Multi Gene Vascular Systems

MGVS

June 15th, 2005

RAC Meeting

Presentation Outline

- Introduction
  - MGVS Overview
  - Peripheral arterial disease (PAD)
  - Angiogenesis, Therapeutic angiogenesis
  - MultiGeneAngio Product
- MultiGeneAngio – Preclinical studies:
  - Toxicity studies
  - Efficacy studies
  - Bio-distribution
- Clinical protocol
- Discussion

MGVS: Overview

- Established - August 2000,
- Based in Carmel Medical Center, Haifa, Israel. 22 full time employees
- Focus on cell and gene therapy for heart and blood vessels disorders

- Pre-IND process initiated November 2002, pre-clinical studies completed June 2005
- Phase I study in collaboration with University of Michigan, Ann Arbor

MGVS Overview

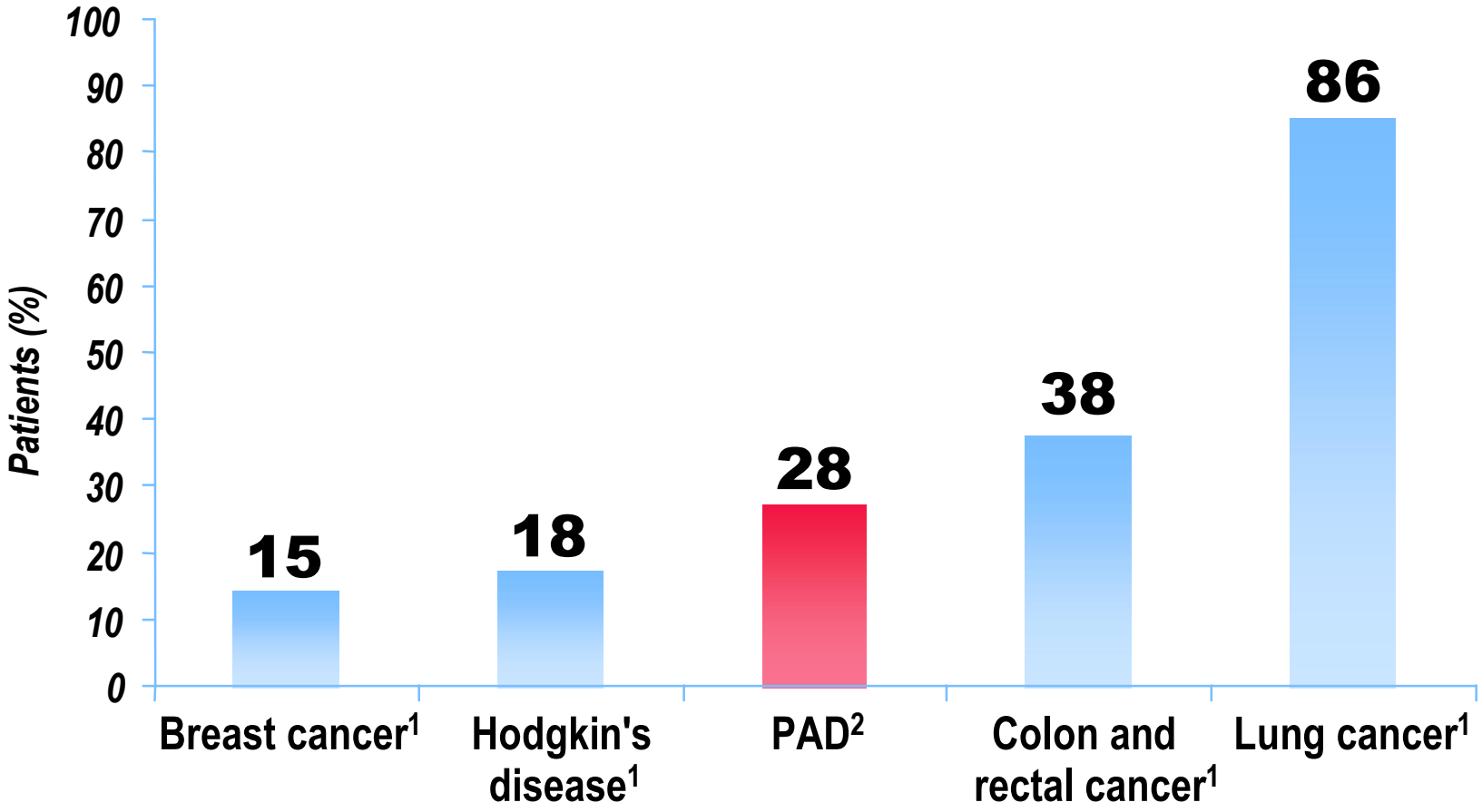
- MGVS products for growing new arteries and tissue engineering are based on cells and genes that are operative in the natural development and maintenance of the arterial tree

- **Scientific Advisory Board:**
  - Prof. Israel Vlodavsky, Haifa, Israel
  - Prof. Gera Neufeld, Haifa, Israel
  - Prof. Basil Lewis, co-founder of MGVS, Haifa, Israel
  - Prof. Jacob Schniederman, Tel Hashomer, Israel
  - Prof. Wolfgang Schaper, Bad Nauheim, Germany
  - Prof. Eli Keshet, Jerusalem, Israel
  - Prof. Eithan Galun, Jerusalem, Israel
  - Dr. F-L Cosset, Lyon, France
  - Prof. Aharon Chiechanover, Haifa, Israel; Nobel Prize Laureate 2004

Peripheral Arterial disease

- Narrowing or occlusion of blood vessels supplying the lower extremity most often due to atherosclerosis
- Annual mortality of PAD is 4%
- Patients with critical limb ischemia have an anuual mortality of 25%
- Symptoms are claudication that may progress to critical limb ischemia manifest by rest pain, tissue loss, and gangrene, that eventually may necessitate amputation

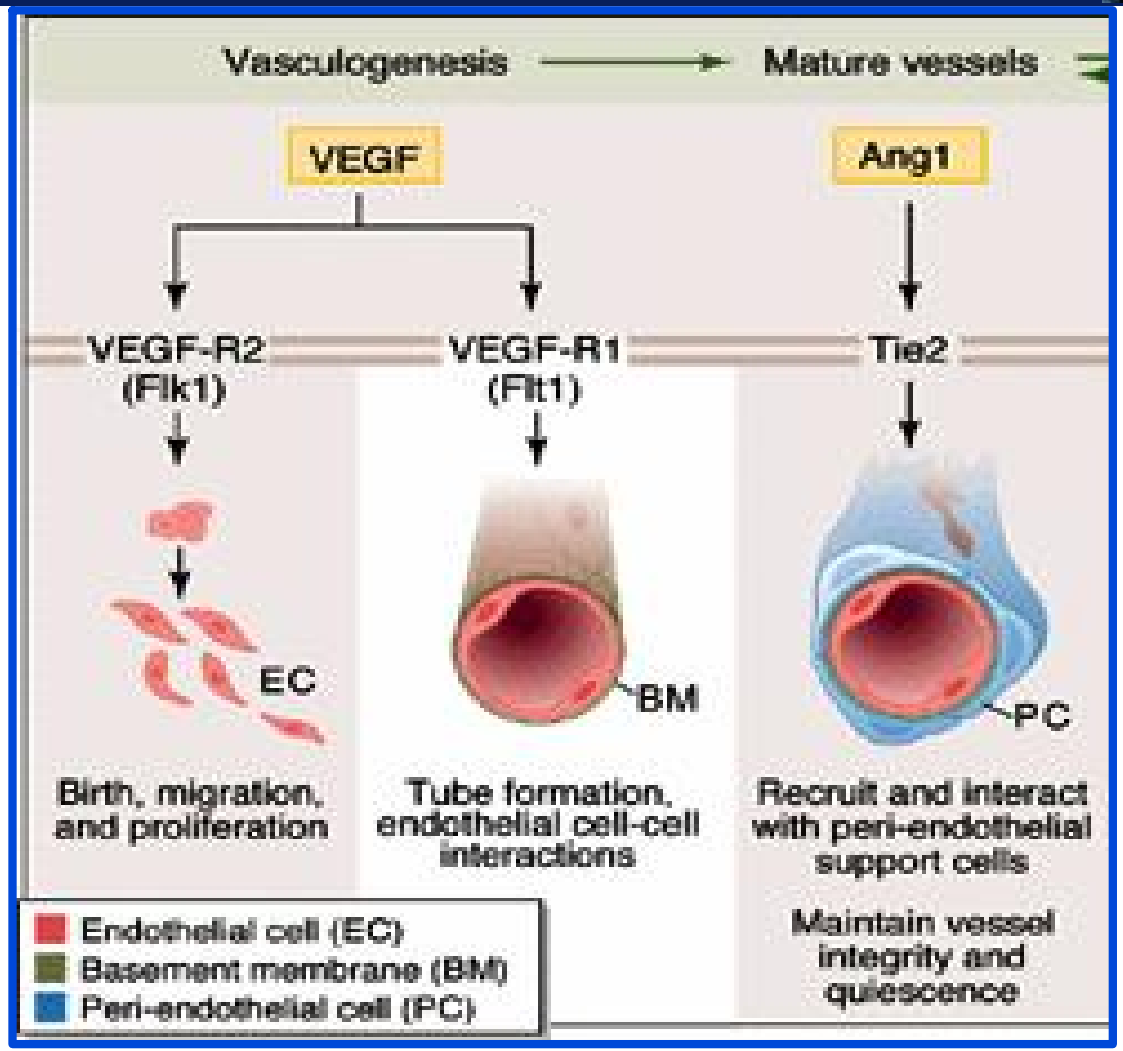
Relative 5-year PAD mortality rates versus other common pathologies



[1]American Cancer Society. Cancer Facts and Figures – 1997.

