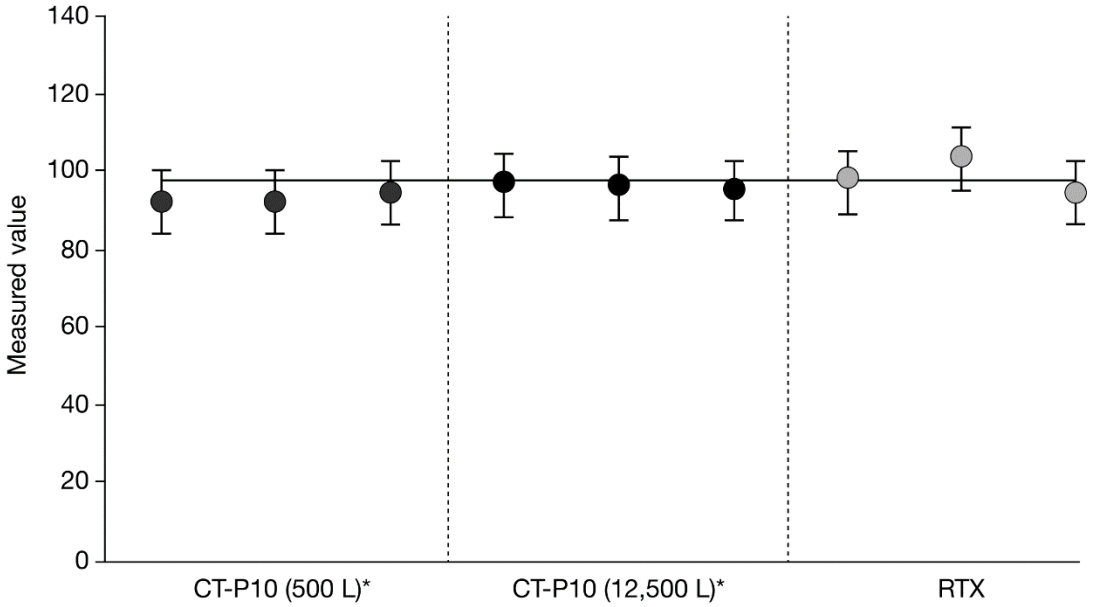
ONLINE SUPPLEMENTARY MATERIAL A

COMPARATIVE BIOLOGICAL DATA FOR CT-P10 AND RTX

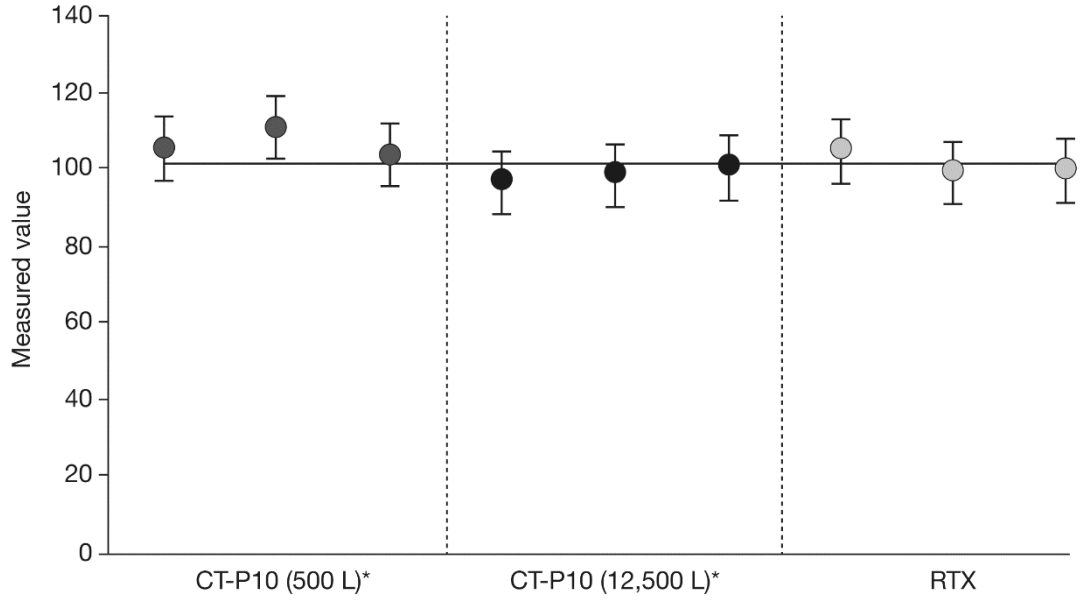
Figure A-1. Complement-dependent cytotoxicity (CDC)



*Values in liters (L) indicate the scale of cultivation (i.e. CT-P10 batches were cultured in a 500 L or 12,500 L reactor). Relative CDC bioactivities were calculated as a percentage of the EC50 of each sample curve versus the EC50 of the in-house reference curve. Error bars represent the standard deviation of each sample analyzed in triplicate. The horizontal line represents the average value for RTX in this assay.

EC50, half maximal effective concentration; RTX, innovator rituximab.

Figure A-2. FcRn binding affinity



*Values in liters (L) indicate the scale of cultivation (i.e. CT-P10 batches were cultured in a 500 L or 12,500 L reactor). FcRn binding affinity of CT-P10 and RTX were compared with surface plasmon resonance. The relative binding affinity of CT-P10 and RTX was calculated as a percentage of the KD of the in-house reference versus the KD value of each sample. Error bars represent the standard deviation of each sample analyzed in triplicate. The horizontal line represents the average value for RTX in this assay.

