ORIGINAL RESEARCH

Baseline serum levels of cross-linked carboxy-terminal telopeptide of type I collagen predict abatacept treatment response in methotrexate-naive, anticitrullinated protein antibody-positive patients with early rheumatoid arthritis

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ABSTRACT

Objective To investigate correlations between biomarkers of bone remodelling and extracellular matrix turnover with baseline disease activity and treatment response in patients with early rheumatoid arthritis (RA).

Methods Assessing Very Early Rheumatoid arthritis Treatment-2 (AVERT-2; NCT02504268) included disease-modifying antirheumatic drug-naive, anti-citrullinated protein antibody (ACPA)-positive patients randomised to weekly subcutaneous abatacept+methotrexate (MTX) or abatacept placebo+MTX for 56 weeks. This post hoc exploratory subanalysis assessed the association between baseline disease activity and eight biomarkers (Spearman's correlation coefficient), and whether baseline biomarkers (continuous or categorical variables) could predict treatment response at weeks 24 and 52 (logistic regression).

Results Patient characteristics were similar between overall (n=752) and biomarker subgroup (n=535) populations and across treatments. At baseline, neoepitopes of matrix metalloproteinase-mediated degradation products of types III and IV collagen and of C reactive protein (CRP) showed the greatest correlations with disease activity; cross-linked carboxy-terminal telopeptide of type I collagen (CTX-I) showed weak correlation. Only CTX-I predicted treatment response; baseline CTX-I levels were significantly associated with achieving Simplified Disease Activity Index remission and Disease Activity Score in 28 joints (DAS28 (CRP)) <2.6 (weeks 24 and 52), and American College of Rheumatology 70 response (week 52), in patients treated with abatacept+MTX but not abatacept placebo+MTX. CTX-I predicted significant differential response between arms for DAS28 (CRP) <2.6 (week 24). Treatment differences were greater for abatacept+MTX in patients with medium/high versus low baseline CTX-I.

Conclusion In MTX-naive, ACPA-positive patients with early RA, baseline CTX-I predicted treatment response to abatacept+MTX but not abatacept placebo+MTX.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The availability of multiple therapeutic agents for the treatment of rheumatoid arthritis (RA) means that clinical remission is an achievable goal for some patients.

⇒ The absence of effective biomarkers to aid clinical decision making for appropriate individualised targeted therapy remains a significant unmet need.

WHAT THIS STUDY ADDS

⇒ We report on the association of bone remodelling biomarkers, extracellular matrix turnover and baseline disease activity in patients with early RA who were anti-citrullinated protein antibody-positive and methotrexate (MTX)-naive, and the ability of such biomarkers to predict clinical treatment response to abatacept+MTX.

⇒ This post hoc analysis of data from a randomised controlled trial showed that biomarkers of synovial (matrix metalloproteinase (MMP)-mediated degradation products of types III and IV collagen (C3M and C4M)) and systemic (MMP-mediated degradation product of C reactive protein) inflammation were associated with disease activity at baseline.

⇒ Baseline cross-linked carboxy-terminal telopeptide of type I collagen (CTX-I), a biomarker of bone remodelling, predicted response to treatment with abatacept+MTX but not abatacept placebo+MTX.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Based on these data, further studies of CTX-I as a predictive biomarker of response to treatment with biologic disease-modifying antirheumatic drugs in patients with early RA are warranted.

INTRODUCTION

Rheumatoid arthritis (RA), a heterogeneous chronic autoimmune inflammatory disease, is characterised by the production of both pathogenic autoantibodies (rheumatoid factor and anti-citrullinated protein antibodies (ACPAs)) and proinflammatory cytokines, leading to the development of synovitis and systemic inflammation.[1–4] Furthermore, the disease is associated with the resorption of cartilage and bone, the development of bone erosions and longer-term destruction of synovial joints.[1–4]

Treatment guidelines from both the American College of Rheumatology (ACR) and the European Alliance of Associations for Rheumatology (EULAR) recommend a treat-to-target approach to managing disease in patients with RA, with clinical remission as the main therapeutic target.[5 6] However, recent guidance from ACR conditionally recommends an initial target of low disease activity, with a subsequent goal of remission, based on factors including patient preference.[6]

The increasing availability of multiple therapeutic agents with different mechanisms of action, which have the potential to be linked to the pathobiology of individual patients, means that clinical remission is now an achievable goal for more patients than was previously considered.[4] Nevertheless, a significant unmet need in the treatment of RA is the absence of effective biomarkers to aid clinical decision making for appropriate individualised targeted therapy.

Patients with RA who are ACPA positive have a poorer prognosis and are more likely to develop severe erosive disease than patients who are ACPA negative.[7 8] Data from clinical trials and real-world studies have shown that responses to treatment may vary based on ACPA status.[9–12] However, despite extensive study of many potential candidates over the years, no effective serum biomarker, other than perhaps ACPA, has been found that reliably predicts disease progression or treatment response to specific medications prior to starting therapy.[2 4] Additionally, no serum biomarker has been identified that can reliably assess treatment response (or lack of response) while receiving therapy in a meaningful number of patients.

Among patients with RA who are ACPA positive, bone loss can occur early and prior to the onset of clinical symptoms (eg, synovitis, arthralgia).[13] Furthermore, loss of bone mineral density in early, undifferentiated arthritis can predict the development of RA.[14] Thus, markers of bone remodelling and turnover of extracellular matrix (ECM) proteins might serve as disease-relevant surrogate biomarkers indicative of synovial joint pathophysiology.[15–17] Type I collagen is a major component of bone matrix, and fragments of type I collagen, known as cross-linked carboxy-terminal telopeptide of type I collagen (CTX-I), can act as specific markers of bone resorption.[18] The breakdown of collagen by matrix metalloproteinases (MMPs) leads to an increase in serum levels of MMP-degradation products of collagen type I (C1M), type III (C3M) and type IV (C4M).[19–24] In addition, MMP-mediated degradation of C reactive protein (CRPM) is a marker of MMP-mediated degradation of the acute phase reactant CRP and is released into the circulation during inflammation.[25] Studies have shown that serum levels of CTX-I, C1M, C3M, C4M and CRPM are elevated in patients with RA and that their production can be significantly reduced in response to treatment.[15–17 20 23 26]

Cathepsin K, released by osteoclasts, is the major producer of CTX-I through receptor activator of nuclear factor κ-B ligand (RANKL)-dependent pathways.[27 28] In patients with RA, RANKL is one of the principal factors involved in the differentiation of osteoclasts and their subsequent invasion of the periosteum.[2 29] Furthermore, in RA, activated T cells produce high levels of RANKL and promote osteoclast differentiation through both RANKL-dependent and RANKL-independent mechanisms.[4 29 30]

