

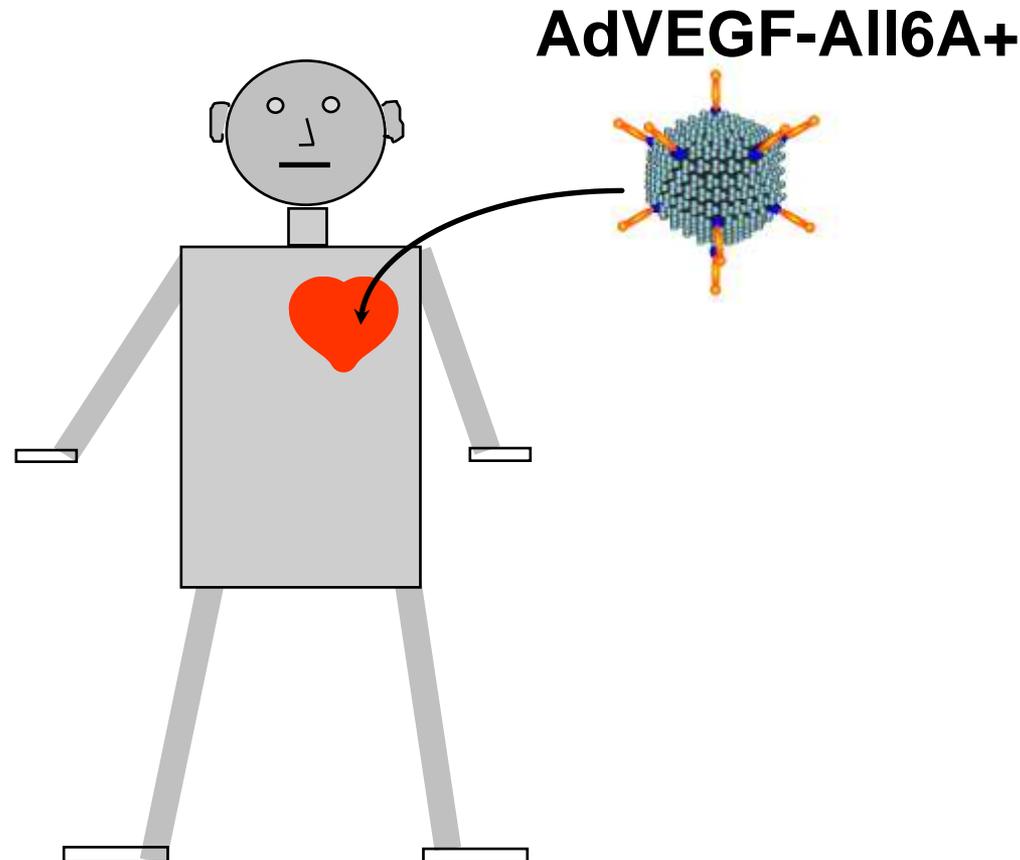
**A Phase I Study of Direct Administration of  
AdVEGF-All6A+, a Replication Deficient  
Adenovirus Vector Expressing a cDNA/Genomic  
Hybrid of Human Vascular Endothelial Growth  
Factor to the Ischemic Myocardium of Individuals  
with Diffuse Coronary Artery Disease via  
Minimally Invasive Surgery**

**RAC #1201-1145**

R. Crystal  
Weill Cornell Medical College

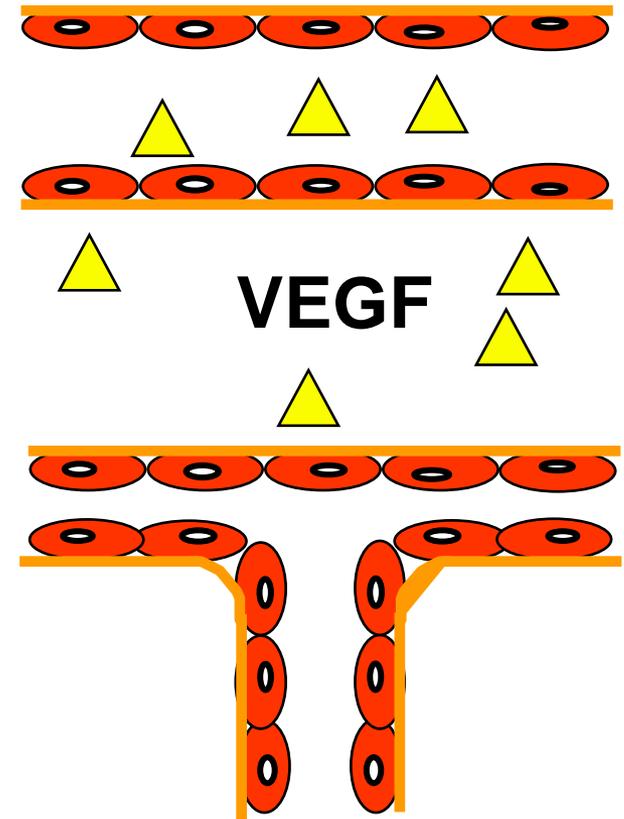
T. Rosengardt  
SUNY-Stony Brook School of Medicine

# AdVEGF-AII6A+ Gene Therapy for Individuals with Moderate to Severe Diffuse Coronary Artery Disease on Optimal Medical Therapy With No Other Therapeutic Options



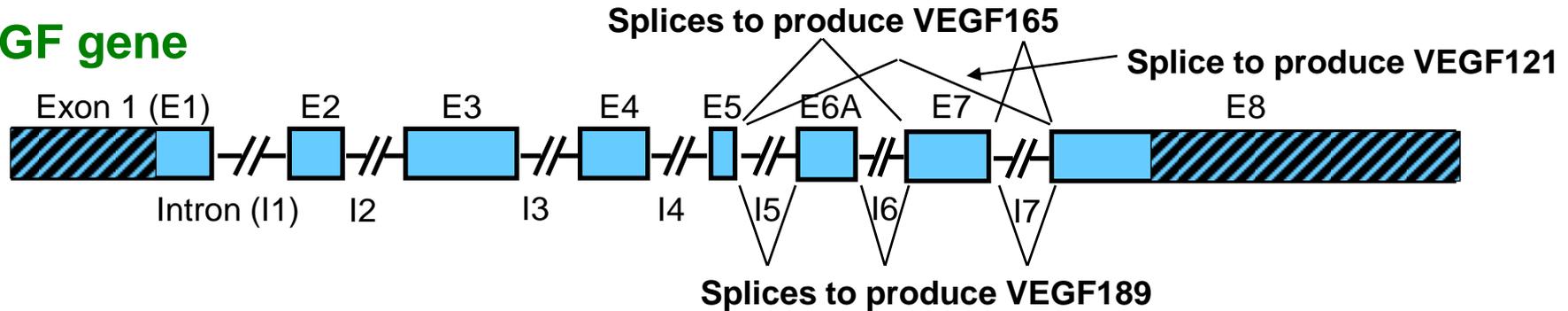
# Vascular Endothelial Growth Factor

- Potent mediator that initiates angiogenesis through tyrosine kinase receptors on endothelium and endothelial progenitors
- Mediates proliferation and mobilization of endothelial cells, basement membrane breakdown and vessel remodeling
- There are 3 major isoforms: VEGF 121, 165, 189
- All prior human cardiac gene therapy studies with VEGF have used only one isoform, either 121 or 165



