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Serological biomarker profiles of rapidly progressive osteoarthritis in tanezumab-treated patients

M.A. Karsdal ‡ *, K.M. Verburg †, C.R. West †, A.C. Bay-Jensen ‡, D.S. Keller †, R.H.G.P. Arends †

† *Pfizer Inc., 445 Eastern Point Road, Groton, CT 06340, USA*
‡ *Nordic Bioscience, Herlev Hovedgade, DK-2730 Herlev, Denmark*

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SUMMARY

There is a need for efficacious and safe pain treatments for OA (osteoarthritis). The nerve growth factor (NGF) antibody tanezumab is associated with high efficacy, but when combined with chronic NSAID treatment shows an increased risk of rapidly progressive osteoarthritis (RPOA) in a small group of patients.

Aim: The aim of this study was to phenotype with biochemical biomarkers of bone, cartilage, soft tissue, synovial metabolism OA patients who are at risk of developing RPOA type-2, for both limited and chronic NSAIDs users.

Material and methods: The dataset consisted of OA patients participating in tanezumab trials who used NSAIDs <90 days (limited NSAID users) or chronic users (NSAIDs ≥90 days) over an average 10 month period. Biomarker data were available for 47 cases (RPOA type-2) and 92 controls. Non-linear and linear multivariable predictive models were developed.

Results: By use of two biomarkers at baseline the receiver operating characteristic (ROC) curve area for RPOA type-2 in limited NSAID users was 71%, [CI] (60–83%). OA subjects with this biomarker phenotype had 8-fold higher confidence interval [CI][(2–33)] relative risk of developing RPOA type-2 as compared to OA patients without this phenotype. The AUC of the model in chronic NSAIDs users based on 5 biomarkers was 78%, [CI](69–88%), with 4-fold [CI (2–6)] relative risk of developing RPOA type-2.

Conclusion: In this hypothesis generating and exploratory study, we identified combinations of biomarkers associated with OA patients who develop RPOA type-2, which may be related to the pathology of the RPOA type-2 joint.

Introduction

Osteoarthritis (OA), a degenerative joint disease, is the most prevalent form of arthritis[1]. OA is partly unpredictable and can remain silent for a long time. However, it can progress rapidly and manifests itself and is caused by cartilage loss, osteophytes and to some extent synovial inflammation, resulting in a reduction of joint space leading to joint function loss. The treatment for joint failure is total joint replacement (TJR). Pain is one common denominator of OA and often the reason for a first patient–doctor interaction. The high efficacy demonstrated with anti-NGF treatments currently in development, that exceeds the efficacy of available treatments, is very encouraging[2–4] as the benefit-risk ratio of available pain treatments is relatively low. During the conduct of Phase 3 clinical OA studies unexpected adverse events of rapidly progressive osteoarthritis (RPOA) were identified which resulted in a clinical hold of the anti-NGF programs.

There is a lack of data in the literature on the rate of rapidly progressive OA in a progressed OA population and the causes of this disease progression. As described by Hochberg and colleagues[5]"RPOA is characterized by pain, with radiographs showing rapid joint space narrowing as a result of chondrolysis (type-1)." Possibly subsequently, these patients progress to an osteolytic phase with severe progressive atrophic bone destruction (type-2).

* Address correspondence and reprint requests to: M.A. Karsdal, Nordic Bioscience A/S, Herlev Hovedgade 207, DK-2730 Herlev, Denmark. Tel.: 45-44-52-52-10.
E-mail addresses: mk@nordicbio.com (M.A. Karsdal), Kenneth.M.Verburg@pfizer.com (K.M. Verburg), Christine.West@pfizer.com (C.R. West), acbj@nordicbio.com (A.C. Bay-Jensen), David.S.Keller@pfizer.com (D.S. Keller), Rosalin.Arends@pfizer.com (R.H.G.P. Arends).

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However, this continuity is not clear due to a lack of longitudinal studies[5].

In the Phase III clinical OA studies of the investigational anti-NGF monoclonal antibody tanezumab there were 67 cases adjudicated as showing RPOA (RPOA type-1 $n = 11$ and RPOA type-2 $n = 56$) adjudicated from 7301 patients treated with tanezumab[5] in either the knee or the hip. These cases were initially reported by clinical investigators as osteonecrosis owing to the radiologic similarities between end stage OA or RPOA type-2 and osteonecrosis and their co-existence at certain stages. Of the 67 RPOA events all were observed in patients treated with tanezumab except for one RPOA type-2 event in a patient who was on NSAIDs only. The greatest risk of RPOA was observed in patients who received tanezumab in combination with oral nonsteroidal anti-inflammatory drugs (NSAIDs), particularly those patients treated with NSAIDs on a daily basis for greater than 90 days. Therefore, to lower the risk of RPOA, future development of tanezumab in the OA population will be as monotherapy. Importantly, tanezumab treatment in combination with NSAIDs did not provide substantial greater pain relief than tanezumab alone[2].