Abatacept, a selective T-cell costimulation modulator that blocks the interaction between cluster of differentiation (CD)80/CD86 on antigen-presenting cells and CD28 on T cells and disrupts naive T-cell activation, is effective in treating patients with RA, including those with early RA,[31 32] when a window of opportunity may exist for improved long-term outcomes.[33 34] The phase 3b AVERT-2 (Assessing Very Early Rheumatoid arthritis Treatment-2) trial (NCT02504268) evaluated the efficacy of subcutaneous (SC) abatacept+methotrexate (MTX) versus abatacept placebo+MTX in treating MTX-naive patients with seropositive, early, active RA.[35] Although the primary study endpoint (Simplified Disease Activity Index (SDAI) remission (≤3.3) at week 24) was not met, during the 56-week induction period of the AVERT-2 trial, more patients achieved SDAI remission with abatacept+MTX versus abatacept placebo+MTX at week 52.[35] In addition, there was a significant difference favouring abatacept+MTX in the proportion of radiographic non-progressors at week 52.[35] This exploratory post hoc analysis used data from AVERT-2 to investigate the correlation between baseline biomarkers of bone remodelling and ECM turnover and baseline disease activity in patients with RA and whether such biomarkers predicted treatment response, and assessed pharmacodynamic changes in biomarkers in response to treatment with abatacept+MTX versus abatacept placebo+MTX.

METHODS
Study design

AVERT-2 (NCT02504268) was a phase 3b, 132-week study of patients with active, early RA who were ACPA positive.[35] This two-phase study consisted of a 56-week double-blind, randomised, placebo-controlled induction period followed by a 48-week treatment de-escalation period. Full details of the study design have been reported previously.[35] At the start of the induction period, patients were randomised (3:2) to weekly SC abatacept 125 mg+MTX (starting dose: 7.5–15 mg/week titrated to ≥15 mg within 8 weeks) versus abatacept placebo+MTX for 56 weeks.[35] The primary endpoint was the proportion of patients in SDAI remission (≤3.3) at week 24 of the induction

period.[35] Other efficacy endpoints included the proportion of patients in SDAI remission at week 52 and with Disease Activity Score in 28 joints using CRP (DAS28 (CRP)) <2.6, in Boolean remission, or who achieved 20%/50%/70% improvement in ACR criteria (ACR20, ACR50 or ACR70) response at weeks 24 and 52.

Patient population

The AVERT-2 trial included adult patients (aged ≥18 years) with RA (defined by ACR/EULAR 2010 criteria[36]) of ≤6 months in duration who were ACPA positive and disease-modifying antirheumatic drug (DMARD)-naive.[31] Eligible patients had SDAI scores >11, ≥3 tender joints and ≥3 swollen joints, high-sensitivity CRP >3 mg/L or erythrocyte sedimentation rate ≥28 mm/hour at baseline. Cohort 1 (the intention-to-treat (ITT) population) included all randomised patients who received at least one dose of the study drug during the 56-week induction period.[35] This study was an exploratory post hoc analysis that included a subgroup of all randomised patients who had blood samples available for biomarker analysis.

Collection of blood samples

Patient blood samples were collected in standard serum separation tubes and prepared into aliquots. Serum samples were stored at ≤−70°C and shipped frozen on dry ice to Nordic Bioscience (Herlev, Denmark) for biomarker assessment.

Assessment of serum biomarkers

None of the biomarkers included in this analysis were prespecified. Baseline serum levels of biomarkers of ECM degradation and osteocalcin were measured at Nordic Bioscience following methods described previously, including: CTX-I; neoepitope of C3M[19]; neoepitope of MMP-2-degraded, MMP-9-degraded and MMP-12-degraded C4M α1 chain[37]; neoepitope of granzyme B-mediated degradation of type IV collagen (C4G)[38]; neoepitope of MMP-1-degraded and MMP-8-degraded CRPM[39]; neoepitope of human neutrophil elastase-mediated degradation of calprotectin (CPa9-HNE; unpublished data); and neoepitope of MMP-2-mediated, MMP-8-mediated and trypsin-mediated degradation of citrullinated vimentin (VICM).[40] CTX-I and osteocalcin (N-MID) were measured using the Roche Diagnostic Cobas e411 platform. C3M, C4M, C4G, CRPM, CPa9-HNE and VICM were measured using manual competitive ELISA or the robotic platform IDS-i10 (Immunodiagnostic Systems, Tyne and Wear, UK). Samples were rerun if duplicate coefficients of variation were higher than 15%. Intra-assay and interassay variation were <10% and <15%, respectively. All runs included three quality control samples, which were accepted within a 20% range of the target value. Serum levels of C3M, C4M and CRPM were also assessed at weeks 24 and 52. In this study, CTX-I levels were only assessed at baseline.

Serum levels of RANKL at baseline and weeks 24 and 52 were tested using Quanterix's (Billerica, Massachusetts, USA) fully automated Simoa HD-1/HD-X immunoassay platform at Rules Based Medicine (Austin, Texas, USA) according to the manufacturer's instruction. Serum levels of ACPA were also assessed at baseline.

Data analysis

Analysis of baseline data

Normality testing was carried out after log2 transformation of baseline levels of ECM biomarkers for all patients included in this study. Baseline values of RANKL and CTX-I were obtained from selected patients with baseline levels of both RANKL and ECM biomarkers available. The associations between baseline ECM biomarkers and baseline disease activity measures were assessed using Spearman's correlation coefficient. Weak, moderate and high correlations were defined as 0 to <0.3, 0.3 to <0.6 and 0.6 to 1, respectively. The impact of sex and corticosteroid use at baseline on baseline levels of CTX-I and the impact of baseline CTX-I levels on baseline levels of RANKL were assessed retrospectively.

Predictive analysis

A logistic regression model was used to assess the ability of baseline levels of ECM biomarkers to predict SDAI remission, DAS28 (CRP) <2.6, ACR70 response or Boolean remission at weeks 24 and 52. Biomarker levels were defined as either a continuous variable or categorically (low, medium and high tertiles). Confounding factors were considered, including baseline disease activity score (SDAI), age, sex, region, baseline ACPA level, baseline erosion score and baseline corticosteroid use. Stepwise regression was used to select significant covariates, resulting in baseline disease activity score, age, and baseline erosion score being included in the final predictive model. In addition to these three covariates, the model also included treatment arm, baseline biomarkers (continuous log2-transformed or categorised into tertiles), and biomarker-by-treatment group interaction. Interaction-effect plots for continuous biomarkers, model estimates (β_{BM}, β_{Trt*BM}) and unadjusted p values were reported.

The estimated proportion of patients (95% CI) achieving SDAI remission, DAS28 (CRP) <2.6, ACR70 response or Boolean remission was assessed at weeks 24 and 52. Point estimates of ORs (95% CI) were assessed by baseline CTX-I and RANKL tertiles: low, medium and high CTX-I tertiles were categorised as 0.028–0.324 ng/mL (n=178), 0.325–0.525 ng/mL (n=177) and 0.526–1.570 ng/mL (n=178), respectively. Low, medium and high RANKL tertiles were categorised as 1.6–9.7 pg/mL (n=180), 9.8–17 pg/mL (n=182) and 18–75 pg/mL (n=171), respectively.

