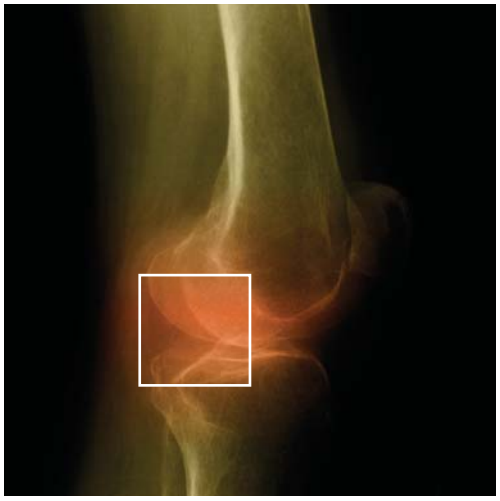




OA BIOMARKERS

Leading-edge Osteoarthritis 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 OA
- Increase sensitivity and predictive power in preclinical OA studies
- Reduce time to completion of studies

Clinical Research:

- Assist in earlier diagnosis of OA and better disease characterization
- Complement data from imaging techniques
- Increase sensitivity and predictive power in OA 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 has pioneered the development and utilization of OA molecular biomarkers based on cartilage matrix turnover.

Utility of IBEX biomarkers

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

IBEX biomarkers accurately measure collagen and proteoglycan synthesis and degradation allowing for timely evaluation of the imbalance of both phenomena as a reliable indicator of early OA and disease progression.

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 OA, 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 OA 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.

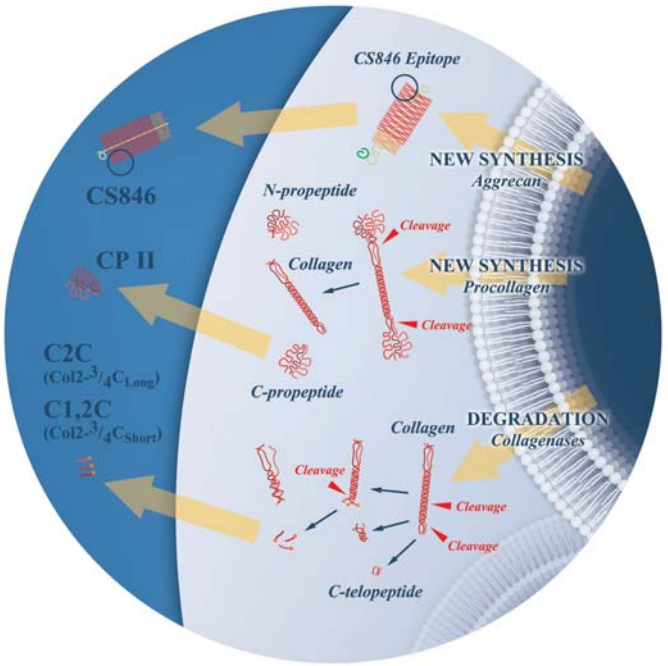
Cartilage Degradation Assays

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

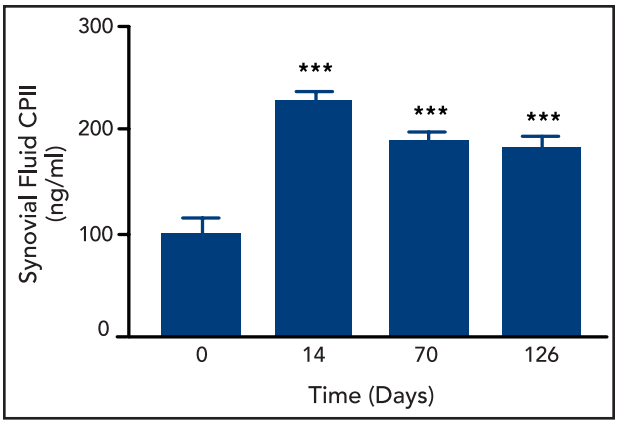
As with the C2C epitope, the C1,2C antibody detects collagen cleavage products. This assay measures the carboxy terminus of the peptide (C1,2C or Col 2 3/4C Short) generated by cleavage of types I and II collagens by the MMP-1, MMP-8 and MMP-13 collagenases.



Animal Studies

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 at al., 2003). See Figure A below.

Figure A
Type II Collagen Synthesis in dog model of OA



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

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 ± 0.010	0.169 ± 0.028 ‡
C2C	10	3 weeks	41.7 ± 13.4	61.0 ± 12.4 ‡
C2C	9	12 weeks	47.9 ± 7.6	81.2 ± 15.2 †

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

Human Studies

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.

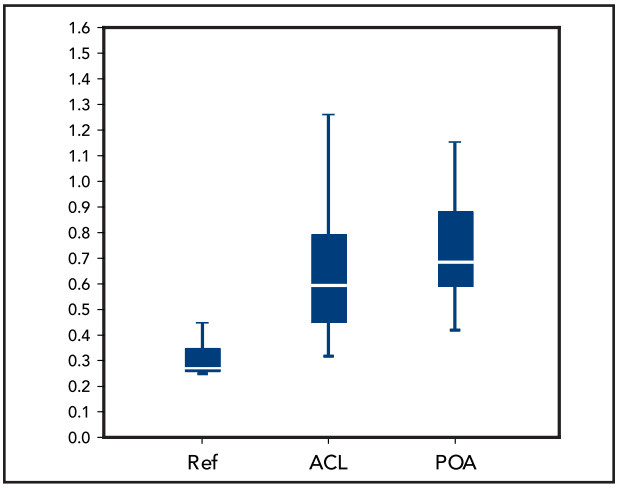
Figure C
Early RA vs. Normals

Marker	Odds Ratio for joint space grade progression (95% CI), adjusted for age, gender, BMI & baseline disease severity.	
	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)
CPII	1.00	(0.997, 1.002)

Odds ratio for each unit of marker increase. Figures adjusted for age, gender, BMI and baseline disease severity.
(Sharma et al., 2004)

Reactivity 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 macro-molecule turnover, which is reflected by measureable changes in aggrecan epitopes in the synovial fluids (Lohmander et al., 1999), see Figure D below.

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



(Lohmander et al., 1999)

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 OA *In Vivo* Studies

Animal Studies

The following studies also support the impact of measuring cartilage synthesis and degradation biomarkers as an indication of OA outcome.

- In a single stifle knee joint following ACL section (rabbit), C2C and CI,2C epitopes are elevated in synovial fluid and this precedes more severe cartilage damage (Laverty et al., unpublished).
- CS846 is elevated in synovial fluid of rabbits following ACL section (Poole & Killar, unpublished).

Human Studies

Other human studies also validate the use of serum biomarkers for evaluating the outcome of OA.

- The increased levels of CPII in SF may reflect an increased rate of synthesis of collagen II in the joint cartilage of patients with knee injury and developing OA. The increase reaches a maximum well before radiographic changes indicative of OA become apparent (Lohmander et al., 1999).
- CPII increases in SF in primary OA (Nelson et al., 1998).
- A combination of hsCRP and CS846 correlated well with early knee OA (Fortin et al., ACR Abstract, 2003).
- C2C/C1,2C ratio is predictive of OA progression (Cerejo et al., ACR Abstract, 2004).
- Serum biomarkers of degradation of cartilage are useful in predicting the development of radiographic knee osteoarthritis (Jordan et al., ACR Abstract, 2004).

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