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Protocol 0307-594

A Phase 1 Study to Determine the Safety and Biological Activity of Cell-Mediated Gene Therapy Using TissueGene-C in Patients with Degenerative Joint Disease of the Knee Prior to Total Knee Arthroplasty

Presentation to the NIH

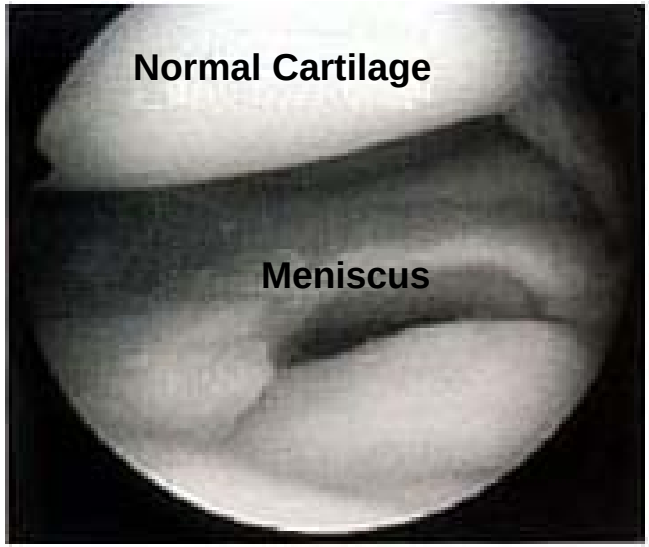
Recombinant DNA Advisory Committee

Bethesda, MD

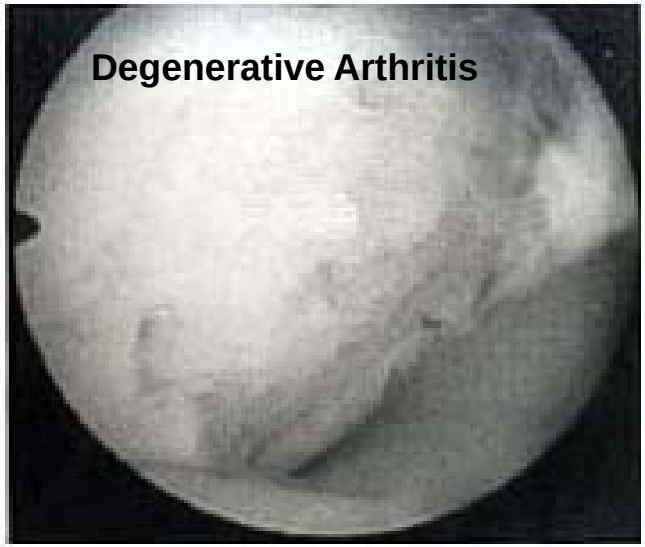
September 17, 2003

Degenerative Arthritis

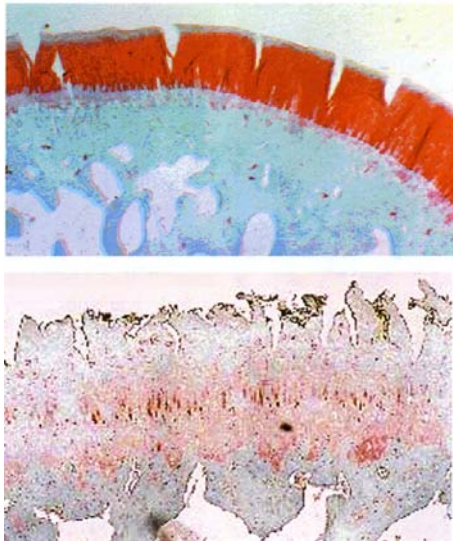
The pathogenesis of this disease is the degeneration of the hyaline articular cartilage in the joint, which becomes deformed, fibrillated, and eventually excavated over time. Treatment methods, until now, primarily have been pharmacological treatments, physical therapy, and surgery.



Arthroscopic image of a healthy knee joint. Healthy articular cartilage is white, shiny and smooth.



Worn and irregular articular cartilage seen in degenerative arthritis patients.



Pathology of degenerative arthritis

Our technology provides a possible treatment for degenerative arthritis, a debilitating orthopedic disease that effects one in seven people.

