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Pre-clinical studies of retrovirally transduced human chondrocytes expressing transforming growth factor-beta-1 (TG-C)

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Abstract

Background aims. The aim was to evaluate cartilage regeneration in animal models involving induced knee joint damage. Through cell-mediated gene therapy methods, a cell mixture comprising a 3:1 ratio of genetically unmodified human chondrocytes and transforming growth factor beta-1 (TGF-β1)-secreting human chondrocytes (TG-C), generated via retroviral transduction, resulted in successful cartilage proliferation in damaged regions. *Methods.* Non-clinical toxicology assessments for efficacy, biodistribution and local/systemic toxicity of single intra-articular administration of the cell mixture in mice, rabbits and goats was conducted. *Results.* Administration of the mixture was tolerated well in all of the species. There was evidence of cartilage proliferation in rabbits and goats. As an additional precautionary step, the efficacy of TGF-β1 secretion in irradiated human chondrocytes was also demonstrated. *Conclusions.* Four studies in rabbits and goats demonstrated the safety and efficacy of TG-C following direct intra-articular administration in animal models involving induced knee joint damage. Based on these pre-clinical studies authorization has been received from the USA Food and Drug Administration (FDA) to proceed with an initial phase I clinical study of TG-C for degenerative arthritis.

Key Words: *cartilage regeneration, cell-mediated therapy, degenerative arthritis, transforming growth factor-beta-1*

Introduction

Degenerative arthritis is caused by the inflammation, breakdown and eventual loss of the cartilage of joints. Among the more than 100 different types of arthritis condition, osteoarthritis is the most common, affecting the hands, feet, spine and large weight-bearing joints such as the hips and knees. The pathogenesis of this disease concerns the degeneration of the hyaline articular cartilage, which becomes deformed, fibrillated and eventually excavated during the course of degenerative arthritis (1). If degenerated cartilage could be regenerated, most patients would be able to function without debilitating pain.

Transforming growth factor-beta proteins (TGF-β) have been reported to induce osteogenesis and chondrogenesis (2,3). Among the TGF-β, TGF-β1 is regarded as one of the most important factors involved in the biologic process of cartilage formation. TGF-β1 plays crucial roles in tissue regeneration, cell differentiation and extracellular matrix protein synthesis (2). Recent studies have suggested that TGF-β1 stimulates proteoglycan synthesis in chondrocytes (4,5) and the growth of articular chondrocyte cells (6–8). In addition to its stimulatory effect on chondrocytes, TGF-β has been shown to possess anti-inflammatory and immune suppressive properties (9). This has led to recent reports detailing the therapeutic value of TGF-β protein in orthopedics (10–13). However, clinical applications employing TGF-β protein have been limited because of its short-term effects resulting from a short half-life. Consequently, a new method for the long-term and effective delivery of TGF-β1 is required for the treatment of degenerative arthritis.

TG-C is a cell-mediated gene therapy employed for the regeneration of cartilage tissue. TG-C comprises a

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3:1 mixture of normal allogeneic human chondrocytes (hChonJ) and irradiated allogeneic human chondrocytes that express TGF-β1 (hChonJb#7) (Figure 1). We have developed proprietary technology to deliver TGF-β1 to degenerative joints in a minimally invasive manner that does not require surgery (14). Cells, including human chondrocytes, are transfected with a viral vector containing the human TGF-β1 gene. When transduced human chondrocytes are injected into the damaged knee joints of rabbits and dogs, constitutive TGF-β1 release and the proliferation of regenerative cartilage are observed (15). Uninfected chondrocytes are included as additional cells for filling the defect site. These cells also provide additional target cells for TGF-β1 expressed from infected cells because TGF-β1 possesses both autocrine and paracrine modes of action (16).

To evaluate the potential of TG-C to regenerate cartilage and its safety and efficacy as a treatment for osteoarthritis, we performed several pre-clinical animal studies. Biodistribution studies were conducted following both systemic and local administration to determine the fate of TG-C cells subsequent to dosing. Studies employing surgically induced cartilage-defect models in rabbits and goats were performed to evaluate the safety and efficacy of TG-C following direct intra-articular administration. Further testing was performed in mice to determine the potential for TG-C cells to differentiate into cartilage and other tissues. Data from these studies suggest that cell-mediated gene therapy using TGF-β1 as a transgene may be a promising treatment option for osteoarthritis. Based on these pre-clinical studies, authorization has been received from the USA Food and Drug Administration (FDA) to proceed with an initial phase I clinical study of TG-C for degenerative arthritis.

