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ORIGINAL RESEARCH

Safety, pharmacokinetics, biomarker response and efficacy of E6742: a dual antagonist of Toll-like receptors 7 and 8, in a first in patient, randomised, double-blind, phase I/II study in systemic lupus erythematosus

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ABSTRACT

Objectives To evaluate the safety, tolerability, pharmacokinetics (PK), biomarker response and efficacy of E6742 in a phase I/II study in patients with systemic lupus erythematosus (SLE).

Methods Two sequential cohorts of patients with SLE were enrolled and randomised to 12 weeks of two times per day treatment with E6742 (100 or 200 mg; n=8 or 9) or placebo (n=9). The primary endpoint was safety, the secondary endpoints were PK and interferon gene signature (IGS), and the exploratory endpoints were efficacy and biomarker.

Results The proportion of patients with any treatment-emergent adverse events (TEAEs) was 58.8% in the E6742 group (37.5% (3/8 patients) for 100 mg; 77.8% (7/9 patients) for 200 mg) and 66.7% (6/9 patients) in the placebo group. No Common Terminology Criteria for Adverse Events≥Grade 3 TEAEs occurred. PK parameters were similar to these in previous phase I studies in healthy adults. The IGS and levels of proinflammatory cytokines after ex-vivo challenge with a Toll-like receptor 7/8 agonist were immediately decreased by E6742 treatment. The response rate of the British Isles Lupus Assessment Group-based Composite Lupus Assessment at week 12 was 37.5% (3/8 patients) for E6742 100 mg, 57.1% (4/7 patients) for E6742 200 mg and 33.3% (3/9 patients) for placebo group.

Conclusions E6742 had a favourable safety profile and was well tolerated, with suppression of IGS responses and preliminary efficacy signals in patients with SLE. These results provide the first clinical evidence to support E6742 in the treatment of SLE, and support larger, longer-term clinical trials.

Trial registration number NCT05278663.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Because of the limited efficacy and safety concerns of current drug therapies, unmet medical needs remain for many patients with systemic lupus erythematosus (SLE), necessitating new, more efficacious drugs.

⇒ There is strong evidence for the relationship between Toll-like receptor (TLR)7/8 and SLE pathophysiology, and two phase I clinical studies of E6742, a small molecular selective dual antagonist of TLR7/8, in healthy adults showed good tolerance without safety issues.

WHAT THIS STUDY ADDS

⇒ E6742 was well tolerated in this phase I/II clinical trial of patients with SLE, demonstrating a favourable safety profile and suppression of interferon gene signature and preliminary efficacy signals.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This study provides the first clinical evidence to suggest that E6742, as a first-in-class TLR7/8 inhibitor, may be beneficial for SLE.

⇒ The study outcomes also support larger, longer-term clinical trials of E6742.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a heterogeneous autoimmune disease characterised by systemic inflammation in various organs, including the skin, joints, kidneys and central nervous system. SLE is more common in young female adults (9:1 ratio of females to males).[1] The reported global prevalence of SLE varies considerably, ranging from 3.2 to 517.5 per 100 000 individuals.[2 3] According to the European Alliance of Associations for Rheumatology (EULAR) recommendations for the management of SLE

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(2023 update), it should be treated as early as possible and aiming for remission or at least low disease activity to avoid organ damage.[4] Hydroxychloroquine (HCQ) is currently recommended for all patients with SLE unless contraindicated, while the use of glucocorticoids has shifted from mainstay of treatment to a 'bridging therapy' limited to minimal use on the basis of detrimental effects. In patients who do not respond to HCQ (alone or in combination with glucocorticoids) or are unable to reduce glucocorticoids, addition of immunosuppressants and/or biologics should be considered. However, most of these conventional drugs cause serious side effects, leading to the anticipation of development of more targeted therapies.[5–7]

Although the pathophysiology of SLE is not fully understood, there is much evidence indicating that autoimmune responses exerted via type I interferons (IFNs) and activation of the interferon gene signature (IGS) are important in the pathogenesis. Plasmacytoid dendritic cells (pDCs) are specialised subsets of dendritic cells that produce large amounts of type I IFNs in response to viral or bacterial pathogens. Although these IFNs represent fundamental defence reactions against infections,[8–10] their dysregulation can drive autoimmunity. Toll-like receptor (TLR)7, which recognises single-stranded RNA, is highly expressed on pDCs and is thought to be a major trigger of type I IFN production in SLE.[11–14] There is also strong evidence for a genetic contribution of TLR7 in the pathogenesis of SLE.[15 16] Compared with TLR7, TLR8 is more abundant in neutrophils, monocytes and myeloid dendritic cells, inducing strong production of inflammatory cytokines via activation of nuclear factor-κB signalling.[17] Growing evidence has demonstrated that dual inhibition of TLR7/8 may have stronger effects than inhibition of either receptor alone in the treatment of SLE, modulating innate immune responses and suppressing the production of many inflammatory cytokines, including IFN-α. Furthermore, preclinical studies revealed that a dual TLR7/8 inhibitor has the potential to increase the effectiveness of glucocorticoids, indicating a glucocorticoid-sparing potential for TLR7/8 inhibition.[18] E6742 is a selective dual antagonist for TLR7/8. TLR7/8 have the ligand-binding hydrophobic pocket which exists only in the inactivated form of TLR7 and 8 dimers. E6742 binds to the same pocket to inhibit the activation by holding the inactive form.

E6742 was evaluated in phase I studies testing a single ascending dose (SAD) and a multiple ascending dose (MAD) in healthy adults. The safety results from the SAD and MAD studies demonstrated that E6742 has an adequate safety and tolerability profile.[19]

Herein, we present the results of a phase I/II, double-blind, placebo-controlled study of E6742 to further confirm its safety, pharmacokinetics (PK), IGS and explore initial clinical efficacy in patients with SLE.