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Traditional Treatments for DA

The traditional treatment methods for degenerative arthritis are unable to provide complete recovery.

- Traditional treatment methods for degenerative arthritis (DA) either involve surgical “smoothing” or only address symptomatic pain
- The time required to produce even partial solutions under traditional methods can be lengthy and the costs typically are high.

Pharmacological treatment

- Acetaminophen and NSAID including COX-2 inhibitors
- Injection of Corticosteroid and Hyaluronic Acid

Physical therapy

- Physiotherapy including diet and massage

Operative treatment

- Microfracture, Arthroscopic debridement, Arthroplasty

Complete and permanent Recovery is not possible

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Recently Tried Technologies

None of the alternative technologies provide complete, permanent solutions to DA.

- Systemic drug therapies
  - Cannot be targeted to specific joints
  - Require high concentrations of the drugs, creating side effects
  - Cannot provide complete tissue regeneration
- Autologous chondrocyte transplantation (ACT)
  - Limited to partial-thickness defects
  - Requires two surgeries
  - Applicable under age 55
- Cartilage polymer matrix transplantation
  - Unable to produce pure hyaline cartilage
  - Polymer compounds can inhibit chondrocyte growth

TissueGene-C Compared

Our solution can be targeted to specific joints and provides the possibility of improving cartilage recovery

No surgery required

- Heterologous application requires no surgery; solution can be mass-produced and mass-packaged
- Autologous application may require one surgery to collect chondrocytes from patient

Regenerated cartilage is fully integrated hyaline

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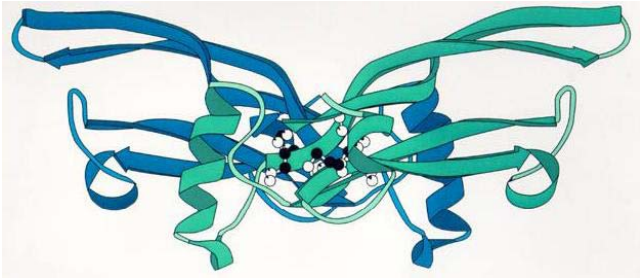
Special Technology Features

Our technology provides a solution to effectively harnessing the regenerative power of growth-factor proteins.



- TGF-βs are well-known growth factor proteins, however, their clinical utility is limited due to very short half-lives and side effects associated with systemic exposure.
- **Local delivery** of TissueGene's product overcomes the half-life and side effect limitations of systemically administered TGF-β.
- **Increase matrix synthesis** while maintaining type-II collagen phenotype
- Increase proteoglycan and collagen synthesis
- **Suppress immune response**

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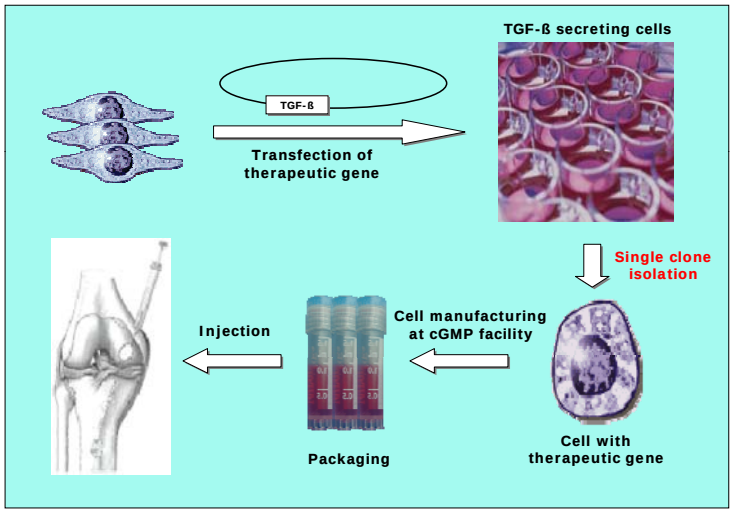
Technology Summary

We have developed a proprietary form of **cell-mediated gene therapy** to deliver a regenerative protein to damaged tissue, which catalyzes rapid repair of injured bone, cartilage and tendons without the need for surgery.

