SUPPLEMENTAL MATERIAL

Data S1.

Supplemental Methods for Handling Biomarkers: Missing Data

Biomarker	Missing/Imputation information
Cytokines/Inflammation	
IL-6	413 (72%) under detection limit – Using Vectra instead -Also dichotomized at detection limit, 2.6
sTNF-R1	Not in Discovery Map, but in Vectra
Serum amyloid A (SAA)	7 (1%) above detection – imputed as highest limit
hsCRP	102 (18%) above detection – imputed as highest limit, comparing with Vectra and BCH
CD-40 ligand	4 (0.7%) below detection – imputed as ½ lower limit
Macrophage Inhibitory Factor (MIF)	83 (14%) below detection – imputed as ½ lower limit
Lipid Parameters	
Apolipoprotein A1	0 missing
Apolipoprotein A2	0 missing
Apolipoprotein B	2 missing (QNS) – removed 2
Apolipoprotein C1	0 missing
Apolipoprotein C3	0 missing
Apolipoprotein H	0 missing
Apolipoprotein(a) aka Lp(a)	0 missing
oxLDL (not measured)	Not in Discovery Map
LOX-1	245 (43%) outside detection -dichotomized at lower detection limit, 0.2
Adipokines	
Adiponectin	0 missing
Leptin	0 missing
Resistin	1 (0.1%) above detection – imputed as highest limit
Atherothrombosis	
Antithrombin III	90 (16%) above detection – imputed as highest limit
PAI-1	0 missing
Miscellaneous	
Cardiac troponin	Not in Discovery map, but in BCH – 46% of values below detection limit, dichotomized at lower detection limit, 6.0
Nt-pro-BNP	0 missing
VCAM-1	0 missing
VEGF	1 below detection – imputed as ½ lowest limit
MMP-1	298 (52%) outside detection – Using Vectra instead -dichotomized at lower limit, 0.31
MMP-3	3 (0.5%) above detection – imputed as highest limit
YKL-40	0 missing
Cystatin-C	0 missing
Osteopontin	1 (0.1%) below detection – imputed as ½ lowest limit

Osteoprotegrin (OPG)	0 missing

Supplemental Methods for Handling Biomarkers: Correlation between Discovery map and Vectra Biomarkers

Biomarker	Spearman Correlation Estimate	Spearman P-value	Pearson Correlation Estimate	Pearson P-value
Leptin	0.96	<0.0001	0.94	<0.0001
SAA	0.95	<0.0001	0.93	<0.0001
MMP3	0.95	<0.0001	0.89	<0.0001
Resistin	0.76	<0.0001	0.79	<0.0001
VCAM1	0.69	<0.0001	0.69	<0.0001
VEGF	0.39	<0.0001	0.36	<0.0001
YKL40	0.95	<0.0001	0.98	<0.0001
hsCRP	0.97	<0.0001	0.55	<0.0001
BCH x DM hsCRP	0.98	<0.0001	0.60	<0.0001

Note – of the 125 individuals with Baseline TBR, 5 of them do not have baseline lab values, and one individual is a TBR outlier, bringing our population down to 119.