Adjusted mean change from baseline over time in SDAI and DAS28 (CRP) scores, in swollen joint counts and in patient pain scores were evaluated for low, medium and high CTX-I tertiles.

Table 1 Baseline characteristics in the overall AVERT-2 cohort 1 (ITT) population and the ECM biomarker subgroup by treatment arm

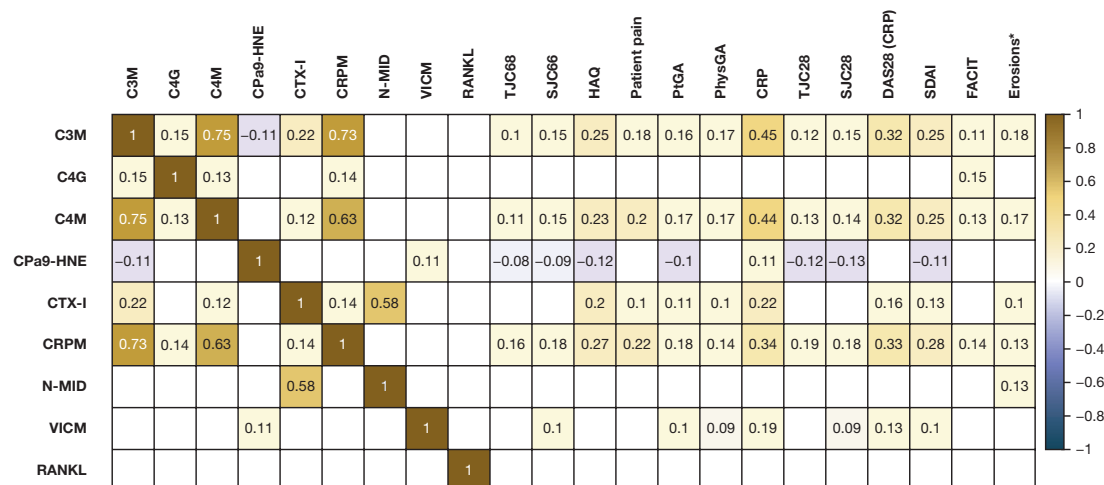|	ITT population			ECM biomarker subgroup		
	Abatacept+MTX (n=451)	Abatacept placebo+MTX (n=301)	p value	Abatacept+MTX (n=330)	Abatacept placebo+MTX (n=205)	p value
Age, years	49 (13)	49 (14)	0.69	48 (13)	47 (14)	0.52
Sex, female, n (%)	349 (77)	243 (81)	0.31	266 (81)	171 (83)	0.48
Race, n (%)						
Asian	77 (17)	52 (17)	0.99	61 (18)	41 (20)	0.96
Black	20 (4)	15 (5)	–	13 (4)	9 (4)	–
Other	39 (9)	25 (8)	–	31 (9)	19 (9)	–
White	315 (70)	209 (69)	–	225 (68)	136 (66)	–
Duration of RA, months	1.2 (1.4)	1.2 (1.4)	0.7	1.2 (1.4)	1.2 (1.4)	0.81
RF-positive, n (%)	421 (93)	279 (93)	N/A	304 (92)	189 (92)	N/A
ACPA titre, U/mL	836 (1.4×10^3)	780 (1.5×10^3)	0.34	931 (1.6×10^3)	766 (1.3×10^3)	0.05
Tender joint count (68 joints)	21 (13.5)	22 (13.1)	0.46	21 (13.1)	22 (13.7)	0.45
Swollen joint count (66 joints)	13 (8.7)	14 (9.4)	0.19	14 (8.4)	15 (10.0)	0.41
Tender joint count (28 joints)	13 (6.8)	14 (6.8)	0.33	13 (6.7)	14 (6.8)	0.79
Swollen joint count (28 joints)	10 (5.7)	11 (5.8)	0.43	10 (5.6)	11 (6.0)	0.61
Erosion score	4.1 (7.0)	5.7 (8.7)	<0.05	3.9 (6.7)	6.0 (8.7)	<0.05
CRP, mg/L	20 (27)	19 (22)	0.34	19 (27)	17 (19)	0.35
Patient Global Assessment of disease activity	66 (23)	63 (24)	0.46	66 (24)	61 (25)	0.29
Physician Global Assessment of disease activity	65 (18)	66 (20)	0.67	64 (19)	63 (20)	0.89
SDAI score	38 (14)	39 (14)	0.25	39 (14)	39 (14)	0.87
DAS28 (CRP) score	5.6 (1.1)	5.6 (1.0)	0.36	5.6 (1.1)	5.6 (1.0)	0.99
HAQ-DI score	1.6 (0.7)	1.6 (0.7)	0.88	1.6 (0.7)	1.6 (0.7)	0.90
Patient assessment of pain (0–100 mm VAS)	66 (23)	65 (22)	0.44	66 (23)	65 (23)	0.55
Corticosteroid dose, mg	7.4 (4.3)	7.9 (6.5)	1	7.1 (4.1)	8.0 (6.9)	0.88
Corticosteroid use, n (%)	212 (47)	95 (32)	N/A	163 (49)	70 (34)	N/A
Biomarker name						
CTX-I, ng/mL	–	–	–	0.47 (0.26)	0.47 (0.25)	0.99
C3M, ng/mL	–	–	–	20 (7.2)	20 (9.7)	0.45
C4M, ng/mL	–	–	–	41 (17)	40 (15)	0.32
C4G, ng/mL	–	–	–	41 (31)	44 (39)	0.40
CPa9-HNE, ng/mL	–	–	–	137 (92)	158 (100)	0.04
CRPM, ng/mL	–	–	–	16 (5.5)	16 (5.3)	0.94
N-MID, ng/mL	–	–	–	18 (7.3)	19 (8.7)	0.97
VICM, ng/mL	–	–	–	16 (23)	16 (23)	0.28
RANKL, pg/mL	–	–	–	16 (9.7)	16 (11.0)	0.35

Data are mean (SD) unless otherwise stated. In this analysis, data for the ITT population (cohort 1) were adjusted to remove duplicate/missing data.

ACPA, anti-citrullinated protein antibody; AVERT-2, Assessing Very Early Rheumatoid arthritis Treatment-2; C4G, neo-epitope of granzyme B-mediated degradation of type IV collagen; C3M, neo-epitope of MMP-9–mediated degradation of type III collagen; C4M, neo-epitope of MMP-2-mediated, MMP-9-mediated and MMP-12-mediated degradation of type IV collagen α1 chain; CPa9-HNE, neoepitope of human neutrophil elastase-mediated degradation of calprotectin; CRP, C reactive protein; CRPM, neo-epitope of MMP-1-mediated and MMP-8-mediated degradation of CRP; CTX-I, cross-linked carboxy-terminal telopeptide of type I collagen; DAS28, Disease Activity Score in 28 joints; ECM, extracellular matrix; HAQ-DI, Health Assessment Questionnaire-Disability Index; ITT, intention-to-treat; MMP, matrix metalloproteinase; MTX, methotrexate; N/A, not available; N-MID, osteocalcin; RA, rheumatoid arthritis; RANKL, receptor activator of nuclear factor κ-B ligand; RF, rheumatoid factor; SD, standard deviation; SDAI, Simplified Disease Activity Index; VAS, visual analogue scale; VICM, neoepitope of MMP-2-mediated, MMP-8-mediated and trypsin-mediated degradation of citrullinated vimentin.

