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Original Article

A multicenter open-label phase I/II study to assess the safety, tolerability, and efficacy of three dose levels of TuNEX in patients with rheumatoid arthritis

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Abstract

Background: Tumor necrosis factor (TNF)-α is a pivotal inflammatory cytokine in the pathogenesis of rheumatoid arthritis (RA). TuNEX, a recombinant TNF-α receptor protein, can effectively bind TNF-α. The purpose of this phase I/II dose-escalation study was to assess the safety and preliminary efficacy of three dose levels of TuNEX in Taiwanese patients with RA.

Methods: Eighteen patients with active RA from three medical centers who had failed previous therapy with at least one disease modifying antirheumatic drug (DMARD) were enrolled. The primary efficacy endpoint was a 20% improvement in the American College of Rheumatology criteria (ACR20) in the fourth week. The occurrence of treatment-emergent adverse events (TEAEs) was the primary safety variable.

Results: The highest percentage of TuNEX 25-mg- and 35-mg-treated patients achieved an ACR20 response (60% and 100%, respectively) for the first time at Week 2 during the 4-week treatment period. There was a strong trend toward a superior ACR20 response rate in the TuNEX 15-mg group (83.3%) in comparison with the TuNEX 25-mg group (40.0%) and the TuNEX 35-mg group (50.0%) at week 4. Patients who received 15-mg TuNEX, 25-mg TuNEX, and 35-mg TuNEX had 35.99%, 16.85%, and 21.86% reduction of disability indices of Health Assessment Questionnaire after drug treatment, respectively. The most commonly reported adverse event was injection-site reaction. The TEAEs were comparable between the three TuNEX-treated groups.

Conclusion: TuNEX reduced the signs and symptoms of RA and improved physical function, with clinically acceptable safety and tolerability in patients who had previously received DMARDs.

Keywords: efficacy; immunology; RA; rheumatoid arthritis; TNF-α; tumor necrosis factor; TuNEX

1. Introduction

Rheumatoid arthritis (RA), which is characterized by a progressive inflammatory synovitis, should be treated early and aggressively to prevent joint destruction and disability.[1,2] Although disease modifying antirheumatic drugs (DMARDs) can inhibit disease progression, many patients fail to achieve an adequate response to therapy due to lack of efficacy or toxicity.[3] Tumor necrosis factor (TNF)-α is a crucial inflammatory mediator in synovitis and subsequent tissue damage in RA, and therefore represents a promising target for therapeutic intervention in this disease.[4−6] Clinical and pharmacologic studies have shown that TNF-α inhibitors can be an effective and well-tolerated therapy for patients with RA.[7−10]

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TuNEX is a dimeric fusion protein consisting of the extracellular domain of the human 75-kilodalton (p75) TNF receptor (TNFR) linked to the constant region of human immunoglobulin (Ig) G1. The extracellular portion of TNFR was also discovered as soluble TNF-α-binding protein in urine and serum and plays a physiologic role by inhibiting TNF-α activity. TuNEX is produced by recombinant DNA technology in a Chinese hamster ovary mammalian cell expression system and was developed by Mycenax Biotech Inc. (Miaoli County, Taiwan). Since TNF-α is an important proinflammatory cytokine in the pathogenesis of RA, it is hypothesized that TuNEX might be useful in the treatment of this disease. TuNEX consists of 934 amino acids, has an apparent molecular weight of approximately 150 kilodaltons, and is a biologic product similar to etanercept, which has been granted market approval in Taiwan. Based on the conclusion of preclinical (phase I) studies (13-week subcutaneous toxicity in cynomolgus monkeys with a 4-week interim sacrifice and a 4-week recovery phase) and the consideration of safety margin in humans, the effective dose range for TuNEX has been judged to be 15–35 mg given subcutaneously (sc) twice weekly.

The present study is the first phase I/II clinical trial to assess the safety and preliminary efficacy of three dose levels of TuNEX in Taiwanese patients with RA. Patients with RA with history of treatment failure with at least one of the DMARDs received one of the following doses: (1) 15 mg TuNEX given (sc) twice weekly, (2) 25 mg TuNEX given sc twice weekly, or (3) 35 mg TuNEX given sc twice weekly. Proportions of patients with dose-limiting toxicity (DLT) were the primary outcome of the study. Efficacy, incidence of adverse events, and immunogenicity were the secondary outcomes planned in the study.