METHODS

Study design

This was a randomised, double-blind, placebo-controlled, multicentre, MAD study of E6742 in two sequential cohorts of patients with SLE from 12 study sites in Japan (online supplemental list of investigators and study sites). The primary endpoint was to evaluate the safety and tolerability of E6742, the secondary endpoints were to evaluate the PK and IGS after the treatment of E6742. In each cohort, patients were randomised to either E6742 or placebo at a ratio of 2:1 and received multiple oral doses of E6742 or placebo for 12 weeks (85 days). Patients received 100 mg E6742 or placebo two times per day in Cohort 1 and 200 mg E6742 or placebo two times per day in Cohort 2. A randomisation list was generated by an interactive web response system using a permuted block design. Subjects and all personnel involved with the conduct and the interpretation of the study, including investigators, site personnel and sponsor staff were blinded to the treatment codes. PK parameters for dose escalation assessment were evaluated by the predesignated unblinded PK analyst, while all other study personnel remained blinded.

This study consisted of screening/baseline period (day -30 to start of study drug dosing on day 1), a treatment period (days 1–85) and a follow-up period of 4 weeks (28 days) after the last dose of study drug. The study was registered at ClinicalTrials.gov (NCT05278663). The protocol was approved by the Institutional Review Board for each study site. All patients gave written informed consent before participation. This study was conducted in accordance with the standard operating procedures of the sponsor, which were designed to ensure adherence to the Declaration of Helsinki and Good Clinical Practice.

Patients

Patients between 18 and 75 years of age who met the 1997 American College of Rheumatology (ACR) criteria, the 2012 Systemic Lupus International Collaborating Clinics (SLICC) criteria or the 2019 EULAR/ACR criteria for SLE at least 6 months before the informed consent deadline were eligible for enrolment if they met all other entry criteria. These criteria included a requirement for active SLE disease at screening, that is, exhibited at least one active clinical symptom of SLE (arthritis, rash, myositis, mucosal ulcer, pleurisy, pericarditis or vasculitis) based on SLE Disease Activity Index 2000 (SLEDAI-2K) assessment. Positive tests for antinuclear autoantibodies, anti-double-stranded DNA (anti-dsDNA) antibodies and/or anti-Smith (anti-Sm) antibodies were also required, and patients had to be on stable standard SLE therapy for at least 4 weeks prior to the first dose of the study medication. Allowable baseline standard SLE therapies included oral glucocorticoids (≤20 mg/day prednisone or equivalent), antimalarials (≤400 mg/day HCQ) and immunosuppressants (one of the following: ≤200 mg/day azathioprine, ≤3 g/day mycophenolate, ≤15 mg/

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week methotrexate, ≤3 mg/day tacrolimus and≤200 mg/day ciclosporin).

Patients were excluded if they had any unstable or progressive manifestation of SLE (eg, active nephritis or active central nervous system involvement) or other inflammatory disease that might confound efficacy assessments. Furthermore, patients were not allowed to have had previous treatment with the following within prespecified timeframes prior to the first dose of the study medication: Belimumab /Anifrolumab (within 24 weeks), oral Janus kinase inhibitors (within 12 weeks) and Rituximab (within 48 weeks). The race and ethnicity of patients were determined based on self-reported method with a fixed set of categories.

Assessments

Safety

Safety was evaluated based on all treatment-emergent adverse events (TEAEs) and serious TEAEs, clinical laboratory parameters (haematology, blood chemistry, urinalysis), vital signs, 12-lead ECG, physical examination results and chest X-rays. TEAEs were coded using the Medical Dictionary for Regulatory Activities (MedDRA), V.26.0. Severity of a TEAE was graded on a 5-point scale according to Common Terminology Criteria for Adverse Events (CTCAE) V.5.0. A TEAE was defined as an adverse event that emerged during the time from the first dose of study drug to 35 days after the participant's last dose, having been absent at pretreatment (baseline) or re-emerged during treatment, present at pretreatment (baseline) but stopped before treatment, or worsened in severity during treatment relative to the pretreatment state, when the AE was continuous.

Pharmacokinetics

On days 1 and 15, plasma samples were collected immediately before administration of the study drug and at 1, 2, 3 and 6 hours postadministration. The plasma concentration of E6742 was measured in patients receiving active treatment using a validated liquid chromatography-tandem mass spectrometry method.

Biomarker response

Blood samples for IGS assessment were collected from all patients at baseline, weeks 2, 4, 8 and 12 and the follow-up visit. RNA was extracted from whole blood for analysis of IGS expression levels by RNA sequencing using validated methods. Based on previous reports of higher expression of IFN-related genes in patients with SLE compared with that in healthy adults,[20–22] a total of 127 IGSs were selected for this study (online supplemental table S1). Pretreatment and post-treatment changes in expression of these 127 genes were evaluated, and the most prevalently expressed 21 genes (*EPSTI1*, *HERC5*, *IFI27*, *IFI44*, *IFI44L*, *IFI6*, *IFIT1*, *IFIT3*, *ISG15*, *LAMP3*, *LY6E*, *MX1*, *OAS1*, *OAS2*, *OAS3*, *PLSCR1*, *RSAD2*, *RTP4*, *SIGLEC1*, *SPATS2L* and *USP18*) were selected for calculation of the 'IGS score'.[20 23]

Blood samples were collected from all patients at five time points on days 1 and 15 (predose and 1, 2, 3 and 6 hours postdose), and then the collected samples in heparinised tubes were used for determination of interleukin (IL)-1β, IL-6 and tumour necrosis factor-α (TNF-α) levels after ex-vivo challenge with a TLR7/8 agonist. Blood samples were incubated with the dual TLR7/8 agonist R848 (2.4 µM) for 24 hours at 37°C and 5% CO_2. After centrifugation of the samples, supernatants were collected and stored at −80°C until the cytokines were measured. Concentrations of cytokines were quantified using the V-PLEX Human Proinflammatory Panel 1 Kit from Meso Scale Discovery, in accordance with the manufacturer's instructions.