Pharmacodynamic analysis

A linear mixed-effects model was used to evaluate changes in ECM biomarker levels over time in response to treatment; all biomarker levels were log2-transformed. The difference in adjusted mean change in biomarker levels (least square means, converted to per cent change from baseline) between abatacept+MTX and abatacept placebo+MTX at the predefined time point, with corresponding 95% CI and p values, was provided.

	C3M	C4G	C4M	CPa9-HNE	CTX-I	CRPM	N-MID	VICM	RANKL	TJC68	SJC66	HAQ	Patient pain	PtGA	PhysGA	CRP	TJC28	SJC28	DAS28 (CRP)	SDAI	FACIT	Erosions*
C3M	1	0.15	0.75	-0.11	0.22	0.73				0.1	0.15	0.25	0.18	0.16	0.17	0.45	0.12	0.15	0.32	0.25	0.11	0.18
C4G	0.15	1	0.13			0.14															0.15	
C4M	0.75	0.13	1		0.12	0.63				0.11	0.15	0.23	0.2	0.17	0.17	0.44	0.13	0.14	0.32	0.25	0.13	0.17
CPa9-HNE	-0.11			1				0.11		-0.08	-0.09	-0.12		-0.1		0.11	-0.12	-0.13		-0.11		
CTX-I	0.22		0.12		1	0.14	0.58						0.2	0.1	0.11	0.1	0.22		0.16	0.13		0.1
CRPM	0.73	0.14	0.63		0.14	1				0.16	0.18	0.27	0.22	0.18	0.14	0.34	0.19	0.18	0.33	0.28	0.14	0.13
N-MID					0.58		1															0.13
VICM				0.11				1					0.1			0.1	0.09	0.19		0.09	0.13	0.1
RANKL									1													

Figure 1 Association between baseline ECM biomarkers and baseline disease activity measures. Spearman's correlation coefficient was shown if p<0.05; empty squares = not significant. Weak, moderate and high correlations were defined as 0 to <0.3, 0.3 to <0.6 and 0.6 to 1, respectively. *Baseline erosion score was taken as an average of two readers. C3M, neoepitope of MMP-9-mediated degradation of type III collagen; C4G, neoepitope of granzyme B-mediated degradation of type IV collagen; C4M, neoepitope of MMP-2-mediated, MMP-9-mediated and MMP-12-mediated degradation of type IV collagen α1 chain; CPa9-HNE, neoepitope of human neutrophil elastase-mediated degradation of calprotectin; CRP, C reactive protein; CRPM, neoepitope of MMP-1-mediated and MMP-8-mediated degradation of CRP; CTX-I, cross-linked carboxy-terminal telopeptide of type I collagen; DAS28, Disease Activity Score in 28 joints; ECM, extracellular matrix; FACIT, functional assessment of chronic illness therapy; HAQ, Health Assessment Questionnaire; MMP, matrix metalloproteinase; N-MID, osteocalcin; PhysGA, Physician Global Assessment of disease activity; PtGA, Patient Global Assessment of Disease Activity; RANKL, receptor activator of nuclear factor κ-B ligand; SDAI, Simplified Disease Activity Index; SJC, swollen joint count; TJC, tender joint count; VICM, neoepitope of MMP-2-mediated, MMP-8-mediated and trypsin-mediated degradation of citrullinated vimentin. Adapted from ACR Convergence held 3–9 November 2021. The American College of Rheumatology does not guarantee, warrant or endorse any commercial products or services. Reprinted by BMJ Publishing Group.

RESULTS

Patients

Cohort 1 (ITT population) in the AVERT-2 trial included 752 patients who were randomised to receive abatacept+MTX (n=451) or abatacept placebo+MTX (n=301).[35] Of these, 330 patients treated with abatacept+MTX and 205 treated with abatacept placebo+MTX had serum samples available for ECM biomarker determination and were included in the present analysis (table 1). A further subset of patients (n=533) had both ECM biomarker and RANKL data available.

Demographics and baseline characteristics were similar between the overall ITT population and the ECM biomarker subgroup, and across treatment arms in these two populations (table 1). There were no significant differences between treatment arms in baseline levels of C3M, C4M, C4G, CRPM, CTX-I, osteocalcin or VICM (table 1). A significant difference was observed in CPa9-HNE (p=0.039; table 1). The sample size was smaller for CPa9-HNE; 26% of patients had missing data compared with other biomarkers studied.

Association between serum biomarkers and disease activity at baseline

Of the ECM biomarkers tested, baseline C3M, C4M and CRPM showed the greatest correlation with baseline measures of disease activity (figure 1); although statistically significant, these correlations were weak to moderate. There was no significant correlation between

CPa9-HNE, N-MID or VICM and measures of disease activity at baseline.

Baseline serum CTX-I levels showed a weak correlation with baseline disease activity measures (figure 1). At baseline, CTX-I levels were significantly lower in females versus males (p=0.0021; online supplemental figure 1A). In addition, there was no significant difference in baseline CTX-I levels based on corticosteroid use at baseline (online supplemental figure 1B).

In addition, there was no significant correlation between serum levels of RANKL at baseline and other baseline ECM biomarkers or measures of disease activity (figure 1).