- We insert a therapeutic “growth factor” (TGF-β) gene into **heterologous cells** using traditional viral methods
- We isolate **single clone expressing TGF-β** by the limit dilution method.
- We inject the stable population of genetically modified cells into the damaged tissue area
- The modified cells secrete growth factor proteins directly into the injured site (like living “**protein factories**”)

TissueGene-C



Potential for significant cartilage recovery

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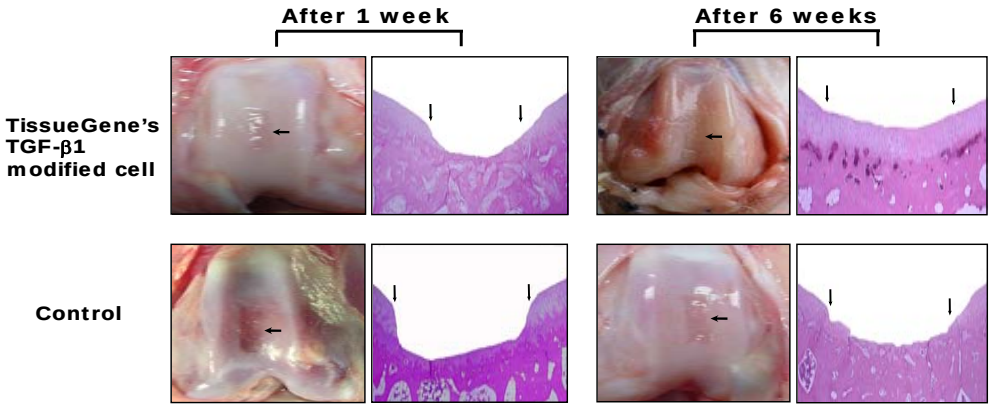
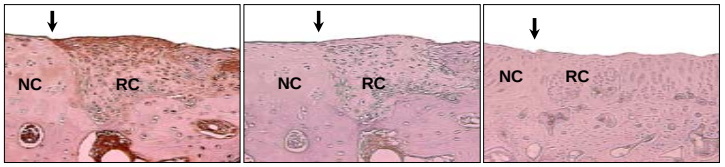
Preclinical Studies

We have demonstrated the therapeutic potential of our technology in animal studies involving rabbits and dogs. In these studies, cartilage has been regenerated.

Cartilage Defect Repair in Rabbits

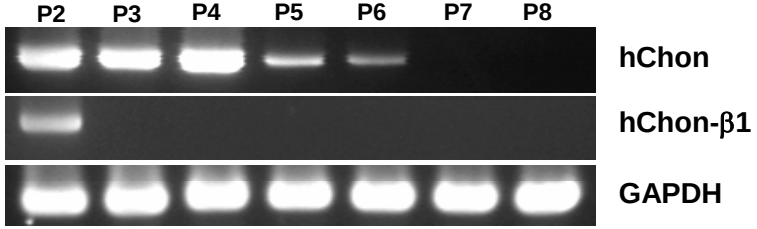


Immunohistochemical staining for MHC class I



3 wks postinjection (x100) | Control (2° Ab only) (x100) | 6 wks postinjection (x40)

RT-PCR analysis for HLA-a



Expression of HLA type-A antigenicity was decreased from passage #5 and disappeared at passage #7. The results of our pre-clinical trials were published in ***Human Gene Therapy*** *(Sept 20, 2001)* and show that we are capable of regenerating cartilage through cell-mediated technologies.

