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ARD Online First, published on December 22, 2016 as 10.1136/annrheumdis-2016-210297

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EXTENDED REPORT

Low immunogenicity of tocilizumab in patients with rheumatoid arthritis

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Handling editor Tore K Kvien

▶ Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2016-210297).

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Received 1 August 2016
Revised 22 November 2016
Accepted 26 November 2016

ABSTRACT

Objective Subcutaneous (SC) and intravenous formulations of tocilizumab (TCZ) are available for the treatment of patients with rheumatoid arthritis (RA), based on the efficacy and safety observed in clinical trials. Anti-TCZ antibody development and its impact on safety and efficacy were evaluated in adult patients with RA treated with intravenous TCZ (TCZ-IV) or TCZ-SC as monotherapy or in combination with conventional synthetic disease-modifying antirheumatic drugs (csDMARDs).

Methods Data from 5 TCZ-SC and 8 TCZ-IV phase III clinical trials and 1 TCZ-IV clinical pharmacology safety study (>50 000 samples) were pooled to assess the immunogenicity profile of TCZ-SC and TCZ-IV (8974 total patients). The analysis included antidrug antibody (ADA) measurement following TCZ-SC or TCZ-IV treatment as monotherapy or in combination with csDMARDs, after dosing interruptions or in TCZ-washout samples, and the correlation of ADAs with clinical response, adverse events or pharmacokinetics (PK).

Results The proportion of patients who developed ADAs following TCZ-SC or TCZ-IV treatment was 1.5% and 1.2%, respectively. ADA development was also comparable between patients who received TCZ monotherapy and those who received concomitant csDMARDs (0.7–2.0%). ADA development did not correlate with PK or safety events, including anaphylaxis, hypersensitivity or injection-site reactions, and no patients who developed ADAs had loss of efficacy.

Conclusions The immunogenicity risk of TCZ-SC and TCZ-IV treatment was low, either as monotherapy or in combination with csDMARDs. Anti-TCZ antibodies developed among the small proportion of patients had no evident impact on PK, efficacy or safety.

INTRODUCTION

For patients with rheumatoid arthritis (RA) who do not respond to or are intolerant of conventional synthetic disease-modifying antirheumatic drugs (csDMARDs), biologic DMARDs (bDMARDs) are recommended.[1] Often, a bDMARD and ≥1 csDMARD are used in combination, but bDMARDs can also be used as monotherapy. The currently approved bDMARDs include antitumour necrosis factor-α agents (aTNFs), anti-interleukin 6 receptor (IL-6R) therapy, anti-CD20 B cell targeted therapy and T cell co-stimulation inhibition. One safety concern of bDMARDs is the development of antidrug antibodies (ADAs).[2] Multiple factors may contribute to ADA development, including structure and idiotype,[3] route of administration,[3] mechanism of action,[4] concomitant csDMARD use,[5 6] disease activity,[7] genetic status,[3 8] patient immunocompetence,[3] treatment duration,[3] the disease itself[9] and drug dose/frequency.[8] ADAs can lead to loss of efficacy[10] and/or immune-mediated adverse reactions, including IgE-mediated or non-IgE-mediated events.[11]

Tocilizumab (TCZ) is a humanised monoclonal antibody (mAb) of the IgG_1 subclass that blocks IL-6 binding to the membrane-bound and soluble IL-6R, consequently inhibiting IL-6 activity. TCZ is approved for adult RA (as intravenous or subcutaneous (SC) formulations) and as intravenous for systemic-course and polyarticular-course juvenile idiopathic arthritis and Castleman disease (Japan only).[12] TCZ has demonstrated efficacy and a well characterised safety profile as monotherapy or in combination with csDMARDs.[13–19]

This study addresses important clinical and scientific questions: Is a therapeutic antibody by SC administration more immunogenic compared with intravenous administration? Is the immunogenic risk of TCZ monotherapy similar to that of co-therapy with methotrexate (MTX)? Here, the immunogenicity of TCZ is assessed in different clinical settings—ADA development following TCZ administration as SC or intravenous formulations as monotherapy or in combination with csDMARDs, after dosing interruptions and in TCZ-washout samples—as well as its correlation with adverse events (AEs), clinical response and pharmacokinetics (PK). Data were derived from five TCZ-SC and nine intravenous TCZ (TCZ-IV) RA trials plus their long-term extensions: SUMMACTA,[20 21] BREVACTA,[22 23] the TCZ-SC long-term extension rollover study of US patients from BREVACTA and SUMMACTA,[24] MUSASHI (Multi-Center Double-Blind Study of Tocilizumab Subcutaneous Injection in Patients Having Rheumatoid Arthritis to Verify Noninferiority Against Intravenous Infusion),[25 26] FUNCTION,[27] AMBITION (Actemra vs Methotrexate Double-Blind Investigative Trial in Monotherapy),[15] TOWARD (Tocilizumab in Combination With Traditional DMARD Therapy),[17] OPTION (Tocilizumab Pivotal Trial in Methotrexate Inadequate Responders),[13] LITHE (Tocilizumab Safety and the Prevention of Structural

To cite: Burmester GR, Choy E, Kivitz A, *et al*. *Ann Rheum Dis* Published Online First: [*please include* Day Month Year] doi:10.1136/annrheumdis-2016-210297