Prediction of treatment response by baseline CTX-I level

Among the ECM biomarkers that were tested, only CTX-I was a significant predictor of response to treatment with abatacept+MTX. Higher baseline levels of CTX-I were significantly associated with a higher probability of achieving SDAI remission and DAS28 (CRP) <2.6 at weeks 24 and 52, and ACR70 response at week 52 in patients treated with abatacept+MTX but not abatacept placebo+MTX (figure 2). The probability of achieving Boolean remission was also associated with baseline CTX-I levels, although this association was not statistically significant (figure 2). This predictive effect of CTX-I to show a differential treatment response between treatment arms was statistically significant for DAS28 (CRP) <2.6 at week 24 (figure 2). In contrast, higher baseline C3M (online
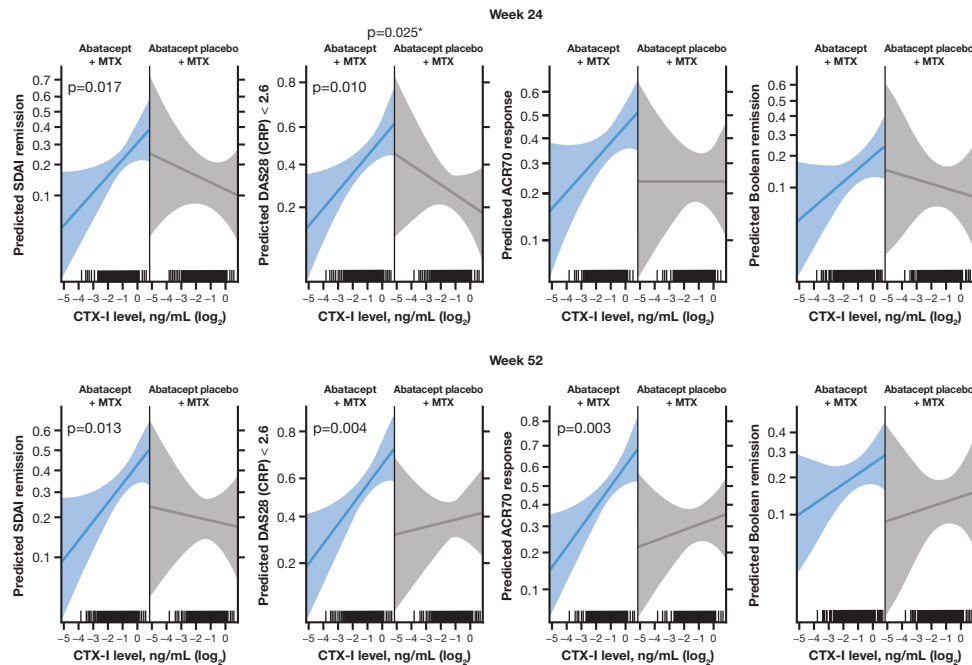
Figure 2 Probability of achieving efficacy outcomes at weeks 24 and 52 with abatacept+MTX or abatacept placebo+MTX as predicted by baseline CTX-I level. The shaded areas on each graph show 95% CIs. Statistically significant p values (p<0.05) are shown for the prognostic value of CTX-I as a predictor of response to a given treatment and for the predictive value of CTX-I to show a differential response between treatment arms (interaction effect); non-significant p values (p>0.05) are not shown. The model included treatment arm, baseline disease activity measure, age, baseline erosion score, baseline biomarkers (continuous log2-transformed) and biomarker-by-treatment group interaction. Covariates were selected based on Akaike Information Criterion in a stepwise regression. *Interaction effect for abatacept+MTX versus abatacept placebo+MTX. ACR70, 70% improvement in American College of Rheumatology criteria; CRP, C reactive protein; CTX-I, cross-linked carboxy-terminal telopeptide of type I collagen; DAS28, Disease Activity Score in 28 joints; MTX, methotrexate; SDAI, Simplified Disease Activity Index. Adapted from ACR Convergence held 3–9 November 2021. The American College of Rheumatology does not guarantee, warrant or endorse any commercial products or services. Reprinted by BMJ Publishing Group.

supplemental figure 2), CRP (online supplemental figure 3) and ACPA (online supplemental figure 4) did not predict a differential treatment response between the two arms; however, higher baseline C4G levels were associated with a greater probability of achieving ACR70 response at week 24 for abatacept+MTX (online supplemental figure 5).

Analysis of categorical variables also showed greater treatment differences between abatacept+MTX and abatacept placebo+MTX in patients with medium and high versus low baseline CTX-I levels (figure 3A). Statistically significant treatment differences between the proportions of patients achieving efficacy outcomes were observed for SDAI remission (week 52), DAS28 (CRP) <2.6 (weeks 24 and 52) and Boolean remission (week 52) in patients with high baseline CTX-I, and for ACR70 response in patients with medium and high baseline CTX-I at weeks 24 and 52 (p<0.05; figure 3B).

Among patients with medium or high baseline CTX-I levels, significantly greater improvements in adjusted mean change from baseline (95% CI) in SDAI and DAS28 (CRP) scores at all post baseline time points (figure 4) and in swollen joint count and patient pain at some post baseline time points (online supplemental figure 6) were observed for abatacept+MTX versus abatacept placebo+MTX (p<0.05); patients with low baseline CTX-I levels generally showed fewer time points with significantly greater improvements.

Prediction of treatment response by baseline RANKL level

The probability of achieving SDAI remission or ACR70 response at week 52, as predicted by baseline RANKL levels, showed a differential treatment response between abatacept+MTX and abatacept placebo+MTX (figure 5). Differential treatment response for the probability of achieving SDAI remission at week 52 was statistically significant (p<0.05 for interaction effect). Similarly, categorical analysis demonstrated greater treatment differences at weeks 24 and 52 for abatacept+MTX versus abatacept placebo+MTX in patients with medium and high versus low baseline RANKL levels (figure 6).

Compared with patients with low baseline CTX-I, those with medium (p=0.059) or high (p=0.081) baseline CTX-I levels had numerically higher levels of RANKL, although statistical significance was not reached (online supplemental figure 7). Higher baseline CTX-I levels were not associated with a greater decrease in RANKL over time in response to treatment (online supplemental figure 8A), and there was no significant correlation between serum RANKL and disease activity measures at week 52

