SUPPLEMENTARY MATERIALS

Title: Effects of BI 655064, an antagonistic anti-CD40 antibody, on clinical and biomarker parameters in patients with active rheumatoid arthritis: a randomised, double-blind, placebo-controlled, Phase IIa study

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Additional methods: exclusion criteria

Patients who had previously received anti-tumour necrosis factor (TNF) drugs should have had the last dose of etanercept at least 28 days before randomisation and the last dose of infliximab, adalimumab, certolizumab or golimumab at least 60 days before randomisation. Exclusion criteria included the use of any standard disease-modifying anti-rheumatic drug except methotrexate (MTX; including but not limited to sulfasalazine, leflunomide, hydroxychloroquine, D-penicillamine, azathioprine, cyclosporine or gold salts) that would have been continued after randomisation; impaired hepatic function (aspartate aminotransferase, alanine aminotransferase, bilirubin or alkaline phosphatase levels >2 times the upper limit of normal); impaired renal function (calculated creatinine clearance <50 mL/min); patients with chronic or relevant acute infection (including but not limited to HIV, hepatitis B, hepatitis C and tuberculosis), severe disability (functional class IV), confirmed severe systemic manifestations (amyloidosis, Felty's syndrome, lymphoproliferative disorders or rheumatoid vasculitis), any active or suspected malignancy or history of documented malignancy within the 5 years prior to screening; or hypersensitivity or previous intolerance to MTX, as the main cause for treatment discontinuation.

Additional methods: sample collection

Blood samples for assessment of anti-BI 655064 antibodies (anti-drug antibodies [ADAs]) were taken at Weeks 0, 4, 8 and 12 after administration of the first dose of BI 655064 and at the end-of-study visit (Week 20).

Blood samples (20 mL) for exploratory clinical biomarkers were taken at Weeks 0, 4, 8 and 12. Biomarkers were analysed either in plasma ethylenediaminetetraacetic acid (EDTA) or serum.

For pharmacogenomic analyses, patients were asked to give four blood samples. The first and second samples (8.5 mL) were taken at Week 0 for genotyping and DNA banking. The third and fourth samples (2.5 mL) were taken at Weeks 0 and 12, respectively, for exploratory RNA gene expression analyses. Provision of all pharmacogenomic samples was voluntary, and samples were taken and processed or stored after patients provided separate informed consent that was in accordance with local ethical and regulatory requirements.

Blood samples for pharmacokinetics, ADAs and exploratory biomarkers were immediately placed on ice after collection, and centrifuged at 4°C for 10 minutes within 30 minutes of sample collection. Plasma was transferred into two polypropylene sample vials (0.5 mL each) and stored at –20°C before shipping to the analytical laboratory.

Serum for exploratory analyses was collected in a serum separator tube and left to clot at room temperature for 30 minutes, then centrifuged at 4°C for 10 minutes. Serum was transferred into 0.5 mL polypropylene tubes, and stored at –20°C before shipping to the analytical laboratory.

Blood samples for genotyping were collected in a PAXGene Blood DNA sampling tube.

Genomic DNA extraction and preparation of reagents was performed by a chemagic Magnetic Separation Module I according to manufacturer's instructions. Genotyping of rs4810485 was performed in a subset of 44 patients who gave informed consent, using an allelic discrimination assay based on TaqMan polymerase chain reaction (PCR; Applied Biosystems).

Additional methods: analytical methods

ADAs were analysed in plasma samples using a validated bridging electrochemiluminescence (ECL) method. All reported sample data met the assay-specific acceptance criteria. Validation of the ADA assay demonstrated that 250 ng/mL of the positive control anti-BI 655064 ADA could be detected in the presence of plasma concentrations of 50 µg/mL BI 655064. A true positive response in a patient was further characterised by additional titre assays. Titres were determined by analysis of serial twofold dilutions of the sample. Reported titres were the highest-fold dilution that produced a mean ECL value that was greater than or equal to the plate-specific cut point. ADA assessments were performed by Covance Laboratories, Inc., USA.