# Alternate Splicing of the VEGF Gene mRNAs Encodes Protein Isoforms with Different Biological Activities

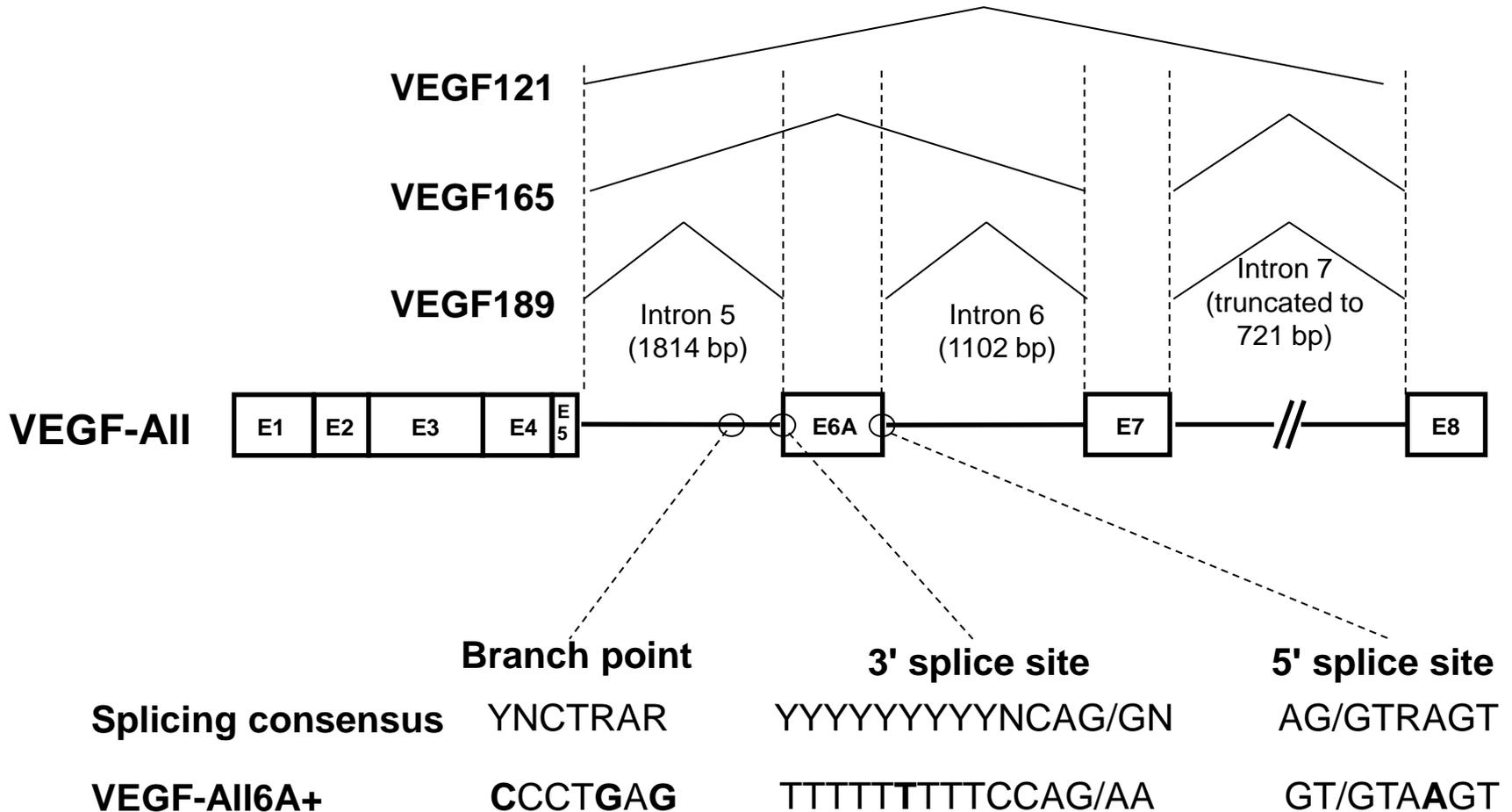
## VEGF gene



## Biological Properties

<u>Isoform</u>	<u>Exons</u>	Angiogenesis	Binding to extra-cellular matrix	Bind to neuropilin co-receptor	Induce vascular permeability
121	1-2-3-4-5-8	+	-	-	+
165	1-2-3-4-5-7-8	+	+/-	+	+
189	1-2-3-4-5-6A-7-8	+	++	+	-

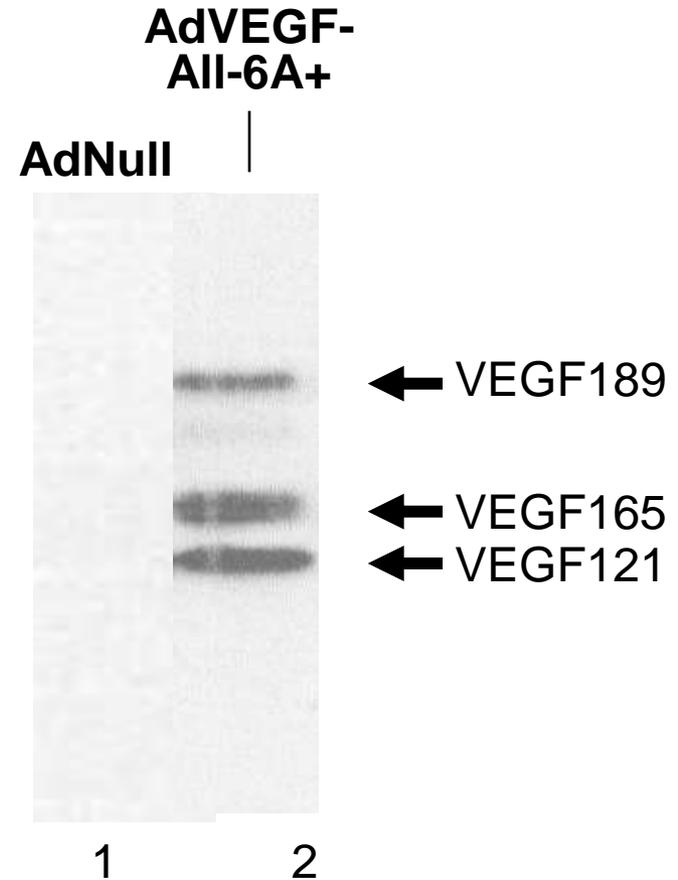
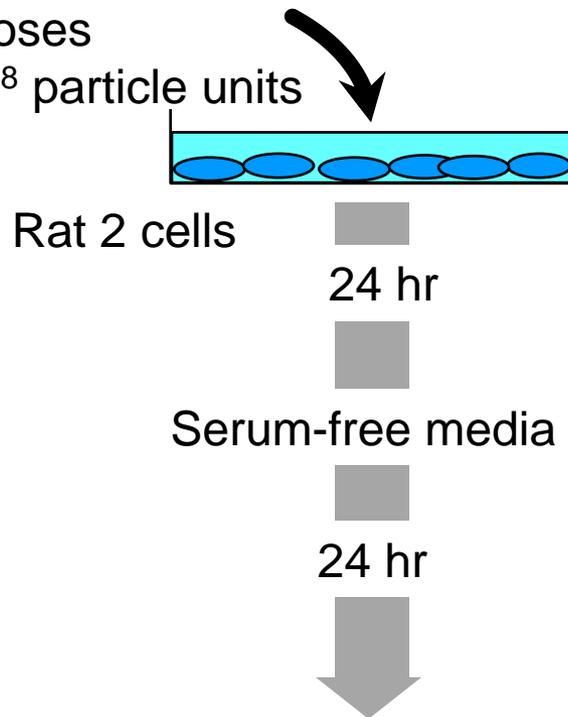
# AdVEGF-AII6A+



# Production of VEGF 121, 165, and 189 by AdVEGF-All6A+

- AdVEGF-All-6A+
- AdNull

All doses  
 $5 \times 10^8$  particle units



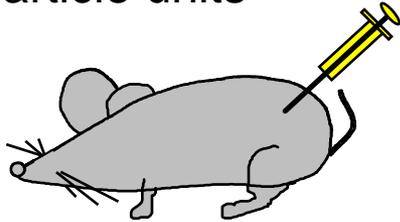
## Evaluate

- VEGF in media (Western)

# AdVEGF-AII6A+ Correction of Hind Limb Ischemia

## Intramuscular

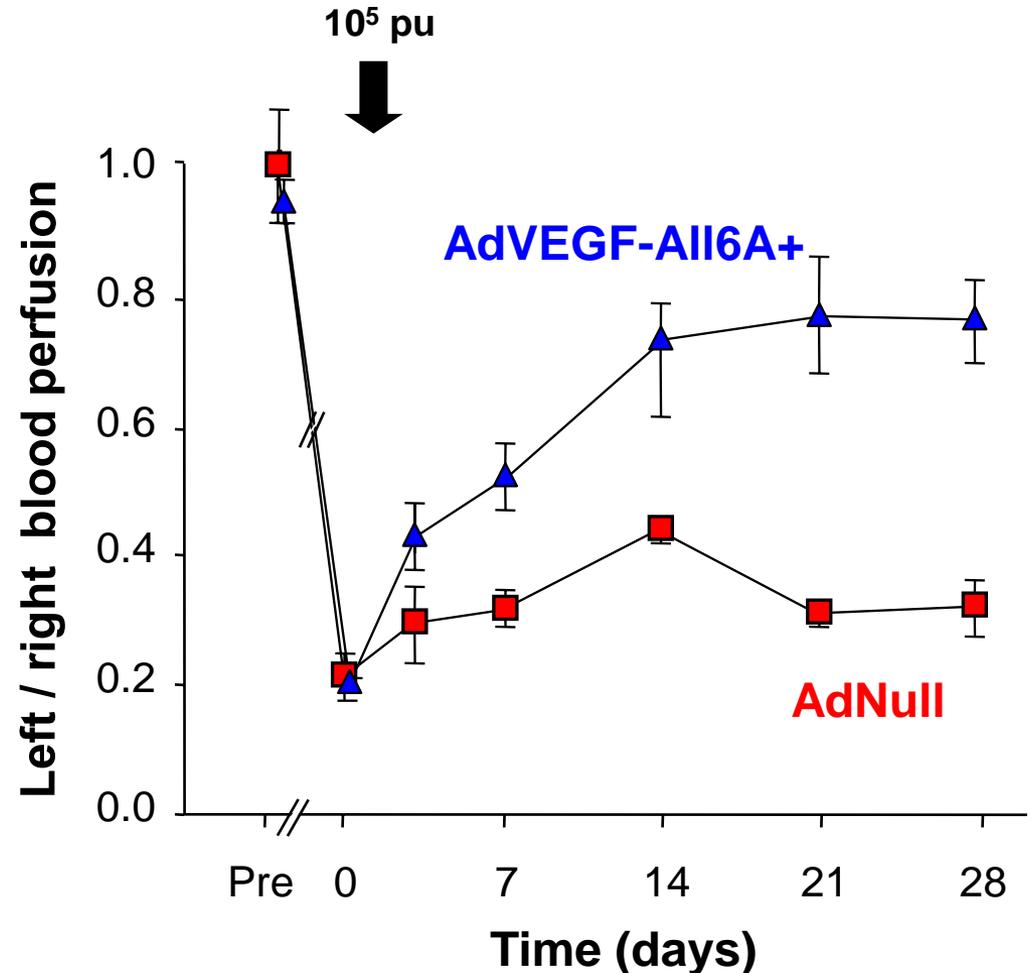
- AdNull
- AdVEGF-AII6A+  
10<sup>5</sup> particle units



