The Development of a Novel Gene Therapy, Flexion's FX201, for the Treatment of Osteoarthritis

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Introduction

The ultimate goal when treating osteoarthritis (OA) is to restore degraded cartilage or decelerate disease progression. Current treatment have failed due to the multifactor etiology of OA. For instance, some patient present with high levels of inflammation while others do not. Making the molecular mechanism of OA poorly understood. High serum levels of interleukin-1β (IL1β) appears to be an important mediator for those with high inflammation.

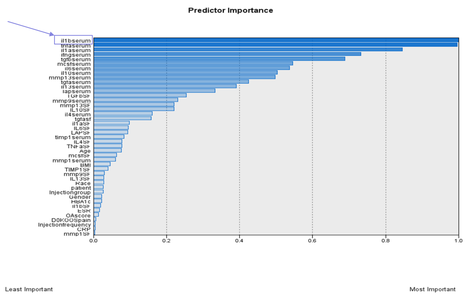


Figure 1. A predictive model of serum cytokines and the importance in osteoarthritis. Cluster analysis reveals two groups of patient. Those with high levels of inflammation and those without. High Serum IL1β has an important predictive values of OA for those clustered into the high inflammatory group

Previous studies have determined that IL1β is a cytokine that triggers the increase in the inflammatory pathway leading to the pathological changes commonly associated with osteoarthritis; cartilage degradation, subchondral bone changes, and thickening of the synovial membrane.

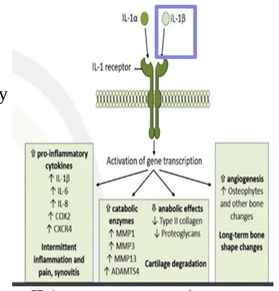


Figure 2. Downstream effects of Interleukin 1β (IL1-β) signaling in osteoarthritis progression. FX201 produces IL1ra under control of inflammatory promoters to block IL1-β binding to IL1 receptor, thus slowing the progression of osteoarthritis

In animal models, recombinant IL1 receptor antagonist (IL1ra) prevented cartilage breakdown and slowed the progression of OA [1-4]. This led to the development of Anakinra and recombinant form of IL1ra for use in humans. Anakinra was shown to safely reduce pain in humans. However, due to the short half life and short duration of joint space availability, Anakinra did not provided long term benefits when compared to a placebo[5]. To address the short comings of Anakinra, Flexion Therapeutics developed FX201, a helper-dependent adenovirus (HDad) vector to carry the coding sequence for IL1ra under the control of an inflammatory responsive promoter. Previous clinical trials proved to be variable.

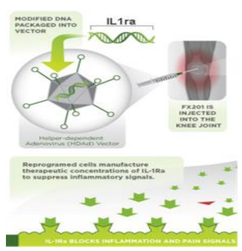


Figure 3. FX201 is a helper-dependent adenovirus vector that has been proven to release IL1ra under conditions of high inflammation conditions and is down regulated under low inflammatory conditions

The purpose of this study is to identify target patient populations for FX201 treatment. LSU Integrated Musculoskeletal Biobank (LIMB) has been collecting de-identified tissue samples from individuals with knee OA undergoing total knee arthroplasty (TKA). Our biorepository has previously clustered patient into two groups, high inflammatory patients and low inflammatory patient, using serum inflammatory profiles. In order to identify patient populations for treatment we are establishing synoviocyte cultures from patient's synovium, that have previously been frozen, for in vitro studies using FX201 while obtain pre and post treatment inflammatory profiles and comparing these values to our patient clusters. We hypothesis that we will be able to identify patient population for treatment with FX201.

Methods

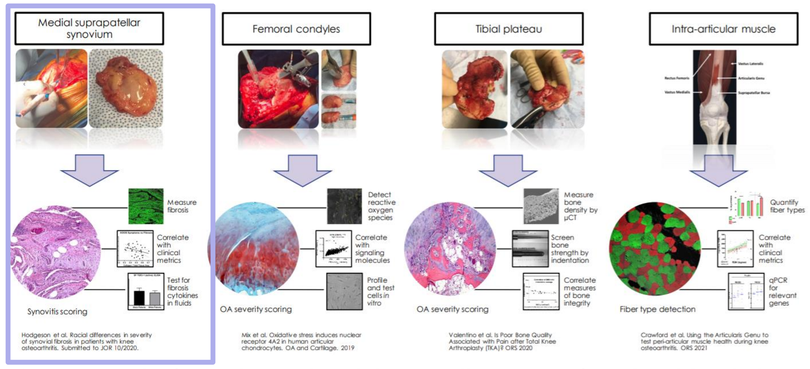


Figure 5. Example of tissues harvested (Medial suprapatellar synovium, femoral condyles, tibial plateau, and intra-articular muscle) during total knee arthroplasty and experiments conducted