Efficacy

The following efficacy outcomes were assessed at week 12: British Isles Lupus Assessment Group-based Composite Lupus Assessment (BICLA) response, SLEDAI-2K, British Isles Lupus Assessment Group Index 2004 (BILAG-2004), Physician Global Assessment (PGA), Cutaneous Lupus Erythematosus Disease Area and Severity Index (CLASI) activity score, CLASI-50 response (decrease of ≥50% from baseline CLASI activity score), tender joint counts (out of 68 joints) and swollen joint counts (out of 66 joints) and serological markers (anti-dsDNA antibody and complement C3/C4 levels). The BICLA response was defined as meeting all of the following criteria: all BILAG-2004 A scores at baseline improved to B, C or D, and all BILAG-2004 B scores improved to C or D; no worsening in disease activity to BILAG-2004 A score and no scores≥2 worsening to BILAG-2004 B score compared with baseline level; no worsening of total SLEDAI-2K score compared with the baseline level; no worsening in the PGA (an increase of <0.3 points from baseline); and no discontinuation of trial medication or use of restricted medications beyond the protocol-allowed threshold.

Statistical analyses

Sample size selection was not based on statistical power. Twelve patients per cohort (eight patients randomised to E6742 and four patients randomised to placebo) were considered adequate to evaluate the safety, tolerability, PK and IGS in patients with SLE. Safety analyses were conducted using the safety analysis set, which included all patients who received at least one dose of the study treatment and had safety data. PK analyses were conducted using the PK analysis set, which included all patients who received at least one dose of the study drug and had sufficient PK data to derive at least one PK parameter. Biomarker analyses were conducted using the full-analysis set, which included all patients who were randomised and received at least one dose of the study treatment and had at least one postdose efficacy measurement. The 21 most prevalently expressed IFN-inducible genes were selected as candidate biomarkers for anti-IFN-α therapy in SLE.[20] To assess the level of expression of this gene set, the IGS score for each patient was calculated as the median

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of the fold change in these 21 genes. The fold change for each gene was calculated as the fold of the level of expression in each subject relative to a pooled healthy adult sample.[23] All efficacy analyses were conducted using the full-analysis set. Efficacy data were summarised using descriptive statistics. BICLA response analyses were performed on patients with at least one BILAG-2004 A or BILAG-2004 B score at baseline. Participants who discontinued treatment were imputed as non-responders for all of the visits following treatment discontinuation. Data were analysed using Statistical Analysis Software (SAS) V.9.4 (SAS Institute, North Carolina, USA).

Patient and public involvement

Patients from the Japanese lupus group were involved to discuss the appropriateness of the trial procedures during the trial. There was no further patient or public involvement in the planning, practice, analysis or reporting process of the trial.

RESULTS

Patient disposition and baseline characteristics

This study was conducted between April 2022 and September 2023. A total of 39 patients were screened, of whom 27 were randomised into the study. Of the 27 randomised patients, 26 received at least one dose of study drug; 9 patients received placebo (4 patients in Cohort 1 and 5 patients in Cohort 2) and 17 patients received E6742 at a dosage of 100 mg two times per day (8 patients in Cohort 1) or 200 mg two times per day (9 patients in Cohort 2). Most of the treated patients completed the study, with one participant each in the placebo and 200 mg two times per day groups withdrawing from the study because of lack of efficacy and an AE, respectively (figure 1).

Baseline demographics and disease characteristics are shown in table 1. Although the 100 mg group had a slightly lower mean age and a shorter disease duration than the other groups, the baseline demographics were generally similar across the treatment groups. Regarding clinical characteristics, some disease index parameters, including SLEDAI-2K scores and tender or swollen joint counts, were slightly lower in the 200 mg group than in the other groups. Among the BILAG-2004 components, the three most common manifestations of active SLE were mucocutaneous (80.8%), musculoskeletal (69.2%) and haematological (57.7%). The most frequently used concomitant medication for SLE at baseline was oral glucocorticoid (96.2%), followed by HCQ (80.8%) for all patients. At baseline, 92.3% of the patients were receiving oral glucocorticoid at doses ≤10 mg/day. Overall, at baseline, 34.6% of the patients were taking three medications

