Biomarkers of Osteoarthritis

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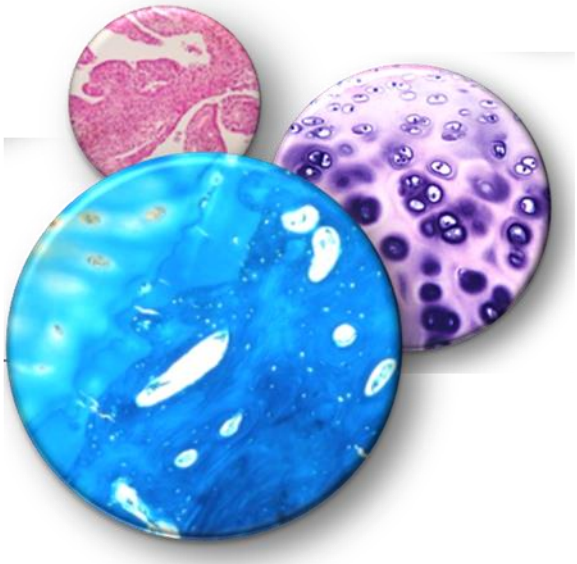
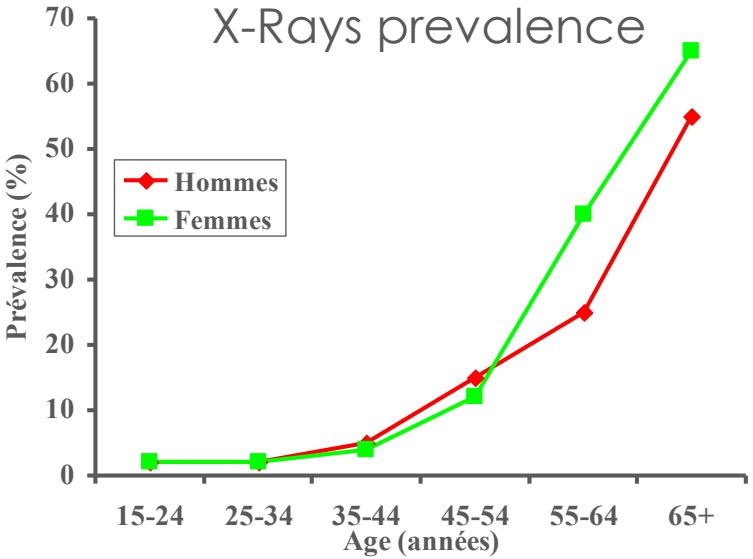
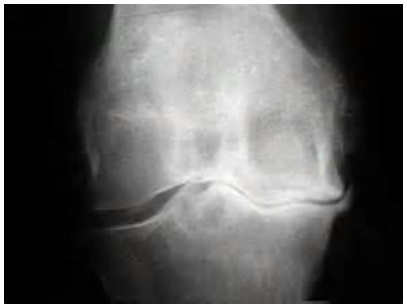


Université de Liège

ARTHROPÔLE LIÈGE

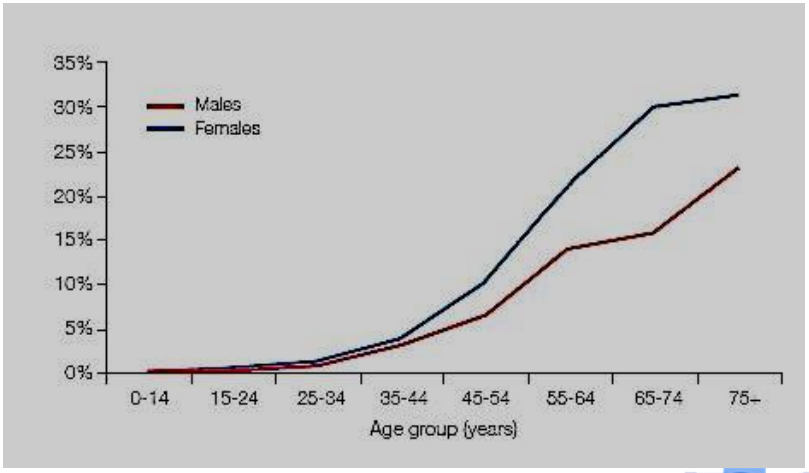
Osteoathritis: an ageing-related disease





A 65 ans, 30% des sujets ont une arthrose invalidante

Symptoms prevalence

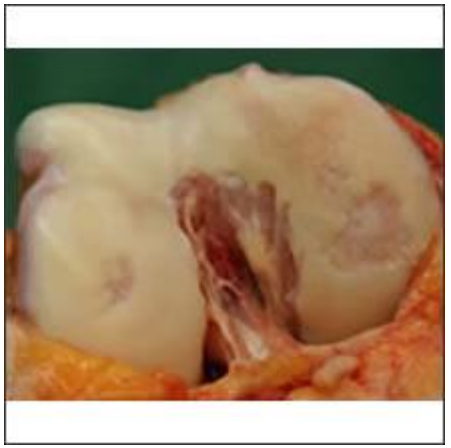


Osteoarthritis an «old disease»

Local mechanical disease

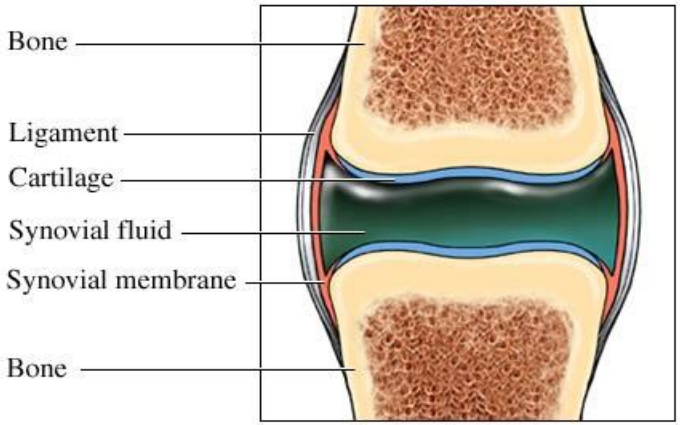
Overloading
Overuse
Joint instability
Malignancy

