Biomarker Qualification Letter of Intent (LOI)

Administrative Information

1. **Submission Title**:
   - Osteoarthritis prognostic biomarkers as assessed by immunoassays
2. **Requesting Organization:**
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3. **Submission Dates:**
   August 30, 2019

Drug Development Need Statement

As recently acknowledged by the FDA[1], osteoarthritis (OA) is a serious disease associated with increased risk of morbidity, disability, and even mortality[2,3]. OA (knee and hip) ranks fifth among all causes of disability worldwide[4], and knee OA affects an estimated 250 million people worldwide[5]. Together, knee and hip OA are estimated to affect 10% of men and 18% of women in the world's population over age 60[6]. The risk of mobility disability (defined as needing help walking or climbing stairs) attributable to knee OA alone is greater than that attributable to any other medical condition in people aged 65 years and older[7,8]. The only approved therapeutics for OA are analgesics. The absence of approved therapies to reduce the risk of OA progression[9] is due, at least in part, to the lack of qualified biomarkers to intelligently guide OA drug development and OA trial design and conduct. The current practice of attempting to identify individuals at high risk of progression based on parameters such as age, knee pain, body mass index, and baseline severity of knee OA is poorly prognostic of OA progression[10-12]. Qualified biomarkers are needed to establish a prognostic enrichment strategy (as defined in FDA guidance[13]) to select patients for trial inclusion with a high likelihood of substantial worsening of OA during the trial period as well as to establish the knowledge to monitor disease progression of patients based on biomarker concentrations over time (see Attachment 1). The FNIH Biomarkers Consortium PROGRESS OA project aims to address that need by

qualifying prognostic biomarkers of OA progression, thereby improving the conduct and eventual success of OA clinical trials.

Biomarker Information and Interpretation

***Biomarker name**:* [urinary=u, serum=s] uCTXII, sPIIANP, and uC2C-HUSA (derived from COL2A1); sNTXI, uNTXI, sCTXI, uCTXIalpha, and uCTXIbeta (derived from COL1A1); and sHyaluronan (PDB name: 3HYA)

Biomarker matrix: Blood (serum or plasma) and urine

Biomarker type: Molecular

Primary biomarker category: Prognostic

***Analytical methods*:** Two of these biomarkers, urine and serum NTXI (Osteomark) and CTXI (Crosslaps), are FDA approved for monitoring bone resorption. The remainder are for research use only (RUO). None are yet qualified for clinical use in OA (see Attachment 2). The biomarkers are measured by a Clinical Laboratory Improvement Amendments (CLIA)–certified laboratory using commercially available enzyme-linked immunosorbent assays (ELISAs) with chromogenic reporters yielding concentration data. Serum and/or urinary biomarker concentrations will be quantified. Urinary biomarkers will be normalized to urine creatinine (Cr) concentrations to control for variation in urine flow rate across samples. The same ELISA kits (same manufacturers) used in the completed Phase I of PROGRESS OA[14] will be used for this Phase II study.

***Measurement units and limit(s) of detection*:** Reported concentration measurements typically range from pg/ml to mg/l; the concentration units for the proposed serum (s) and urine (u) biomarkers are as follows: ng/mmol Cr (uCTXII and C2C HUSA); µg/mmol Cr (uCTXIalpha, uCTXIbeta); nM bone collagen equivalents (BCE)/mmol Cr (uNTXI); ng/ml (sPIIANP, sHyaluronan, sCTXI); nM BCE (sNTXI). Limits of detection (LODs) vary by ELISA kit lot but are relatively similar lot to lot. LODs for each proposed biomarker were published in the Phase I study results (Attachment 3). Just as in Phase I, sample analyses in Phase II of the project will use a uniform (single) lot of kits for each biomarker. Biomarker concentration Phase I results from Osteoarthritis Initiative (OAI) cases and controls and "multijoint supercontrols" are provided in Attachment 4.

Biomarker interpretation and utility:

Post-analytical application/conversion of biomarker raw measure to the applied measure. The raw biomarker measures will be used independently and in combination with the other biochemical markers and, in future, radiographic biomarkers (bone trabecular indices) to identify the most predictive algorithm for knee OA progression (see example of combinatorial analysis in Attachment 4). To compare the biomarkers head to head, concentrations are converted to z-scores. To evaluate short-term values as prognostic indicators of OA progression, time-integrated concentrations (TICs) will be used. As has been recognized for cancer biomarkers, due to their dynamic nature[15,16] the use of TICs (or kinetic response measures[17]) is well suited for evaluating the biomarker information content or activity over time, particularly in non-treatment (placebo) arms of trials as in this Phase II study.