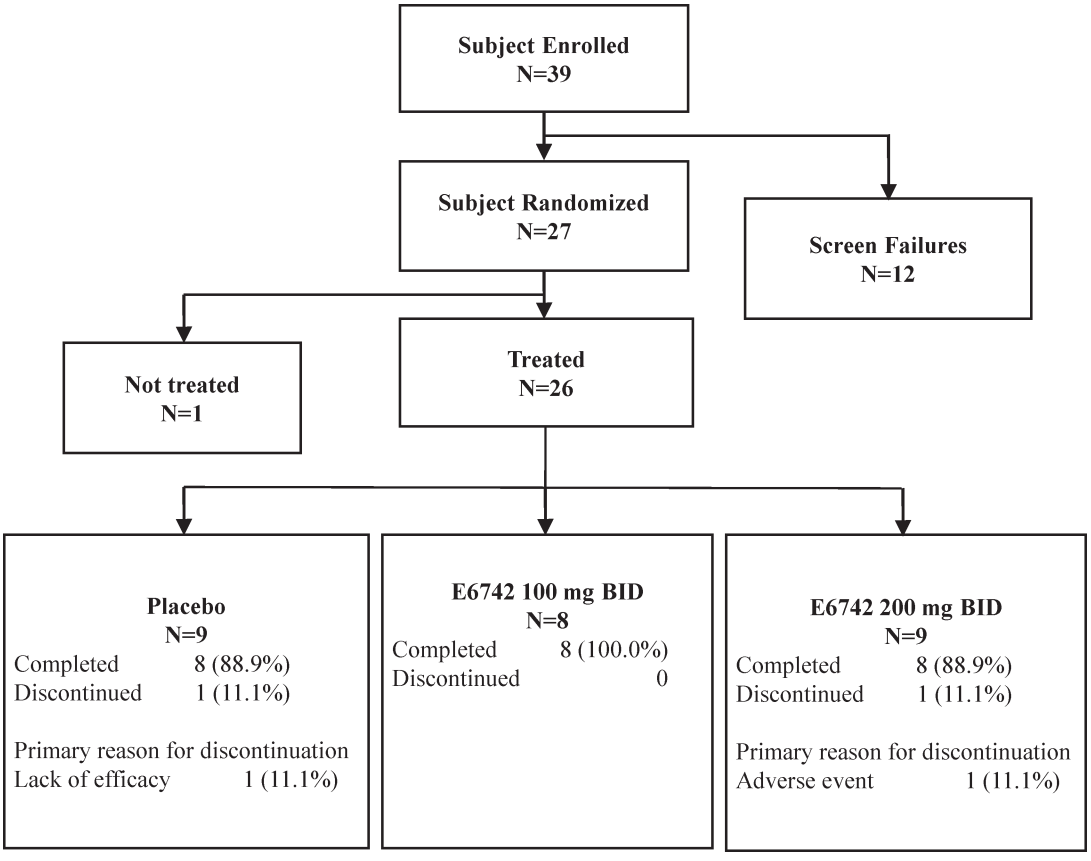

Figure 1 Trial patient disposition. One subject randomised to 200 mg two times per day treatment was withdrawn from the study after randomisation without any study treatment due to ineligibility (a concomitant medical condition that in the opinion of the investigator would compromise the subject's ability to safely complete the study) from the baseline assessment. Of the nine patients in placebo, four patients received placebo in Cohort 1 and five patients received placebo in Cohort 2. BID, two times per day.

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Table 1 Baseline demographics and disease characteristics

	Placebo (n=9)	E6742 100 mg (n=8)	E6742 200 mg (n=9)	Total (n=26)
Age (years), mean (SD)	38.9 (9.20)	33.6 (12.97)	40.4 (11.17)	37.8 (11.07)
Female, n (%)	8 (88.9)	8 (100.0)	8 (88.9)	24 (92.3)
Ethnic origin (Asian), n (%)	9 (100.0)	8 (100.0)	9 (100.0)	26 (100.0)
Weight (kg), mean (SD)	53.6 (13.74)	53.2 (6.34)	51.8 (8.26)	52.9 (9.70)
BMI (kg/m^2), mean (SD)	21.1 (4.58)	20.9 (2.55)	21.1 (2.73)	21.1 (3.31)
Disease duration of SLE (years), mean (SD)	7.7 (6.10)	4.5 (3.62)	8.2 (6.96)	6.9 (5.81)
SLEDAI-2K total score, mean (SD)	7.8 (2.91)	8.6 (4.69)	6.7 (2.83)	7.7 (3.47)
BILAG-2004 components, n (%)				
Mucocutaneous, A/B/C	0/6/0 (66.7)	0/6/1 (87.5)	1/4/3 (88.9)	1/16/4 (80.8)
Musculoskeletal, A/B/C	0/4/3 (77.8)	0/5/1 (75.0)	0/4/1 (55.6)	0/13/5 (69.2)
Haematological, A/B/C	0/0/5 (55.6)	0/0/5 (62.5)	0/0/5 (55.6)	0/0/15 (57.7)
PGA (0–3) score, mean (SD)	1.3 (0.50)	1.0 (0.27)	1.1 (0.40)	1.2 (0.41)
CLASI activity score, mean (SD)	3.7 (4.69)	3.5 (3.30)	3.7 (4.77)	3.6 (4.17)
Tender joint count (68), mean (SD)	9.6 (15.88)	9.1 (12.38)	5.4 (7.13)	8.0 (11.98)
Swollen joint count (66), mean (SD)	2.9 (5.30)	3.8 (4.98)	1.8 (2.33)	2.8 (4.28)
Autoantibody titre, n (%)				
ANA positive (≥80 titre) at the time of study entry	7 (77.8)	7 (87.5)	7 (77.8)	21 (80.8)
Anti-dsDNA positive (IgG 12 IU/mL or IgM ≥6 U/mL)	6 (66.7)	5 (62.5)	8 (88.9)	19 (73.1)
Anti-Sm positive (≥10 U/mL)	3 (33.3)	0	1 (11.1)	4 (15.4)
Complement level, n (%)				
Low C3 (<0.65 g/L)	4 (44.4)	3 (37.5)	2 (22.2)	9 (34.6)
Low C4 (<0.13 g/L)	5 (55.6)	4 (50.0)	6 (66.7)	15 (57.7)
Concomitant medication for SLE, n (%)				
Oral glucocorticoids, n (%)	8 (88.9)	8 (100.0)	9 (100.0)	25 (96.2)
Dose (mg/day)*, mean (SD)	4.8 (3.45)	5.6 (2.15)	6.2 (2.99)	5.6 (2.85)
<=10 mg/day, n (%)	7 (77.8)	8 (100.0)	9 (100.0)	24 (92.3)
>10 mg/day, n (%)	1 (11.1)	0	0	1 (3.8)
Hydroxychloroquine, n (%)	8 (88.9)	7 (87.5)	6 (66.7)	21 (80.8)
Immunosuppressants, n (%)	4 (44.4)	4 (50.0)	4 (44.4)	12 (46.2)
Mycophenolate mofetil	0	0	1 (11.1)	1 (3.8)
Methotrexate	0	2 (25.0)	1 (11.1)	3 (11.5)
Tacrolimus	4 (44.4)	2 (25.0)	2 (22.2)	8 (30.8)

Data expressed as n (%) or mean (SD).
*Prednisolone or equivalent.
ANA, antinuclear antibody; anti-dsDNA, anti-double-stranded DNA; anti-Sm, anti-Smith; BILAG, British Isles Lupus Assessment Group; BMI, body mass index; CLASI, Cutaneous Lupus Erythematosus Disease Area and Severity Index; PGA, Physician's Global Assessment; SLE, systemic lupus erythematosus; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index.

concomitantly and 53.8% of patients were taking two medications concomitantly for SLE: HCQ, oral glucocorticoid, or an immunosuppressant.