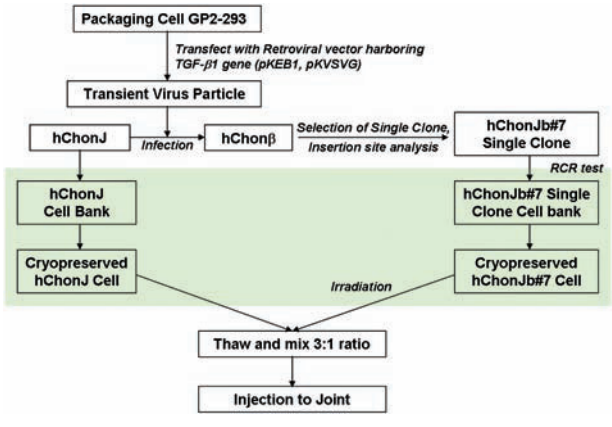

Figure 1. Production scheme of TG-C. The cells used to create the cell banks for human chondrocytes were derived from a human donor. hChonJb#7 was isolated according to the limiting dilution method. Cryopreserved cells were thawed, washed and mixed to a 3:1 ratio (hChonJ:hChonJb#7) at the planned dose.

Methods

TG-C was made using a retroviral vector to create a TGF-β1-expressing chondrocyte cell line. Subsequently these cells were tested in an intravenous (i.v.) biodistribution study in SCID mice, cartilage-induced defect models in rabbits and goats, and tissue differentiation studies in mice.

Isolation of a single clone

Virus construction, infection and single-clone isolation methods were performed as described previously (15,16), using a retroviral vector. A limiting dilution method was used for the single-clone isolation. Transduced chondrocytes were diluted and cultured in 96-well plates. Wells containing more than two colonies were excluded. Selected colonies were transferred to 24- or six-well plates and cultured to approximately 2×10^5 cells. Each colony was tested for TGF-β1 production by an enzyme-linked immunosorbent assay (ELISA) using a Quantikine kit (R&D Systems, Minneapolis, MN, USA).

Biodistribution in SCID mice

A 90-day i.v. biodistribution study was performed using SCID mice. Groups of 24 mice of each sex per group were administered either control media (group 1) or TG-C cells at 4×10^5 (group 2) or 4×10^6 (group 3) cells/mouse. Following i.v. dosing with a high dose level of 4×10^6 cells/mouse, mortality was observed in group 3 female mice, and subsequently the dose levels were split to include lower dose levels of 1×10^5 and 1×10^6 cells. Thus half of the group 2 mice were treated with 4×10^5 cells and half with 1×10^5 cells, and half of the group 3 mice were treated with 4×10^6 cells and half with 1×10^6 cells. Mice were dosed i.v. with either control media or TG-C at a dose volume of 200 μL. Physical examinations (clinical observations) and body weight assessments were performed prior to dosing, at weekly intervals and immediately prior to killing. Blood samples for polymerase chain reaction (PCR) analysis were obtained prior to termination on days 2, 15, 30 and 90 and snap-frozen. Subsequently, five mice/sex/group were killed and tissues obtained for quantitative (Q)-PCR biodistribution analysis. Following blood collection, samples of liver, thymus, heart, lung, kidney, spleen, pancreas, iliac lymph nodes, bone marrow, brain and ovaries or testes were collected from each mouse, snap-frozen and then stored at –70°C. All tissues from mice killed on days 2 and 15, in addition to lung tissue from mice killed on day 30, were evaluated for the presence of TGF-β1 cDNA using the Q-PCR technique described below.

Total DNA was isolated from 50 μL SCID mouse blood using a QIAmp DNA mini blood kit (Qiagen, Valencia, CA, USA). Total DNA was isolated from SCID mouse tissues using a DNeasy tissue kit (Qiagen). The DNA was extracted directly from the initial sample vessel for tissue samples containing less than 30 mg of material, or 15 mg for spleen. For samples containing greater amounts, the tissue was minced and a portion was placed into a sterile tube for DNA extraction. All DNA samples were eluted in a final volume of 200 μL elution buffer provided with the Qiagen kit. The absorbance at 260 nm (A_{260}) was measured for a 1:20 dilution of each blood and tissue DNA sample using a SpectraMax384 spectrophotometer (Molecular Devices, Sunnyvale, CA, USA) and the DNA concentration for each sample was calculated.

91-day and 6-month safety and efficacy study of TG-C in New Zealand white rabbits

One-hundred and twenty rabbits underwent surgical induction of a single partial cartilage defect in the patellar groove of the right (femorotibial) joint with control media or TG-C applied intra-articularly using doses of 1.8×10^5, 1.8×10^6 and 9.0×10^6 cells. Clinical observations, body weight, food consumption and ophthalmic examinations were performed. Clinical pathology, PCR and antibody assessments were obtained. Four animals/sex were killed for the low- and mid-dose groups and six animals/sex were killed for the control and high-dose groups at either 30 days, 91 days or 6 months post-dosing. Full necropsy with organ weight and a full panel of tissues, as well as bone, ligament, muscle and synovium associated with the right joint, were collected. Histopathology was performed on control and high-dose groups.

8-week pilot safety and efficacy study of TG-C in goats

Six goats had a single full-thickness cartilage defect surgically induced in the patellar groove of both hind legs. Group 1 animals were dosed with a single intra-articular injection of vehicle (media) control in the left hind leg and received no treatment to the right hind leg. Group 2 animals were dosed with a single intra-articular injection of TG-C (1×10^6 cells) in the joint of the left hind leg and injection of vehicle control (media) in the right hind leg. Similarly, group 3 animals were administered TG-C (1×10^7 cells). One leg was weight bearing and the other was splinted to simulate non-weight bearing in one animal in each group. At 8 weeks, animals were subjected to a full necropsy. Clinical pathology, hematologic and PCR assessments were performed.