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Joint Damage),[14] RADIATE (Research on Actemra Determining Efficacy After Anti-TNF Failures),[16] TOZURA global umbrella study (interim analysis) and a clinical pharmacology study.[28]

PATIENTS AND METHODS

Study designs

The study designs of the TCZ trials are summarised (see online supplementary table S1).[13–17 20 22 24 25 27]

Sampling

Blood samples for ADA detection and PK analysis were collected at baseline and regularly predose (TCZ trough level) throughout the studies and at the study completion or early withdrawal visit. Furthermore, patients who withdrew due to hypersensitivity reactions in five of the studies had additional samples for ADAs collected at the time of the event and at least 4–8 weeks after the last treatment.[20 22 24 27] To minimise potential TCZ interference in the immunogenicity assay, in the TCZ-IV versus TCZ-SC study, TCZ-washout samples (at least 4 weeks or 8 weeks after the last treatment, or predose samples after treatment interruptions during the study) were evaluated.[20]

Immunogenicity assessment strategy and assays

In all studies, consistent assay methodology was applied,[29] and a sequential testing strategy was adopted (figure 1). All samples were initially screened for antibodies, and positive samples were analysed by a confirmation assay for specificity. Characterisation of any samples with confirmed anti-TCZ antibodies was performed to detect neutralising potential and IgE isotype. In three studies, an IgE assay was also conducted in patients who withdrew because of hypersensitivity reactions, regardless of their confirmation assay status.[20 22 24 27] The IgE assay was not performed in the TCZ-IV studies consistently; therefore, results were not available. Clinical AEs and efficacy measures were evaluated in association with ADA development.

The screening assay employed a bridging ELISA and used biotinylated TCZ from different labelling preparations immobilised on streptavidin-coated microtitre plates. Anti-TCZ antibodies form a complex of TCZ-biotin/anti-TCZ antibody/TCZ-digoxigenin, captured by immobilised streptavidin and then detected by an antidigoxigenin-peroxidase antibody (figure 2A). An assay cut point was determined from serum samples from patients with RA, containing various levels of rheumatoid factor in order to minimise its interference. The confirmation assay was conducted the same as the screening assay except the preincubation of test or control samples with digoxigenylated TCZ was performed in parallel in the presence and absence of excess free TCZ, which competes with digoxigenylated TCZ and biotinylated TCZ for binding to anti-TCZ antibodies (figure 2B).

To detect neutralising potential of ADAs, an inhibition ELISA was performed for all studies except the Japanese study (figure 2C). The neutralising assay evaluates whether anti-TCZ antibodies competitively interfere with the binding of TCZ to immobilised soluble IL-6R. Blocking the binding of TCZ to IL-6R, resulting in a decrease in assay signal, is indicative that anti-TCZ antibodies can neutralise the therapeutic effect of TCZ. In the Japanese study, an antigen-binding fragment (Fab) assay in a bridging ELISA format that can detect anti-TCZ antibodies that bind to the Fab fragment of TCZ was applied as the neutralising assay.[25 26] IgE isotype antibodies were detected using the ImmunoCAP assay system (Quest Diagnostics) (figure 2D).[29] Anti-TCZ-IgE antibodies captured by immobilised TCZ were detected by an antihuman IgE-specific antibody.

PK assay

TCZ serum concentrations were determined by ELISA. The lower limit of quantitation was 100 ng/mL. The impact of ADAs on PK was formally evaluated in three intravenous studies and two SC studies.[13 16 17 20 22]

Analyses

In all studies except the Japanese study, hypersensitivity events were conservatively defined as all AEs (excluding injection-site reactions (ISRs)) that occurred during or within 24 hours of an infusion or injection and were not judged unrelated to

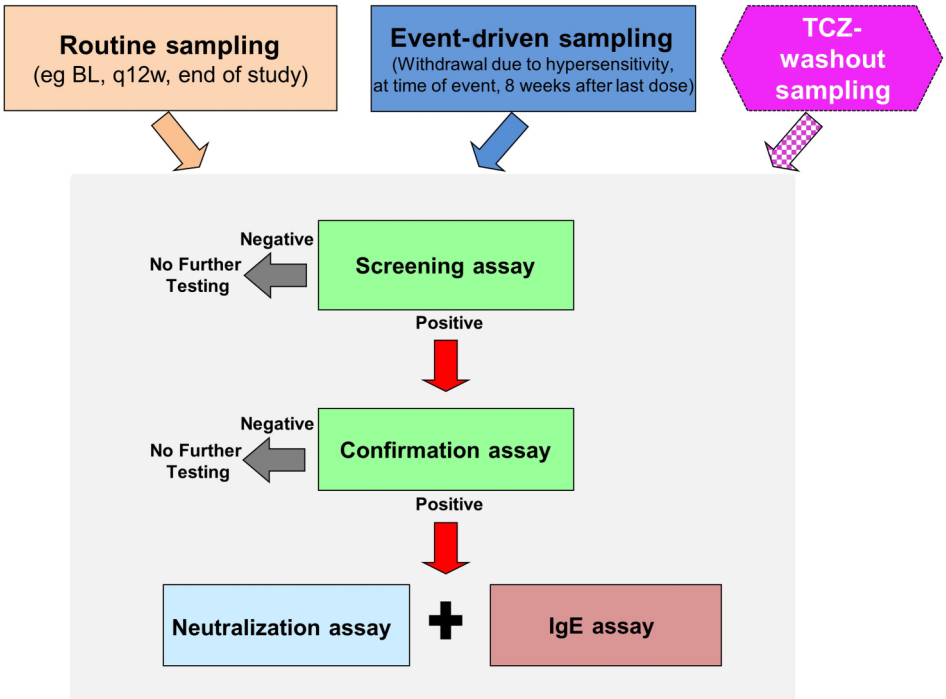

Figure 1 Tocilizumab (TCZ) immunogenicity assessment strategy*. *Blood samples were taken at baseline (BL) and regularly prior to dosing throughout the studies. q12w, every 12 weeks.