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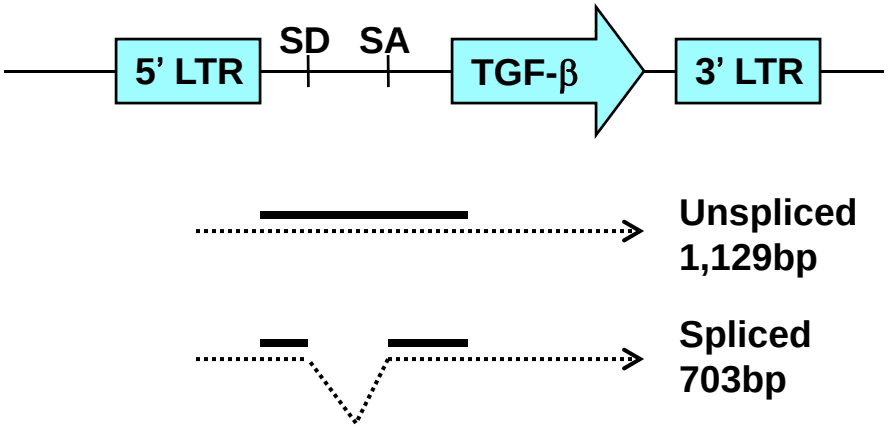
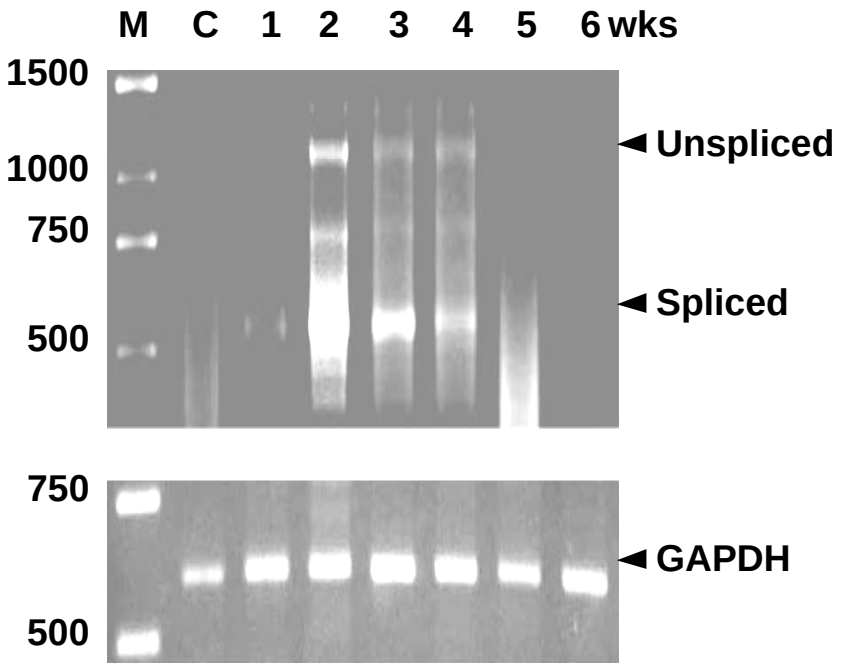
Preclinical Studies

The results of RT-PCR analysis of the regenerated tissue showed that the expression of TGF-β transgene peaked at 2 weeks post injection and lasted up to 4 weeks.





We think that the regeneration mechanism is both autocrine and paracrine mode of activation