FcRn, neonatal Fc receptor; KD, equilibrium dissociation constant; RTX, innovator rituximab.

ONLINE SUPPLEMENTARY MATERIAL B: PATIENTS AND METHODS

Patients

Patients aged 18–75 years and diagnosed with rheumatoid arthritis (RA) (according to the revised 1987 American College of Rheumatology [ACR] classification criteria [Arnett et al. *Arthritis Rheum* 1988;31:315–24]) for ≥6 months before randomization were recruited. Eligible patients had active disease—defined as ≥6 swollen joints (of 66 assessed) and ≥6 tender joints (of 68 assessed) plus either serum C-reactive protein (CRP) ≥1.5 mg/dL or erythrocyte sedimentation rate (ESR) ≥28 mm/hour—and had received oral or parenteral methotrexate (MTX; 10–25 mg/week) for ≥12 weeks before screening (last 4 weeks at a stable dose). All patients had previously shown an inadequate response, or intolerance to, anti-tumor necrosis factor (TNF) agents (adalimumab, certolizumab, etanercept, golimumab, or infliximab).

Exclusion criteria included prior treatment with more than two biologic drugs; allergies or hypersensitivity to murine, chimeric, human, or humanized proteins (as reported by the patient or determined from the patient's medical history); receipt of oral or parenterally injected antibiotics ≤2 weeks or ≤4 weeks before randomization, respectively, or other serious infection ≤6 months before randomization; history of recurrent herpes zoster or other chronic or recurrent infection; history of rheumatic autoimmune disease other than RA (except secondary Sjögren's syndrome); and significant systemic involvement secondary to RA (vasculitis, pulmonary fibrosis, or Felty's syndrome).

Trial ethics

The trial was conducted in accordance with the Declaration of Helsinki and International Conference on Harmonization Good Clinical Practice guidelines. The trial protocol was reviewed and approved by each center's ethics committee and the relevant regulatory authorities. All patients provided written informed consent.

Randomization

Patients were randomly assigned 2:1 to receive CT-P10 or innovator rituximab (RTX). Randomization was performed using a computer-generated randomization schedule and stratified by region (Europe versus non-Europe) and prior anti-TNF agent status (failure versus intolerance).

Concomitant treatments

All patients continued treatment with MTX (10–25 mg/week) and oral folic acid (≥5 mg/week) with doses of these drugs stably maintained throughout the study. Patients could receive glucocorticoids (prednisone/prednisolone ≤10 mg/day or equivalent) and nonsteroidal anti-inflammatory drugs if they were on stable doses of these drugs for ≥4 weeks before randomization until study end. Paracetamol and/or tramadol were allowed as rescue therapy but not in the 24 hours before joint evaluation.

Methylprednisolone (100 mg intravenously), acetaminophen/paracetamol (usually 500–1,000 mg) and an antihistamine (chlorpheniramine [2–4 mg or dose equivalent]) were given 30–60 minutes before each infusion of study drug.

Assessments and endpoints

Patients underwent clinical assessments at screening, day 0, and then every 8 weeks thereafter. Blood samples for pharmacokinetic (PK) and B-cell assessments were obtained 15 times between screening and week 24 for each patient. Analyses of blood samples for routine laboratory parameters, CRP, ESR, immunoglobulins (IgM, IgG, and IgA), and anti-drug antibodies (ADAs) were also performed throughout the study.

Primary PK endpoints are defined in the main paper. Secondary PK endpoints included maximum serum concentration after first infusion ($C_{max,\ 1}$); trough serum concentration before second infusion (C_{trough}); volume of distribution (V_d); total body clearance over both infusions (CL); terminal elimination half-life after second infusion ($T_{1/2}$); and time to C_{max} after first and second infusions (T_{max}).

An electrochemiluminescent (ECL) bridging assay was developed and validated for analysis of RTX and CT-P10 concentrations in human serum. With this method, human serum samples containing RTX/CT-P10 (after 1:25 dilution in assay buffer containing 3% bovine serum albumin) are incubated together with biotinylated rat anti-rituximab antibody (biotin) and ruthenium-labelled rat anti-rituximab antibody, and an antibody–complex bridge is formed. The biotin portion of the complex then binds to a streptavidin-coated Meso Scale Discovery (MSD) plate when it is transferred to the plate. A washing step is then performed to remove unbound material. After the addition of read buffer containing tripropylamine, the plates are read on an MSD SECTOR Imager 6000. The voltage applied by the reader triggers the ruthenium to produce a chemiluminescence signal that is directly proportional to the concentration of RTX/CT-P10.

Clinical assessments of disease activity included ACR 20%, 50%, and 70% (ACR20, ACR50, and ACR70) response rates, time-to-onset of ACR20, ACR individual component scores, European League Against Rheumatism (EULAR) response criteria, mean

decrease in Disease Activity Score 28 (DAS28), Clinical Disease Activity Index (CDAI), Simplified Disease Activity Index (SDAI), functional disability (Health Assessment Questionnaire Disability Index), and general health status (Medical Outcomes Study Short-Form Health Survey [SF-36]).

