Neovasculaization in Ischemic Hindlimb

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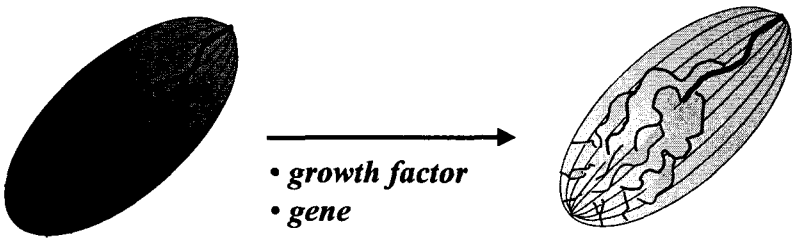
Background

- **Increase 500~1,000 new PAOD patients annually, & 150,000 patients should be taken amputation in USA.**
- **Leading pathophysiology is atherosclerotic PAOD as seen in coronary and cerebral arterial disease.**
- **Drug treatment, angioplasty(PTA), surgical procedure (Bypass) were performed, but disappointing severe cases.... About 10~25%**

Therapeutic angiogenesis !

Therapeutic angiogenesis



Muscle Ischemia
due to vessel occlusion

Improvement of
blood flow & function

Endogenous angiogenic factors

Factor	MW	Endothelial mitogen	Year reported
Fibroblast growth factor			
acidic	18,000	yes	1984
basic	16,400	yes	1984
Angiogenin	14,100	no	1985
TGFα	5,500	yes	1986
TGFβ	25,000	no	1986
TNFα	17,000	no	1987
VEGF	45,000	yes	1983
PDEGF	45,000	-	1989
GCF	17,000	yes	1991
Placental growth factor	25,000	weak	1991
Interleukin 8	40,000	yes	1992
Hepatocyte growth factor	92,000	yes	1993
Proliferin	35,000	yes	1994

Folkman J, N Engl J Med 1995;333:1757

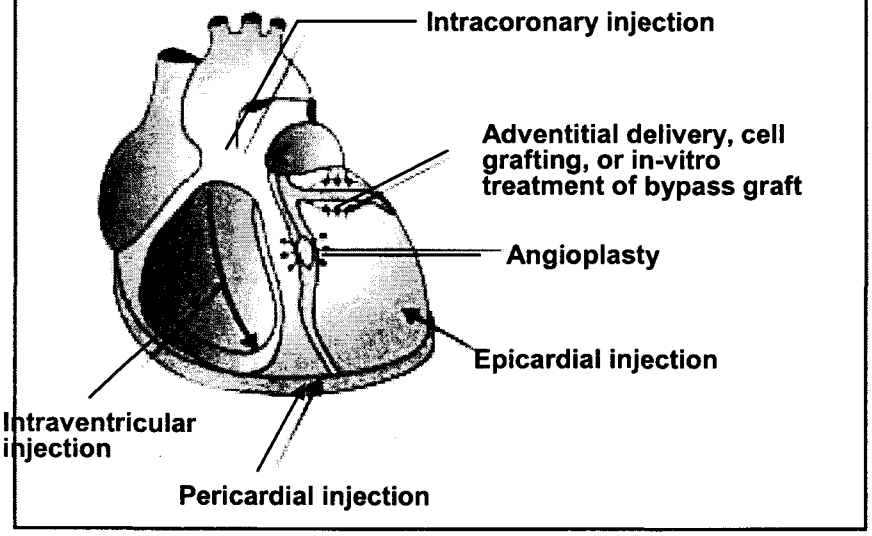
Ideal agent for therapeutic angiogenesis

- Potent angiogenesis
- Sustained clinical benefit
- Specific to targeted ischemic tissue
- Absence of unwanted angiogenesis
- High local concentrations
- Adequate exposure time
- Re-administration feasible
- Non-invasive method of delivery (oral or intravenous)
- Inexpensive (cost effective)

