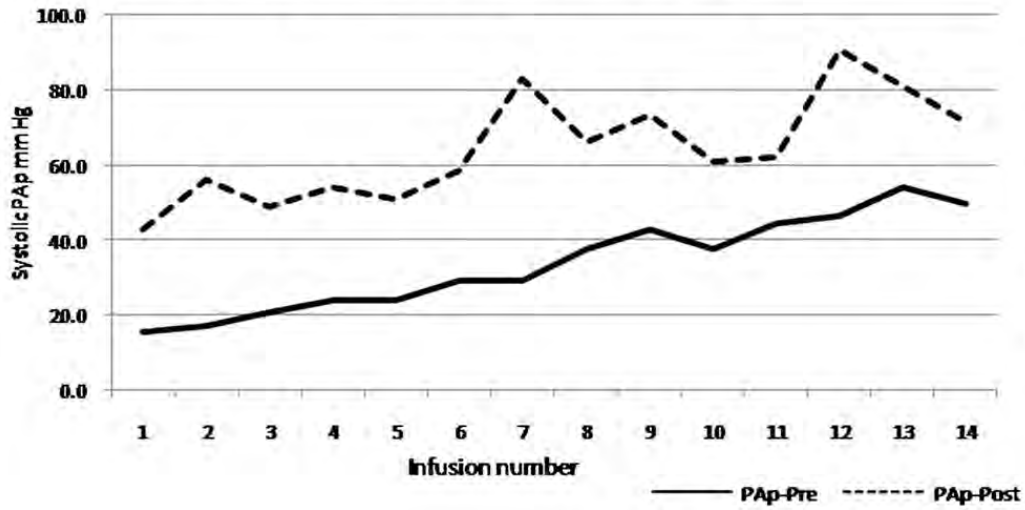
(a)



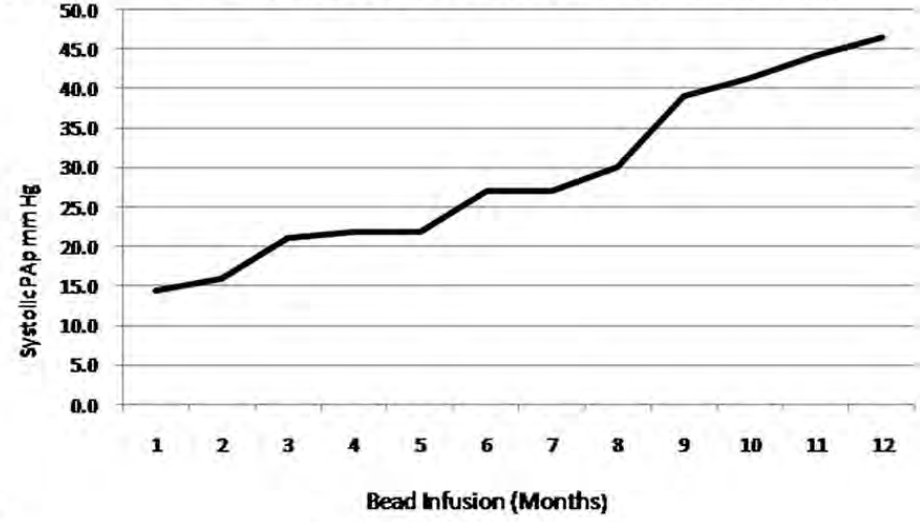
(b)

Canine bead model

(a)

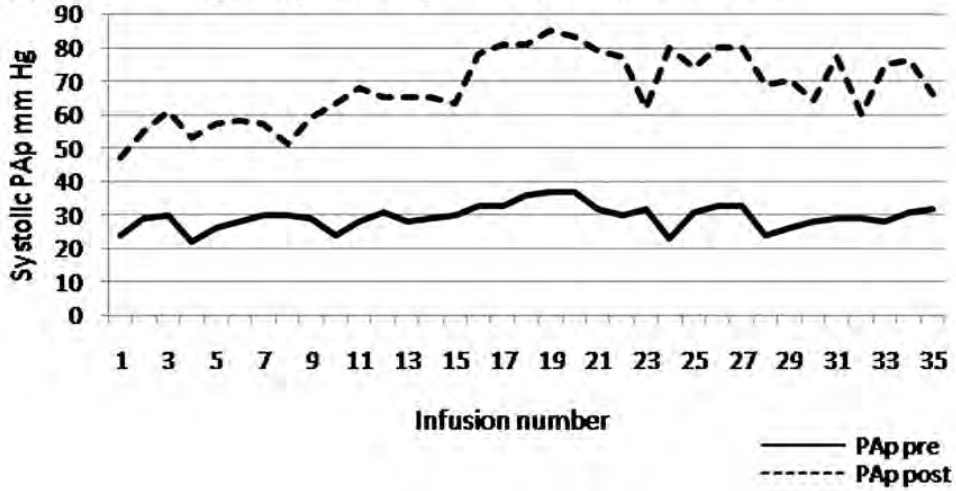


(b)



Porcine bead model

(a)