Cartilage degradation

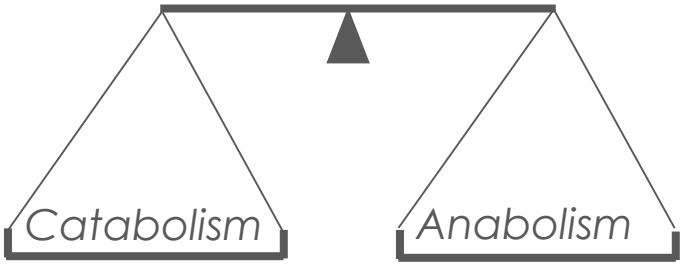


Adysregulation of chondrocyte metabolism

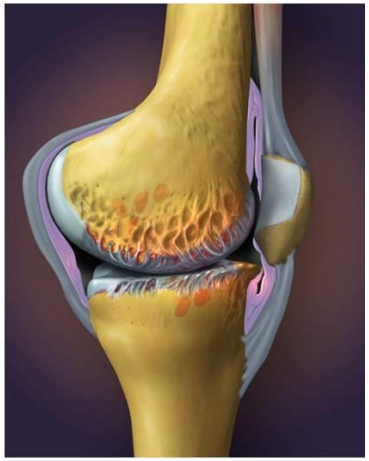
Healthy

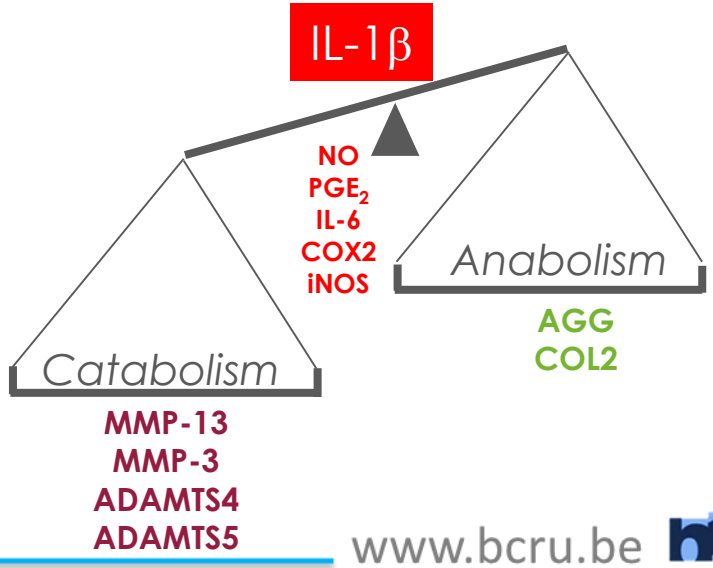


Cross section of a healthy joint

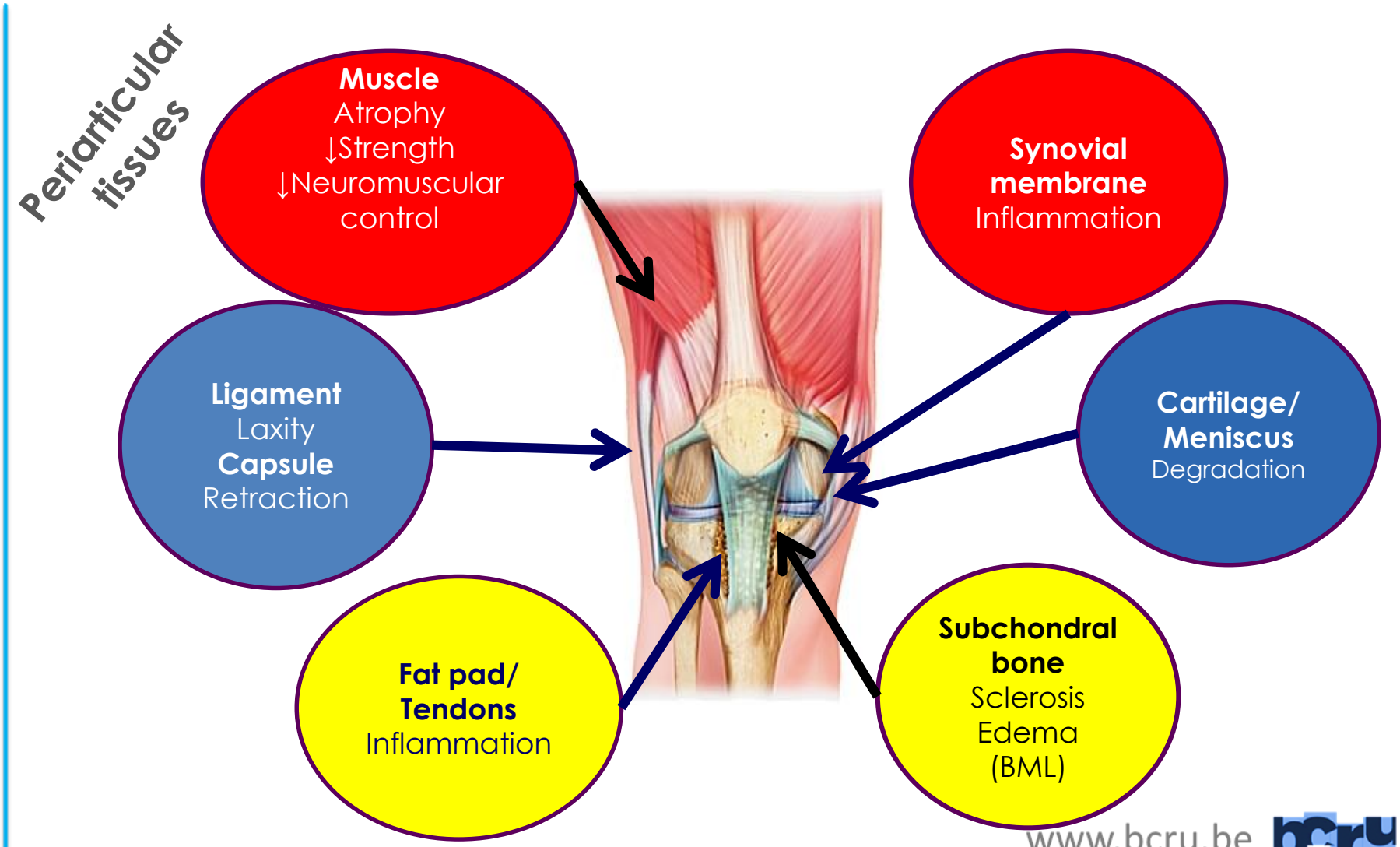


osteoarthritis



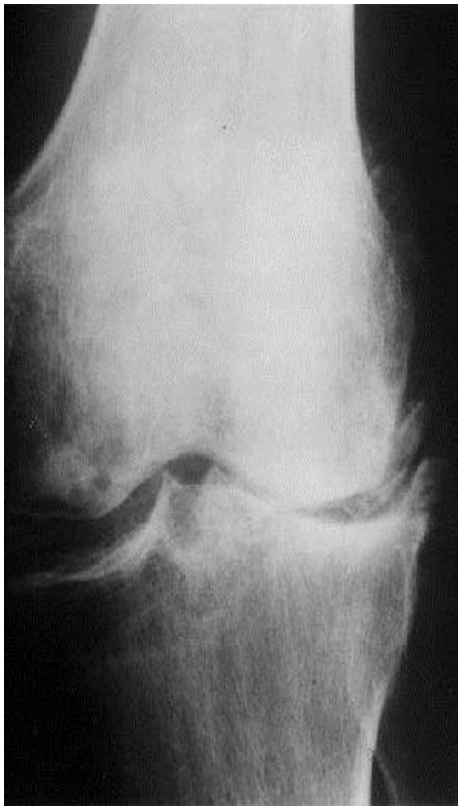


OA affects the whole joint and surrounding tissue



OA diagnosis : symptoms and standard radiography

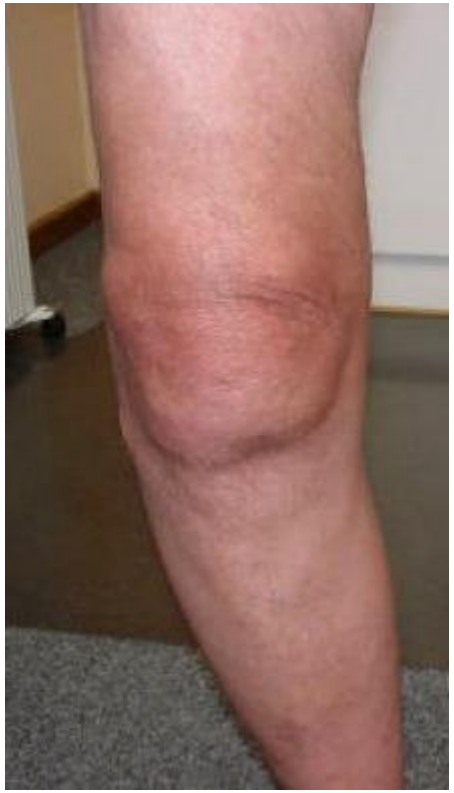
X-ray



Osteophytes
Joint space narrowing
Bone sclerosis
Attrition
Geodes

Symptoms

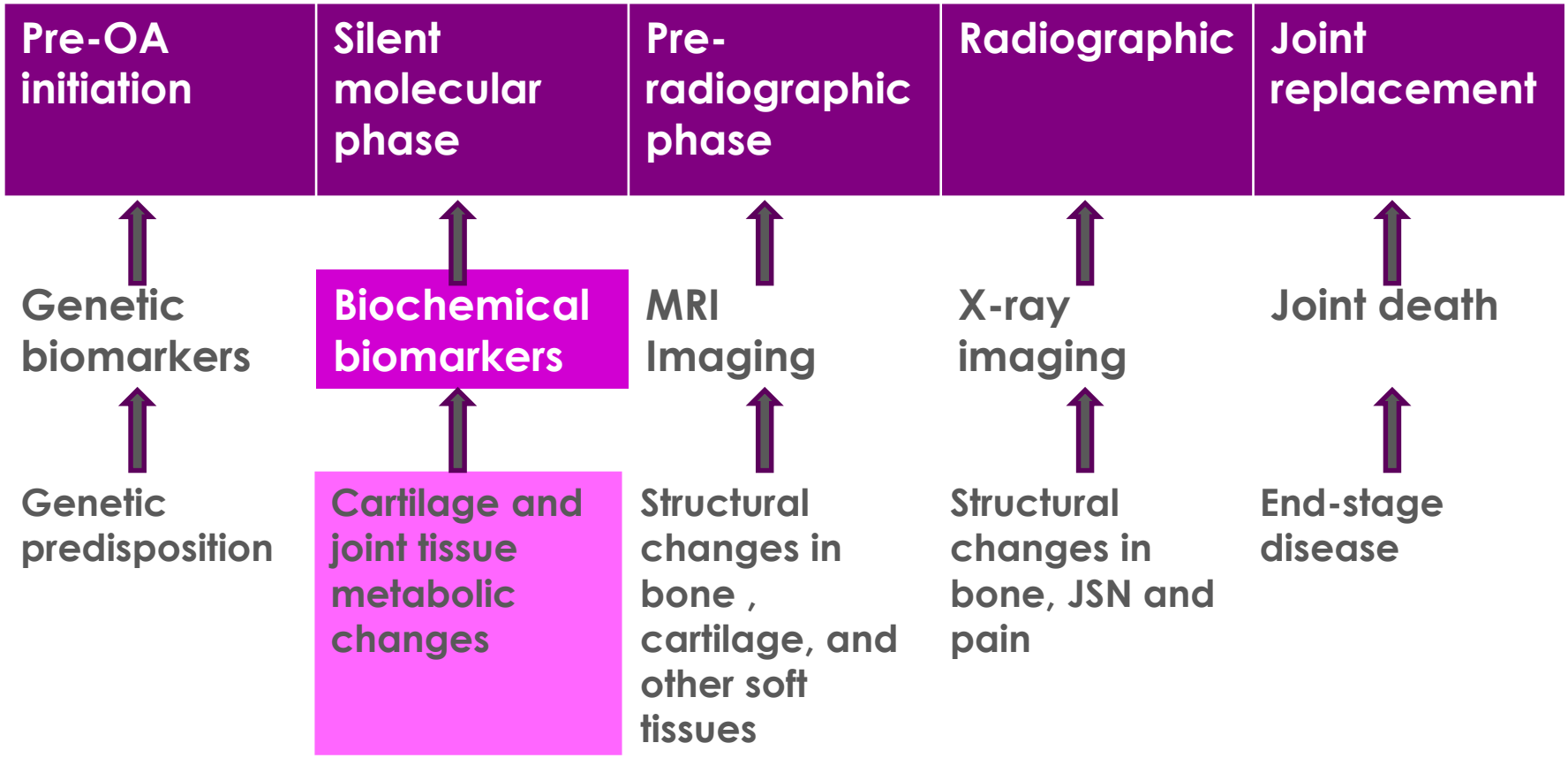
Pain
Stiffness
Swelling
Cracks
Deformity
Malalingment