[2]Kampozinski RF, Bernhard VM. In: Vascular Surgery (Rutherford RB, ed). Philadelphia, PA: WB Saunders: 1989;chap 53.

Key genes in angiogenesis

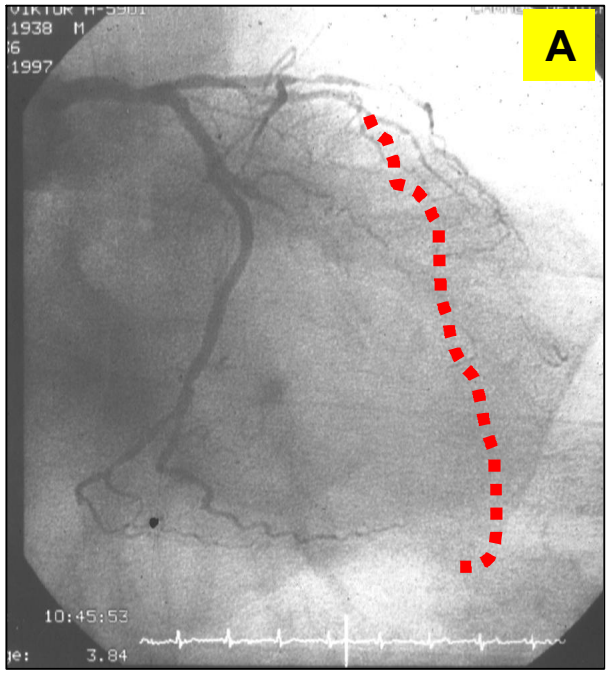


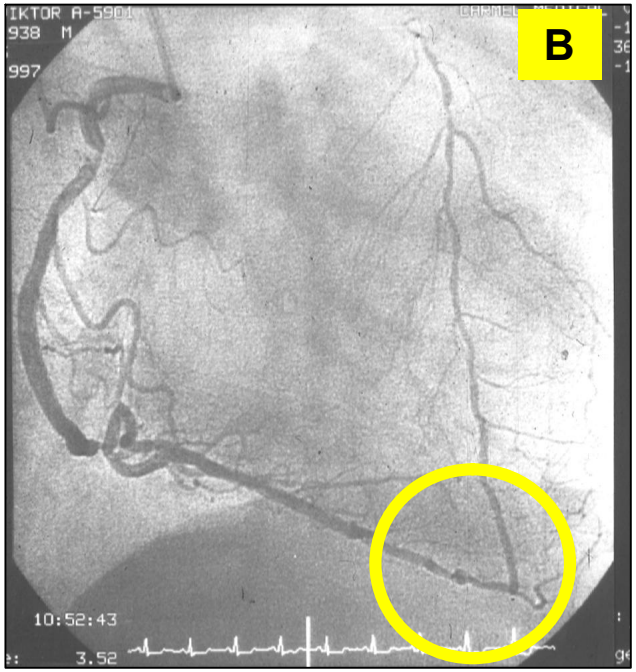
Hanahan, Science 1997

Angiogenesis studies – Phase II and III

	Disease	Gene/ Protein	Growth factor	Delivery	Pt. # included	Result
VIVA	CAD	Protein	VEGF	Intracoronary +I.V.	178	negative (high dose pos. at 120 days)
FIRST	CAD	protein	FGF-2	intracoronary	337	negative
GM-CSF	CAD	protein	GM-CSF	Intracoronary + subcutaneous	21	positive
TRAFFIC	PAD	protein	FGF-2	intraarterial	190	positive (neg. at 180 days)
AGENT	CAD	gene	FGF-4	intracoronary	79	positive (one dose only)
KAT	CAD	gene	$VEGF_{165}$	intracoronary	103	positive (Ad only)
REVASC	CAD	gene	$VEGF_{121}$	intramyocardial surgery	67	positive (neg. at 3 months)
Euroinject One	CAD	gene	$VEGF_{165}$	intramyocardial catheter-based	74	negative (pos. after exclusion of 2 centers)
VEGF PVD	PAD	gene	$VEGF_{165}$	Local, catheter-mediated	54	positive
RAVE	PAD	gene	$VEGF_{121}$	intramuscular	105	negative

The goal for therapeutic angiogenesis





Angiogenic factors & cytokines

Relevant cells

Flow mediated shear stress

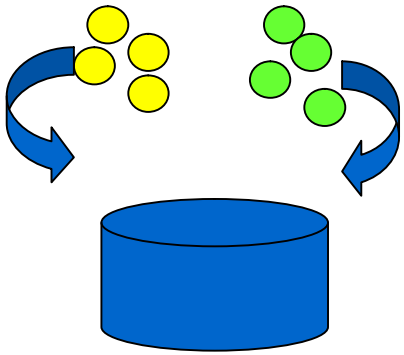
} Successful therapeutic angiogenesis

Rationale for use of cells and genes

- Use of a single angiogenic protein or gene to induce therapeutic angiogenesis failed to show efficacy
- Angiogenesis is complex biological process involving multiple cell types and proteins, all operating in coordination
- Many investigators focus on stem cell therapy but the cells lack complete characterization and required growth factors for differentiation
- Use of autologous endothelial and smooth muscle cells activated by VEGF and Ang-1 genes injected intra-arterially to produce hemodynamically significant collateral arteries

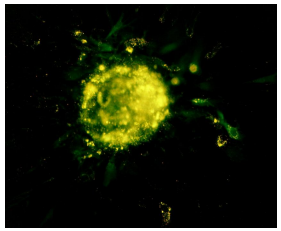
In-vitro angiogenesis 3-D spheroid model

EC and SMC 1:1 mixture (750 cells/well)



