RAC protocol 0307-594

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(TherImmune Research Corp., regulatory agent)

Ad hoc Reviewer: Robert H. Carter, M.D.

Summary statement

- This protocol addresses a major clinical need - the ability to restore cartilage to damaged joints.

- The use of injected cells minimizes exposure to viral agents and inadvertent transgene exposure, although with the risk of allosensitization (and chronic rejection).

- The follow-up arthroplasty removes the long-term local (but not systemic) risk, and allows for careful evaluation of local effects of the therapy.

Potential Concerns:

1. Risks associated with administered transgenic cells
2. Effects of expressed TGF-β
3. Disease-specific

Potential Concerns:

1. Transgenic cells

A. Evaluation of safety of cells

B. Systemic exposure to cells

C. Immune response to cells

Potential Concerns:

2. **Effects of expressed TGF-β**

A. Chondrocyte overgrowth

B. Increased susceptibility to local infection

Potential Concerns:

3. Disease-specific

   A. How will cells be prepared and administered

Initial Critique and Responses

(taken in order of written comments)

Initial **Critique** and Responses

I. To what extent are chondrocytes altered by passage in culture? Is there any change in growth properties, compared to chondrocytes directly ex vivo? Are there any changes in chromosomes?

I. Are chondrocytes altered by passage?

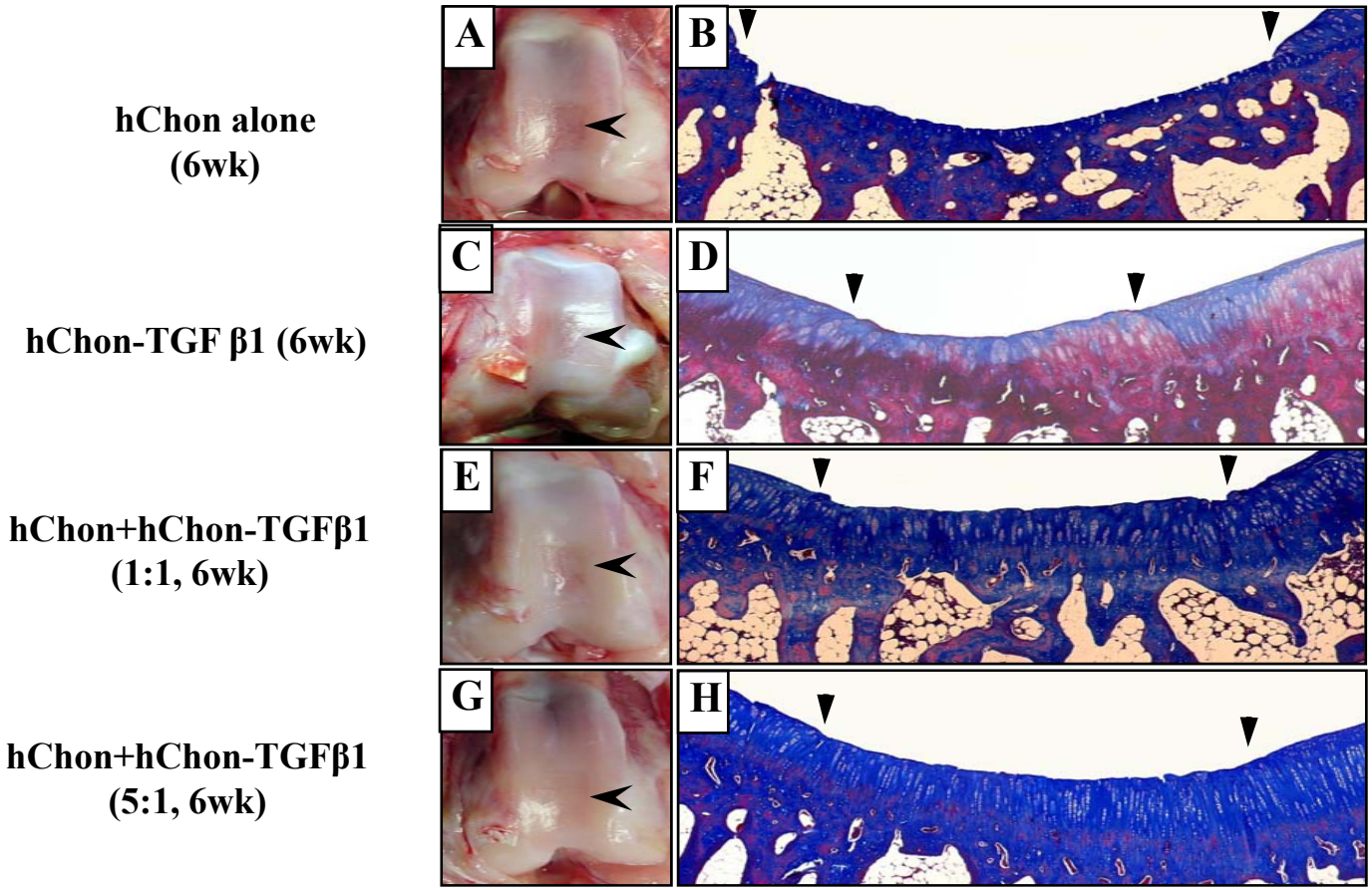
The cell line was selected based on its ability to maintain the characteristics of hyaline cartilage after numerous passages. … The cultured cell product will be in the range of 10 to 15 passages. An analysis of changes in the chromosome has not yet been performed. As suggested, a karyotypic analysis will be conducted to address chromosomal changes, including the potential for transformation.