A Estimated proportion of patients (95% CI) achieving SDAI remission, DAS28 (CRP) <2.6, ACR70 response and Boolean remission
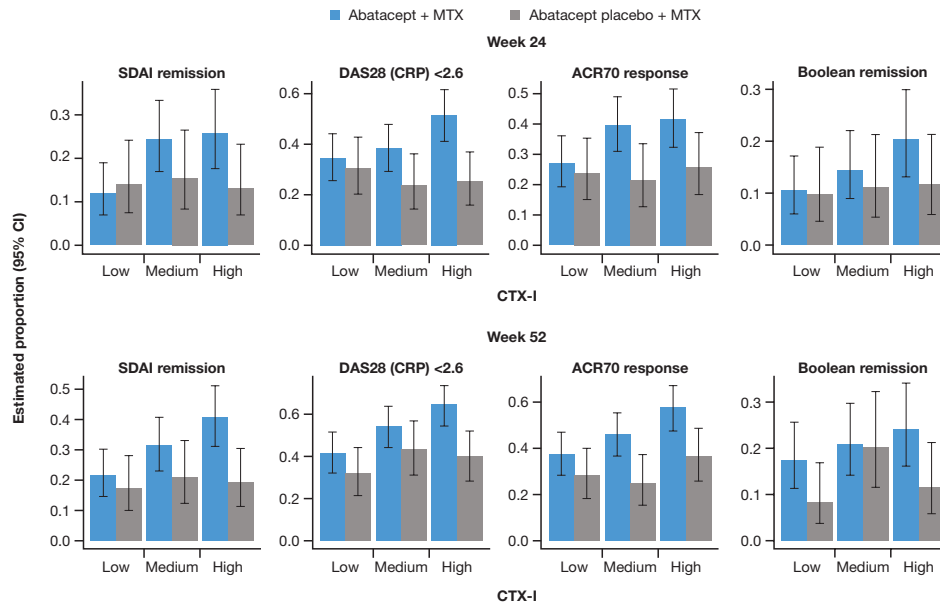
B Treatment difference in SDAI remission, DAS28 (CRP) <2.6, ACR70 response and Boolean remission
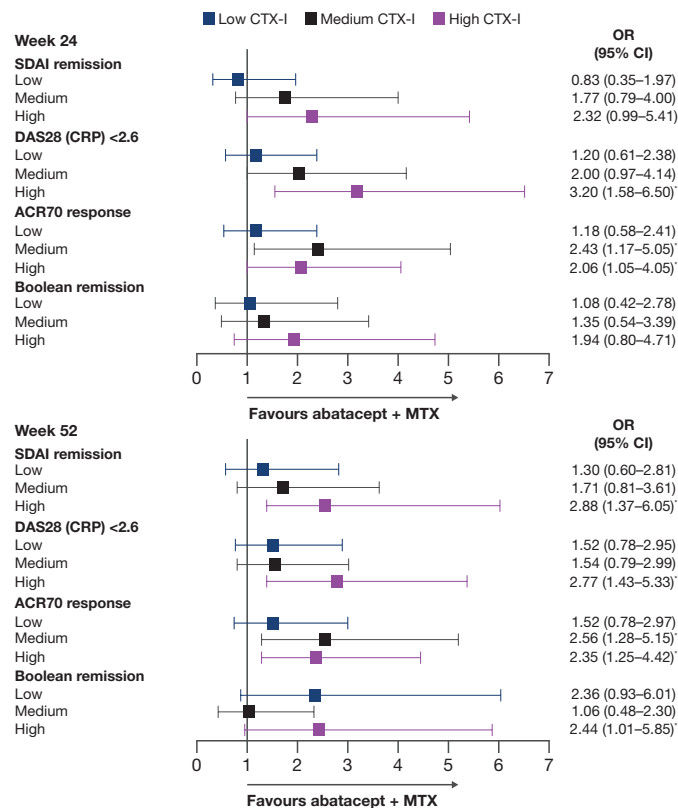
Figure 3 Treatment differences between abatacept+MTX and abatacept placebo+MTX in efficacy outcomes at weeks 24 and 52 by baseline CTX-I levels (low, medium and high). The model included treatment arm, baseline disease activity measure, age, baseline erosion score, baseline biomarkers (categorised into tertiles) and biomarker-by-treatment group interaction. Low, medium and high CTX-I categories are based on tertiles 1 (n=178, 0.028–0.324 ng/mL), 2 (n=177, 0.325–0.525 ng/mL) and 3 (n=178, 0.526–1.570 ng/mL), respectively. Reference ranges for normal levels of CTX-I in serum are: female (premenopausal), 0.040–0.465 ng/mL; female (postmenopausal), 0.104–1.008 ng/mL; and male, 0.060–0.700 ng/mL.[43] *p<0.05 for comparison of abatacept+MTX versus abatacept placebo+MTX. Adjusted p values are shown in online supplemental table S1. ACR70, 70% improvement in American College of Rheumatology criteria; CRP, C reactive protein; CTX-I, cross-linked carboxy-terminal telopeptide of type I collagen; DAS28, Disease Activity Score in 28 joints; MTX, methotrexate; SDAI, Simplified Disease Activity Index. Adapted from ACR Convergence held 3–9 November 2021. The American College of Rheumatology does not guarantee, warrant or endorse any commercial products or services. Reprinted by BMJ Publishing Group.
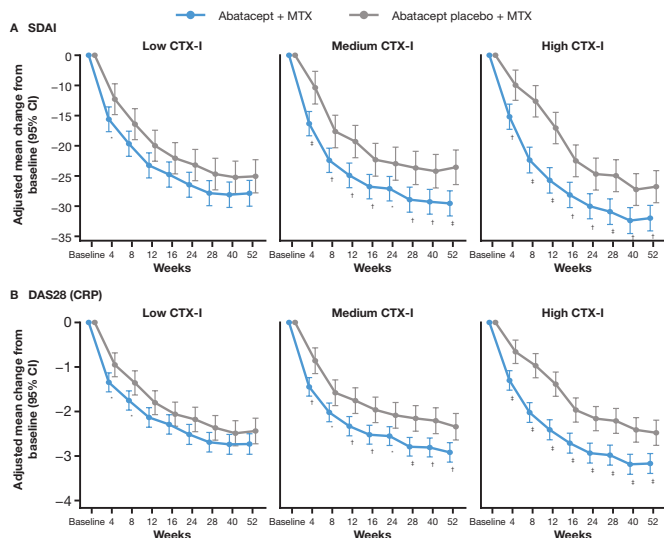
Figure 4 Adjusted mean change from baseline (95% CI) in SDAI and DAS28 (CRP) scores over time in patients receiving abatacept+MTX versus abatacept placebo+MTX by baseline CTX-I tertile. Low, medium and high CTX-I categories are based on tertiles 1 (n=178, 0.028–0.324 ng/mL), 2 (n=177, 0.325–0.525 ng/mL) and 3 (n=178, 0.526–1.570 ng/mL), respectively. *p<0.05; †p<0.01; ‡p<0.001. CRP, C reactive protein; CTX-I, cross-linked carboxy-terminal telopeptide of type I collagen; DAS28, Disease Activity Score in 28 joints; MTX, methotrexate; SDAI, Simplified Disease Activity Index. Reprinted from ACR Convergence held 3–9 November 2021. The American College of Rheumatology does not guarantee, warrant or endorse any commercial products or services. Reprinted by BMJ Publishing Group.

in patients treated with abatacept+MTX (online supplemental figure 8B).

Pharmacodynamic changes in bone remodelling/ECM biomarkers

Compared with patients receiving abatacept placebo+MTX, patients treated with abatacept+MTX demonstrated significantly greater adjusted mean percent change from baseline (95% CI) in C3M, C4M and RANKL at weeks 24 and 52 (p<0.01) and in CRPM at week 24 (p<0.05; figure 7).

DISCUSSION

In MTX-naive patients with early RA who were ACPA positive, baseline levels of CTX-I, a biomarker of bone resorption, predicted differential SDAI remission and DAS28 (CRP) <2.6 in response to treatment with abatacept+MTX versus abatacept placebo+MTX. Higher baseline CTX-I levels were associated with a greater probability of achieving efficacy endpoints for abatacept+MTX at weeks 24 and 52 of treatment. This relationship was not observed with abatacept placebo+MTX. In addition, differences between the two treatment groups were greatest among patients with medium or high baseline CTX-I levels.

Similarly, baseline levels of RANKL, which modulates differentiation and activation of osteoclasts, predicted

a differential treatment response between abatacept+MTX and abatacept placebo+MTX in terms of the proportion of patients achieving SDAI remission. Again, this differential treatment response was greatest among patients with medium or high baseline RANKL levels. Treatment with abatacept+MTX also demonstrated significant pharmacodynamic effects on biomarkers of bone remodelling and ECM turnover, with statistically significantly greater reductions in RANKL, C3M, C4M and CRPM over time compared with abatacept placebo+MTX.