Selected tissues were collected for histopathologic examination and PCR analysis. In particular, the bone, ligament, muscle and synovium associated with the defect site were examined for cartilage repair and histopathologic changes. Defect sites were stained using hematoxylin-eosin, toluidine blue, safranin-O and Masson's trichrome. Immunohistochemical staining for type II collagen, TGF-β1 and mammalian major histocompatibility complex (MHC) class I was also performed.

1-year safety and efficacy study of TG-C in goats

Each animal had a single full-thickness cartilage defect surgically induced in the patellar groove of both hind legs. Ten control (group 1) animals were dosed with a single intra-articular injection of vehicle (media) in the left hind leg and received no treatment to the right hind leg. Twenty test (group 2) animals were dosed with a single intra-articular injection of TG-C (3×10^7 cells) in the joint of the left hind leg and injection of vehicle control (media) in the right hind leg.

At 6 and 12 months, respectively, half of the animals were subjected to a full necropsy, clinical pathology, hematologic and antibody assessments. A full tissue list was collected for histopathologic examination and PCR analysis. In particular, the bone, ligament, muscle and synovium associated with the defect site were examined for cartilage repair and histopathologic changes. Staining was performed as described for the 8-week goat study.

DNA extraction

DNA was extracted from tissue specimens using the BioRobot M48 nucleic acid extraction system (Qiagen). A naive tissue was included with each batch of specimens to serve as an extraction contamination control (negative extraction control; NEC). Purified DNA from tissue was quantitated using a SpectraMax 190 Spectrophotometer (Molecular Devices) and adjusted to 100 ng/μL with water. Ten microliters of purified DNA (1 μg DNA) was used for each Q-PCR.

Q-PCR analysis

All real-time PCR analyses were performed in 96-well optical grade plates on an Applied Biosystems ABI 7900HT sequence detection instrument (Applied Biosystems, Foster City, CA, USA). For the quantification of TGF-β1 cDNA sequences, plasmid DNA or DNA extracted from cells or tissues was subjected to real-time PCR analysis (5 min at 50°C, 10 min at 94°C, followed by 40 cycles of 30 s at 94°C and 1 min

at 60°C) using TaqMan master mix reagents (Applied Biosystems), TGF-β1-specific primers 1270-F (100 nM, 5′-CGAGCCTGAGGCCGACTAC-3′) and 1270-R (100 nM, 5′-TGTCATAGATTTCGTT-GTGGGTTT-3′) and TaqMan probe 1270-P (150 nM, 5′-[dFAM]CCAAGGAGGTCACCCGCGTG C[TAMRA]-3′). Real-time PCR analysis of serial dilutions of plasmid pKEB-1 was used to generate a standard curve for the quantification of TGFβ-1 cDNA sequences.

For the biodistribution studies, three replicates of each tissue sample containing 1.0 μg, 500 ng or 100 ng DNA/replicate were analyzed. One-hundred or 50 copies of pKEB-1 plasmid were added to one of the three replicates for each tissue sample, as a spiked control. A lower limit of quantification (LOQ) was set at 10 copies for all samples.

Differentiation study

A non-clinical GLP tissue differentiation study was conducted in an attempt to evaluate the potential of TG-C I and TG-C II to differentiate following subcutaneous (s.c.) implantation into male SCID mice. The two forms of test article TG-C I and II represent mixtures of untransduced chondrocytes and TGF-β1-transduced chondrocytes with high and low expression levels of TGF-β1, respectively. TG-C I is the intended clinical product (comprising hChonJ + hChonJb#7 cells at a 3:1 ratio), whereas TG-C II utilizes a different clone that expresses TGF-β1 at a lower level (comprising hChonJ + hChonJb#55 at a 3:1 ratio). Vehicle (media) and untransduced chondrocytes (hChonJ) were also obtained and implanted into SCID mice as controls. SCID mice were implanted with 2×10^6 TG-C or control cells once on day 1. Assessments included cage-side observations and weekly body weight measurements. Injection site observations were performed for erythema and edema pre-dose on day 1 and at day 30. Groups of five mice were killed on days 60 and 180, and the remaining mice were killed on day 199. Each mouse was subjected to a full gross necropsy. The injection site and underlying skin and muscle tissues were preserved in formalin and subsequently examined microscopically.

Cell irradiation study

In all of the aforementioned *in vivo* non-clinical studies, the hChonJb#7 cells in TG-C were not irradiated prior to administration. As a safety measure for clinical use, the hChonJb#7 cells are irradiated with 15 Gy X-rays prior to mixing with the hChonJ cells, to render them replication incompetent. An *in vitro* study was performed to assess the viability and TGF-β1 expression levels (via ELISA assay using the Quantikine kit; R&D Systems) of hChonJb#7 cells following irradiation (using a Varian CL2100 C/D linear accelerator) with 10, 15, 20 or 25 Gy of X-ray radiation and to determine the replication competence of the cells using the MTT assay. Assays were performed every 2–3 days (up to 28 days) to determine the radiation dose necessary to render the cells replication incompetent.

