Efficacy and safety of an interleukin-6 monoclonal antibody for the treatment of systemic lupus erythematosus: a phase 2 dose-ranging randomised controlled trial

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SUPPLEMENTARY MATERIALS

Exclusion criteria

Key exclusion criteria were prior treatment with an anti-interleukin-6 (IL-6) agent; ≥3 courses of corticosteroids required for concomitant conditions within 1 year; treatment with any B-cell-depleting agents; cyclophosphamide or belimumab within 180 days; tumour necrosis factor inhibitors, anakinra, intravenous (IV) immunoglobulin, high-dose corticosteroids (>100 mg/day prednisone or equivalent) or pulse IV doses, or plasmapheresis within 90 days; intramuscular or IV corticosteroids or any new immunosuppressive, immunomodulatory or antimalarial agent within 60 days; immunoglobulin A level below the lower limit of normal; history of thrombosis (venous or arterial) or other vascular complications within 180 days due to antiphospholipid syndrome or anticardiolipin antibodies; or severe systemic lupus erythematosus (SLE) renal or active central nervous system disease. Patients with positive hepatitis B or hepatitis C tests, known history of human immunodeficiency virus, history of active or untreated latent tuberculosis, and women who were pregnant or breastfeeding were also excluded.

Pharmacokinetic, pharmacodynamics, and biomarker assessments

Blood samples were obtained for measurement of PF-04236921, IL-6 and C-reactive protein (CRP). Serum samples were analysed for PF-04236921 concentrations using a validated, sensitive and specific enzyme-linked immunosorbent assay. Serum CRP was the primary pharmacology marker of the anti-IL-6 activity of PF-04236921 in this trial.

Extractable nuclear antigens (anti-SSA/Ro, anti-SSB/La, anti-RNP and anti-SM) and anti-double-stranded DNA (anti-dsDNA) were measured using a bead-based immunoassay (AtheNA Multi-Lyte ANA Test System), with a negative threshold of <100 IU/mL and positive for >120

IU/mL (values between 100 and 120 were considered equivocal). Antinuclear antibodies (ANA) were measured with the Kallestad human epithelial type 2 (HEp-2) Cell Line Substrate with a 1:40 threshold for positivity. The complement C3 and C4 assays were performed by immunonephelometry using the Siemens BNII Nephelometer (normal range for adults is 90.0–180.0 mg/mL for C3 and 10.0–40.0 mg/mL for C4). These values were all reported by the Covance Central Laboratory.

Safety assessments

Adverse events (AEs), vital signs, haematology, chemistry and urinalysis were monitored. As a secondary endpoint, blood samples were collected for analysis of antidrug antibodies (ADAbs) using electrochemiluminescent immunoassays; samples that were positive for ADAbs were further tested for neutralising antibodies.

Additional efficacy analyses

At Week 24, reductions in anti-dsDNA antibodies ≥10% from baseline were greater for 10 and 50 mg than placebo, although sample sizes were small (14–18 patients in each group had measurable anti-dsDNA antibody levels). More patients in the 10 mg group achieved ≥30% and ≥50% reductions than the 50 mg group.

More patients receiving 10 mg (26.7%) or 50 mg (20.8%) achieved reductions in corticosteroid doses ≥25% from baseline, and to ≤7.5 mg/day, for at least one visit up to and including Week 24, compared with placebo (8.7%), although, again, sample sizes were small (14–24 patients in each group were receiving corticosteroid doses of >7.5 mg/day at baseline). Of the four patients fulfilling these criteria in the 10 mg group, three had reductions initiated at Week 16 and sustained through Week 24.

Sensitivity analysis

As a sensitivity analysis, the SLE Responder Index (SRI) and British Isles Lupus Assessment Group-based Composite Lupus Assessment (BICLA) were analysed using the logistic regression model using the last observation carried forward for the missing data at each visit with treatment and stratification variables (baseline disease severity and anti-dsDNA status) as factors.

A greater percentage of 10 mg-treated subjects (49.4%) compared with placebo-treated subjects (36.8%) had SRI responses using logistic regression at Week 24 (p=0.116). In addition, a greater percentage of 10 mg-treated subjects (44.4%) compared with placebo-treated subjects (26.7%) had BICLA responses using logistic regression. Details of both analyses are included in supplementary table S1. The proportion of subjects achieving SRI and BICLA responses compared to placebo using the logistic regression model was numerically less than the SRI response from the GLMM, though the confidence intervals widely overlapped.

The proportion of 50 mg-treated subjects who had logistic regression SRI and BICLA responses at Week 24 was not significantly different than placebo.

Supplementary table S1 Sensitivity analysis of efficacy outcomes at Week 24

	Placebo (n=45)	**10 mg (n=45)**	**50 mg (n=47)**
SRI response rate, n/N (%)*	17/45 (36.8)	23/45 (49.4)	15/47 (30.9)
Odds ratio vs placebo (90% CI)		1.68 (0.82, 3.42)	0.77 (0.37, 1.59)
p value		0.116	0.725
BICLA response rate, n/N (%)*	12/45 (24.9)	20/45 (41.4)	18/47 (35.3)
Odds ratio vs placebo (90% CI)		2.13 (1.00, 4.51)	1.64 (0.78, 3.46)
p value		0.05	0.138

*Estimates from the logistic regression model with last observation carried forward for missing data.