Accuracy of Dichotomized Variables in Discovery Map vs Vectra (MMP1 and IL6)
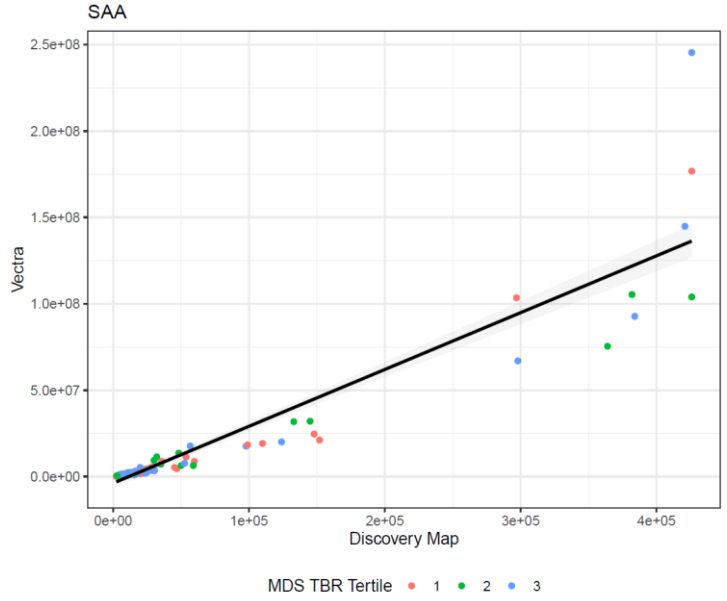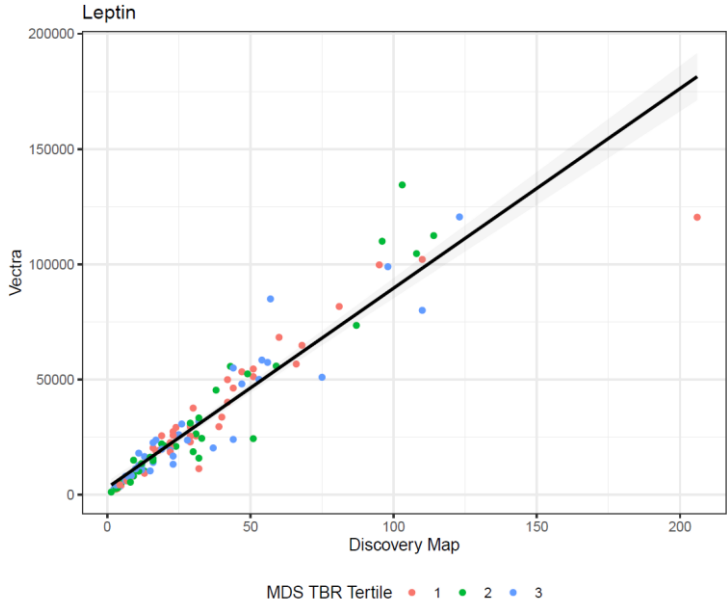-In Discovery Map dichotomized at limit of detection, in Vectra dichotomized at MEDIAN

Discovery Map MMP1	Vectra MMP1 (Median = 7872)	
	Above Median	Below Median
Above Detection Limit	41	24
Below Detection Limit	19	35

Accuracy: 0.64

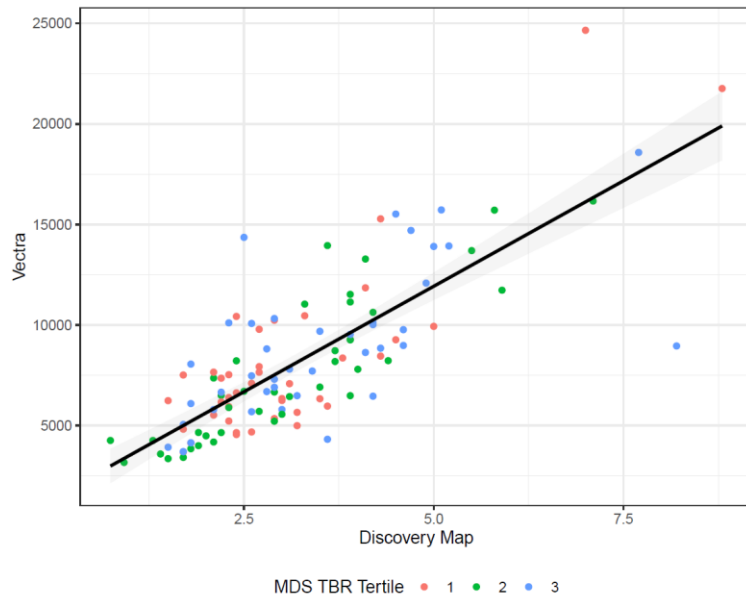| Discovery Map IL6	Vectra IL6 (Median = 10.4)	
	Above Median	Below Median
Above Detection Limit	35	5
Below Detection Limit	25	54

Accuracy: 0.75

Supplemental Methods for Handling Biomarkers: Scatterplots of Biomarkers in Multiple Platforms