4.8% Methyl Cellulose

24h

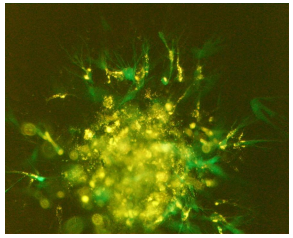


Spheroid

Collagen embedding

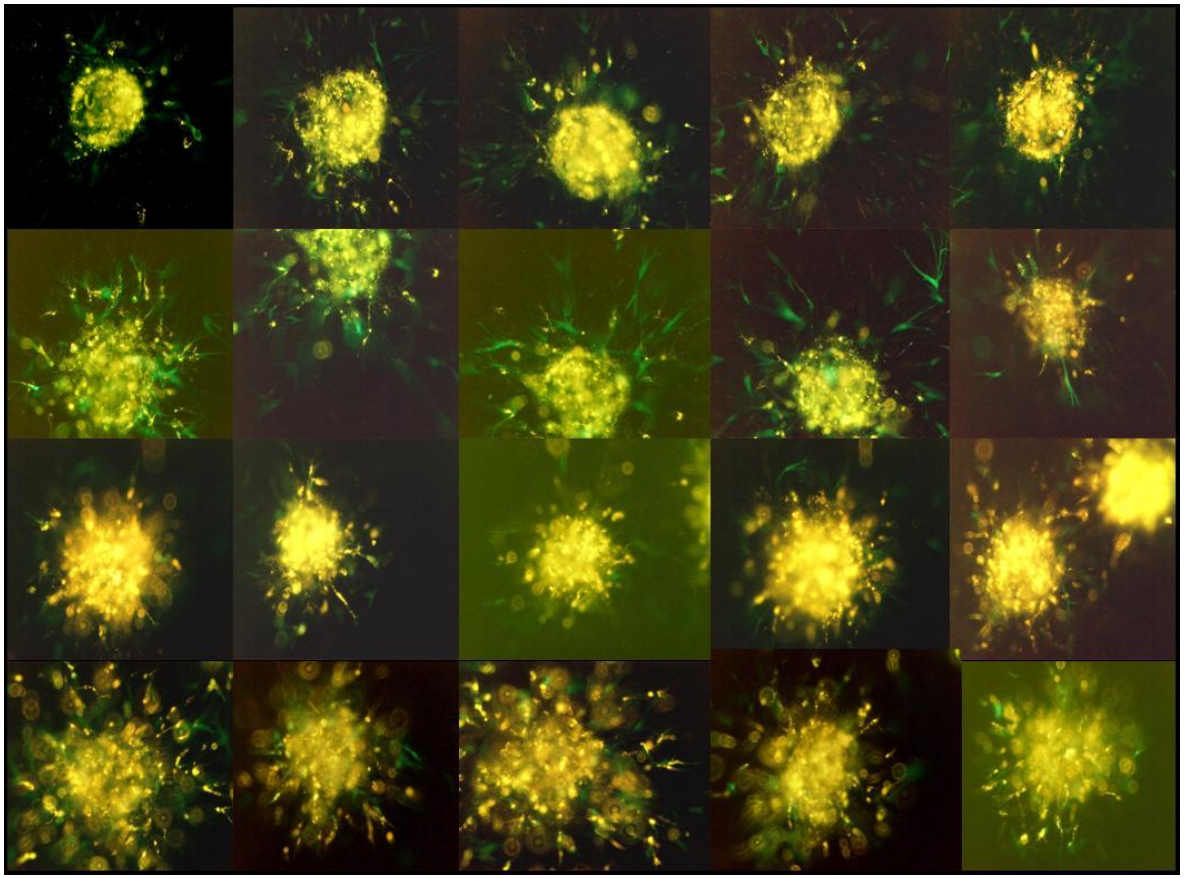
24-72h

Sprouting



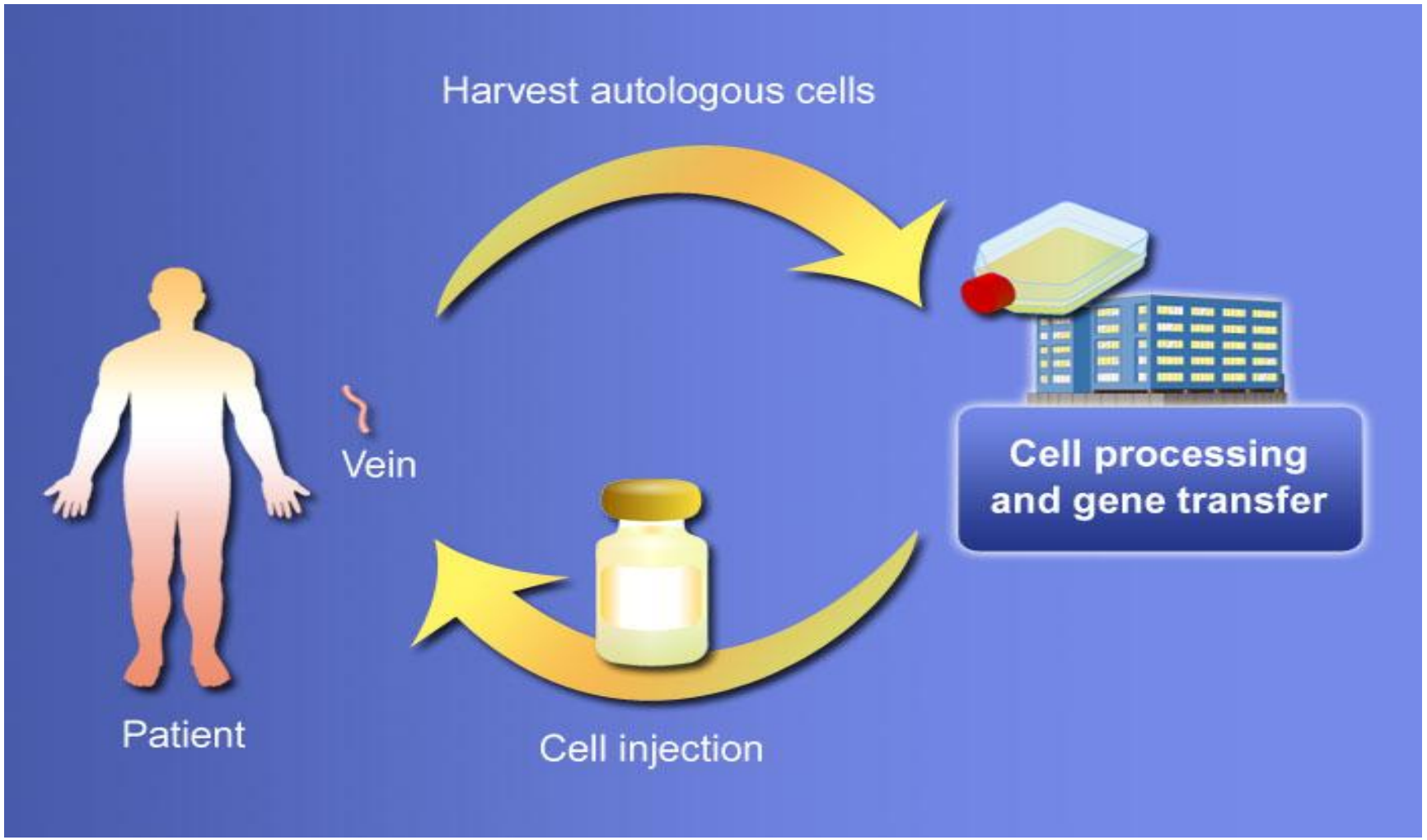
In-vitro angiogenesis assay

SMC	EC
G	G
V	G
G	A
V	A



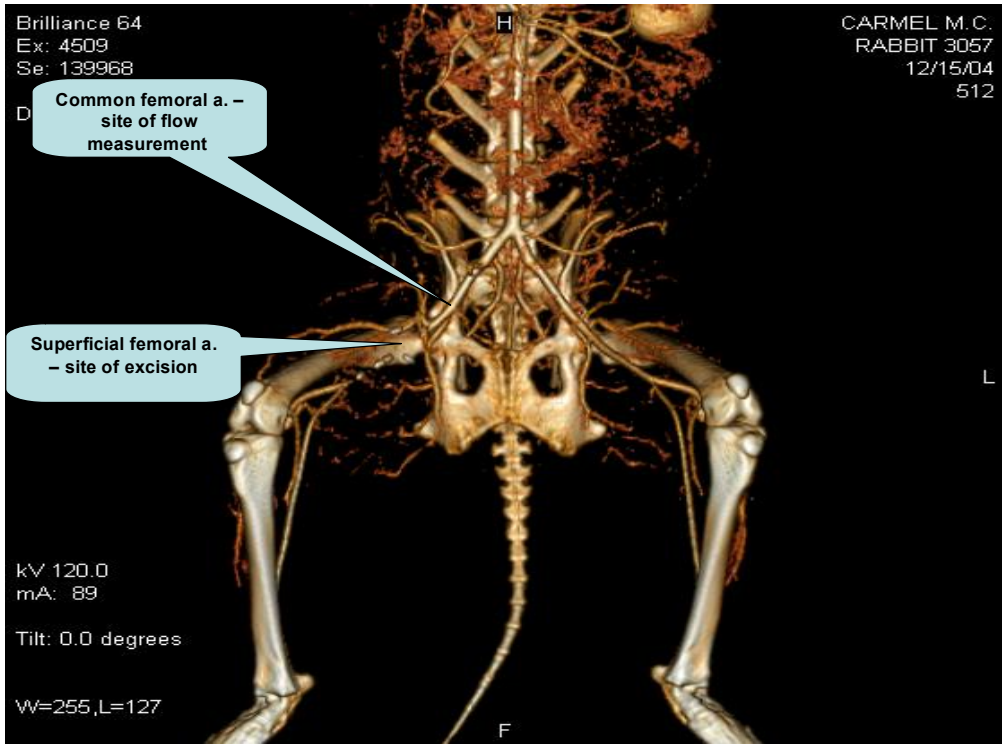
G=GFP, V=VEGF, A=Ang1

MultiGeneAngio



Rabbit hind limb ischemic model

- Safety
  - Cell embolizaton
  - Toxicity (Tumorgenesis, Retinopathy), hemangiomas
- Efficacy
  - Feeder artery Flow
  - Muscle Perfusion
  - Angiography
- Bio-Distribution
  - Tissue and temporal kinetics
- Production and QC



Production & safety *human primary cells*

- Production
  - EC and SMC isolation and expansion
  - Cell characterization and gene transfer efficiency
- Safety
  - Hematopoetic cultures
  - Telomerase activity
  - Number of transgene copies

Safety - statements

Summary of Gross Pathology[1]:

- "No gross lesions related to the treatment were observed in any organ or tissue of any of the experimental rabbits at any time point."