Excise 5 mm of left iliac artery

## Evaluate

- Blood flow (Doppler laser, 3 wk)



# Safety/Toxicology Assessment of Cardiac Administration of AdVEGF-All6A+

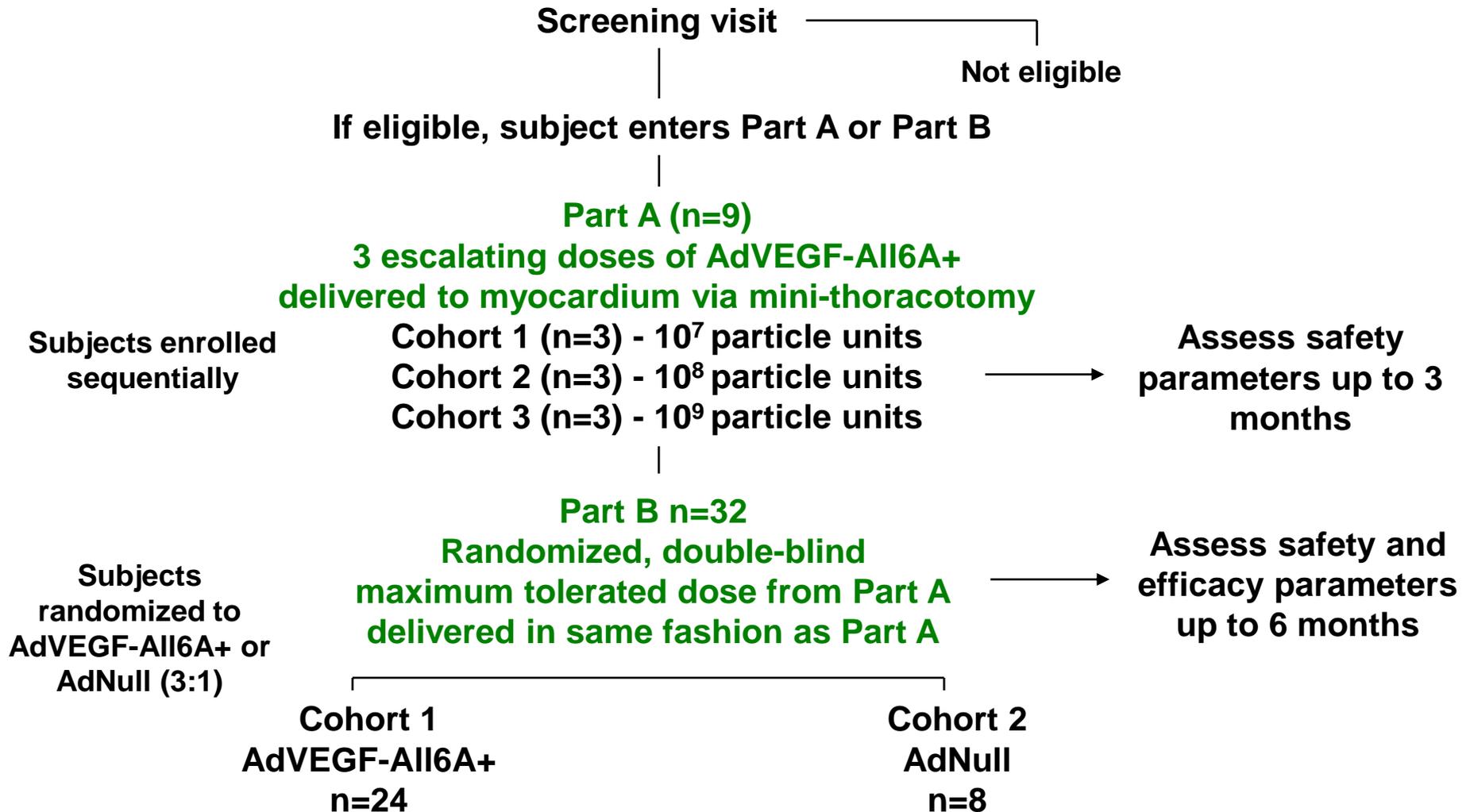
Rat cohort <sup>1</sup>			Ligation of left anterior descending coronary artery	Time point for evaluation <sup>2</sup>			
Vector	Dose	Total n		5 d	14 d	30 d	1yr
PBS	—	30	Yes	•	•	•	
		20	No			•	•
AdNull	10 <sup>7</sup> pu	30	Yes	•	•	•	
AdVEGF-All6A+	10 <sup>5</sup> pu	30	Yes	•	•	•	
		10	No			•	
	10 <sup>6</sup> pu	30	Yes	•	•	•	
		10	No			•	
	10 <sup>7</sup> pu	30	Yes	•	•	•	
		20	No			•	•

<sup>1</sup> n=210 total; 50% male / 50% female

<sup>2</sup> Mortality, general safety, complete blood count, serum chemistry, gross and histopathology, anti Ad antibody

# Overall Study Design

Subjects with diffuse moderate to severe coronary artery disease on optimal medical therapy with no other therapeutic options



# Clinical Endpoints

## Primary

- Exercise tolerance testing – time to 1 mm ST depression or time to termination of exercise (testing -30 and -15 days pre-rx, and 90 and 180 days post-rx)

## Secondary

- Dobutamine stress echocardiogram (pre, 90, 180 days)
- Angina (Canadian Cardiovascular Society Classification; pre, 30, 90, 180 days)
- Computed tomography coronary angiogram (pre, 180 days)

# Koch Review

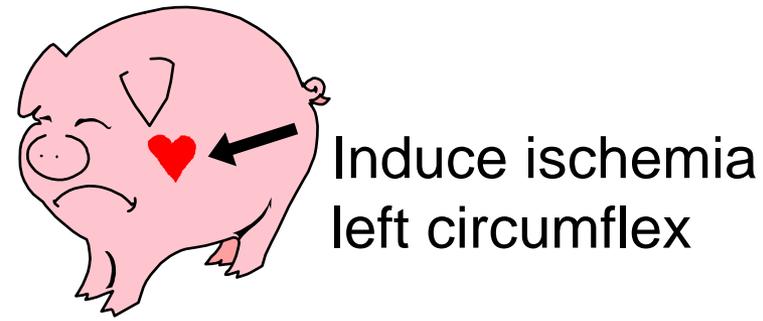
## Major

- Use of a pig model?
- Route of administration?

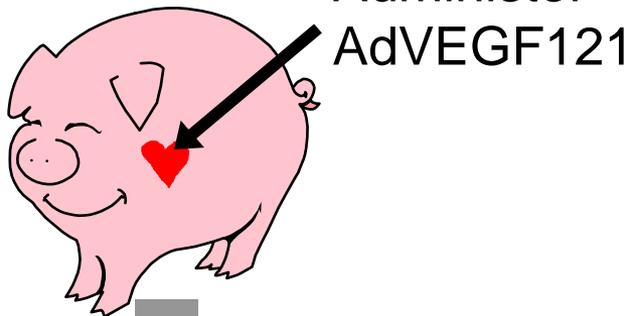
## Other

- Why not use VEGF121?
- Why not steroids and immunosuppressants?
- Angina as an outcome variable?
- Consent form issues?
- % cells transduced?