Leptin

MDS TBR Tertile ● 1 ● 2 ● 3

SAA

MDS TBR Tertile ● 1 ● 2 ● 3

MMP3

MDS TBR Tertile ● 1 ● 2 ● 3

Resistin

MDS TBR Tertile ● 1 ● 2 ● 3

VCAM1

MDS TBR Tertile   ● 1   ● 2   ● 3

VEGF

MDS TBR Tertile   ● 1   ● 2   ● 3

YKL 40

MDS TBR Tertile ● 1 ● 2 ● 3

hsCRP

MDS TBR Tertile ● 1 ● 2 ● 3
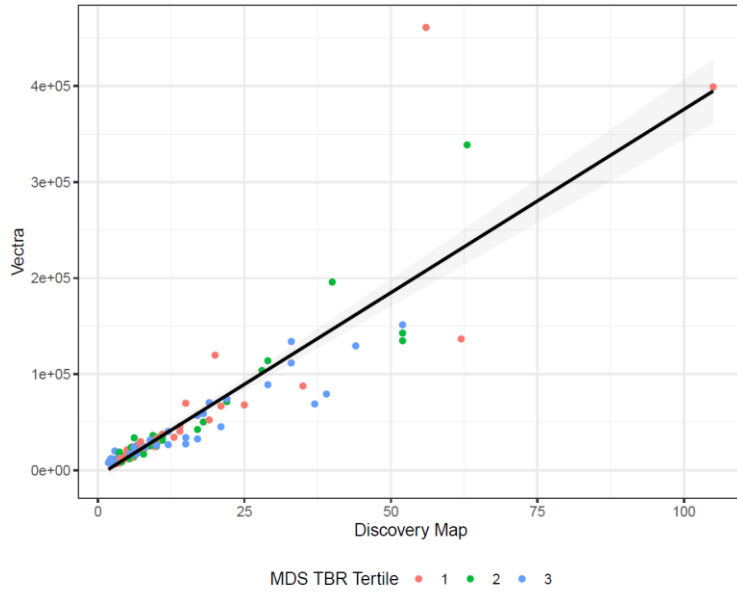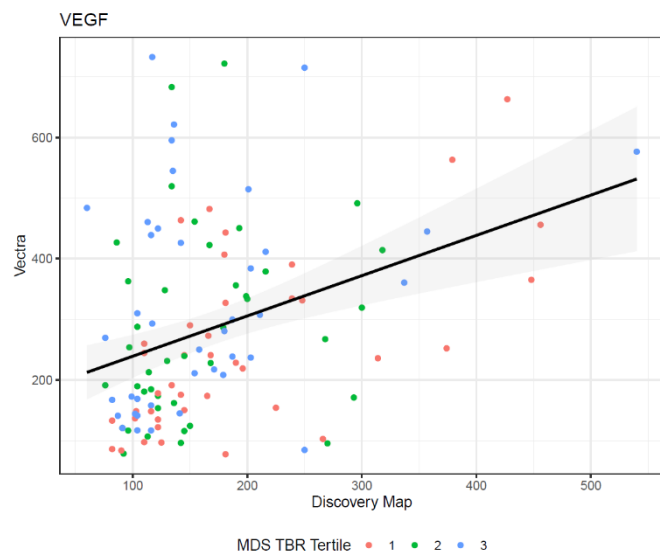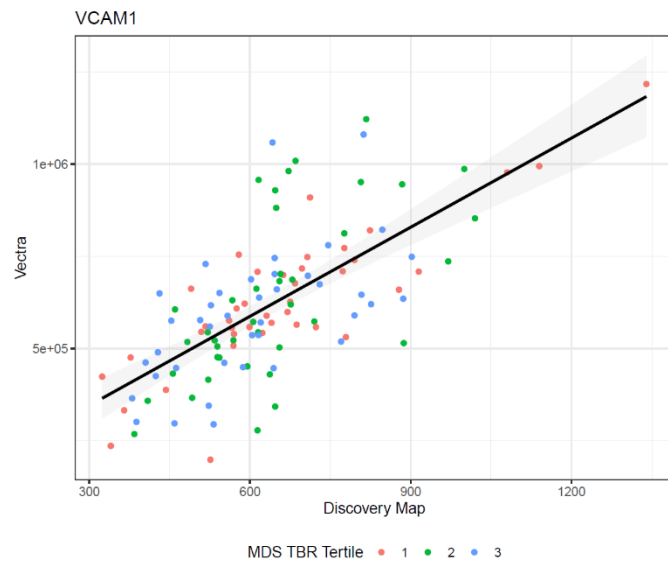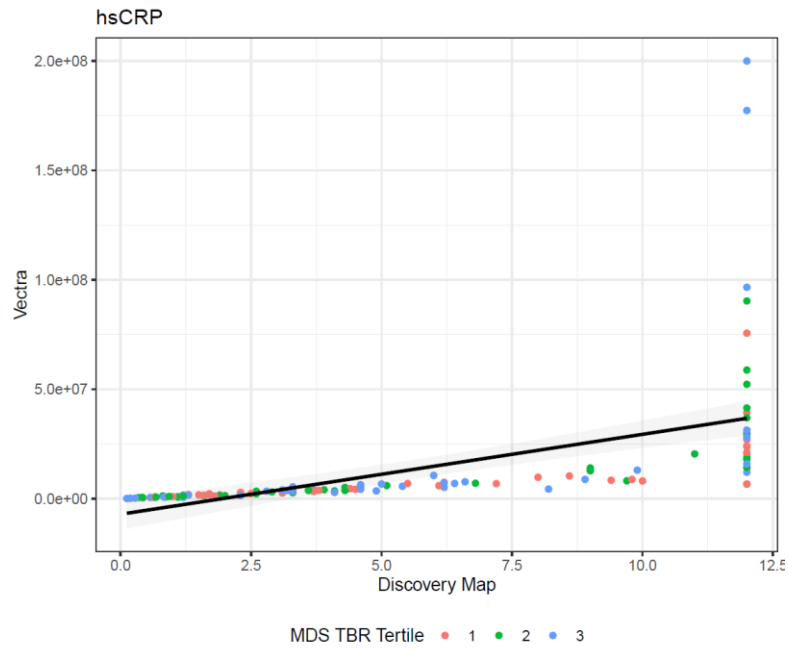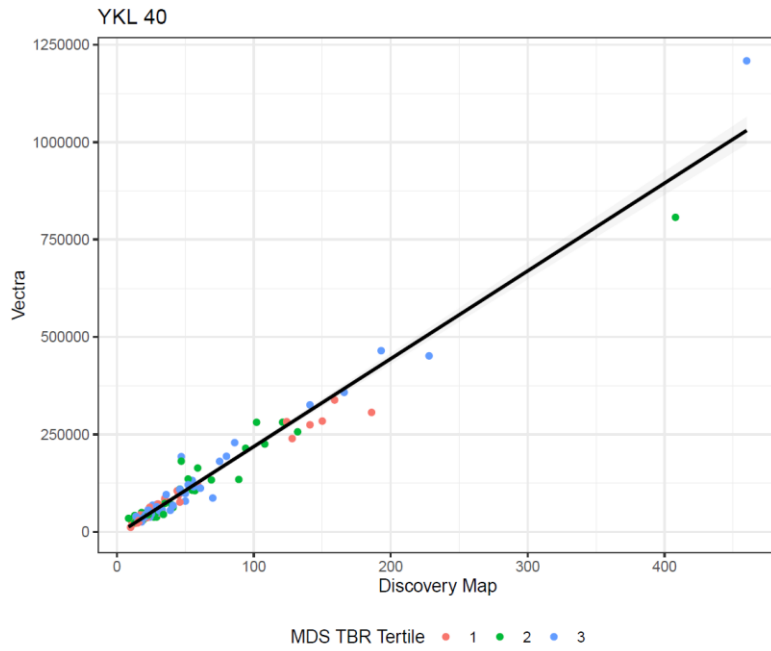
Table S1. Selected Biomarkers and Selected References Describing their Relationships with RA and CVD.