Protein and cellular biomarkers were assessed in whole blood. Protein and cellular biomarkers were measured by Q2 Solutions, USA. Serum IgG ACPA was assessed using the EliA™ CCP. Serum IgG rheumatoid factor (RF), IgA RF and IgM RF levels were assessed using the COBAS Tina-quant RF II immunoturbidimetric assay. Plasma CD40L was assessed using the validated Quantikine® Human CD40 Ligand/TNFSF5 immunoassay kit.

TNFα, IL-6 and RANKL were measured by Q2 Solutions, UK; RANKL was measured by Q2 Solutions, Singapore. Serum TNFα and IL-6 were assessed using the QuantiGlo ELISA kit (R&D Systems). Serum MMP3 was assessed using custom arrays (Randox Laboratories Limited). Soluble RANKL levels were assessed using the total sRANKL ELISA kit (Immundiagnostik AG).

B-cell subsets (listed below) were analysed using the BD FACS Canto II Flow cytometry system using the following antibodies: anti-IgD-FITC, anti-CD27-PE, anti-CD19-PerCP-Cy5.5,

anti-IgG1-APC, anti-CD40-APC, anti-IgG1-BV421, anti-CD95-BV421, anti-CD45, anti-V500 (BD Biosciences, BioLegend). B-cell subsets were determined by Q2 Solutions, UK.

rs4810485 was genotyped in a subset of 44 patients who gave informed consent, by an allelic discrimination assay based on TaqMan PCR (Applied Biosystems) and using a validated primer and probe set from Applied Biosystems (C_1260190_10). All single nucleotide polymorphisms were genotyped using a TaqMan assay in a 7900HT Sequence Detection System, and analysed using SDS 2.4 (Applied Biosystems). Reactions were then subjected to: 95°C, 10 min; 40 cycles 95°C, 15 s; 60°C, 60 s.

B-cell subsets
B cells
CD19+ (%)
Naive B cells
CD19+IgD+CD27-CD40+ (%)
CD19+IgD+CD27-CD95+ (%)
Memory B cells
CD19+IgD-CD27+CD40+ (%)
CD19+IgD-CD27+CD95+ (%)
Double-negative B cells
CD19+IgD-CD27-CD40+ (%)
CD19+IgD-CD27-CD95+ (%)
Pre-switch memory B cells
CD19+IgD+CD27+CD40+ (%)
CD19+IgD+CD27+CD95+ (%)

Additional methods: analysis of primary endpoint

The primary endpoint (ACR20 at Week 12) was evaluated using a Bayesian approach that was applied separately to each of the two treatment groups. For the BI 655064 treatment group, a non-informative beta (0,0) prior was used; no assumptions were made regarding the response rate for the BI 655064 treatment group. For the placebo group, an informative beta (5.5, 16.5) prior was used; the expected placebo response, based on historical data, was assumed to be 25%. The effective sample size was chosen to be 22, which equalled the actual number of patients in the placebo group. The difference in ACR20 response rates was evaluated by simulating the posterior distribution for each treatment group 1,000,000 times.

For exploratory reasons, subgroup analyses were performed on the same population as the primary analysis for various baseline characteristics. Unadjusted absolute risk differences for ACR20, along with the corresponding confidence intervals, are provided in a forest plot for illustration purposes.

Additional methods: statistical analysis of secondary endpoints, clinical biomarkers and pharmacogenomic analyses

The rs4810485 G/T-allele was genotyped in all 46 patients who provided informed consent. Patients who were homozygous for T/T and heterozygous for G/T were grouped together and compared with patients homozygous for G/G. Statistical analysis included unadjusted absolute risk differences of ACR20 and ACR50 response rates at Week 12 between BI 655064 and placebo for each genotype group. The proportion of responders in each arm, the risk difference and 95% exact unconditional confidence intervals were calculated together with a corresponding (one-sided) p-value to investigate if active treatment has a higher response rate than placebo.

Comparisons of clinical biomarkers between treatment groups overall and within each rs4810485 G/T-allele group, as described earlier, were performed. Median changes or percent changes from baseline at each visit were compared using a Wilcoxon two-sample test and nominal p-values were calculated. No adjustments for multiple comparisons were made. Similar comparisons were made for ACR50 responders versus non-responders at Week 12 for patients who received BI 655064.

