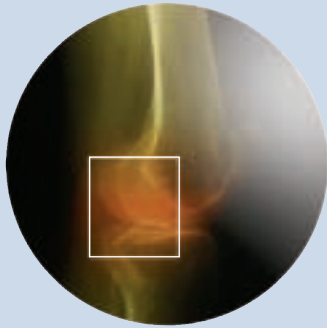


# Arthritis Biomarkers

## CARTILAGE SYNTHESIS AND DEGRADATION



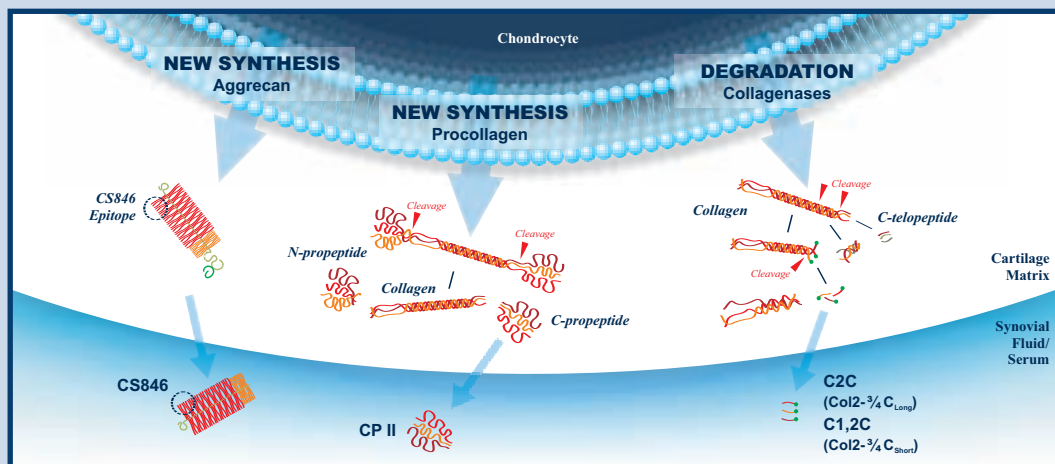
Osteoarthritis



Rheumatoid Arthritis



The change in matrix composition in arthritic joints is due to abnormal matrix turnover. IBEX biomarker assays accurately measure both type II collagen and aggrecan synthesis and degradation by detecting cartilage matrix fragments in blood, synovial fluid and urine. These biomarkers provide a timely and direct measure of the disease process, are important in developing new treatments and in assessing their effectiveness.



# RA *In vivo* Studies (selected)

## Animal Studies

**Study A** Zack *et al.* (2003): Inhibition of C2C production correlated with reduced destruction of joint cartilage in response to combination therapy with anti-IL-1 and anti-TNF, suggesting that this biomarker can be used to evaluate the benefit of therapeutic intervention as demonstrated in Figures A and B below.

Figure A  
Histology Cartilage Score

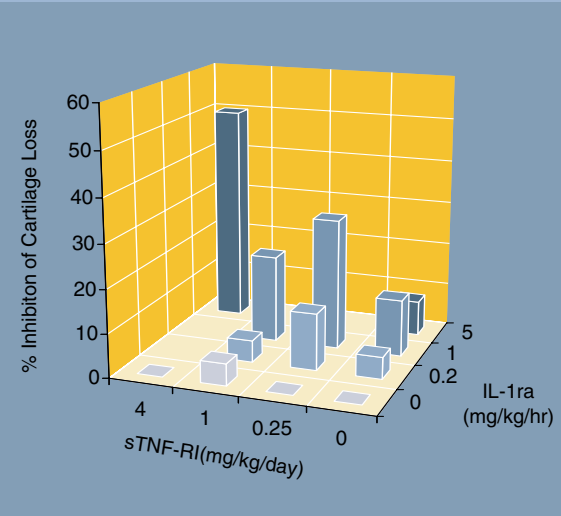
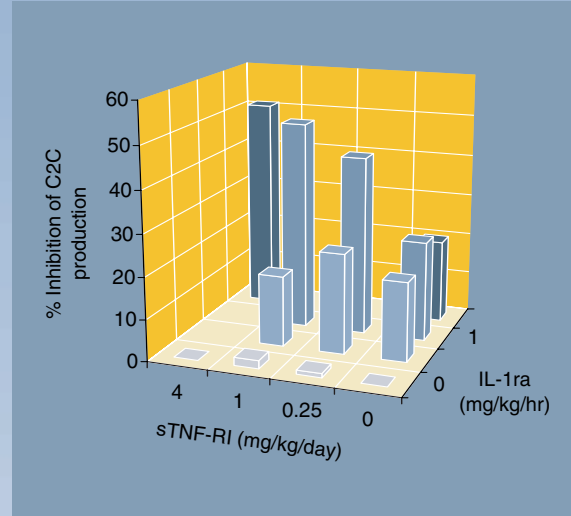