Describe rationale for post-analytical elements used as inputs in application or conversion of the raw biomarker measurement. This proposal focuses on molecular biomarkers for enrichment of trials with patients likely to progress; therefore, predictive models will be used. Development of predictive models will follow accepted model development strategy[18]. Each biomarker's predictive association to each outcome will be explored individually. Multivariable models with all candidate biomarkers will be constructed, using backward selection to remove variables not meeting nominal inclusion criteria (α=0.05). Final model performance will be assessed via discriminatory ability (C-statistic or Area Under a Receiver Operating Characteristic curve) and calibration. For identification of a combinatorial model, nomograms will be created based upon final multivariable models to

provide a risk generation tool to assist in the identification of high-risk progression. Clinical nomograms are a pictorial representation of a complex mathematical formula and have been used in the OA literature to predict nonresponse to total knee replacement[19]. *Clinical interpretive criteria*. It is still to be determined what will be used as the cutoff values, cut-points/thresholds, or boundaries/limits of the biomarkers to draw an actionable conclusion based on the biomarker result. These will be determined by analyzing the existing data from Phase I, including biomarker data generated in Phase I for n=129 elder multijoint supercontrols (see Attachment 5), and the data to be generated from the Phase II study. Based on Phase I results, both high concentrations of type I and II collagen biomarkers in serum or urine were indicative of higher risk of OA progression. High serum hyaluronan concentrations, associated with synovial inflammation, were also associated with higher risk of OA progression. In contrast, low serum PIIANP concentrations, representing failure to produce a novel isoform of type II collagen associated with cartilage repair, were indicative of higher risk of OA progression. This interpretation was recently borne out in a re-analysis of Phase I data using a machine learning approach[20]; using all biochemical and imaging biomarker observations (73 baseline variables), baseline variables with the greatest contribution to non-progression at 48 months included WOMAC pain, lateral meniscal extrusion, and serum PIIANP, while those contributing to progression included bone marrow lesions, osteophytes, medial meniscal extrusion, and urinary CTX-II.

Context of Use Statement (500 characters)

Primary COU:

Prognostic enrichment molecular biomarkers for use in phase 2 and 3 clinical trials to identify individuals with a diagnosis of knee osteoarthritis who are likely to experience disease progression within the subsequent 48 months based on the WOMAC pain subscale and/or radiographic joint space width loss and/or joint replacement.

Secondary or allied COUs:

Prognostic biomarkers based on time-integrated concentrations (TICs) from baseline to 12 months, to provide a method for early identification of osteoarthritis patients to define who are likely to experience disease progression within the subsequent 48 months based on the WOMAC pain subscale and/or radiographic joint space width loss and/or joint replacement.

Analytical Considerations

Please provide the following information (if applicable or available):

- *General description of what aspect of the biomarker is being measured*. Baseline concentration of serum and/or urine biomarkers for the primary COU; TIC of serum and/or urine biomarkers from baseline to 12 months for the secondary COU.
- *Index/scoring system*. To predict knee OA progression, we aim to identify cutoffs for optimizing the positive predictive value of biomarkers used singly or in combination, as it is possible that a combination of markers will be more useful for predicting knee OA progression than a single marker. In addition, we aim to investigate different definitions of knee OA progression: radiographic only, clinical only (pain progression and/or diminished function), radiographic *and* clinical (pain progression or diminished function), and knee joint replacement.
- *Description of sample source*. Biospecimens (serum and urine) from the placebo arms of three completed randomized placebo-controlled clinical trials will be used; namely, two calcitonin trials[21] (NCT00486434 and NCT00704847, Attachment 6) and the VIDEO trial of vitamin D[22] (ISRCTN94818153, Attachment 7). The pertinent details regarding the primary trials can be found in Attachments 6 and 7. The biospecimens for the calcitonin trials have been stored at -20 °C for 8–10 years; the biospecimens for the VIDEO trial

have been stored at -80 °C for 12 years. A total of 809 and 237 baseline serum samples are available for Phase II from the calcitonin and VIDEO trials, respectively.

