Update on Molecular Biomarkers

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Disclosure: I do not have a financial relationship with any commercial interest with respect to the content of this talk.

Overview

- Overview on recent updates in biochemical biomarkers.
- What studies have examined the relation between imaging and biochemical markers.
- Context for the future unique role of biochemical markers in OA clinical trials and where they may enrich and enhance the application of imaging.

GOAL FOR OA BIOMARKERS

Contribute to strategies that improve the lives of people with OA or at risk of OA

Multiple actions are urgently needed

- education campaigns for OA prevention
- research funding to identify treatments that address the causes rather than [simply the] symptoms of OA

De Ceuninck 2011 Drug Discovery Today

GOAL FOR OA BIOMARKERS

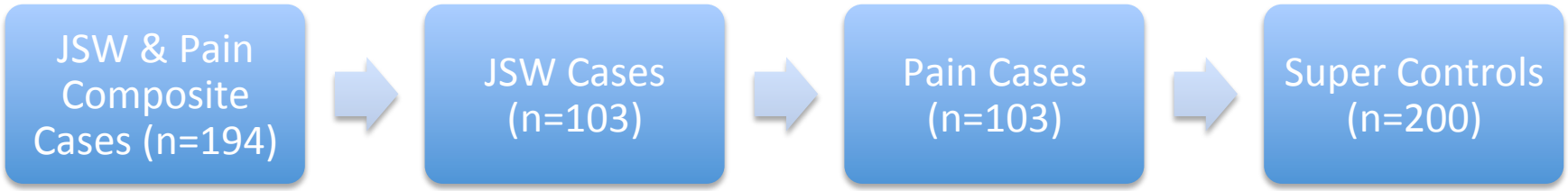
Contribute to strategies that improve the lives of people with OA or at risk of OA

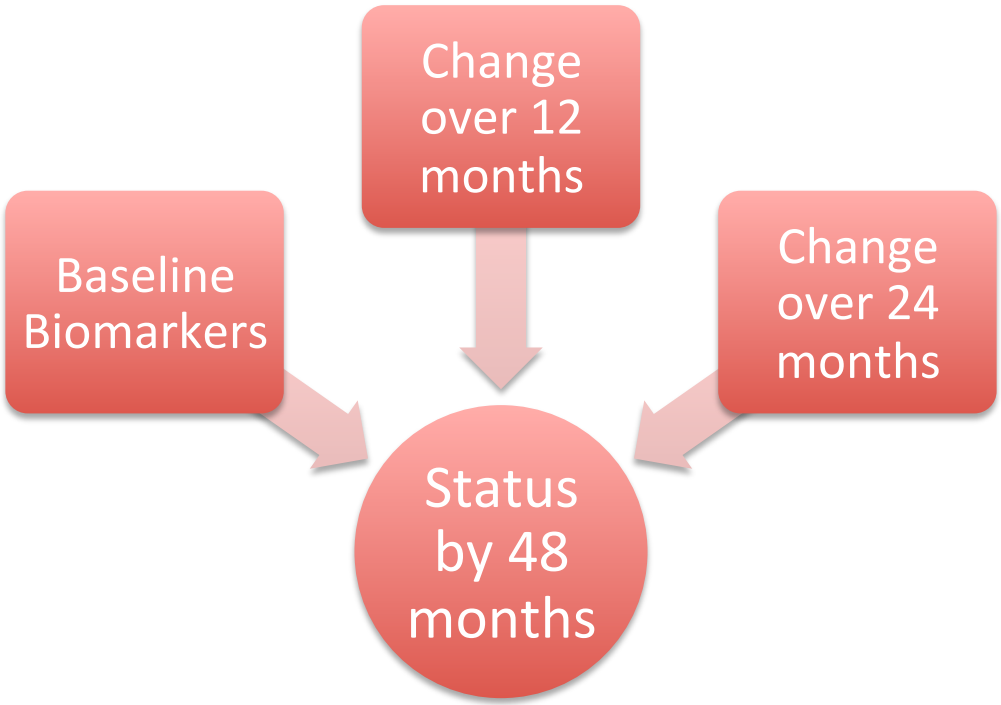
- Drug development
- Treatment monitoring
- Disease monitoring
- Prognosis
- Reducing psychological distress
- Personalized evidence-based action plan

