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Identification of serological biomarker profiles associated with total joint replacement in osteoarthritis patients

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SUMMARY

Objective: Establish a biomarker panel associated with all-cause total joint replacement (TJR) through identification of patients with osteoarthritis (OA) who do or do not progress to TJR and investigate effects of nonsteroidal anti-inflammatory drugs (NSAIDs).
Design: Serum samples from patients enrolled in phase III trials of tanezumab who experienced TJR ($n = 174$) or matched patients who did not ($n = 321$) were analyzed for bone, cartilage, soft tissue, and inflammation markers. Classification and Regression Tree (CART) analysis was used to identify biomarker phenotypes associated with TJR.
Results: At baseline, biomarker combinations for patients who did not use NSAIDs before starting tanezumab and used NSAIDs during tanezumab treatment <90 days ("nonNSAID"), identified 77% (95% confidence interval [CI]: 71–84%) of patients who experienced TJR and 77% (95% CI: 65–86%) who did not over a 6-month study period (on average). These biomarker combinations increased odds of identifying patients to remain free of a TJR by 3.3-fold. In patients who used NSAIDs continuously (during screening and ≥90 days during tanezumab treatment), 64% (95% CI: 54–73%) who had TJR and 75% (95% CI: 68–83%) who did not were identified by biomarker combinations different from nonNSAID patients, with an increase in odds of identifying patients to remain free of a TJR by two-fold.
Conclusions: Although validation on other cohorts is necessary, biomarkers may assist in identifying patients who will need TJR. The profiles suggest NSAID use increases importance of bone metabolism in TJR pathology.

Introduction

Osteoarthritis (OA) is the most common form of arthritis[1]. Hallmarks of OA include progressive degeneration of articular cartilage, generation of osteophytes and subsequent joint space narrowing, leading to joint function loss and total joint replacement (TJR). A relationship exists between subchondral bone and articular cartilage, but synovial inflammation also contributes significantly to OA structural changes and pain[2–6]. OA is starting to be considered as a candidate for personalized health care, in which biomarkers could assist in identifying which intervention may be optimal for treating specific phenotypes of OA[7].

The end stage of joint failure is TJR. No serologic profile exists for prediction or coincident detection of TJR that could guide more objective decisions and provide further insights into pathways and biological processes leading to TJR[8,9]. If upregulated biological processes are predictive and diagnostic for TJR, interventions that attenuate these processes and delay TJR may be identified.

Serologic biochemical markers (biomarkers) are receiving increased attention in drug development and for guiding evidence-based patient management[10]. Different markers reflect pathophysiologic processes that may be quantified and interpreted. Of key importance for OA, and possibly the inflammatory-driven phenotype of OA since a higher percentage of those patients use nonsteroidal anti-inflammatory drugs (NSAIDs), is local tissue

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damage and tissue inflammation[11]. The interstitial membrane of the synovial lining is the main local area affected by inflammation and is mainly composed of types I and III collagen[12–15]. Matrix metalloproteinase (MMP)-2 and -9 have been implicated in the degradation of extracellular matrix components and are expressed by inflammatory cells such as macrophages[16]. Upon tissue inflammation, MMP expression and proteolytic activity is upregulated, causing protein fragments to be released into the circulation where they may serve as tissue-specific biomarkers. Type III collagen degraded by MMP (C3M) is a neo-epitope of type III collagen generated by both MMP-2 and -9 and has been shown to be elevated in OA, rheumatoid arthritis, and spondyloarthritis[16–23]. Importantly for this analysis, C3M has been shown to originate in the inflamed synovium in OA and is associated with the inflammatory phenotype in OA[24].

In the present study, we investigated a large panel ($n = 15$) of biomarkers of inflammation, bone, cartilage, and soft tissue remodeling reflective of the complexity of endstage joint disease. This panel of biomarkers was measured in a subset of patients who participated in phase III trials and received treatment with the investigational drug tanezumab for pain associated with moderate-to-severe OA. Tanezumab is a monoclonal antibody that binds to and inhibits the actions of nerve growth factor. Nerve growth factor inhibition significantly lowers pain in preclinical and clinical studies[25–36]. The patient subset in which biomarkers were measured included those patients who underwent all-cause TJR (investigator-reported adverse event of osteonecrosis (ON) and/or TJR surgery) and for whom biomarker samples were available and those who did not undergo TJR during the course of the trials (matched controls).

The aims of this analysis were two-fold: (1) Derive biomarker phenotypes in patients who undergo an all-cause TJR; (2) Understand how chronic NSAID use (≥90 days) alter the biomarker phenotypes that are associated with an all-cause TJR. This second aim was particularly pertinent since across the phase III tanezumab trials, the rate of all-cause TJR in patients treated with tanezumab combined with NSAIDs was over two-fold greater than the rate in patients treated with tanezumab monotherapy, NSAIDs, or placebo[33].