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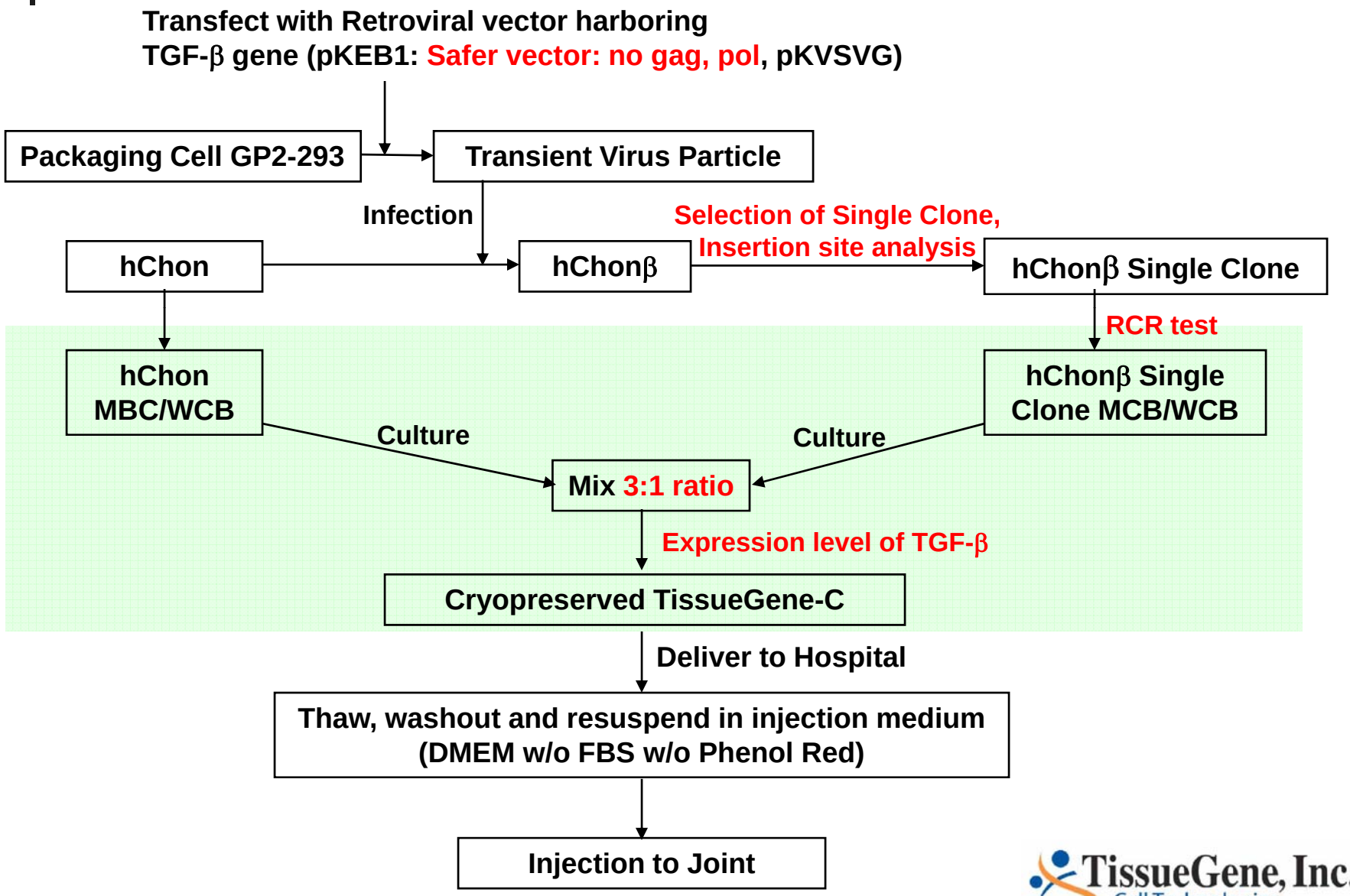
Production of TissueGene-C in BioReliance



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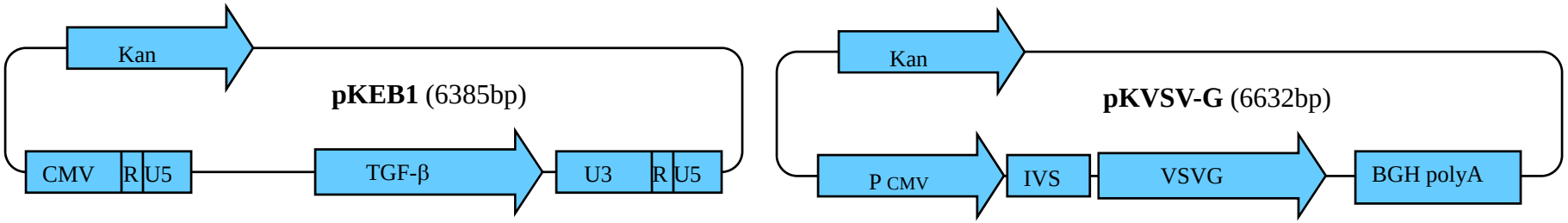
Genetic modification



•Safer vector: No gag, pol and env gene in the retroviral vector
RCR (Replication Competent Retrovirus) is theoretically impossible.
No RCR will be confirmed with hChonβ single clone.

• MLV based retroviral vector was used for the introduction of TGF-β gene to hChon cell line.
: Construction of hChonβ was done in BioReliance (Rockville, MD)
: Plasmid production was performed in Altheatech (San Diego, CA)
-Bacterial bank for each plasmid was constructed with cGMP.