# Evaluation of AdVEGF121-Induced Angiogenesis in the Ischemic Pig Myocardium



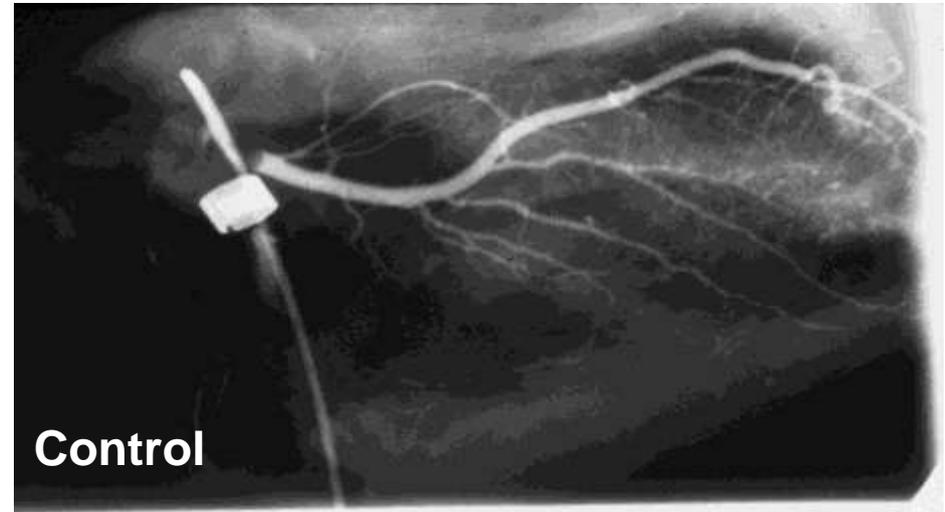
3 wk



4 wk

**Evaluate**

- Safety
- Efficacy



# Why Do a Pig Ameroid Constrictor Model?

## Arguments why it should be done

- The pig heart is similar to the human heart
- The ameroid constrictor coronary occlusion model mimics isolated coronary artery disease evolving over several weeks

# Why Do a Pig Ameroid Constrictor Model?

## Arguments as to why it should not be done for safety issues

- Extensive safety data in rats, pigs and humans of the identical Ad5 vector coding for VEGF121 at higher doses than proposed in the AdVEGF-All6A+ trial
- Rat myocardial safety data with AdVEGF-All6A+

# Why Do a Pig Ameroid Constrictor Model?

## Arguments as to why it should not be done for efficacy issues

- AdVEGF121 is efficacious in the pig ameroid constrictor model
- AdVEGF-All6A+
  - Splices to form all 3 major VEGF isoforms *in vivo*
  - Efficacious in a rat hind limb ischemia model
  - Experimental models with pigs, rats and mice are not humans with diffuse coronary artery disease

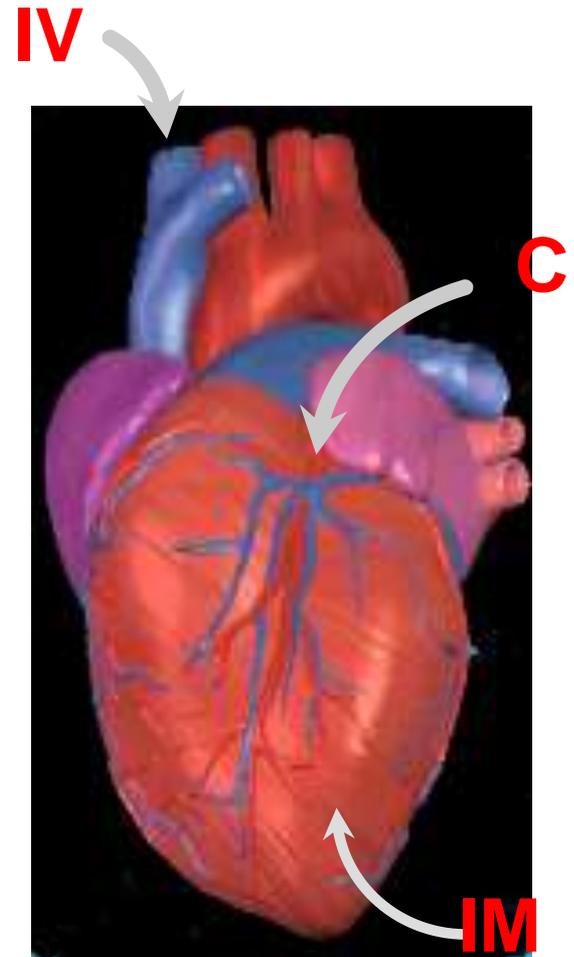
# Why Do a Pig Ameroid Constrictor Model?

## Summary

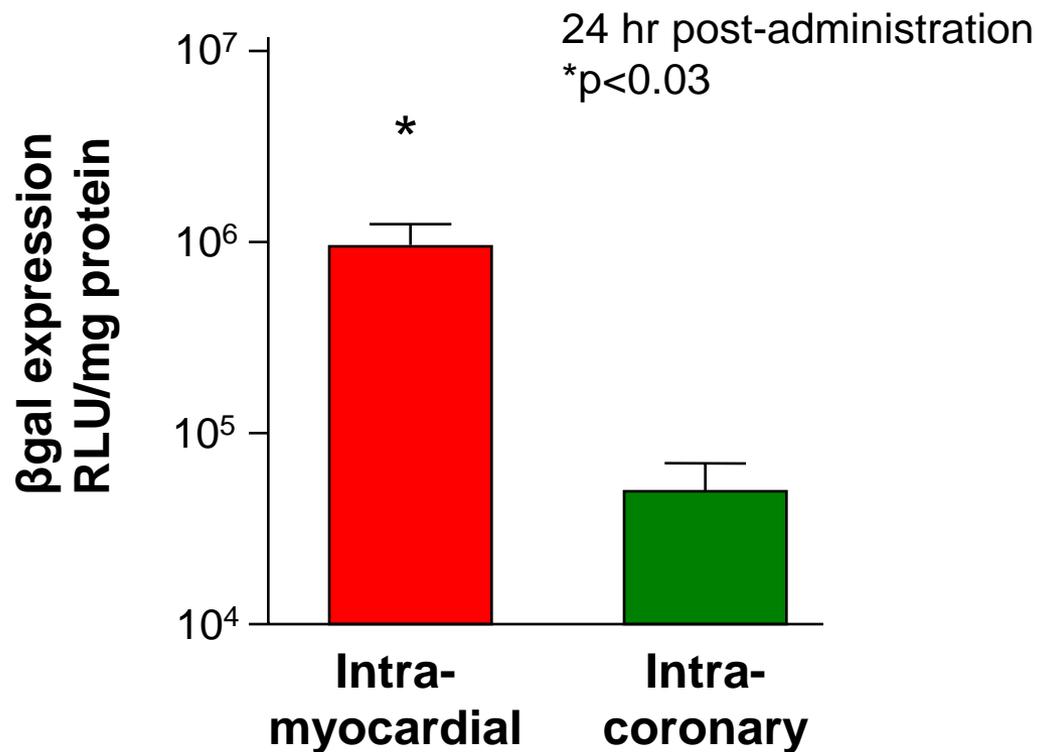
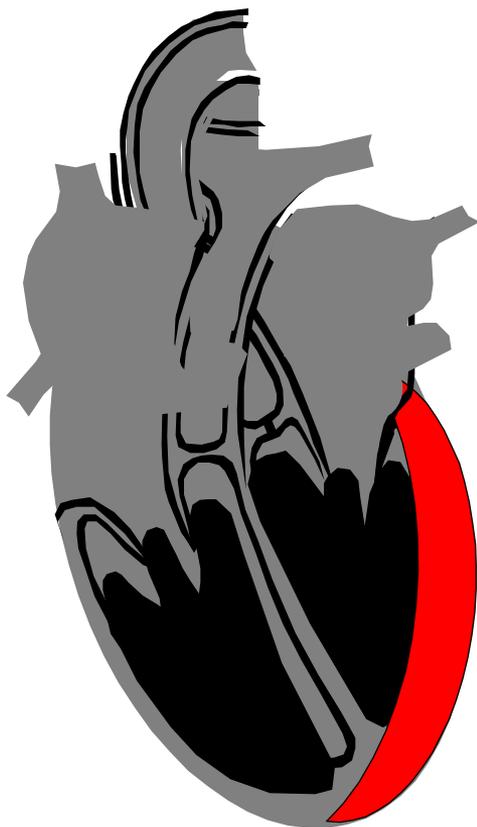
- While of academic interest, a pig ameroid constrictor model is not on the clinical development path for AdVEGF-All6A+ therapy of diffuse coronary artery disease
- Doing a pig ameroid constrictor model would take 1-2 years and cost several 100 thousand dollars
- We do not have the funding to carry out such as study
- In the current era of limited funding resources, the arguments for doing such a model are far out weighed by the arguments against, and it is very unlikely such as study would be funded
- Unless we move to humans, we will learn little, and this class of therapies will not move forward