- *Description of pre-analytical factors and quality assurance/quality control (QA/QC) plans*. Only archived biospecimens will be used for this research. Unthawed aliquots of serum are available from the VIDEO trial, but samples from the calcitonin trials have been previously thawed. Many of the biomarkers proposed for qualification in Phase II are cross-linked collagen epitopes, and these in particular are robust to sample freeze-thaw cycles. The sample provenance is well known and can be assessed statistically if major differences in results by cohort are observed.
- *Analytical validation plan*. Biomarker technical performance was extensively assessed in Phase I (these validation reports are provided as Attachments 8–17 and are publicly available upon request from KAI Research, Inc.). Each of the biochemical markers proposed herein for qualification have good analytical validity based on the extensive analytical validation conducted within the Phase I PROGRESS OA project. The analytical validity data generated previously for each biomarker will apply to Phase II because the same ELISA reagents/kits will be used for Phase II analyses as were used in Phase I. In brief, during Phase I of the project, the following assay performance metrics were assessed by LabCorp Clinical Trials (San Leandro, CA), a CLIA- and College of American Pathologists–certified division within LabCorp:
    1. Appropriateness of the calibration algorithm
    2. Sensitivity: lower limit of quantitation (LLOQ) confirmation
    3. Sensitivity: limit of detection (LOD) or blank (LOD) determination
    4. Upper limit of quantitation (ULOQ) confirmation
    5. Accuracy: method comparison
    6. Spike and recovery
    7. Precision
        A. Intra-assay precision
        B. Inter-assay precision
    8. Analytical specificity
    9. Establishing reference interval
    10. Stability (to freeze-thaw cycle)
    11. Linearity (dilutional recovery)
- *Once the SOP and analytical validation plan is finalized, describe how you will use this process to validate the final version of the measurement tool*. The analytical validation results, together with the published results of their clinical utility, have already been used to select a subset of 9 biomarkers for analysis in Phase II out of a total of 18 assessed in Phase I.

Clinical Considerations

- *Describe how the biomarker measurement is used to inform drug development. Please provide a decision tree to guide how the biomarker information would be used in drug development or a clinical trial*. See Attachment 18.
- *Describe patient population or drug development setting in which the biomarker will be used*. The biomarkers are intended to identify individuals with radiographic knee OA who are at high risk of progression for enrollment in OA clinical trials and, in future, for treatment with disease-modifying OA drugs (DMOADs) once available.
- *Clinical validation: provide information to support biological and clinical relevance of the biomarker as applied in the COU. Describe how normal or other reference values are established, provide study design(s), analytical plan, etc.* OA is characterized by an active and complex process involving mechanical, inflammatory, and metabolic alterations that may affect multiple structures of the joint organ[23], as demonstrated by magnetic resonance imaging (MRI), including the hyaline articular cartilage, subchondral bone, synovium, and soft tissue structures such as the menisci[24]. Change in all these structures has been

shown to be associated with clinically relevant progression of the disease. The serum (s) and urinary (u) biochemical biomarkers proposed herein reflect the multitude of simultaneous biological processes involved in disease progression, including cartilage degradation (uCTXII and uC2C-HUSA), cartilage synthesis (sPIIANP), synovial inflammation (sHyaluronan), and subchondral bone remodeling (sNTXI, uNTXI, sCTXI, uCTXIalpha, uCTXIbeta). A wealth of literature informed the choice and supported the informativeness of this set of biomarkers for predicting knee OA progression[25]. A recent study provides support for the prognostic value of this set of biochemical markers for predicting clinically relevant progression (pain *and* radiographic worsening) of knee OA over 48 months[14]. In this study, all uCTXII time points (baseline, 12-month TIC, and 24-month TIC) predicted clinically relevant progression. From among the 18 biomarkers tested, uCTXII TIC over 24 months, used alone, yielded the highest odds ratios (OR=1.72) for predicting clinically relevant progression compared with non-progressors (without pain *or* radiographic progression). The most predictive and parsimonious combinatorial model for predicting clinically relevant knee OA progression over 48 months consisted of TICs over 24 months of uCTXII, sHyaluronan, and sNTXI (area under the curve [AUC]=0.667 with covariates adjusted[14]). Baseline urinary CTXII and CTXIalpha both significantly predicted clinically relevant progression (OR 1.29 and 1.20, respectively). Normal reference values and optimal cutoffs will be determined using the reference multijoint supercontrol biomarker data[26].