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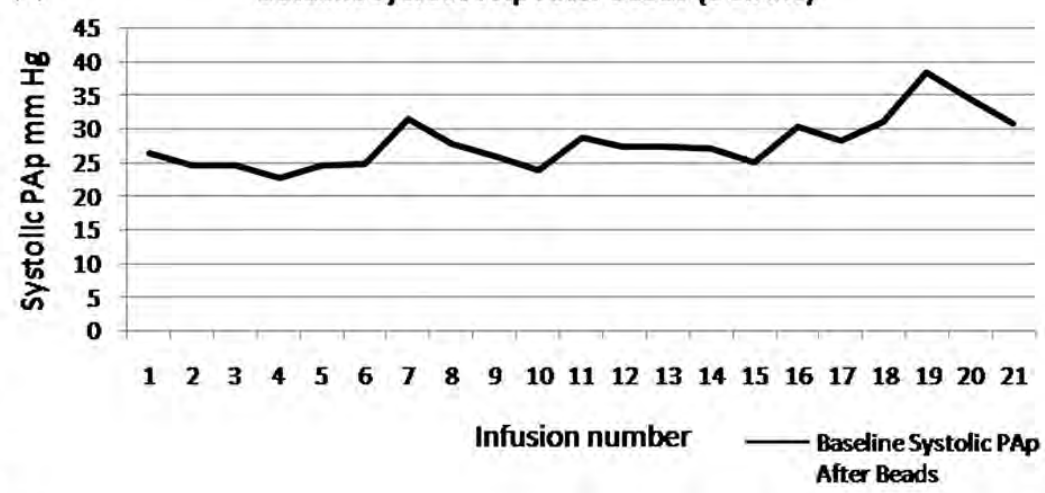
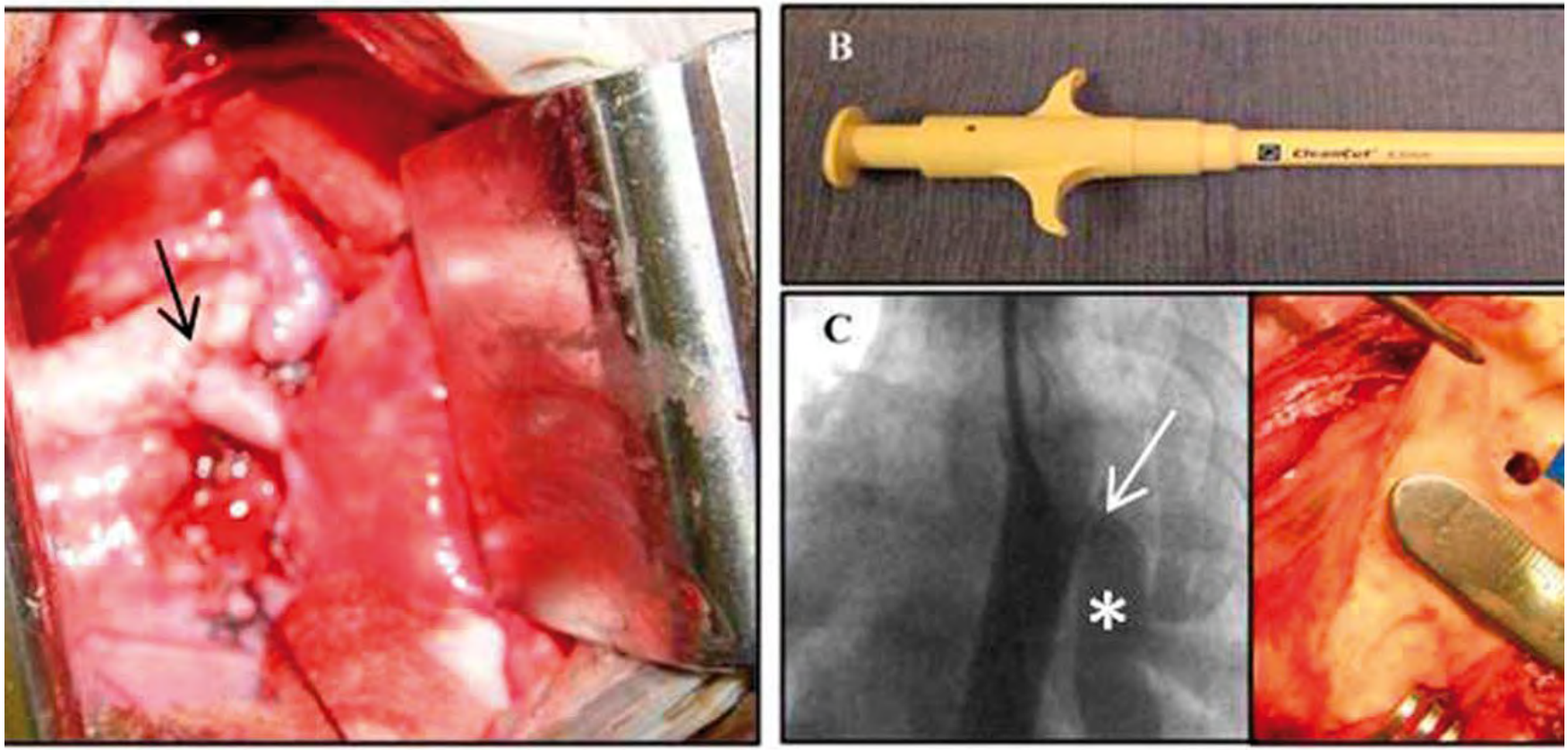
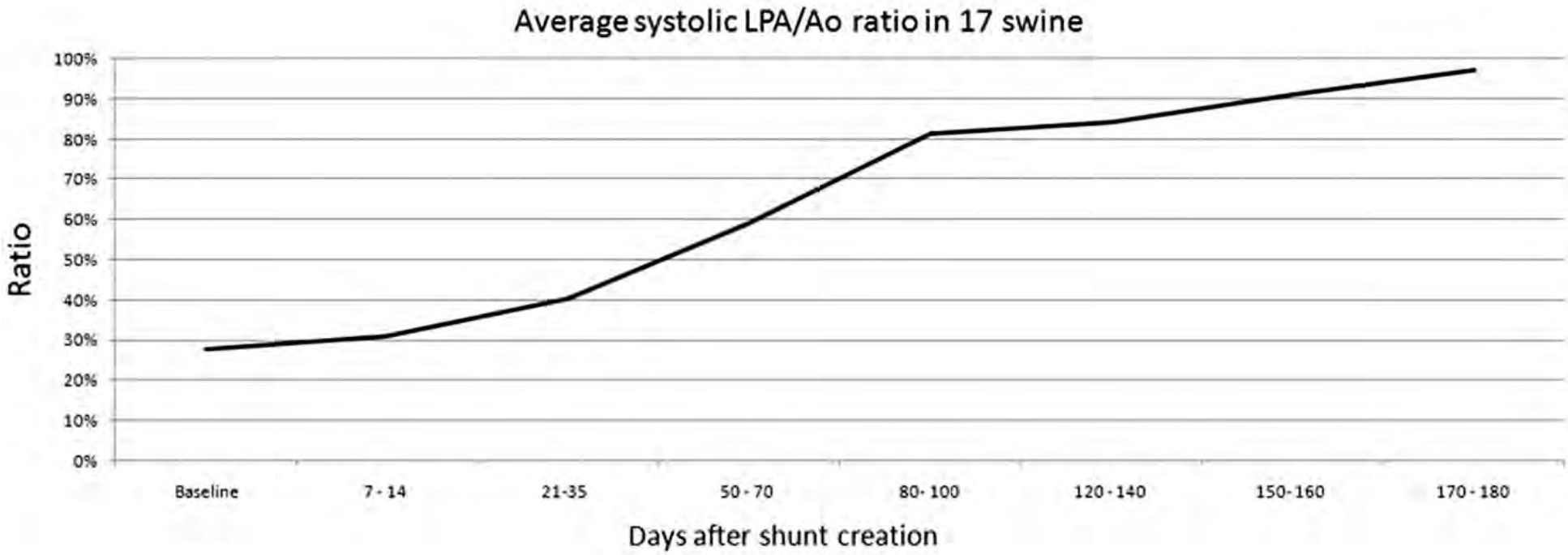