The biomarkers measured in this study reflect different components of the inflammatory response and joint destruction: CTX-I is a marker of bone resorption and CRPM is a marker of systemic inflammation, while C3M and C4M are markers of synovial inflammation. Previous studies have shown that such markers of bone remodelling and ECM turnover may be useful as disease-relevant surrogate biomarkers indicative of synovial joint pathophysiology and treatment response. An evaluation of clusters of biomarkers using data from two RA studies (LITHE (NCT00106535) and OSKIRA-1 (NCT01197521)) and two osteoarthritis studies demonstrated that clusters comprised predominantly of patients with RA showed high levels of cartilage turnover (MMP-degraded type II collagen (C2M)), CRP metabolism (CRPM), interstitial matrix turnover (C1M and C3M) and bone turnover (CTX-I and procollagen type I N-terminal propeptide (PINP)).[15] Another study demonstrated significantly higher serum levels of both C2M and C3M in patients with RA (n=47) compared with healthy controls (n=56),[20] and a combination of these two markers predicted disease progression both in patients with RA and in those with ankylosing spondylitis with 80% sensitivity and 61% specificity. Furthermore, biomarkers of ECM turnover and systemic inflammation can be suppressed in response to treatment. In a prospective study of 149 Japanese patients with RA, serum levels of C1M, C3M, C4M and CRPM were elevated at baseline compared with healthy individuals, and all four markers were significantly correlated with baseline DAS28 score (p<0.0001), demonstrating enhanced turnover of major collagen constituents of the synovial membrane in patients with active disease.[16] In addition, levels of all four biomarkers were attenuated in response to treatment with several different drugs with varying mechanisms of action, including MTX, adalimumab, tocilizumab and tofacitinib.[16] Recently, Jura-Półtorak et al[17] reported significantly lower pretreatment levels of serum PINP (a marker of bone formation) and higher levels of CTX-I and soluble RANKL (bone resorption) in 31 females with RA compared with healthy controls. Following 15 months of treatment with a tumour necrosis factor (TNF) inhibitor, they observed a decrease in CTX-I (partly due to the RANKL/osteoprotegerin reduction) and a concomitant increase in PINP levels.[17]

Building on such previous work, this study shows that baseline serum levels of C3M and C4M (markers of synovial inflammation) and CRPM (a marker of systemic
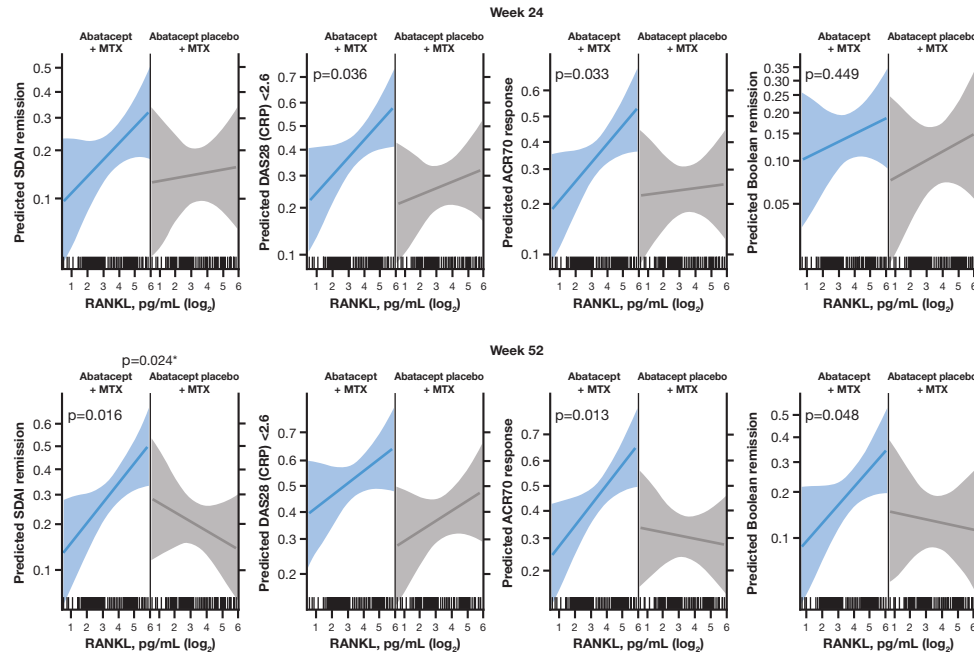
Figure 5 Probability of achieving efficacy outcomes at weeks 24 and 52 with abatacept+MTX or abatacept placebo+MTX as predicted by baseline RANKL level. The shaded areas on each graph show 95% CI. Statistically significant p values (p<0.05) are shown for the prognostic value of RANKL as a predictor of response to a given treatment and for the predictive value of RANKL to show a differential response between treatment arms (interaction effect); non-significant p values (p>0.05) are not shown. The model included treatment arm, baseline disease activity measure, age, baseline erosion score, baseline biomarkers (continuous log2-transformed) and biomarker-by-treatment group interaction. Covariates were selected based on Akaike Information Criterion in a stepwise regression. *Interaction effect for abatacept+MTX versus abatacept placebo+MTX. ACR70, 70% improvement in American College of Rheumatology criteria; CRP, C reactive protein; DAS28, Disease Activity Score in 28 joints; MTX, methotrexate; RANKL, receptor activator of nuclear factor κ-B ligand; SDAI, Simplified Disease Activity Index.

Figure 6 Treatment differences between abatacept+MTX and abatacept placebo+MTX in efficacy outcomes at weeks 24 and 52 by baseline RANKL levels (low, medium and high). Low, medium and high RANKL categories are based on tertiles 1 (n=180, 1.6–9.7 pg/mL), 2 (n=182, 9.8–17 pg/mL) and 3 (n=171, 18–75 pg/mL), respectively. ACR70, 70% improvement in American College of Rheumatology criteria; CRP, C reactive protein; DAS28, Disease Activity Score in 28 joints; MTX, methotrexate; RANKL, receptor activator of nuclear factor κ-B ligand; SDAI, Simplified Disease Activity Index.
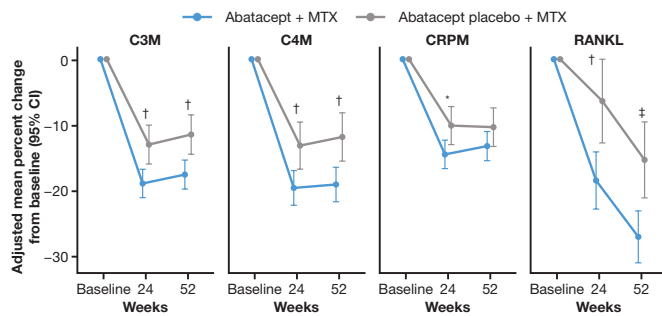
Figure 7 Adjusted mean percent change from baseline (95% CI) in C3M, C4M, CRPM and RANKL at weeks 24 and 52. A linear mixed-effects model was used to evaluate change in ECM biomarker levels over time in response to treatment; all biomarker levels were log2-transformed. The model included change from baseline as the repeated measure, with fixed effects of baseline value, visit, treatment and visit-by-treatment interaction. The parameter estimations were made using the method of restricted maximum likelihood. *p<0.05; †p<0.01; ‡p<0.001. C3M, neoepitope of MMP-9–mediated degradation of type III collagen; C4M, neoepitope of MMP-2-mediated, MMP-9-mediated and MMP-12-mediated degradation of type IV collagen α1 chain; CRP, C reactive protein; CRPM, neoepitope of MMP-1-mediated and MMP-8-mediated degradation of CRP; ECM, extracellular matrix; MMP, matrix metalloproteinase; MTX, methotrexate; RANKL, receptor activator of nuclear factor κ-B ligand. Reprinted from ACR Convergence held 3–9 November 2021. The American College of Rheumatology does not guarantee, warrant or endorse any commercial products or services. Reprinted by BMJ Publishing Group.

