Introduction:
Lack of expression of (endogenous) IL-1β antagonizing molecules (IL-1RII and IL-1ra) in human chondrocytes using gene chip arrays. Alternatively, a ten fold higher expression of IL-1β mRNA was observed, using Real Time PCR, in Osteoarthritic (OA) cartilage as compared to normal cartilage. Functional genomic analysis of soluble (s) IL-1RII, at pico-nano gram concentrations, (but not soluble TNF receptor: Fc) significantly inhibited IL-1β induced nitric oxide (NO) and/or prostaglandin (PG) E2 production in chondrocytes, synovial and epithelial cells. In osteoarthritic, deficiency expression of innate antagonists regulators of IL-1β by chondrocytes such as IL-1ra and IL-1RII (soluble or membrane form) may allow the catabolic effects of IL-1β to proceed unopposed. The inhibition of IL-1β action by sIL-1RII has therapeutic implications that could be directed towards correcting this unfavorable tissue dependent imbalance. Therefore, we examined the antagonist role of IL-1 type II decoy receptor in chondrocytes using gene therapy approach.

Materials and Methods:
Procurement of Human cartilage. Cartilage slices were taken from the knees of patients with the diagnosis of advanced OA (age: 50-70 yr) who were undergoing knee replacement surgery. The use of discarded human cartilage was approved by appropriate institutional review boards and consents.

Chondrocyte culture in alginate beads. Chondrocytes were immobilized in alginate beads, as reported previously by Attur et al (2000).

Preparation of Adenoviral vectors. The full length human IL-1RII was sub-cloned from pFAST-BAC1 to pAd1/RSV vector at Hind III - Not I site. The adenoviral vector used in this is deleted of E1A and E1B and a portion of E3 region.

Transduction of various cell types, 10^3 moi/cell was used. Functional genomic analysis of soluble (s) IL-1RII, at pico-nano gram concentrations, (but not soluble TNF receptor: Fc) significantly inhibited IL-1β induced nitric oxide (NO) and/or prostaglandin (PG) E2 production in chondrocytes, synovial and epithelial cells. In osteoarthritic, deficiency expression of innate antagonists regulators of IL-1β by chondrocytes such as IL-1ra and IL-1RII (soluble or membrane form) may allow the catabolic effects of IL-1β to proceed unopposed. The inhibition of IL-1β action by sIL-1RII has therapeutic implications that could be directed towards correcting this unfavorable tissue dependent imbalance. Therefore, we examined the antagonist role of IL-1 type II decoy receptor in chondrocytes using gene therapy approach.

Discussion:
Recombinant IL-1RII by recombinant adenovirus in human chondrocytes, which lack IL-1RII led to the functional expression of both membrane and soluble IL-1RII proteins. Our studies suggest that IL-1RII cells were resistant to the insults of IL-1β with respect to the production of PGE2, NO, IL-6 and IL-8 which have been implicated in inflammation and cartilage destruction. Adenoviral mediated expression of IL-1RII decoy receptor was significantly effective in neutralizing the effects of IL-1β. IL-1RII chondrocytes (as a “modified cartisel approach”) may be significantly effective for cartilage repair, may protect against the insults of IL-1β. These experiments also give insight on the mechanism of action of IL-1RII in joint cells (Fig 1). The potent IL-1β neutralizing property may be due to the multifunctional effects of IL-1RII as compared to IL-1RI. IL-1RII inhibitory functions of IL-1RII has been demonstrated for the soluble and membrane form of the receptor. This study shows similar observations, where the presence of both soluble and membrane forms of IL-1RII significantly inhibited IL-1β effects versus the soluble form alone.

Results:
Transduction of chondrocytes with the AdMock virus demonstrated no detectable levels of IL-1RII in the medium or significant effect on the spontaneous production of PGE2. Transduction of these cells with adenovirus (AdRSVRII) [at moi of 100, 1,000 and 10,000] released sIL-1RII at a concentration of 60 ± 10, 594 ± 100 and 630 ± 70 pg/ml respectively in the supernatant. Stimulation of untransduced cells with IL-1β showed a significant augmentation in the production of PGE2 (5.2 ng/ml). Human chondrocytes transduced with AdRSVRII showed 50-60% inhibition of IL-1β induced PGE2 accumulation.

Specificity of IL-1RII. The ability of IL-1RII to neutralize the effects of IL-1β and TNFα was studied in chondrocytes. Primary chondrocytes were transduced with AdMock and AdRSVRII. The AdRSVRII transduced chondrocytes were resistant to the effects of IL-1β but not TNFα with respect to NO and PGE2 production which is indicative of the specificity of IL-1RII expressed.

Regulation of Proteoglycan synthesis in IL-1RII transduced chondrocytes. Earlier studies have shown that IL-1β, NO and PGE2 influence matrix homeostasis either directly or indirectly in cartilage. Chondrocytes in alginate beads (to maintain their dedifferentiated phenotype) were transduced with AdMock and AdRSVRII. In the presence of IL-1β, the AdRSVRII transduced cells expressed similar amounts of NO (~ 5 uM) and PGE2 (~2.2 ng/ml) as in monolayer culture. Additionally, a significant decrease in the amount of 35S incorporation into proteoglycan was observed in AdRSVRII transduced as compared to the control.

Regulation of IL-1β mRNA by IL-1RII. Production of inflammatory mediators such as NO, PGE2, IL-6 and MPPs in chondrocytes are known to be induced by autocrine IL-1β. IL-1β (10 ng/ml) augmented IL-1β mRNA both in AdMock and untransduced cells but in IL-1RII transduced chondrocytes, the augmentation was 70% less as compared to AdMock and control cells. Thereby, suggesting that transient expression of sIL-1RII not only “sponges” the IL-1, but the IL-1RII inhibits signal transduction and subsequent induction of IL-1β mRNA.

Transplantation of autologous IL-1RII+ chondrocytes on human cartilage in vitro. Previous studies have shown that autologous transplantation of chondrocytes in vivo and in vitro has a significant effect on cartilage repair. Human OA-affected cartilage obtained from surgery was divided into two parts. Part of it was used to isolate chondrocytes and transduced them with AdMock or AdRSVRII and the other piece of cartilage was incubated in F-12 medium. The IL-1RII+ chondrocytes were then transplanted on cartilage chips. Cartilage transplanted with IL-1RII+ cell showed spontaneous production of NO and PGE2, which could be augmented by IL-1β, whereas chips transplanted with IL-1RII+ cells showed inhibition of spontaneous and IL-1β production of NO and PGE2.

Discussion:
Recombinant IL-1RII by recombinant adenovirus in human chondrocytes, which lack IL-1RII led to the functional expression of both membrane and soluble IL-1RII proteins. Our studies suggest that IL-1RII cells were resistant to the insults of IL-1β with respect to the production of PGE2, NO, IL-6 and IL-8 which have been implicated in inflammation and cartilage destruction. Adenoviral mediated expression of IL-1RII decoy receptor was significantly effective in neutralizing the effects of IL-1β. IL-1RII chondrocytes (as a “modified cartisel approach”) may be significantly effective for cartilage repair, may protect against the insults of IL-1β. These experiments also give insight on the mechanism of action of IL-1RII in joint cells (Fig 1). The potent IL-1β neutralizing property may be due to the multifunctional effects of IL-1RII as compared to IL-1RI. IL-1RII inhibitory functions of IL-1RII has been demonstrated for the soluble and membrane form of the receptor. This study shows similar observations, where the presence of both soluble and membrane forms of IL-1RII significantly inhibited IL-1β effects versus the soluble form alone.