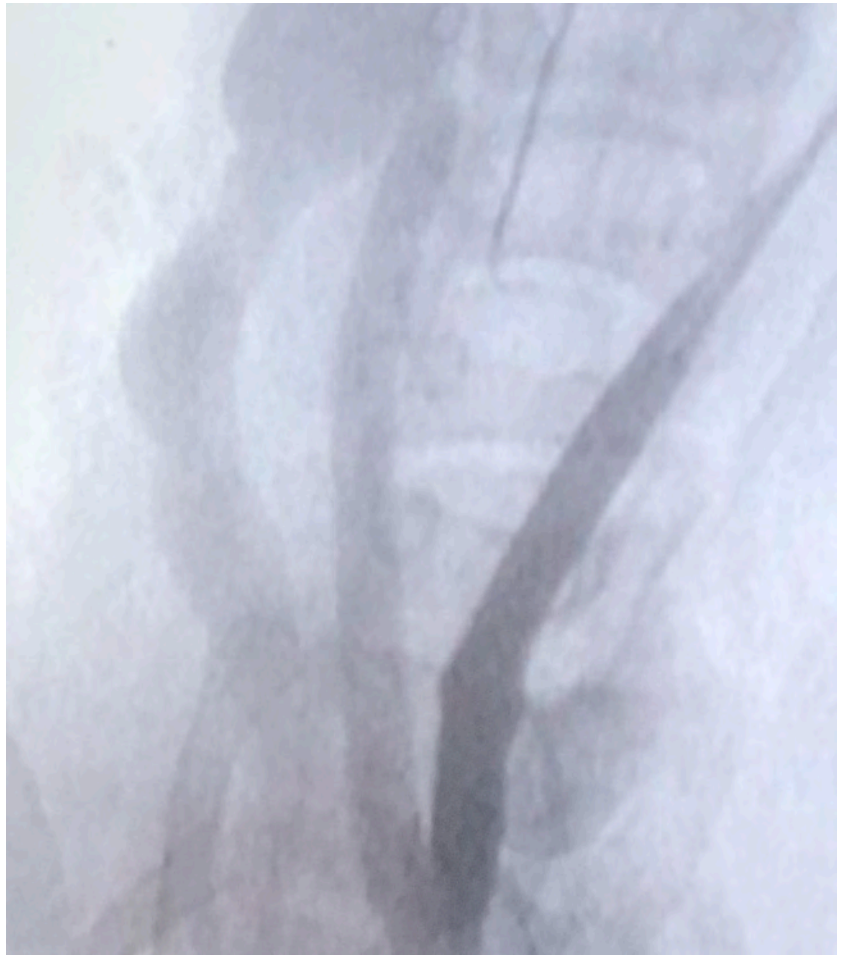
Porcine shunt model

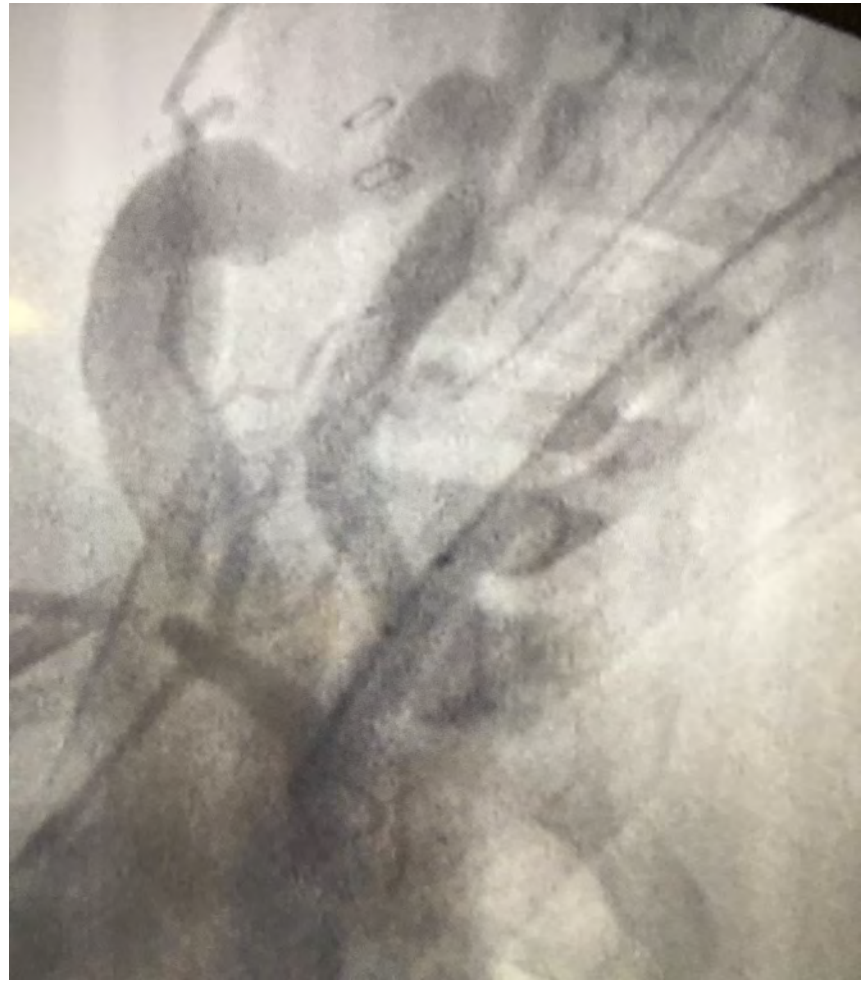


Porcine LPA shunt model



Porcine neck shunt





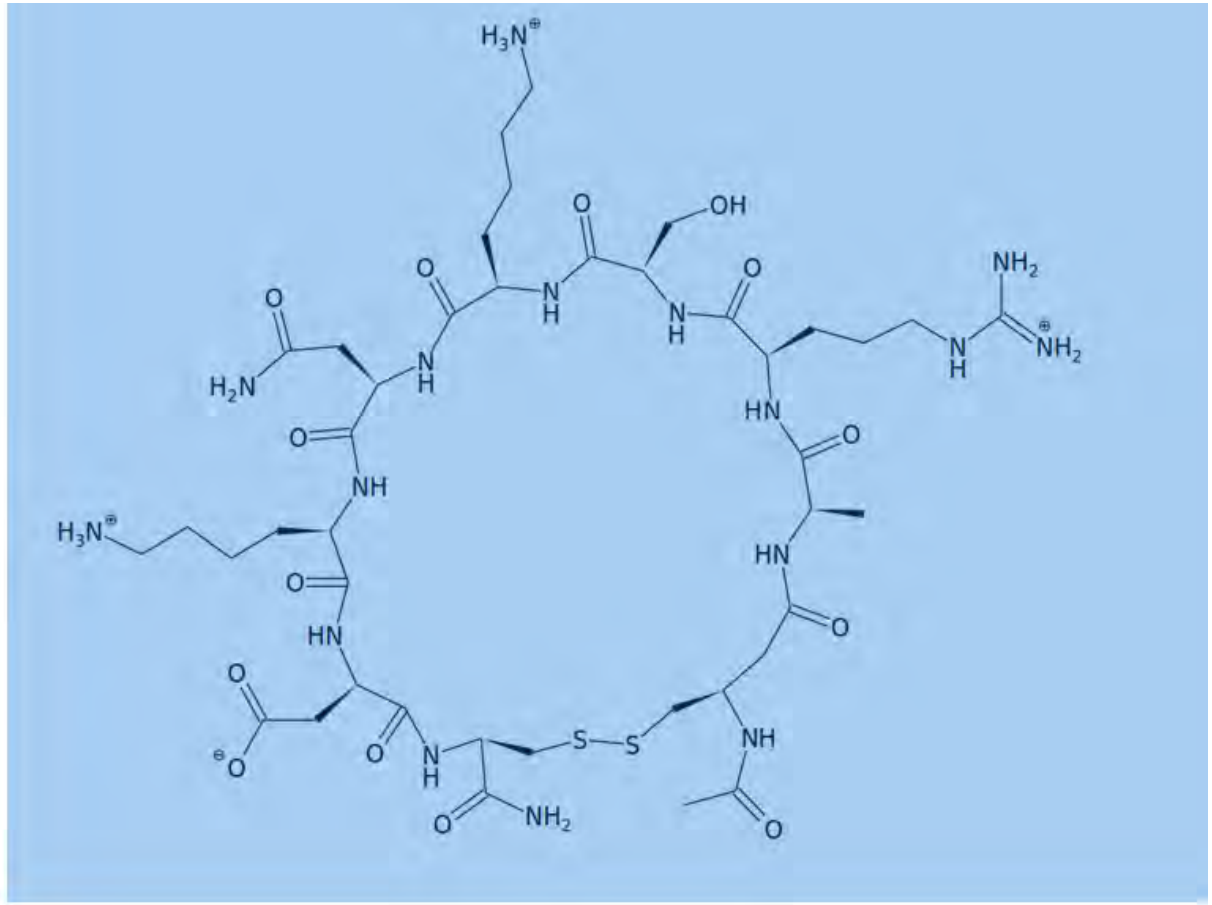
Porcine neck shunt + L-NAME

- **Exercise Facilitates Early Recognition of Cardiac and Vascular Remodeling in Chronic Thrombo-Embolic Pulmonary Hypertension in a Novel CTEPH Swine Model.**
- Stam K1, van Duin RWB2, Uitterdijk A2, Cai Z2, Duncker DJ3, Merkus D2.
- Am J Physiol Heart Circ Physiol. 2017 Nov 22
- Chronic thrombo-embolic pulmonary hypertension (CTEPH) develops in 4% of patients after pulmonary embolism and is accompanied by an impaired exercise tolerance, which is ascribed to the increased right ventricular (RV) afterload and a ventilation/perfusion (V/Q) mismatch in the lungs. This study investigated changes in arterial PO2 and hemodynamics in response to graded treadmill exercise during development and progression of CTEPH in a swine model. Swine were chronically instrumented and received multiple pulmonary embolisms by (i) microsphere infusion (Spheres) over five weeks, (ii) endothelial dysfunction by administration of eNOS inhibitor L-Nω-Nitroarginine methyl ester (LNAME) during seven weeks, (iii) **combined pulmonary embolisms and endothelial dysfunction (LNAME+Spheres**), or (iv) served as sham-operated controls (Sham). After nine weeks follow-up, embolization combined with endothelial dysfunction resulted in CTEPH as evidenced by a mean pulmonary artery pressure of 39.5±5.1mmHg versus 19.1±1.5mmHg (Spheres, $p<0.001$), 22.7±2.0mmHg (LNAME, $p<0.001$) and 20.1±1.5mmHg (Sham, $p<0.001$), and a decrease in arterial PO2 that was exacerbated during exercise, indicating a V/Q-mismatch. RV dysfunction was present after five weeks of embolization, both at rest (trend towards increased RV end systolic lumen area, $p=0.085$ and decreased SVi $p=0.042$) and during exercise (decreased SVi vs Control $p=0.040$). With sustained PH, RV hypertrophy (Fulton index $p=0.022$) improved RV function at rest and during exercise, but this improvement was insufficient in the CTEPH swine to result in an exercise-induced increase in cardiac index. In conclusion, embolization in combination with endothelial dysfunction results in CTEPH in swine. Exercise increased RV afterload, exacerbated V/Q mismatch and unmasked RV dysfunction.