Results

Biodistribution

A 90-day study of TG-C was conducted to establish the biodistribution of TG-C following a single i.v. administration in male and female SCID mice. Analysis of the PCR data indicated that TG-C was found in the lung (in 13 of 20 animals) and heart tissues (in six of 20 animals) in mice killed on day 2, but by day 15 only a single lung specimen tested positive for TG-C by PCR (Figure 2). The biodistribution analysis revealed that TG-C was retained for only a short time in the lung; by day 30 following administration there was no sign of TG-C DNA in the lung, nor did there appear to be adverse growths or nodules present in the fully immunocompromised mice. Thus, following a single i.v. administration, TG-C appeared to be present in the heart and lung but was completely cleared from all other tissues by day 15 and from the lung by day 30. TG-C was not detected in the lung from treated mice killed on day 30, nor was there any positive signal or persistence

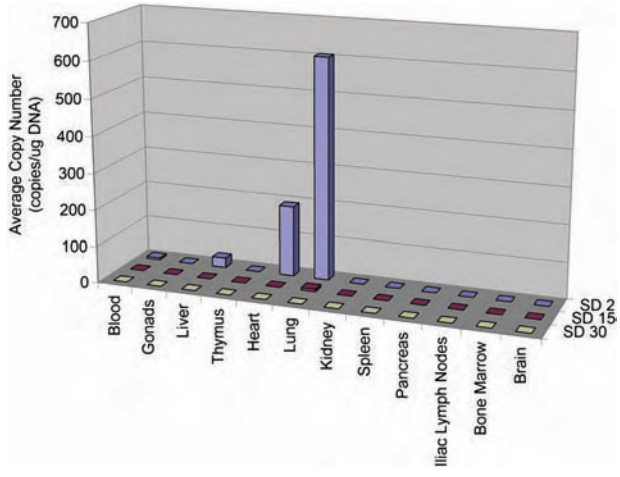

Figure 2. Biodistribution of TG-C following single i.v. administration in male and female SCID mice. TG-C was found in the lung, heart and liver tissues in mice killed on day 2. By day 15, only a single lung specimen was positive. By day 30 following administration, there was no sign of TG-C DNA in the lung in these fully immunocompromised mice.

of TG-C cDNA in any other tissues or blood beyond day 2 following treatment. Q-PCR analysis of tissues obtained from rabbits killed on day 30 indicated that no TG-C DNA was evident in either the blood or tissues of male and female rabbits following a single intra-articular dose 30 days following treatment (data not shown).

A Q-PCR assay was used to measure the persistence of TG-C in a safety and efficacy study conducted on female goats. The results indicated that 8 weeks following intra-articular administration, TG-C was undetected in the tissues of treated animals.

91-day and 6-month safety and efficacy study of TG-C in New Zealand white rabbits

No test article-related changes in mortality, clinical observation, body, body weight, food consumption, ophthalmology, clinical pathology, organ weight, organ pathology and antibody analysis were observed. Examination of the surgical defect/TG-C dose site suggested a relationship between intra-articular TG-C and speed of cartilage regeneration (Figures 3 and 4). Administration of 9×10^6 cells/animal resulted in the fastest cartilage regeneration time, with signs of regeneration clearly present at terminal necropsy. In animals treated with 1.8×10^6 cells/animal, signs of cartilage regeneration were mostly noted at terminal necropsy. There were no signs of cartilage regeneration associated with the low-dose and control groups.

8-week pilot safety and efficacy study of TG-C in goats

The findings suggested that both the low- and high-dose gene therapies resulted in foci of proliferation