inflammation) were all strongly correlated with measures of baseline disease activity in MTX-naive patients with early RA who were ACPA positive. In contrast, at baseline, CTX-I—a marker of bone turnover—was less strongly correlated with baseline disease activity and showed relatively weak association with baseline erosion score (Spearman's correlation coefficient=0.1). Although they had very severe disease, patients enrolled in the AVERT-2 trial had early or very early RA (disease duration ≤6 months). Therefore, it is possible that the CTX-I levels observed in this population may reflect active bone remodelling rather than the more severe bone erosion associated with more established disease. This idea of active bone remodelling is supported by a positive correlation between baseline CTX-I and osteocalcin (N-MID) levels observed in this study (Spearman's correlation coefficient=0.58). Osteocalcin is secreted by active osteoblasts.

CTX-I levels can also be affected by osteoclast activity in patients with osteoporosis, and osteoporosis is common among patients with RA. Although osteoporosis at baseline was not measured in this study, CTX-I levels at baseline were shown to be higher in males than females, and there was no significant difference in CTX-I levels by baseline corticosteroid use, which may alleviate concerns about confounding effects of baseline osteoporosis due to corticosteroid use on the study results.

In this study, baseline levels of CTX-I were significantly associated with the probability of achieving SDAI remission and DAS28 (CRP) <2.6 at weeks 24 and 52, and ACR70 response at week 52 in patients treated with abatacept+MTX but not abatacept placebo+MTX. Furthermore, the ability of CTX-I to predict a differential treatment response in DAS28 (CRP) <2.6 between abatacept+MTX and abatacept placebo+MTX was statistically significant at week 24. This differential treatment response was greatest among patients with medium or high baseline CTX-I. In patients with RA who have active disease, high levels of CTX-I may reflect increased activation of T lymphocytes. T-cell activation induces the formation of human osteoclasts, which leads to cathepsin K-mediated production of CTX-I[2 4] and the breakdown of bone tissue via both RANKL-dependent and RANKL-independent mechanisms.[29 30] In patients with RA, activated T cells express high levels of soluble RANKL, and RANKL signalling pathways play a major role in the differentiation of osteoclast progenitors and expansion of mature osteoclasts.[4 29 30] Activated T cells also secrete proresorptive cytokines, such as interleukin (IL)-1, IL-6 and IL-17, which stimulate expression of RANKL on the cell surface of osteoblasts and fibroblasts, activating osteoclast formation through a contact-dependent process.[29 30] In this study, the association observed between baseline CTX-I and disease outcomes, in response to treatment with abatacept+MTX, suggests a possible role for the modulation of T-cell activation and subsequent effects on osteoclast differentiation. This is supported by the fact that higher C4G levels (a marker of T-cell activity) were also modestly correlated with a differential treatment response between abatacept+MTX and abatacept placebo+MTX. These data suggest that, following an inadequate response to MTX, patients with high serum levels of CTX-I could perhaps benefit from preferential treatment with abatacept.

Furthermore, these findings suggest that abatacept may work better in DMARD-naive patients with early RA who have high levels of T-cell activation and are undergoing active bone remodelling, as shown by a significantly greater reduction in RANKL, C3M, C4M and CRPM over 52 weeks of treatment with abatacept+MTX versus abatacept placebo+MTX. In vitro studies have shown that cytotoxic T-lymphocyte-associated antigen 4 dependently inhibits RANKL-mediated and TNF-mediated osteoclastogenesis, further supporting a role for T-cell modulation in regulating RANKL-mediated bone destruction.[41 42]

There were some limitations to this study. This was a retrospective, hypothesis-driven exploratory analysis and none of the biomarkers studied were prespecified in the clinical protocol. This raises the question of which other biomarkers could have been assessed that might or might not predict response to treatment with abatacept+MTX or abatacept placebo+MTX. Not all patients randomised to the AVERT-2 ITT cohort had samples available for biomarker analysis. While the model suggests that there is potential predictive value of CTX-I and RANKL in the specific population of patients studied, this requires further evaluation in prospective clinical studies with

larger populations of patients with RA who have varying disease characteristics, including different stages of disease. In addition, CTX-I levels were not measured at time points beyond baseline (ie, on treatment). Future studies should assess CTX-I levels over the course of treatment to provide data on changes in CTX-I levels in response to treatment with abatacept and possible implications for joint erosions. While baseline CTX-I levels predicted treatment response to abatacept+MTX but not to abatacept placebo+MTX, it is not possible to discern whether it was predictive of response to treatment with abatacept alone or abatacept in combination with MTX. Although not assessed in this study, it would be of interest to investigate whether there is any correlation between reduction in markers of synovial inflammation, such as C3M and C4M, and reduction in synovitis in response to treatment. Finally, as with all biomarkers, to be of practicable use to clinicians, an easy-to-perform, validated test for CTX-I would need to be readily available.

To conclude, in this study of MTX-naive patients with early RA who were ACPA-positive, baseline levels of the CTX-I bone remodelling biomarker were significantly associated with the probability of achieving SDAI remission and DAS28 (CRP) <2.6 at weeks 24 and 52, and ACR70 response at week 52 in patients treated with abatacept+MTX but not abatacept placebo+MTX. The predictive effect of CTX-I to show a differential treatment response between the two treatment arms was statistically significant for DAS28 (CRP) <2.6 at week 24. This difference between the two treatment arms was greatest among patients who had medium or high baseline CTX-I levels. Thus, CTX-I may be useful as a predictive biomarker to select populations (eg, patients with early RA with an inadequate response to MTX) who might achieve early SDAI remission on treatment with abatacept+MTX. In addition, significant pharmacodynamic effects on serum levels of C3M, C4M, CRPM and RANKL were observed after 24 and 52 weeks of treatment with abatacept+MTX. Further studies of baseline CTX-I as a predictive biomarker of response to treatment with abatacept in patients with early and very early RA, as well as for predicting those at high risk of developing RA likely to respond to disease intervention by abatacept treatment, are warranted.

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