It is important that the population of patients that developed the bone destruction which is specific for the RPOA type-2 phenotype is carefully investigated as well as the role NSAIDs play in the development of this pathology. Therefore, in RPOA type-2 subjects who did or did not use NSAIDs chronically and for whom biomarker samples were available, biomarkers of inflammation, bone, cartilage, and soft tissue remodeling reflective of the complexity of end-stage joint disease[6] were measured.

Biomarkers of joint tissue turnover have been measured before in the tanezumab Phase 3 program[7]. However, the population evaluated was broad ($n = 174$) including any patient who underwent all-cause TJR (investigator-reported adverse event of osteonecrosis (ON) and/or TJR surgery) and both adjudicated and non-adjudicated TJR cases. The results supported that OA consists of a mixture of biomarker phenotypes reflecting the known heterogeneity of the underlying pathophysiology the OA disease. The focus of this paper is just the adjudicated group of patients with RPOA type-2 which due to the uniqueness of its clinical OA pathology would be expected to be associated with a more specific biomarker profile of the joint tissues. Also, in this more unique group not just the baseline data but also post-dose data were assessed for biomarker profiles. Therefore, the aims of the described analyses are to: 1. Derive predisposition biomarker phenotype(s) for patients who developed RPOA type-2. 2. Assess if the RPOA type-2 biomarker phenotypes are different between patients with limited NSAID use vs patients who use NSAIDs chronically, 3. Evaluate if the biomarker phenotypes change from baseline with tanezumab treatment.

Methods

Studies and samples

The blood samples used to measure the biomarkers were retention samples collected across 6 clinical Phase 3 trials that were designed to investigate efficacy and safety of tanezumab when treating pain in patients with moderate-to-severe knee or hip OA[2–4,8,9]. In these trials (A4091014; NCT00744471, A4091016; NCT00809783, A4091017; NCT00864097, A4091025; NCT00809354, A4091030; NCT00985621, A4091043; NCT00994890), patients were randomized to one of the following treatments: tanezumab monotherapy, tanezumab + NSAIDs, NSAID monotherapy, controlled release oxycodone, or placebo (Table I). But for the biomarker data analysis the patients across these treatment were reclassified into two categories (groups) based on patient NSAID use during the duration of the treatment which was on average 10 months long: (1) NSAID use for <90 days during tanezumab treatment (2) NSAID use for ≥90 days during treatment.

In total, 56 OA patients were adjudicated with rapidly progressive OA type-2[5] and of these subjects samples for biomarker analysis were available in 47 patients at one or more time points during study duration. In this group of 47 patients, baseline biomarker data were available in 11 of the 13 patients who were limited NSAID users and in 26 of the 34 patients who were chronic NSAID users.

The control group for the RPOA type-2 cases consisted of OA patients who did not experience a joint-related safety event. These controls were selected using propensity scores estimated from a logistic regression model, to match them to the cases on as many

Table I
Demographics in Subjects used in the Baseline Biomarker Analysis

Characteristic	Cases (RPOA type-2)		Controls (mOA)	
	Limited NSAID user	Chronic NSAID user	Limited NSAID user	Chronic NSAID user
N	11	26	36	33
Gender				
Female	9 (81.8%)	22 (84.6%)	32 (88.9%)	28 (84.8%)
Male	2 (18.2%)	4 (15.4%)	4 (11.1%)	5 (15.2%)
Age				
Mean (SD)	63.4 (9.44)	61.3 (7.69)	63.9 (12.6)	62.0 (6.81)
BMI	30.4 (5.77)	32.0 (5.29)	31.6 (5.39)	32.6 (5.27)
WOMAC Pain score				
Mean (SD)	7.4 (1.67)	7.3 (1.69)	7.5 (1.84)	6.9 (1.66)
KL Grade				
2	1 (9.1%)	6 (23.1%)	8 (22.2%)	7 (21.1%)
3	7 (63.6%)	13 (50.0%)	20 (55.6%)	14 (42.4%)
4	3 (27.3%)	7 (26.9%)	8 (22.2%)	12 (36.4%)
Randomized Treatment				
Placebo	0	0	1 (2.8%)	0
Tanezumab	9 (81.8%)	0	29 (80.6%)	0
Tanezumab + NSAID	2 (18.2%)	25 (96.2%)	4 (11.1%)	27 (81.8%)
Active Comparator	0	1 (3.8%)	2 (5.6%)	6 (18.2%)
NSAID use during treatment				
yes	5 (45.5)	26 (100%)	14 (38.9)	33 (100%)
no	6 (54.5)	0 (0%)	22 (61.1)	0 (0%)
Time on Study (weeks)				
Mean (SD)	32.7 (17.7)	35.5 (20.4)	38.7 (23.9)	41.2 (14.1)

Table II
Mean (95% CI) and [min, max] biomarker concentrations at baseline in limited and chronic NSAID users*

