Osteoarthritis Biomarkers Consortium FNIH Project: Study Design

1. Project Objective
To establish the predictive and concurrent validity and responsiveness of disease prognostic and efficacy of intervention imaging and biochemical biomarkers pertinent to knee osteoarthritis (OA) progression.

2. Summary
600 subjects, and one index knee per subject, were selected for a nested case-control study of potential prognostic and efficacy of intervention biomarkers of knee OA progression. Subjects, imaging and molecular biomarker data were from the NIH Osteoarthritis Initiative (OAI), a unique longitudinal cohort (4,796 men and women ages 45–79) that contains a longitudinal repository of imaging data, and blood and urine biospecimens together with extensive clinical profile data. For molecular biomarkers, serum and urine biospecimens were sent to selected vendors who performed the assays. For imaging biomarkers, knee images were sent to selected vendors for image analysis. The project will determine the biomarkers from these sets with the best predictive and concurrent validity and responsiveness for knee OA progression. The results will facilitate taking the next step toward regulatory qualification of these biomarkers, which will be to evaluate them in a clinical trial setting either prospectively or using data from completed clinical trials.

IMPORTANT: BLINDING BIOMARKER MEASUREMENT TO OUTCOME STATUS: All image analyses and biochemical assay measurements in this study were performed blinded to the knee OA progression outcome status of the sample. Investigators intending to perform additional measurements on images or biospecimens of subjects in this sample should undertake the necessary procedures for performing these assessments blinded to outcome status, and describe these procedures in manuscripts intended for publication. If additional measurements are not performed blinded to outcome status, this limitation should be acknowledged in the manuscript.

Upon request, users may obtain biospecimens (https://www.oai.ucsf.edu/datarelease/biospecimens.asp) and images (https://www.oai.ucsf.edu/datarelease/DataImaging.asp) for the study knees identified by blinded IDs and the data will be unblinded when measurements are complete.

IMPORTANT: Use of the data intended for publication should follow OAI publication guidelines (https://www.oai.ucsf.edu/datarelease/Docs/OAIPubsGuidePublicData.pdf). Please use the following language to acknowledge the OA Biomarkers Consortium FNIH Project in publications using data from the OA Biomarkers Consortium FNIH Project public use datasets:

"Data provided from the FNIH OA Biomarkers Consortium Project made possible through grants and direct or in-kind contributions by: AbbVie; Amgen; Arthritis Foundation; Artialis; Bioiberica; BioVendor; DePuy; Flexion Therapeutics; GSK; IBEX; IDS; Merck Serono; Quidel; Rottapharm | Madaus; Sanofi; Stryker; the Pivotal OAI MRI Analyses (POMA) study, NIH HHSN2682010000 21C; and the Osteoarthritis Research Society International.

The OAI is a public-private partnership comprised of five contracts (N01-AR-2-2258; N01-AR-2-2259; N01-AR-2-2260; N01-AR-2-2261; N01-AR-2-2262) funded by the National Institutes of Health. Funding partners
include Merck Research Laboratories; Novartis Pharmaceuticals Corporation, GlaxoSmithKline; and Pfizer, Inc. Private sector funding for the Consortium and OAI is managed by the Foundation for the National Institutes of Health.”

3. Aims
The project has three primary aims:

Aim 1. To examine the relationship between putative efficacy of intervention biomarkers (biochemical markers, imaging features on x-ray and MRI and their progression over 1 and 2 years) and clinically relevant outcomes over a 4-year follow-up period.

Aim 2. To identify the most responsive biomarker(s) of OA progression.

Aim 3. To develop a risk score based on baseline values of selected biomarkers that predict clinically relevant outcomes.

4. Osteoarthritis Initiative (OAI)
The study used public data and images from the Osteoarthritis Initiative (OAI), an ongoing multi-center observational cohort study of knee and hip OA (http://www.oai.ucsf.edu/) designed to identify and help qualify imaging, biochemical and genetic biomarkers for the onset and/or progression of knee OA and funded by the NIH and industry (1). The OAI enrolled 4,796 subjects ages 45-79 at four clinical centers. At enrollment participants either had, or were at increased risk for, symptomatic knee OA. Symptomatic knee OA was defined as frequent knee pain and radiographic OA in the same knee. The presence of radiographic OA was defined as a baseline Kellgren Lawrence grade (2) (KLG) ≥ 2. Increased risk was defined by the presence of two or more risk factors for knee OA.