Evaluation of Clinical Utility

FNIH/OARSI Osteoarthritis Biomarkers Consortium Project

Status based on change in knee OA parameters over 48 months including persistent pain increase for Composite and Pain Cases





Biochemical Assays:
FNIH/OARSI Osteoarthritis Biomarkers Consortium Project

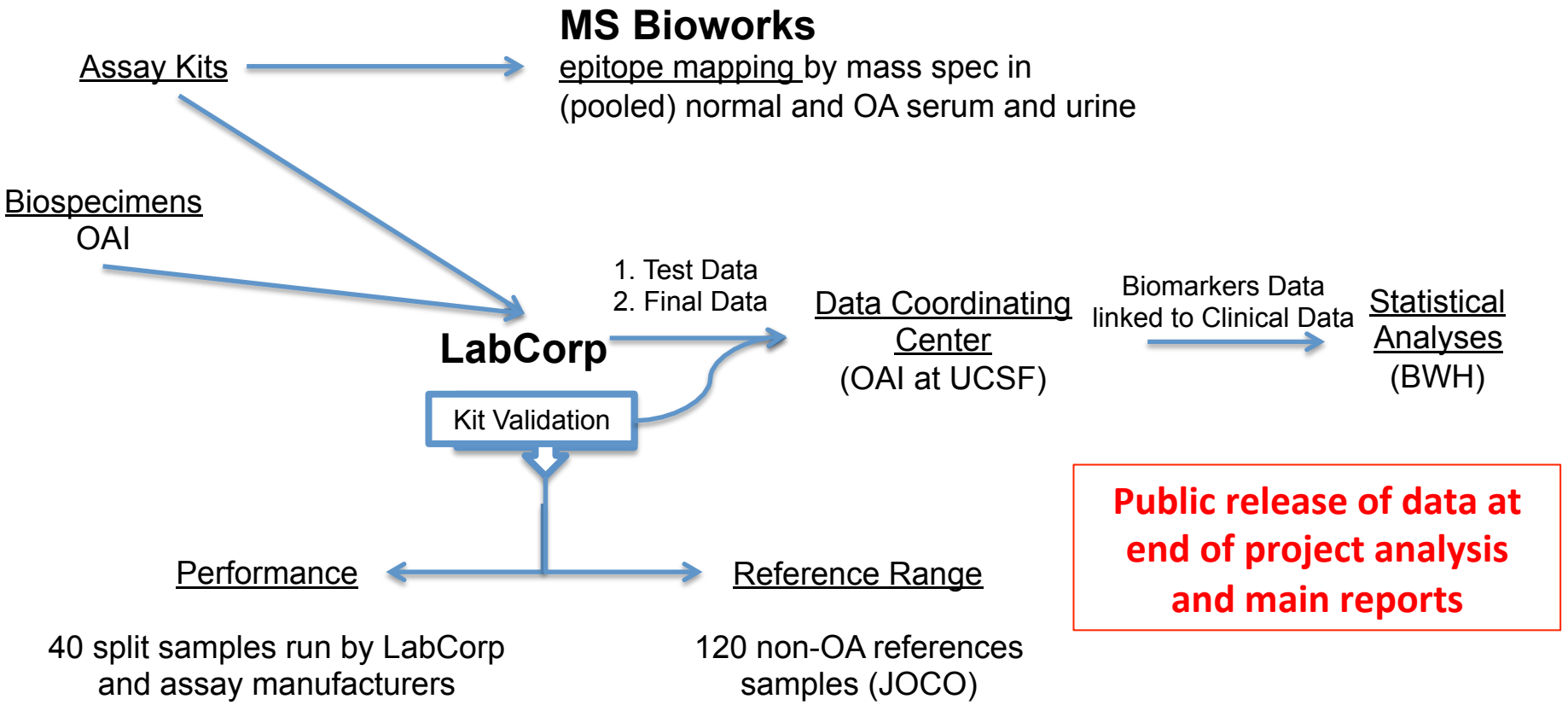
Biomarker Assay	Manufacturer (In-kind donation/Cost Reduction)
Urinary CTX-II	IDS
Serum COMP	BioVendor (in-kind donation)
Serum HA	Corgenix (reduced cost)
Serum C1,2C	IBEX (in-kind and reduced cost)
Urine C1,2C	IBEX (in-kind and reduced cost)
Serum C2C	IBEX (in-kind and reduced cost)
Urine C2C HUSA	IBEX (in-kind and reduced cost)
Serum CPII	IBEX (in-kind and reduced cost)
Serum PIIANP	Merck Group/Millipore (reduced cost)
Serum NTX-1	Alere (in-kind donation)
Urine NTX-1	Alere (in-kind donation)
Serum CTX-1	IDS (in-kind donation)
Urine (alpha) CTX-1	IDS (in-kind donation)
Urine (beta) CTX-1	IDS (in-kind donation)
Serum CS846	IBEX (in-kind and reduced cost)
Serum MMP-3	Invitrogen (reduced cost; additional kits TBD)
Urine Creatinine	Quidel (in-kind donation)
Urine Col-2-1NO2	Artialis (in-kind donation)