Cytokine/Inflammation	Results related to RA	Results related to CVD	
IL-6	Multifactorial roles[63, 64]; antagonists effective for RA treatment[65]	Strong association with CV events[66, 67]; associated with atherosclerosis[68]	pg/mL
sTNF-R1 and R2	Important in rheumatoid synovitis[69]; antagonists effective for RA treatment[70]	Association with CV events[71, 72]	pg/mL
SAA	Produced by rheumatoid synovium[73]	Association with CAD[74]	ng/mL
CRP	Strong association with disease activity[75] and radiographic progression[76]	Association with CV risk factors[77]; strong and consistent association with CV risk[78]	ug/mL
CD-40 ligand	Strong association with disease activity[79]; over-expressed in rheumatoid synovium[80]	Strong association with CV events[81]; CD40 ligand stabilizes plaques[82]	ng/mL
Adipokines			
Adiponectin	Associated with BMI and x-ray damage[83]; associated with disease activity[84]	Associated with reduced CV risk[85]	ug/mL
Leptin	Associated with disease activity[84]	Association with CV risk[86]	ng/mL
Resistin	Associated with disease activity[84]	Associated with CV risk[87]	ng/mL
Atherothrombosis			
Antithrombin III	Elevated levels found in rheumatoid synovial fluid[88]	Strong association with CV events[89]	ug/mL
PAI 1	Elevated levels found in rheumatoid synovial fluid[90]	Localized in atherosclerotic plaque[91]	ng/mL
Lipids Parameters			
Apolipoproteins	Predicts response to TNFi treatment[92]	Apolipoprotein sub-fractions predict CV events[93]	A1: mg/mL A2: ng/mL B: ug/mL
Lp (a)	Strongly associated with inflammation in RA[94]	Lp(a) associates with CVD progression/events[95]	ug/mL
LDL	Associated with radiographic progression[96]	Strong, consistent relationship with CV events[97]	mg/dL
Other Analytes			
Cardiac troponins	Associated with disease activity[98]	hsTroponin associated with future CV events[99]	ng/L
nt-pro-BNP	Associated with disease activity[100]	nt-pro-BNP associated with CV ischemia[101]	pg/mL
VCAM-1	Expressed in rheumatoid synovium[102]	VCAM-1 associated with future CV events[103]	ng/mL
VEGF-A	Associated with disease activity[104]	VEGF regulates ischemia in CVD[105]	pg/mL
MMP-1 and MMP-3	Both associated with disease progression[106]	MMP-1 associated with plaque burden[107]	ng/mL
YKL-40	Elevated levels associated with disease activity[108]	Elevations associated with strokes on women[109]	ng/mL
Cystatin-C	Elevated in RA with CVD[110]	Elevations in high risk patients associated with CV events[111]	ng/mL
Osteopontin (OPN)	Reduced OPN associated with bone destruction[112]	Increased OPN levels associated with increased CV events[113]	ng/mL
Osteoprotegrin (OPG)	Reduced OPG/RANKL associated with bone destruction[114]	OPG increased in CVD[115]	pM