These signs and symptoms occur in the late stage of the disease

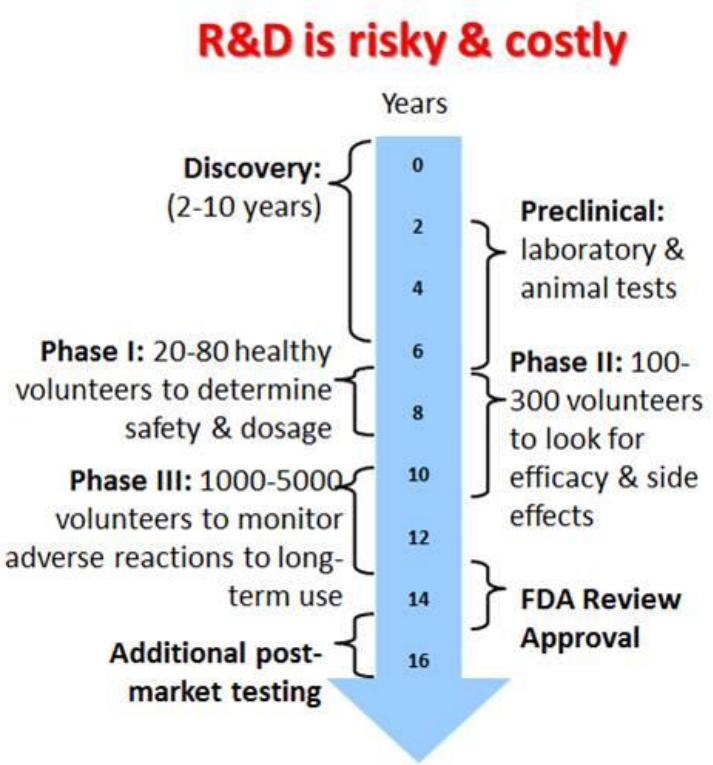
Radiographic and clinical signs are preceeded by a silent molecular phase

(D Patra & L Sandell, J Knee Surg, 2011)



...To diagnose the disease at the silent molecular phase

Drug discovery is protracted, risky and costly



Nothing new to offer at the patients and the OA research community

Clinical trials end-point

- **Symptoms modification** (3 to 6 months)

Pain

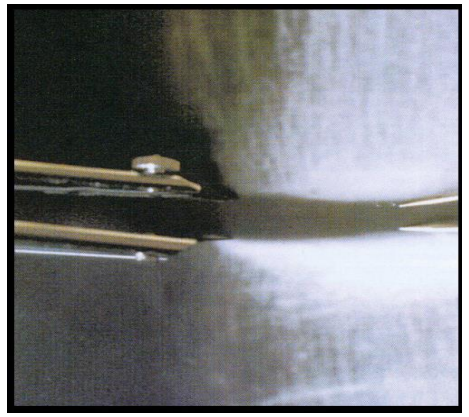
Physical function

Patient global assessment

- **Structure modification** (1 to 3 years)

Imaging outcomes

Joint Space Narrowing



The main limitations of JSN

- Indirect measure of the alteration in articular cartilage.
- Fails to measure a dynamic process
- Confounded by the presence of meniscal lesions and extrusion.
- Changes overtime are small, and occur in only a subset (progressors) of patients.
- Poorly reproducible (full extension).
- Poorly correlated with joint function and pain.

Why do we need of biological markers?

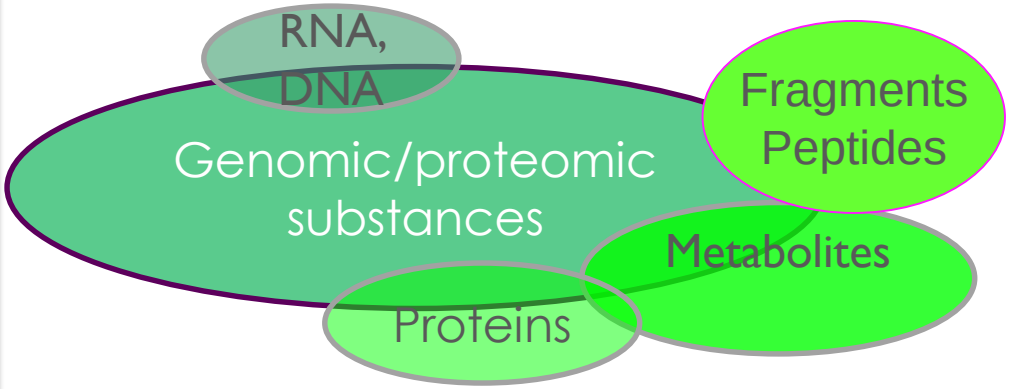
- To enrich our understanding of OA pathogenesis
- To detect early OA
- To discriminate progressor and non progressor
- To monitor progression of OA and efficacy of treatment
- To surrogate clinical end-point
- To decrease the length and cost of trials

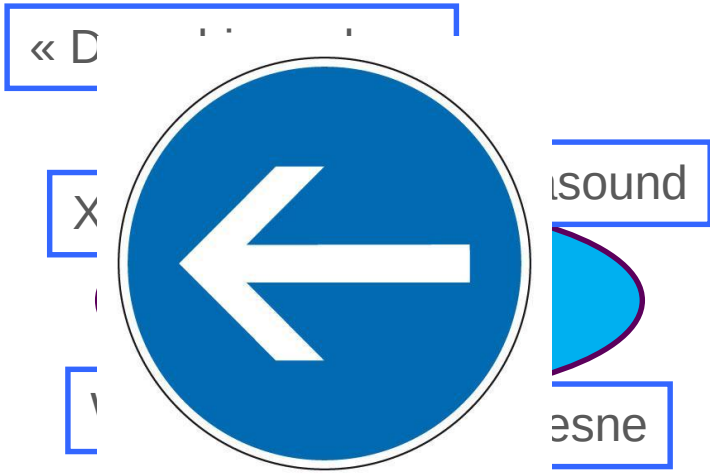
Definition - Classification

A biomarker is a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention. »

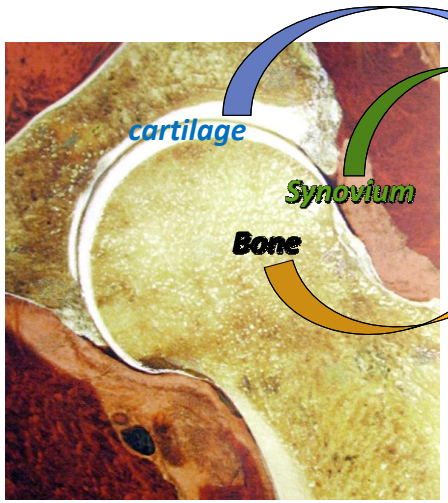
Biomarkers Definitions Working Group I. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther 2001; 69: 89-95.