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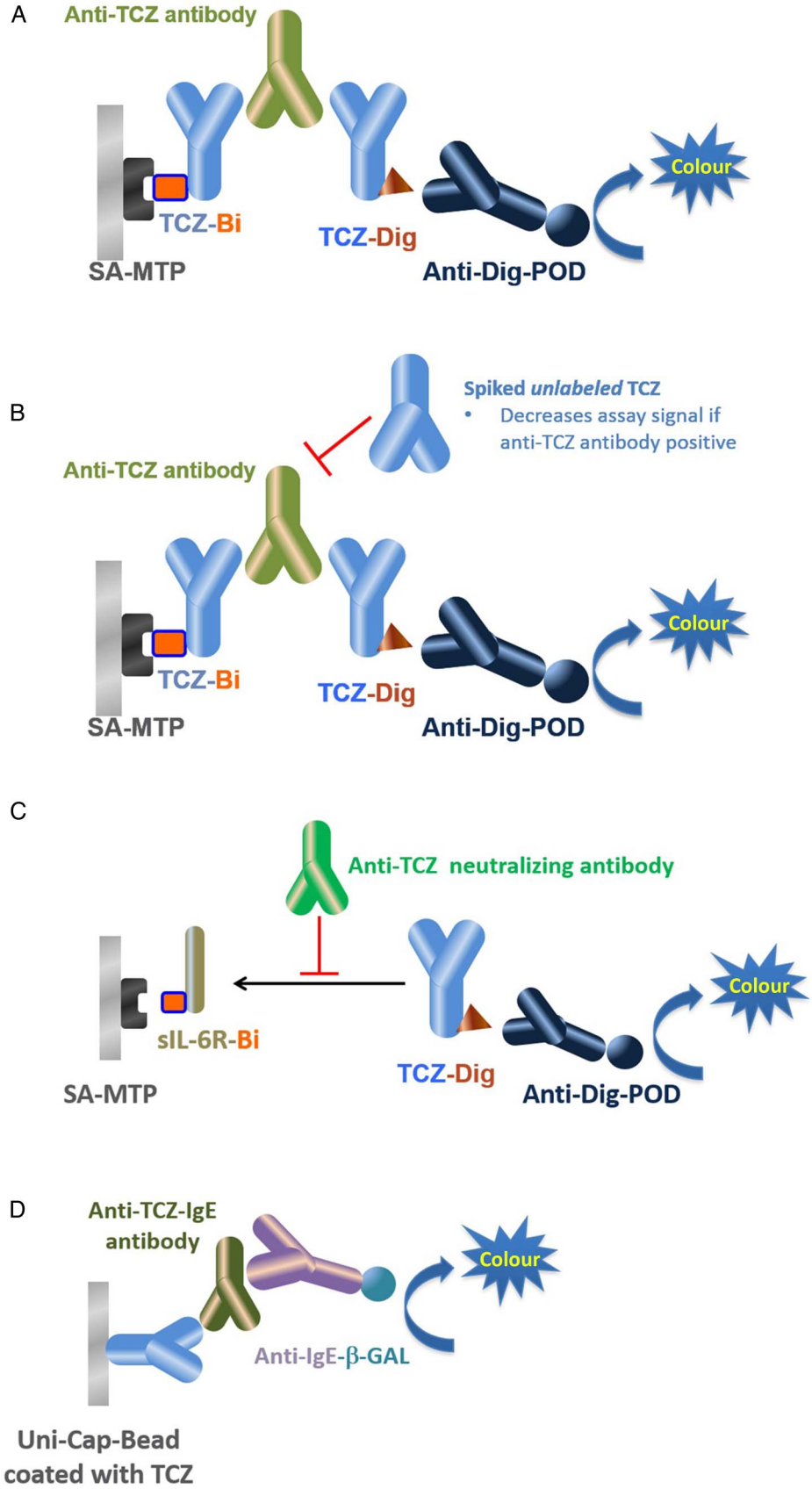

Figure 2 Anti-tocilizumab (anti-TCZ) antibody assay. (A) The screening assay employed bridging ELISAs and used biotinylated TCZ from different labelling preparations immobilised on streptavidin-coated microtitre plates. Bi, biotin; Dig, digoxigenin; SA-MTP, streptavidin-coated microtitre plate; POD, peroxidase. (B) For samples positive from the screening assay, an additional competitive displacement step was used for the confirmation assay, where unlabelled TCZ inhibited the formation of TCZ-Bi/anti-TCZ antibody/TCZ-Dig complexes. (C) An inhibition ELISA was adopted to detect the neutralising potential of anti-TCZ antibodies (whether anti-TCZ antibodies competitively interfere with the binding of TCZ to immobilised soluble IL-6 receptor (sIL-6R)). (D) IgE isotype antibodies were detected using the ImmunoCAP assay system (Quest Diagnostics).