2. Methods

2.1. Patients

This multicenter study was conducted at Taichung Veterans General Hospital, Kaohsiung Veterans General Hospital, and Buddhist Dalin Tzu Chi Hospital in Taiwan. Twenty-seven patients who fulfilled the American College of Rheumatology (ACR) 1987 revised criteria for RA[11] and had failed previous therapy with at least one of the DMARDs were enrolled at the time of screening. Treatment failure was defined as either intolerance or discontinuation due to lack of efficacy determined by investigator. DMARDs include methotrexate, hydroxychloroquine, gold preparations, azathioprine, D-penicillamine, sulfasalazine, and cyclosporine. Patients receiving DMARDs at the time of screening were required to undergo a washout for 4 weeks before start of study medication. Active disease at the time of screening was defined as six or more swollen joints and six or more tender joints, and presence of at least one of the following criteria: erythrocyte sedimentation rate (ESR) $\geq$ 28 mm/h, C-reactive protein (CRP) $\geq$ 2.0 mg/dL, and duration of morning stiffness for at least 45 minutes.

Patients were excluded if they had received any of the following: biologic products (such as etanercept, infliximab, and adalimumab) within 4 weeks prior to planned start of trial treatment (Day 0), and/or exposure to anti-CD20 antibodies within 2 years before screening for this trial; any use of cyclophosphamide, nitrogen mustard, chlorambucil, or other alkylating agents within 5 years before screening for this trial; use of leflunomide $\leq$12 weeks prior to planned start of trial treatment (Day 0); intra-articular treatment with corticosteroids within 4 weeks before screening; or live vaccine within 3 months prior to study. Other criteria for exclusion were as follows: past or current malignancy, except for resected cervical carcinoma Stage 1B or less, resected noninvasive basal cell and squamous cell skin carcinoma, malignant melanoma with a complete response of a duration of >10 years, and other cancer diagnoses with a complete response of a duration of >5 years; history of infected joint prosthesis within 5 years before screening and infected native joints within 1 year before screening; patients known or suspected to be unable to comply with this trial protocol (such as due to alcoholism, drug dependency, or psychological disorder); patients with clinically active tuberculosis (TB) or radiographic evidence of old pulmonary TB; patients with renal impairment (serum creatinine >2.0 mg/dL) and hepatic impairment (alanine aminotransferase, asparate transaminase values >2 times the upper limit of normal range); patients with laboratory test abnormality (blood chemistry or hematology) that, in the investigator's opinion, might put the patient at a higher risk if treated with study medication; pregnant and nursing mothers; patients with positive serology for human immunodeficiency virus antibody, hepatitis B surface antigen (HBsAg) or hepatitis C (HCV) antibody; history of another collagen-vascular disease; preexisting or recent onset of central nervous system demyelinating disorders; patients with significant medical diseases, including uncompensated congestive heart failure, severe myocardial infarction within 6 months, uncontrolled hypertension, poorly controlled diabetes mellitus, and chronic or active infection; and patients with any condition that might cause their participation in this study to be detrimental, as judged by a physician. In addition, concomitant use of DMARDs or any other investigational drugs were prohibited during this study. However, stable doses of oral corticosteroids ($\leq$ 10 mg prednisone or equivalent), nonsteroidal anti-inflammatory drugs were permitted, but the dose could not be greater than the maximum level recommended by the manufacturer. Patients could receive analgesics during the study except for the 24-hour period before scheduled joint examination. This study was approved by the Clinical Research Ethics Committee of Taichung Veterans General Hospital, Kaohsiung Veterans General Hospital and Buddhist Dalin Tzu Chi Hospital, and informed consent was obtained from each participant.

2.2. Study design

This was an open-labeled, nonrandomized, sequential dose-escalating clinical exploratory study to assess the safety and

efficacy of TuNEX in patients with RA. As illustrated in Fig. 1, six patients with RA received the lowest dose level of TuNEX 15 mg sc twice weekly at the start of the study (Arm A). A safety review was conducted at the end treatment period of Arm A. If less than one-third of patients in Arm A were reported as DLT, the study continued to treat an additional six patients with the medium dose levels of TuNEX 25 mg sc twice weekly (Arm B). A safety review was conducted at the end-treatment period of Arm B. If less than one-third of patients in Arm B were reported as DLT, the study continued to treat 6 more patients with the maximal dose level of TuNEX 35 mg sc twice weekly (Arm C).