Summary of histopathology[2]:

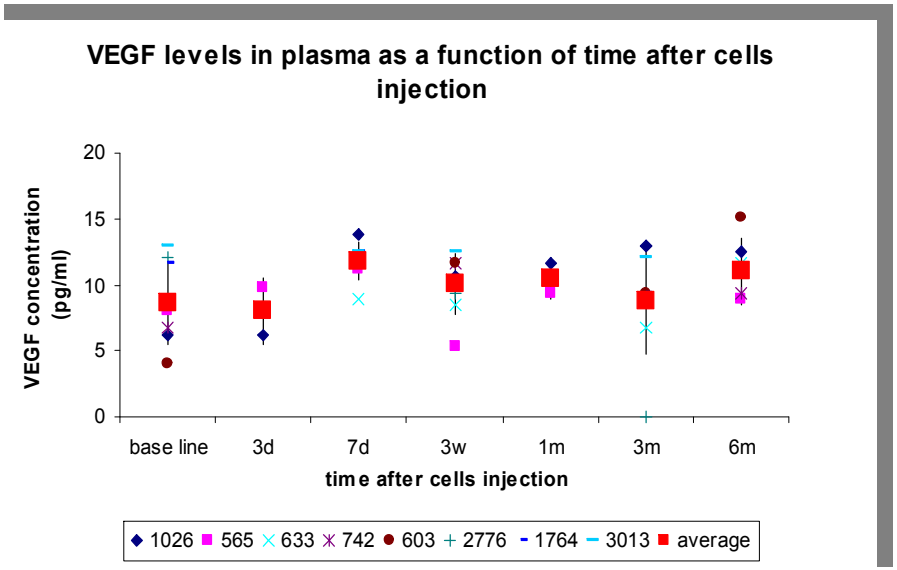
- "There was no evidence of local or systemic toxicity after administration of the MultiGeneAngio product, or its component parts, to the rabbit ischaemia model."

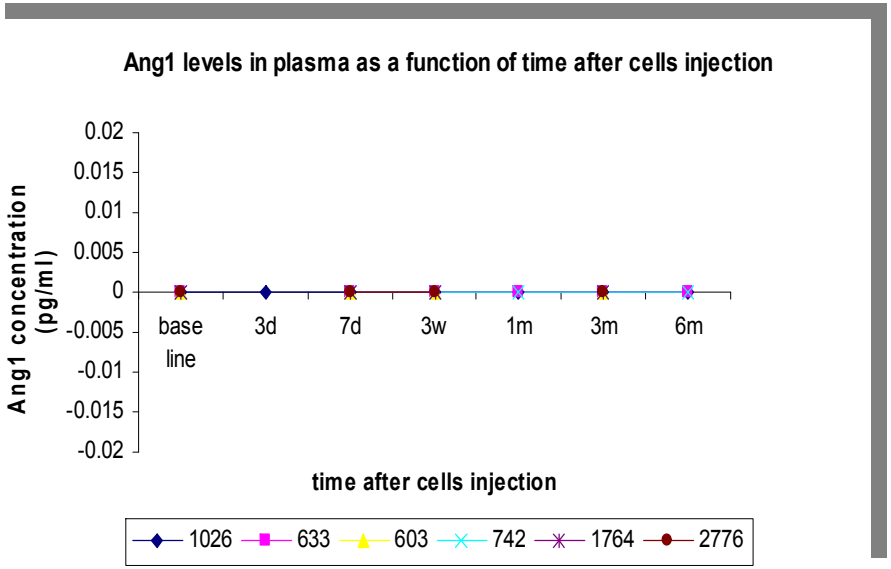
Summary of ophtalmology[3]:

- "There was no evidence of retinal neo vascularization, blood vessels tortuosity or dilatation noticed by the ophthalmological evaluation before sacrifice in all study animals at all time points."

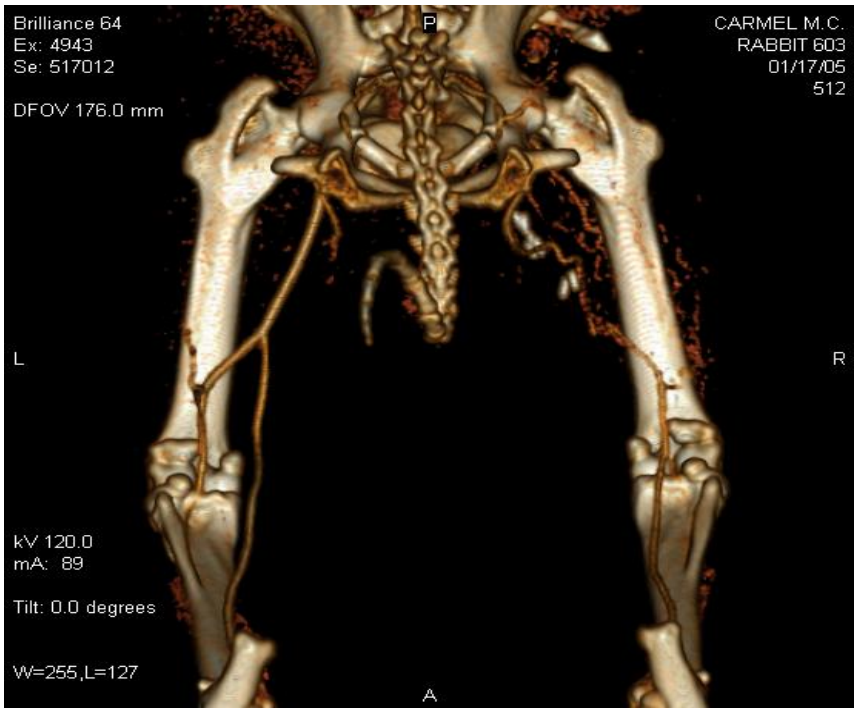
- 1-Ori Brenner, BVSc, Weitzman Institute of Science, Rehovot, Israel
- 2-W.J. Henderson, BVM&S, MRCVS; Quintiles, Scotland
- 3-Yaron Lang, MD, Haemek Medical center, Afula, Israel