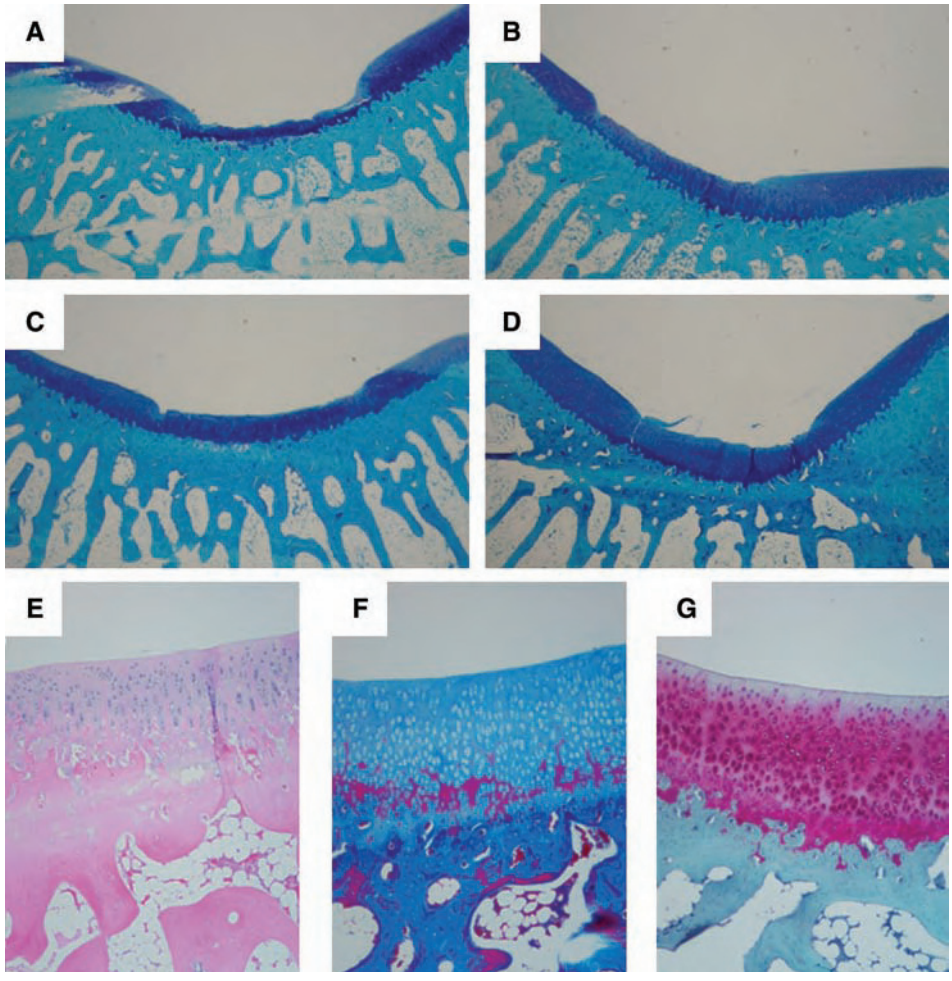

Figure 3. Representative results of histologic staining of regenerated hyaline cartilage formed 30 days following administration of a control or TG-C in the rabbit study. (A–D) Toluidine blue staining in defect areas treated with media control (A) or at a dose of 1.8×10^5 (B), 1.8×10^6 (C) or 9.0×10^6 (D) cells. (E–G) Histologic staining in defect areas treated with 9.0×10^6 cells using hematoxylin-eosin (E), toluidine blue (F) or Masson's trichrome (G). Original magnification (A–D) × 40, (E–G) × 100.