Biomarker	Limited NSAID users		Chronic NSAID users		Reference value† 5th-95th percentile
	Control patients	RPOA type-2 (cases)	Control patients	RPOA type-2 (cases)	
CTX-I, ng/mL	*n* = 35	*n* = 11	*n* = 30	*n* = 26	*n* = 430
	0.321 (0.258–0.384) [0.044, 0.725]	0.273 (0.187–0.359) [0.100, 0.522]	0.269 (0.214, 0.325) [0.044, 0.629]	0.250 (0.205–0.295) [0.044, 0.535]	0.259–0.950
Total OC, ng/mL	*n* = 34	*n* = 11	*n* = 30	*n* = 26	*n* = 192
	23.4 (19.4–27.5) [11.3, 59.4]	21.1 (16.6–25.7) [9.88, 32.4]	19.9 (17.7, 22.0) [8.30, 33.8]	21.2 (17.9, 24.4) [6.74, 36.8]	16.5–51.3
PINP, ng/mL	*n* = 34	*n* = 11	*N* = 30	*N* = 26	*n* = 216
	52.8 (43.9–61.6) [18.9, 117]	49.1 (39.1–59.0) [21.6, 73.5]	48.9 (41.1, 56.4) [4.60, 106]	50.4 (41.3, 59.5) [22.5, 113]	24.9–90.3
SOST, ng/mL	*n* = 19	*n* = 4	*n* = 28	*n* = 20	*n* = 30
	0.777 (0.653–0.900) [0.352, 1.24]	0.748 (0.595–0.902) [0.619, 0.853]	0.824 (0.741, 0.907) [0.505, 1.26]	0.710 (0.619, 0.800) [0.422, 1.06]	0.531–1.19
Dkk1, pg/mL	*n* = 21	*n* = 5	*n* = 28	*n* = 20	*n* = 29
	773 (458–1090) [177, 3180]	525 (260–789) [231, 804]	780 (532, 1030) [180, 3350]	664 (387, 941) [83.5, 2800]	481–1650
ICTP, ng/mL	*n* = 32	*n* = 10	*n* = 29	*n* = 26	*n* = 28
	5.24 (4.56–5.91) [0.785§, 10.7]	4.29 (3.43–5.14) [2.36, 6.63]	4.65 (4.06, 5.23) [1.79, 8.07]	4.20 (3.68, 4.71) [2.21, 6.92]	2.62–6.57
C1M, ng/mL	*n* = 31	*n* = 11	*n* = 29	*n* = 25	*n* = 30
	277 (228–326) [78.2, 500]	261 (178–343) [63.3, 462]	323 (273, 374) [133, 676]	288 (250, 326) [125, 486]	144–369
MMP-9, ng/mL	*n* = 34	*n* = 11	*n* = 31	*n* = 26	*n* = 30
	428 (322–535) [33.5, 1430]	442 (331–551) [253, 734]	361 (300, 422) [33.5, 715]	397 (287, 505) [33.5, 1140]	289–1090
C2M, ng/mL	*n* = 34	*n* = 11	*n* = 29	*n* = 26	*n* = 31
	0.322 (0.241–0.404) [0.091, 1.31]	0.502 (0.351–0.653) [0.190, 0.934]	0.447 (0.324, 0.570) [0.091, 1.48]	0.483 (0.344, 0.622) [0.091, 1.55]	0.113–0.603
PIIANP, ng/mL	*n* = 32	*n* = 11	*n* = 27	*n* = 19	*n* = 49
	1750 (1310–2190) [181, 5070]	1390 (939–1840) [515, 2550]	1630 (1390, 1880) [680, 2970]	1820 (1430, 2210) [816, 4160]	425–2050
COMP, U/L	*n* = 34	*n* = 11	*n* = 30	*n* = 26	*n* = 187
	9.58 (8.35–10.8) [3.69, 15.9]	9.21 (7.42–11.0) [5.76, 15.5]	10.0 (8.83, 11.2) [4.18, 17.0]	9.78 (8.69, 10.9) [5.49, 14.8]	6.90–16.0
C3M, ng/mL	*n* = 34	*n* = 11	*n* = 30	*n* = 26	*n* = 30
	41.1 (35.3–46.9) [16.8, 86.2]	31.3 (24.5–38.0) [21.0, 52.6]	41.9 (35.1, 48.6) [19.0, 93.3]	35.7 (31.4, 40.1) [13.8, 55.9]	14.9–34.4
VEGF, pg/mL	*n* = 22	*n* = 5	*n* = 27	*n* = 18	*n* = 24
	94.0 (59.9–128) [18.7, 327]	44.9 (23.0–66.9) [18.7, 66.6]	97.1 (70.3, 124) [18.7, 229]	67.6 (41.2, 94.0) [18.7, 174]	22.4–249
IL-6, pg/mL	*n* = 30	*n* = 10	*n* = 26	*n* = 25	*n* = 32
	3.49 (1.74–5.25) [0.468, 20.0‡]	3.60 (−0.575–7.77) [0.642, 20.0]	2.03 (1.53, 2.53) [0.648, 5.87]	2.02 (1.36, 2.68) [0.444, 6.746]	0.390–1.71
hsCRP, mg/L	*n* = 28	*n* = 10	*n* = 26	*n* = 24	*n* = 173
	3.78 (2.30–5.27) [0.040, 16.5]	2.99 (0.253–5.731) [0.680, 13.0]	5.67 (3.43, 7.91) [0.480, 20.0]	3.17 (2.01, 4.33) [0.350, 11.6]	0.15–4.13
PINP:total OC	*n* = 34	*n* = 11	*n* = 30	*n* = 26	NA
	1.09 (0.758–1.42) [0.158, 5.86]	1.60 (−0.06–3.25) [0.500, 9.00]	1.76 (0.220, 3.31) [0.043, 23.5]	1.29 (0.698, 1.88) [0.255, 8.00]	
CTX-I:total OC	*n* = 34	*n* = 11	*n* = 30	*n* = 26	NA
	1.19 (0.852–1.53) [0.304, 5.15]	1.29 (0.101–2.48) [0.212, 6.50]	1.01 (0.624, 1.40) [0.123, 5.63]	0.908 (0.670, 1.15) [0.200, 2.71]	