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Release Testing for hChonβ Master Cell Bank

Concern	Assay	Specification
Identity	Cell Culture Identification and Characterization	human origin
Potency	TGF-β ELISA (Level of expression will be controlled for Safety and efficacy)	5-30 ng/10^5 cells/24hr
Safety	Mycoplasma	Negative
	Sterility (Direct Inoculation Method)	Negative
	Test for Inapparent Viruses	Negative
	Porcine Parvovirus	Negative
	PCR for HIV-1/2	Negative
	PCR for HBV	Negative
	PCR for Hepatitis C Virus	Negative
	PCR for HHV-7	Negative
	PCR for EBV	Negative
	PCR for CMV	Negative
	PCR for HHV-6	Negative
	In Vitro Assay for Bovine Virus	Negative
	In Vitro Assay for Viral Contaminants	Negative
	PCR for HTLV-I/II	Negative
	PCR for Human Parvovirus B19	Negative
	PCR for AAV	Negative
	Transmission Electron Microscopic Evaluation of Cultured Cells	No identifiable virus-like particles nor any microbial agents
	RCR (Co-culture of cells and supernatant)	No RCR detected

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TissueGene-C Release Testing

Concern	Method	Specification
Identity	Immunostaining RT-PCR	Type II Collagen Presence TGF-β Presence
Potency	TGF-β ELISA Assay	2-10 ng/10^5 cells/24hr
Viability	Trypan blue dye exclusion	>70%
RCR	Co-culture of end-of production cells and supernatant amplification	No replication competent retrovirus detected
Mycoplasma	1993 Points to Consider	Negative
Endotoxin	LAL	To be determined
Sterility	21 CFR 610.12	No Growth

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Preclinical Study Plan to Support Phase I

90-Day Biodistribution Study in SCID Mice

This study is designed as **a worst-case scenario**, as we anticipate cells will distribute throughout the body with **I.V. injection** in contrast to distribution from a model of local cartilage injury as will be assessed in the rabbit intraarticular safety study.

Rabbit Intraarticular Safety and Efficacy Study

The nonclinical safety assessment of TissueGene-C is focused upon the **local administration** of human chondrocytes expressing TGF-β into degenerative knee joints. Thus, an animal model of knee joint injury will be utilized to obtain efficacy, toxicity and biodistribution data under the conditions of use of this product.

90-day Tissue Differentiation Study

A cellular differentiation study is planned to determine the potential for cellular **overgrowth, differentiation or transformation** of TissueGene-C following a single subcutaneous administration to male SCID mice.

In addition, to support future clinical development, safety and efficacy studies in a large animal model are being designed.

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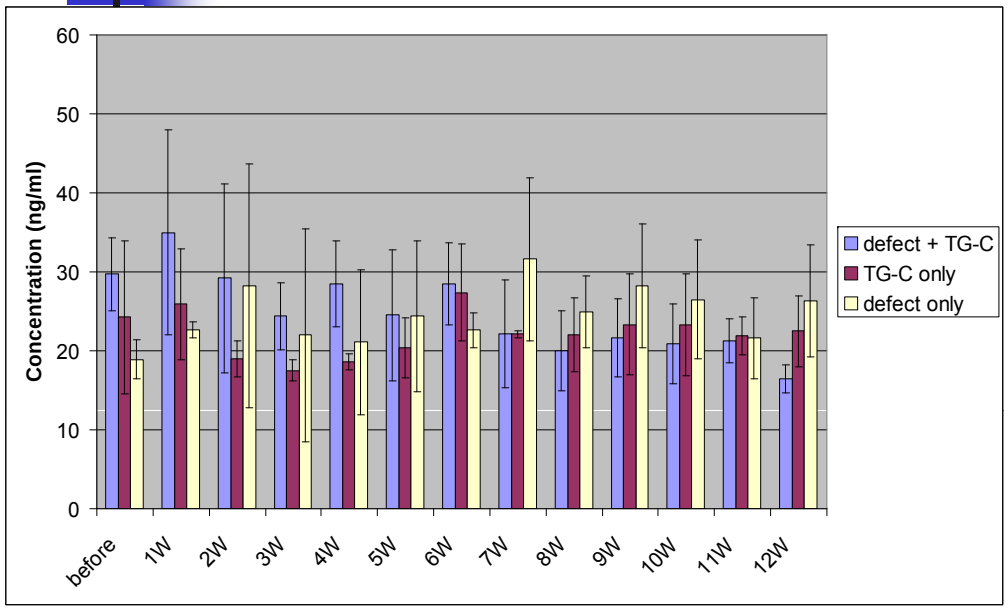
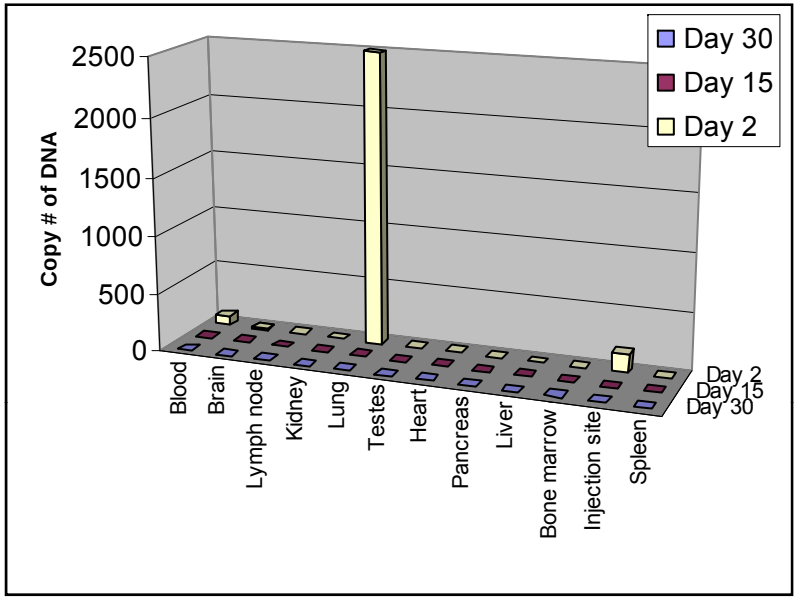
Biodistribution of TissueGene-C