Table S2. Baseline Characteristics of TARGET Study Subjects Included in the Current Analysis.

	All Patients (n=109)	MTX + TNF inhibitor (n = 55)	Triple therapy (n = 54)
	N (%) or median (interquartile range)		
Age, years	58 (54, 65)	58 (54, 66)	58 (54, 64)
Sex, female	77 (71)	36 (65)	41 (76)
Race			
White	86 (81)	48 (91)	38 (72)
Black	10 (9.4)	4 (7.5)	6 (11)
Other	10 (9.4)	1 (1.9)	9 (17)
Ethnicity			
Hispanic	31 (28)	15 (27)	16 (30)
Non-Hispanic	78 (72)	40 (73)	38 (70)
RA disease duration, years	1.6 (0.5, 6.8)	1.6 (0.5, 7.4)	1.5 (0.6, 5.3)
Serologic status, positive	71 (65)	35 (64)	36 (67)
DAS28-CRP	4.8 (4.0, 5.6)	4.9 (4.0, 5.6)	4.6 (3.7, 5.5)
hsCRP (mg/L)	4 (2, 9)	4 (1, 7)	4 (2, 10)
Glucocorticoid use	38 (35)	17 (31)	21 (39)
NSAID use	45 (41)	25 (45)	20 (37)
Aspirin use	27 (25)	18 (33)	9 (17)
Methotrexate weekly dose (mg)	20.0 (17.5, 25.0)	20.0 (20.0, 25.0)	20.0 (15.0, 25.0)
Heath assessment questionnaire	1.25 (0.50, 1.75)	1.13 (0.50, 1.75)	1.25 (0.63, 1.72)
Body mass index (kg/m2)	29.2 (25.7, 33.2)	29.4 (25.9, 33.6)	28.6 (25.8, 33.1)
Hypertension	50 (46)	26 (47)	24 (44)
Hyperlipidemia	23 (21)	12 (22)	11 (20)
Diabetes mellitus	2 (1.8)	2 (3.6)	0 (0.0)
Tobacco use			
Current	12 (11)	2 (3.6)	10 (19)
Past	28 (26)	18 (33)	10 (28)
Never	69 (63)	35 (64)	34 (63)
Statin use			
Low-moderate intensity	22 (20)	11 (20)	11 (20)

Adapted and used with permission from Annals of the Rheumatic Diseases 2023; 82:324-330.