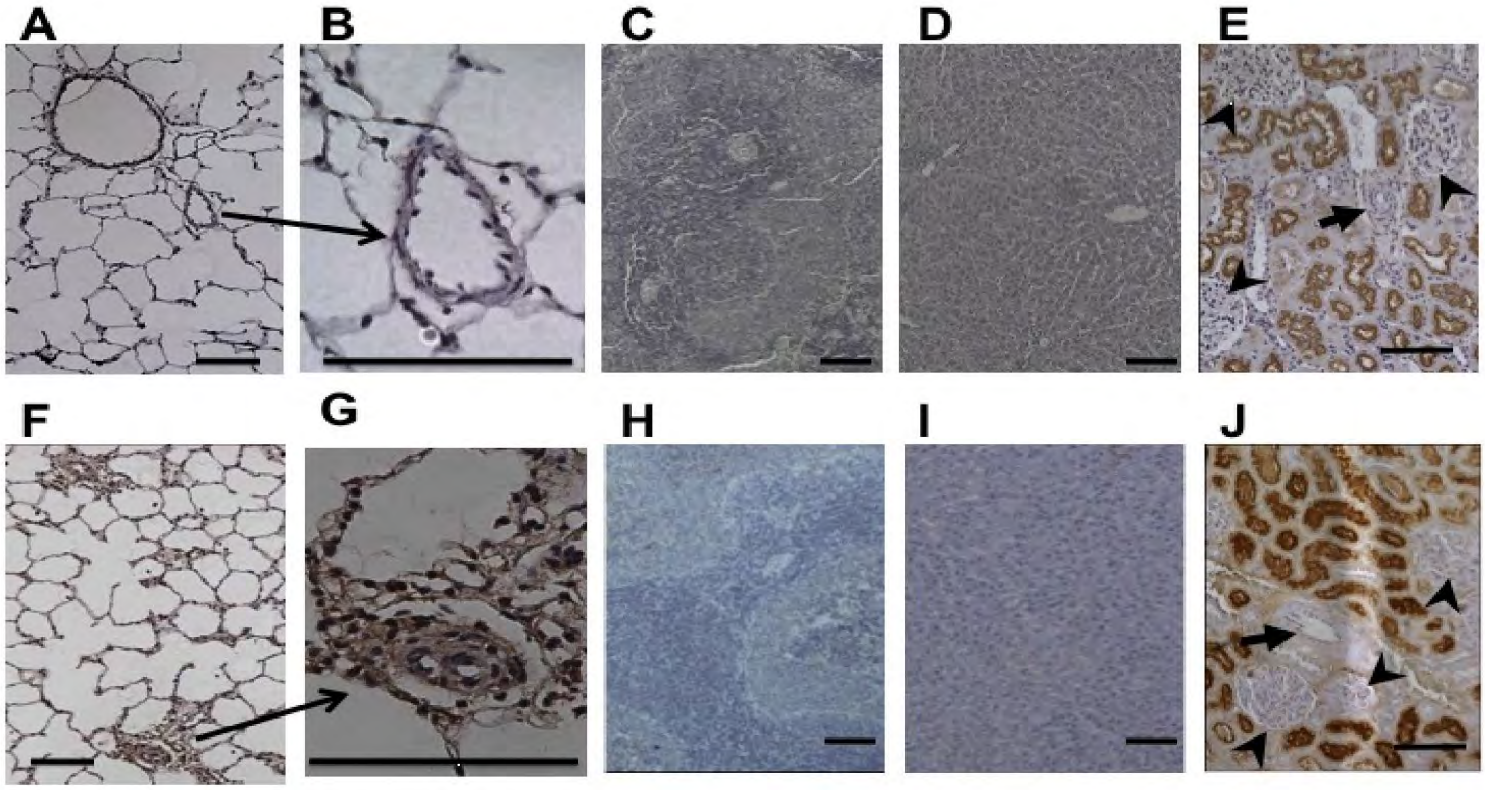
Testing a homing peptide - CAR

- Limitation of PAH medications, systemic effects
- Aim to target the drug more exclusively to the pulmonary vasculature
- In our models: High levels of inflammatory markers in PAH
- Peptides target “Tissues with high inflammation”
- Peptides have been tested which improve targeting of chemotherapeutic agents to tumors but not normal tissues

CAR



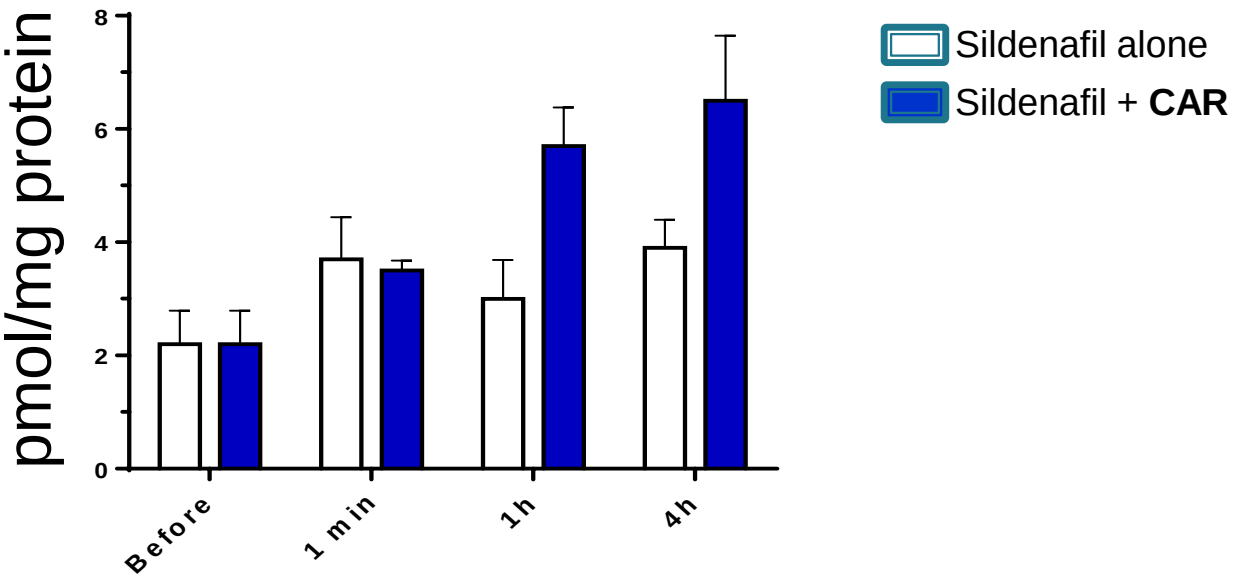
CAR: A Pulmonary Hypertensive Homing Peptide



Distribution of CAR in tissues of a normal (A-E) and a SU/Hx/Nx PAH rat (F-J)

Toba et al., A Novel Vascular Homing Peptide Strategy to Selectively Enhance Pulmonary Drug Efficacy in Pulmonary Arterial Hypertension, *Am J Path.* 2014.

cGMP Increase in the Lung

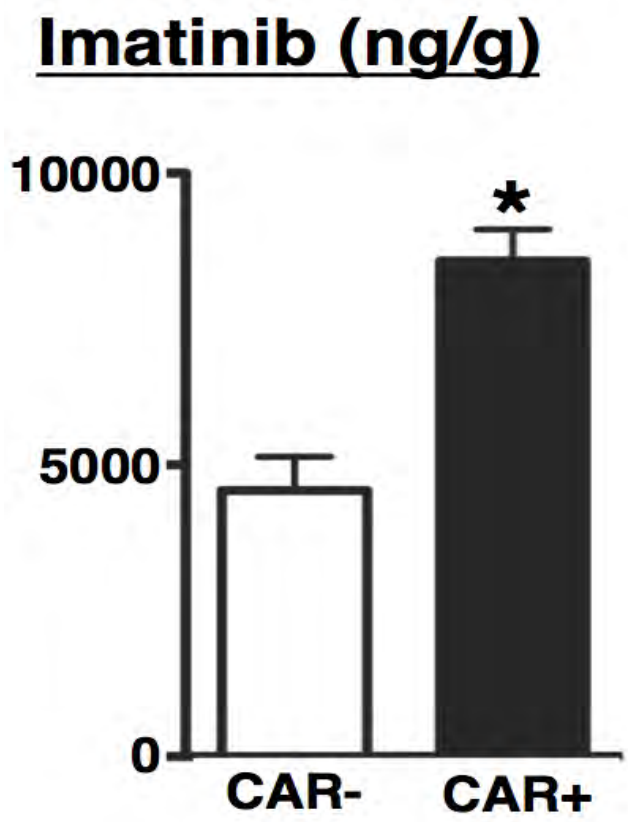


Sildenafil 100 µg/kg, i.v.