B-cell kinetics, including depletion and recovery, were evaluated using absolute numbers of $CD19^+$ and $CD20^+$ cells with a FACScalibur flow cytometer (Becton Dickinson and Company; San Jose, CA, USA).

An ECL immunoassay method (MSD, Rockville, Maryland, USA) was used to measure ADAs. This method uses the MSD SECTOR Imager 6000 platform and follows a two-tiered assay approach consisting of a screening assay and specificity/confirmatory assay (see online supplementary material C). The assays were first evaluated with a surrogate positive control consisting of affinity-purified rat anti-human CT-P10/RTX antibody. The screening assay was then performed on collected samples in order to detect the presence of CT-10-binding antibodies. Samples with a signal-to-noise value greater than or equal to the assay cut-point were then analyzed to confirm specificity of the response. Drug-treated samples analyzed in the specificity/confirmatory assay that depleted in the presence of excess soluble drug were then reported as positive for the presence of anti-drug antibodies. During validation of the ECL immunoassay method, no effects of rheumatoid factor were observed during interference testing.

Neutralizing antibodies against CT-P10/RTX were measured in human serum using a complement-dependent cytotoxicity (CDC) assay. This assay is dependent on the ability of the complement system to be activated by the binding of CT-P10 or RTX (i.e. monoclonal anti-CD20 antibodies) to the CD20 antigen on B-cells. If a serum sample contains detectable levels of antibodies that can neutralize CT-P10 or RTX by binding to their F(ab')2 region, this results in an increased output signal in the cytotoxicity assay

that is directly proportional to number of viable cells. In the absence of neutralizing antibodies, CT-P10 or RTX can bind CD20, resulting in a decreased output signal that is inversely proportional to CDC activity.

Safety evaluations included monitoring of adverse events, serious adverse events, and adverse events of special interest (e.g. infections, infusion-related reactions including hypersensitivity, and malignancies or lymphomas), clinical laboratory analyses, general physical examination, vital signs, and electrocardiogram. An interferon-γ release assay was used to monitor for latent tuberculosis infection at baseline and week 24.

Statistical analysis

Sample size calculations were based on an assumption of equivalence in the primary endpoints ($AUC_{0–last}$ and C_{max}) between CT-P10 and RTX groups. Based on 90% power, a type error of 0.1, an inter-patient coefficient of variation in $AUC_{0–last}$ of 35%, a true ratio of means of 1.0, and a 2-sided equivalence margin of 80–125% for $AUC_{0–last}$ and C_{max}, 78 and 39 patients in the CT-P10 and RTX groups, respectively, were required. Allowing for a 20% drop-out rate, 147 patients were needed for randomization.

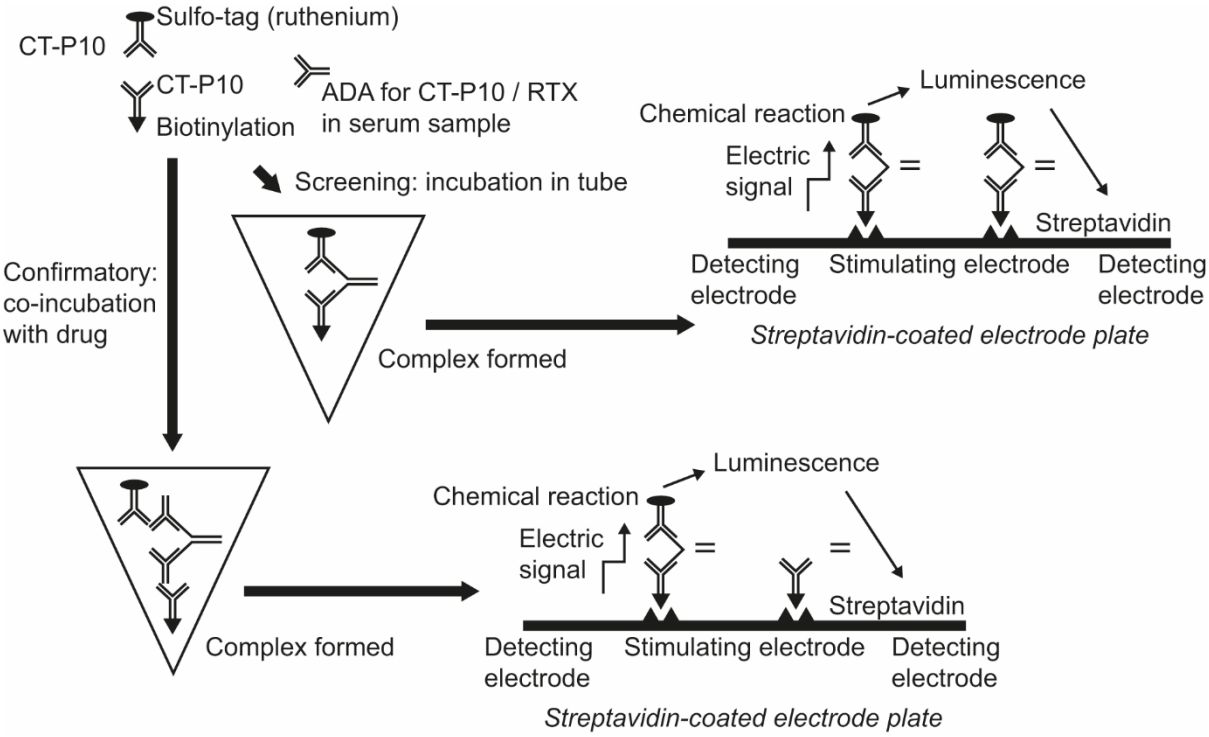
The primary statistical analysis for the study is described in the main paper. Secondary PK parameters were summarized using descriptive statistics. A t-test was used to compare efficacy data at week 24 across treatment groups. Pharmacodynamic (PD) parameters and safety evaluations were described descriptively.