Therapeutic angiogenesis
Considerations

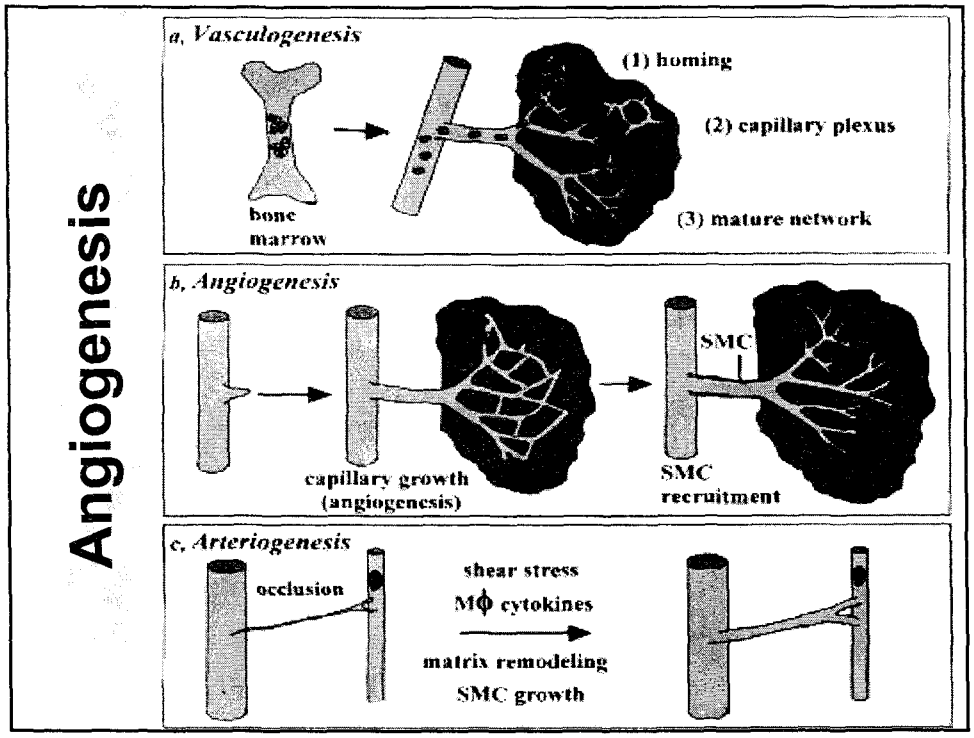
- **Which angiogenic factor, or combination of factors, is the most effective in inducing the formation of new blood vessels?**
- **Which delivery system is best suited to induce therapeutic angiogenesis?**
- **Is therapeutic angiogenesis safe for clinical use?**
- **How long do the newly formed blood vessel persist?**

Gene therapy Route
for treatment of myocardium and CAD

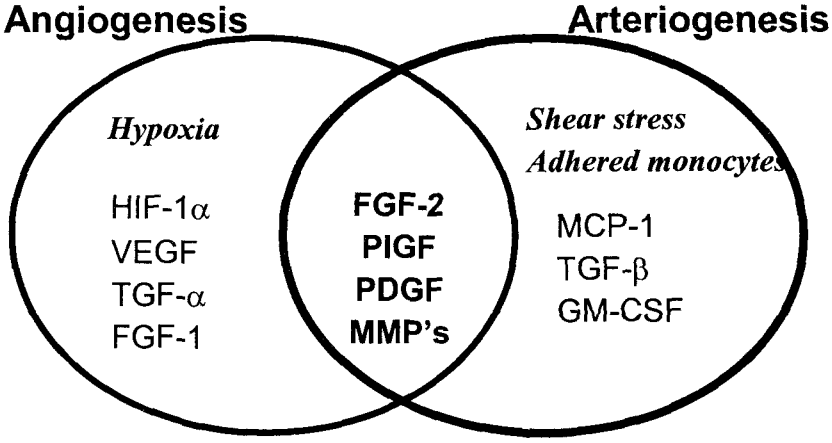


Angiogenesis
Differences between gene and protein therapy

	Gene therapy	Protein therapy
Temporal exposure	sustained	Finite
Dose response	Unpredictable	Defined
Administration	Single	Repeated
Targeting	Possible	Possible
Slow release	Yes	Possible
Inflammatory response	Yes	No
Foreign material	Yes	No
Serum half-life	Long	short
Tissue half-life	Unpredictable	Short



Why FGF-2 gene therapy ?