CAR 3 mg/kg, i.v.

N = 4

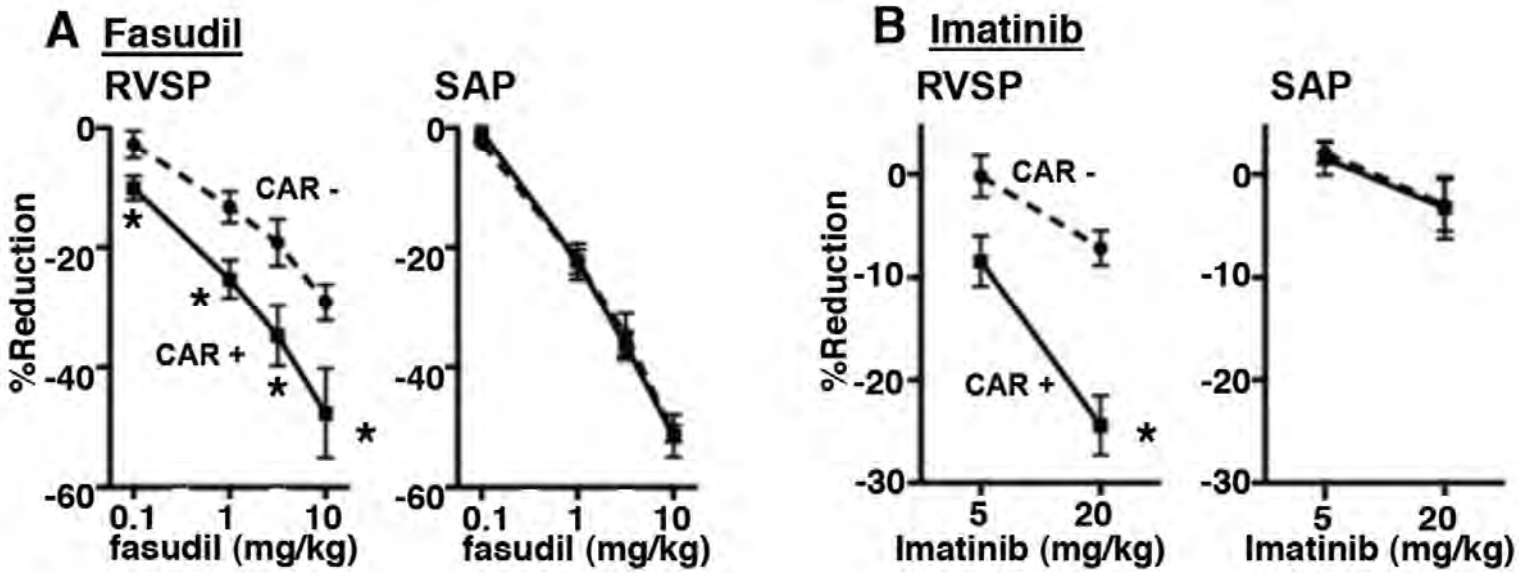
CAR Increases Imatinib Concentration in Isolated PAH Lungs



To examine if CAR increases drug transportation into the hypertensive lungs, tissue concentrations of imatinib were measured in isolated salt solution-perfused PAH lungs.

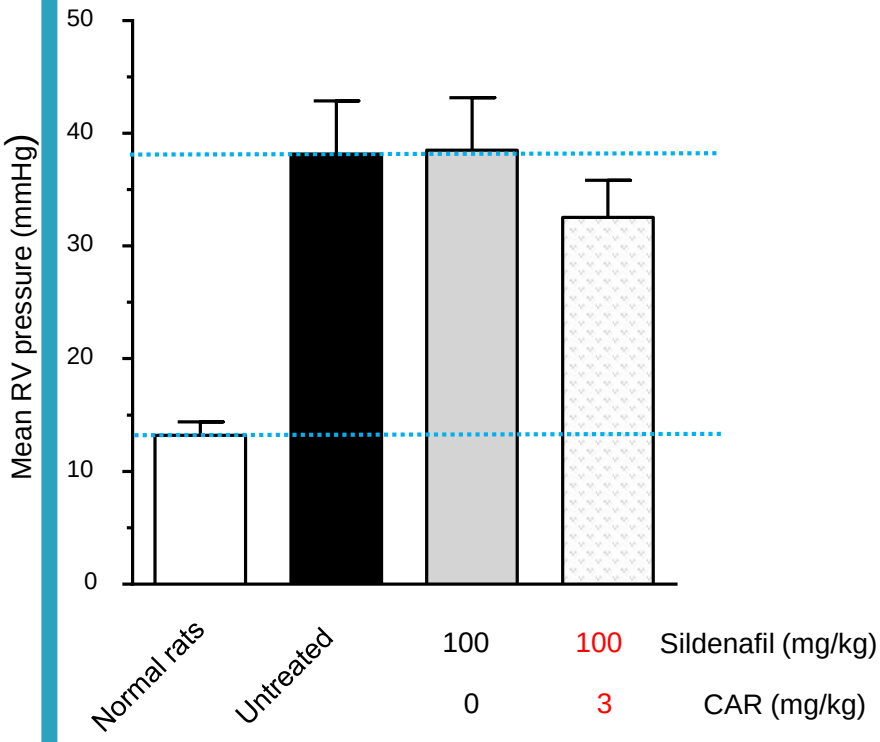
Co-administered CAR markedly increased imatinib levels in PAH lung tissues compared to those without CAR.