Safety

TEAEs occurred in six (66.7%) of nine patients in the placebo group, three (37.5%) of eight patients in the 100 mg group and seven (77.8%) of nine patients in the 200 mg group, with a similar incidence between the treatment groups (table 2). The TEAEs reported in two or more patients in either of the E6742 treatment groups, categorised as MedDRA Preferred Terms, were nasopharyngitis (25.0% (2/8 patients)) in the 100 mg group and headache (22.2% (2/9 patients)) in the 200 mg group. Although these TEAEs only occurred in E6742-treated

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Table 2 Summary of safety data

	Placebo (n=9) n (%)	E6742 100 mg (n=8) n (%)	E6742 200 mg (n=9) n (%)	E6742 total (n=17) n (%)
TEAEs	6 (66.7)	3 (37.5)	7 (77.8)	10 (58.8)
Treatment-related TEAEs*	2 (22.2)	1 (12.5)	1 (11.1)	2 (11.8)
TEAEs by worst CTCAE grade of				
Grade 1	1 (11.1)	2 (25.0)	5 (55.6)	7 (41.2)
Grade 2	5 (55.6)	1 (12.5)	2 (22.2)	3 (17.6)
Grade ≥3	0	0	0	0
Serious TEAEs	0	0	1 (11.1)‡	1 (5.9)
Deaths†	0	0	0	0
TEAEs leading to withdrawal	0	0	1 (11.1)‡	1 (5.9)
TEAEs that occurred in ≥2 reported by MedDRA System Organ Class				
Infections and infestations	3 (33.3)	3 (37.5)	3 (33.3)	6 (35.3)
Nasopharyngitis	0	2 (25.0)	1 (11.1)	3 (17.6)
COVID-19	0	1 (12.5)	1 (11.1)	2 (11.8)
Pneumonia	0	0	1 (11.1)	1 (5.9)
Upper respiratory tract infection	0	0	1 (11.1)	1 (5.9)
Bronchitis	1 (11.1)	0	0	0
Cystitis	1 (11.1)	0	0	0
Gastroenteritis	1 (11.1)	0	0	0
Nervous system disorders	0	0	2 (22.2)	2 (11.8)
Headache	0	0	2 (22.2)	2 (11.8)
Respiratory, thoracic and mediastinal disorders	1 (11.1)	0	2 (22.2)	2 (11.8)
Cough	1 (11.1)	0	1 (11.1)	1 (5.9)
Hyperventilation	0	0	1 (11.1)	1 (5.9)

A TEAE was defined as an AE that emerged during the time from the first dose of study drug to 35 days after the participant's last dose, having been absent at pretreatment (baseline) or re-emerged during treatment, present at pretreatment (baseline) but stopped before treatment, or worsened in severity during treatment relative to the pretreatment state, when the AE was continuous.
Participants with ≥2 TEAEs in the same system organ class (or with the same MedDRA Preferred Term) were counted only once for that system organ class (or preferred term).
*Included treatment-related TEAEs considered by the investigator to be related to study drug or TEAEs with missing causality.
†Includes all patients with serious TEAE resulting in death.
‡A serious TEAE occurred in one participant in the 200 mg group, resulting in withdrawal from study treatment.
AE, adverse event; CTCAE, Common Terminology Criteria for Adverse Events; MedDRA, Medical Dictionary for Regulatory Activities; TEAE, treatment-emergent adverse event.

patients, all but one (a case of nasopharyngitis in the 100 mg group) were considered unrelated to the study drug. All TEAEs were CTCAE Grade 1 or 2 in severity; no Grade 3 or higher TEAEs occurred. No deaths were reported during the study. One participant in the 200 mg group experienced pneumonia (serious TEAE, CTCAE Grade 2) that led to withdrawal of the study treatment, but recovered within 1 week after hospitalisation. At each patient visit, 12-lead ECG assessments were performed. On days 1 and 15, ECGs were measured at 3 hours post-dose, which roughly corresponded to the peak concentration (C_{max}) of E6742. No patients in any treatment group had a clinically meaningful postbaseline corrected QT (QTc) interval using Fridericia's formula exceeding 480 msec or an increase exceeding 60 msec (online supplemental figure S1). No clinically significant findings in clinical laboratory tests, vital signs or chest X-rays were observed in E6742-treated patients.

PHARMACOKINETICS

The median time for E6742 to reach C_{max} (t_{max}) and median time to steady-state maximum concentration ($t_{ss,max}$) following oral dosing at 100 and 200 mg two times per day were 1.44 and 1.87 hours, and 1.11 and 1.42 hours, respectively. Plasma concentrations of E6742 increased with increasing doses. The geometric mean accumulation ratio of C_{max} and area under the curve of E6742 following oral dosing at 100 and 200 mg two times