treatment by the investigator; those events may or may not be consistent with hypersensitivity clinically. Anaphylactic reactions were events that occurred during or within 24 hours of an infusion or injection and met Sampson criteria.[30] Serious hypersensitivity events were hypersensitivity events that were also reported as serious AEs, and clinically significant hypersensitivity events were hypersensitivity events that led to study withdrawal. ISRs were AEs occurring at the local injection

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sites following SC administration. In the Japanese study, hypersensitivity events were defined as AEs (excluding ISRs) that occurred during or within 24 hours of an infusion or injection and were also judged to be a hypersensitivity event by the clinical expert.[25 26]

Assay results were also evaluated for patients who met the criteria for loss of efficacy, defined as those who withdrew from the study prematurely due to insufficient therapeutic response after experiencing an American College of Rheumatology criteria for 50% improvement or a European League Against Rheumatism good response.

RESULTS

Patient population

The TCZ-SC all-exposure population consisted of 3099 patients from the clinical trials, including 616 patients who received TCZ-SC as monotherapy and 2483 who received TCZ-SC in combination with csDMARDs (figure 3A). TCZ-SC treatment was administered for up to 3.5 years. The TCZ-IV all-exposure population consisted of 5875 patients, with 753 patients who received TCZ-IV as monotherapy and 5122 who received TCZ-IV in combination with csDMARDs (figure 3B). TCZ-IV treatment was administered for up to 5 years.

Incidence of ADA development and effect on safety and efficacy following TCZ-SC or TCZ-IV all-exposure

Of the patients who received TCZ-SC or TCZ-IV and were screened for ADAs (99.8% and 98.8%, respectively), the proportion of patients who developed ADAs following either TCZ treatment was low and comparable (1.5% (47 patients) and 1.2% (69 patients), respectively; table 1). Among the patients who developed ADAs, 40 (85.1%) who received TCZ-SC and 54 (78.3%) who received TCZ-IV were also positive for the neutralising assay. Of the patients who were screened for ADAs, 9 (0.3%) who received TCZ-SC developed IgE antibodies; results for IgE antibodies were not available for TCZ-IV. In all studies, most detected ADAs were transient and did not occur at all time points (see online supplementary table S2).