All PK analyses were performed in the PK population (all patients who received two doses of study drug [at weeks 0 and 2 with a total dose of 2,000 mg] and provided sufficient blood concentration data). Efficacy and PD analysis populations included all patients who received at least one full dose of CT-P10 or RTX and provided at least one

post-treatment efficacy or PD result, respectively. The safety population included all patients who received at least one (full or partial) dose of CT-P10 or RTX.

ONLINE SUPPLEMENTARY MATERIAL C: CHEMOLUMINESCENT ASSAY USED TO DETECT ANTI-DRUG ANTIBODIES

Figure C-1. Chemiluminescent assay used to detect anti-drug antibodies

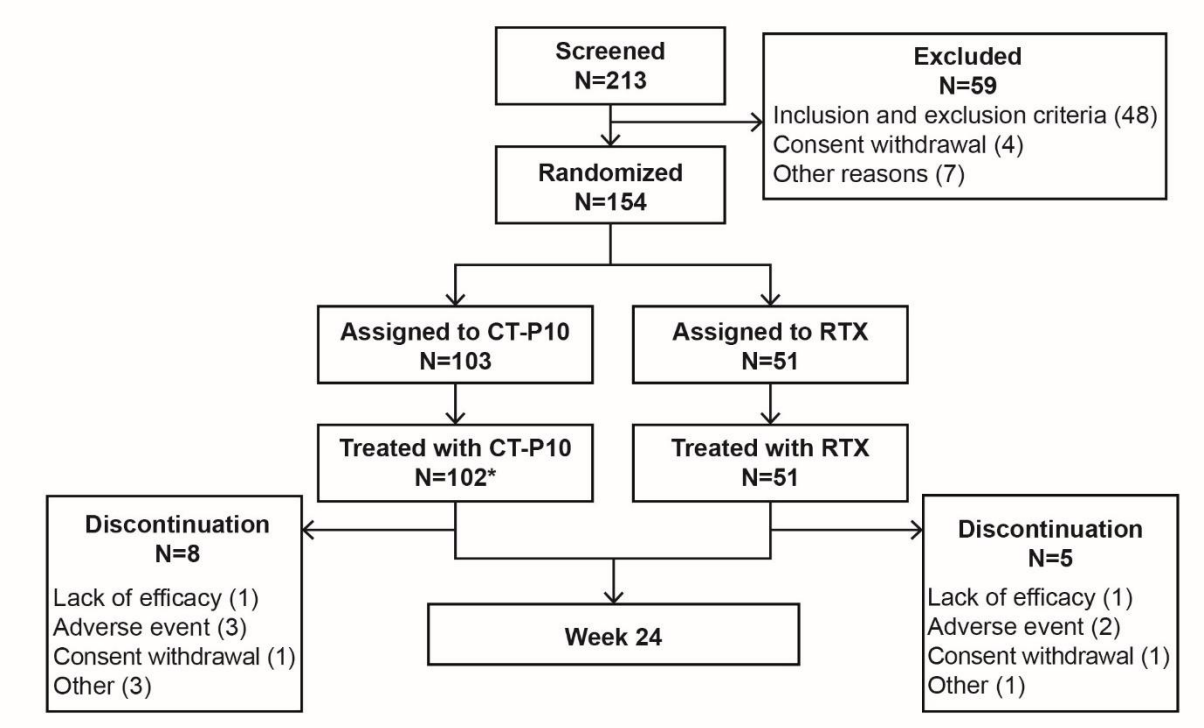


A bridging ELISA format was used for the ADA assay employed in the study. In this assay, CT-P10 is labeled with sulfo-tag and biotin. The biotin-labeled CT-P10 acts as the capture molecule and the sulfo-tag-labeled CT-P10 acts as the detection molecule. For the screening assay, labeled CT-P10 is incubated with serum samples that may contain ADA to CT-P10 or RTX. If the serum sample contains ADA, immune complexes composed of labeled CT-P10 and ADA are formed. The sample is then incubated on a streptavidin-coated plate and immune complexes present in the sample bind to the bottom of the plate. When the reaction buffer is added, the sulfo-tag shows luminescence. The luminescence signal is proportional to the amount of ADA in the serum sample. If the signal is higher than the cut-point, the sample is regarded as a potentially positive sample. For the confirmatory assay, non-labeled CT-P10 is co-incubated with labeled CT-P10 and serum samples. The non-labeled CT-P10 competes with the labeled CT-P10 when ADA is present in the serum sample. This results in a decrease in the luminescence signal. The percentage decrease in luminescence signal is used for the evaluation of confirmed positives. If the percentage decrease in the signal is higher than the confirmatory cut-point, the sample is regarded as a confirmed positive sample.

ADA, anti-drug antibody; ELISA, enzyme-linked immunosorbent assay.