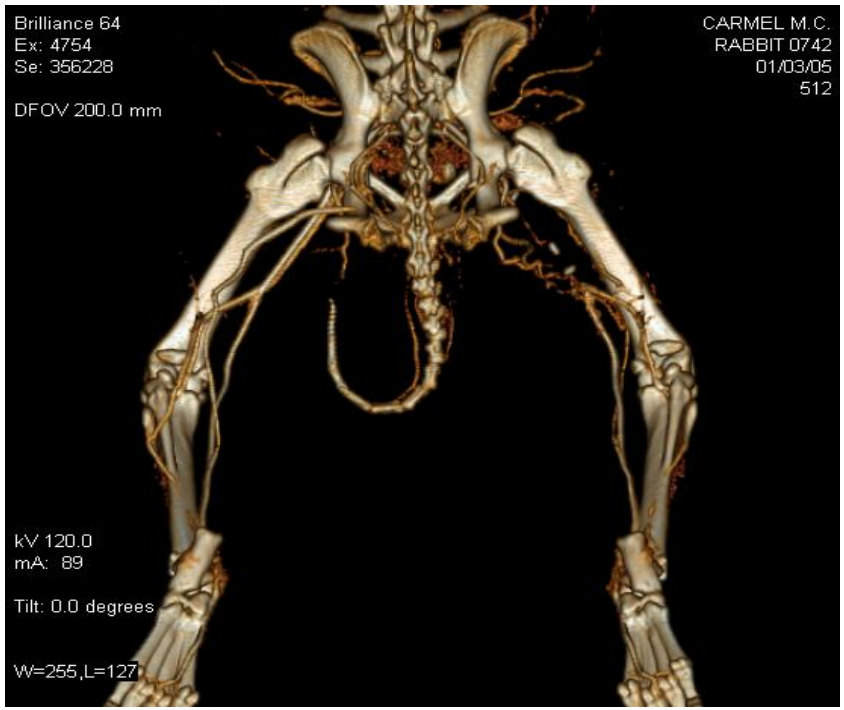
Transgene expression





Hemangiomas



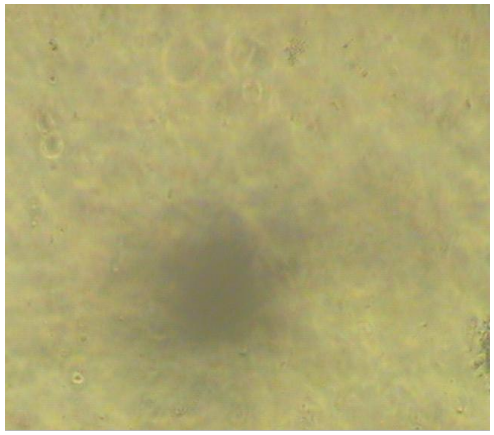
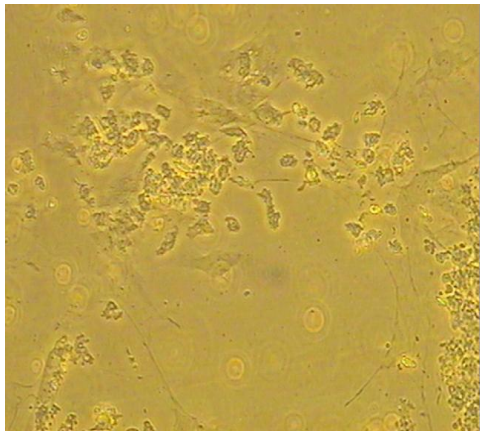
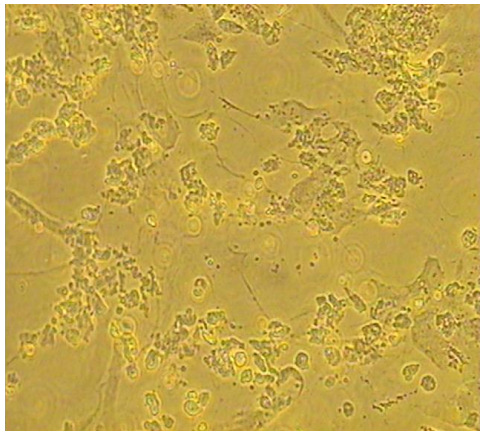
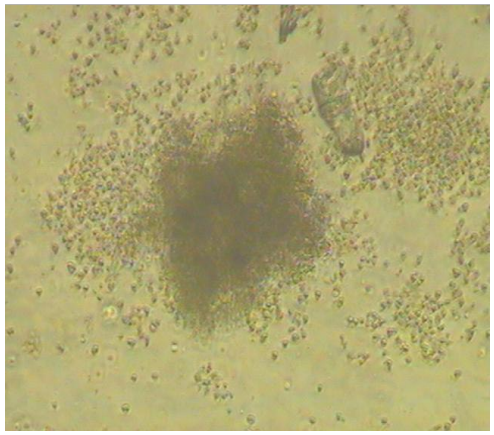
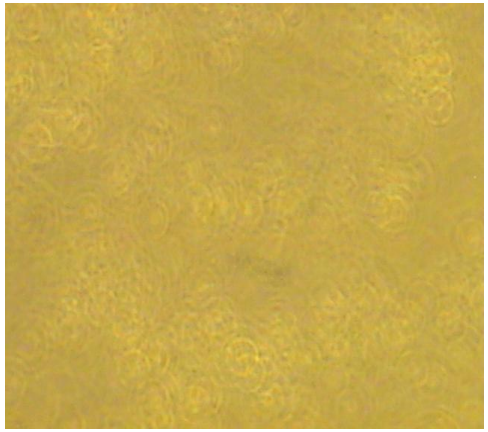
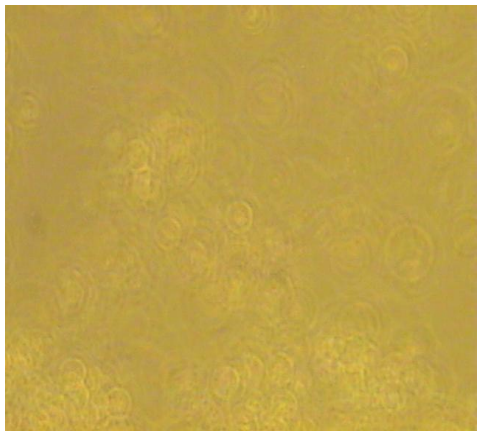


- CT angio 6 month following high or mid dose injection:

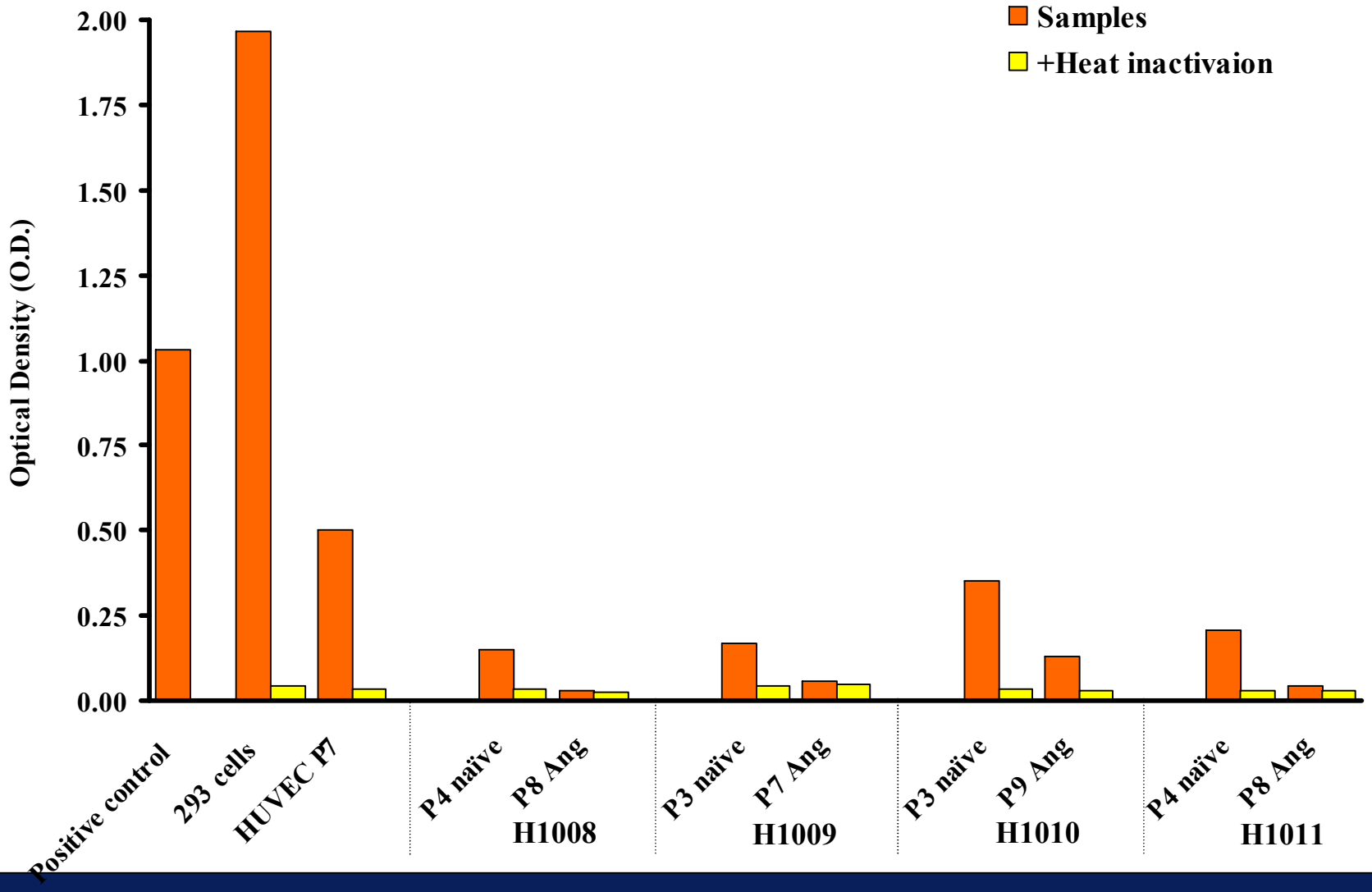
no signs of hemangioma

- 15 muscle biopsies from the treated limb – no signs of hemangioma

Hematopoietic culture – human EC

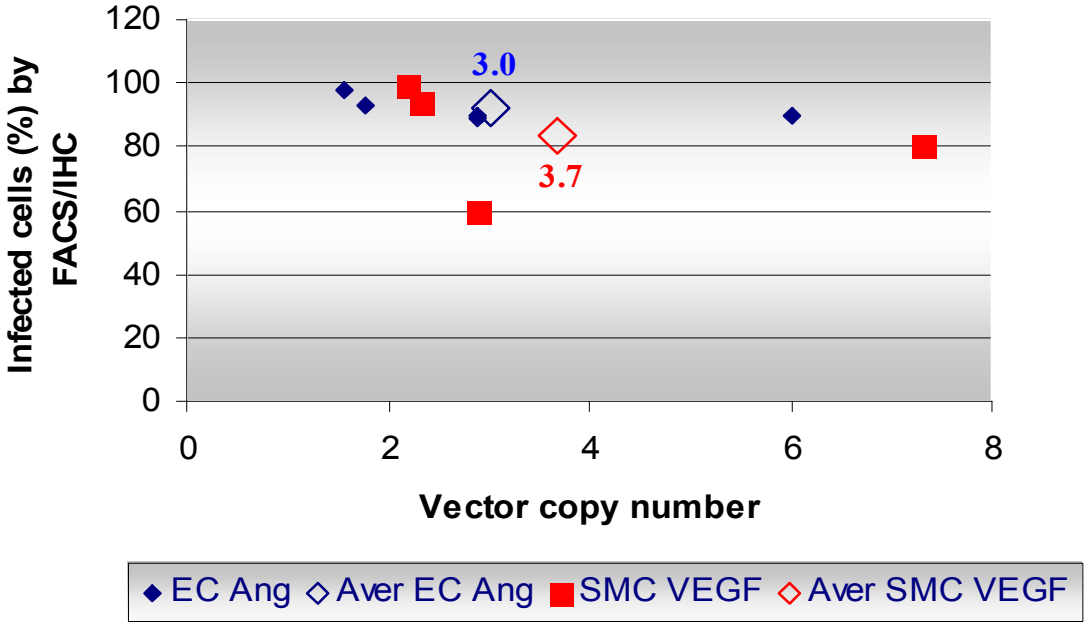
	Human peripheral blood culture - PC	Human saphenous vein Endothelial cell -1, P6	Human saphenous vein Endothelial cell -2, P6
Adherent cells 14 days			
Non-adherent cells 14 days			