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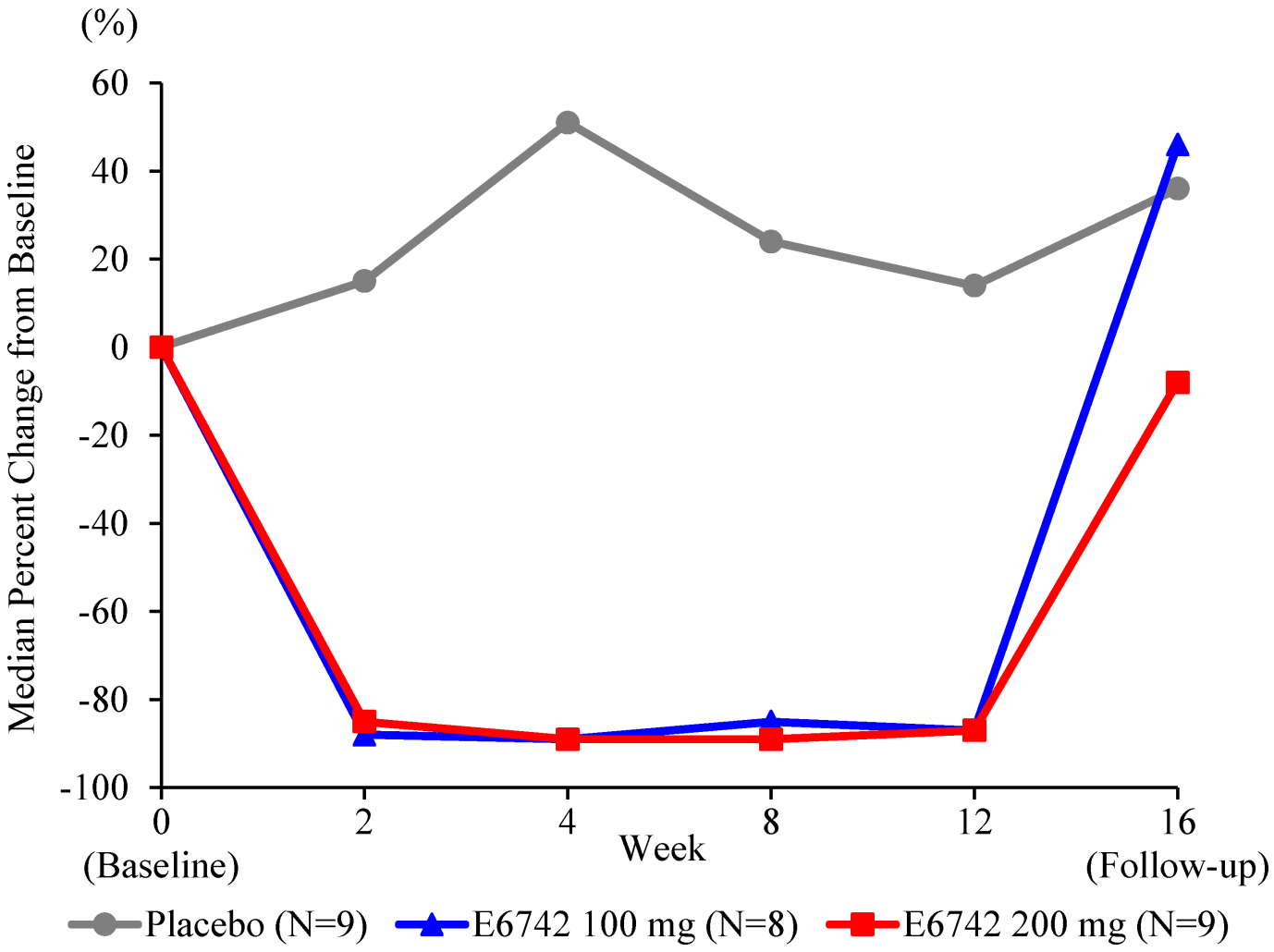

Figure 2 Effect of E6742 on IGS scores in patients with SLE. The IGS scores were calculated from the fold change in expression of a set of 21 IGSs. Only patients with non-missing data at both baseline and the relevant postbaseline visits were included in the calculation. Results were expressed as the median percent change from baseline. The actual data of each plot are included in online supplemental table S3. IGS, interferon gene signature; SLE, systemic lupus erythematosus.

per day were 1.03 and 1.32, and 1.04 and 1.28, respectively (online supplemental figure S2).

Biomarker response

The 127 IGSs which have been reported as higher expression in patients with SLE[20–22] were evaluated in this study. An early decrease in the expression of the majority of these genes was observed from 2 weeks post-treatment onwards, with the reductions maintained throughout the treatment with E6742. For example, the expression levels of *MX1*, *IFIT1* and *IFI44L* were markedly reduced at week 12 in the E6742-treated groups, showing the following median percent changes from baseline: –87.6% in the 100 mg group, –86.1% in the 200 mg group and 6.83% in the placebo group for *MX1*; –88.5% in the 100 mg group, –90.7% in the 200 mg group and 14.8% in the placebo group for *IFIT1*; and –95.5% in the 100 mg group, –95.9% in the 200 mg group and 14.8% in the placebo group for *IFI44L* (the median percent change from baseline of all IGSs were listed in online supplemental table S2). Furthermore, IGS scores based on the major 21 genes dropped under treatment with E6742 (figure 2), with both the 100 mg and 200 mg groups showing approximately 90% reductions by 2 weeks post-treatment; these reductions were maintained throughout the treatment period.

Pharmacodynamic biomarkers were also assessed using an ex-vivo assay. R848, a TLR7/8 agonist, was incubated with blood samples collected on days 1 and 15 to evaluate the inhibitory effect of E6742 on the levels of R848-induced cytokines (IL-1β, IL-6 and TNF-α). The mean concentrations of IL-1β, IL-6 and TNF-α were significantly decreased from baseline in E6742-treated participants at 1 hour postdose on day 1, with mean percent changes of >95%. These reductions were generally maintained until day 15 (figure 3).

Efficacy

At week 12, a BICLA response was observed in 33.3% (3/9) of patients in the placebo group, 37.5% (3/8) in the 100 mg group and 57.1% (4/7) in the 200 mg group, respectively (figure 4). Two patients in the 200 mg group