Note: Refer to Table II, column 1 for definitions of abbreviations.
All ratios are calculated as the ratio of the individual percentile transformed values based on the distribution over all available data.
Values have been rounded to 3 significant digits.

* limited NSAID users: NSAID use <90 days during treatment. Chronic NSAID users: NSAID use ≥90 days during treatment.
† From a healthy, post-menopausal population. Measured in same assays kits and in the same assay lab as the values measured in the samples collected in the investigated OA subgroups.
‡ upper limit of quantitation of the assay after diluting at the maximum allowed dilution of 1:2. Values above the limit of quantitation were set to the upper limit value.
§ lower limit of quantitation of the assay: Values below the limit of quantitation were set to half the lower limit value.

Table III
In limited NSAID users: Diagnostic measures of model performance at baseline, more than 3 months (antecedent) and less or equal than 3 months (coincident) from the rapidly progressive osteoarthritis (RPOA) type-2 event

Time point	Cases/controls	Biomarker	AUC (%) (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Baseline	11/36	C2M, C3M	71 (60–83)	86 (64–100)	56 (42–70)
>3 months	8/31	PINP/OC, CTX-I, PINP	82 (72–91)	91 (73–100)	68 (51–81)
≤3 months	7/14	PINP/OC	78 (62–95)	60 (30–90)	96 (89–100)

variables as possible from the following list, as previously described[7]: age (<65 or ≥65 years), Kellgren–Lawrence (KL) grade, gender, body mass index (<30 or ≥30 kg/m2), 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". In addition, controls were matched to the RPOA type-2 cases on NSAID use (<90 days or >90 days). In total, the matching criteria resulted in 92 patients suitable as controls for the RPOA type-2 cases. Given the large number of biomarker determinations per sample, we limited the number of matches to ~2 which greatly improved the precision of estimates relative to a single matching control and a pragmatic balance between statistical power and cost.

Given the large number of biomarker determinations per sample, we limited the number of matches to 2 which greatly improved the precision of estimates relative to single matching control and a pragmatic balance between statistical power and cost. For the data analysis, for the RPOA type-2 cases, the retention samples were binned according to the collection time relative to the timing of the RPOA type-2 event: baseline, antecedent (>3 months prior to the RPOA type-2 event) and coincident (within 3 months before the RPOA type-2 event)[10]. Post-dose control samples to match the cases had to be collected 21–182 days postdose for the antecedent analysis and >183 days postdose for the coincidence analysis.

The biomarker data which have been published and described[10], were reanalyzed in this sub analysis of RPOA type-2.

Statistical analysis

Classification and Regression Tree (CART)[11,12] methods that were used in the previous work[7] were also applied to develop multivariable predictive models in this subset of cases and controls. Models were fit including baseline, antecedent or coincident biomarker data for the limited or chronic NSAID users during tanezumab treatment. To avoid over-fitting, we used the one minus standard error rule for pruning the number of tree splits. Complexity parameters and standard errors were derived from cross-validation over 10 subsets of the original dataset. This methodology led to extreme over-pruning with this limited dataset (eliminating all biomarkers but C2M for the limited NSAID group), so the final pruning method was simply set to a minimum of 10 observations per tree node.

Bayesian priors were used to assign equal weight to cases and controls for assessing misclassification loss. In addition to each individual biomarker the ratio of procollagen type I N-propeptide (PINP):total osteocalcin (OC) and degradation products of c-terminal telopeptides of type I collagen (CTX-I):total OC were selected as possible predictor variables. Ratios were computed based on percentile transformation of each variable.