Blood level of TGF-β
(local injection)



Detection of therapeutic cell
(I.V. injection)

- No elevation of serum TGF-β level was observed after injection of transduced TGF-β producing cells by intra-particularly**.**
- The presence of TissueGene-C DNA in the lungs and injection sites was not persistent and dissipated by day 15 of the treatment, as did its presence in the blood, brain, and bone marrow.
- Two of five animals analyzed at days 15 and 30-post administration had detectable TGF-β in the spleen, and one had detectable TGF-β in the heart 30 days post treatment**.**

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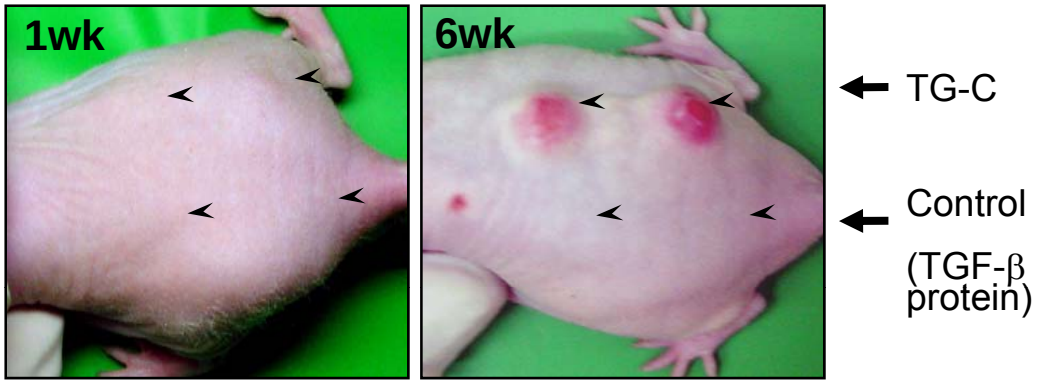
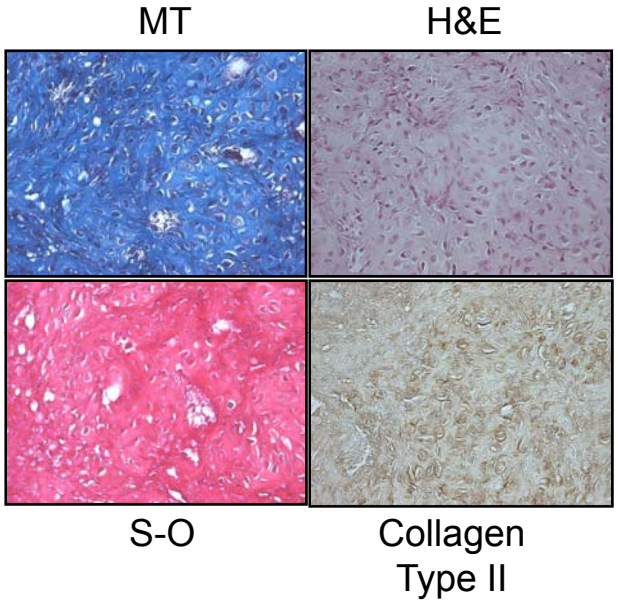
Differentiation study