The therapeutic response was defined according to the ACR Response Criteria.[12] Visual analogue scale (VAS) was used to assess pain and global assessment. The Chinese version of Health Assessment Questionnaire (HAQ)-Disability Index was used to assess disability.[13] Clinical and laboratory assessment were performed before the start of treatment, 72 hours after the first dose, and at weekly intervals. Data on demographic characteristics, contact history with active TB disease, medical history, and prior medications were collected. Body weight and height were measured, physical examination was performed, and vital signs, including blood pressure and heart rate, were taken. Laboratory examinations including hematology, blood chemistry, CRP, rheumatoid factor, HBsAg, HCV antibody, urinalysis, and a pregnancy test in women were performed during a fasting state. A complete joint assessment (68-joint version) was performed for measurement of a tender joint count (TJC), swollen joint count (SJC), and duration of morning stiffness was recorded by an experienced rheumatologist who was blinded to the results of other evaluations. Each patient underwent chest X-ray and 12-lead electrocardiogram. Initially, all patients underwent a 4-week washout period prior to commencement of treatment with trial medications. After the washout period, baseline laboratory data were checked to determine eligibility for participation.

In addition, all patients received baseline TB screening using tuberculin skin test (TST) according to the Mantoux method (intradermal injection of 2 tuberculin units of PPD RT-23).[14] The two-step TST was not performed in any of our patients at baseline screening. The size of induration was measured 48–72 hours later, and a positive result was defined as the induration diameter $\geq$5 mm.[15]

3. Study objectives

3.1. Primary

The primary objective of the study was to evaluate the safety and tolerability of three fixed doses of TuNEX in patients with RA. The occurrence of treatment-emergent adverse events (TEAEs) was the primary safety variable. The proportion of patients reporting DLT at each dose level was the primary safety endpoint.

3.2. Secondary

The key secondary objective was to determine the most effective dose of TuNEX in patients with RA according to the ACR response criteria. In addition, immunogenicity of TuNEX in patients with RA was also determined.

The proportions of patients with ACR20 and ACR50 response for each dose level were the efficacy endpoints of this study. To avoid interobserver variability in the evaluation of ACR response, there was only one well-trained rheumatologist who examined the therapeutic responses for all enrolled

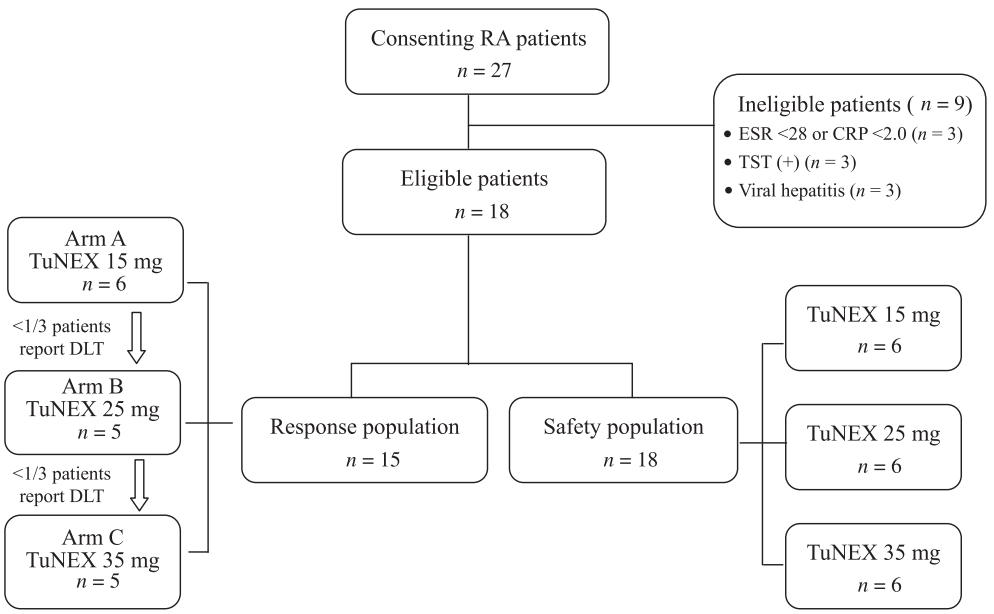

Fig. 1. Flow chart showing the enrolled patients with active rheumatoid arthritis (RA) for this phase I/II clinical trial to assess the safety and preliminary efficacy of three dose levels of TuNEX. This was an open-labeled, nonrandomized, sequential dose escalating (TuNEX 15 mg, 25 mg, and then 35 mg twice weekly) clinical study. CRP = C-reactive protein; DLT = dose-limiting toxicity; ESR = erythrocyte sedimentation rate; TST = tuberculin skin test.

patients at each site. In addition, the examiner observed the response in a blind way with respect to the laboratory results. Patients were assessed for the primary efficacy endpoint.