# Delivery of Gene Transfer Vectors to the Myocardium

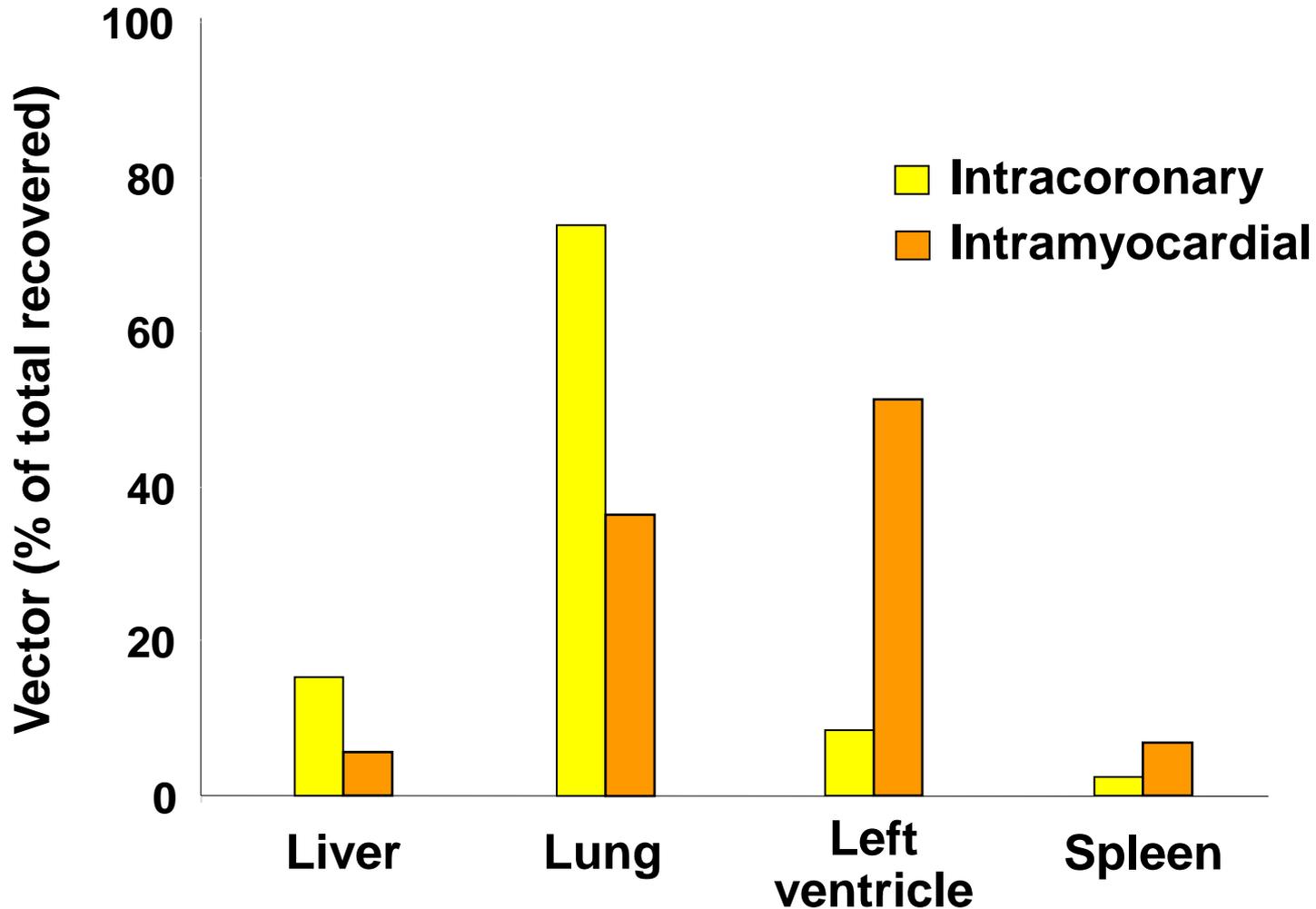
- Intravenous
- Intracoronary
- Intramyocardial
  - epicardial
  - endocardial



# Pharmacokinetics of Adenovirus Vector Intracoronary vs Intramyocardial Administration



# Tissue Distribution of Adenovirus Vectors 24 hr Following Intramyocardial vs Intracoronary Administration in the Pig



# Route of Delivery – Why Not Intracoronary or Intraventricular?

## Intracoronary

- Multiple studies failed to demonstrate efficacy (rVEGF165, rFGF2, AdFGF4), likely because of low level delivery to the myocardium

## Intraventricular (NOGA – cardiac navigation system)

- No NOGA-based angiogenesis trial yielded positive results
- NORTHERN trial<sup>1</sup> (double blind, randomized, placebo controlled pVEGF165 NOGA intraventricular) “use of a percutaneous catheter system ... makes it difficult to be certain that the DNA product was indeed injected into the myocardium”
- Euroinject ONE<sup>2</sup> (double blind, randomized, placebo controlled pVEGF165 NOGA intraventricular) “... the whole extent of the area intended to be treated...was not used...only 60% of the area intended to be treated was injected”

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<sup>1</sup> Stewart D et al, Mol Ther 2009; 17: 1109

<sup>2</sup> Gyöngyösi M et al, Circulation 2005; 112: I-157

# Route of Delivery – Why Not Intracoronary or Intraventricular?

## Summary

- Intracoronary is inefficient
- NOGA intraventricular
  - cannot insure that the vector is injected into the entire wall of the left ventricle
  - no control as to fraction of the myocardium that is treated
  - no control as to whether the vector is injected intravascular with the attendant high risk of anti-vector innate immunity

## Mini-thoracotomy / epicardial

- Absolute control of site of injection
- Limits of intravascular administration
- Shown to be safe in our prior experience with AdVEGF121

# Major Adverse Events Following Myocardial Administration of AdVEGF121 vs Routine CABG for Coronary Artery Disease

Group	n	Age (yr)	Co-morbid index	Major adverse events*	
				per individual	% within 0-7 d
Mini-thoracotomy + AdVEGF121	16	60±11	3.1±1.4	1.0	38
CABG, no gene therapy	8	71±8	2.1±1.9	1.1	89

\* "Major" adverse event = 3 or 4 on a scale of 0 - 4

# Ornelles Review

## Major

- Safety of the vector?

## Other

- Promote tumor growth?
- Species variability of human VEGF?
- Significance of changes in outcome variables?
- Other therapies?
- Consequences of immune clearance?
- Consent form issues?

# Safety of the Ad5 Component of the Vector

- Extensive safety data of intramyocardial administration of Ad5VEGF121 in rats and pigs
- Humans with the identical phenotype of moderate to severe diffuse coronary artery disease received Ad5VEGF121 with no major events related to the vector
- AdVEGF-All6A+ is safe in a formal toxicology study in the rat myocardium