BICLA, British Isles Lupus Assessment Group-based Composite Lupus Assessment; CI, confidence interval; SRI, Systemic Lupus Erythematosus Responder Index.

Study subjects

Patients were enrolled from 65 centres in 11 countries (Argentina, Chile, Columbia, Germany, Hungary, Korea, Moldova, Peru, Poland, Romania, USA). Patient visits took place between December 2011 and March 2014.

Pharmacokinetic and pharmacodynamic outcomes

Serum levels of PF-04236921 increased in a dose-proportional manner (supplementary figure S1).

Following administration of PF-04236921, serum CRP levels were continuously suppressed from Week 2 through Week 24 (supplementary figure S2). Reductions in CRP levels appeared to increase monotonically with dose.

Safety

Treatment-emergent AEs (TEAEs), infectious AEs, and discontinuations due to AEs were comparable across treatment groups. The most frequent TEAEs (excluding infections and injection-site reactions) were headache, nausea and diarrhoea (nausea and diarrhoea were most commonly reported with placebo), and the most frequent infectious TEAEs were upper respiratory infection, cystitis and pharyngitis/laryngitis.

Four deaths occurred during the study. The first was a 32-year-old Hispanic female subject from Colombia with SLE who received a single 10 mg dose of study medication. She was taking daily doses of 15 mg oral prednisone, 50 mg azathioprine and 250 mg chloroquine. Relevant medical

history included hypertension. On Study Day 20, the subject called the centre complaining of pain in right leg. The investigator instructed subject to go to the emergency room (ER) due to the possibility of a deep venous thrombosis (DVT). The subject's relative informed the site that she went the same day but she was diagnosed with a muscle tear and was sent home. On Study Day 22, due to persisting symptoms of malaise, dyspnoea and adynamia, the subject went to the ER again and was diagnosed with DVT with pulmonary embolism (PE). The subject arrived at the hospital with shortness of breath and chest pain. The medical analysis showed that the subject experienced chest pain, tachycardia (showed by electrocardiogram [ECG]), shortness of breath, hypocalcaemia and creatinine elevation. X-ray showed evidence of diffuse micronodules on both lungs. The subject died that day due to a suspected PE, considering the acute episode, chest pain, shortness of breath and ECG tachycardia. An autopsy was not performed.

The remaining three deaths all occurred in the 200 mg arm. The first was a 54-year-old Black female subject from the USA who received a single 200 mg dose of study medication. Her relevant medical history included hypertension, anaemia, obesity, sleep apnoea and former tobacco abuse. The subject was originally diagnosed with rheumatoid arthritis based upon arthralgias and weakly positive rheumatoid factor and treated with methotrexate. The subject had a history of recurring bouts of pleuritic-type chest pain accompanied by shortness of breath that required an extensive cardiology work-up and was diagnosed with cardiac disease secondary to SLE. At study entry, the subject had an ANA >1:2560 and was negative for anticardiolipin antibodies. On Study Day 8, the subject experienced severe shortness of breath and collapsed on the way to the ER, where she was pronounced dead on arrival of acute cardiorespiratory arrest. Troponin levels measured in the ER were elevated, although it is not known if this was after attempted resuscitation. An autopsy was not performed.