II. The “optimal” mixture of untransfected and transduced chondrocytes rests on a subcutaneous injection model in SCID mice.

Initial Critique and **Responses**

II. mixture of untransfected and transduced chondrocytes

[The requirement for the mixture has been confirmed in other experiments done in joints in other animals. Such an experiment is included in the (recently provided) submitted manuscript.]



Initial **Critique** and Responses

III. The intent of the protocol is to deliver chondrocytes “into the defect” by positioning the knee before injection. Some skepticism that this results in delivery “into the defect” seems appropriate, unless the procedure was performed under radiologic guidance.

III. the protocol is to deliver chondrocytes "into the defect"

The injection will be performed with arthroscopic guidance.

IV. This is a single blinded study. Assurances should also be given that those interpreting the MRI and the joint pathology will also be blinded.

IV. interpreting the MRI and the joint pathology will also be blinded

The sponsor confirms that those interpreting the MRI and the joint pathology will also be blinded.

V. Could joint fluid be obtained at the time of surgery for assay of TGFβ levels?

V. joint fluid be obtained at the time of surgery for assay of TGFβ

In humans, it is not clear what amount of fluid will be present in the knee joint at the time of surgery; some patients may have a “dry” joint. The protocol will be modified to direct that, when available, fluid in the knee joint will be obtained at the time of surgery.

VI. The appendix M answers (M-II-B-1-b, page 61) indicate that the cells will be washed after thawing before injection, although this is not mentioned in the protocol (page 15). If the cells are washed, that would seem to increase the risk of infection.

VI. … cells will be washed after thawing

The cells will be washed with DMEM (without phenol red) after thawing and before injection to remove FBS. A specific procedure is in development that will utilize a closed system to minimize the risk of infection.

VII. What is the basis for the statement that all hChonB1 express TGFβ? Simply clonal derivation from an expressing precursor would seem insufficient for such a conclusion.

VII. "all hChonB1 express TGFβ"

Expression of TGFβ1 by the clonal hChonβ1 cells is confirmed during manufacturing of the Master Cell Bank as described in Table 3 (M-II-B-1-B-iv, page 62) of the Appendix M document. Further, expression of TGFβ1 is confirmed for the release of each batch of hChonβ1 cells as described in Table 5 (M-II-B-1-B-iv, page 65) of the Appendix M document.