Telomerase activity in human endothelial cells



Number of viral copies in human cells

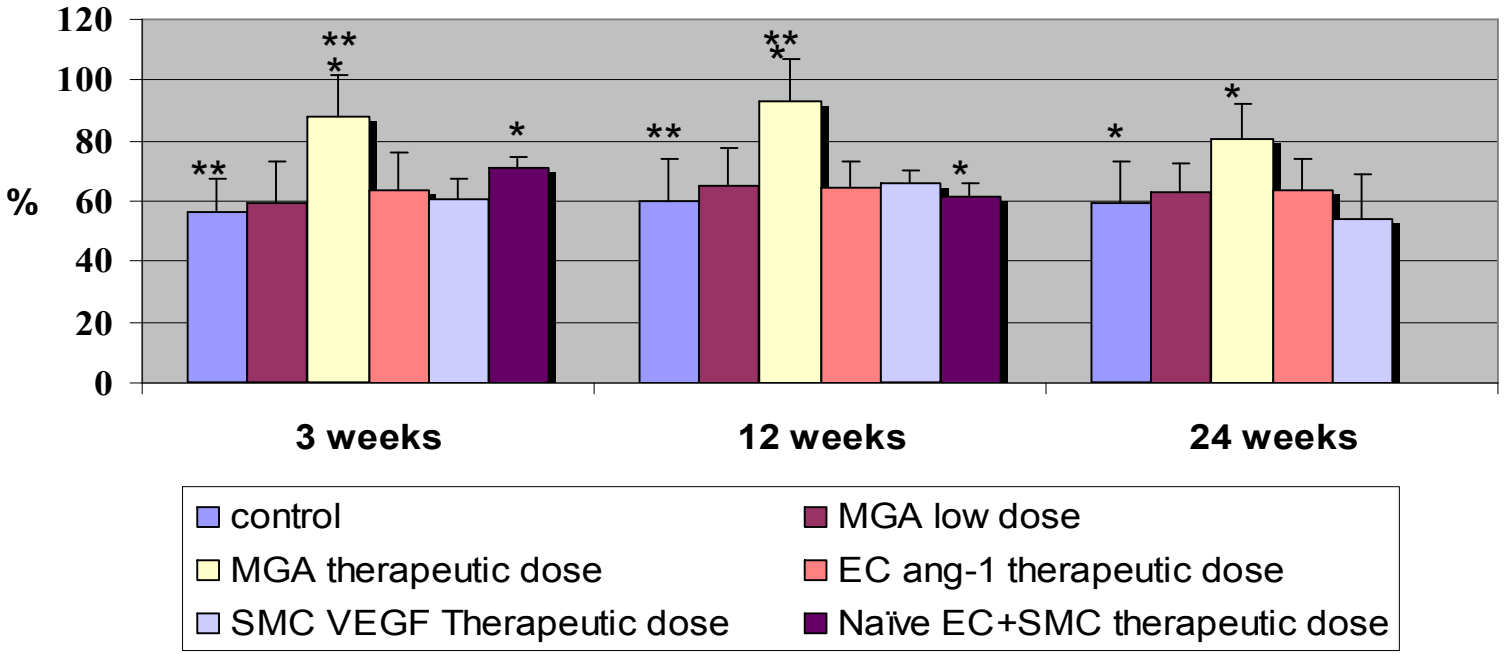
- Average copy number in HSVEC Ang 1 is 3.0 copy/cell
- Average copy number in HSVSMC VEGF is 3.67 copy/cell.



Leukemic complications in mice were observed when typically > 10 per clone were found (Bunting KD, et al., Blood. 1998; Bunting KD et al., Blood. 2000). Modlich U et al (Blood 2005) showed that viral-transduction-related leukemia developed in mice when transgene copy numer was more than 5. Cells studied in the above reports were bone marrow cells.

Efficacy, Mid (therapeutic) Dose: Flow

Femoral a. blood flow ratio
ischemic, treated limb to control non treated limb



*p<0.06

**p<0.001

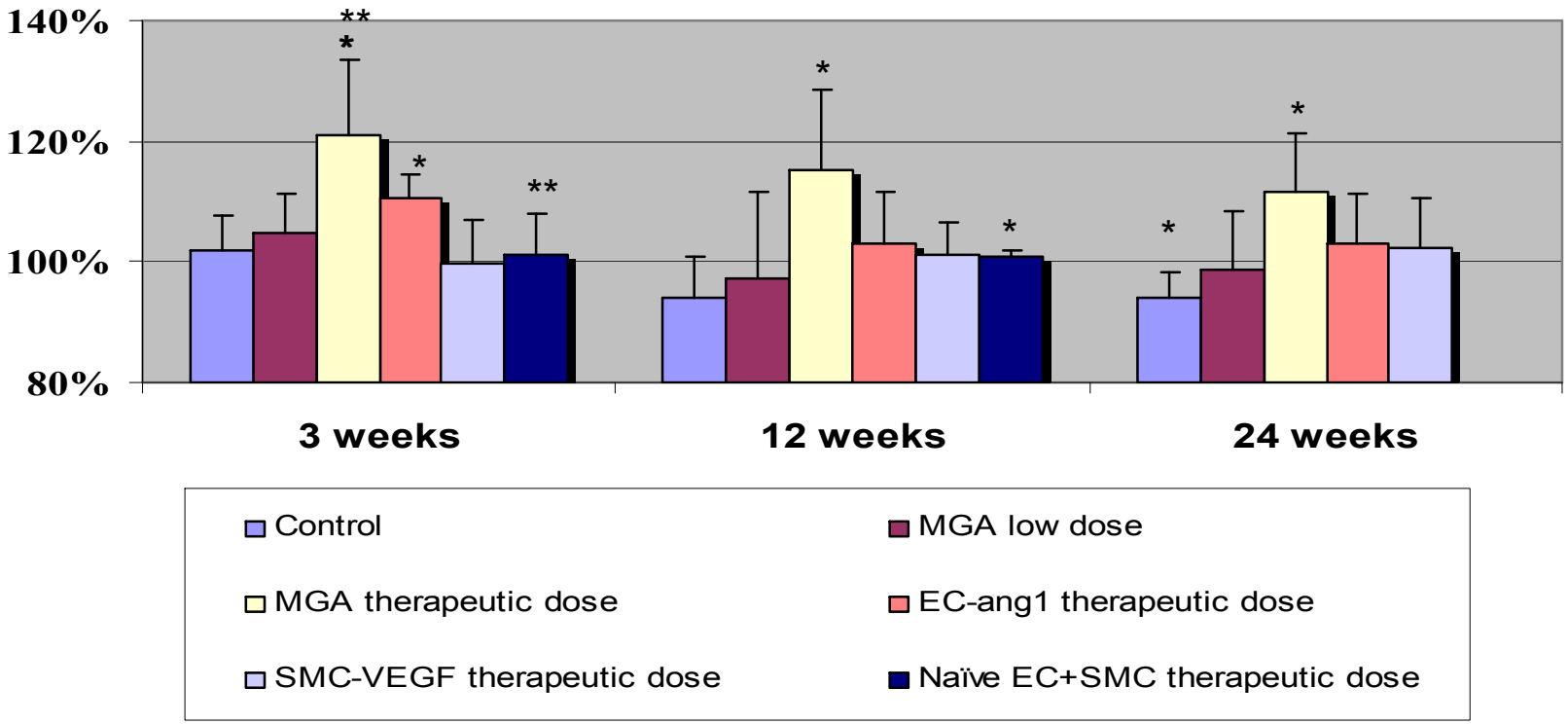
*p<0.01

**p <0.008

*p<0.02

Efficacy, Mid (therapeutic) Dose: Perfusion

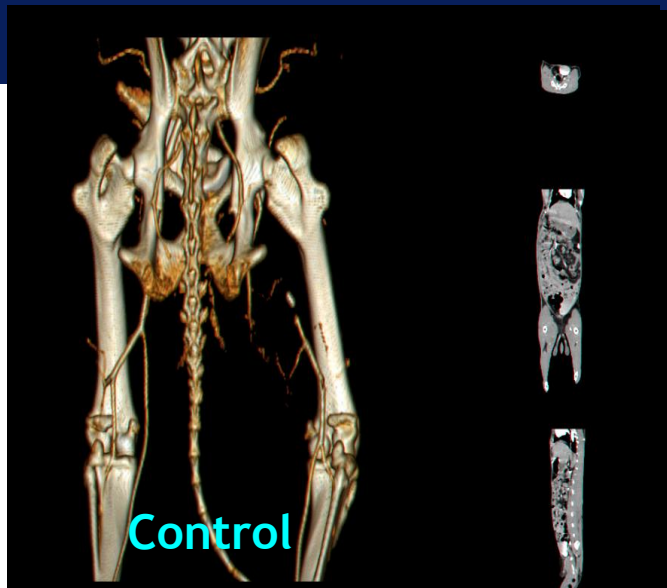
Muscle perfusion ratio
ischemic, treated limb to control non treated limb