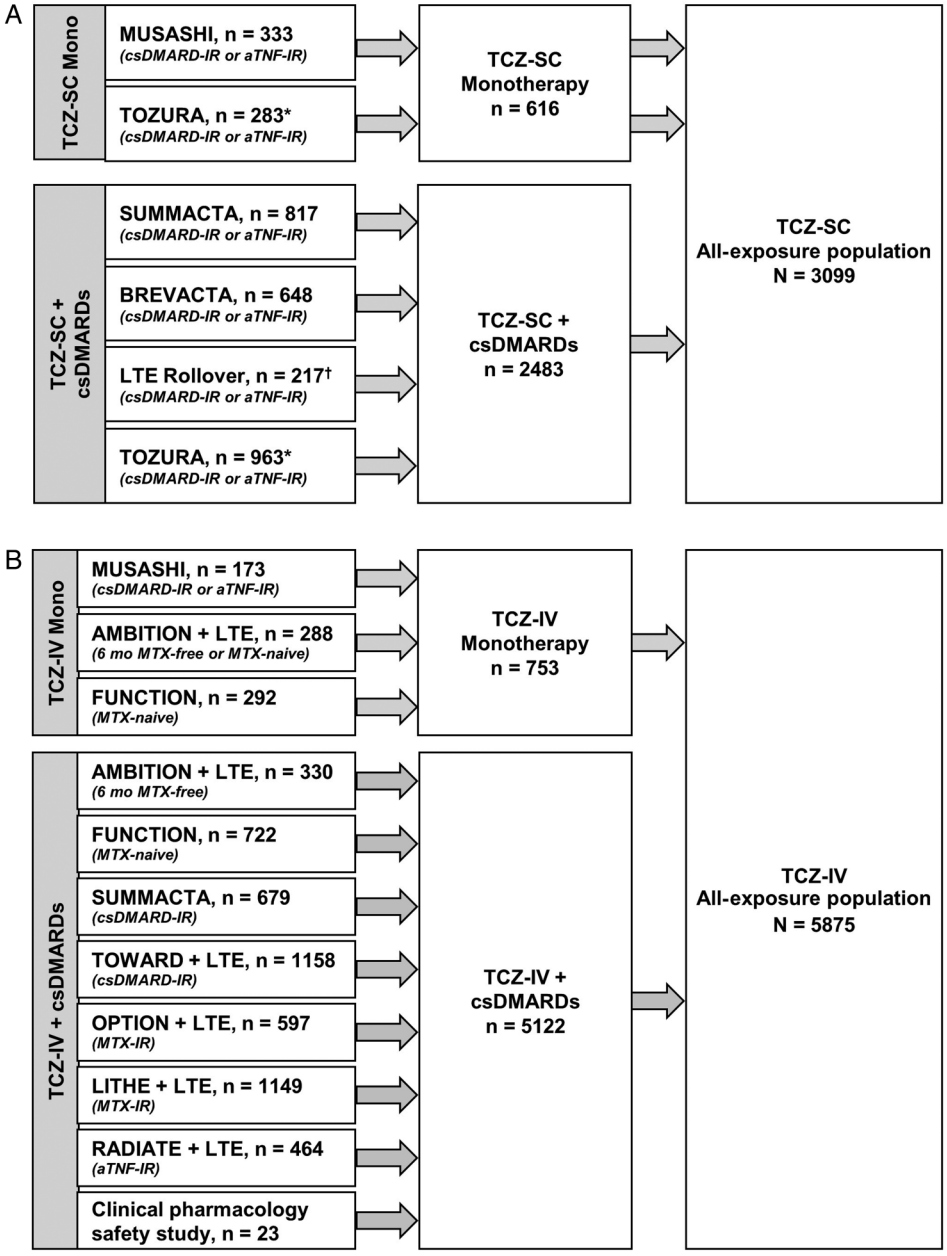

Figure 3 Patient disposition. Immunogenicity was assessed from the clinical trials following treatment in patients with rheumatoid arthritis. (A) Treatment with subcutaneous tocilizumab (TCZ-SC). Mono, monotherapy; csDMARD, conventional synthetic disease-modifying antirheumatic drug; IR. inadequate responder; aTNF, antitumour necrosis factor-α agent; LTE, long-term extension. (B) Treatment with intravenous tocilizumab (TCZ-IV). MTX, methotrexate.
*TOZURA is a multinational, open-label, single-arm global umbrella study comprising 11 protocols from different countries/regions.
†There were a total of 217 patients who received TCZ-SC treatment in the long-term extension (LTE) rollover study, including 55 patients who switched from TCZ-IV (in SUMMACTA) to TCZ-SC for the LTE rollover period.

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Among the all-exposure safety populations, no patients who received TCZ-SC experienced anaphylaxis, whereas 10 patients (0.2%) who received TCZ-IV had anaphylaxis (table 1). Clinically significant hypersensitivity (leading to study withdrawal) occurred in 31 patients (1.0%) who received TCZ-SC and in 91 patients (1.5%) who received TCZ-IV; 10 patients (0.3%) in the TCZ-SC group and 51 (0.9%) in the TCZ-IV group had serious hypersensitivity (hypersensitivity events meeting seriousness criteria). Of the 47 patients who received TCZ-SC and developed ADAs, 1 (2.1%) experienced clinically significant hypersensitivity, but none had serious hypersensitivity. Of the 69 patients who received TCZ-IV and developed ADAs, 5 (7.2%) experienced anaphylaxis, 10 (14.5%) had clinically significant hypersensitivity, and 6 (8.7%) had serious hypersensitivity, including the 5 patients with anaphylaxis. Among the patients who received TCZ-SC, a total of 310 (10.0%) experienced ISRs. Of the 47 patients who received TCZ-SC and developed ADAs, 4 (8.5%) experienced ISRs; all events resolved without sequelae.

Among all patients who developed ADAs with neutralising potential following TCZ treatment, none experienced loss of efficacy, regardless of formulation (table 1).

Incidence of ADA development and effect on safety and efficacy following TCZ monotherapy or in combination with csDMARDs

The overall incidence of ADA development was low in the 1360 patients treated with TCZ monotherapy (intravenous: 0.7%; SC: 2.0%) and the 7540 patients treated with TCZ+csDMARDs (intravenous: 1.3%; SC: 1.4%), regardless of formulation (table 2).

Among the safety population, the incidences of hypersensitivity events were consistent between patients who received TCZ monotherapy or TCZ + csDMARDs (table 2). No patients experienced anaphylaxis with TCZ-SC compared with 1 patient (0.1%) who received TCZ-IV monotherapy and 9 patients (0.2%) who received TCZ-IV+csDMARDs. Clinically significant hypersensitivity occurred in 6 patients (1.0%) who received TCZ-SC monotherapy and in 25 patients (1.0%) who received TCZ-SC+csDMARDs. Serious hypersensitivity occurred in one patient (0.2%) in the TCZ-SC monotherapy group and in nine patients (0.4%) in the TCZ-SC+csDMARDs group. Twelve patients (1.6%) who received TCZ-IV monotherapy and 79 (1.5%) who received TCZ-IV+csDMARDs had clinically significant hypersensitivity events. Nine patients (1.2%) who received TCZ-IV monotherapy and 42 (0.8%) who received TCZ-IV +csDMARDs had serious hypersensitivity events.

There was no clear impact of ADA development on safety, regardless of TCZ administration as monotherapy or in combination with csDMARDs (table 2). Of the five patients who received TCZ-IV monotherapy and developed ADAs, one had clinically significant hypersensitivity and none had serious hypersensitivity or anaphylaxis. Of the 64 patients who received TCZ-IV+csDMARDs and developed ADAs, 9 experienced clinically significant hypersensitivity and 6 had serious hypersensitivity events, including the 5 anaphylaxis cases. Of the 12 patients who received TCZ-SC monotherapy and developed ADAs, 1 had clinically significant hypersensitivity and none had serious hypersensitivity or anaphylaxis. Of the 35 patients who received TCZ-SC+csDMARDs and developed ADAs, none experienced anaphylaxis, serious hypersensitivity or clinically significant hypersensitivity.