ONLINE SUPPLEMENTARY MATERIAL D: PATIENT DISPOSITION

Figure D-1. Patient disposition



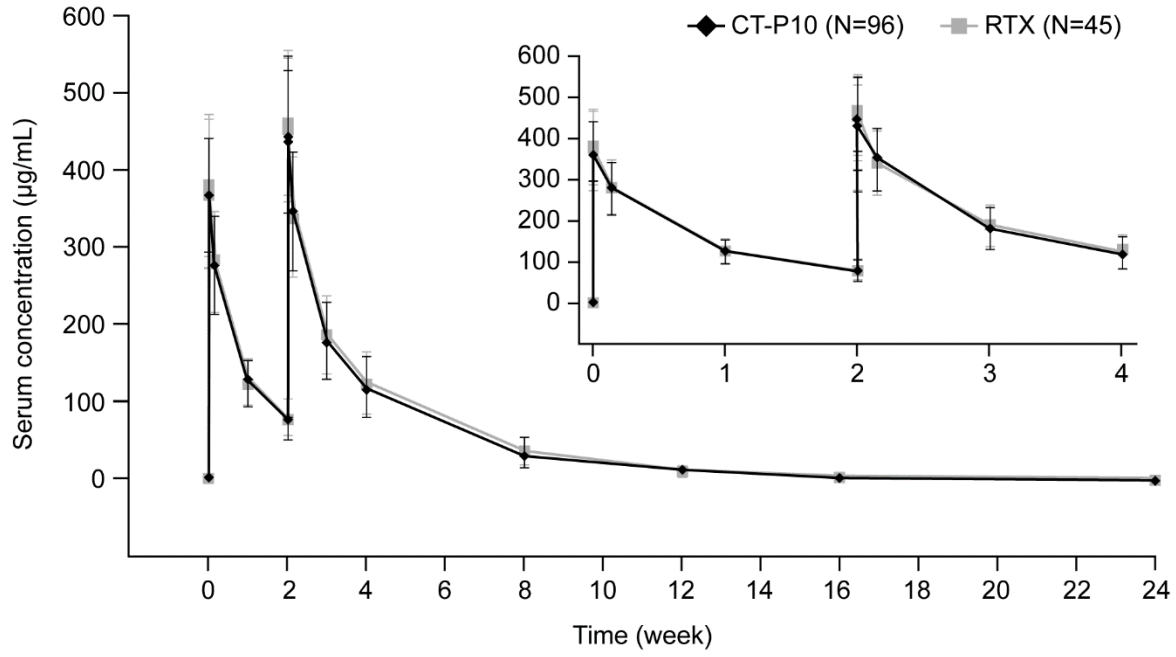
*One patient was not treated with the study drug due to difficulty in finding a vein for intravenous infusion. This patient was therefore excluded from the safety population.

RTX; innovator rituximab.

ONLINE SUPPLEMENTARY MATERIAL E:

SERUM CONCENTRATIONS OF CT-P10 AND RTX VERSUS TIME

Figure E-1. Mean serum concentrations of CT-P10 and RTX versus time up to week 24 with magnified linear concentration–time up to week 4 (PK population)



PK; pharmacokinetic; RTX, innovator rituximab.

ONLINE SUPPLEMENTARY MATERIAL F: ASSESSMENT OF PHARMACOKINETICS BY ANTI-DRUG ANTIBODY STATUS

Table F-1. Analysis of primary PK endpoints by ADA status

Parameter	Treatment	N	Geometric mean	Ratio (%) of geometric mean	P-value*
AUC_{0-last} (day × μg/mL)					
ADA-positive	CT-P10	18	6636.0	110.7	0.5156
	RTX	8**	5996.1		
ADA-negative	CT-P10	78	8262.4	96.2	0.5213
	RTX	37	8586.9		
C_{max} (μg/mL)					
ADA-positive	CT-P10	18	422.4	106.4	0.6303
	RTX	8**	396.9		
ADA-negative	CT-P10	78	480.6	95.8	0.3706
	RTX	37	501.8		

The primary PK endpoints were analyzed using an analysis of variance model with treatment, region and prior anti-TNF blocker status as fixed effects.

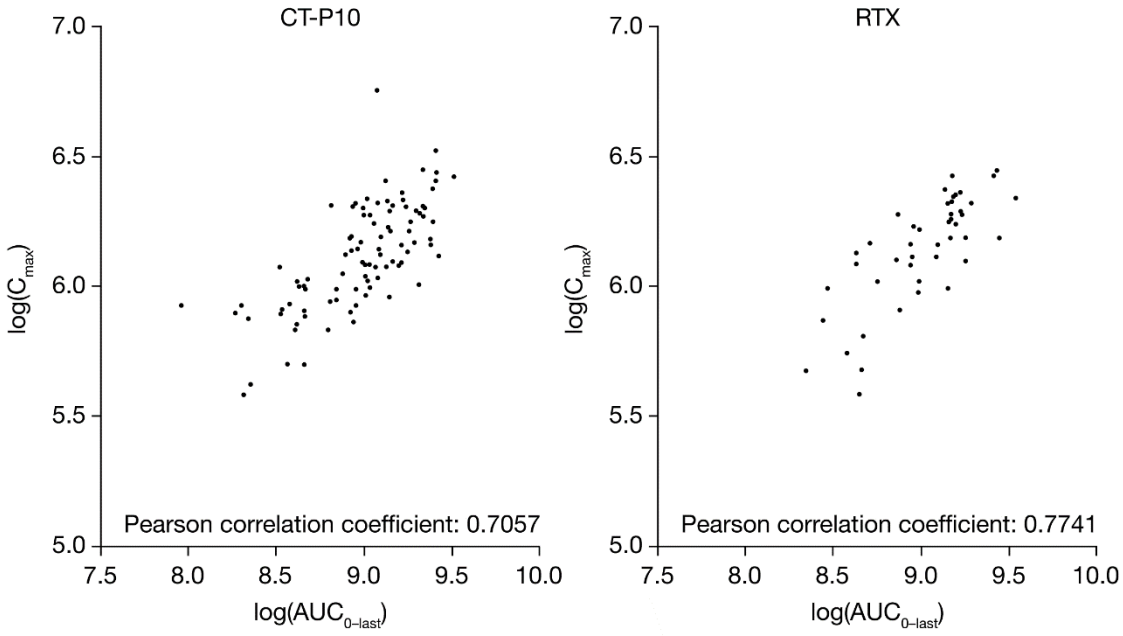
*P-value (CT-P10 versus RTX) calculated using the log-normal t-test. The test is significant at the 10% level.