Model performance was summarized by receiver operating characteristic (ROC) curves. The 95% confidence intervals (CIs) for the area under the curve were estimated using a non-parametric approach[13]. The 95% confidence interval [CI]s for sensitivity and specificity, using the 50% threshold, were approximated using a stratified non-parametric percentile method (over 2000 bootstrapped samples) as programmed in pROC open source package for R[14,15].

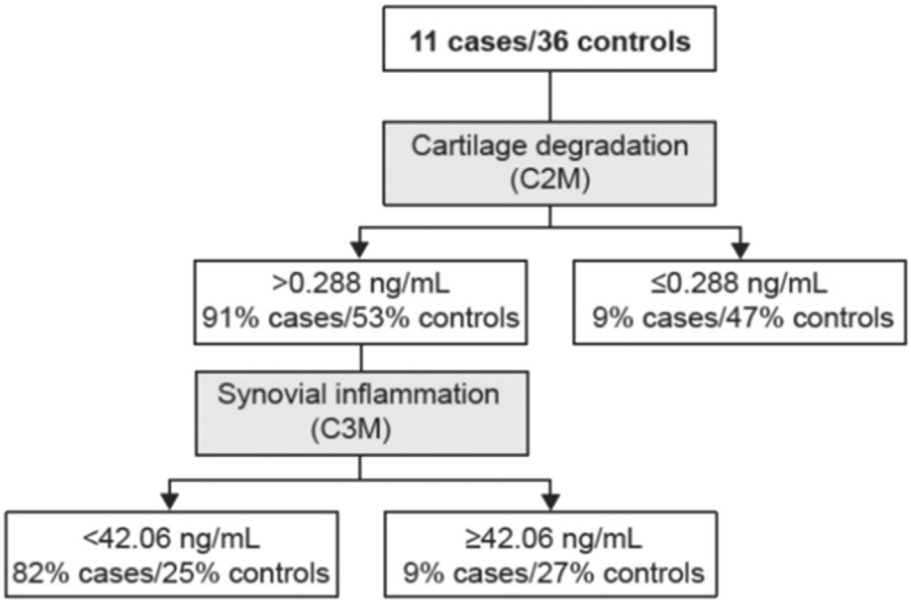

Fig. 1. In limited NSAID users: Decision tree as a predictive model (CART) that maps the biomarker data that result in a rapidly progressive osteoarthritis (RPOA) type-2 event or not.

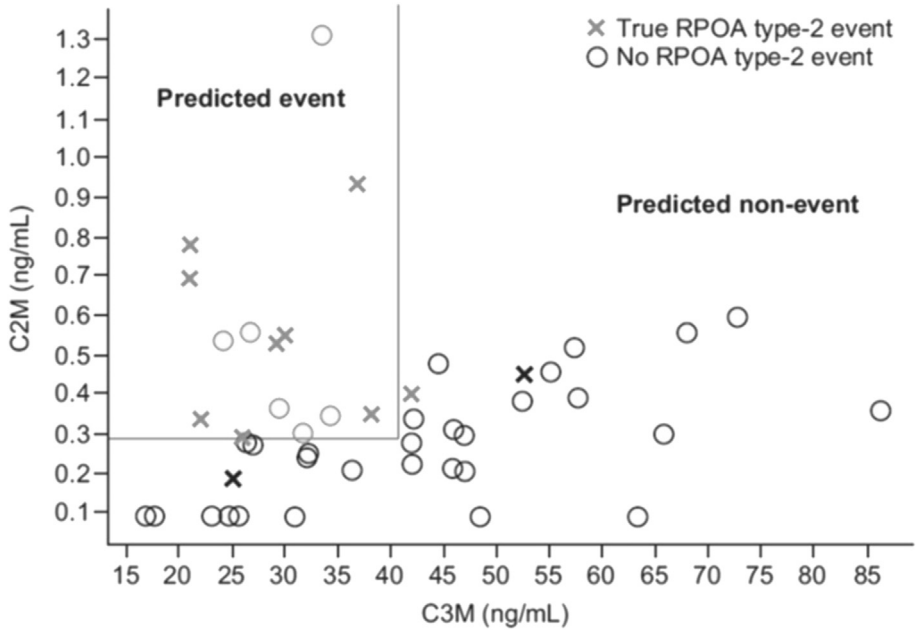

Fig. 2. Scatterplot of C2M vs C3M at baseline with Classification and Regression Tree (CART) predicted classification regions and true observed case status overlaid.

For the limited NSAID users' biomarker data, we also used poisson regression in addition to the CART method to confirm the robustness of our results to the somewhat arbitrary binning into time categories described above. Collapsed over sampling times by selecting the result from the sample furthest in time from the event of interest we included data from all 13 cases and 48 controls and incorporated an offset equal to the natural log of the number of days between the sample collection and either RPOA type-2 event or the end of follow-up for patients not having events. Biomarkers included were the subset of biomarkers identified in the sample time specific CART models.

Results

The demographics for the subjects included in the baseline CART analysis are reported in Table I. The groups were well balanced. Baseline biomarker values are provided in Table II.