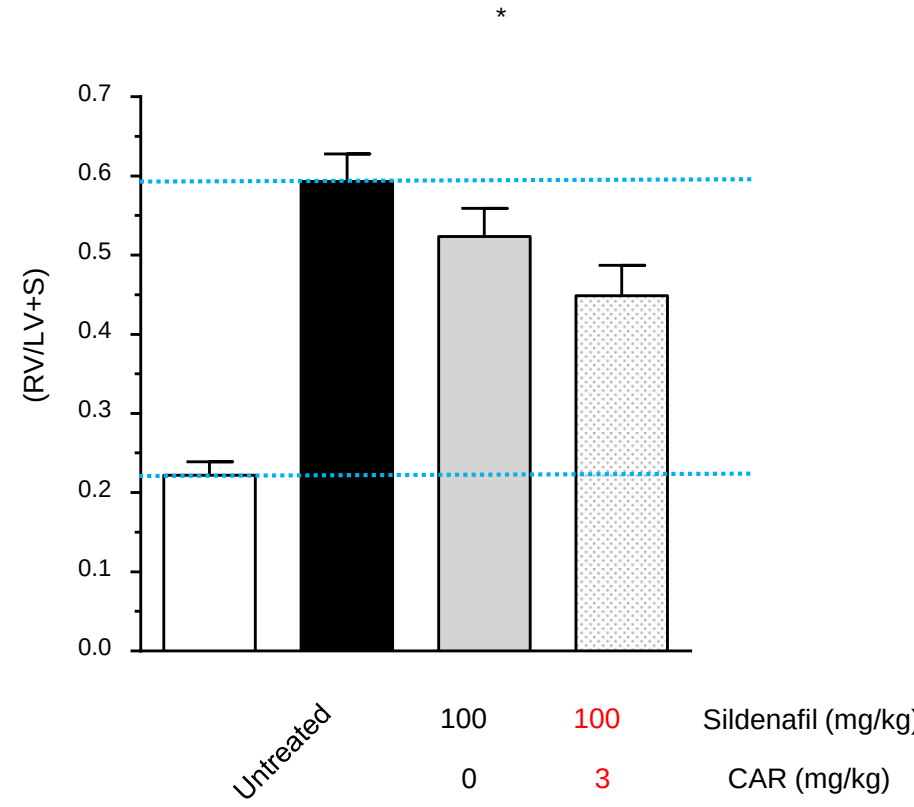
Acute Hemodynamic Effects of CAR on Vasodilators



Dose-response curves to fasudil (A) and imatinib (B) (iv, bolus) mixed with and without CAR (3 mg/kg) for right ventricular systolic pressure (RVSP) and systemic arterial pressure (SAP) in PAH rats. Values are means ± SE. N = 5-6. *p < 0.05 vs. without CAR.