Soluble or « wet » biomarkers





OA Biomarker candidates



lymphatics

Blood
~5 liters

kidney

urines

bone
- *sPINP*
- *uCTX-I*

Cartilage
- *sColl2-1*
- *sFibulin3*
- *sCOMP*
- *sYKL40*
- *sC2C*
- *uCTX-II*
- *PIIANP*
- *CPII*
- *Agrec-1*

Synovium & inflammation
- *sColl2-1NO2*
- *sPIIINP*
- *sHA*
- *sCRP ultrasensible*
- *Cytokines, MPO*

Metalloproteases
- *sMMP1(collagenase)*
- *sMMP3 (stromelysin*

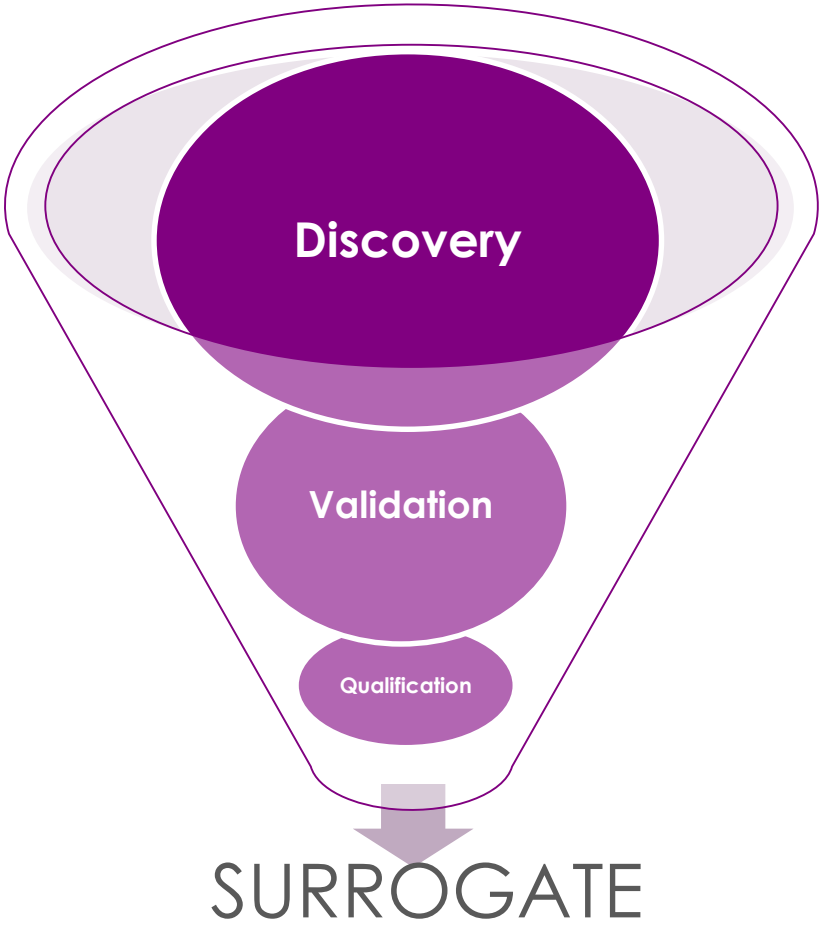
The long and winding road…

Process of soluble biomarkers development

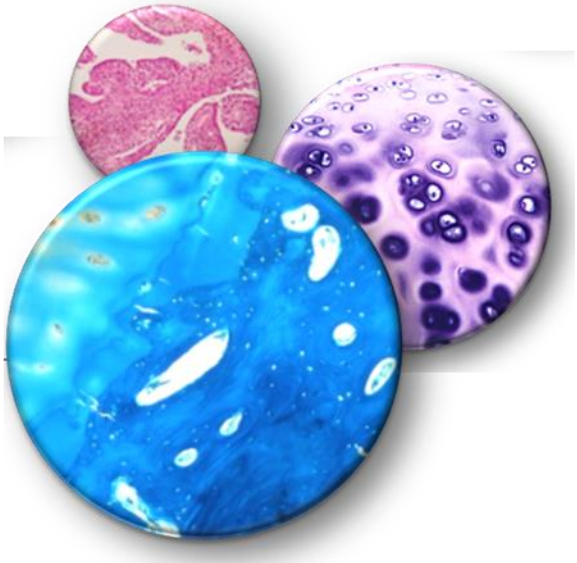
Brain/OMICS technics

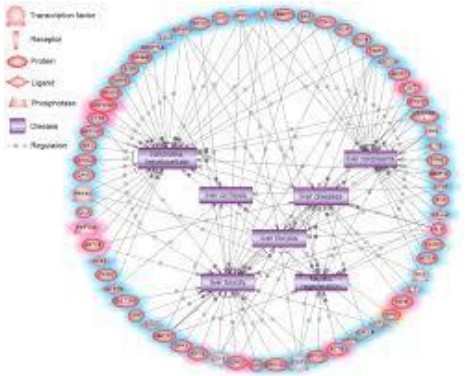
Analytical performance of assay

Assessing clinical value



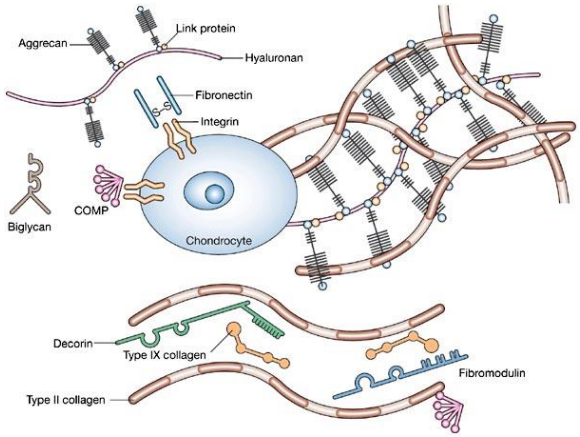
Discovery

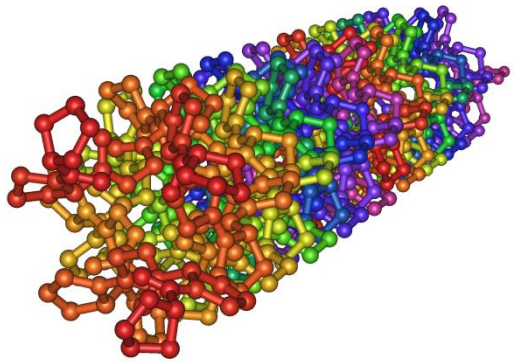




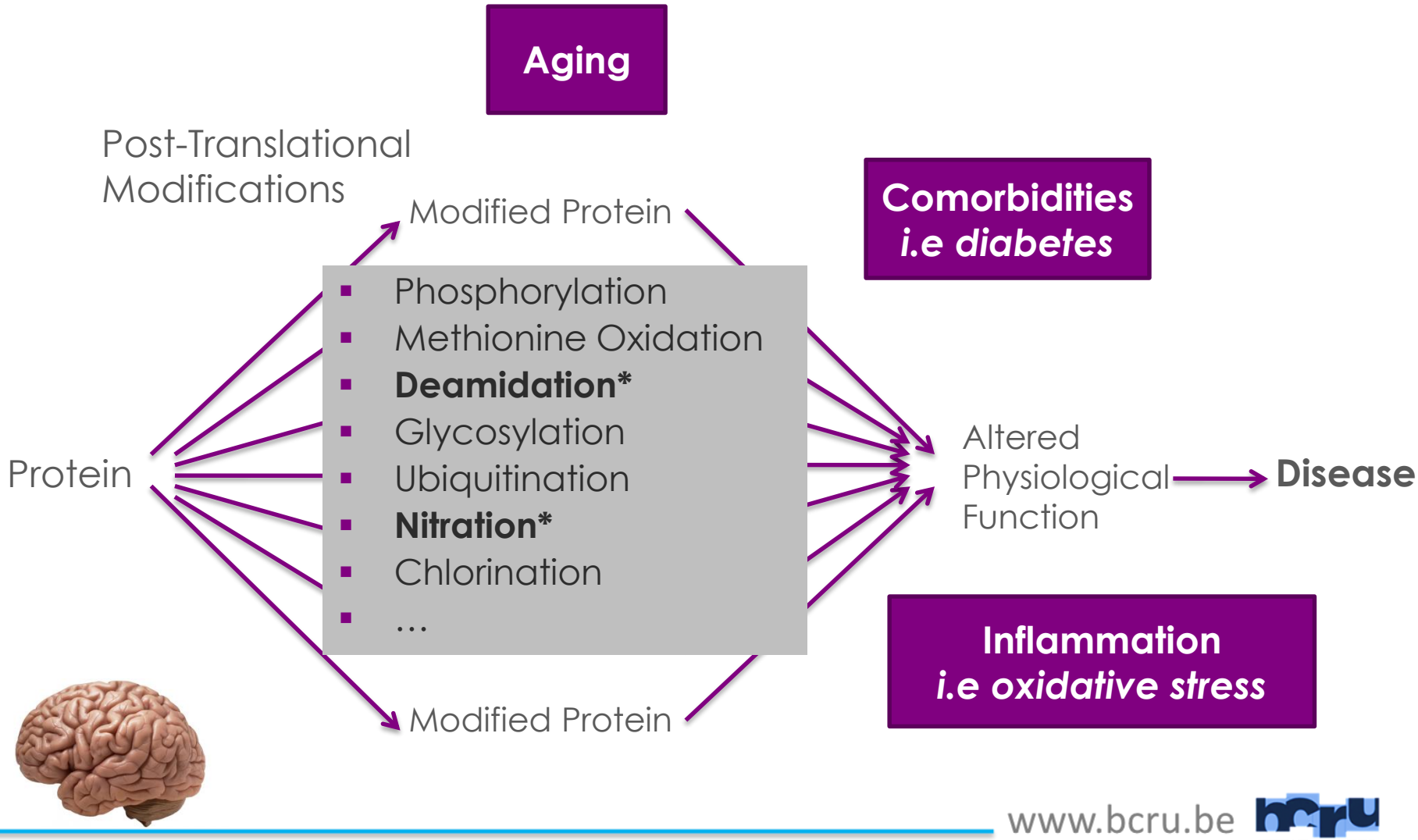
The rationale

- The most abundant protein in cartilage
- Relatively specific of hyaline cartilage
- Makes up only 1% of all collagens
- Collagen breakdown is a critical event in the pathology of osteoarthritis