Study of 12 distinct entities, 17 total biomarkers Recommended by the OARSI/ FDA Biomarkers Working Group

Kraus VB, et al. Application of biomarkers in the development of drugs intended for the treatment of osteoarthritis. Osteoarthritis Cartilage 2011;19(5):515-42.

THE biomarkers CONSORTIUM | FNIH Foundation for the National Institutes of Health

Work Plan Biochemical Biomarker Analysis



Public release of data at end of project analysis and main reports

Assay Kit Validation Plan

- Intra-assay precision: QC samples plus three levels of spiked serum or urine matrix assayed a minimum of 20 replicates in a single assay
- Inter-assay precision: QC samples plus three levels of spiked serum or urine matrix assayed a minimum of four replicates times five independent assays
- Accuracy: Assay method comparison of 40 split samples assayed by LabCorp and the assay kit manufacturer
- Accuracy: Spike recovery
- Sensitivity: LOD and LLOQ
- Interference/specificity: Hemoglobin, triglycerides and bilirubin interferents will be assayed, additional homologous antigens may be assayed

Assay Kit Validation Plan (cont.)

- QC preparation/qualification
- Stability
  - -70°C or below (Evaluated at day 0, 1, 3, 6, 9 and 12 months)
  - -20°C (Evaluated at day 0, 1, 3, 6, 9 and 12 months)
  - +2-8°C (Evaluated at 1 hour, 3 hours, 6 hours, 1 day, 2 days, 3 days, 4 days and 7 days)
  - Room temperature (Evaluated at 1 hour, 3 hours, 6 hours, 1 day, 2 days, 3 days, 4 days and 7 days
  - Freeze/thaw (Evaluated up to 6 freeze/thaw cycles)
- Reportable Range: Linearity
- Reference Interval
  - Confirm (40 samples used when an existing manufacturer's reference interval exists)
  - Establish (120 samples from radiographically well-characterized non-OA healthy subjects)

Biochemical Studies Timeline

Activity	2012				2013				2014	
Quarter	1	2	3	4	1	2	3	4	1	2
Define Study population - COMPLETED										
OAI to ship biospecimens to LabCorp										
Duke University to ship biospecimens to MSBioworks										
Biochemical Biomarker Measurements										
Epitope Mapping Studies										
Conduct QC/QA Activities, Integrate Data										
Conduct Statistical Analyses										
Manuscript preparation and Data Release										

Institute of Medicine (IOM) Biomarker Report

- Cheerios. “You Can Lower Your Cholesterol 4% in 6 weeks.”
- FDA sent a letter to the chair of General Mills, the producer of Cheerios. “based on claims made on your product’s label, we have determined that your Cheerios® Toasted Whole Grain Oat Cereal is promoted for conditions that cause it to be a drug because the product is intended for use in the prevention, mitigation, and treatment of disease.”
- IOM report initiated in 2007 by the Center for Food Safety and Applied Nutrition of the FDA, which has received dozens of applications for approval of health claims for foods, most of which reflected claims of effects on a biomarker.
- The Omnibus Appropriations Act of 2009, included funds for an Institute of Medicine (IOM) study to examine and make recommendations regarding Front of Package nutrition symbols.