A patient was defined as "responder" in the ACR20 response at Week 4 in the modified full analysis set if the following three criteria were met: a $\geq$ 20% improvement in TJC; a $\geq$ 20% improvement in SJC; and a $\geq$ 20% improvement in at least three of the following assessments: pain visual analog scale (VAS, 0 being no pain and 100 being severe pain); patient's global assessment of disease activity (0 being no disease activity and 10 being extreme disease activity); physician's global assessment of disease activity (0 being no disease activity and 10 being extreme disease activity); the disability index of HAQ (0 being without difficulty, 1 being with some difficulty, 2 being with much difficulty, and 3 being unable to do so), and CRP values. A patient was considered an ACR 50% responder if there was at least 50% reduction of the modified full analysis set at Week 4 from baseline.

Safety was evaluated by (1) proportion of patients reporting DLT at each dose level. DLT was defined (according to Common Terminology Criteria for Adverse Events, Version 3.0) as any treatment-related adverse event (AE) $\geq$ grade 2 or any AE $\geq$ grade 3, (2) maximum tolerated dose (MTD), which was defined as the maximal dose with less than one-third of patients demonstrating DLT, (3) proportion of patients with antibodies to TuNEX, (4) clinically significant changes in vital signs, physical examination or laboratory parameters, and (5) incidence of adverse events.

Auto-antibody tests [antinuclear antibodies (ANA), anti-double-stranded-DNA (anti-dsDNA) Ab, anti-cardiolipin Ab (ACA), anti-β2-glycoprotein I Ab (ABGI Ab), anti-phosphatidylserine Ab (APTS Ab), lupus anticoagulant (LAC) screening ratio] were performed at baseline before TuNEX therapy and the end of therapy or upon early withdrawal. Anti-TuNEX antibody was determined by enzyme-linked immunosorbent assay.

3.3. Statistical analysis

The efficacy analysis was performed on an intent-to-treat population, which was defined as all patients with baseline data and at least one post-treatment evaluation. The last observation carried forward method was used to substitute for missing data. Primary and secondary efficacy variables were analyzed by calculating the change and percentage change from baseline at weeks 4, 8, and 12 (endpoint). The change and percent change from baseline in the treatment group were determined by nonparametric Wilcoxon signed rank test. The differences between treatment groups for the efficacy endpoints were compared by nonparametric Wilcoxon rank sum test. The differences in the ACR 20% and 50% response rate were analyzed by Fisher's exact test.

The safety analysis was performed on all patients who received randomized study medication. TEAEs included all adverse events that either began on or after administration of study drugs or pre-existing conditions that worsened on or after study drug administration. The number and percentage of subjects reporting TEAEs were tabulated by MedDRA[16] preferred terms and system organ class. Vital signs and laboratory data profiles were analyzed based on change from baseline using nonparametric Wilcoxon rank sum test for the analysis between treatment groups and Wilcoxon signed rank test for the analysis within treatment groups. The number of patients with adverse events was compared between treatment groups using Fisher's exact test. Ordinal variables were analyzed using the Cochran-Mantel-Haenszel test.

4. Results

4.1. Demographics and baseline characteristics

In this study, 18 patients were assigned to treatment, and nine patients were not eligible after screening (ESR$<$ 28 or CRP $<$ 2.0 mg/dL in three patients, positive results for TST in three patients, and viral hepatitis in three patients). All patients with RA who received TuNEX treatment were women (100%) with a mean age of 53.1 (range, 38.4–63.0 years). As illustrated in Table 1, there were no significant differences in demographic data, baseline characteristics, including values for body mass index, disease activity, disability index of the HAQ, and baseline chemistry values among the three TuNEX-treated groups.

4.2. Efficacy assessment: ACR20 and ACR50 response

The number of patients who discontinued treatment was three (one patient in the TuNEX 25-mg group, two patients in the TuNEX 35-mg group). Of these three patients, one patient

Table 1
Demographic and baseline characteristics of patients with rheumatoid arthritis receiving different dose levels of TuNEX.