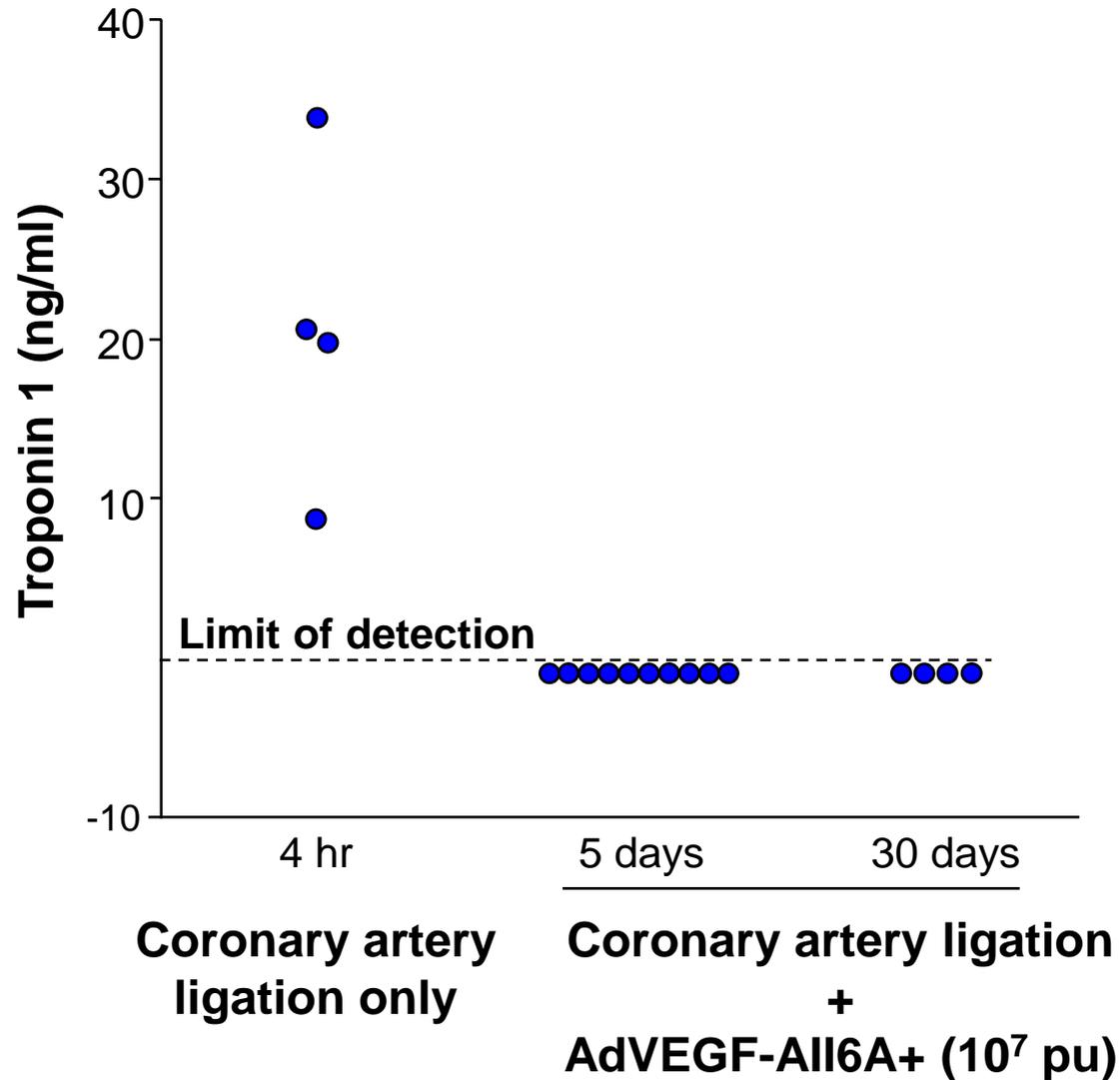
# Toxicology Assessment of Cardiac Administration of AdVEGF-All6A+

Rat cohort <sup>1</sup>			Ligation of left anterior descending coronary artery	Time point for evaluation <sup>2</sup>			
Vector	Dose	Total n		5 d	14 d	30 d	1yr
PBS	—	30	Yes	•	•	•	
		20	No			•	•
AdNull	10 <sup>7</sup> pu	30	Yes	•	•	•	
AdVEGF-All6A+	10 <sup>5</sup> pu	30	Yes	•	•	•	
		10	No			•	
	10 <sup>6</sup> pu	30	Yes	•	•	•	
		10	No			•	
	10 <sup>7</sup> pu	30	Yes	•	•	•	
		20	No			•	•

<sup>1</sup> n=210; 50% male / 50% female

<sup>2</sup> Mortality, general safety, complete blood count, serum chemistry, gross and histopathology, anti Ad antibody

# AdVEGF-AII6A+ Administration into the Left Ventricle of Rats – Myocardial Infarction Model

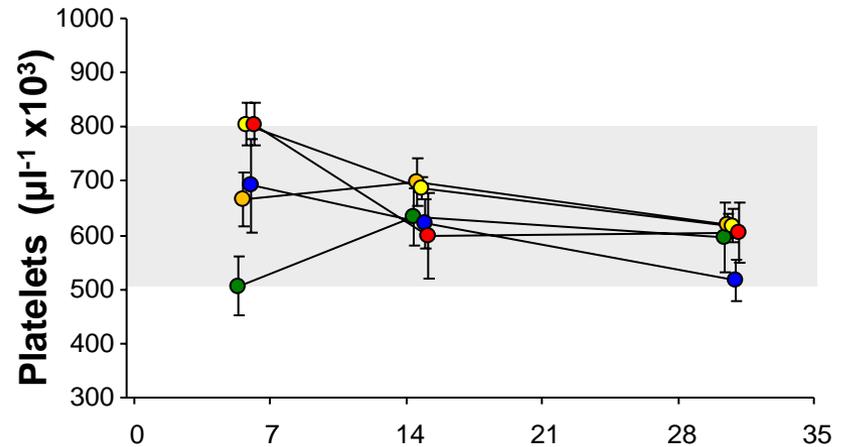
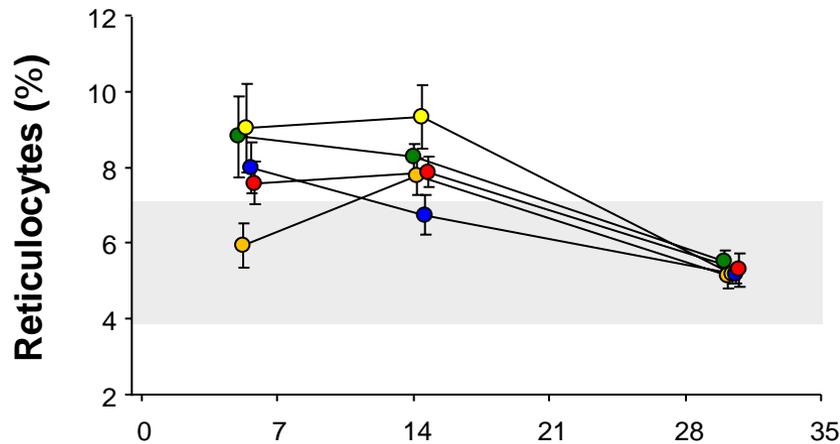
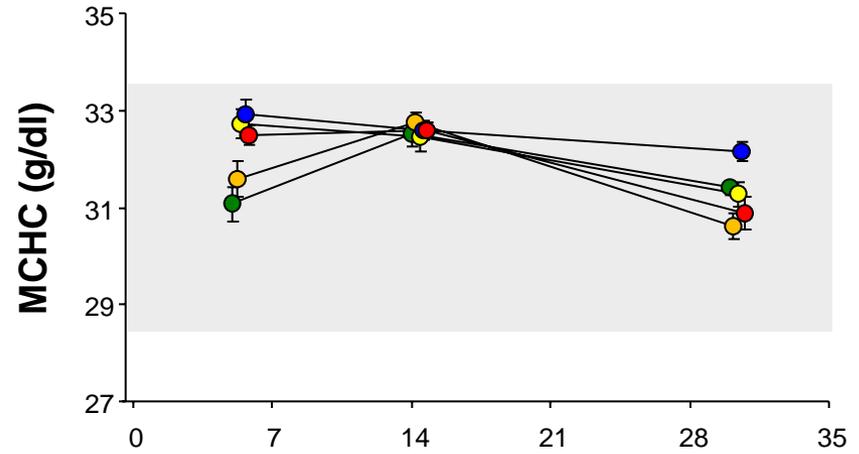
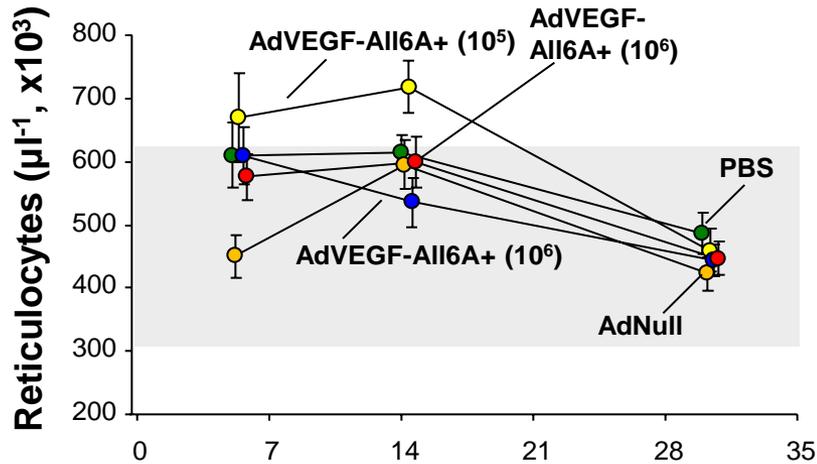