SUPPLEMENTARY TABLES

Table S1 Efficacy analyses at Week 12 by baseline CRP subgroup

	BI 655064	Placebo
Median CRP >7.5 mg/L	n=17	n=16
ACR20, n (%)*	11 (64.7)	8 (50.0)
ACR50, n (%)*	7 (41.2)	3 (18.8)
Change from baseline		
DAS28-CRP, mean (SE)[†‡]	–1.83 (0.26)	–1.38 (0.27)
CRP, mean (SE)[†§]	–10.45 (3.54)	–3.90 (3.65)
Median CRP ≤7.5 mg/L	n=27	n=6
ACR20, n (%)*	19 (70.4)	2 (33.3)
ACR50, n (%)*	9 (33.3)	1 (16.7)
Change from baseline		
DAS28-CRP, mean (SE)[†]	–1.49 (0.19)	–1.58 (0.40)
CRP, mean (SE)[†]	0.13 (0.57)	1.26 (1.23)

*FAS, non-responder imputation.
[†]FAS, last observation carried forward.
[‡]ANCOVA analysis adjusted for region, anti-TNF history and baseline DAS28−CRP.
[§]ANCOVA analysis adjusted for region, anti-TNF history and baseline CRP.
ACR20/50, American College of Rheumatology 20/50% improvement criteria; ANCOVA, analysis of covariance; CRP, C-reactive protein; DAS28, Disease Activity Score in 28 joints; FAS, full analysis set; SE, standard error.

Table S2 Median change from baseline at Week 12 in ESR, CRP and RF

	BI 655064	**Placebo**
ESR (mm/h)	–18 (n=39)	–9 (n=19)
CRP (mg/L)	–0.5 (n=39)	–1.5 (n=20)
RF (IU/mL)	–49.3 (n=33)	–8.6 (n=18)

Data are median.
CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; FAS, full analysis set; RF, rheumatoid factor.

Table S3 Mean change from baseline to Week 12 in ACR core measures

	BI 655064 (n=39)	Placebo (n=20)
SJC 66/68	–8.55 (7.57)	–6.65 (4.07)
TJC 68/68	–11.09 (10.19)	–10.35 (9.23)
CRP (mg/L)*†	–4.81 (1.62)	–0.78 (2.34)
ESR (mg/L)*†	–17.17 (1.98)	–9.21 (2.89)
PtGA VAS (0−100 mm)	–26.36 (23.30)	–16.45 (29.37)
PGA VAS (0−100 mm)	–35.54 (19.98)	–31.70 (24.17)
HAQ	–0.38 (0.48)	–0.42 (0.42)
Pain VAS (0−100 mm)	–26.82 (24.09)	–11.90 (30.86)

*ANCOVA analysis adjusted for baseline values, treatment, region and anti-TNF history.
†FAS, last observation carried forward.
Data are mean (SD). FAS, observed.
CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; FAS, full analysis set; HAQ, Health Assessment Questionnaire; PGA, Physician Global Assessment; PtGA, Patient Global Assessment; SD, standard deviation; SJC, swollen joint count; TJC, tender joint count; TNF, tumour necrosis factor; VAS, visual analogue scale.

Table S4 Median percent change from baseline to Week 12 of IL-6, RANKL and MMP3 in all patients, ACR50 responders and ACR50 non-responders

	All patients		ACR50 responders		ACR50 non-responders	
	BI 655064 (n=44)	**Placebo (n=22)**	**BI 655064 (n=16)**	**Placebo (n=4)**	**BI 655064 (n=23)**	**Placebo (n=16)**
IL-6 (%)						
Week 4	–12.0	–13.5	–13.3	–30.9	–10.1	–3.6
Week 8	–28.1	–2.8	–32.2	–47.3	–18.9	5.0
Week 12	–17.3	18.7	–39.0	3.1	–4.7	18.7
RANKL (%)						
Week 4	–4.2	–5.7	–4.8	13.6	–4.2	–9.4
Week 8	–19.6	1.8	–12.5	11.0	–19.6	4.6
Week 12	–29.4	0	–21.8	53.1	–35.5	–1.3
MMP3 (%)						
Week 4	1.8	–4.2	–1.4	–4.6	16.0	1.4
Week 8	–7.5	4.6	–19.6	–13.5	18.2	12.1
Week 12	–7.8	2.3	–25.2	–15.3	14.2	12.1

Median percent change from baseline to Week 12 of IL-6, RANKL and MMP3 in all patients and ACR50 responders and non-responders. Data are presented in figure S2. FAS, observed.