Angiogenesis, & Arteriogenesis

- **Angiogenesis**
  - **Formation of capillary network**
  - **Increased expression of the VEGF gene**
- **Arteriogenesis**
  - **Growth of preexisting collateral arteries**
  - **Initiated when shear stress increase in the preexistent collateral pathways upon a narrowing a main artery**
  - **Shear stress-activated endothelial cells with upregulate transcription and translation of the MCP-1 gene and the adhesion molecule ICAM-1.**

Arteriogenesis

- Can arteriogenesis be stimulated when the collateral circulation has already matured?
- Arteriogenesis and atherosclerosis
  - Invasion of monocyte, the inflammatory environment, the migration of smooth muscle cell, upregulation of adhesion molecules

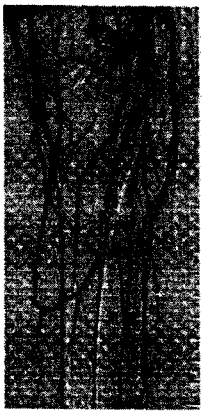
Constitutive Expression of phVEGF$_{165}$ After Intramuscular Gene Transfer Promotes Collateral Vessel Development in Patients With Critical Limb Ischemia

Iris Baumgartner, MD; Ann Pieczek, RN; Orit Manor, PhD; Richard Blair, MD; Marianne Kearney, BS; Kenneth Walsh, PhD; Jeffrey M. Isner, MD

Circulation 1998;97:1114

A

Before gene therapy



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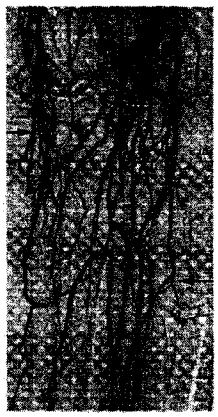


After gene therapy (8wks)

Clinical trials
Therapeutic angiogenesis, CAD

Authors	n	Angiogenic factors	Route	Results
Schumacher et al.	20	aFGF protein	IM (R)	+
Seike et al.	8	bFGF-protein	IM	+
Laham et al	24	bFGF-protein	IM (R)	+
Unger et al.	25	bFGF-protein	IC (R)	safe
Laham et al.	52	bFGF-protein	IC	+
Simmons et al.	337	bFGF-protein	IC (R)	-
Rogengart et al.	21	Ad/VEGF121	IM	+
Losordo et al.	5	VEGF165-plasmid	IM	+
Gibson et al.	28	VEGF165-protein	IV	+
Henry et al.	178	VEGF165-protein	IC+ IV (R)	-

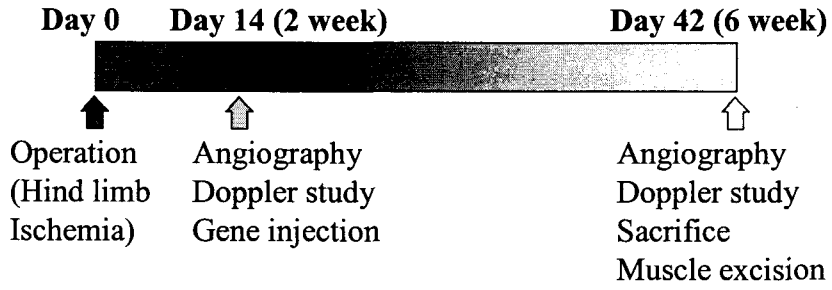
Epstein et al. Cardiovascular research 2001

FGF-2 in endothelial cells

- **18, 22, 22.5, and 24 kDa**
- **Mitogen for both EC and SMC**
- **modulation of integrin expression (Klein et al., 1993)**
- **gap-junctional intercellular communication (Pepper and Meda, 1992)**
- **urokinase receptor upregulation (Mignatti et al., 1991a).**