# Toxicology Assessment of Cardiac Administration of AdVEGF-AII6A+

Parameter	p value <sup>1</sup>	Parameter	p value <sup>1</sup>	Parameter	p value <sup>1</sup>
White blood cells	0.78	ALP	0.40	Creatinine kinase	0.99
Red blood cells	0.56	ALT	0.23	Amylase	0.96
Hemoglobin	0.18	AST	0.58	Lipase	0.24
Hematocrit	0.56	GGT	0.79	Sodium	0.74
MCV	0.52	Albumin	0.44	Chloride	0.96
<b>MCHC</b>	<b>0.003</b>	Total protein	0.45	Potassium	0.39
Neutrophils (%)	0.42	Globulin	0.47	Na/K ratio	0.28
Lymphocyte %	0.47	Total bilirubin	0.80	A/G ratio	0.12
Monocyte %	0.47	BUN	0.61	B/C ratio	0.93
Eosinophil %	0.32	Creatinine	0.77	Calculated osmolarity	0.54
Basophil %	0.03	Cholesterol	0.97	Anion gap	0.98
Neutrophil absolute	0.79	Triglyceride	0.90		
Lymphocyte absolute	0.63	Glucose	0.04		
Monocyte absolute	0.65	LDH	0.53		
Eosinophil absolute	0.61	Calcium	0.44		
Basophil absolute	0.03	Phosphorus	0.28		
<b>Platelets</b>	<b>0.004</b>	Bicarbonate	0.90		
<b>Reticulocytes</b>	<b>0.006</b>				
<b>Reticulocytes absolute</b>	<b>0.009</b>				

<sup>1</sup> ANCOVA for treatment group with time (5,14,30 days) as covariate

# Toxicology Assessment of Cardiac Administration of AdVEGF-AII6A+



Time post-vector (days)

# Dresser Review

## Major

- Ethics of the placebo control?

## Other

- Qatar site issues?
- Consent form issues?

HMC Approved  
Consent, page 1

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Version



<p><input type="radio"/> HGH   <input type="radio"/> WH   <input type="radio"/> RH   <input type="radio"/> AAH <input type="radio"/> AKH   <input type="radio"/> OTHERS</p> <p>HC NO: PATIENT NAME: DOB: GENDER: NATIONALITY:</p> <p>Identifiable samples but NO intent to Disclose the Testing Results</p>	<p><input type="radio"/> م. حمل العام   <input type="radio"/> م. النساء   <input type="radio"/> م. الرميّة   <input type="radio"/> م. الأمل <input type="radio"/> م. أخرى   <input type="radio"/> م. الغور</p> <p>رقم السجل: إسم المشارك: تاريخ الميلاد: النوع (ذكر   أنثى) : الجنسية:</p> <p>نموذج موافقة مستبينة للمشاركة بدراسة تتطوى على الإختبارات الجينية</p> <p>(استخدام عينات معرفة دون الكشف عن نتائج الإختبارات أو الفحص</p>
<p>You are free to ask as many questions as you like before, during or after in this research, you decide to give consent to participate in this research study. The information in this form is only meant to better inform you of all possible risks or benefits. Your participation in this study is voluntary. You do not have to take part in this study, and your refusal to participate will involve no penalty or loss of rights to which you are entitled. You may withdraw from this study at any time without penalty or loss of rights or other benefits to which you are entitled.</p>	<p>كمشارك في هذا البحث العلمي لك مطلق الحرية في طرح أى سؤال أو إستفسار عن هذا البحث وذلك قبل أو أثناء أو بعد إكمال إجراء البحث. الهدف الرئيسي من المعلومات الواردة في هذا النموذج هو أن نقدم لكم الشرح اللوالب والمستفيض عن كل الأخطار والفوائد التي يمكن أن تتمخض عن إجراء هذا البحث. المشاركة في هذا البحث عمل طوعي خالص وبالتالي لكم مطلق الحرية بعدم المشاركة. قراركم بعدم المشاركة في هذا البحث العلمي لا يترتب عليه أى تبعات أو حرمان من حقوقكم المستحقة. أيضا يمكنكم الانسحاب وعدم مواصلة المشاركة في هذا البحث في أى وقت أو مرحلة دون أن يؤثر ذلك في حقوقكم أو فوائدهم المستحقة.</p>
<p><b>Note: This template is for genetic studies</b> Using identifiable samples but there is no intention to disclose results of the genetic testing to subjects or research participants, families, or participants' health care providers.</p> <p>PLEASE DO NOT CHANGE THE FORMAT OF THE TEMPLATE</p>	<p><b>ملاحظة :</b> هذا النموذج يستخدم في دراسات بحث ينطوي على الإختبارات الجينية لعينات المشارك المعرفة ولكن ليس هناك نية لطريق البحث الكشف عن نتائج هذه الإختبارات أو الفحوصات للأفراد المشاركين في الدراسة، أو لعائلاتهم، أو حتى للطبيب أو الأطباء الذين يقدمون للمشارك رعاية طبية ليست ذات صلة بهذه الدراسة.</p> <p>فضلا لا تغيير في الشكل العام لهذا النموذج</p>

Signed Consent Form / موافقة خطية للمشاركة بدراسة بحث طبي

HMC Research Committee  
Approved  
Approval Date : 3/11/11  
Expiry Date : 2/11/12

# Study Design Regarding Active Drug vs Placebo

## Part B, at the highest tolerable dose from part A

- 3:1 AdVEGF-All6A+ to AdNull randomized, blinded
- Patients, treating physicians and the study team will be blinded to the active drug vs placebo
- Both vectors will be delivered to the myocardium by the epicardial route via a mini-thoracotomy

# What Are the Ethical Issues?

- Discomfort and risk of the general anesthesia and mini-thoracotomy with a 1 of 4 chance of receiving an adenovirus gene transfer vector with no VEGF-All6A+ transgene

# Why do a Randomized, Double Blind, Placebo Design?

- Complex disease with many factors modulating the endpoints
- Sham surgery itself is a “placebo therapy” (Parkinson’s, arthroscopy)<sup>1-3</sup>
- Needle sticks to the heart can induce angiogenesis
- Ad vectors will attract monocytes and monocytes express VEGF
- AdNull recruits cardiac progenitor cells to the myocardium

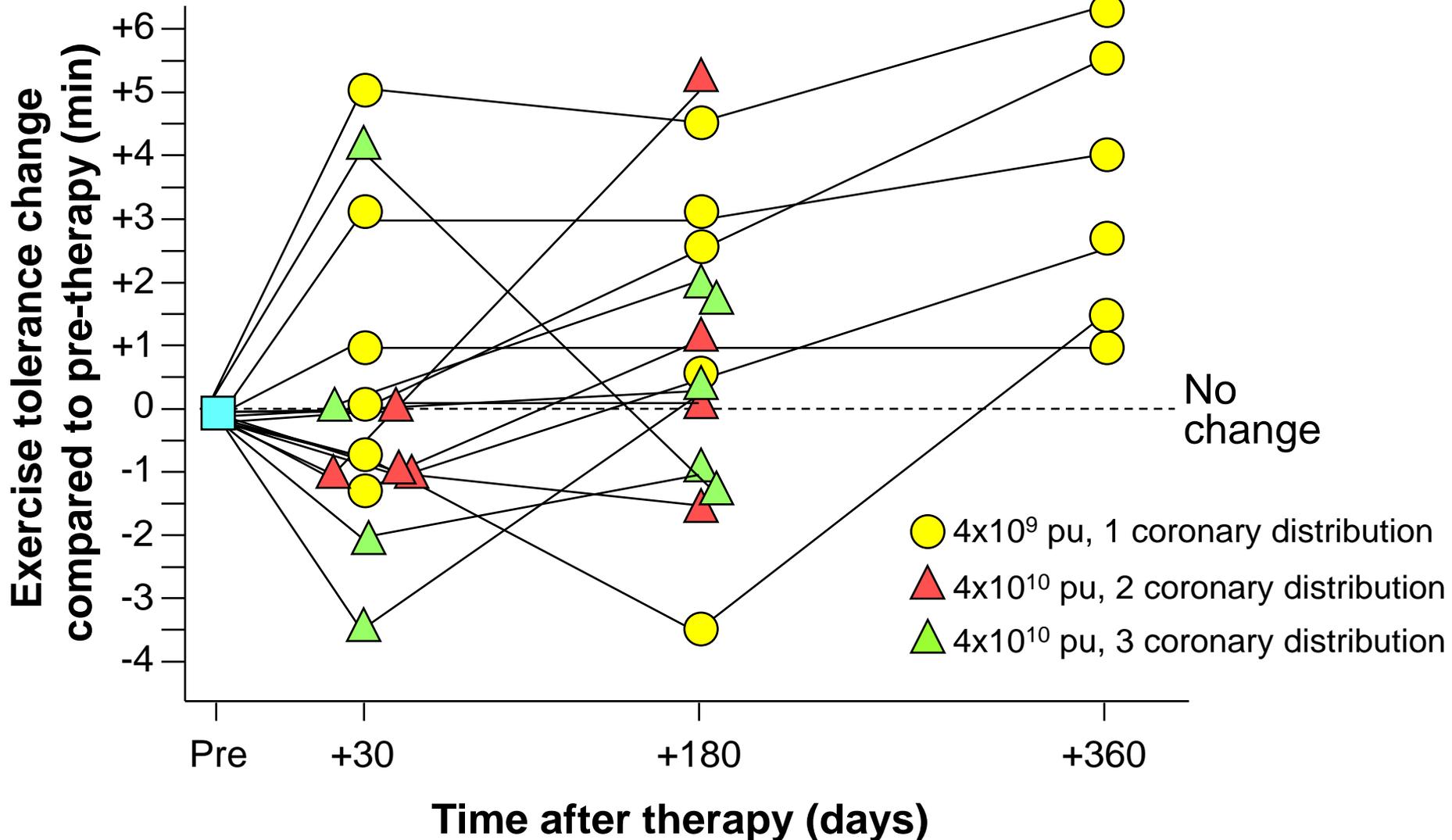
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<sup>1</sup> Freed et al, N Engl J Med 2001; 344:710