ISRs were reported in 81 patients (13.1%) who received TCZ-SC monotherapy compared with 229 (9.2%) who received TCZ-SC+csDMARDs (table 2). One patient (0.2%) who received TCZ-SC monotherapy and developed ADAs had an ISR; three (0.1%) of the patients who received TCZ-SC +csDMARDs and developed ADAs had ISRs.

Among all patients who developed ADAs with neutralising potential following TCZ treatment, none experienced loss of efficacy, regardless of whether it was administered as monotherapy or in combination with csDMARDs (table 2).

Table 1 Immunogenicity rates and safety and efficacy in patients who developed anti-TCZ antibodies following TCZ-SC or TCZ-IV treatment

	TCZ-SC 162 mg qw or q2w all-exposure (n=3099)	TCZ-IV 4 mg/kg or 8 mg/kg q4w all-exposure (n=5875)
Anaphylaxis, n (%)*	0	10 (0.2)
Clinically significant hypersensitivity (leading to withdrawal), n (%)†	31 (1.0)	91 (1.5)
Serious hypersensitivity (reported as SAE), n (%)‡	10 (0.3)	51 (0.9)
Injection-site reactions, n (%)	310 (10.0)	N/A
Total patients screened for ADAs, n (%)	3094 (99.8)	5806 (98.8)
Total patients who developed ADAs, n (%)§	47 (1.5)	69 (1.2)
Positive neutralisation assay, n (%)§¶	40 (1.3)	54 (0.9)
Positive IgE assay, n (%)§	9 (0.3)	N/A
Anaphylaxis, n (%)*§	0	5 (0.1)
Clinically significant hypersensitivity (leading to withdrawal), n (%)†§	1 (0.03)	10 (0.2)
Serious hypersensitivity (reported as SAE), n (%)‡§	0	6 (0.1)
Injection-site reactions, n (%)§	4 (0.1)	N/A
Loss of efficacy, n (%)§**	0	0

*Anaphylactic reactions were events that occurred during or within 24 hours of an infusion or injection and met Sampson criteria.
†Clinically significant hypersensitivity events were defined as any events that occurred during or within 24 hours of an infusion or injection and led to withdrawal from treatment.
‡Serious hypersensitivity events were defined as any events that occurred during or within 24 hours of an infusion or injection and were reported as SAEs.
§Denominator is total patients screened for ADAs.
¶The Fab assay was applied in the MUSASHI study to measure neutralisation potential.
**Loss of efficacy was defined as patients who withdrew from the study prematurely due to insufficient therapeutic response after experiencing an American College of Rheumatology criteria for 50% improvement (ACR50) or European League Against Rheumatism good response.
ADA, antidrug antibody; N/A, not available; q2w, every other week; q4w, every 4 weeks; qw, every week; SAE, serious adverse event; TCZ, tocilizumab; TCZ-IV, intravenous TCZ; TCZ-SC, subcutaneous TCZ.

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Table 2 Safety, immunogenicity and effect of ADAs on safety and efficacy following TCZ as monotherapy or in combination with conventional synthetic disease-modifying antirheumatic drugs (csDMARDs)

	TCZ-SC mono 162 mg qw or q2w (n=616)	TCZ-SC+csDMARDs 162 mg qw or q2w (n=2483)	TCZ-IV mono 4 mg/kg or 8 mg/kg q4w (n=753)	TCZ-IV+csDMARDs 4 mg/kg or 8 mg/kg q4w (n=5122)
Anaphylaxis, n (%)*	0	0	1 (0.1)	9 (0.2)
Clinically significant hypersensitivity (leading to withdrawal), n (%)†	6 (1.0)	25 (1.0)	12 (1.6)	79 (1.5)
Serious hypersensitivity (reported as SAE), n (%)‡	1 (0.2)	9 (0.4)	9 (1.2)	42 (0.8)
Injection-site reactions, n (%)	81 (13.1)	229 (9.2)	N/A	N/A
Total patients screened for ADAs, n (%)	615 (99.8)	2479 (99.8)	745 (98.9)	5061 (98.8)
Total patients who developed ADAs, n (%)§	12 (2.0)	35 (1.4)	5 (0.7)	64 (1.3)
Positive neutralisation assay, n (%)§¶	7 (1.1)	33 (1.3)	4 (0.5)	50 (1.0)
Positive IgE assay, n (%)§	3 (0.5)	6 (0.2)	N/A	N/A
Anaphylaxis, n (%)*§	0	0	0	5 (0.1)
Clinically significant hypersensitivity (leading to withdrawal), n (%)†§	1 (0.2)	0	1 (0.1)	9 (0.2)
Serious hypersensitivity (reported as SAE), n (%)‡§	0	0	0	6 (0.1)
Injection-site reactions, n (%)§	1 (0.2)	3 (0.1)	N/A	N/A
Loss of efficacy, n (%)§**	0	0	0	0

*Anaphylactic reactions were events that occurred during or within 24 hours of an infusion or injection and met Sampson criteria.
†Clinically significant hypersensitivity events were defined as any events that occurred during or within 24 hours of an infusion or injection and led to withdrawal from treatment.
‡Serious hypersensitivity events were defined as any events that occurred during or within 24 hours of an infusion or injection and were reported as SAEs.
§Denominator is total patients screened for ADAs.
¶The Fab assay was applied in the MUSASHI study to measure neutralisation potential.
**Loss of efficacy was defined as patients who withdrew from the study prematurely due to insufficient therapeutic response after experiencing an American College of Rheumatology criteria for 50% improvement (ACR50) or European League Against Rheumatism good response.
ADA, antidrug antibody; N/A, not available; q2w, every other week; q4w, every 4 weeks; qw, every week; SAE, serious adverse event; TCZ, tocilizumab; TCZ-IV, intravenous TCZ; TCZ-SC, subcutaneous TCZ.

