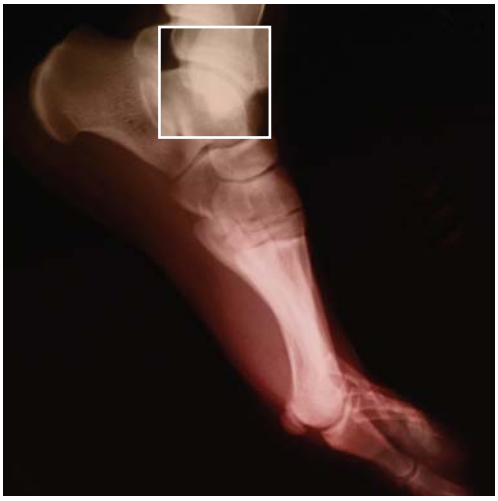
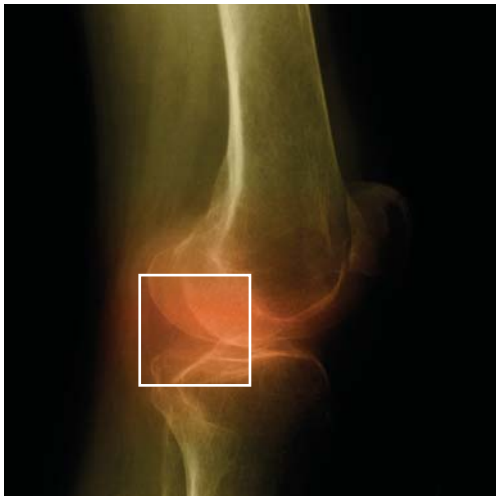
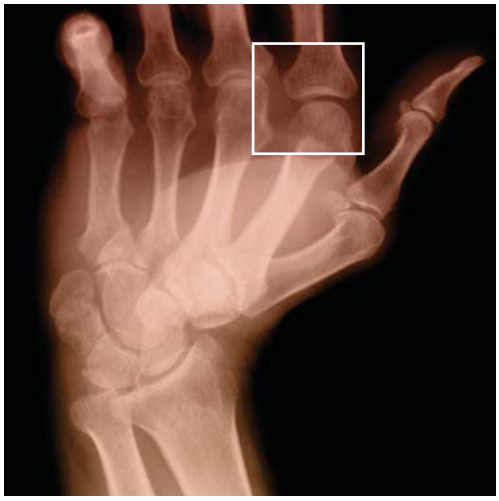




RA
BIOMARKERS

Leading-edge Rheumatoid Arthritis biomarkers accelerating decision-making and reducing the cost of pre-clinical and clinical research.

Preclinical Research:

- Assist in the design of better animal studies of RA
- Increase sensitivity and predictive power in preclinical RA studies
- Reduce time to completion of studies

Clinical Research:

- Assist in the early diagnosis of RA and better disease characterization
- Complement the data from imaging techniques
- Increase sensitivity and predictive power in RA clinical trials
- Help identify patients who are at a high risk for disease progression
- Monitor patient response to drug therapy and define drug efficiency
- Reduce time to completion of clinical trials and accelerate time to market

IBEX

IBEX biomarkers represent powerful tools providing a direct means for investigating joint damage and better characterizing rheumatoid arthritis

Utility of IBEX biomarkers

- Early detection of RA
- Determine the rate of disease progression
- Rapidly evaluate effectiveness of therapeutic intervention
- Identify effective drug candidates in animals (preclinical studies) and human (clinical studies)

The IBEX approach specifically measures cartilage metabolism, which is altered in arthritis patients.

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 critical in diagnosing and monitoring RA, in developing new treatments, and in assessing their effectiveness.

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 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 RA 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.

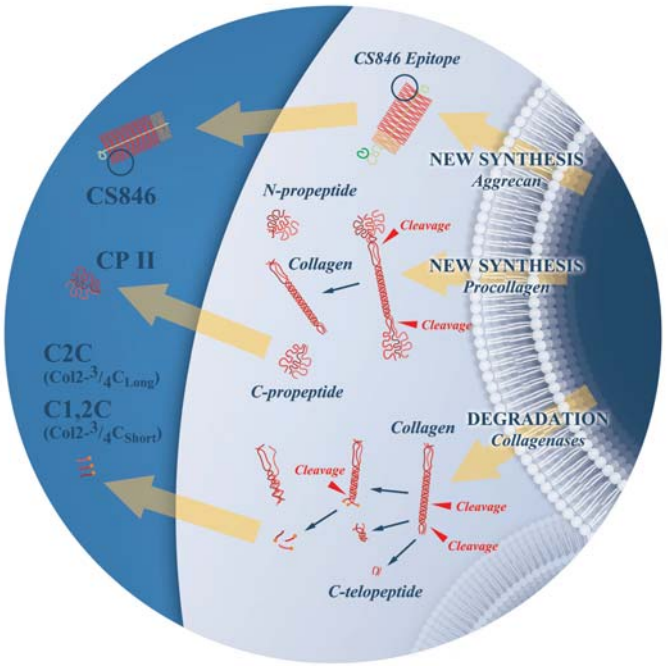
Cartilage Degradation Assays

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.

Cl,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 the MMP-1, MMP-8 and MMP-13 collagenases.



Biomarkers & Outcome in RA *In Vivo* Studies

Animal Studies

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 (Zack et al., 2003).