- **Cartilage formation can be achieved by injection of TGF-β producing cells.**
  - TGF-β protein mixed with hChon showed no cartilage formation even at high concentration (Top: 50 ng, Bottom: 200 ng).
  - Cell mediated gene therapy is the only way to form cartilage in immunodeficient mice.
- **In addition, cellular proliferation and differentiation are being explored.**

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Clinical Trial Synopsis

- **Title:** A Phase 1 Study to Determine the **Safety** and Biological Activity of Cell-Mediated Gene Therapy Using TissueGene-C in Patients with Degenerative Joint Disease of the Knee Prior to Total Knee Arthroplasty
- **Objectives:**

  Primary: to evaluate the safety and activity of intra-articularly administered TissueGene-C.

  Secondary:

  - To evaluate dose response of the hChonβ cells in engrafting at the defect.
  - To evaluate distribution of hChonβ cells out of the injection site.
  - To evaluate the regeneration of hyaline cartilage as determined by the histological analysis of the resected knee tissue.
  - To evaluate the joint for evidence of tissue overgrowth or transformation.
  - To evaluate the resected knee tissue for expression of a panel of specific genes associated with the biological activity of TissueGene-C.

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Clinical Trial Synopsis (cont'd)

- **Study Population:** 12 male or female patients, age 18 and older, with degenerative arthritis (DA) of the knee joint refractory to existing therapies, who are scheduled for surgical replacement of the knee. Must provide written informed consent.
- **Exclusion Criteria:** HIV positive, clinically significant cardiovascular, renal, hepatic, endocrine disease, cancer, Type I diabetes, women who are pregnant or breast feeding.
- **Treatment:** TissueGene-C (N = 3) or placebo (N = 1) at 3 dose levels using a dose escalation design. The dose of cells will be increased to the next dose level following completion of dosing and 14 days of demonstrated safety at the previous dose level (total 42 days post TissueGene-C administration)
- **Safety Criteria:** Observation of the injection site for irritation or other abnormalities, the incidence and severity of adverse events, and the changes in physical examination findings and laboratory tests.
- **Pharmacokinetic Criteria:** Blood samples will be taken during evaluations at baseline, 24 hours following dosing, then at days 3, 10, 21, and day 28 (prior to surgery), and day 29 (one day post-surgery) following dosing and analyzed for TGF-β expression by ELISA and by PCR for vector DNA.

Clinical Trial Synopsis (cont'd)

- **Evaluation Schedule:** Patients will be administered TissueGene-C or placebo four weeks prior to scheduled knee replacement surgery. Patients will be evaluated during screening, immediately prior to dosing, at 24 hours following dosing, then at days 3, 10, 21, and day 28 (prior to surgery), and day 29 (one day post-surgery) following dosing. Follow-up patient monitoring will be performed also at 3, 6, and 12 months and annually thereafter for up to 15 years.
- **Biological Evaluation Criteria:** Histological analysis of resected knee tissue and observation for regenerated cartilage tissue. MRI imaging at baseline and prior to surgery on day 28. Examinations prior to surgery on days 3, 10, 21, and 28 to include evaluation of joint pain, range of motion, and functionality. Gene expression of resected knee tissue.
- **Anticipated Study Duration:** Approximately 12 months

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Consent Form

- **The Informed Consent Form has been revised to include:**
  - Simplified Purpose section
  - Addition of statement that surgery will occur as scheduled
  - Schedule of compensation
  - Statement that investigator has no financial interest in the company
  - Statement that some patients will receive placebo
  - Statements on risk of overgrowth, transformation, insertional mutagenesis and appearance of T-cell leukemia in Paris study

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Summary

- TissueGene's technology offers potential for complete recovery of degenerated cartilage, offering hope to DA patients.
- The technology leverages known attributes of an endogenous growth factor.
- The focus of the Phase I trial is safety.

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