TCZ-washout samples

To minimise the potential TCZ interference in the immunogenicity assay, additional samples for ADA measurements were obtained from the TCZ-SC versus TCZ-IV study[20] at the follow-up visits after treatment completion or after dosing interruption. In total, 928 samples were collected from 879 patients (table 3). Among them, 549 samples (59.2%) from 503 patients were TCZ-free (TCZ serum levels below the limit of quantitation) and 239 samples (25.8%) from 238 patients had low TCZ concentration (<10 µg/mL). Of the 503 patients who provided TCZ-free samples, which allows for TCZ interference in the immunogenicity assay to be excluded, only one patient (0.2%) was positive for ADAs. Another two samples from two patients who were positive for ADAs had TCZ concentrations of 0.2 µg/mL and 18.1 µg/mL. All three patients who developed ADAs did not experience hypersensitivity reactions or ISRs and did not withdraw due to insufficient therapeutic response or meet the criteria for loss of efficacy. None of the three patients who were determined as ADA-positive in washout samples were positive at the regular sampling time points.

Table 3 TCZ-washout samples by TCZ concentration (SUMMACTA)

Total: 928 total samples from 879 patients	TCZ BLQ	TCZ <10 µg/mL	TCZ ≥10 µg/mL
Washout samples, n (%)*	549 (59.2)	239 (25.8)	140 (15.1)
Patients, n	503	238	138
Total patients who developed ADAs, n (%)†	1 (0.2)	1 (0.4)	1 (0.7)
Positive neutralisation assay†	0	0	1 (0.7)
Positive IgE assay	0	0	0

Note: Multiple samples (with different TCZ concentrations) could be provided by a single patient.
*Denominator is total sample number.
†Denominator is total number of patients who provided washout samples.
ADA, antidrug antibody; BLQ, below the lower limit of quantitation (TCZ concentration, 100 ng/mL); TCZ, tocilizumab.

Immunogenicity in patients who missed doses

ADA development after dose interruption was analysed in three TCZ-SC studies. In the TCZ-SC versus TCZ-IV study,[31] 179 patients from the TCZ-SC once-weekly (qw) group and 40 patients from the TCZ-IV-switch-to-TCZ-SC group missed ≥3 consecutive TCZ-SC qw injections, and 241 patients from the TCZ-IV every-4-weeks and TCZ-SC-switch-to-TCZ-IV groups missed ≥1 TCZ-IV infusion during the study; among these patients, two in the TCZ-SC arm and two in the TCZ-IV arm had negative screening assay results before the first missed dose and then were positive for confirmation and neutralising assays after dosing was resumed. In the TCZ-SC versus placebo study,[31] 188 patients in the TCZ-SC every-other-week group and 48 patients in the placebo-switch-to-TCZ-SC group missed ≥1 dose during the treatment period and had negative ADA assays before the missed injection. One patient in the placebo-switch-to-TCZ-SC arm was positive for ADAs by the confirmation and neutralising assays after dosing was resumed. In the Japanese study,[25 26] 247 patients in the safety population who received TCZ-SC had an injection interval of >21 days between doses and were negative for ADAs prior to the dosing interval. Among them, one patient in the TCZ-SC arm developed ADAs after reinitiating TCZ treatment. For all TCZ-SC studies, no impact of ADAs on efficacy or safety was observed in patients who developed ADAs after dose interruption.

Pharmacokinetics

There was no obvious trend of reduced serum TCZ levels in the patients who tested positive for ADAs, including those with neutralising potential. A graphical analysis of apparent clearance estimated by population PK analysis for patients with positive

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ADA compared with patients with negative ADA status showed no differences in intravenous studies (see online supplementary figure S1) or SC studies (see online supplementary figure S2). Moreover, no correlation was observed between relative ADA concentration and TCZ values among ADA-positive patients in the intravenous versus SC study (see online supplementary figure S3).

In the TCZ-IV monotherapy versus TCZ-IV+MTX study in patients with early RA, no overall trends of decreasing concentrations were noted for up to 2 years of treatment[27] (in preparation/to be submitted). Similarly, in the TCZ-SC+csDMARDs versus TCZ-IV+csDMARDs study, once steady state was reached, mean TCZ concentrations in patients from both groups remained stable up to week 97.[21]

DISCUSSION

Our pooled results from 8974 patients treated with TCZ indicated that the incidence of ADA development was low, regardless of intravenous or SC formulation and whether it was administered as monotherapy or in combination with csDMARDs. In patients who did develop ADAs, ADAs were mostly transient and no correlation to PK, safety events or loss of efficacy was observed. The precise mechanism of the observed low immunogenicity in patients treated with TCZ has not been fully elucidated; the immunogenic potential of a biologic treatment is affected by several factors, including molecule-related factors (eg, mechanisms of action, molecular structure and manufacturing process) and patient characteristics. ADA incidence is also dependent on the assay itself (eg, assay sensitivity, specificity and methodology).