IQR, inter-quartile range; RA, rheumatoid arthritis; IQR, interquartile range; hsCRP, high sensitivity C-reactive protein; NSAID, non-steroidal anti-inflammatory drugs. DAS28-CRP, disease activity score in 28 joints.

Table S3. Covariates Stratified by Vascular TBR for TARGET Final Population.

	Tertile 1 (1.65 – 2.41) (N=35)	Tertile 2 (2.41 – 2.73) (N=37)	Tertile 3 (2.73 – 4.44) (N=37)	P-value for trend
	N (%) or median (IQR)			
Sociodemographic				
Age, years	61 (56, 65)	56 (52, 62)	60 (55, 66)	0.96
Sex, female	30 (86%)	23 (62%)	24 (65%)	0.43
Race				
Black	5 (14%)	3 (8.6%)	2 (5.6%)	0.02
White	26 (74%)	31 (89%)	29 (81%)	0.68
Other	4 (11%)	1 (2.9%)	5 (14%)	0.90
Ethnicity				
Hispanic	9 (26%)	10 (27%)	12 (32%)	0.22
Non-Hispanic	26 (74%)	27 (73%)	25 (68%)	0.22
Clinical CV Risk Factors				
Blood pressure, systolic	126 (117, 133)	129 (121, 142)	130 (116, 145)	0.36
Blood pressure, diastolic	74 (68, 80)	79 (75, 84)	76 (70, 86)	0.12
Hypertension treatment, current	17 (49%)	14 (38%)	15 (41%)	0.53
Total cholesterol, mg/dl	198 (179, 234)	202 (172, 225)	208 (179, 228)	0.59
High density lipoprotein, mg/dl	56 (50, 70)	59 (45, 70)	58 (44, 68)	0.60
Tobacco use, current	4 (11%)	3 (8.1%)	5 (14%)	0.70
Body mass index, kg/m2	28.5 (25.6, 34.4)	28.2 (26.2, 34.7)	29.8 (27.0, 33.1)	0.90
Diabetes	0 (0%)	0 (0%)	2 (5.4%)	…
Statin use, any	6 (17%)	6 (16%)	10 (27%)	0.40

Tests for trend were performed using tertiles as an ordinal variable for continuous demographic characteristics, and using a weighted test of trend of proportions for categorical demographic characteristics.

Table S4. High Value Candidate Biomarker Values at Baseline Stratified by Baseline Vascular TBR for TARGET Final Population.