Characteristics	TuNEX 15 mg (n = 6)	TuNEX 25 mg (n = 6)	TuNEX 35 mg (n = 6)
Age, yrs			
Mean (SD)	52.2 (10.16)	52.30 (8.32)	53.19 (8.57)
Sex			
Women	6 (100%)	6 (100%)	6 (100%)
BMI, kg/m^2			
Mean (SD)	24.15 (2.45)	19.73 (2.14)	24.32 (4.06)
Tender joints			
Median	7.50	8.00	22.50
IQR	(4.97, 15.37)	(5.26, 13.74)	(9.53, 37.47)
Swollen joints			
Median	6.00	8.00	15.50
IQR	(4.12, 13.88)	(5.87, 11.13)	(9.88, 20.45)
ESR, mm/h			
Median	45.00	37.50	57.50
IQR	(25.59, 62.41)	(16.05, 70.95)	(34.48, 82.85)
CRP, mg/L			
Median	0.96	2.17	1.49
IQR	(0.17, 2.09)	(0.54, 4.00)	(0.42, 2.78)

BMI = body mass index; CRP = C-reactive protei; ESR = erythrocyte sedimentation rate; IQR = interquartile range; SD = standard deviation.

in the 25-mg group received one injection of TuNEX and discontinued after 2 days due to abnormal results of chest X-ray screening (a nodular lesion 2.5 × 2.0 cm over the right upper lung lobe), one patient in the 35-mg group received four courses of TuNEX and discontinued due to the emergence of maculopapular rashes over four extremities in moderate degree, and the other patient in the 35-mg group received four courses of TuNEX and discontinued due to misuse dosage. Therefore, a total of 15 patients were evaluated for efficacy at Week 4.

The primary efficacy endpoint with RA was the proportion of patients with ACR20 at the end of treatment. As illustrated in Table 2, more patients in the TuNEX 15-mg-treated group achieved ACR20 and ACR50 responses compared with those in the TuNEX 25-mg-treated group and TuNEX 35-mg-treated group after 4 weeks of treatment, although these differences were not statistically significant in this study. As shown in Fig. 2A, the highest percentages of TuNEX 25-mg- and 35-mg-treated patients achieved an ACR20 response for the first time at Week 2 during the 4-week treatment period. There was a trend toward a superior ACR20 response rate in the TuNEX 15-mg group (83.3%) in comparison with the TuNEX 25-mg group (40.0%) and TuNEX 35-mg group (50.0%) at week 4. As shown in Fig. 2B, the highest percentages of TuNEX 25-mg- and 35-mg-treated patients achieved an ACR50 response for the first time at Week 3 during the 4-week treatment period. No significant difference in the proportion of patients who achieved ACR50 response after completion of treatment was observed in the TuNEX 15-mg group (33.3%) in comparison with the TuNEX 25-mg group (20.0%) and TuNEX 35-mg group (25.0%). Individual measurement of ACR items showed a marked improvement of all the dimensions.

At baseline, higher values of ESR and CRP in the TuNEX-25 mg group and TuNEX-35 mg group were observed in comparison with the TuNEX-15 mg group. At follow-up of TuNEX treatment, the mean values of ESR and CRP improved after all three dosages of treatment. Patients who received 15 mg, 25 mg, and 35 mg had 43.3%, 11.1% and 38.6% reductions in ESR, respectively, after the completion of TuNEX treatment. Similarly, patients who received 15 mg, 25 mg, and 35 mg had 68.1%, 30.0%, and 85.0% reductions in CRP levels, respectively, at the end of the therapeutic course of TuNEX.