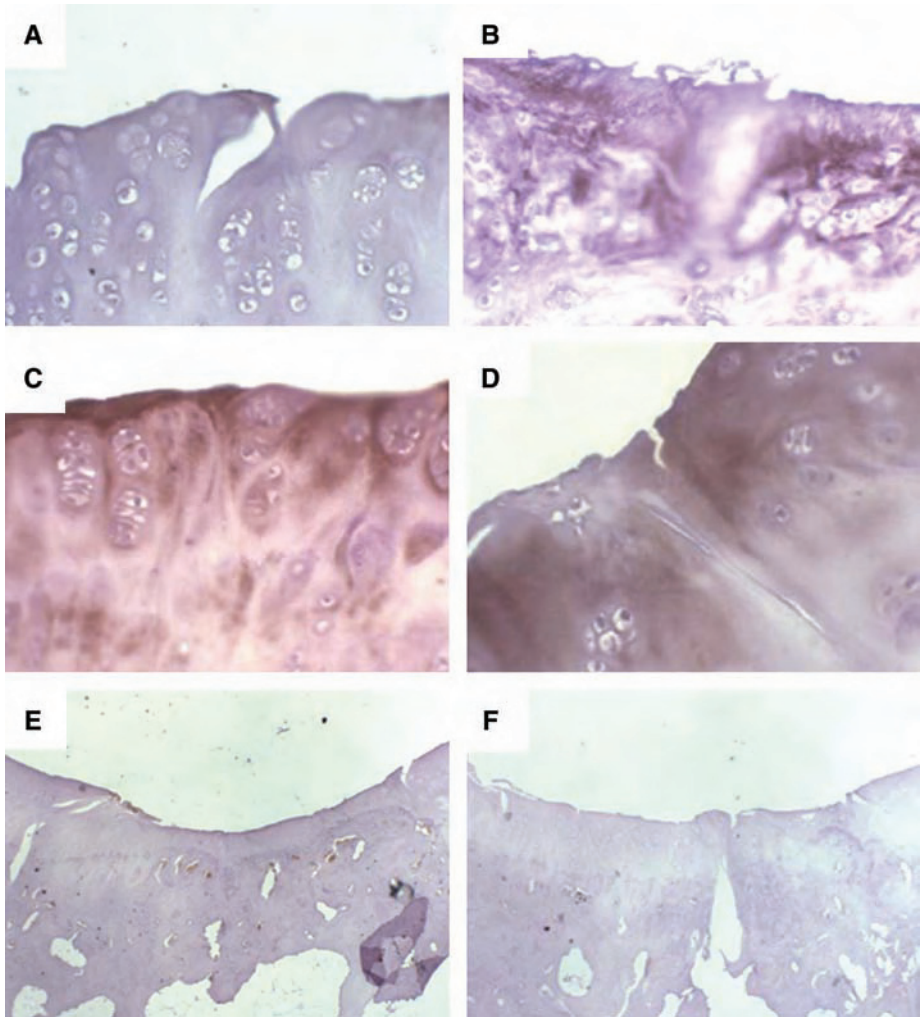

Figure 4. Representative results showing immunohistologic staining of regenerated hyaline cartilage formed 6 months following administration of a control or TG-C in the rabbit study. (A–D) Type II collagen staining in defect areas 6 months following injection with media control (A) or at a dose of 1.8×10^5 (B), 1.8×10^6 (C) or 9.0×10^6 (D) cells. (E, F) TGF-β1 staining in defect areas 6 months following injection with media control (E) or at a dose of 9.0×10^6 cells (F). Original magnification (A-D) × 200, (E, F) × 100.

of new chondrocytes in the hyalinecartilage matrix at the base of the surgical defects in both animals (Figure 5). These findings consisted of foci of young chondrocyte proliferation in the hyaline cartilage matrix that stained positive with toluidine blue and type II collagen, although proteoglycan staining with safranin-O was not marked. Staining for human chondrocytes (TGF-β1) indicated that there were small focal areas of positive staining at the defect sites, but not in other areas or the vehicle-treated joints. The morphology of the defect foci and staining characteristics indicated that hyaline cartilage consistent with articular cartilage was present. There were no differences between the exercise-restricted and non-restricted groups. No test article-related changes in mortality, clinical observation, body, body weight and clinical pathology were observed.

1-year safety and efficacy study of TG-C in goats

No significant test article-related changes in mortality, clinical observations, body, body weight, clinical pathology and antibody analysis were observed. Three group 2 animals died on days 15, 16 and 17, respectively. Examination revealed that the surgical sites were infected and death was attributed to infection induced during the surgical procedure. Positive immunohistochemical staining for type II collagen was present in the proliferating cartilage at 6 months but not at 12 months. There was positive staining in the proliferating cartilage in the defect of the treated joint of five out of eight group 2 animals, indicating that the new proliferating cartilage was elaborating type II collagen. Additionally, there was a small amount of positive staining for TGF-β1 in one treated joint in this group at 6 months.