Figure B  
Inhibition of C2C Production

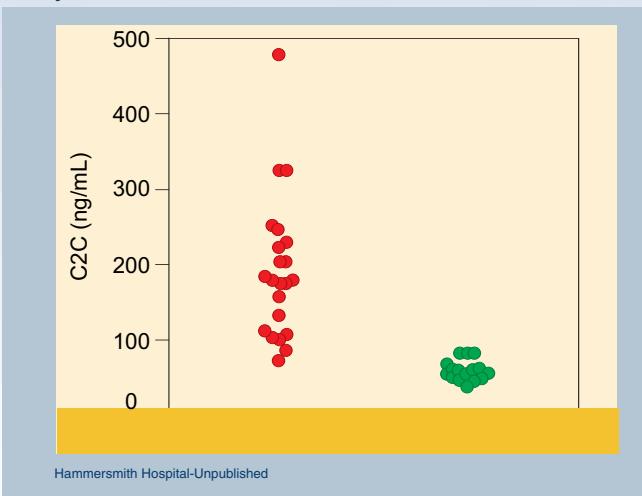


## Human Studies

**Study A** (unpublished data): The C2C epitope also showed a high correlation to early RA vs. normals in a study conducted at Hammersmith Hospital (See figure C below).

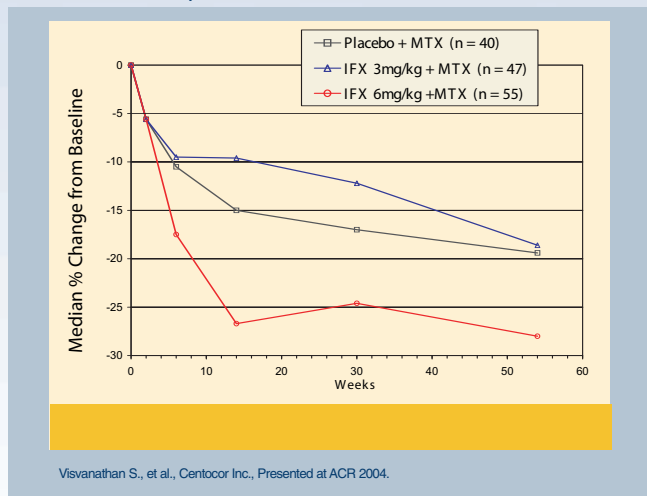
**Study B** Visvanathan *et al.* (ASPIRE trial) demonstrated that combination therapy with Infliximab (IFX) and Methotrexate (MTX) resulted in the reduction of C2C in serum, which correlated with improved symptoms (See figure D below).

Figure C  
Early RA vs. Normals



Hammersmith Hospital-Unpublished

Figure D  
Serum C2C Response to Treatment in RA Patients

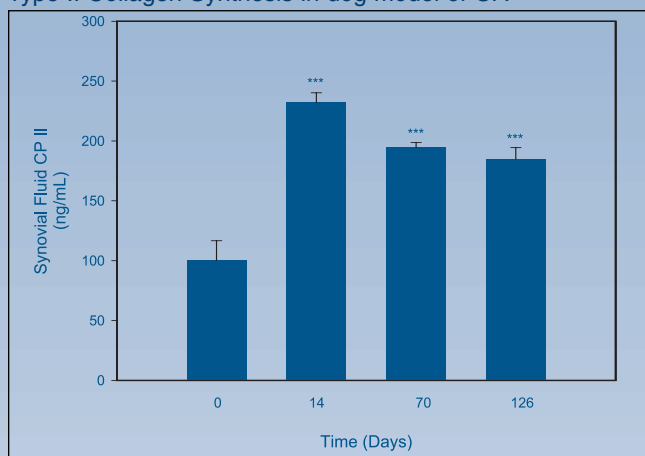


Visvanathan S., et al., Centocor Inc., Presented at ACR 2004.

## Animal Studies

**Study A:** As shown in a dog CCL preclinical model of osteoarthritis, CPII analysis in the synovial fluid (SF) demonstrates that type II collagen synthesis significantly increases above baseline after 14 days in an attempt at repair (Trumble *et al.*, 2003; see figure A below).

Figure A  
Type II Collagen Synthesis in dog model of OA



\*\*\*p<0.001  
(Trumble *et al.*, 2003)

**Study B:** In a dog ACL section model of osteoarthritis, serum and urine elevations of the C2C epitope are seen at 12 weeks when early damage is observed. This is also reflected in an earlier elevation of the CS846 seen in serum at 3 and 12 weeks after surgery. This reveals a very sensitive response to early injury (Matyas *et al.*, 2003; see figure B below).