Micheel 2012 (IOM report)

Project Meets Institute of Medicine Framework

- Task: recommend a framework for the evaluation of biomarkers; additionally, make ancillary recommendations for their application.
- The committee met its principal task by recommending a three-part framework for biomarker evaluation:

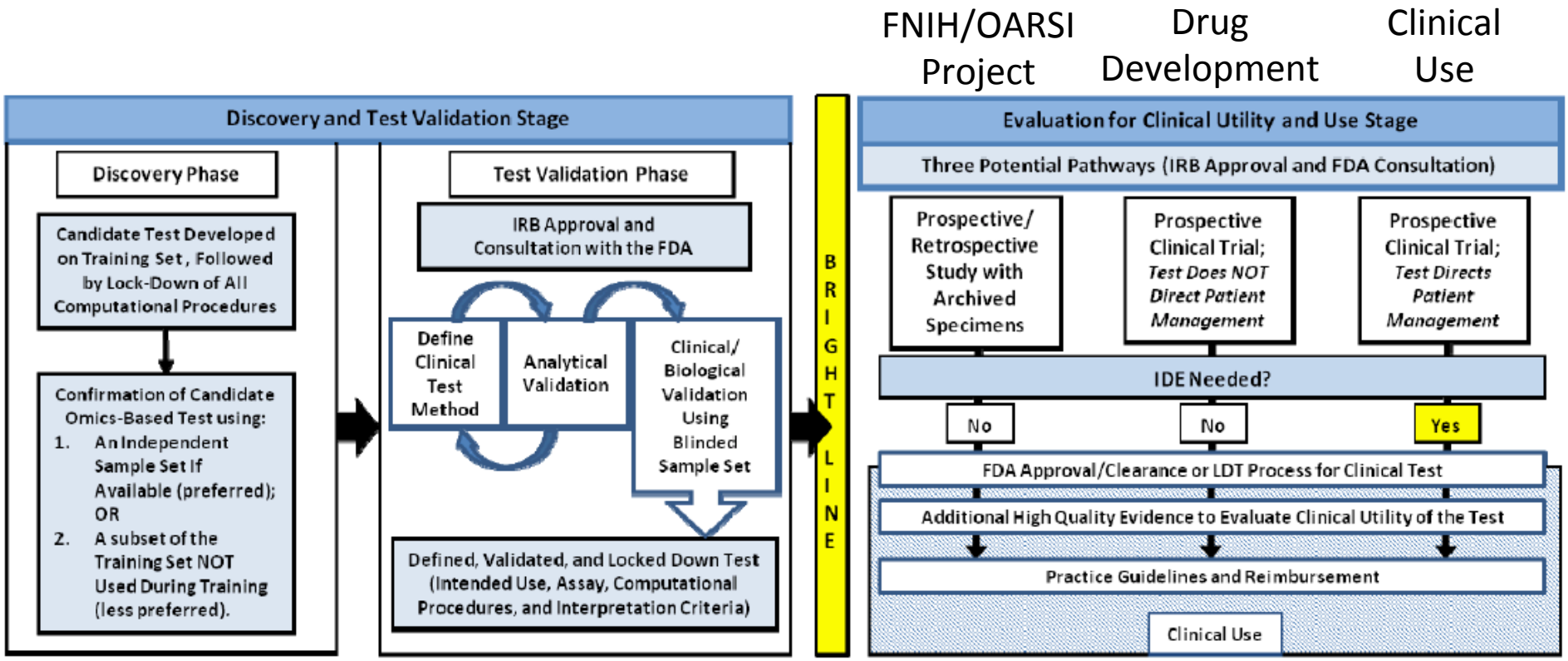
  (1) Analytical validation—is the biomarker able to be accurately measured?