- *Benefits and risks of applying the biomarker in drug development or a clinical trial*. Current OA trials suffer from low power due to inability to identify a subset of patients likely to undergo OA progression during the course of the trial. The proposed biomarkers for qualification are intended to overcome this major challenge to drug development by providing a cost-effective screening strategy (based on soluble biomarkers in serum and/or urine) for enriching a trial with individuals likely to progress over the 24 months following enrollment in the trial. A strategy for even modest enrichment of a trial for OA progressors or for reducing screen failure rates (e.g., risedronate trials for OA had screen failure rates of 73%[27]) could have significant beneficial cost implications.
- *Describe any current knowledge gaps, limitations and assumptions in applying the biomarker in drug development or a clinical trial*. Phase II will be powered on the ability of a baseline biomarker to predict radiographic knee OA progression. The serial knee radiographs of all individuals used for these analyses will be re-evaluated for joint space width using a uniform software and protocol to harmonize the joint space width loss outcome across all cohorts. Current estimates of power are based on radiographic progression determinations made in the primary studies and so represent an educated approximation of the radiographic progression outcome that will be used for Phase II. Presuming an OA progression rate of 11% (i.e., 110 subjects out of 1,000 progress), a prognostic biomarker would have 88.4% power to be detected if its true underlying odds of impacting progression was 1.4 (i.e., 40% increase in odds of having clinical OA progression). Any increase in either progression or underlying odds of clinical progression increases power. Based on available data, we anticipate a 15% rate of progression in the extant trials used for these analyses, so these power estimates are likely conservative. We will generate a uniform measure of radiographic joint space width using KneeTool software (Optasia Medical) for standardization of the radiographic progression metric across studies. Funding is currently available for baseline and 12-month quantification of biomarkers; funding permitting, we will evaluate the 24-month biospecimens in future.

Supporting Information

- *Underlying biological process or supporting evidence of association of the biological process with the biomarker*. Each of the nine biomarkers selected for Phase II fulfills one or more of the BIPEDS categories[25,28] corresponding to Burden of disease, Investigational, Prognostic, Efficacy of intervention, Diagnostic, and Safety biomarkers. The original 18 biochemical biomarkers from which these 9 were selected were agreed upon through consensus of the Osteoarthritis Research Society International/FDA Biomarkers Working Group[25].
- *Summary of existing preclinical or clinical data to support the biomarker in its COU (e.g., summaries of literature findings, previously conducted studies)*. The biochemical markers (Attachment 4) proposed for

qualification include 9 of the 18 biomarkers that performed well in Phase I to predict the longer term outcome of clinically relevant knee OA progression (defined as pain and radiographic worsening)[14]. Biomarkers (baseline and TICs of biomarkers over 12 and 24 months) were evaluated individually and in combination, in multivariable logistic regression models, for associations with knee OA progression over 24–48 months (adjusted for sex, race, baseline minimum joint space width, baseline WOMAC pain score, age, body mass index, Kellgren-Lawrence radiographic OA severity[29], and use of pain medications). The nine biomarkers selected to carry forward to Phase II validation represent the best qualified OA-related biomarkers from Phase I of the study; they include serum and urinary markers of synthesis and degradation of cartilage, resorption of new and old bone, and inflammation of synovium. Statistically significant odds for predicting any radiographic OA progression based on joint space width loss were achieved by uCTXII, uC2C-HUSA, uC1,2C, uCTXIbeta, uCTXIalpha, uNTXI, sCTXI, sNTXI, sHyaluronan (OR 1.24 –1.72), and sPIIANP (OR 0.79–0.70). All uCTXII time points (baseline, 12-M and 24-M TICs) predicted clinically relevant progression. In addition, all of these biomarkers have been quantified in "multijoint supercontrols" (n=129)—elders (aged 45 to 95 years) who at baseline and follow-up were free of radiographic hand, hip, knee, and lumbar spine OA, without knee or hip symptoms and with minimal or no hand and spine symptoms at all available time points; the majority (79%) had one or more follow-up evaluations 5–15 years later[26].

- *Summary of any planned studies to support the biomarker and COU*. We will perform a retrospective analysis of Phase I results (generated from OAI biospecimens) to identify cut-point thresholds to optimize the positive predictive value. These cut-points will be assessed and tested in the new clinical trial validation data sets (calcitonin and VIDEO biospecimens) to determine their positive predictive value for knee OA progression.
- *Please describe alternative comparator, current standard(s), or approaches*. This project will assess molecular (soluble) biochemical markers. The current approach of identifying progressors relies on patient characteristics such as age, gender, and body mass index; these characteristics have been shown repeatedly to be poor prognostic indicators of risk for knee OA progression[11,30]. Alternative approaches are being developed in parallel to qualify MRI and radiographic (trabecular bone texture [TBT]) biomarkers as prognostic indicators of knee OA progression. Biochemical and radiographic biomarkers provide an attractive first-stage screening approach to identifying progressors due to their cost effectiveness and, in the case of radiographic measures, their derivation from a radiograph, which has become the standard for all trials.