<sup>2</sup> Moseley et al, N Engl J Med 2002; 347:81

<sup>3</sup> Campbell et al, Health Tech Assessment 2010; 14:1

# Exercise Duration Following Myocardial Adenovirus VEGF121-Mediated Gene Transfer



# Does the AdNull Placebo Have Any Chance of Efficacy?

- KAT RCT trial (Hedman et al Circ 2003, 107: 2677-2683) with intracoronary AdVEGF165 vs pVEGF165

“We cannot fully exclude the possibility that the adenoviral vector itself could have contributed to the results”

# Does the AdNull Placebo Have any Chance of Efficacy?

- AdNull, the placebo, is identical to AdVEGF-All6A+ except it does not contain the VEGF-All6A+ transgene
- Does AdNull have any possibility of efficacy?
  - Needle sticks to the myocardium induces angiogenesis<sup>1-3</sup>
  - Ad vectors attract inflammatory cells, including monocytes; monocytes express VEGF<sup>4</sup>
  - In experimental animals, AdNull administration to the heart recruits cardiac progenitor cells to the peri-infarct area after myocardial infarction<sup>5</sup>

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<sup>1</sup> Chu et al, J Thorac Cardiovasc Surg, 1999; 118: 849

<sup>2</sup> Mueller et al, Heart, 2001; 85:697

<sup>3</sup> Pelletier et al, Ann Thorac Surg 1998; 66:12

<sup>4</sup> Harvey B-G et al, J Clin Invest 1999; 104:1245

<sup>5</sup> Spillmann F et al, Regenerative Med 2006; 1:235

# What is the Risk of the Mini-thoracotomy in This Population?

- Requires general anesthesia
- Procedure takes ~ 1 hr
- Post-op chest tube <24 hr
- Discharge 2-3 days
- Mini-thoracotomy in the identical study population for sole therapy with AdVEGF121 in n=16 subjects was not associated with unanticipated serious adverse events and no deaths

# Could We Do Only a Skin Incision as “Sham Surgery”?

- No, because the patients and the physicians would immediately know who got what because of lack of chest tubes and length of recovery

# Why Would Potential Participants Want to Participate in This Trial?

## Arguments AGAINST

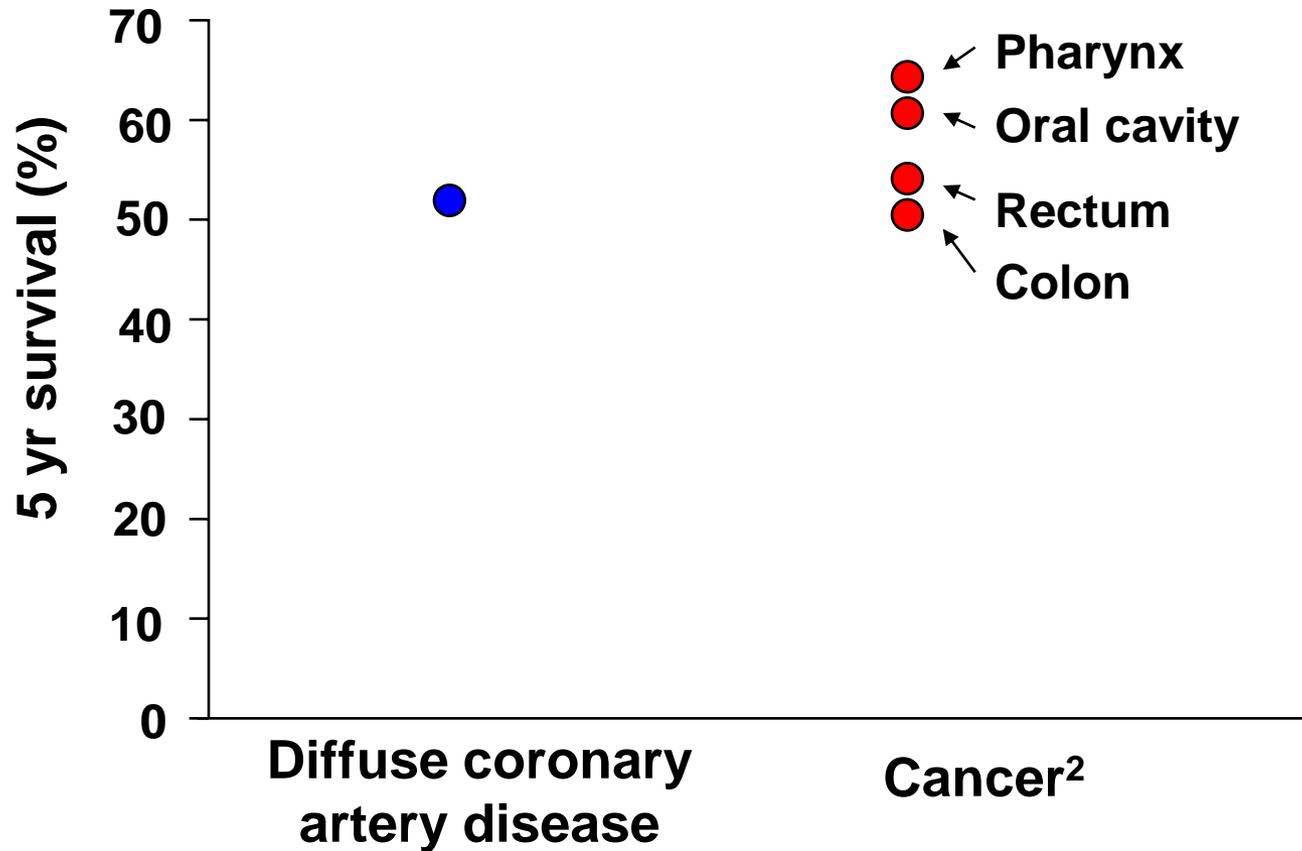
- Unproven therapy
- Discomfort and risk of general anesthesia and mini-thoracotomy
- Risk of cardiac administration of the AdVEGF-All6A+ vector

# Why Would Potential Participants Want to Participate in This Trial?

## Arguments FOR

- On maximal medical therapy for diffuse coronary artery disease
- Not eligible for bypass surgery or stenting
- Quality of life – Angina Class III – symptoms with everyday living activity or Class IV – inability to perform any activity without angina or angina at rest
- Estimated 5 yr life span 52%, similar to cancers of the oral cavity, pharynx, colon, rectum, retroperitoneum and leukemias

# Survival for Diffuse Coronary Artery Disease



<sup>1</sup> Allen KB et al, Ann Thorac Surg 2004; 77:1228

<sup>2</sup> Surveillance, epidemiology and end results, NCI (2011) [www.seer.cancer.gov](http://www.seer.cancer.gov)

# Consent Documents

- We agree with all of the suggested changes, including Dr. Dresser's suggestion to have the participant write the explicit acknowledgment:

**“I realize that I might receive only placebo surgery. This surgery will likely not benefit my diffuse coronary artery disease.”**

# Ethical Considerations (1)

- For the clinical development of therapies for disabling, potentially fatal disorders for which the phenotype is complex (i.e., not a simple blood biomarker), the direct way to prove or disprove efficacy is to carry out a placebo controlled, randomized, blinded trial
- There is no difference between what is proposed and the assessment of new chemotherapeutic agents for many fatal cancers
  - There is discomfort and risk, including the risk of death from the therapy
  - The therapy being tested is superimposed on standard, optimal medical therapy

# Ethical Considerations (2)

- This is research; no matter how rational and no matter how many positive experimental animal studies, if we knew the drug was efficacious, the drug would be approved
- Gene therapy has matured as a clinical development field where it is critical that we design our trials as placebo controlled blinded studies