Methods

Studies and samples

Serum or plasma samples were collected as retention samples in phase III studies designed to investigate efficacy and safety of tanezumab in treating pain in patients with moderate-to-severe knee or hip OA (Supplemental Table I)[25–29,31,34–36]. During the clinical trials, patients were randomized to tanezumab monotherapy, tanezumab + NSAIDs, NSAID monotherapy, oxycodone, or placebo. Regardless of randomized treatment in the clinical trials, patients included in this analysis were reclassified into three categories (groups) based on patient NSAID use before and during tanezumab treatment: (1) no NSAID use during screening and for <90 days during tanezumab treatment ("nonNSAID user"); (2) NSAID use during screening and for <90 days during treatment ("scrNSAID user"); and (3) NSAID use during screening and for ≥90 days during treatment ("NSAID user"). NSAID use during screening only (i.e., scrNSAID users) was assessed as a separate category from nonNSAID users since taking an NSAID during screening could, comparatively, reflect a different disposition in these patients, which could result in different baseline biomarker phenotypes for the two groups. Contrasting the scrNSAID group (used <90 days during tanezumab treatment) with the NSAID-user group (used ≥90 days with tanezumab) allows for investigating whether combining an NSAID with tanezumab changes the baseline phenotype predictive of TJR.

An adjudication committee was formed to categorize the joint arthropathies in patients who underwent a TJR or had an investigator-reported adverse event of ON which was not always associated with a TJR[32]. Sufficient radiographic information was available for the adjudication committee to examine 249 of the 386 patients with an investigator-reported adverse event of ON and/or TJR (all-cause TJR) including all 87 adverse events described by investigators as ON[32]. Across the three NSAID groups in the dataset, samples for biomarker analysis were available from 174 of the 386 patients who underwent an all-cause TJR. Of these 174 patients, 26% were not adjudicated while 60% were adjudicated as either rapidly progressive OA (RPOA; type 1 [significant loss of joint space width (≥1 mm) in <1 year] or type 2 [abnormal loss and/or destruction of bone not normally present in endstage OA which, in the most severe form, was catastrophic bone failure and joint destruction][32]) or normal progression of OA (NPOA) and 0.6% ($n = 1$) as primary ON. The remaining 14% was not adjudicated to a specific category: 8% were adjudicated as "other", and 3.5% as either RPOA, NPOA or ON or "other" due to lack of info, and 2.3% for whom there was a lack of consensus (Table I). The TJR dataset had 14 patients who were reported by investigators to have ON (of which only one patient was adjudicated to ON) and did not undergo a TJR.

Samples were also collected from patients who did not experience an all-cause TJR, effectively serving as the control group. Controls were selected for inclusion in the analysis using propensity scores estimated from a logistic regression model to match them to the cases in a ratio of 2:1 of controls:cases based on the following covariates: age (<65 or ≥65 years), Kellgren–Lawrence (K-L) grade, gender, body mass index (<30 or ≥30 kg/m^2), number of doses of study medication received and baseline OA severity. Severe OA was defined as Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) Pain and Physical Function subscale scores ≥7 (on an 11 point numeric rating scale) and Patient Global Assessment score of "poor" or "very poor;" otherwise, OA was classified as "not severe". From the pool of identified controls, a total of 321 patients were selected that met the maximal number of matching criteria.

Samples were collected at baseline, mid-study (antecedent) and end-of-study (coincident) with data analysis conducted using sampling windows relative to the TJR event (Supplemental Table II). Considering the wide range in the timing of the antecedent (3–14 months) and coincident (0–2.7 months) sample collection before occurrence of the TJR event, biomarker data from these post-dose samples are presented in a graphical descriptive fashion only.

Samples were analyzed for 15 biomarkers according to manufacturer directions at BioClinica (formerly Synarc Laboratories; Lyon, France) using Certified Authorization Professional (CAP®; ISC^2, Clearwater, FL) certified standards (Table II). Samples were analyzed as single samples or in duplicate depending on the assay. Specifically, the assays for c-terminal telopeptides of type I collagen (CTX-I), total osteocalcin (OC), procollagen type I N-propeptide (PINP) and high-sensitivity C-reactive protein (hsCRP) were performed on single samples; in all other assays samples were run in duplicate. If the standard dilution resulted in a non-quantifiable result due to values exceeding the upper limit of the assay range, samples were run a second time at double the standard dilution factor as long as sufficient sample volume was available. Samples were pre-diluted in the assays for Dkk1 (1:4), MMP-9 (1:100), PIIANP (1:2), COMP (1:10), C3M (1:4), and VEGF (1:2).

Statistical analysis

Mean and 95% confidence interval (CI) were determined for the 15 biomarkers measured in baseline samples. Comparisons