**One of the nine patients in the RTX group who were ADA-positive was excluded from the PK population due to a major protocol deviation.

ADA, anti-drug antibody; AUC_{0-last}, area under the serum concentration–time curve from time zero to last quantifiable concentration; C_{max}, maximum serum concentration (after second infusion); PK, pharmacokinetic; RTX, innovator rituximab.

ONLINE SUPPLEMENTARY MATERIAL G: PLOTS OF LOG($AUC_{0–LAST}$) AND LOG(C_{MAX}): CORRELATION ANALYSES

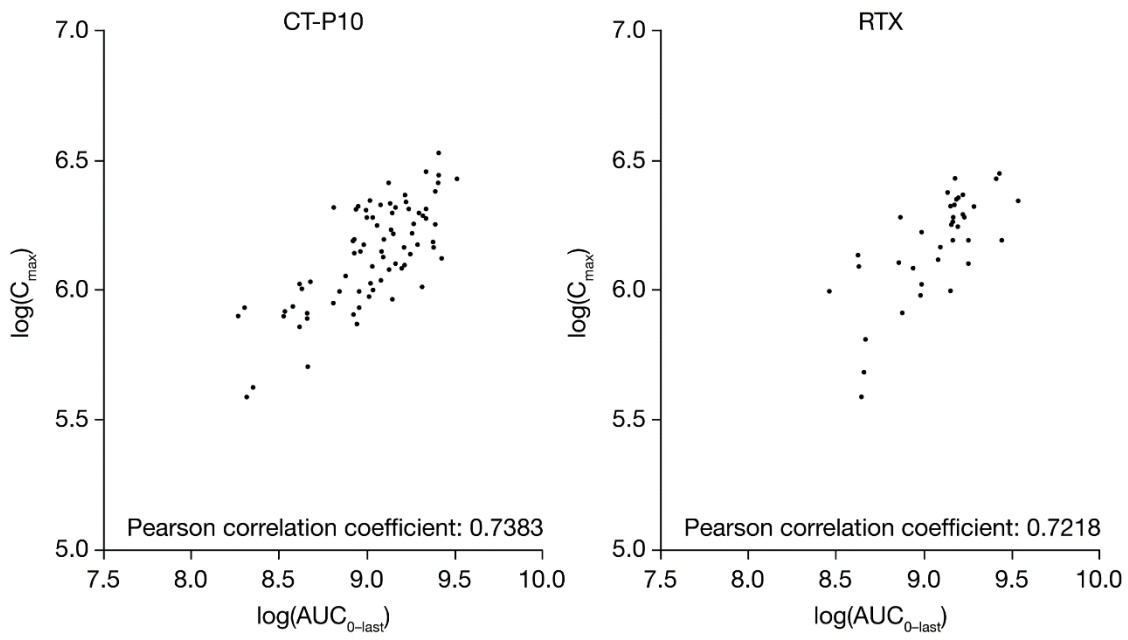
*Figure G-1. Log($AUC_{0–last}$) versus log(C_{max}) in each patient**



*$AUC_{0–last}$ and C_{max} assessed at week 24.

$AUC_{0–last}$, area under the serum concentration–time curve from time zero to last quantifiable concentration; C_{max}, maximum serum concentration after second infusion; RTX, innovator rituximab.