  (2) Qualification—is the biomarker associated with the clinical endpoint of concern?

  (3) Utilization—what is the specific context of the proposed use?

Micheel 2012 (IOM report)

BIOMARKER PIPELINE



Micheel 2012 (IOM report Fig 1)

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Imaging and Biochemical Biomarkers--Friends or Foes?

- Molecular markers need good outcomes for qualification. Imaging markers 'think' they can stand alone but consider the fact that molecular markers may provide more objective data than patient reported outcomes!

Non-MRI (Rad, Arthroscopy) Related Studies

Results/Cohort	Reference
inverse relation between cartilage **AGEs** and actual cartilage damage at time of knee replacement by histology and macroscopic grading	Vos 2012 OAC
Decreasing **sfARGS** after meniscectomy (18 and 26 years) associated with progressive knee rOA JSN	Larsson 2012 OAC
Sf and plasma **CTGF** (connective tissue growth factor) correlated with each other and knee rOA severity	Honsawek 2012 Biomarkers
5 clusters identified in knee and hip rOA cohort: 'bone-CTX-II', 'inflammation', 'synovium', 'C1,2C-adipokines', and 'cartilage synthesis' cluster; uCTX-II clustered with biomarkers of bone metabolism, while sCOMP clustered with biomarkers of synovial activity	Van Spil 2012 OAC
SF **visfatin** higher in KL 4 vs 3 knee rOA and positively correlated with CTX-II, AGG1 and AGG2	Duan 2012 Rheum Int
Clusterin, hemopexin (glycation), alpha-1 acid glycoprotein-2, and macrophage stimulating protein differentially expressed in knee rOA progressors	Fukuda 2012 Proteome Sci
The two highest basline tertiles of **adiponectin** had a decreased risk of 70% (RR=0.3 (0.2 to 0.7) for hand rOA progression in comparison with patients in the lowest tertile. Leptin and resistin levels were not associated with progression.	Yusuf 2011 Ann Rheum Dis
No association between 17 inflammatory markers and the presence of knee hand and/or knee rOA in the Framingham cohort (**adiponectin, plasma and serum CD40 ligand, CRP, fibrinogen, ICAM-1, IL-6, lipoprotein-associated phospholipase A2 activity and mass, MCP-1, MPO, osteoprotegerin, P-selectin, resistin, TNF-α, TNF-RII and urine isoprostane**)	Vlad 2011 J Rheumatol
Data suggest a molecular basis of knee OA progression, with higher levels **of IL-2, IL-5, MCP-1, and MIP-1** indicative of greater degrees of OA based on arthroscopic grading	Vangsness 2011 Bull NYU Hosp Jt Dis
sHA, sCOMP and uCTX2 correlated positively with total rOA OST burden; sCOMP correlated negatively with total JSN burden	Kraus 2010 PLoSOne
Dkk-1 is lower in rOA than control and lower in sf than plasma; both inversely associated with knee rOA severity	Honsawek 2010 BMC Musculoskeletal Disord
Tendon calcification by knee ultrasound was related to cartilage synthesis (based on **PIIANP** levels) in men and to cartilage degradation (based on u**CTx-II** concentrations) in women.	Kumm 2009 Calcif Tissue Int

Non-MRI (Ultrasound) Related Studies

Results/Cohort	Reference
Tendon calcification by knee ultrasound was related to cartilage synthesis (based on **PIIANP** levels) in men and to cartilage degradation (based on u**CTx-II** concentrations) in women.	Kumm 2009 Calcif Tissue Int
YKL-40 associated with oteophyte size by knee ultrasound	Zivanovic 2009 Int Orthop
COMP and HA associated with size of medial osteophytes and capsular distention; sHA associated with effusion and/or synovial proliferation by knee ultrasound	Jung 2006 Clin Exp Rheumatol