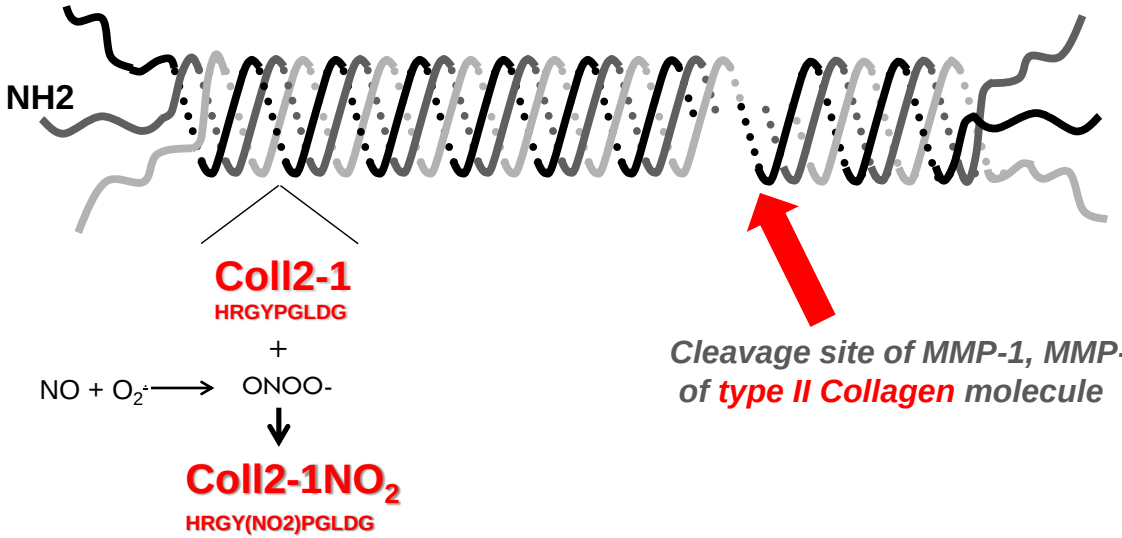


Post-translational modifications



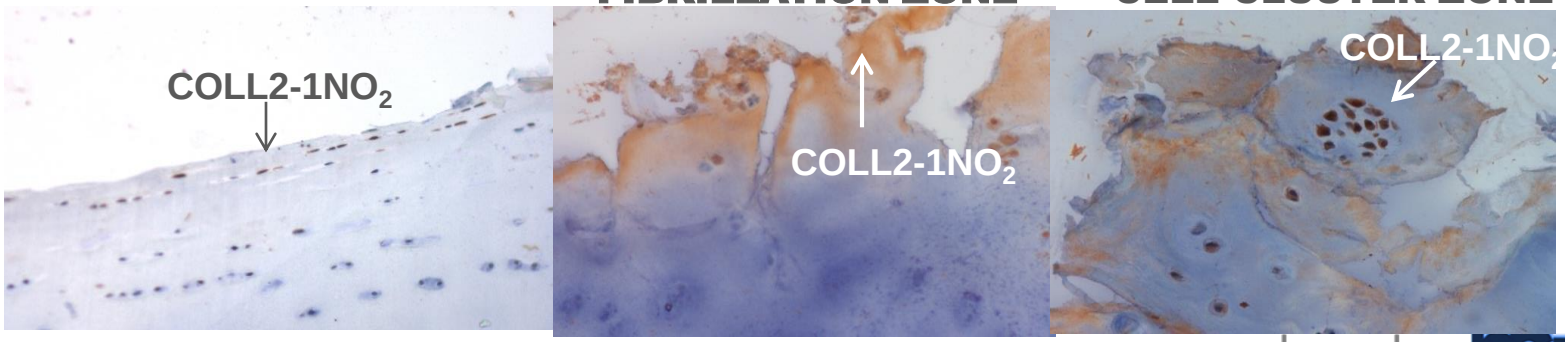
Coll2-1NO2: a joint inflammation related biomarkers