Using the CART analysis approach, Table III provides the measures of the diagnostic performance of the model at baseline, >3 months and ≤3 months relative to the timing of the RPOA type-2 event. The accuracy of predicting subjects who experienced RPOA type-2 and who did not is reflected by the AUC of the ROC curve. When using baseline biomarker data exclusively the accuracy was 71% (95% CI: 60–83%) for the limited NSAID using group. The derived baseline model consisted of the two biomarkers C2M and C3M, which represent cartilage tissue degradation and synovial inflammation, respectively (Table III, Fig. 1). Figure 2 shows the prediction of this non-linear model and the overlap between CART predicted classification regions and true observed case status. The two markers resulted in one biomarker phenotype for RPOA type-2 and two biomarker phenotypes for the OA controls (Fig. 1). The phenotype for RPOA type-2 was one of high cartilage degradation and synovial inflammation in the upper range of what is observed in healthy non-OA subjects. The phenotypes for OA controls were one of high cartilage degradation in the presence of synovial inflammation in the upper range of what is typical in OA patients and one of just low cartilage degradation. Despite the fact that the model still misclassifies 14% of RPOA type-2 cases (the median sensitivity was 86% (95% CI: 64–100%)) the odds of RPOA type-2 was clearly higher (8-fold, 95% CI: 2–33)) in the subjects with the phenotype of high cartilage degradation and elevated synovial inflammation (relative to healthy non-OA subjects) compared to

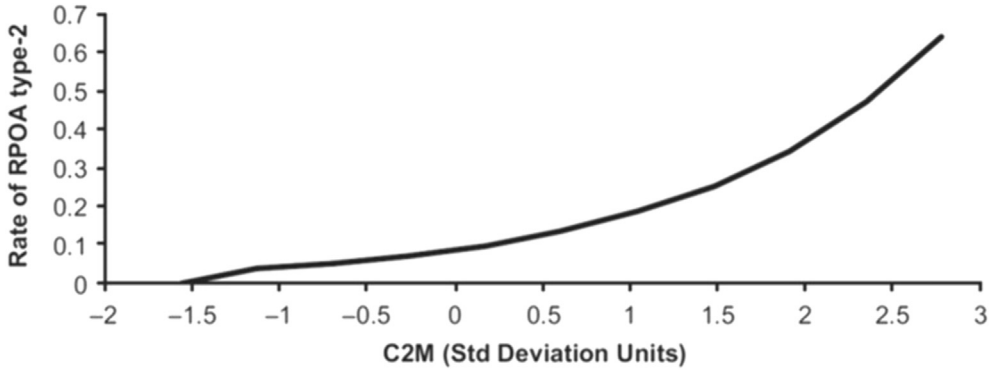

Fig. 3. Poisson regression predicted rate of RPOA-2 as a function of baseline C2M level expressed as the standardized difference from overall baseline mean*. *Standardized: the difference between the individual value and the overall mean was divided by the standard deviation.

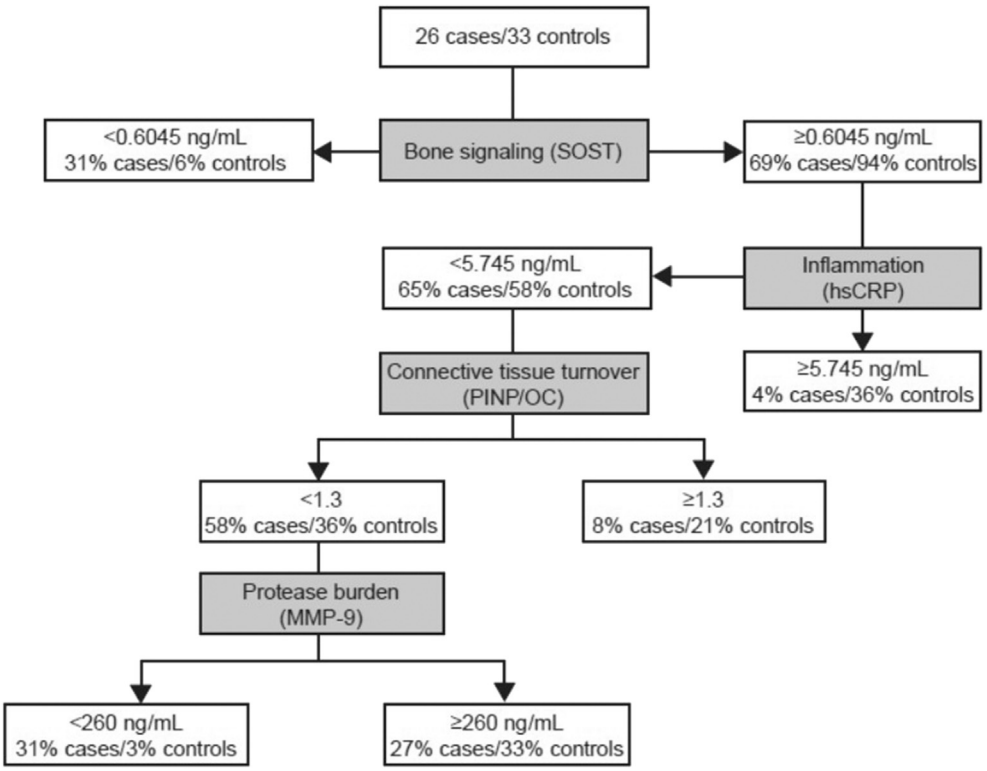

Fig. 4. In chronic NSAID users: Decision tree as a predictive model (CART) that maps the biomarker data that result in a RPOA type-2 event or not.