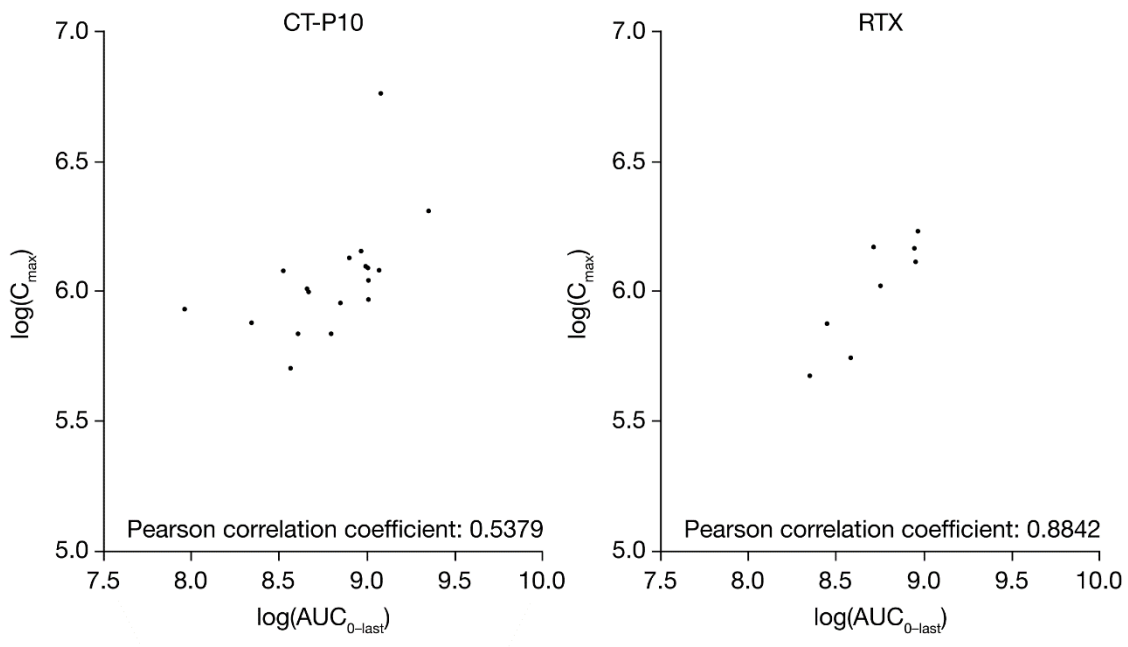
*Figure G-2. Log($AUC_{0–last}$) versus log(C_{max}) in ADA-negative patients**



*$AUC_{0–last}$, C_{max} and ADA status assessed at week 24.

ADA, anti-drug antibody; $AUC_{0–last}$, area under the serum concentration–time curve from time zero to last quantifiable concentration; C_{max}, maximum serum concentration after second infusion; RTX, innovator rituximab.

*Figure G-3. Log(AUC_{0-last}) versus log(C_{max}) in ADA-positive patients**



*AUC_{0-last}, C_{max} and ADA status assessed at week 24.

ADA, anti-drug antibody; AUC_{0-last}, area under the serum concentration–time curve from time zero to last quantifiable concentration; C_{max}, maximum serum concentration after second infusion; RTX, innovator rituximab.

ONLINE SUPPLEMENTARY MATERIAL H: ACR IMPROVEMENT CRITERIA AT WEEK 24

*Table H-1. Change from baseline in parameters of the ACR improvement criteria at week 24 (efficacy population)**

Individual parameters of the ACR improvement criteria**	CT-P10 (N=100)	RTX (N=48)	P-value
Swollen joint count (66 joints)	-11.3 ± 7.6	-10.0 ± 7.7	0.3545
Tender joint count (68 joints)	-15.4 ± 12.4	-16.1 ± 15.2	0.7569
Patient's global assessment of disease activity, mm (0–100 mm VAS)	-28.7 ± 25.4	-31.5 ± 27.9	0.5644
Physician's global assessment of disease activity, mm (0–100 mm VAS)	-34.9 ± 21.7	-34.3 ± 24.6	0.8929
Health Assessment Questionnaire Disability Index	-0.5 ± 0.6	-0.4 ± 0.6	0.4278
Patient's assessment of pain, mm (0–100 mm VAS)	-29.1 ± 25.2	-29.5 ± 26.2	0.9325
C-reactive protein, mg/dL	-0.8 ± 1.3	-1.1 ± 2.4	0.2827
Erythrocyte sedimentation rate, mm/hour	-16.5 ± 22.1	-14.5 ± 20.7	0.6188

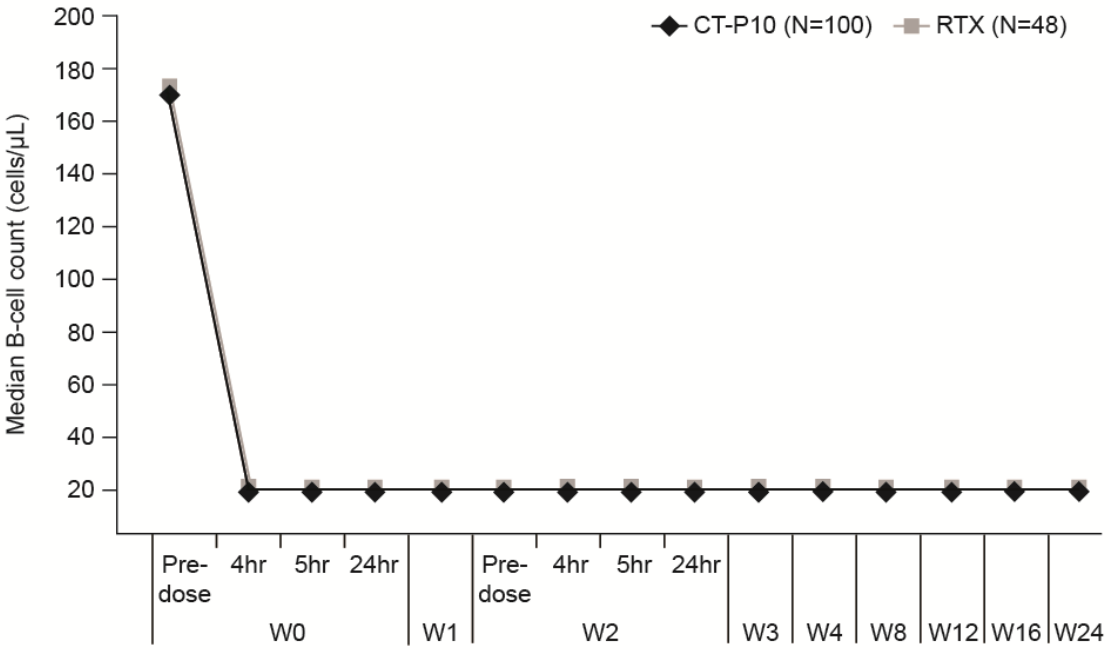
*All values are mean ± standard deviation change from baseline.