Immunogenicity assays are challenged and complicated by drug interference, and the observed low incidence of ADA might be a reflection of the assay used. To minimise TCZ interference, TCZ-washout samples were collected and evaluated in the TCZ-SC versus TCZ-IV study.[20] Among the 503 patients who provided TCZ-free samples, the proportion who developed ADAs was low (0.2%) across treatment arms, confirming a low incidence of ADA development when drug interference is ruled out. Moreover, the observed low incidence of ADA development is consistent with three independently published studies that examined the immunogenicity of TCZ using commercially available immunogenicity assays; in those studies, 0% to 3.3% of patients treated with TCZ developed ADAs.[32–34]

One possible mechanism of the observed low immunogenicity of TCZ might be related to the downregulation of B cell activities due to the blocking of IL-6 signalling (a different mechanism of action from that of aTNFs). Our findings and a recent study[33] indicate no increased risk of ADA development and no clear impact on TCZ trough level in either TCZ monotherapy or combination therapy settings[21 27] (in preparation/to be submitted). Consistently, similar efficacy has been observed with TCZ monotherapy compared with TCZ in combination with csDMARDs (either intravenous or SC).[35–38] In contrast, it has been reported that with two aTNFs (adalimumab and infliximab), concomitant administration of MTX suppresses immunogenicity and maximises efficacy.[7 39 40] Development of ADAs against adalimumab and infliximab may correlate with the disappearance of drug from the blood and may decrease efficacy by neutralising the drug or by creating immune complexes.[10 41] In this study, patients who were positive for neutralising assay did not experience a loss of efficacy; it is possible that while the neutralising antibodies were able to block TCZ in vitro, they may not function as such in vivo (eg, are not at sufficient concentration and/or affinity) to affect TCZ levels or efficacy. It is unclear why in three patients, ADA became present after drug washout, and the release of the inhibition of B cell activity after TCZ washout leading to ADA development might be a possible explanation; however, most of the detected anti-TCZ antibodies were transient in this study.

Other possible factors contributing to low immunogenicity might be molecule-related factors, including mAb structure (eg, a specific molecular structure with an idiotype of low immunogenic potential) and manufacturing processes. In general, it is not clear whether a humanised mAb treatment is more immunogenic than a fully human mAb. ADA development has been reported for fully human mAbs (eg, adalimumab and golimumab).[42] ADAs against the fully human adalimumab induced neutralising responses that varied by disease and therapy (5–89%), and ADAs correlate with a lack of efficacy in some adalimumab-treated patients.[7 41 43]

To our knowledge, this study, including data from >8900 patients, is the most robust and comprehensive clinical trial-based assessment addressing immunogenicity compared with published data for a biologic RA treatment. In the small proportion of patients who developed ADAs following administration of TCZ-SC or TCZ-IV, no clear correlation of ADA development to PK, clinical response or AEs was observed. Further, administration of TCZ as monotherapy did not increase the risk of immunogenicity and had no impact on the TCZ trough level. However, the limitation due to the low number of ADA-positive patients is acknowledged, especially between subgroups such as TCZ monotherapy versus TCZ in combination with MTX. Overall, our data suggest that routine ADA testing is unnecessary for the clinical use of TCZ in treating adult RA.

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Acknowledgements The authors thank all investigators and patients who participated in the study and all members of the clinical trial teams. Support for third-party writing assistance for this article, furnished by Denise Kenski, PhD, and Ellen Mercado, PhD, of Health Interactions, was provided by F. Hoffmann-La Roche Ltd. The authors also thank Caroline Kreuzer of Roche, who contributed to the immunogenicity assays and sample analyses.

Contributors All authors contributed to the development of this manuscript.

Funding Clinical trials were funded by F. Hoffmann-La Roche, Ltd (Roche). MUSASHI was funded by Chugai Pharmaceuticals Co., Ltd. Roche sponsored the study; participated in the design of the study along with the academic centres involved in the trials; and participated in the collection, analysis and interpretation of the data. This article was reviewed by Roche, but the decision to submit was contingent only on the approval of the lead author and coauthors, including those employed by Roche and Chugai.

Competing interests MB, WR, TW, JP and MM are employed by Genentech, Inc. SL, AP-S, HB, KS and NLM are employed by F. Hoffmann-La Roche. AN is employed by Chugai Pharmaceutical. GRB has received grants and honoraria for consulting and lectures from Roche. EC has received research grant, speaker and consultancy

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fees from Roche and Chugai Pharmaceutical. AK has received research grants and consulted for Genentech, Inc. AO has received consulting fees, speaking fees and/or honoraria from Chugai Pharmaceutical. MCG has received grants and consulting fees and/or honoraria from Roche.

Provenance and peer review Not commissioned; externally peer reviewed.

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Low immunogenicity of tocilizumab in patients with rheumatoid arthritis

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Ann Rheum Dis published online December 22, 2016

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