Table IV
In chronic NSAID users: Diagnostic measures of model performance at baseline, more than 3 months and less or equal than 3 months from the RPOA type-2 event

Time point	Cases/controls	Biomarker	AUC (%) (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Baseline	26/33	SOST, hsCRP, PINP/OC, MMP9	78 (69–88)	47 (32–62)	93 (84–100)
>3 months	17/23	MMP9, COMP, SOST, OC	79 (70–89)	48 (32–68)	83 (69–94)
≤3 months	18/14	ICTP CTX-I/OC,COMP	84 (77–92)	58 (38–77)	100 (100–100)

the subjects who had one of the two control phenotypes. The predictive biomarkers changed over time from cartilage and synovial biomarkers (C2M and C3M) to bone biomarkers (PINP, CTX-1, OC) (Table III).

Results from the poisson regression model indicated that only C2M was a borderline significant ($p = 0.087$) individual predictor of RPOA type-2. Figure 3 shows the increase in the poisson predicted rate of RPOA type-2 as the baseline C2M value increases. This analysis confirmed the importance of C2M as an important early predictor of RPOA type-2.

Baseline CART analysis for chronic NSAIDs users is visualized in Fig. 4. The odds of RPOA type-2 was 4-fold (95% CI: 2–6) higher in the subjects with the phenotype of low bone signaling or low connective tissue turnover compared to the subjects who had one of the three controls phenotypes. The predictive biomarkers changed over time from bone and connective turnover markers (SOST, MMP-9), to markers of bone and connective tissue degradation (ICTP, CTX-I) (Table IV).

Discussion

Development of novel treatments for OA increasingly involves precision medicine tools. To this end, biomarkers can help encode and divide patient phenotypes hereby identifying and giving priority to the patient group that would gain the most from various programs of intervention[16,17]. The aim of this current analysis was to identify serum biomarker phenotypes that are associated with the clinical event of RPOA type-2 in a symptomatic OA population who are treated with tanezumab and use NSAIDs either in a limited fashion or chronically.

The main findings of the current analyses were that a baseline biomarker phenotype associated with RPOA type-2 was identified with good accuracy in both the limited and chronic NSAID users (71% and 78% respectively) using the CART approach. This phenotype was associated with respectively 8-fold or 4-fold higher relative risk of experiencing a RPOA type-2 event compared to when this profile was not present.

In the limited NSAID users, the biomarker phenotype of RPOA type-2 predisposition support a profile of high cartilage degradation (C2M) in the presence of elevated (upper normal range) synovial tissue inflammation (C3M) and reflects the interplay of tissues. Consistent with the pathophysiology of this joint-related safety event, the biomarkers important for distinguishing cases from controls changed to biomarkers of bone tissue turnover (PINP, OC, CTX-I) advancing from pre-treatment to treatment. In the chronic NSAID users, the phenotype of RPOA type-2 predisposition reflected low tissue turnover, either specifically of bone (SOST) or more general of connective tissue (MMP-9). Over time the chronic NSAID phenotypes changed to a more destructive profile with higher levels of degradation markers (MMP-9, 1-CTP, CTX-I). It is

not surprising that the limited and chronic NSAID using phenotypes are different, as NSAID use has been shown to affect the outcome of tanezumab studies, and affects bone health[2,10,18]. In general, the biomarker profiles seem to reflect inflammation and cartilage initially, whereas at the coincident time point, biomarkers were more related to bone. This may be intuitively correct, as the RPOA phenotypes are associated with bone pathology.

We used an advanced multivariable non-linear statistical approach as a first choice to develop prediction models. Interestingly, the traditional linear method that was subsequently evaluated for the limited NSAID users only identified C2M (cartilage degradation), not synovial inflammation (C3M) as a biomarker of interest associated with RPOA type-2. The difference in the results between the two methods could be partly due to the relatively small sample population but suggests that more advanced methods such as CART are needed to address complex relationships between biological changes. Linear regression methods test simple direct effects of biomarkers and cannot derive more than one biomarker combination that is associated with a clinical outcome. These differences are visualized in Figs. 2 and 3.