Figure A
Histology Cartilage Score

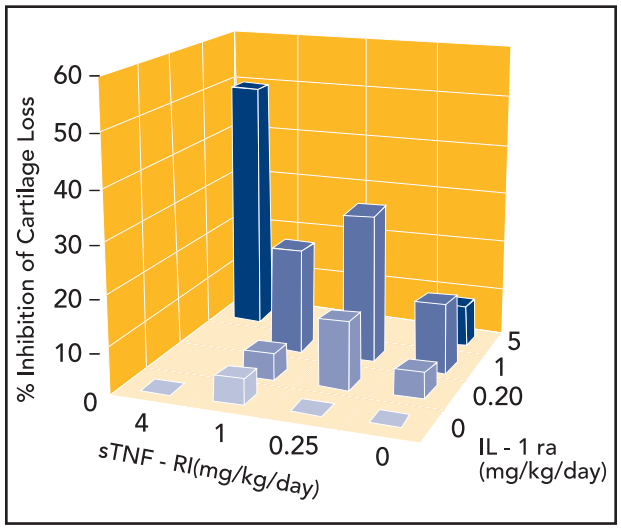
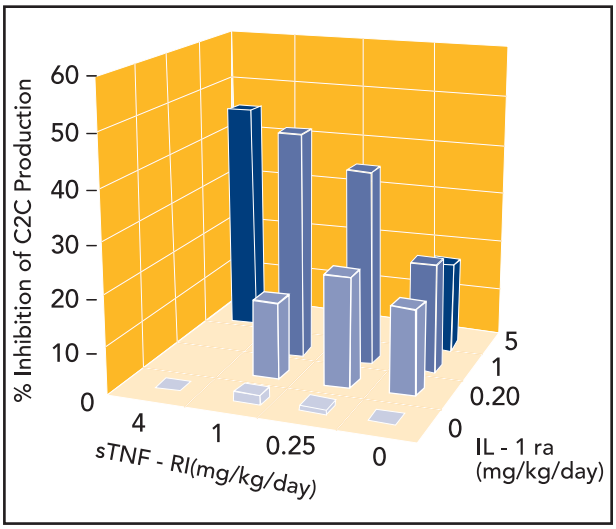


Figure B
Inhibition of C2C Production

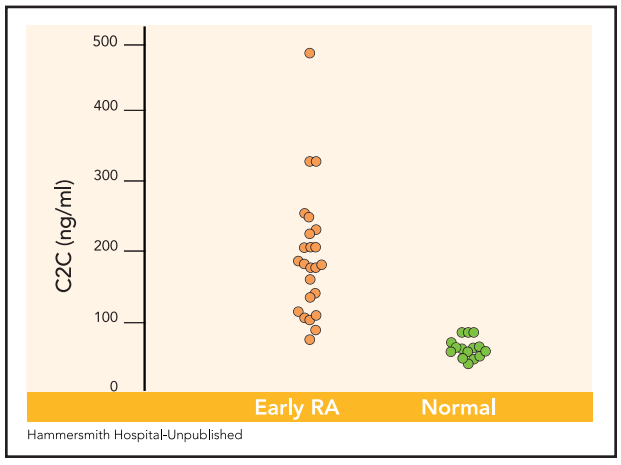


Human Studies

The C2C epitope also showed a high correlation to early RA vs. normals in a study conducted at Hammersmith Hospital (unpublished data), see Figure C below.

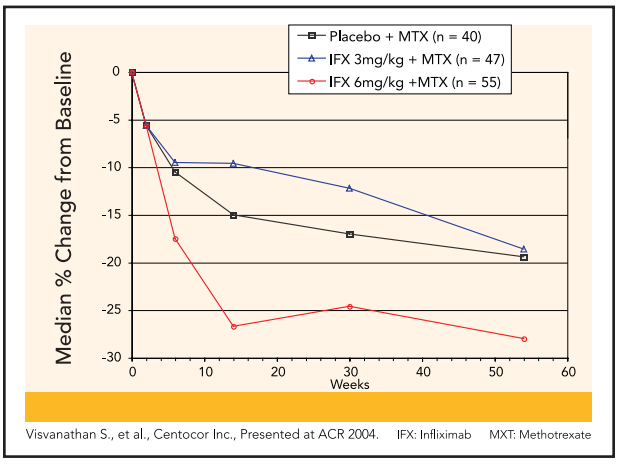
Visvanathan et al. (ASPIRE trial) demonstrated that combination therapy with infliximab and methotrexate resulted in the reduction of C2C in serum, which correlated with improved symptoms. See Figure D below.

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. IFX: Infliximab MXT: Methotrexate

See reverse for additional data supporting the use of biomarkers in animals and humans.

Utility of Ibex Arthritis Assays

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

• validated * potential utility

Biomarkers & Outcome in RA *In Vivo* Studies

Animal Studies

- In a rat model for RA, C2C concentration is elevated in serum at onset and C2C concentration correlates with disease severity and reflects efficacy of treatment (Song et al., 1999).

Human Studies

- The CS846 epitope is increased in arthritis patients. In RA, the epitope is elevated in serum in chronic disease and depressed in rapid progressive disease (Mansson et al., 1995).
- CPII content is directly correlated to type II collagen synthesis (Nelson et al., 1998).
- The increase in serum CP II in RA (Nelson et al., 1998; Mansson et al., 1995) and decrease in serum CP II in OA (Nelson et al., 1998) support that measurement of serum CP II can be useful in the diagnosis of RA, distinguishing RA from OA in humans.
- The ratio of C2C/CPII, appears to a good predictor of early responses to anti-TNF therapy (Mullan et al., 2004).
- The relationship of biomarkers to clinical outcome in a group of 62 RA patients under various treatment regimens demonstrated that changes in C2C/CP II ratio from time 0 to 1 month significantly correlated to a disease activity score at 3 months (Mullan et al., 2004).
- Baseline marker C2C/CPII ratio was shown to significantly correlate to RA disease progression (Verstapen et al., 2004).
- RA responders to Enbrel treatment were shown to have lower C2C and elevated CS846 epitope levels (Maksymowych et al., 2004).

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