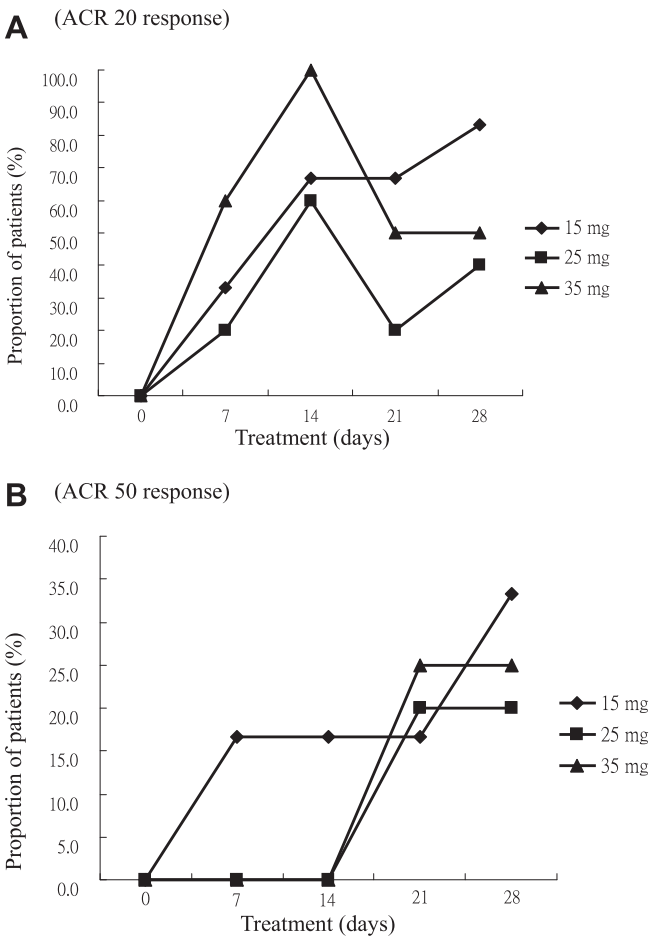

Fig. 2. The proportion of patients with rheumatoid arthritis who met the American College of Rheumatology (ACR) criteria for (A) 20%; and (B) 50% improvement (ACR20 and ACR50, respectively).

Table 2
Therapeutic response to TuNEX in patients with rheumatoid arthritis who previously were inadequately responding to DMARDs.[a]

	TuNEX 15 mg ($n = 6$)	TuNEX 25 mg ($n = 6$)	TuNEX 35 mg ($n = 6$)
ACR20 at wk 4	5/6 (83.3%)	2/5 (40.0%)	2/4 (50.0%)
ACR50 at wk 4	2/6 (33.3%)	1/5 (20.0%)	1/4 (25.0%)
ESR reduction at wk 4 (%)	43.3%	11.1%	38.6%
CRP reduction at wk 4 (%)	68.1%	30.0%	85.0%
Daily steroid dose (mg)	8.3 ± 2.0	6.3 ± 3.1	7.5 ± 2.2
Used DMARDs			
Methotrexate	4 (66.7%)	4 (66.7%)	5 (83.3%)
Sulfasalazine	3 (50.0%)	4 (66.7%)	5 (83.3%)
Hydroxychloroquine	3 (50.0%)	4 (66.7%)	5 (83.3%)
Azathioprine	4 (66.7%)	1 (16.7%)	0 (0.0%)
Leflunomide	4 (66.7%)	5 (83.3%)	2 (33.3%)
Cyclosporine	0 (0.0%)	0 (0.0%)	1 (16.7%)

ACR20 = 20% improvement based on American College of Rheumatology (ACR) response criteria; ACR50 = 50% improvement based on ACR response criteria; CRP = C-reactive protein; DMARDs = disease-modifying anti-rheumatic drugs; ESR: erythrocyte sedimentation rate.

[a] Data are presented as mean ±SD or number (percentage).

The HAQ is the most common assessment tool for functional disability. In the present study, patients who received 15 mg TuNEX, 25 mg TuNEX, and 35 mg TuNEX had 35.99%, 16.85%, and 21.86% reductions of HAQ score after drug treatment, respectively (Fig. 3). Our results indicate that disability scores were improved in all TuNEX dosage groups.

4.3. The occurrence of TEAEs

The safety population was defined as all eligible patients who received at least one injection of TuNEX. All of the 18 eligible patients were included in the safety analysis (Table 3).

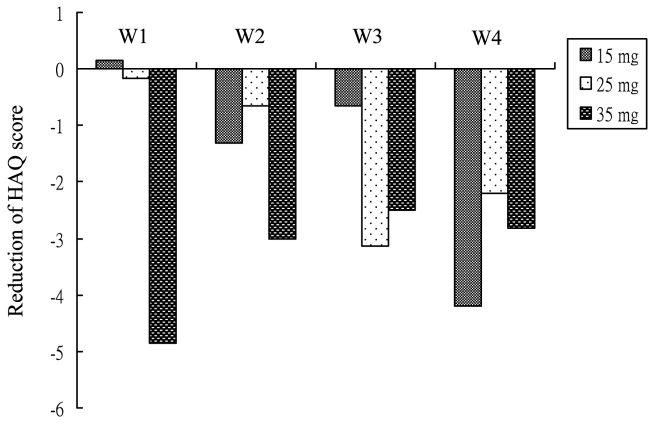

Fig. 3. Reduction of health assessment questionnaire (HAQ) disability score was observed in all TuNEX dosage groups. W1 = first week; W2 = second week; W3 = third week; W4 = fourth week.