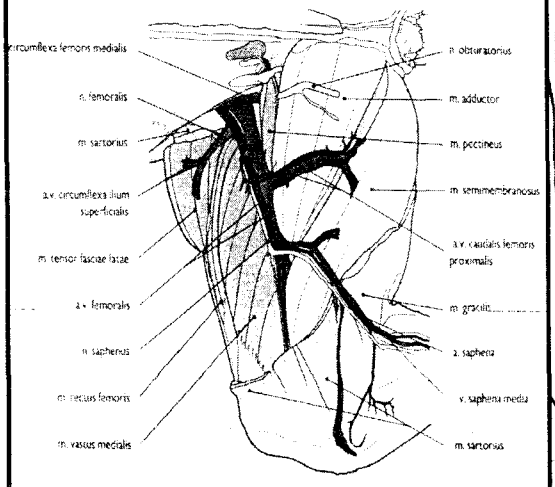
Methods

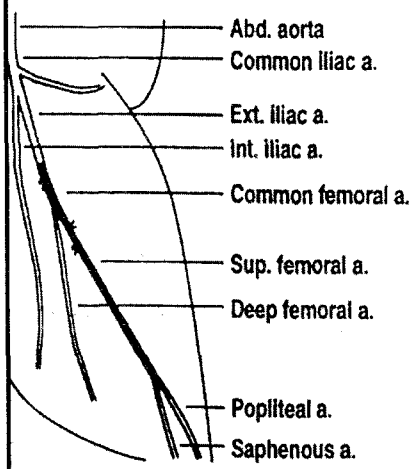
- New Zealand white rabbit (male, 3.5 –4.0Kg)
- Group; FGF, LacZ for control
- FGF; unique 4sFGF-2 recombinant gene, Vector; Adenovirus



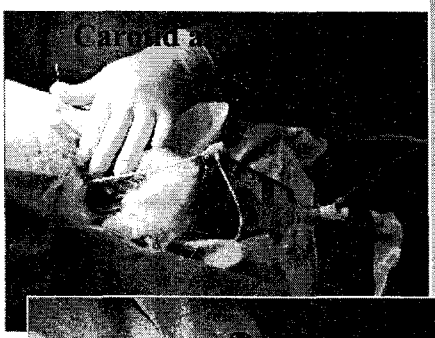
Methods

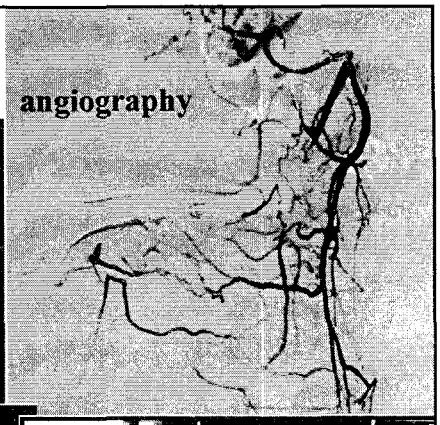
Rabbit Hind Limb ischemic Model

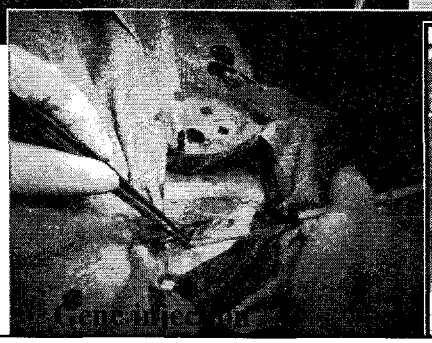


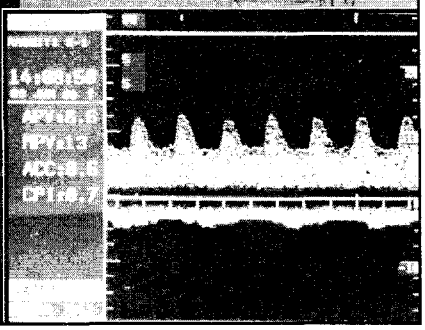


Methods









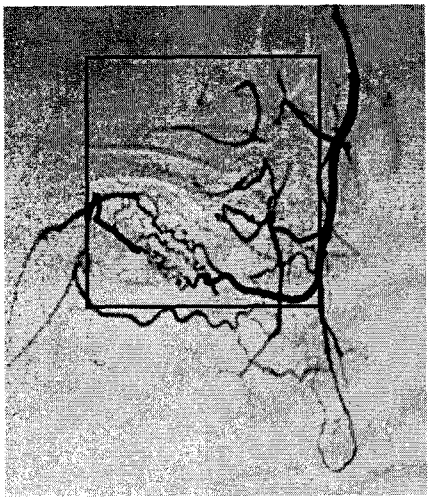
Methods

- **Anatomic assessment of angiogenesis**
  - Gross: angiographic vessel count (>1mm)
    - : by using electric caliper
  - Micro: tissue analysis
    - vessel count under routine H-E stain
    - evaluate angiogenesis, arteriogenesis under HIS with Flk-1 for eary and VWF for late angiogenesis.
- **Functional assessment of angiogenesis**
  - APV, MPV ratio between internal iliac a. / abd. aorta
    - : Can solve the problem of anatomic, physiologic differences between individuals