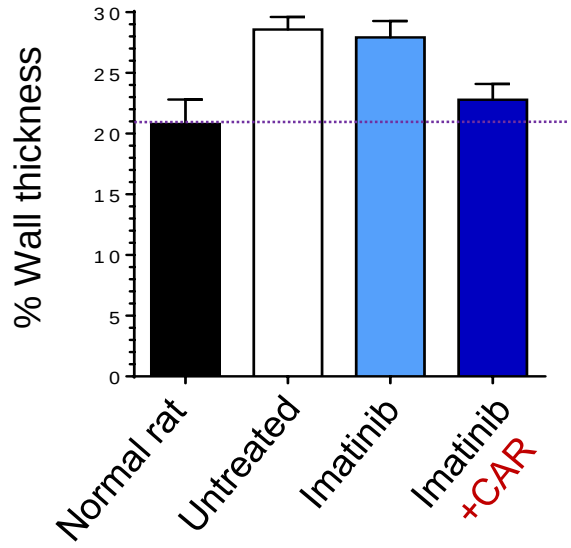
Effect on RV Pressure and Hypertrophy



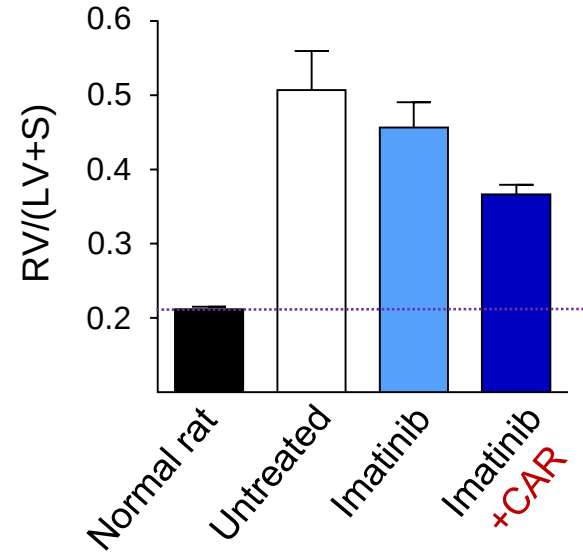


CAR Adjuvant Enhances Therapeutic Effect of Imatinib

Pulmonary artery muscularization

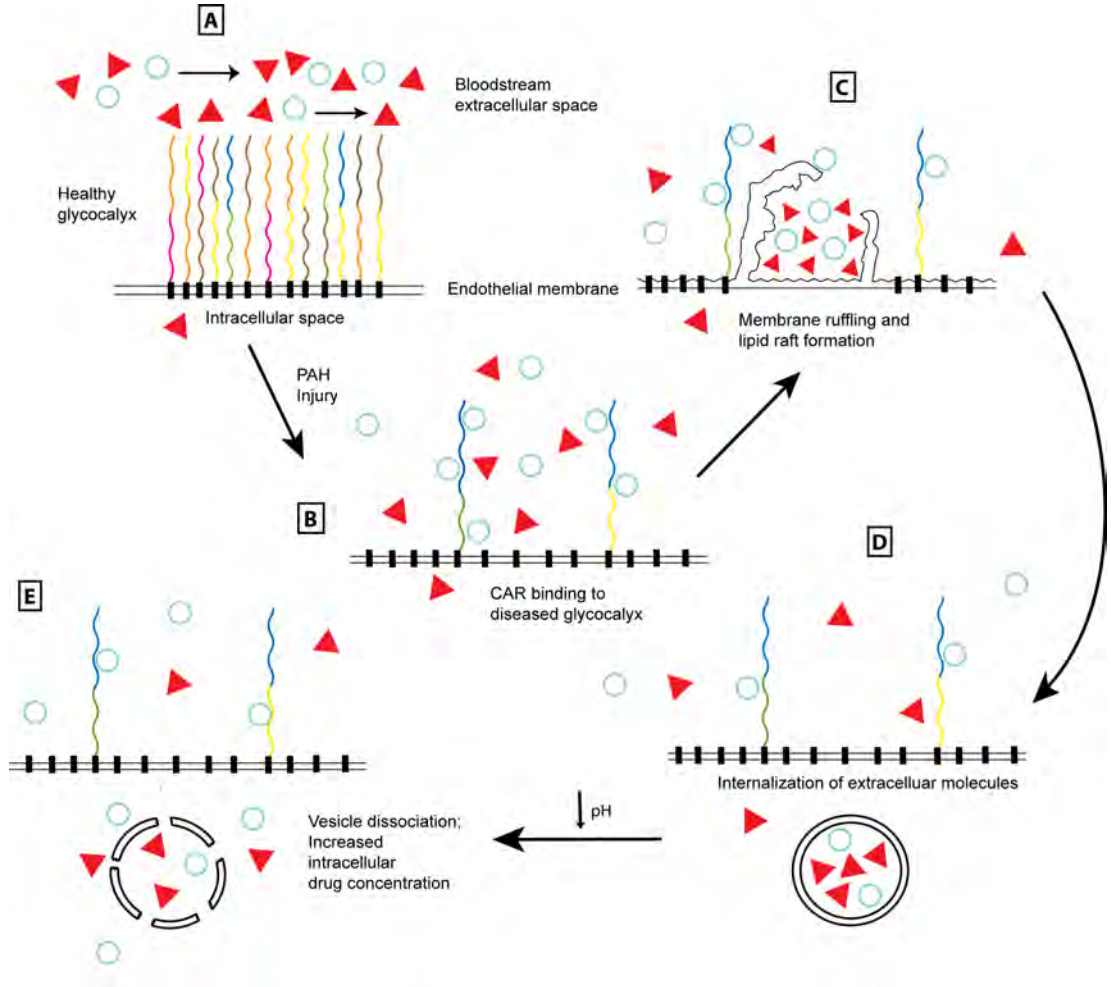


Right heart hypertrophy



Imatinib: 10 mg/kg (low dose)
CAR: 3 mg/kg

Hypothesized Mechanism of Action for CAR

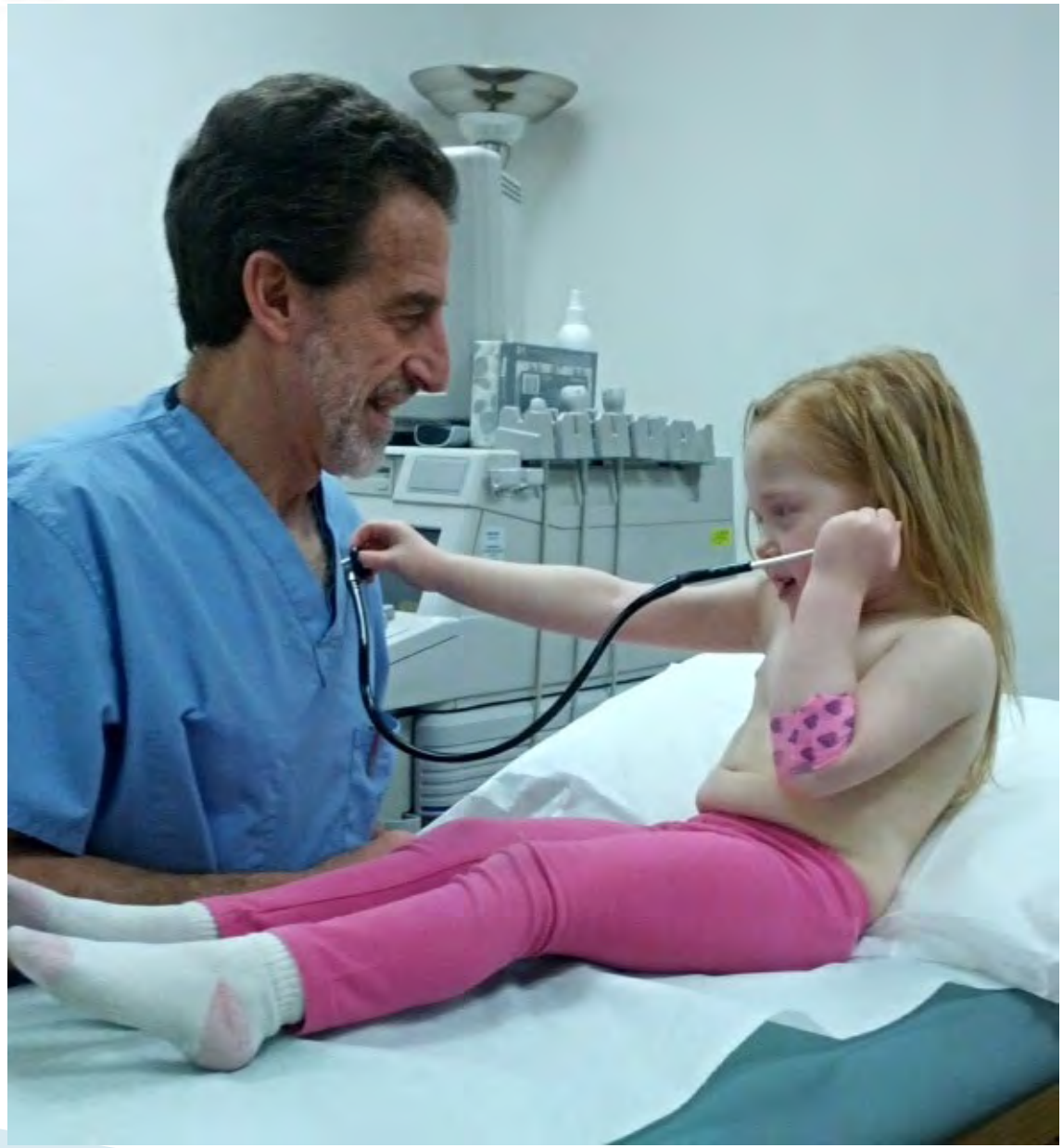


CAR - summary

- CAR is an effective homing peptide to hypertensive pulmonary vasculature in PAH
- CAR binds selectively to pulmonary arteries
- CAR facilitates the effect of sildenafil and immatinib in acute and chronic small animal models of PAH
- CAR may have beneficial effects on other diseases associated with inflammation: tumor regression, sepsis, CF, cachexia
- Ongoing CAR studies in large animal models
- Eventual use in patients

The Future

- Continued progress in understanding the cellular and molecular events associated with pulmonary hypertension – cellular studies, endoarterial biopsy
- Better pulmonary vasodilators, growth inhibitors
- Improved drugs for and surveillance of lung transplant rejection
- Ultimate aim: relieve symptoms and prolong survival, cure PAH



Acknowledgements

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Humberto Restrepo, Val Sarukhanov, David Mann
UNLV students