TEAEs were reported in 4 of the 18 patients (22.2%) and were predominantly mild to moderate in intensity. The most commonly reported TEAE in TuNEX-treated groups was injection-site reaction with manifestation of mild urticaria, erythema, and pruritus. The only DLT occurring in the study was observed in the highest TuNEX dosage group. This patient, a woman 38 years of age, developed maculopapular rashes over four extremities and her trunk along with elevated anti-dsDNA antibody after four injections of TuNEX. The adverse reaction was resolved following discontinuation of TuNEX. No other clinical signs of systemic lupus erythematosus (SLE) were found in this patient. This reported DLT, with "moderate" severity, was judged by the investigators as "highly probably related to study drugs." However, TuNEX treatment did not result in any severe adverse event or occurrence of infections. No deaths or malignancies were reported during the study.

Evaluation of laboratory changes, including ANA and anti-dsDNA antibodies, did not demonstrate any clinically relevant differences among the three treatment groups. The presence of antiphospholipid antibodies, including ACA (IgA, IgM, and IgG), ABGI antibodies, or LAC after TuNEX treatment did not reach a significant level in any patient with RA. One patient in the TuNEX 35-mg-treated group had newly developed APTS antibodies (IgG and IgM) at low titers at the end of the study, but these were not considered as clinically significant. This suggests that the proposed maximum tolerated dose (MTD) allows continuous treatment over 4 weeks. In addition, none of patients receiving any of the three dose levels of TuNEX in the present study developed a positive anti-TuNEX antibody during the 4-week treatment and 2-week follow-up period.

5. Discussion

The present study combined the objectives of phase I and II to provide some evidence that TuNEX can be tolerated by Taiwanese patients with RA. Therefore, an open-label, noncomparative study design was considered to obtain safety data. This was a noncomparative study; therefore, neither randomization nor a control group was used. Our results showed that TuNEX could reduce the signs and symptoms of active RA and improve physical function, with a clinically acceptable safety and tolerability profile in patients who had previously received DMARDs.

TuNEX (a recombinant TNF receptor protein) acts by binding TNF-α, which plays a pivotal role in the pathogenesis of RA.[4–6] Consistent with previous clinical trials,[7–10] the present study showed that TuNEX alone in three different doses was well tolerated and provided clinical efficacy in Taiwanese patients with RA. During the 4-week treatment period, signs and symptoms of RA were relieved as early as

Table 3
Treatment-emergent adverse events observed in patients with rheumatoid arthritis receiving different dose levels of TuNEX.

Characteristic	TuNEX 15 mg	TuNEX 25 mg	TuNEX 35 mg
Patients with AE (no.)	2/6 (33.33%)	2/6 (33.33%)	2/6 (33.33%)
Patients with treatment-related AE (no.)	1/6 (16.67%)	2/6 (33.33%)	1/6 (16.67%)
Patients with SAE (no.)	0/6 (0.0%)	0/6 (0.0%)	0/6 (0.0%)
Patients with treatment-related SAE (no.)	0/6 (0.0%)	0/6 (0.0%)	0/6 (0.0%)
Patients who discontinued due to AE (no.)	0/6 (0.0%)	0/6 (0.0%)	1/6 (16.7%)
Patients who discontinued due to SAE (no.)	0/6 (0.0%)	0/6 (0.0%)	0/6 (0.0%)
Patients who died due to AE (no.)			
Patients who died due to AE (no.)	0/6 (0.0%)	0/6 (0.0%)	0/6 (0.0%)
Adverse events (AEs)			
Skin reaction	1/6 (16.7%)	2/6 (33.3%)	1/6 (16.7%)
Feature	Urticaria	Erythema, pruritus	Maculopapular rash
Severity	Mild	Mild	Moderate
Constipation (mild)	0/6 (0.0%)	0/6 (0.0%)	1/6 (16.7%)
Myofascial pain (mild)	1/6 (16.7%)	0/6 (0.0%)	0/6 (0.0%)
Treatment-related AE			
Skin reaction	1/6 (16.7%)	2/6 (33.3%)	1/6 (16.7%)
Feature	Urticaria	Erythema, pruritis	Maculopapular rash
Severity	Mild	Mild	Moderate

AE = adverse events; SAE = serious adverse events.