ACR50, American College of Rheumatology 50% improvement criteria; FAS, full analysis set; IL, interleukin; MMP3, matrix metalloproteinase-3; RANKL, receptor activator of nuclear factor-κB ligand.

Table S5 Median percent change from baseline to Week 12 in CD19+ B-cell subsets in rs4810485 T-allele and non-T-allele carriers

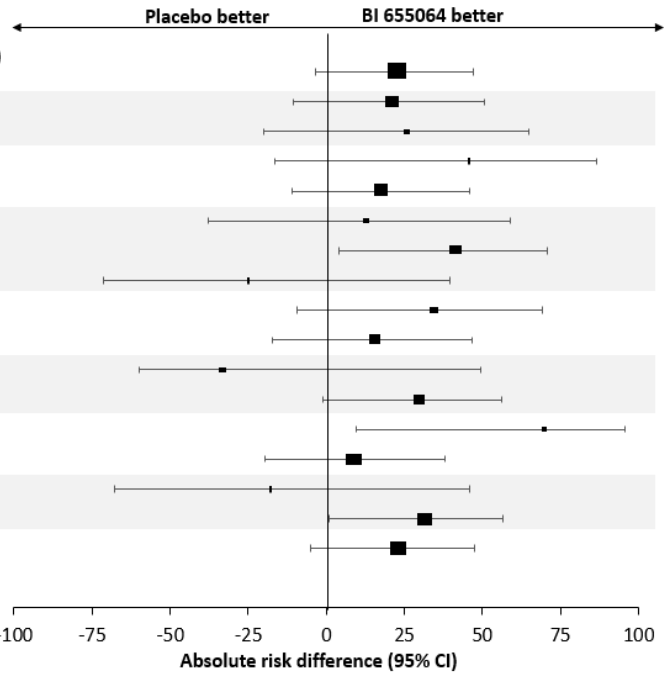
	BI 655064	Placebo	p-value
TT/GT-allele (%)			
n	8	7	
CD19+IgD+CD27+CD95+	–16.8	6.0	0.0728
CD19+IgD−CD27+CD95+	–24.2	4.6	0.0032
CD19+IgD−CD27−CD95+	–18.6	7.0	0.0022
GG-allele (%)			
n	8	5	
CD19+IgD+CD27+CD95+	–20.15	–15.8	1.0000
CD19+IgD−CD27+CD95+	–10.3	–20.2	0.1243
CD19+IgD−CD27−CD95+	–9.4	–9.6	0.8262

Data are median. FAS.
FAS, full analysis set.