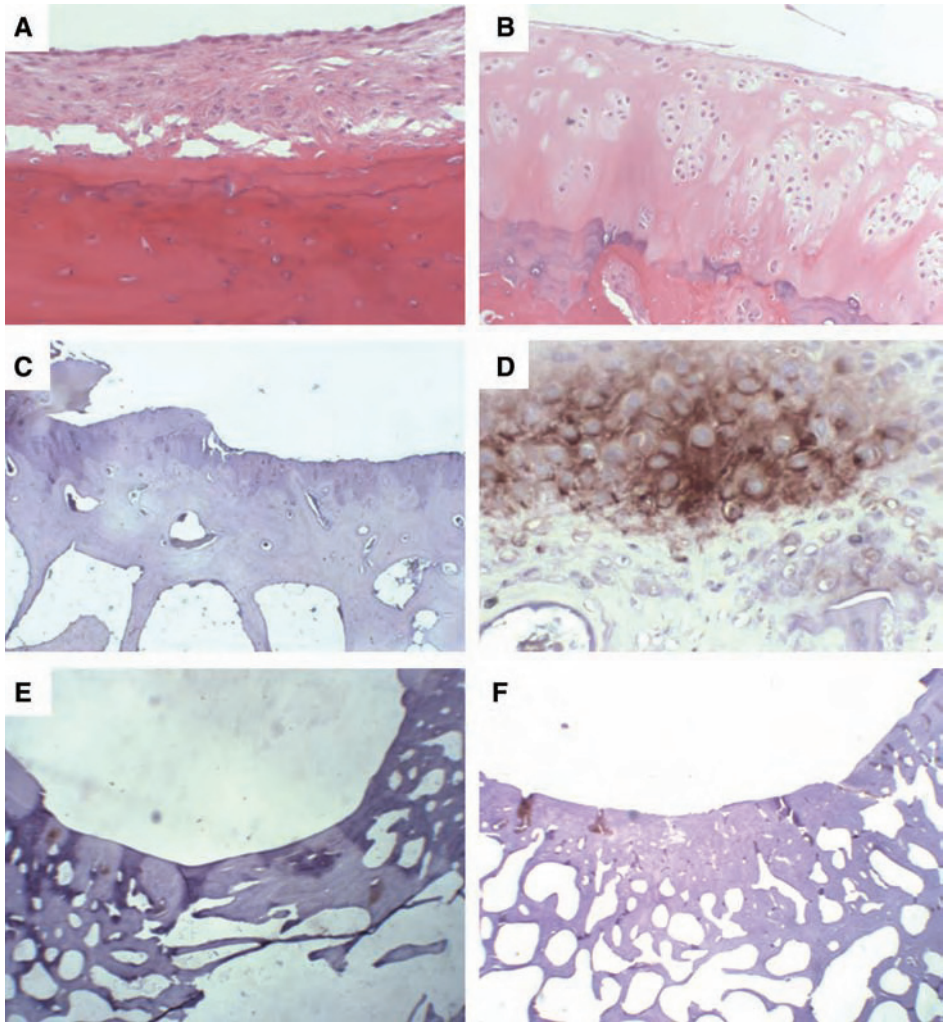

Figure 5. Representative results showing hyaline cartilage regeneration in the goat study. (A, B) Hematoxylin-eosin staining of regenerated cartilage at 8 weeks following administration of media control (A) and TG-C at a dose of 1.0×10^7 cells (B). (C, D) Type II collagen staining of regenerated cartilage at 8-weeks following administration of media control (C) and TG-C at a dose of 1.0×10^7 cells (D). (E, F) Hematoxylin staining of regenerated cartilage at 1-year following administration of media control (E) and TG-C at a dose of 1.0×10^7 cells (F). Original magnification (A–C) × 40, (D) × 200, (E and F) × 10.

At 12 months, there were no significant differences in the staining between the treated and untreated groups when using either the immunohistochemical stains or the special stains. These findings suggested that a positive effect on the joint cartilage may be present at 6 months and diminish at 12 months, although the test article was tolerated well at both time-points.

Differentiation

Treatment-related clinical signs were evident in TG-C-treated mice. Large and small movable and stationary tissue nodules were noted on the dorsal thoracic region of five (29%) treated males from day 15 to 29. The tissue nodules were transient in nature and were not observed after 29 days following injection. No nodules were present or apparent in mice treated with media or cellular controls and no toxicologically significant clinical observations or gross pathologic findings were noted. There were no microscopic histologic findings in male SCID mice following a single s.c. injection of either control cells or TG-C at any time-point. Thus treatment with either control cells or TG-C did not elicit any treatment-related behavioral or pathologic effects nor result in the production of any lasting or overt tissue nodules. Furthermore, the TG-C cells did not appear to differentiate within the first 6 months following s.c. administration of up to 2×10^6 cells.

Cell irradiation study

The results indicated that 15 Gy were sufficient to render the cells replication incompetent. The

in vitro data showed that the hChonJb#7 cells in TG-C continued to express TGF-β1 for at least 8 days following irradiation with 15 Gy X-rays at a declining rate corresponding to the viability of the cells (Figure 6). The irradiation study results indicated that total TGF-β1 production was lower given the presence of fewer viable cells. However, additional analysis showed that the TGF-β1 release was actually higher for irradiated cells in comparison with non-irradiated cells on a per cell basis. One explanation for this may be the release of stored TGF-β1 from the cell following cell lysis and death.

Discussion

The ability of TG-C and related cellular products to promote regeneration of hyaline cartilage has been studied using a number of non-clinical animal models. TG-C has been evaluated in terms of tissue formation following s.c. administration to immunocompromised mice. Additionally, a determination of the optimal ratio of hChonJ to hChonJb#7 for cartilage regeneration has been performed using a rabbit model (surgically induced partial cartilage defect) comprising degenerative arthritis. The normal human chondrocytes in TG-C were included as additional cells to fill the defect site. These cells also provided target cells for TGF-β1 expressed from infected cells, as TGF-β1 possesses a paracrine mode of action. It has been demonstrated that hChonJb#7 cells express type II collagen and high levels of both type 1 and 2 TGF-β1 receptors in culture, and are capable of forming hyaline cartilage in pre-clinical models of degenerative arthritis (5). Additional efficacy studies in the context of the pre-clinical safety studies described above demonstrated that TG-C can form regenerated cartilage in rabbits at doses comparable to the starting clinical dose of 3×10^6 cells. In the 8-week pilot study in goats, TG-C promoted cartilage growth, chondrocyte proliferation and deposition of type II collagen, as well as formation of hyaline cartilage, within the 8-week time frame.

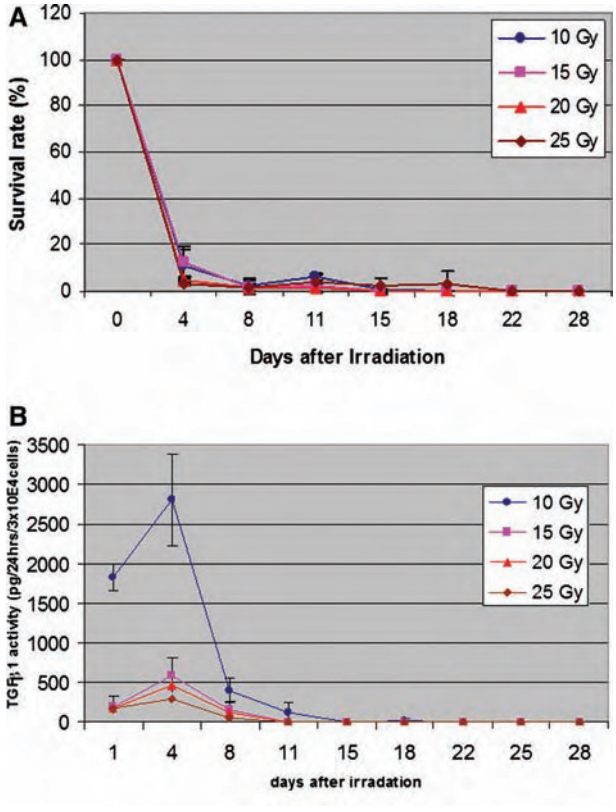

Figure 6. (A) The survival rate (%) of transduced cells irradiated with 10, 15, 20 or 25 Gy of X-ray radiation. For all radiation doses the survival rate dropped significantly in the first 4 days and no surviving cells were detected by day 22. (B) The TGF-β1 expression level increased at day 4 and subsequently declined until reaching undetectable levels by day 22.