Deberg et al. O&C 2008



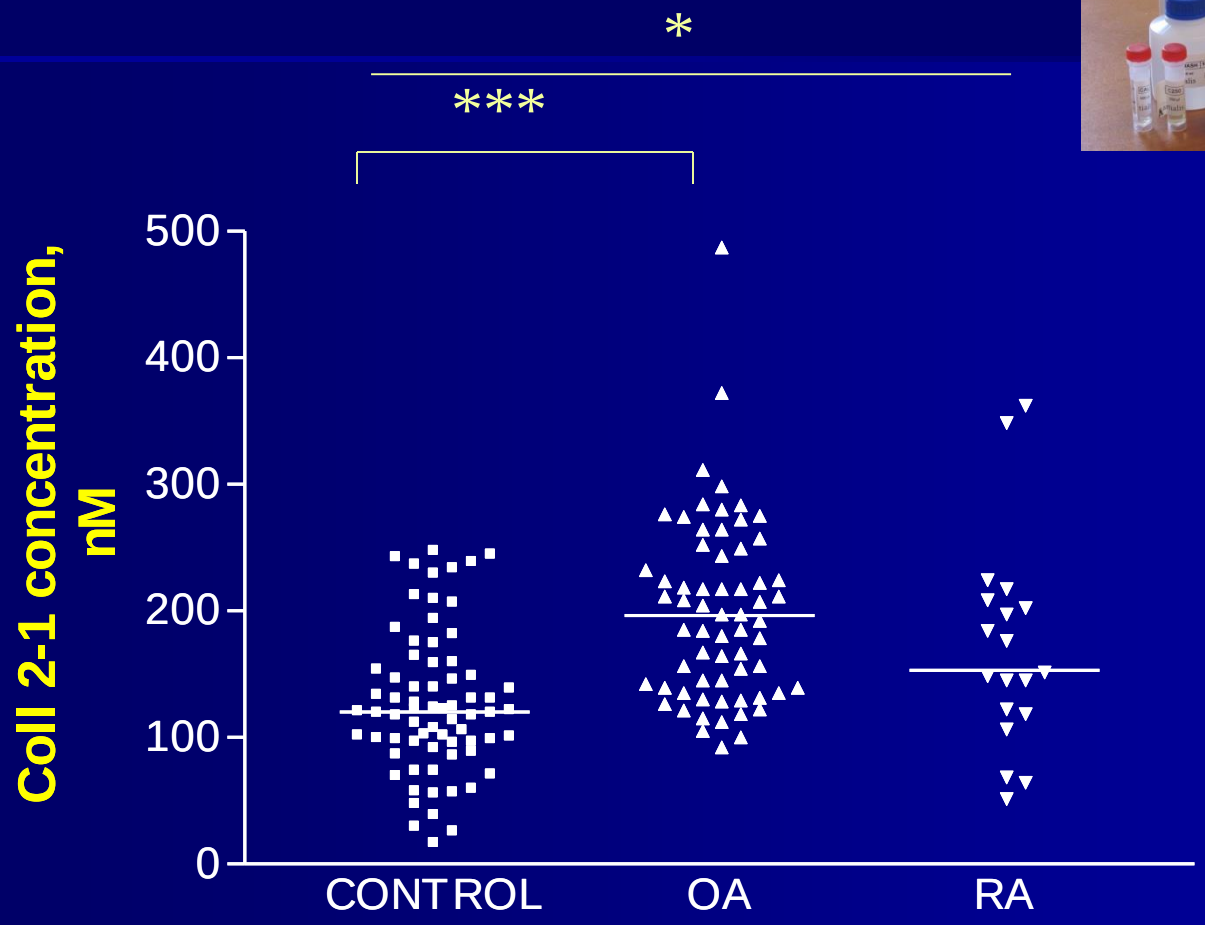
HEALTHY CARTILAGE

DAMAGED CARTILAGE



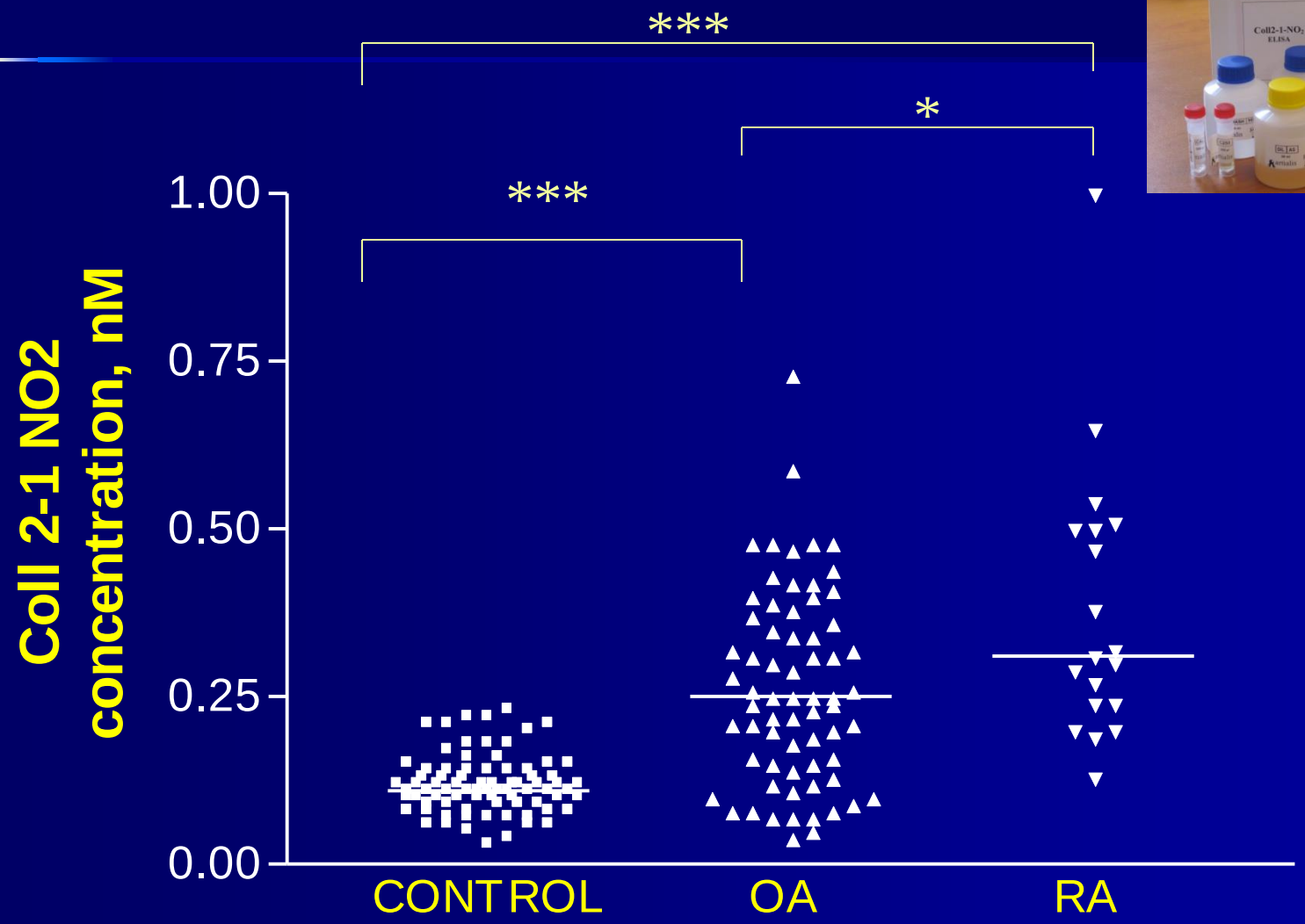
COLL 2-1 IN OA AND RA

Serum levels

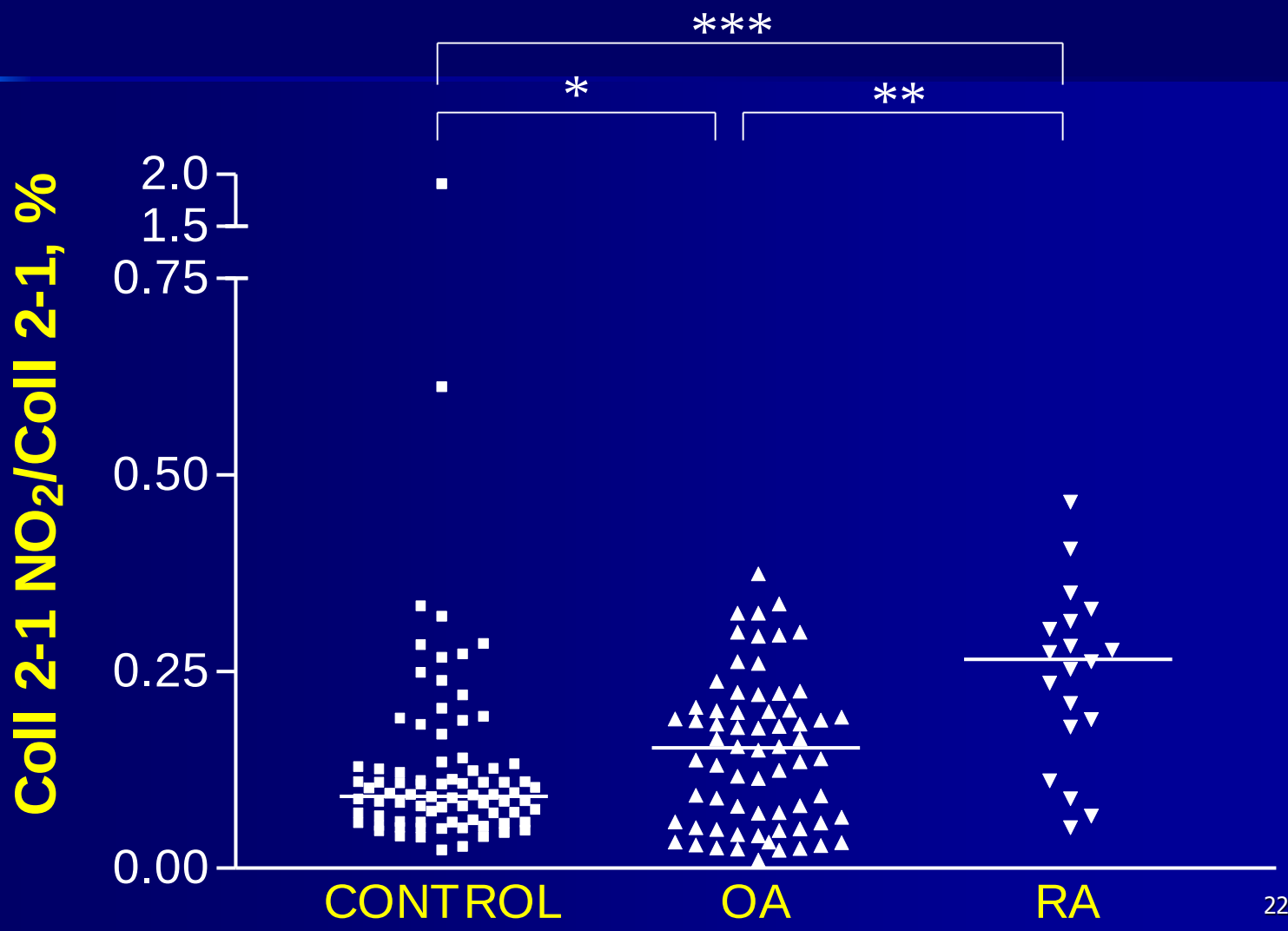


Deberg M *et al. New serum biochemical markers (Coll2-1 and Coll2-1NO2) for studying oxidative-related type II collagen network degradation in patients with OA and RA.* Osteoarthritis Cart 2005; 13: 258-65.