VIII. How definitive are the assays used to make the statement that the cells are free of retrovirus?

VIII. cells are free of retrovirus

The assays used to determine that the cells are free of retrovirus were developed and are conducted in accordance with FDA guidance "Supplemental Guidance on Testing for Replication Competent Retrovirus in Retroviral Vector Based Gene Therapy Products and During Follow-up of Patients in Clinical Trials Using Retroviral Vectors" (FDA/CBER October 2000).

IX. How the investigators will assay for cells, as opposed to TGFβ1, should be clarified.

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A quantitative PCR assay was developed that was specific for the amplification and detection of human TGF β 1 cDNA sequences. … The primer/probe set was designed to span an intron region of the human TGF β 1 sequence located on human chromosome 19 (NCBI accession number AC011462) to limit the possibility of amplification of endogenous TGF β 1 sequences contained in the chondrocyte genome.

Initial **Critique** and Responses

X. The results of an additional biodistribution study, which the protocol describes as "pivotal" were pending at the time of submission….

X. additional biodistribution study

The full 90-day biodistribution study has not yet been initiated…. The safety and biodistribution of TissueGene-C cells administered via intraarticular injection will be determined in the planned rabbit study (M-II-B-2-d, page 82).

Initial **Critique** and Responses

XI. The risk section of the informed consent is minimal.

XI. The risk section of the informed consent is minimal.

The risk section of the Informed Consent form has been modified to add the potential for overgrowth, transformation or insertional mutagenesis. The revised Informed Consent Form is attached. The risk section may be further modified based on the results of the planned safety study in rabbits.

Initial **Critique** and Responses

XII. Although no immune response was detected locally in injected joints in animal studies, and similar studies will be done in subjects' knees after arthroplasty, other evidence of immunization should be sought. The simplest approach would be to test the activation/proliferation of peripheral blood mononuclear cells to irradiated chondrocytes or hChonβ, from the same clones used for inoculation into the joint, with analysis of neutralizing anti-TGF, if available.

XII. other evidence of immunization should be sought.

Anti-TGF production will be checked in animal studies. If possible, we will check the antibody production of TGFβ1 in vitro as you recommended.

XIII. What happens if participants who, for whatever reason, do not undergo the scheduled arthroplasty?

XI. participants who do not undergo the scheduled arthroplasty.

In accordance with the informed consent regulations (21 CFR 50.25) and as noted in the informed consent form, participants may withdraw from the study at any time. In such a case, the participant will be monitored annually for up to 15 years to include blood testing and physical examinations.

Potential Concerns:

1. Risks associated with administered transgenic cells
2. Effects of expressed TGF-β
3. Disease-specific

Summary:

1. transgenic cells
   A. Evaluation of safety of cells
      - Initial preclinical data suggest low risk at injection site, but longer term risk is unknown.

Summary:

1. transgenic cells

   B. Systemic exposure to cells

   - Further preclinical data needed to define systemic exposure risk

Summary:

1. transgenic cells

   C. Immune response to cells

   - Passaged cells have low MHC Class I, and no evidence of local allogenic reaction, but more sensitive assays are needed - suggest *in vitro* activation studies of recipients' PBMC to hChon and hChonβ

Summary:

2. Effects of expressed TGF-β

A. Chondrocyte overgrowth

- For this trial, only a problem if either injected chondrocytes can seed other joints (which needs to be defined), or if participant refuses arthroplasty (which should be added to consent).

Summary:

2. Effects of expressed TGF-β

B. Increased susceptibility to local infection

- Data from primate trials will be critical

Summary:

3. Protocol-specific

   A. How will cells be prepared?

      - Development and testing of safe washing protocol critical

Summary:

3. Protocol-specific

   B. How will cells be administered?

- Concept of injection into the defect remains problematic. Studies to define localization of injected cells besides “the defect” should be included in animal models. Arthroscopic guidance also problematic.