MRI-Related Studies

Results/Cohort	Reference
Fasting plasma (proinflammatory) **phospholipid n-6 and arachidonic acid** correlated with knee synovial thickening on contrast enhanced (CE) knee MRI	Baker 2012 OAC
sfFibronectin-aggrecan complex distinguished knee MRI meniscal injury study group from control group	Scuderi 2011 J Bone Joint Surg Am
Baseline **soluble leptin receptor** was associated with reduced levels of the cartilage formation biomarker **PIIANP**, increased cartilage defects score, and increased cartilage volume loss over 2 years (independent of age, sex, and body mass Index); PIIANP was associated with a reduced risk of joint replacement	Berry 2011 Arthritis Rheum; Berry 2010 Ann Rheum Dis
uCTX-I, sCOMP and uCTX-II correlated with MRI parameters in an ACL rupture cohort. CTX-I correlated with VC (cartilage volume) and AC (cartilage area) of the whole knee joint	Streich 2011 Int Orthop
Baseline serum **IL-6**, and change in IL-6 and serum **TNF-alpha** were associated with (concurrent) cartilage volume loss by MRI over ~3 years	Stannus 2010 OAC
Reduced **CTX-1** (AUC=0.65) and low dGEMRIC index (AUC=0.68)) in the medial tibia were associated with longitudinal cartilage thinning (n=16 with thinning) (NS correcting for multiple testing) by knee MRI	Eckstein 2011 Ann Rheum Dis
In subgroups with higher **PINP** or **Osteocalcin** bone formation markers (based on >or= mean) there was associated higher bone resorption markers **CTX-I** and **NTX-**I and reduced cartilage loss based on MRI cartilage volume.	Berry 2010 J Rheum

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Desirable Biomarker Profile

TABLE 1

Desirable biomarker profile for drug-induced kidney, liver and vascular injuries

	Drug-induced kidney injury (DIKI)	Drug-induced liver injury (DILI)	Drug-induced vascular injury (DIVI)
Type of biomarkers to be clinically qualified	Preclinically qualified and exploratory biomarkers	Exploratory preclinical and clinical biomarkers	Exploratory biomarkers for DIVI and biomarkers for human vascular disorders
Definition of purpose	Risk prediction Early diagnostic Prognostic	Risk prediction Early diagnostic Prognostic	Risk prediction Early diagnostic Prognostic
Contexts of use	Preclinical, early clinical and clinical Candidate TSBMs will be evaluated in clinical studies	Preclinical, early clinical and clinical Candidate TSBMs will be evaluated in clinical studies	Preclinical, early clinical and clinical Parallel 'forward and reverse qualification' required
Current standards	Specific but not sensitive enough, lack of predictivity Current standards include serum creatinine and blood urea nitrogen	Sensitive but not specific enough, lack of predictivity and prognostic value Current standards include liver enzymes and bilirubin	Not sensitive nor specific enough Absence of standards for preclinical DIVI DIVI-like pathologies in human are diagnosed using clinical, pathological and biological criteria

Matheis 2011 Drug Discovery Today

Desirable Biomarker Profile for an Efficacy of Intervention Biomarker

- To demonstrate that a drug is having the desired immediate downstream biochemical effect;
- To understand the pharmacodynamics of a drug intervention and the relationship between pharmacodynamics and pharmacokinetics;
- To provide a basis for the selection of lead candidates for clinical trials;
- To contribute to the understanding of the pharmacology of candidates;
- To characterize subtypes of disease for which a therapeutic intervention is most appropriate;
- To choose a dose and dose schedule via ex vivo and in vivo studies;
- To support an efficacy endpoint;
- To support go/no go decisions in advance of preclinical and clinical studies and trials;
- To serve as a surrogate biomarker for delay of structural worsening, reduction of pain, or improvement in function.