Result

LacZ group

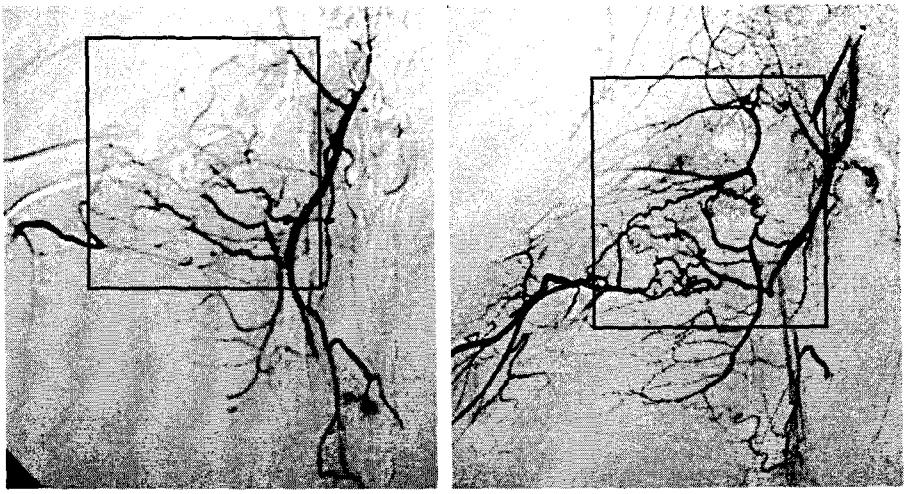
Day 14



Day 30

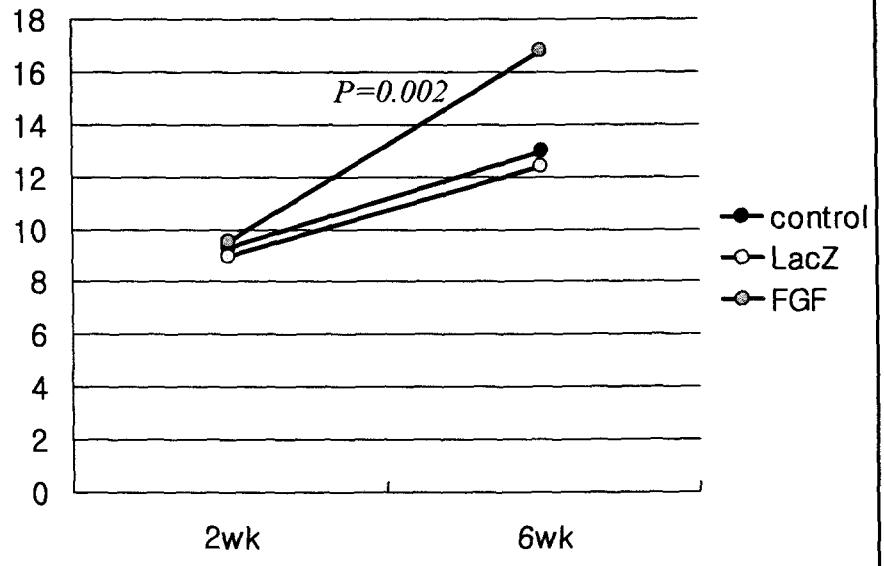
Result

FGF group



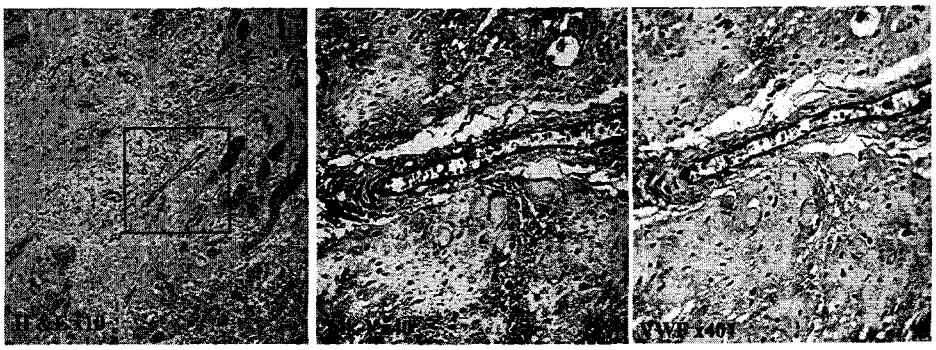
Day 14 Day 30

Vessel count on Angiography



Tissue Study

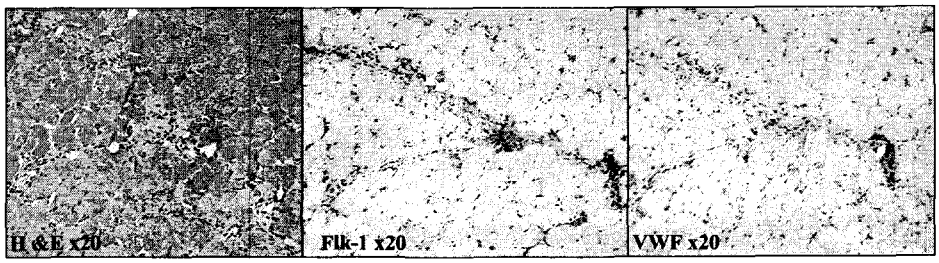
Fibrotic region : angiogenesis



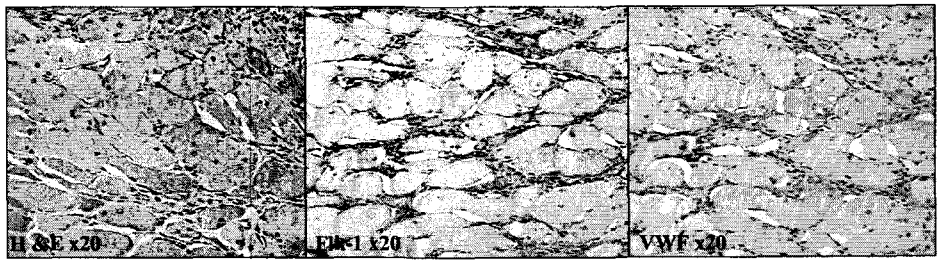
Massive infiltration of firoblasts and neovascularization with interstitial fibrosis