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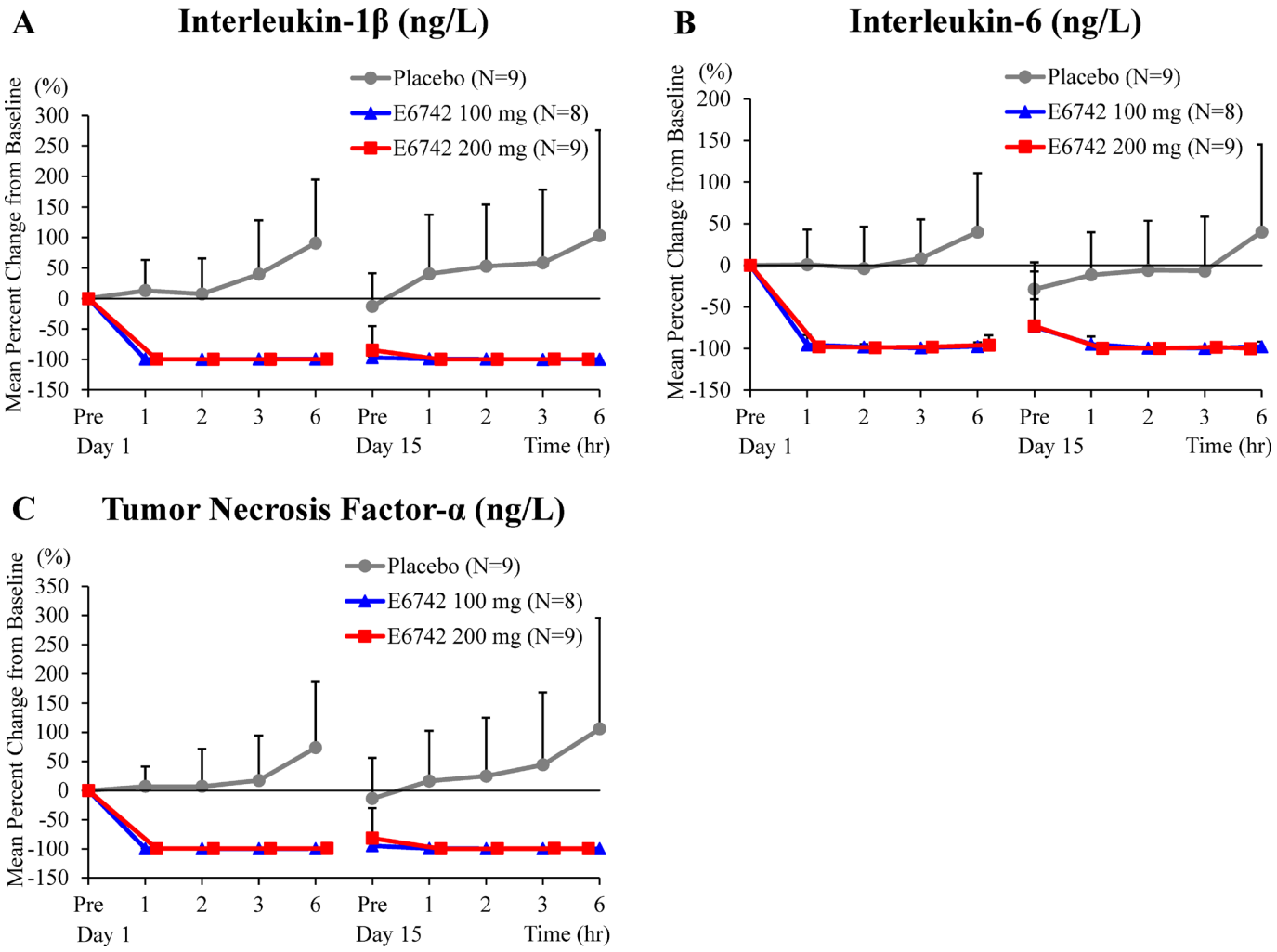

Figure 3 Effects of E6742 on R848-mediated ex-vivo induction of blood cytokines: (A) interleukin-1β, (B) interleukin-6 and (C) tumour necrosis factor-α. Linear plots of mean (+SD) percent change from baseline of the concentrations of the indicated cytokines following study drug treatment of day 1 and day 15 blood samples with Toll-like receptor 7/8 agonist R848. All descriptive statistics were calculated using the percent change from baseline of the median measured concentration in the analytical sample (n=3). If 1/3 measurements were BLQ, the BLQ was assigned as 0 and the median value was used; if 2/3 were BLQ, the lower limit of quantification was used; if all measurements were BLQ, 0 was used. The actual data of each plot are included in online supplemental table S4. BLQ, below the limit of quantification; Pre, predose.

who had no A or B category BILAG-2004 scores at baseline were excluded from the BICLA response calculation.

Clinical outcomes, including PGA scores, CLASI activity scores and joint counts, generally improved from baseline in E6742-treated patients (online supplemental table S5). Additionally, serological parameters including anti-dsDNA antibodies and complement C3 and C4 levels showed improvements in the E6742-treated group. The CLASI-50 response rates were 33.3% (2/6) in the placebo group, 57.1% (4/7) in the 100 mg and 50.0% (4/8) in the 200 mg group, respectively. The reductions from baseline in tender joint counts were –0.9 in the placebo group, –2.4 in the 100 mg and –5.3 in the 200 mg group at week 12. The reduction of swollen joint counts were 0.1 (increase) in the placebo group, –3.8 in the 100 mg, –1.5 in the 200 mg group at week 12. In both E6742-treated patients who had swollen joint counts at baseline, all joint swelling had disappeared at week 12. Reductions in anti-dsDNA immunoglobulin G antibodies were also observed in the E6742-treated groups (–7.7 at 100 mg and –2.7 at 200 mg) at week 12.

DISCUSSION

E6742 is a small molecule compound that directly binds to the single-stranded RNA-sensing receptors TLR7 and TLR8, inhibiting activation of the respective signalling pathways. This multicentre, randomised, double-blind, placebo-controlled study provides the first clinical evidence of E6742 in the treatment of SLE.