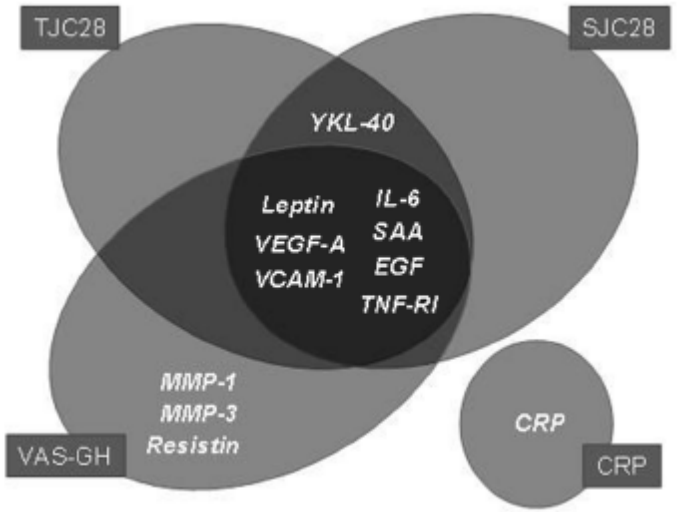
Kraus et al 2011 OAC (OARSI/FDA Biomarker Working Group)

AREA of CONCERN	PROGRESS
DISEASE HETEROGENEITY	• Pro-inflammatory subsets based on peripheral blood leukocyte gene expression identifies severity and progression subgroups
IMAGING FOCUSED ON MODERATE TO SEVERE DISEASE	• Advances in quantitative imaging provide more sensitive monitoring of OA joints
SYSTEMIC LEVELS of BIOMARKERS REFLECT a SUMMATION of CONTRIBUTIONS FROM ALL JOINTS	• First generation biomarkers in qualification study (OARSI/FNIH) • Second generation biomarkers in development

Inspired by Kapoor 2011

Power in Combinatorial Biomarkers VectraDA Score in Rheumatoid Arthritis

- Correlation with the disease activity score (changed with disease activity)
- distinguished remission/low from moderate/high disease activity
- independent predictor of disease activity measures.



Bakker doi 10.1136/annrheumdis-2011-200963 and Curtis…

VectraDA Score in Rheumatoid Arthritis

- “while the MBDA score does not reflect signs and symptoms, the 12 biomarkers are functionally diverse and more biologically comprehensive than current laboratory tests and clinical tools”

- “hypothesis that integration of information from multiple pathways can enhance disease activity assessment”

- -Curtis 2012 doi 10.1002/cr.21767

Next Steps for OA Biomarkers

- Implement evaluation framework for biomarkers for OA
- Test their ability to support evidence-based decision making (singly and in combination)
- Test their ability to contribute to the promotion of public health (singly and in combination)

Conclusions
Recommendations of the Institute of Medicine

- 1. The biomarker evaluation process should consist of the following three steps:
  - 1a. Analytical validation: analyses of available evidence on the analytical performance of an assay;
  - 1b. Qualification: assessment of available evidence on associations between the biomarker and disease states, including data showing effects of interventions on both the biomarker and clinical outcomes; and
  - 1c. Utilization: contextual analysis based on the specific use proposed and the applicability of available evidence to this use. This includes a determination of whether the validation and qualification conducted provide sufficient support for the use proposed.
- 2. For biomarkers with regulatory impact, the Food and Drug Administration (FDA) should convene expert panels to evaluate biomarkers and biomarker tests.
- 3. Initial evaluation of analytical validation and qualification should be conducted separately from a particular context of use.
- 4. The expert panels should reevaluate analytical validation, qualification, and utilization on a continual and a case-by-case basis.

Micheel 2012 (IOM report)

Conclusions

- There are a diversity of marker types under study.
- A panel of biomarkers chosen by a consensus of experts is undergoing qualification in the OAI sample database. As recommended by the Institute of Medicine, this study includes both biomarker validation and qualification and will serve as a valuable paradigm for pursuing qualification of additional next generation OA-related biomarkers.
- There is a great need for more published studies of biomarkers in clinical trials.