SUPPLEMENTARY FIGURES

Figure S1 Risk difference of ACR20 at Week 12

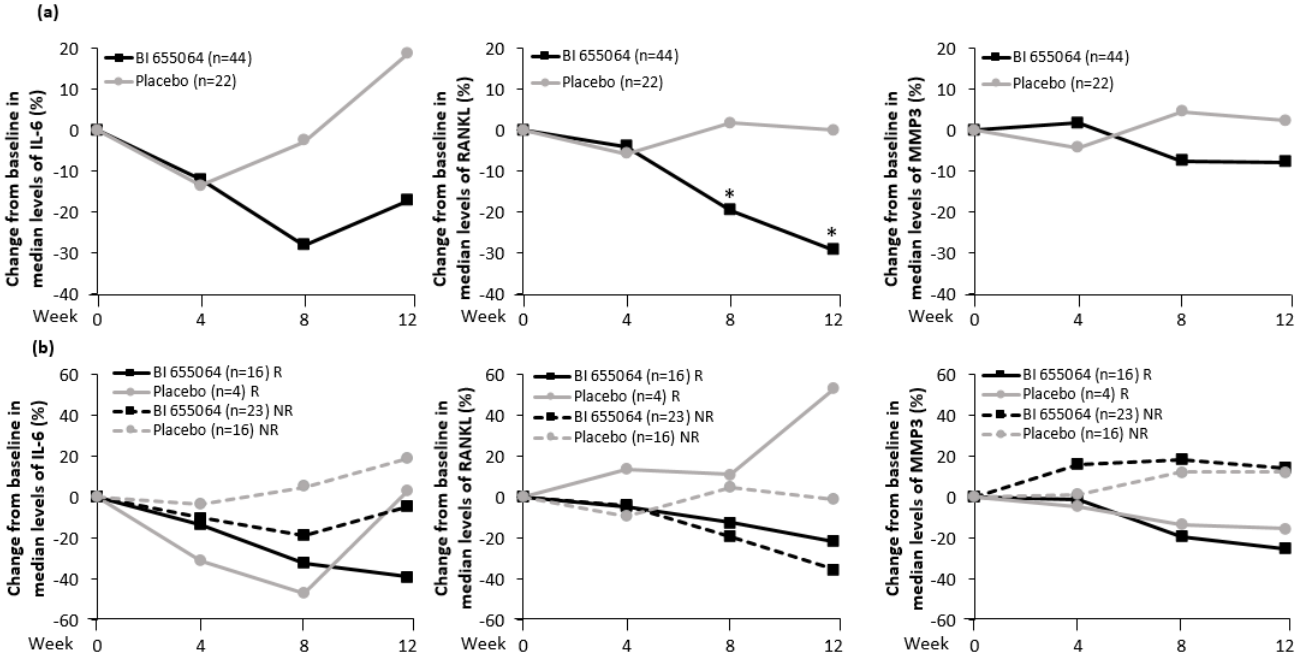
Subgroup	Category	N analysed, BI 655064/Placebo	Absolute risk difference (95% CI)
	Total	44/22	22.7 (–3.3, 46.9)
Region	Eastern Europe	28/15	21.2 (–10.7, 50.5)
	Rest of world	16/7	25.9 (–19.9, 64.9)
Sex	Male	7/5	45.7 (–16.6, 86.6)
	Female	37/17	17.8 (–11.1, 45.8)
Age	<50 years	9/7	12.7 (–37.8, 58.7)
	50 – <65 years	27/11	41.4 (4.0, 70.7)
	≥65 years	8/4	–25.0 (–71.4, 39.7)
DAS28-CRP at baseline	<5.1	18/8	34.7 (–9.3, 69.3)
	≥5.1	26/14	15.4 (–17.3, 46.5)
BMI	<25 kg/m²	21/2	–33.3 (–59.8, 49.5)
	≥25 kg/m²	23/20	29.6 (–1.3, 56.3)
Time since diagnosis	<2.5 years	10/5	70.0 (9.4, 95.7)
	≥2.5 years	34/17	8.8 (–19.8, 37.8)
Renal impairment using CKD-EPI	<75 mg/mL	7/4	–17.9 (–67.9, 45.9)
	≥75 mg/mL	37/18	31.4 (0.8, 56.5)
Anti-TNFα history	Anti-TNFα naïve	38/22	23.0 (–4.9, 47.5)
	Anti-TNFα exposed	6/0	N/A



Unadjusted absolute risk differences of ACR20 response rates at Week 12. FAS, non-completer considered failure.

ACR, American College of Rheumatology; BMI, body mass index; CI, confidence interval; CKD-EPI, Chronic Kidney Disease-Epidemiology Collaboration; CRP, C-reactive protein; DAS28, Disease Activity Score in 28 joints; FAS, full analysis set; TNF, tumour necrosis factor.

Figure S2 Median percent change from baseline to Week 12 in levels of IL-6, RANKL and MMP3



*p≤0.01.

Median percent change from baseline to Week 12 of IL-6, RANKL and MMP3 in (a) all patients and (b) ACR50 responders and non-responders. Data presented in Supplementary Table S5. FAS, observed.

ACR50, American College of Rheumatology 50% improvement criteria; FAS, full analysis set; IL, interleukin; MMP3, matrix metalloproteinase-3; NR, non-responder; R, responder; RANKL, receptor activator of nuclear factor-κB ligand.