	Tertile 1	Tertile 2	Tertile 3	P-value for trend
	N (%) or median (IQR)			
Cytokines/Inflammation				
IL-6	11 (8, 21)	10 (5, 19)	9 (7, 21)	0.31
sTNF-R1	1,344 (1,087, 1,885)	1,283 (1,001, 1,680)	1,487 (1,130, 1,801)	0.67
Serum amyloid A (SAA)	19,950 (11,150, 38,150)	9,940 (6,135, 31,100)	15,300 (7,800, 26,050)	0.67
hsCRP	4.3 (1.9, 9.9)	4.1 (2.0, 12.0)	4.6 (1.8, 7.4)	0.53
CD-40 ligand	0.13 (0.07, 0.29)	0.08 (0.06, 0.24)	0.10 (0.05, 0.13)	0.09
Macrophage Inhibitory Factor (MIF)	0.19 (0.14, 0.25)	0.16 (0.11, 0.24)	0.17 (0.10, 0.25)	
Lipid Parameters				
Apolipoprotein A1	2.30 (1.98, 2.92)	2.10 (1.70, 2.55)	2.10 (1.70, 2.70)	0.02
Apolipoprotein A2	313 (243, 389)	346 (260, 412)	326 (294, 406)	0.04
Apolipoprotein B	910 (690, 1,218)	973 (670, 1,075)	942 (570, 1,175)	0.79
Apolipoprotein C1	360 (274, 418)	348 (244, 402)	354 (296, 397)	0.95
Apolipoprotein C3	320 (251, 470)	299 (216, 378)	302 (240, 436)	0.24
Apolipoprotein H	468 (380, 639)	465 (382, 616)	491 (426, 610)	0.11
Apolipoprotein(a) aka Lp(a)	120 (46, 249)	89 (48, 232)	140 (76, 350)	0.73
Adipokines				
Adiponectin	7.2 (4.4, 9.8)	7.4 (4.8, 10.5)	6.0 (4.4, 8.7)	0.51
Leptin	29 (19, 48)	16 (9, 32)	22 (11, 44)	0.11
Resistin	2.70 (2.30, 3.53)	2.90 (1.95, 3.90)	3.20 (2.60, 4.40)	0.11
Atherothrombosis				
Antithrombin III	448 (388, 490)	466 (415, 542)	420 (378, 493)	0.95
PAI-1	74 (61, 111)	77 (44, 117)	61 (35, 104)	0.26
Miscellaneous				
hsTnT	6.3 (3.0, 8.3)	3.0 (3.0, 8.7)	6.5 (3.0, 9.9)	0.50
Nt-pro-BNP	352 (155, 715)	434 (239, 1,115)	334 (196, 565)	0.93
VCAM-1	636 (565, 736)	616 (539, 678)	604 (520, 719)	0.83
VEGF-A	166 (120, 228)	145 (114, 192)	135 (104, 194)	0.67
MMP-1	6,480 (4,142, 9,339)	8,295 (4,640, 12,338)	8,338 (5,607, 12,989)	0.07
MMP-3	7 (5, 14)	6 (5, 11)	8 (4, 18)	0.55
YKL-40	23 (17, 45)	28 (20, 54)	39 (25, 58)	0.30
Cystatin-C	1,165 (1,008, 1,398)	1,060 (960, 1,310)	1,200 (1,110, 1,315)	0.71
Osteopontin	33 (24, 40)	37 (24, 47)	31 (24, 44)	0.78
Osteoprotegrin (OPG)	8.00 (6.47, 9.62)	7.80 (6.50, 9.65)	8.50 (6.90, 11.00)	0.24

Tests for trend were performed using tertiles as an ordinal variable for continuous biomarkers.

Table S5. Sensitivity Analysis of Linear Regression Results Assessing Relationship Between Z-Score of Candidate Biomarker at Baseline and Change in Target to Background Ratio in the TARGET Trial Cohort, with RA Treatment and Corticosteroids Included.

	Selected biomarkers + PCE + Baseline TBR + RA Treatment + Corticosteroid Use
Cytokine/Inflammation	**Beta estimate (95% CI)**
IL-6	…
sTNF-R1	**-0.09 (-0.18, 0.01)**
SAA	**-0.21 (-0.38, -0.03)**
hsCRP	**0.23 (0.06, 0.40)**
CD-40 ligand	…
Adipokines	
Adiponectin	**-0.07 (-0.16, 0.02)**
Leptin	…
Resistin	…
Atherothrombosis	
Antithrombin III	…
PAI-1	…
Lipids Parameters	
Apolipoprotein A1	…
Apolipoprotein A2	…
Apolipoprotein B	…
Lp (a)	…
LDL	…
Other Analytes	
hsTnT	…
nt-pro-BNP	…
VCAM-1	…
VEGF-A	…
MMP-1	…
MMP-3	…
YKL-40	**0.11 (0.02, 0.20)**
Cystatin-C	…
Osteopontin (OPN)	…
Osteoprotegrin (OPG)	**-0.11 (-0.20, -0.03)**
Pooled cohort equation	0.00 (-0.01, 0.01)
RA treatment	0.00 (-0.17, 0.17)
Corticosteroid use	0.04 (-0.15, 0.24)
Model Fit Statistics	
Adjusted R^2	0.31
RMSE	0.40
NOTES: All models include the baseline target to background ratios values from the most diseased segment (MDS TBR). PCE, Pooled Cohort Equation, includes age, sex, race, diabetes status, cigarette use, systolic blood pressure, treatment for hypertension, and high-density lipoprotein value. R^2 indicates better model fit when larger and RMSE, Root Mean Square Error, is better when smaller. Bolded values have p ≤0.10 and were advanced to the selected biomarkers model. RA treatments included TNFi or triple therapy.	