A list of well-defined biomarkers which includes novel serological markers was quantified in the current study. This comprehensive list reflects joint tissue turnover, including cartilage turnover (C2M[19], PIIANP[20] and COMP[21], bone turnover (CTX-I, PINP, OC[22]), inflammation (hsCRP, IL-6[23,24,25]) and connective tissue turnover (C3M, C1M, ICTP and MMP-9[26]). Moreover, it consists of bone-related Wnt signaling biomarkers (SOST and Dkk1)[27]. These molecular serological biomarkers have been shown to be associated with different pathophysiological features of OA and joint diseases. Unfortunately urinary CTX-II was not quantified due to lack of samples, albeit urine CTX-II is the cartilage degradation marker that has been shown to be the best prognostic biomarker in the field[28,29]. Recently bone and cartilage biomarkers in the FNIH/OAI cohorts were shown to be prognostic for structural progression which included the biomarkers CTX-I, NTX, alpha-CTX-I, CTX-II and PIIANP[28,30]. C2M, PIIANP and COMP specifically have been associated with incidence and progression of OA[21]. CTX-I is a biomarkers of bone resorption and bone loss whereas PINP and OC is indicative of bone formation and bone gain[31]. C3M and C1M are biomarkers of respectively type 3 and 1 collagen destruction by MMPs and are reflective of connective tissue inflammation which is associated with synovial inflammation and systemic tissue inflammation. ICTP and MMP-9 reflect MMP mediated tissue turnover[32,33,34]. Consequently, the panel we selected reflects the state of the art in joint turnover and inflammation biomarkers that have been used in other studies[35,36,37,38] of which some were measured in the OA initiative (CTX-I, CTX-II)[28,30] and include certain novel markers associated with joint biology[39,33].

For the all-cause TJR endpoint previously analyzed, biomarkers of inflammation (C3M, IL-6) were the most impactful biomarkers that distinguished patients who progressed to an all-cause TJR as compared to OA patients who did not have a joint-related safety event (10). Inflammation was higher in the overall tested OA population compared to what is typically measured in healthy subjects. OA patients whose inflammatory markers were in the upper 25th percentile were less likely to progress to a TJR, possibly reflecting the capacity to repair. In the RPOA type-2 populations, inflammation was part of the RPOA type-2 biomarker phenotypes (C3M, hsCRP) but secondary to high cartilage degradation and low bone signaling which were the most important variables in the limited and chronic NSAID users respectively.

This analysis is confounded by several limitations. Considering the small data set due to the rarity of the RPOA type-2 event it is notable that statistically significant biomarkers were identified that can distinguish RPOA type-2 patients from patients without a joint-related safety event. Still, the cut-off values of the biomarkers that separate the cases from the controls need to be regarded with caution, and for actual clinical utility need to be validated in more clinical studies, with full scale validation of the analytical tests as required by the FDA. These cut-off values are dataset-dependent and would have to be confirmed in independent and preferably larger datasets. Phase IIÍ studies are under way which may be used for this, and the current hypothesis generating analysis may inform multiple stakeholders, both regulatory and different companies engaged in nerve growth factor (NGF) research, with a testable hypothesis. Publications are not available on selected or validated OA biomarkers, or even biomarkers that would predict RPOA type-2. In the present study we used a set of biomarkers that have been used in rheumatology research[32,26,40,41], rather than a hypothesis free set. Had we used a hypothesis free approach, a different outcome may have resulted. Event though considered standard in clinical biomarker research, another important limitation this study is the case controlled nature, which may overestimate the AUC[42,43],.

In conclusion, in this hypothesis generating and exploratory study, biomarkers associated with a high risk of experiencing a RPOA type-2 were identified in patients who are limited or chronic NSAID users. The results suggest that in a progressed OA population in which RPOA type-2 develops, a predisposition of high cartilage degradation in combination with elevated synovial tissue inflammation is more prevalent in limited NSAID using subjects and a predisposition of low tissue turnover rate is more prevalent in chronic NSAID using subjects compared to OA subjects who do not develop RPOA type-2.

Author contributions

The authors declare their contributions to this manuscript as follows:

(1) The conception and design of the study, or acquisition of data, or analysis and interpretation of data: RHGP Arends, MA Karsdal, KM Verburg, CR West, AC Bay-Jensen, DS Keller
(2) Drafting the article or revising if critical for important intellectual content: RHGP Arends, MA Karsdal, KM Verburg, CR West, AC Bay-Jensen, DS Keller
(3) Final approval of the version to be submitted: RHGP Arends, MA Karsdal, KM Verburg, CR West, AC Bay-Jensen, DS Keller
(4) The following author takes responsibility for the integrity of the work as a whole, from inception to finished article: Rosalin H. Arends (Rosalin.Arends@pfizer.com).

Competing interests statement

Morten A. Karsdal and Anne C. Bay-Jensen are full-time employees of and hold stock in Nordic Bioscience, a company engaged in the discovery, development and utilization of biomarkers.

Rosalin H.G.P. Arends, Kenneth M. Verburg, Christine R. West, and David S. Keller are employees of and hold stock or stock options in Pfizer Inc.

Role of the funding source

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