Figure B  
Dog model of OA

Marker	Number of animals	Time of necropsy	Pre-operative (baseline value ng/mL)	Necropsy (value ng/mL)
CS846	6	3 weeks	0.129 ± 0.049	0.248 ± 0.050 †
CS846	8	12 weeks	0.062 ± 0.049	0.169 ± 0.028 ‡
C2C	10	3 weeks	74.7 ± 7.7	72.8 ± 11.7 ‡
C2C	9	12 weeks	77.1 ± 7.4	93.1 ± 11.2 †

‡ p<0.01 †p<0.05  
(Matyas *et al.*, 2003)

## Human Studies

**Study A:** Sharma *et al.* (2004), demonstrated a relationship between the ratio of degradation to synthesis and OA progression. The following table clearly indicates that the predictive value of the ratios surpasses that of the individual markers alone, reinforcing the concept that it is not the absolute level of synthesis or degradation that influences progression but rather the balance between the two phenomena (See figure C below).

Figure C  
Early OA vs. Normals  
Prediction of OA progression

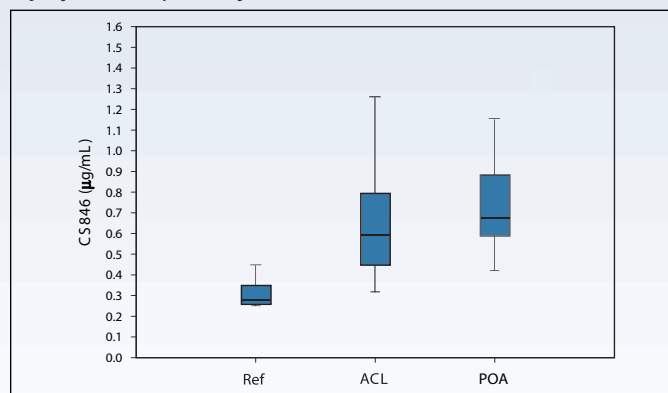
Marker	Odds Ratio	(95% CI) for Progression
C2C: CP II	3.15	(0.91, 10.85)
C1,2C: CP II	1.79	(0.87, 3.69)
C2C	1.00	(0.996, 1.011)
C1,2C	1.00	(0.995, 1.005)
CP II	1.00	(0.997, 1.002)

Odds Ratio for each unit of marker increase. Figure adjusted for age, gender, BMI & baseline disease severity.

(Sharma *et al.*, 2004)

**Study B:** Lohmander *et al.* (1999) showed that activity of the CS846 epitope was increased in all groups compared with the healthy knee reference group and was highest in the primary OA group, as observed in the box and whisker plot below. OA is thus a disease characterized by dynamic changes in tissue macromolecule turnover, which is reflected by measurable changes in aggrecan epitopes in the synovial fluids (See figure D below).

Figure D  
CS846 in joint fluids after injury and in primary osteoarthritis



(Lohmander *et al.*, 1999)

# IBEX Assays

## CARTILAGE SYNTHESIS ASSAYS

### CP II - Procollagen II C-Propeptide

Type II collagen is synthesized as procollagen which contains amino and carboxy propeptides. These are removed extracellularly by amino and carboxy proteases as collagen is incorporated into the fibril. CPII content is directly related to type II collagen synthesis. The CPII assay measures carboxy propeptides released during the formation of type II collagen.

### CS846 - Aggrecan Chondroitin Sulfate 846 Epitope

In arthritic joints the collagen matrix is disrupted, leading to new synthesis and degradation of a fetal form of aggrecan containing the CS846 epitope. Turnover of aggrecan in osteoarthritis releases the CS846 epitope into the bloodstream. In normal adult serum, the concentration of this epitope is very low. The CS846 assay specifically measures this epitope.

## CARTILAGE DEGRADATION ASSAYS

### C2C - Collagen Type II Cleavage

Joint cartilage is composed of a type II collagen-based fibrillar network complexed to proteoglycans. In arthritis, type II collagen is extensively cleaved and destroyed by the activity of collagenases, namely MMP-1, MMP-8 and MMP-13, and serum levels of the cleavage products are increased. The C2C assay measures a neoepitope created by the cleavage of type II collagen by collagenases. This neoepitope is at the C terminus of the 3/4 length type II collagen cleavage product.

### C1,2C - Collagen Type I and II Cleavage

As with the C2C epitope, the C1,2C antibody detects collagen cleavage products. This assay measures the carboxy terminus of the peptide (C1,2C or Col 2 3/4C Short) generated by cleavage of types I and II collagens by MMP-1, MMP-8 and MMP-13 collagenases.

## Characteristics and Utility of IBEX Biomarker Assays

### Characteristics

Assay type and format	Competitive ELISA in a 96-well plate
Sample type	Serum*
Sample size	10-50 µL
Turnaround time	3-4 hours (depending on the assay)

\* Some assays can be used in synovial fluid, plasma, urine, bronchoalveolar lavage, cartilage extracts, culture media.

### Utility

	CP II	CS846	C2C	C1,2C
Human	•	•	•	•
Monkey	•	•	•	•
Dog	•	•	•	•
Rabbit	•	•	•	•
Rat	•	•	•	•
Mouse	•	•	•	•
Guinea pig	•	•	•	•
Horse	•	•	•	•
Cow	•	•	•	•

• potential utility