Previous Qualification Interactions and Other Approvals (if applicable)

- *Qualification submissions to any other regulatory agencies with submission number*. None
- *Prior or current regulatory submissions to Center for Biologics Evaluation and Research (CBER), Center for Drug Evaluation and Research (CDER), and Center for Devices and Radiological Health (CDRH).* DDTBMQ000038 and update submission for DDTBMQ000038 submitted 11.28.2018 related to MRI biomarkers as prognostic biomarkers for knee OA progression. In addition, a companion LOI is submitted concurrently for qualification of subchondral bone TBT biomarkers in the PROGRESS OA Phase II study.

Attachments

- *Please provide a list of publications most relevant to this biomarker development proposal*. See Attachment 20.

- *Optional: If this biomarker development effort is part of a longer-term goal, please summarize your long-term objectives*. We plan retrospective analyses of existing Phase I PROGRESS OA data to inform Phase II analyses when the new data are available. For these analyses we will simulate a clinical trial screening process with biochemical and radiographic criteria as a first screen to simulate identification of trial participants for subsequent MRI with modeling of screening costs based on different strategies. Because

the Phase I study included biochemical, radiographic (TBT), and MRI markers, we will evaluate scenarios for their use in combination to optimize trial costs and performance; a draft manuscript is provided (Attachment 19) in which all three domains of biomarkers were evaluated using a multivariable regression approach. We expect to complete these analyses over the next 3 years in keeping with the FNIH Phase II PROGRESS OA project plan timeline.

- *Optional: If you have other supporting information you would like to provide, please submit as attachment(s):*
    - **Attachment 1:** Study design summary for biochemical biomarker qualification
    - **Attachment 2:** Description of FDA-approved uses of biomarkers NTX and CTXI for monitoring bone resorption
    - **Attachment 3:** Phase I study of biochemical markers in the osteoarthritis initiative (OAI) cohort reported in Kraus et al. Predictive validity of biochemical biomarkers in knee osteoarthritis: data from the FNIH OA Biomarkers Consortium. *Ann Rheum Dis*. 2017;76:186-195.
    - **Attachment 4:** Table listing concentrations and performance of the panel of soluble biomarkers analyzed for the FNIH Biomarkers Consortium PROGRESS OA Project and proposed for FDA qualification.
    - **Attachment 5:** Phase I study of biochemical markers in an elder reference control population without osteoarthritis reported in Kraus et al. Establishment of reference intervals for osteoarthritis-related soluble biomarkers: the FNIH/OARSI OA Biomarkers Consortium. *Ann Rheum Dis*. 2017;76:179-185.
    - **Attachment 6:** Study results of two oral salmon calcitonin trials (NCT00486434 and NCT00704847) reported in Karsdal et al. Treatment of symptomatic knee osteoarthritis with oral salmon calcitonin: results from two phase 3 trials. *Osteoarthritis Cartilage*. 2015;23(4):532-43.
    - **Attachment 7:** Study results of main VIDEO trial (ISRCTN94818153), reported in Arden et al. The effect of vitamin D supplementation on knee osteoarthritis, the VIDEO study: a randomised controlled trial. *Osteoarthritis Cartilage*. 2016;24(11):1858-1866.
    - **Attachment 8**: Validation Report for Quantitative Measurement of C-Terminal Telopeptides of Type II collagen in Urine (uCTXII)
    - **Attachment 9**: Validation Report for Quantitative Measurement of Type IIA N-propeptide in Serum (sPIIANP)
    - **Attachment 10**: Validation Report for Quantitative Measurement of Collagen Type II Cleavage in Urine (uC2C-HUSA)
    - **Attachment 11**: Validation Report for Quantitative Measurement of Cross-linked N-telopeptides of Type I collagen in Serum (sNTXI)
    - **Attachment 12**: Validation Report for Quantitative Measurement of Cross-linked N-telopeptides of Type I collagen in Urine (uNTXI)
    - **Attachment 13**: Validation Report for Quantitative Measurement of Cross-linked C-telopeptides of Type I collagen (CrossLaps) in Serum (uCTXI)
    - **Attachment 14**: Validation Report for Quantitative Measurement of Alpha C- Terminal Telopeptides of Type- I collagen in Urine (uCTXIalpha)
    - **Attachment 15**: Validation Repot for Quantitative Measurement of Beta C- Terminal Telopeptides of Beta Type- I collagen in Urine (uCTXIbeta)
    - **Attachment 16**: Validation Report for Quantitative Measurement of Hyaluronic Acid in Serum (sHA)
    - **Attachment 17**: Retrospective Validation Report for Quantitative Measurement of Creatinine in Urine (uCr)
    - **Attachment 18:** Decision tree describing use of biochemical biomarkers to inform drug development and clinical trials
    - **Attachment 19:** Multivariable Phase I Results—DRAFT manuscript

- **Attachment 20:** References list