Imaging of both knees of participants, blood and urine specimens and clinical data were obtained during in-clinic exams at baseline and annually at 12, 24, 36, and 48 months of follow up (1), with an additional four annual assessments (in-clinic exams and telephone interviews in alternate years) obtained in subjects who consented for an extension of the study. Four identical Siemens 3T MR imaging scanners were dedicated to obtaining state-of-the-art MR images of both knees in all OAI participants at each clinic visit. The comprehensive annual assessments during the first four years of follow up also included knee radiographs, blood and urine collection for storage, demographic and risk factor information, vital clinical measures, a knee exam, questionnaires on joint pain (global, WOMAC, KOOS), physical function (WOMAC, KOOS), quality of life, health status (SF-12), and physical function performance tests (timed walk tests, chair rises) among other measures. Data collected less frequently included knee alignment by full limb radiograph and goniometer, hand and hip radiographs, quadriceps strength, knee examinations, MRI of the thigh and walking endurance (see Tables 5.1 and 5.2 of the OAI protocol pp. 31–38, http://www.oai.ucsf.edu/datarelease/About.asp).

5. Nested Case-Control Study of Knee OA Progression Biomarkers (see Figures 1-3 for schematics of study design)
600 subjects, and one index knee per subject, were selected for a nested case-control study of potential efficacy of intervention biomarkers of knee OA progression. Eligible subjects were those with at least one knee with a KLG of 1, 2 or 3 at baseline from central reading (3,4) and availability at baseline and 24 months of knee radiographs, knee magnetic resonance images (MRI) without artifacts that would interfere with image analysis, stored serum and urine specimens and clinical data. We excluded subjects having a total knee or hip replacement or metal implants in bone from baseline through 24 months due to potential effects on biochemical markers.

Eligible knees were classified for radiographic and pain progression from baseline to 24, 36 and 48 months. We excluded knees that were unable to meet criteria for radiographic or pain progression due to ceiling effects at baseline (minimum medial joint space width <1.0mm and / or WOMAC pain >91 on 0-100 scale).
Radiographic (X-ray) progression definition. Radiography of both knees was performed at all clinic visits using the non-fluoroscopic fixed flexion protocol (5). Radiographs were assessed by central reading (3,4,6) for KLG (2) and semi-quantitative joint space narrowing (JSN) based on the OARSI atlas (2). The minimum joint space width (minJSW) in the medial femorotibial compartment (MFTC) was measured using automated software (8,9). Radiographic progression was defined as a decrease from baseline to 24, 36 or 48 months in minJSW of ≥0.7 mm. This cutoff was determined based on the mean and SD of one year changes in medial minJSW in 90 OAI reference control group knees with a KLG of 0 and WOMAC pain scores of 0 at both time points. A decrease of ≥0.7 mm has a 10% probability of being due to measurement error and is consistent with values for the minimum detectable differences in medial minJSW using other methods (10). The ICC for test-retest reliability of change in medial minJSW in OAI knees over 36 months (the median duration over which radiographic progression was assessed) is 0.96.

Since radiographic progression was determined from changes in MFTC minJSW, knees were excluded if they had poor and/or inconsistent positioning (defined in terms of MFTC tibial plateau rim distance) on knee radiographs at one or more visits that would make measurement of MFTC JSW unreliable. In addition, knees with predominantly lateral compartment joint space narrowing at baseline or during follow-up, based on semi-quantitative assessments (7), were excluded to avoid misclassification on radiographic progression when based only on minJSW changes in the medial compartment.

Pain progression definition. Knee pain was assessed using the WOMAC pain subscale (11). Pain progression was defined as a persistent increase from baseline to 24, 36 or 48 months of ≥9 points on a 0-100 normalized score, based on the literature for a minimum clinically important difference (MCID) for pain worsening (12,13). Pain persistence required a pain increase of ≥9 points at two or more time points from the 24 to 60 month pain assessment, so knees were excluded if there were not enough follow-up time points after the first increase in WOMAC pain data above the threshold to determine if this increase was persistent.

Study subjects and index knees. For the nested case-control study, eligible subjects were classified into one of four groups based on the outcome in an index knee, with one index knee identified per subject. Imaging biomarkers, which are knee-specific, were measured in index knees. The four knee outcomes were:

1) Both radiographic and pain progression (Progressor /Composite Case);
2) Radiographic (X-ray) progression but not pain progression (Radiographic/X-ray Only Progressor);
3) Pain progression but not radiographic progression (Pain Only Progressor); and
4) Neither radiographic nor pain progression (Nonprogressor/ Supercontrol).

Since the primary predictors were biomarker changes during the first 24 months of follow up and outcomes were defined by changes occurring at 24 months and after, subjects with a knee that already met the criteria for radiographic and pain progression at 12 months were excluded.

When a subject had two knees with the same outcome, one was randomly selected as the index knee. Since molecular biomarkers are person-level exposures measured systemically in serum or urine, subjects were excluded if they had outcomes that were inconsistent between knees. The requirements for consistency between outcomes in an index knee and its contralateral knee were as follows:

1) Index knee with both radiographic and pain progression (Progressor/Composite Case);
   • Contralateral knee can have radiographic progression, pain progression, both or neither
2) Index knee with radiographic but not pain progression (Radiographic/X-ray Only Progressor);
   • Contralateral knee cannot have pain progression, but may have radiographic or no progression
3) Index knee with pain but not radiographic progression (Pain Only Progressor);
   • Contralateral knee cannot have radiographic progression, but may have pain or no progression
4) Index knee with neither radiographic nor pain progression (Nonprogressor/Supercontrol);
   • Contralateral knee cannot have either radiographic or pain progression.