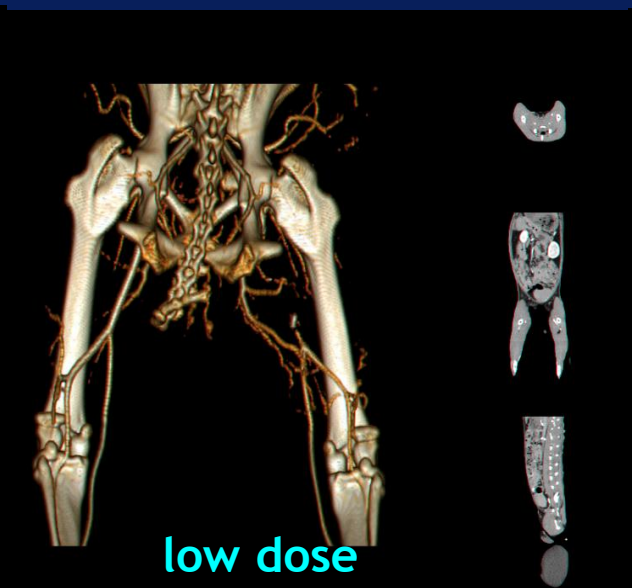


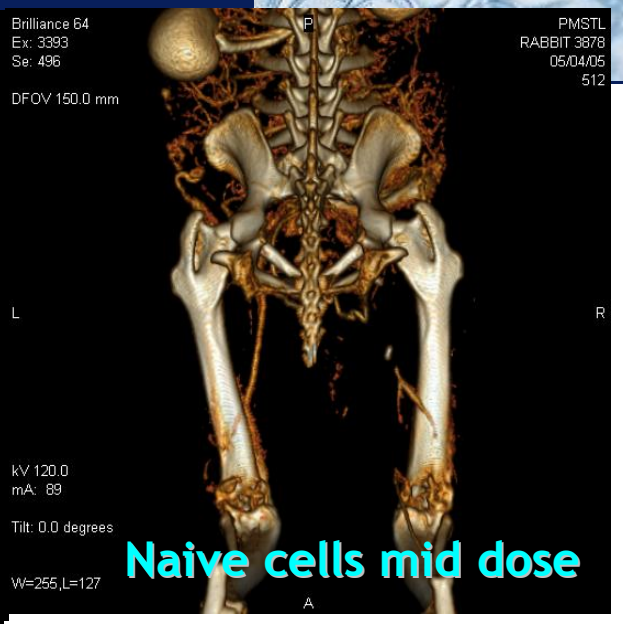
*p<0.04 *p<0.04 *p<0.003

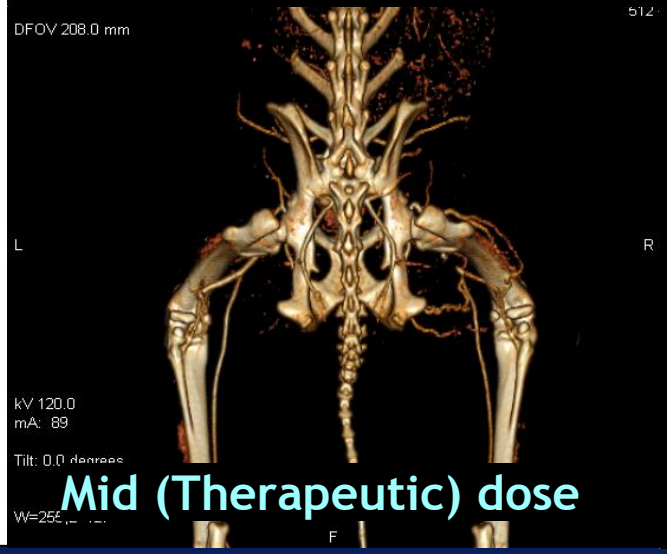
**p<0.03

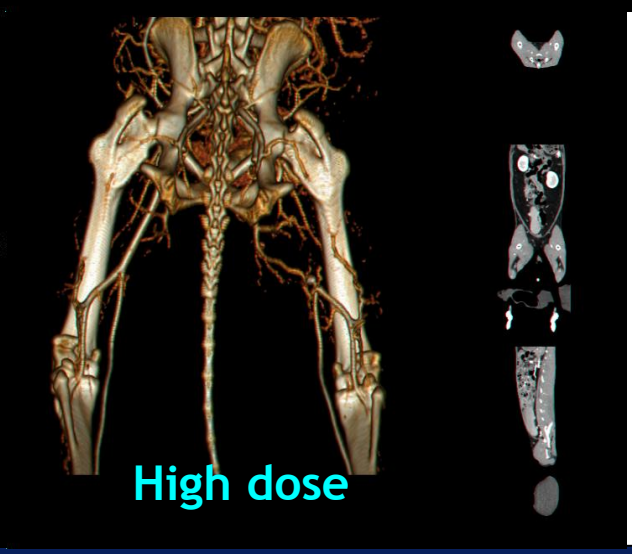
CT angio – 3 weeks following dosing



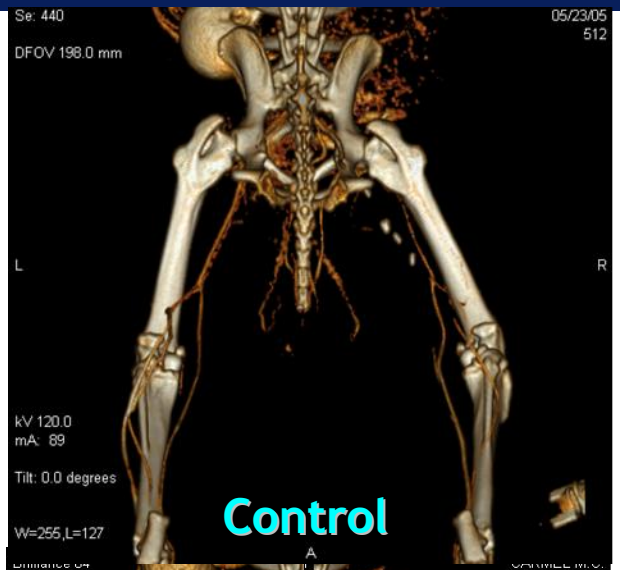




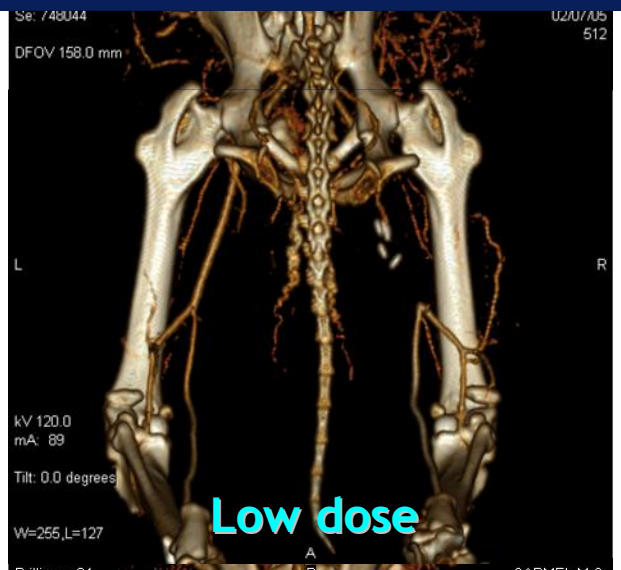




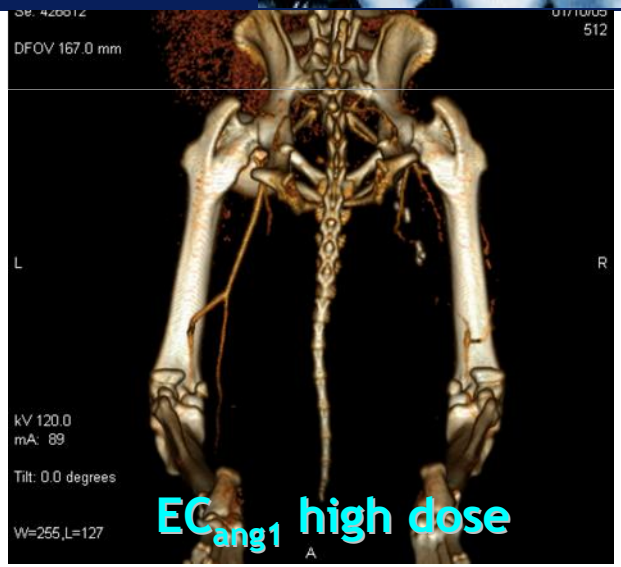
CT angio – 6 month following dosing



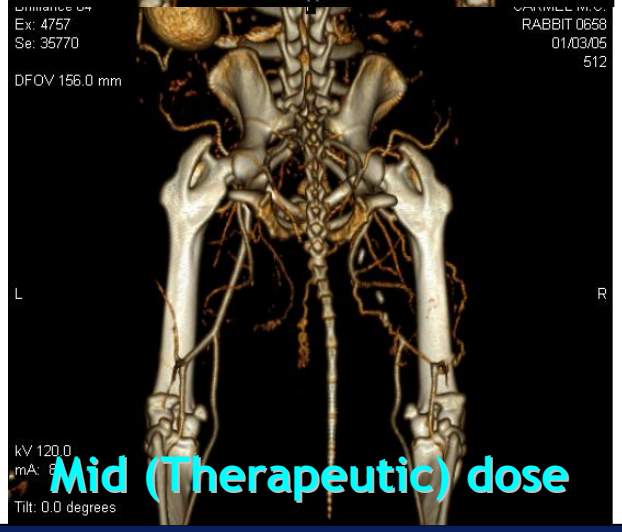
Control



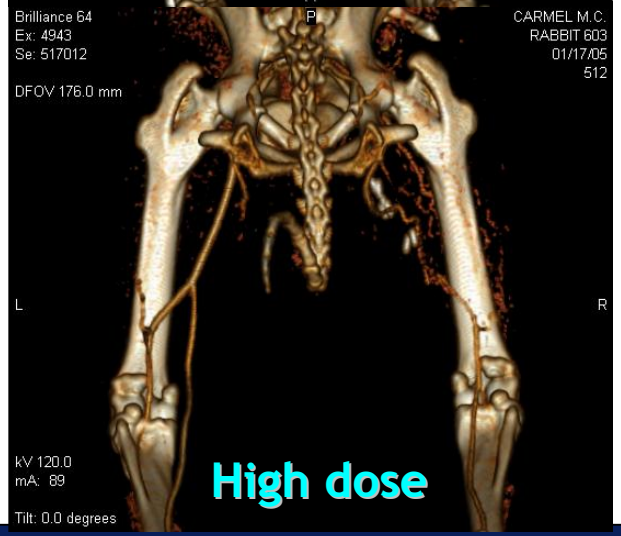
Low dose



EC_{ang1} high dose

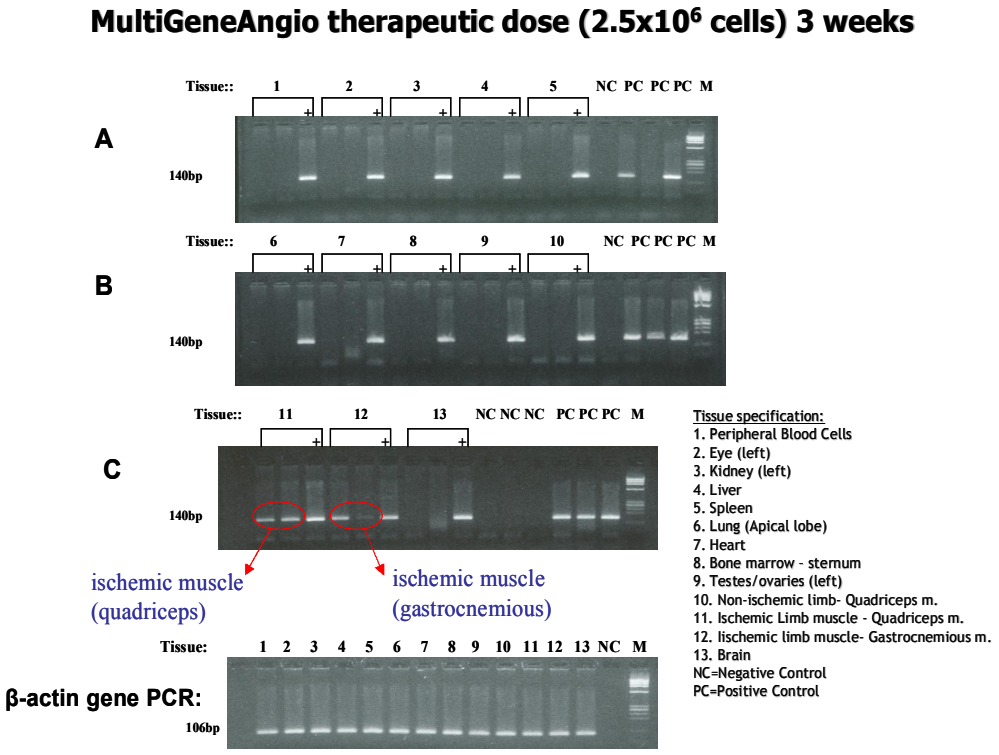


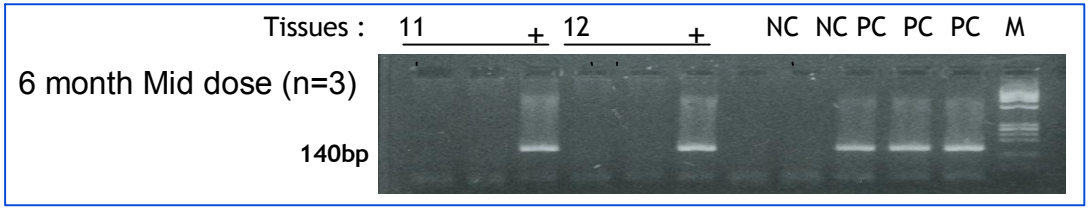
Mid (Therapeutic) dose



High dose

Bio-distribution: summary





Group	Organ	3weeks	24weeks
Control	ALL	Negative	
Mid Dose	Ischemic muscles	3/3 positive	negative
	Lung		
High dose	Lung	2/4 positive	1/4 positive
	Ischemic muscles	4/4 positive	4/4 positive
Intra-Venous	Lung	2/2	

Protocol 0501-0703

Phase I safety, dose escalating study of MultiGeneAngio in patients with PAD

- The primary objective of this trial is to evaluate the safety of MultiGeneAngio in the treatment of patients with PAD
- The secondary objective is to obtain preliminary efficacy information of MultiGeneAngio in patients with PAD

Protocol overview

	Visit 1	Visit 2, 3	Visit 4	Visit 5	Visit 6-9	Visit 10	Visit 11	Visit 12	Visit 13
Time (days)	-35	-28	-21	0	1-14	30	90	180	365
Procedure	Informed consent Screening	Qualification ETT, ABI	Vein Harvest	MGA injection	Follow up	ABI ETT	ABI ETT	ABI ETT	ABI ETT

Enrollment:

- Principal investigator will determine enrollment based upon inclusion and exclusion criteria
- Study product dose cohorts are predetermined
- At the conclusion of every dose cohort, 14 day safety data will be reviewed by the DSMB
- Movement from one dose cohort to higher dose cohort will be dependent on the approval the DSMB

Inclusion criteria:

- Male or female, ≥55 and ≤80 years old, able and willing to give written informed consent.
  - If female, must be (a) postmenopausal, (b) surgically sterile, or (c) use adequate birth control and have a negative pregnancy test within 72 hours prior to administration of study drug
  - should not be breastfeeding
  - males must use an accepted and effective form of barrier birth control
- History of exercise-limiting intermittent claudication (IC) and peripheral arterial disease
  - symptoms in one or both lower extremities,
  - stable symptoms in the 2 months prior to screening

Inclusion criteria:

- Diagnosis of PAD at the screening visit
  - A Doppler-measured ankle-brachial index (ABI) of ≤0.80 in both lower extremities after 10 minutes of rest
  - For subjects with an ABI of >1.3 (noncompressible arteries), a toe-brachial index (TBI) of <0.70 in both lower extremities is required
- Subject must have limitation in walking secondary to claudication with a mean peak walking time (PWT) of between 1 and 10 minutes on 2 standardized Gardner protocol exercise tests
  - ≤ 25% variability between tests

Exclusion criteria:

- Presence of significant inflow disease [defined as >50% stenosis] in the distal aorta, common or external iliac on imaging performed < 1 year prior to screening
  - conventional angiogram or digital subtraction angiography (DSA)
  - magnetic resonance angiography (MRA)
- Documentation of graft patency is required within 6 months prior to enrollment

Exclusion criteria:

- Critical limb ischemia, either chronic or acute ischemia
  - rest pain, ulceration, or gangrene (Category 4 through 6 of Society for Vascular Surgery [SVS] classification [Rutherford]).
- History of malignant neoplasm
  - except cured nonmelanoma skin malignancies
- Renal failure defined as a serum creatinine >2.0 mg/dL
- Ophthalmologic conditions
  - Preclude retinal photography
  - Vascular lesions of the anterior segment of the eye
  - Retinopathy (proliferative or severe nonproliferative)
- Severe congestive heart failure
- Immunodeficient conditions

Patient population

- This is a phase I safety study of MultiGeneAngio in patients with claudication
  - Enrolling patients in this population will allow follow-up for at least one full year and potentially for a longer period of time
- Patients with critical limb ischemia and no option for revascularization may be a future primary target population for the MGA product, however,
  - These patients have high morbidity and up to a 25% one year mortality, which would make collection and interpretation of data difficult and may confound the determination of long term (1 year or more) safety

Safety Analysis

- Adverse events and serious adverse events will be summarized by body system and treatment group
  - Chemistry, hematology and vital sign measurements presented using summary statistics
  - Significant findings from physical examination and electrocardiograms
- Concomitant medications
- Demographics collected
- All data will be reviewed by the DSMB as outlined in the protocol

Thank You