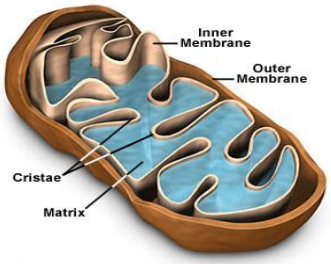
Diagnosis: COLL 2-1NO_2 discriminates OA and RA patients



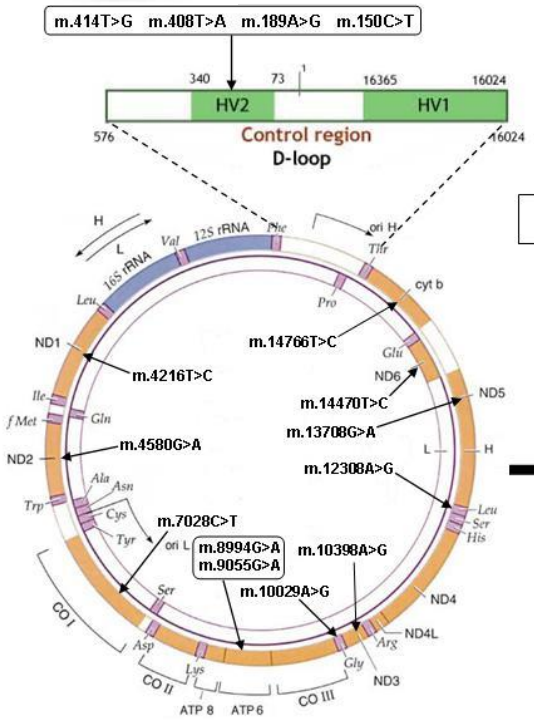
COLL 2-1 NO_2 / COLL 2-1 RATIO
Serum levels



Diagnostic: Coll2-1NO2 discriminates Mitochondrial Haplogroups



Comparison of biomarkers serum levels in haplogroups H and J



Mitochondrial Haplogroups

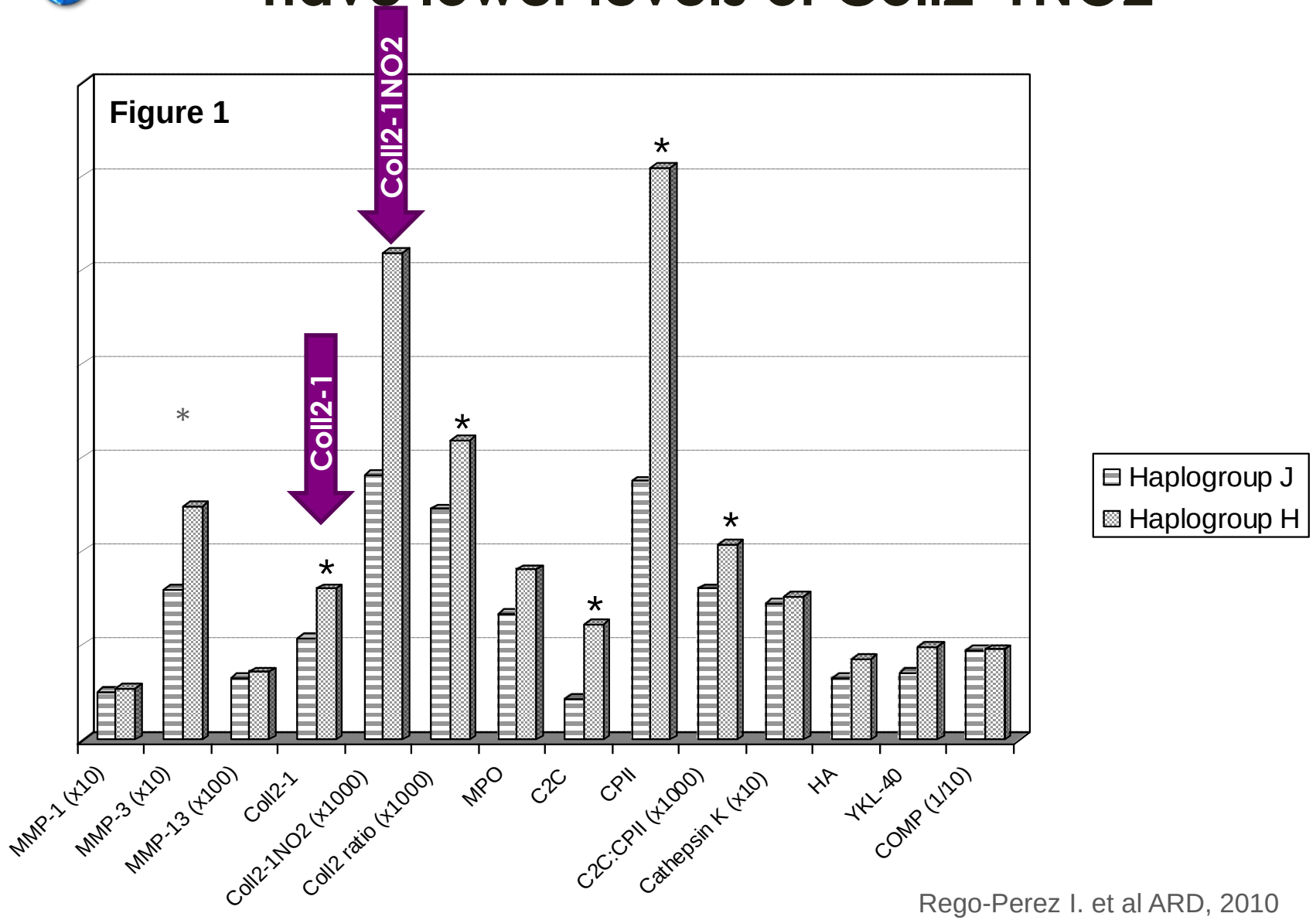
H
V
HV
U
K
T
J
I
X
W

Haplogroups J

Lower probability to develop OA
Lower X-ray OA severity
Higher time to prothesis

Blanco FJ et al. Nature Rheumatology Review, 2010

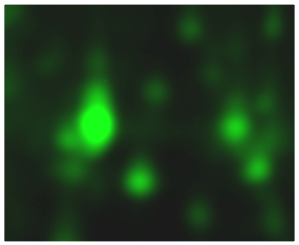
Diagnosis: OA patients with haplogroup J have lower levels of ColI2-1NO2



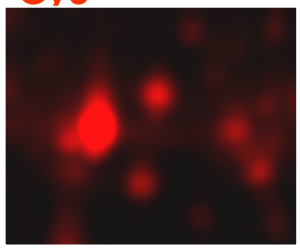
Rego-Perez I. et al ARD, 2010

Proteomic analysis: classical workflow of protein identification

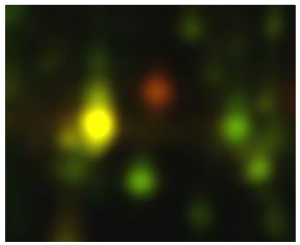
10 OA patients and 5 healthy subjects



Cy3



Cy5



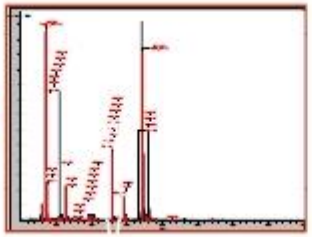
Cy3 + Cy5

Spot extraction and in-gel digestion

Mass Spectrometry

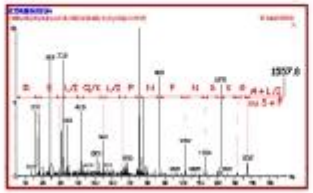
With/Without Liquid chromatography Separation

Empreinte peptidique massique



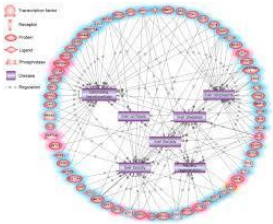
Analyse MS

IDENTIFICATION



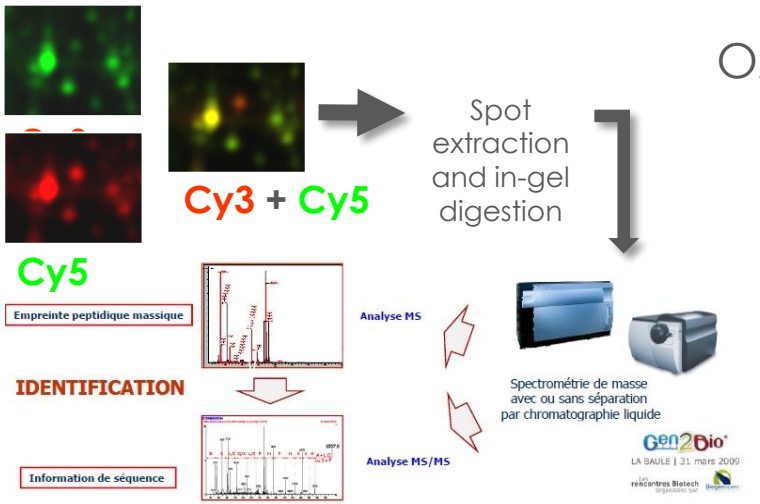
Analyse MS/MS

Information de séquence



Urinary proteome

Henrotin et al. Arthritis Rheum 2012



OA/N > 1.5

Increased (9)
B-actin
α1-microglobulin
Fibulin-3 fragments
Apoptosis inducing factor-2

OA/N > 0.7

Decreased (9)
Serpins β1 et β3
Mannan binding lectin serum proteases-2
Kinnogen 1

FBLN3_HUMAN Mass: 54604 Score: 130 Queries matched: 2

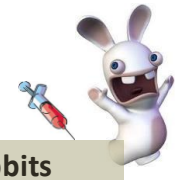
EGF-containing fibulin-like extracellular matrix protein 1 precursor (Fibulin-3) (FIBL-3)

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
149	685.33	1368.64	1368.54	0.09	0	58	0.00027	1	R.CVCPVSNAMCR.E
444	965.32	1928.63	1928.73	-0.10	0	72	4.2e-06	1	R.TCQDINECETTNECR.E