For outcome groups 3 and 4, radiographic progression in the contralateral knee included lateral compartment semi-quantitative JSN progression (7), and if a contralateral knee did not have the medial compartment JSW data needed to determine MFTC progression, this was based on MFTC semi-quantitative JSN data (7). For groups 2, 3 and 4, both radiographic and pain progression in the contralateral knee included having a knee replacement at 36 or 48 months.

A pre-specified number of subjects, with one index knee per subject, were selected in each of the four outcome groups. The sample size goals for the four groups were 200, 100, 100 and 200, respectively. For better covariate balance among the groups, to the extent feasible index knees selected for the four groups were frequency matched for 15 strata of KLG (1 or 2 or 3) by BMI (kg/m²) category (<25; 25 to <27.5; 27.5 to <30; 30 to <35; ≥35). Knee MRIs of the selected index knees were reviewed for artifacts that would interfere with image analysis, and if present this knee and subject were excluded and a replacement selected. The achieved sample sizes in the four groups were 194, 103, 103 and 200 respectively. (See Figure 3 for subject selection flow chart.)

**MRI acquisition sequence parameters.** MRI acquisition was performed using a 3 Tesla MRI system (Trio, Siemens Healthcare, Erlangen, Germany) at the four OAI clinical sites. The MRI pulse sequence protocol included a coronal two-dimensional intermediate-weighted (IW) turbo spin-echo, sagittal three-dimensional (3D) dual-echo at steady-state (DESS), coronal and axial multiplanar reformations of the 3D DESS and sagittal IW fat saturated (FS) TSE sequences. Additional details on the full OAI pulse sequence protocol and parameters (14) and performance of the sagittal 3D DESS and coronal T1 3D FLASH WE sequences for cartilage quantitation (15,16) have been reported.

**Serum and Urine Specimens.** Morning blood and second morning void urine specimens were collected after an overnight fast using a uniform protocol at all clinic visits. The majority of blood was processed and saved as serum for use in biomarker assays for cartilage and bone turnover and proteomic studies and smaller amounts processed for plasma-based assays. Urine specimens were obtained by providing participants with a collection cup and instructions for collecting a second morning void at home on the day of their clinic visit and bringing the specimen to the clinic. Additional details on specimen collection and processing methods can be found in the OAI operations manuals ([http://www.oai.ucsf.edu/datarelease/OperationsManuals.asp](http://www.oai.ucsf.edu/datarelease/OperationsManuals.asp)).

**Analysis.** The study was designed to support the following main analyses for investigating differences in biomarker performance between different progression outcome groups:

1) Group 1 (Progressors/Composite Cases) vs. the combined groups 2 (Radiographic Only progressor), 3 (Pain Only progressor) and 4 (Nonprogressor/Supercontrol);
2) Each of groups 1, 2 and 3 separately vs. group 4;
3) Groups 1, 2 and 3 combined (all Progressors) vs. group 4;
4) Groups 1 and 2 combined (all Radiographic progressors) vs. group 3 and 4 combined (no radiographic progression);
5) Groups 1 and 3 combined (all Pain progressors) vs. group 2 and 4 combined (no pain progression).

**IMPORTANT, BLINDING BIOMARKER MEASUREMENT TO OUTCOME STATUS:** All image analyses and biochemical assay measurements in this study were performed blinded to the knee OA progression outcome status of the sample. Investigators intending to perform additional measurements on images or biospecimens of subjects in this sample should undertake the necessary procedures for performing these assessments blinded to outcome status, and describe these procedures in manuscripts intended for
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Figure 2. Study Design

- Biomarker predictors (imaging, biochemical) assessed using data from BL, 12 mo and 24 mo visits
- Radiographic and pain progression outcomes assessed using data from 24, 36, 48 mo (and for pain, 60 mo) compared to baseline

\[ \Delta \text{Biomarker predictors} \quad \text{Radiographic and pain outcomes compared to baseline levels} \]

Baseline \( \quad \) 12 mo \( \quad \) 24 mo \( \quad \) 36 mo \( \quad \) 48 mo \( \quad \) 60 mo

OAI clinic visits

Figure 3. Participant Flow Diagram

BL KLG 1-3 and biomarker data 3,481 knees (2,246 subjects)

Exclusions, or don’t meet criteria for any of the four progression outcome groups

1,908 (1,519)

- 252 (234)
- 444 (377)
- 269 (236)
- 943 (672)

- Radiographic and Pain progressor (Composite Case)
- Radiographic Only progressor
- Pain Only progressor
- Nonprogressor (Super Control)

* Frequency matching for 15 KLG by BMI strata, with random selection
** Image artifact exclusions

Eligible subjects (knees)

Selected sample for each outcome group
6. References