As the TG-C cells used in these studies represent genetically modified human cells expressing TGF-β1, there were clear reasons to examine closely both the biodistribution and safety of these cells in appropriately sensitive animal models. Biodistribution analyzes in SCID mice, rabbits and goats established that TG-C does not persist in tissues following single intra-articular or i.v. administration. Preliminary efficacy studies in rabbits and dogs demonstrated that administration of TGF-β1-expressing human chondrocytes could promote cartilage regrowth without overgrowth of the cells or tissue. Subsequent studies in rabbits and goats also demonstrated the safety and efficacy of TG-C following direct intra-articular administration.

The systemic biodistribution study was designed to determine the biodistribution of TGF-β1-expressing human chondrocytes following single i.v. administration to male SCID mice. Intravenous administration was intended to represent a 'worst-case' scenario in terms of a potential adverse distribution of TGF-β1-expressing human chondrocytes to peripheral tissues. TG-C is intended for direct intra-articular administration to the knee joint. A Q-PCR-based methodology was developed and utilized to detect the presence of TGF-β1 cDNA sequences in tissues from mice at three defined intervals following injection. In this 90-day biodistribution study, the high dose used represents approximately 3000 times the highest dose level proposed for the initial phase I clinical trial when adjusted for mouse body weight.

Four non-clinical toxicology studies were conducted in animals: 91-day and 6-month safety and efficacy studies in rabbits, and 8-week and 12-month safety and efficacy studies in goats. In general, the administration of TC-G was tolerated well in both

species, and there was evidence of dose-related cartilage proliferation in rabbits at 1 and 6 months, and in goats at 8 weeks and 6 months, but not at 12 months, indicating a positive therapeutic response to treatment with TC-G. Intra-articular doses of TG-C up to 9×10^6 cells in rabbits resulted in improved cartilage regeneration compared with the control, and did not result in any treatment-related mortality or significant dose-related toxicity. Thus, based on the results of this study, the NOAEL for TG-C in male and female rabbits and goats subsequent to a unilateral articular cartilage defect was 9×10^6 cells and 3×10^7 cells, respectively, delivered directly into the stifle joint. This dose is approximately three-fold higher than the starting dose proposed for the initial phase I trial in which patients will undergo a full knee replacement 30 days following dose administration.

From a clinical perspective, therapies utilizing transduced allogeneic cells have been employed in trials for the treatment for cancer (17). Up to 10^8 cells were delivered systemically in these trials. In contrast, the starting dose (number of cells) proposed for the TG-C phase I trial is 30-fold lower, and will be delivered locally (to the knee joint) rather than systemically. The knee joint space represents a relatively avascular site that would not be readily accessible and therefore susceptible to immunologic stimulation. Additionally, while systemic circulation of TGF-β1 is unlikely, TGF-β1 is a potent immunomodulatory molecule that suppresses the proliferation and differentiation of most cells of B- and T-cell lineages *in vitro*, antagonizing the effects of inflammatory effector cytokines such as interleukin (IL)-1β, tumor necrosis factor (TNF)-α and interferon (IFN)-γ, and suppressing the expression of receptors for IL-1β and IL-2 on cells (18,19). It should also be noted that, in early rabbit and dog studies, human chondrocytes did not induce any histologically evident immunogenic responses, while at the same time promoted cartilage regrowth. Current evidence suggests that chondrocytes can inhibit the proliferation of T lymphocytes and are indeed immunosuppressive themselves (20).

There are several additional considerations in the potential utilization of these cells in humans. For example, it is feasible that non-irradiated chondrocytes transplanted into a human host could persist for longer periods of time than seen in animal models. However, as described above, no non-irradiated transduced chondrocytes will be administered to human patients. Therefore, the replication incompetence of the cells will limit their persistence in humans. There is also the potential, however small, that cell tissue distribution and growth potential may differ in the human versus animal host. Accordingly, the initial phase I study in humans has been designed to limit the time of exposure of the cells to the patient (patients will undergo total knee arthroplasty 28 days after dosing) and assays will be performed on blood samples to test for transgene expression (TGF-β1 ELISA) and the presence of the transgene (PCR), and the removed knee joint tissue will be examined histopathologically for possible overgrowth or abnormal growth of the administered cells. These observations and experiments may provide a rationale for the progress and development of novel allogeneic cellular therapies in humans.

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