E6742 was generally well tolerated throughout the 12-week treatment period. There was no clear difference in the incidence of TEAEs among the 100 mg, 200 mg and placebo groups (table 2). A non-fatal but serious TEAE of pneumonia (CTCAE Grade 2) occurred in one participant in the 200 mg group, who recovered within

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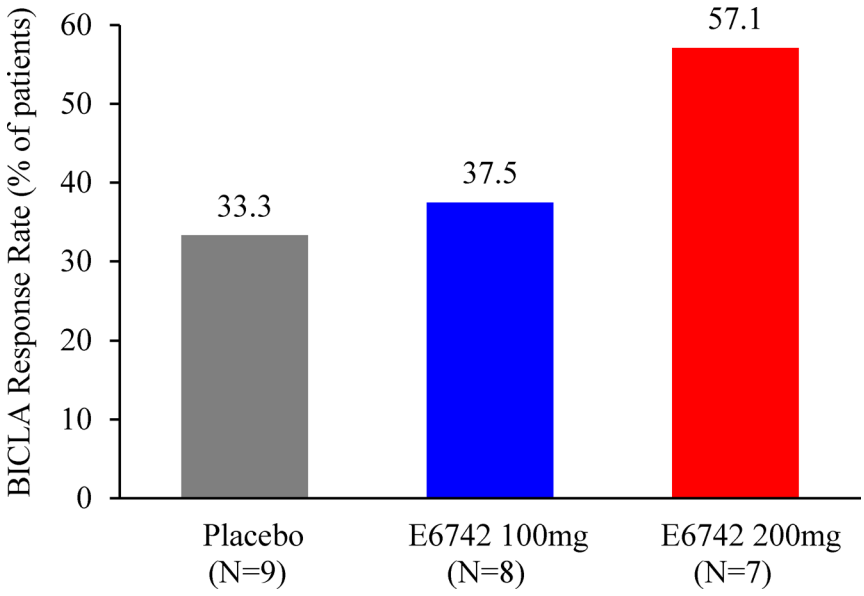

Figure 4 BICLA response at Week 12 of treatment with E6742. Response rate analysed for patients with at least 1 A or B category score in the British Isles Lupus Assessment Group Index 2004 (BILAG-2004) at baseline. Participants who discontinued treatment were imputed as non-responders for all of the visits following treatment discontinuation. BICLA, British Isles Lupus Assessment Group-based Composite Lupus Assessment.

about 1 week after hospitalisation. Because the case of pneumonia observed in this trial was bacterial in nature, the possible relationship between this pneumonia and inhibition of TLR7/8, as a sensor of virus, is inconclusive. The overall incidences of infection were generally comparable in the placebo (33.3%) and E6742 total (35.3%) groups (table 2), and there were no changes in laboratory values related to infection risk, including neutrophil and white blood cell counts, after E6742 administration.

Because multiple drugs are often used concomitantly in the treatment of SLE, it was important to evaluate the safety of E6742 when used in combination with other drugs. Considering the results of our previous phase I studies of E6742, the non-linear PK at high doses and the serum concentration-dependent risk of QT prolongation of E6742 were flagged as potential safety concerns.[19] According to the US Food and Drug Administration, HCQ can cause abnormal heart rhythms such as QT interval prolongation and a dangerously rapid heart rate called ventricular tachycardia.[24] In this trial, 87.5% of patients in the 100 mg group and 66.7% of patients in the 200 mg group concomitantly used HCQ. However, there were no specific concerns regarding PK parameters after multiple oral doses of E6742 in patients with SLE, which were similar to those in healthy adults. Likewise, there were no QTc safety signals after E6742 treatment. However, there are three main limitations regarding the interpretations of E6742 safety data from this trial: (1) only Japanese patients were enrolled; (2) patients with specific complications, such as severe lupus nephritis and central nervous system lupus, were excluded; and (3) the sample size and evaluation period were limited. Therefore, a longer and more detailed evaluation is required to fully derive the safety profile of E6742.

The heterogeneity of disease expression in SLE has made it challenging to find treatments with clear clinical efficacy. Therefore, as a secondary endpoint of this study we evaluated changes in biomarkers in response to E6742 treatment. Activation of the type I IFN system plays an important role in the immune system and is associated with the pathogenesis of SLE.[25] Specifically, patients with high IFN signalling are more likely to exhibit a disease flare of SLE[26] and IFN pathway activation has been shown to impart resistance to glucocorticoids.[18 25 27] TLR7 is highly expressed in pDCs and is thought to be a major trigger for type I IFN production in SLE,[26] which is quantified using the IGS.[28] In this trial, IGSs were clearly downregulated under E6742 treatment. Furthermore, although a direct comparison is not appropriate, the magnitude of change in IGS score from baseline (figure 2) was comparable to that reported for anti-IFN-α receptor antibodies, which directly suppresses IFN signalling.[29] In addition, the concentrations of proinflammatory cytokines (IL-1β, IL-6 and TNF-α) were substantially (>95%) reduced in the ex-vivo assay of TLR7/8 agonist challenge. With regard to clinical efficacy parameters, numerically efficacy signals were observed in the E6742 treated groups. These beneficial effects of E6742 in SLE may suggest that E6742 indirectly regulates T-cell activation and B-cell differentiation by regulating the expression of IFN-related genes and proinflammatory cytokines.[5]

Despite some limitations, including the short-term evaluation of efficacy, lack of disease activity score specified as an inclusion criterion and the lack of stratification by baseline disease activity, the efficacy signals obtained in this trial were encouraging. Furthermore, the significant change in IGS in response to E6742 treatment warrants the evaluation of E6742 efficacy in additional patient

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populations with more active disease and diverse clinical phenotypes.

In conclusion, this phase I/II trial evaluated the safety, tolerability, PK, biomarker response and efficacy profile of E6742 in patients with SLE. These findings support further investigations of E6742 in the treatment of SLE.

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Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by (1) Hospital of the University of Occupational and Environmental Health, Japan Institutional Review Board. (2) Japan Community Health Care Organization Chukyo Hospital Institutional Review Board. (3) Daido Hospital Institutional Review Board. (4) Institutional Review Board of Matsuyama Red Cross Hospital. (5) Hokkaido University Hospital Institutional Review Board. (6) Tohoku University Hospital Institutional Review Board. (7) Tokyo Metropolitan Hospital Organization Tokyo Metropolitan Tama Medical Center Institutional Review Board. (8) National Hospital Organization Kyushu Medical Center Institutional Review Board. (9) Osaka University Hospital Institutional Review Board. (10) St. Luke's International Hospital Institutional Review Board. (11) Certified Review Board of National Center for Global Health and Medicine. (12) Juntendo University Hospital Institutional Review Board. Participants gave informed consent to participate in the study before taking part.

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