**Negative changes represent improvement in the individual parameters of the ACR improvement criteria.

ACR, American College of Rheumatology; RTX, innovator rituximab; VAS, visual analog scale.

ONLINE SUPPLEMENTARY MATERIAL I: MEDIAN B-CELL COUNT

Figure I-1. Median B-cell count up to week 24 (PD population)



PD, pharmacodynamic; RTX; innovator rituximab; W, week.

ONLINE SUPPLEMENTARY MATERIAL J: IMMUNOGLOBULIN LEVELS

Despite complete B-cell depletion up to week 16 (see online supplementary material I), there were no significant reductions in immunoglobulin (Ig) levels. IgM, IgA, and IgG levels were below the lower limit of normal (LLN) on at least one occasion after the start of treatment in 6 (5.9%), 2 (2.0%), and 5 (4.9%) patients, respectively, in the CT-P10 group and in 4 (7.8%), 0, and 4 (7.8%) patients in the innovator rituximab (RTX) group (at baseline, the number [%] of patients with levels below the LLN was 0, 2 [2.0%], and 2 [2.0%] in the CT-P10 group and 1 [2.0%], 0 and 0 in the RTX group).

ONLINE SUPPLEMENTARY MATERIAL K: SAFETY

Table K-1. Treatment-related adverse events, adverse events due to infusion-related reactions, and adverse events due to infections and infestations (safety population)

Number of patients (%)*	CT-P10 (N=102)	RTX (N=51)
Patients with ≥1 of the following treatment-related events:		
Adverse events	37 (36.3)	29 (56.9)
Serious adverse events	1 (1.0)	2 (3.9)
Adverse events leading to withdrawal	1 (1.0)	1 (2.0)
Malignancy	0	0
Deaths	0	0
Adverse events due to infusion-related reactions	17 (16.7)	10 (19.6)
Headache	4 (3.9)	2 (3.9)
Urticaria	4 (3.9)	1 (2.0)
Tachycardia	3 (2.9)	0
Dermatitis	2 (2.0)	1 (2.0)
Dry mouth	2 (2.0)	1 (2.0)
Dizziness	2 (2.0)	0
Feeling cold	1 (1.0)	1 (2.0)
Pruritus	1 (1.0)	1 (2.0)
Throat irritation	1 (1.0)	1 (2.0)
Hypersensitivity	0	2 (3.9)
Dyspepsia	0	1 (2.0)
Adverse events due to infections and infestations	24 (23.5)	13 (25.5)
Upper respiratory tract infection	7 (6.9)	5 (9.8)
Urinary tract infection	3 (2.9)	2 (3.9)
Respiratory tract infection	2 (2.0)	2 (3.9)
Herpes virus infection	1 (1.0)	3 (5.9)
Abscess	1 (1.0)	0
Enterocolitis	1 (1.0)	0
Erysipelas	1 (1.0)	0
Fungal infection	1 (1.0)	0
Hordeolum	1 (1.0)	0
Paronychia	1 (1.0)	0
Ear infection	0	1 (2.0)
Gastroenteritis	0	1 (2.0)
Pneumonia	0	1 (2.0)
Sialoadenitis	0	1 (2.0)

*For each event included in the table, a patient was counted once if he/she reported one or more event. If an event occurred more than once in a patient, only the most severe event was counted.

RTX, innovator rituximab.

Patient withdrawals due to adverse events

Three patients (2.9%) treated with CT-P10 withdrew from the study due to adverse events: hepatic steatosis (considered related to treatment), pericardial effusion (unrelated), and diverticulitis (unrelated). Two patients (3.9%) treated with innovator rituximab (RTX) withdrew due to adverse events: neutropenia (related) and decreased B-lymphocyte count (unrelated [this patient had low levels of $CD19^+$ and $CD20^+$ lymphocytes before RTX treatment and only received the first infusion of RTX]).

Infections

The proportion of patients who experienced at least one infection was similar between the CT-P10 (23.5%) and RTX (25.5%) groups (Table K-1). The most frequently reported treatment-related infection in both treatment groups was upper respiratory tract infection (n=7 [6.9%] and n=5 [9.8%] for CT-P10 and RTX, respectively). Serious infection was reported by one patient in each group (CT-P10: diverticulitis, unrelated to treatment; RTX: lobar pneumonia, possibly related to treatment); both events were resolved. The proportion of patients testing positive on the interferon-γ release assay was similar between the two groups at baseline and week 24.

Other safety assessments

Changes from baseline in hematology, clinical chemistry, and urinalysis parameters were small and comparable between groups. The findings of general physical examinations, vital sign measurements and electrocardiograms were also similar after CT-P10 and RTX treatment.