Fibulin-3 fragments (Fib3-1 and Fib3-2): potential diagnostic biomarkers

Fib3-1: **TCQDINECETTNECR**

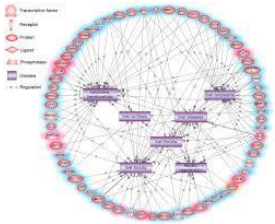
Fib3-2: **CVCPVSNAMCR**

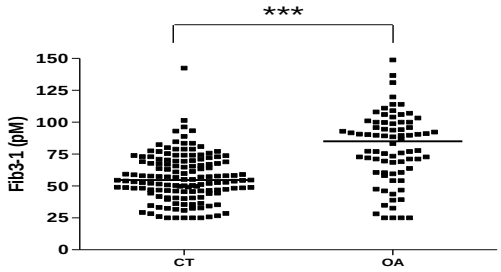


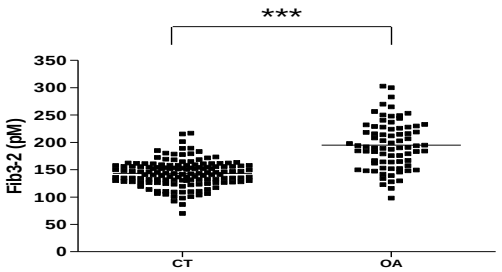
Immunization of rabbits

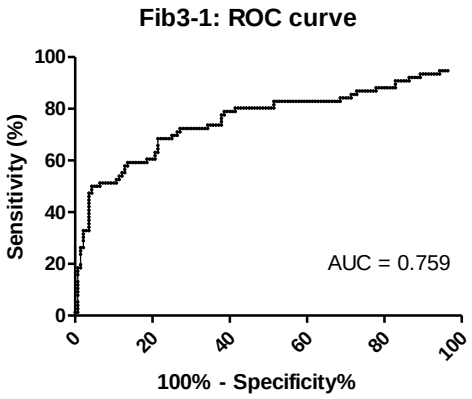
Antiserum production

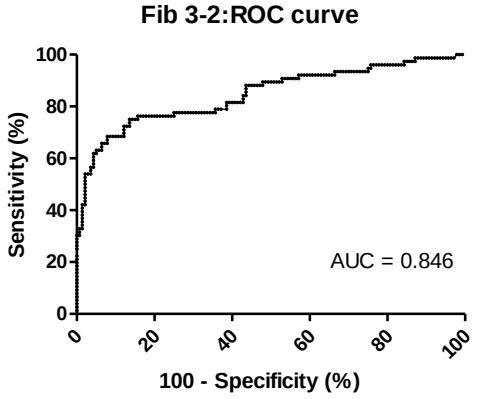
Specific immunoassays of Fib3-1 and Fib3-2 development and validation in human serum







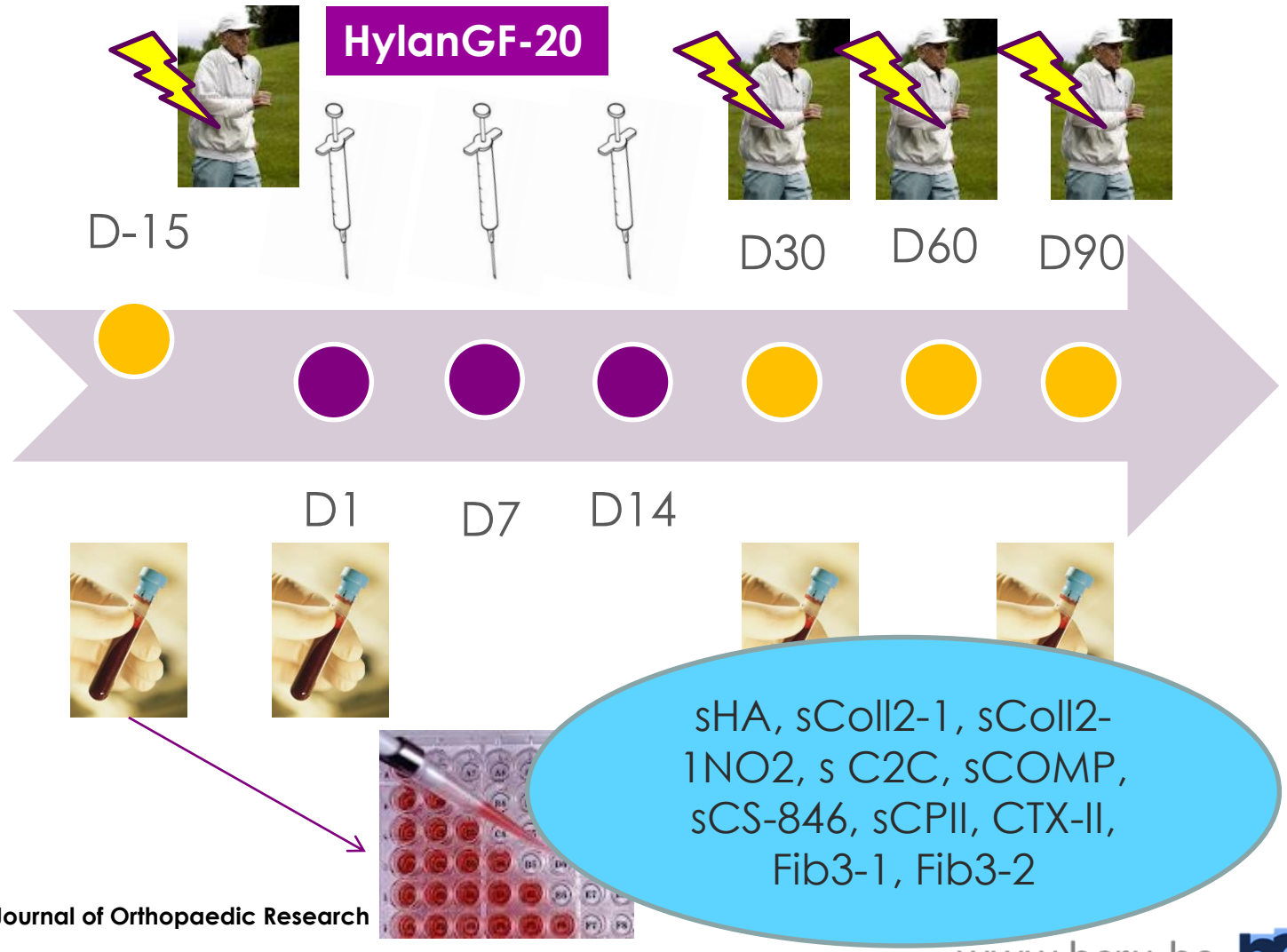




	Sensibility	Specificity
Fib3-1 (cut-off: 71.1 pM)	78.5%	68.4%
Fib3-2 (cut-off: 163.7 pM)	75.0%	86.4%

BIOVISCO study: Study design

Open-label, observational prospective study



Henrotin Y et al. Journal of Orthopaedic Research
19 FEB 2013

BIOVISCO study

An open label observational prospective study

Conrozier et al, J Orthp Res, 2012; Henrotin et al, J Orthp Res,2013.

- ✓ 45 patients with unilateral symptomatic tibiofemoral and/or patellofemoral OA
- ✓ 3-weekly intraarticular injection of hyalan G20 (Synvisc®)
- ✓ Follow-up D1, D30 and D90 after the last injection

	D1 (after the last injection)	90 days (after the last injection)	p-Value D1 vs D90
sColl2-1 (nM)	**140.34(882.44-285.32)**	**128.41 (85.6-241.34)**	**0.05***
sColl2-1NO2 (nM)	**0.400 (0.050-1.010)**	**0.370 (0.14-0.870)**	**0.025***
uCTX-II (ng/nmolcreat)	**392.7 (90.0-816.4)**	**306.0 (90-1123.9)**	**0.02***
sPIICP (ng/ml)	817.9 (131.4-1848.6)	874.8.3 (326.4-1435.0)	0.41
sC2C (ng/ml)	223.6 (99.4-329)	209.5 (135.9-291.7)	0.11
sCOMP (U/L)	10.9 (6.0-20.2)	10.5 (6.0-20.0)	0.82
sCS846 (ng/ml)	99.8 (45.9-172.3)	102.2 (53.0-190)	0.38
sHA (ng/ml)	34.1 (15.4-211)	33.3 (9.5-230.1)	0.38

Future

Critical needs!

- Drugs that can impact the disease progression
- Large cohorts representative of the general population and designed for the qualification of the biomarkers
- A sensitive imaging gold standard detecting early structural changing in joint tissues
- The inclusion of biomarkers as secondary end-point in clinical trials

Short term perspectives

- New technologies adapted to a personalized management
- Combination of biomarkers in multiplex tests
- Agregate score including clinical, imaging and biological parameters
- Companion biomarkers for drugs (Theranostic)

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Bone and Cartilage Research Unit

Thank you for your attention !

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