2 weeks, and the highest percentage (100%) of TuNEX 35-mg-treated patients achieved an ACR20 response for the first time at Week 2. More patients in the TuNEX 15-mg-treated group achieved ACR20 and ACR50 responses compared with those in the TuNEX 25-mg-treated group and TuNEX 35-mg-treated group after 4 weeks of treatment, although these differences were not statistically significant in this exploratory study. Our results are much different from the findings of previous studies showing a dose-dependent response to TNF-α inhibitors in patients with RA or psoriasis.[17,18] This discrepancy may be explained by dissimilar disease activities at baseline, different proportions of patients treated with DMARDs among the three dose groups, a small number of enrolled patients, short duration of treatment, and individual differences in response to treatment in our study. Moreover, the ACR response criteria are only a relative measure and not necessarily a realistic tool for the definition of therapeutic success.[19] Larger and longer phase III study using additional response criteria, such as the European League Against Rheumatism response criteria,[20] to demonstrate the efficacy of TuNEX in patients with RA will be needed in the future. In addition, the ACR20 response rate of TuNEX 35 mg-treated patients was lower in the present study than the rate observed in previous studies using similar biologics.[7,8] This disparity may be due to the differences in the sample size, the baseline characteristics, the used concomitant therapy (without versus with methotrexate), the biologic agents, and the duration of anti−TNF-α therapy.

The time courses of clinical response are different among the three dose-level groups: both the TuNEX 25 mg and 35 mg groups showed a decrement in clinical response between the second and third weeks, but the 15-mg group did not (Fig. 2). Although the development of antibodies against TNF-α blockade may occur in dose- or duration-dependent manner and affect the clinical response,[21,22] none of our patients receiving any of the three dose levels of TuNEX developed a positive anti-TuNEX antibody during the 4-week treatment. In view of the short duration of treatment, a longer phase III study will be required to clarify the association of immunogenicity with clinical response.

The HAQ, the most common assessment tool for functional disability, is a 20-item questionnaire for which scores range from 0 to 3. Wolfe and colleagues[23] reexamined the hypothesis and showed that the HAQ disability index is a good model of disability, using data from a large, prospective, long-term study of disability in RA. As shown in Fig. 3, our results indicate that disability scores improved in all TuNEX dosage groups.

In the present study, there were no differences in the incidence of adverse events among the three dose levels of TuNEX-treated groups. The most commonly reported TEAE was injection-site reactions with a median duration of 3 days. Our results were similar to the findings of previous clinical trials with sc TNF-α inhibitors showing that the occurrence rate of injection-site reactions from 10.6% to 37.0%.[24,25] Maculopapular rash over four extremities and trunk was the only DLT that required interruption of treatment in one patient; she was treated with highest dose of TuNEX. Therefore, maximum tolerated dose MTD was presumably higher than 35 mg of TuMEX.

Induction of autoantibodies is a predictable consequence of TNF-α inhibitors,[26–28] which could (1) promote humoral autoimmunity by inhibiting cytotoxic T-lymphocyte response that normally suppresses autoreactive B cells, and (2) enhance apoptotic processes that increase the release intracellular autoantigens and then development of autoantibodies against nuclear and cytoplasmic components.[26] In the present study, the emergence of newly developed ANA, anti-dsDNA, and APTS antibodies was mainly observed in the highest TuNEX dosage group. Our results were consistent with the findings of previous studies showing that the prevalence of ANA induction ranges from 23% to 57%, and the prevalence of anti-dsDNA antibody induction ranges from 9% to 33% in patients receiving anti-TNF-α therapy.[27] Although none of our patients developed signs/symptoms of lupus-like syndrome or specific autoimmune disease, one patient developed maculopapular rashes along with elevated anti-dsDNA antibody after TuNEX treatment, and resolution of lesions followed withdrawal of this agent. Our finding was similar to the results of a systemic review showing that cutaneous involvement as well as anti-dsDNA antibodies is more common in anti-TNF–induced lupus compared to classical drug-induced lupus.[28] No evidence has been shown to support a positive relationship between the presence of autoantibodies and RA improvement by TuNEX treatment. In the present study, the duration of treatment was too short to draw a definitive conclusion regarding safety profile. Although preclinical pharmacology and toxicology (phase I) studies have been conducted to demonstrate the safety of TuNEX, longer phase III study to demonstrate the safety of TuNEX in patients with RA will be needed.

In conclusion, three dose levels of TuNEX were effective in the treatment of RA with clinical and functional improvement during the 4-week treatment period. Considering the adverse event and severe adverse event incidences and premature discontinuation, TuNEX was shown to have clinically acceptable safety and tolerability profile over a period of 4 weeks. This favorable safety profile of TuNEX clearly implies an improved adherence to therapy. This study has demonstrated that treatment of Taiwanese patients who have RA with TuNEX has a favorable benefit-risk ratio. In light of the small number of enrolled patients and short duration of treatment in our study, the favorable benefit/risk relationship of TuNEX warrant a larger and longer phase III study to demonstrate the efficacy and safety of TuNEX in Chinese patients with RA.

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