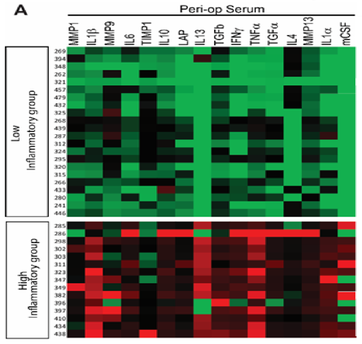
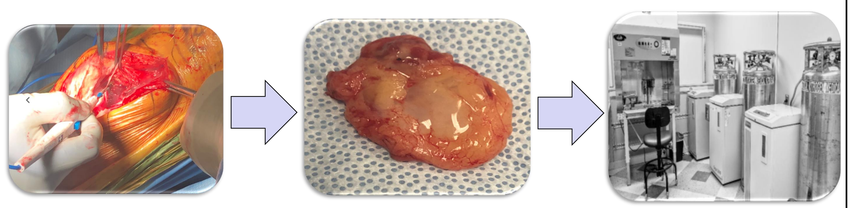
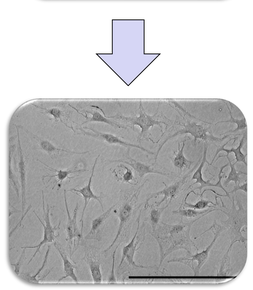


Figure 6 . Patients are clustered into two groups based on expression of cytokines. Patients are clustered according to patterns of circulating cytokines. Red= high levels of cytokines. Green= low levels of cytokines. Black=median levels of cytokines. Patients in high inflammation group have a concentration of IL1β>20.82 pg/ml.



Cell Culturing

Synovial tissue is harvested from patient during total knee replacement then processed for freezing in liquid nitrogen. Synovial tissue is thawed when ready to be used experimentally then placed in collagenase and for 90 minutes at 37ºC. The mixture is pipetted through a 70 µm mesh cell strainer into a fresh 50-mL conical tube. The strained cells are centrifuged at 1500 rpm for 10 minutes. The supernatant is removed and 1 mL Dulbecco's Modified Eagle Medium (DMEM), with , 10% FBS, 1% Zonker. 0.1% Amphotericin. Cells are then counted. 14 mL of DMEM is added to the 50 mL conical tube and the mixture is transferred to T75 flask and incubated at 37ºC and 5% CO_2



Current and Future Experiments

- Currently we are working on creating a standard operating procedure to verify synoviocytes were extracted from patient's synovium and are still viable to use for in vitro studies.
- In the future we will obtain an inflammatory profile from our synoviocytes cell cultures using a multiplex analysis containing 11 pro and anti-inflammatory cytokines, and 6 proteins involved in cartilage turnover.
- We will then treat synoviocytes with FX201 and measure cytokine release by the cells before and after infection.
- Quantitative PCR will be used to confirm decrease in cytokine production.
- ANOVA analysis will identify significant changes in fibrotic and inflammatory output after FX201 treatment.

Expected Results

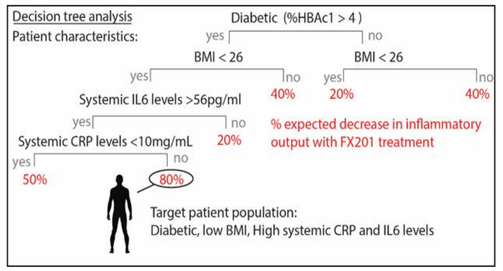


Figure 7. Example of expected output with completion of these aims. We will test the ability for FX201 to reduce inflammatory output and fibrotic output in patient synoviocytes and improve survival of patient chondrocytes. This data will be analyzed by decision tree analysis to identify patterns in patient characteristics that are linked to FX201 efficacy. This data can then be used to select patients for targeted clinical trials.

These results will be fed through a random forest decision tree analysis. Patterns in the characteristics of patients who show high or low response to FX201 treatment. We expect this map will be able to identify patients for clinical trial and to help speed up the development of this therapy

References

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