Tissue Study

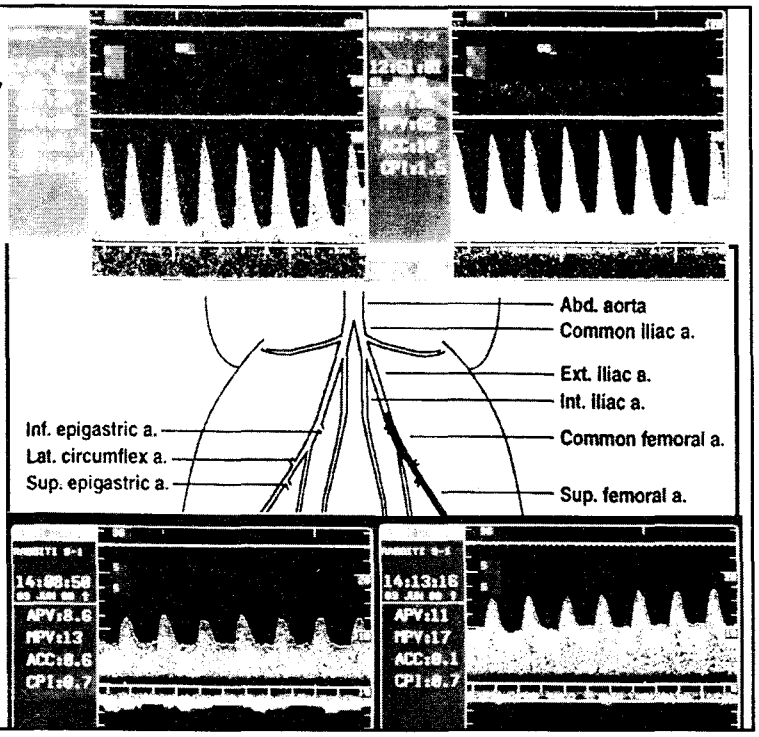
Neighboring region: control vs FGF-2



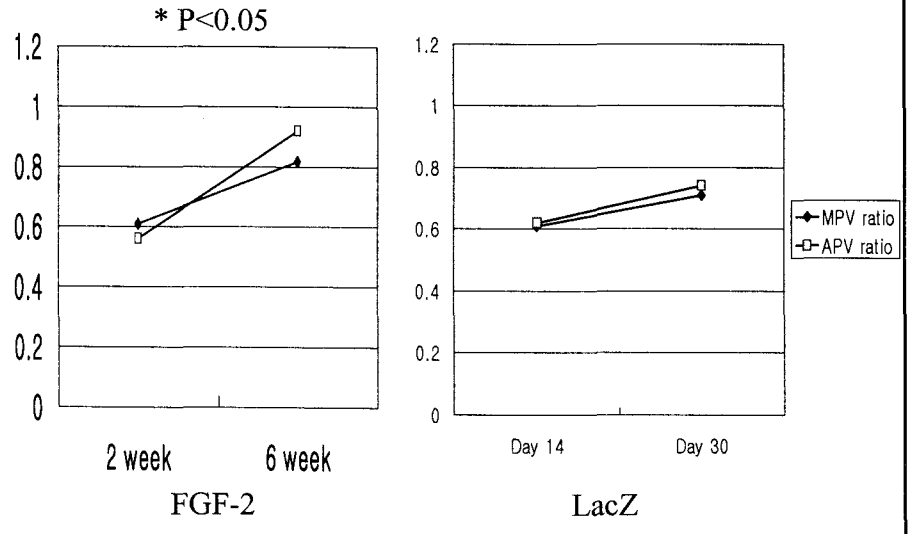
Prominent widened interstitial septa and endothelial proliferation with Hypertrophic change of muscle cells



Doppler Result



Doppler study result



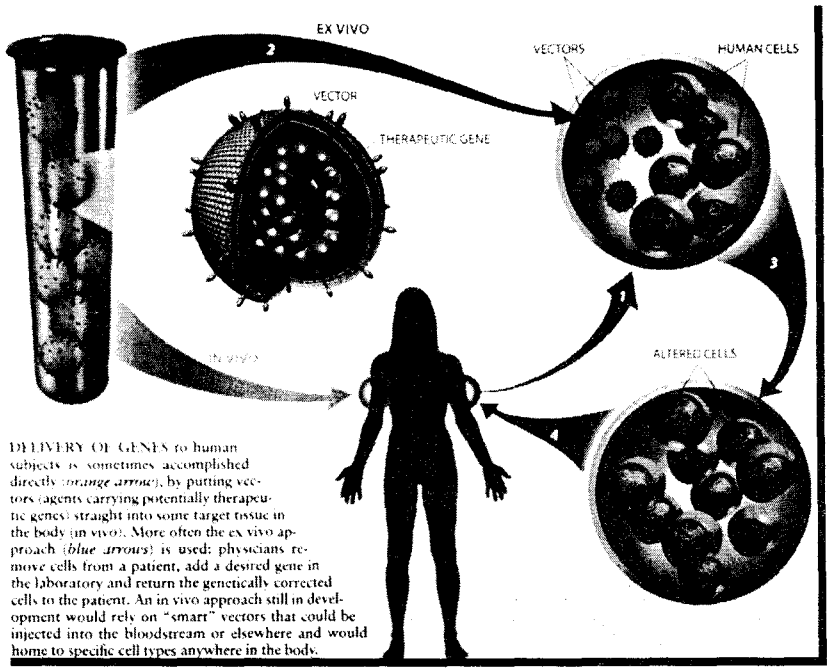
Conclusion

- Anatomic & Functional study is essential for assessment of angiogenesis.
- Prominent widened interstitial septa and endothelial proliferation with Hypertrophic change of muscle cells in tissue study.
- The method for evaluation of systemic effect will be required. (e.g. peripheral FGF conc.).

Conclusion

Problems to be solved

- Which angiogenic factor will turn out to produce an optimal effect?
- Will the protein or gene be a superior means of delivering the factor?
- Dose optimization of response require a multiple-factor strategy?
- What is the optimal delivery strategies?
- Will there be serious side effects?



DELIVERY OF GENES to human subjects is sometimes accomplished directly (*orange arrow*), by putting vectors (agents carrying potentially therapeutic genes) straight into some target tissue in the body (in vivo). More often the ex vivo approach (*blue arrows*) is used: physicians remove cells from a patient, add a desired gene in the laboratory and return the genetically corrected cells to the patient. An in vivo approach still in development would rely on "smart" vectors that could be injected into the bloodstream or elsewhere and would home to specific cell types anywhere in the body.

Cell-Based Gene Transfer

- A relatively homogenous population of genetically modified cells can be developed.
- Transfer and expression of transgene can be confirmed and optimized.
- *Immune-inflammatory responses to vector may be minimized.*
- Myocardial cell transplantation