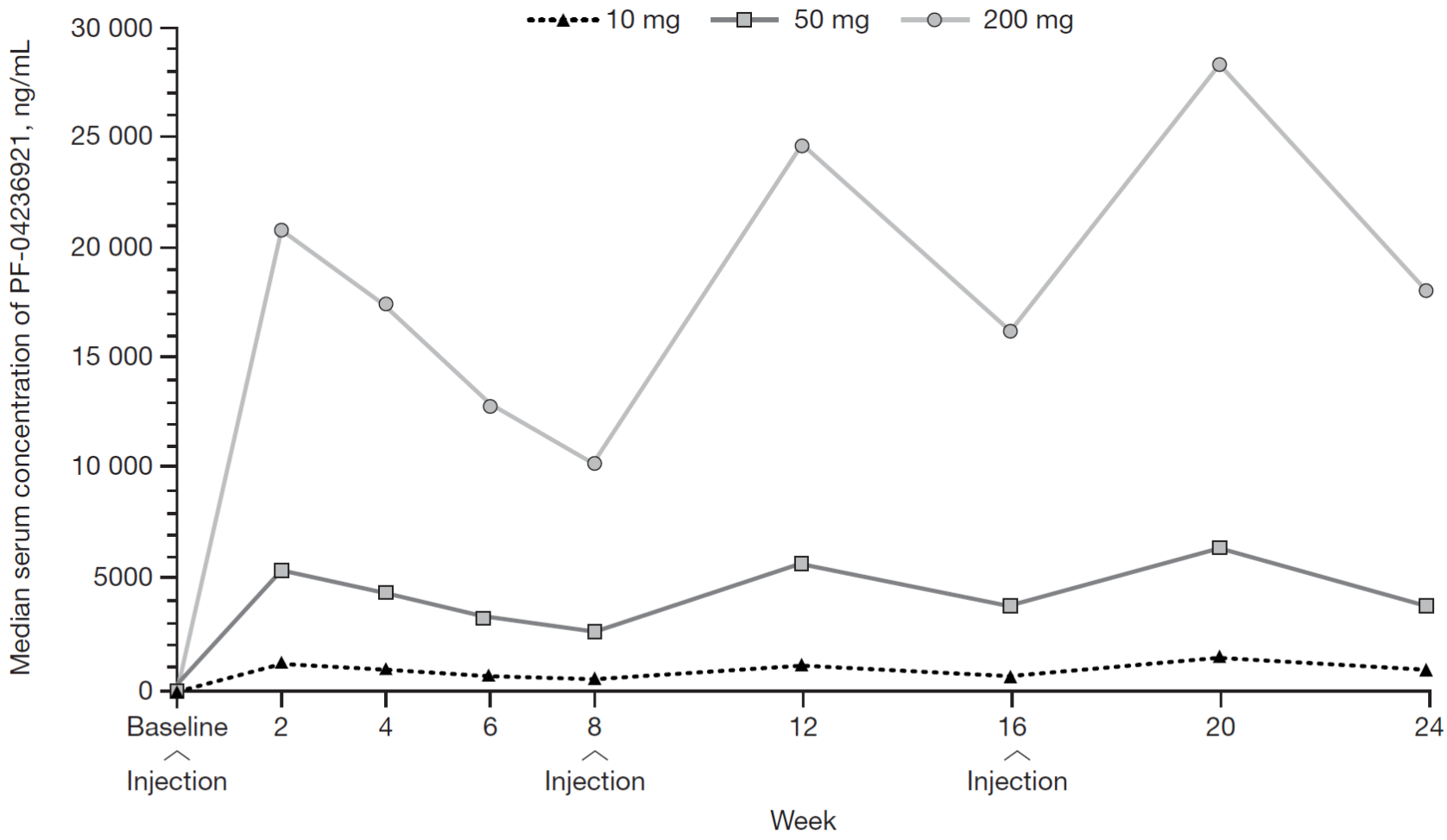
The second subject was a 24-year-old Caucasian female subject with SLE from Moldova who received two 200 mg doses of study medication. At the time of randomisation, she was receiving 20 mg of methylprednisone. She had a 3-year history of lupus characterised by recurrent episodes of refractory pleuropericarditis and had been treated with high-dose corticosteroids and cyclophosphamide. During this 3-year period (prior to enrolment in this study), the subject was evaluated for *Mycobacterium tuberculosis* (MTB) with tuberculin skin test (TST), radiographs and pericardial fluid cultures, all of which were reported as negative, although she was treated with isoniazid and rifampicin for 3 months. During screening for this study, the subject tested negative for MTB using TST and quantiferon tests, and the chest X-ray was reported as normal. She presented with progressive dyspnoea at her Week 12 visit, was hospitalised, and died, in spite of supportive therapy. The preliminary post-mortem report showed features of pulmonary emboli, non-caseating bilateral pulmonary granulomas that were considered to be consistent with disseminated pulmonary tuberculosis. The preliminary results of acid-fast bacillus staining were negative. Polymerase chain reaction (PCR) results confirmed the diagnosis of infection with MTB.

The third death was a 61-year-old Caucasian female subject with SLE from Hungary who received two 200 mg doses of study medication. The subject was treated with 8 mg methylprednisolone and 5 mg methotrexate four times per week. Her relevant medical history included Sjogren's syndrome, hypertension, hypothyroidism, obesity, type 2 diabetes mellitus and diabetes nephropathy. The subject had been hospitalised for pain of lumbar spine, compression fracture of vertebrae L2–5, and protrusion of intervertebral discs T12–L3, thought to be due to long-term corticosteroid use. The subject developed pain in the left leg on Study Day 89 and was hospitalised 3 days later with DVT of the left leg. Doppler imaging confirmed

the presence of a DVT in the left leg and the subject was treated with subcutaneous heparin (80 mg twice a day) and oral hesperidin (flavanone glycoside with possible cardioprotective and anti-inflammatory effects used for blood vessel conditions). Anticoagulation status of this subject following this therapy is not known. The subject then experienced sepsis, which required prolonged hospitalisation. Urine test and blood culture revealed *Escherichia coli (E. coli).* Treatment in the intensive care department included meropenem, ciprofloxacin, methylprednisolone and fluconazole. The *E. coli* was susceptible to meropenem. The sepsis worsened and the subject died on Study Day 106. An autopsy was performed and the pathologic diagnosis was consistent with SLE, Sjogren's syndrome, sepsis, diabetes mellitus and PE. The immediate cause of death was considered to be PE.

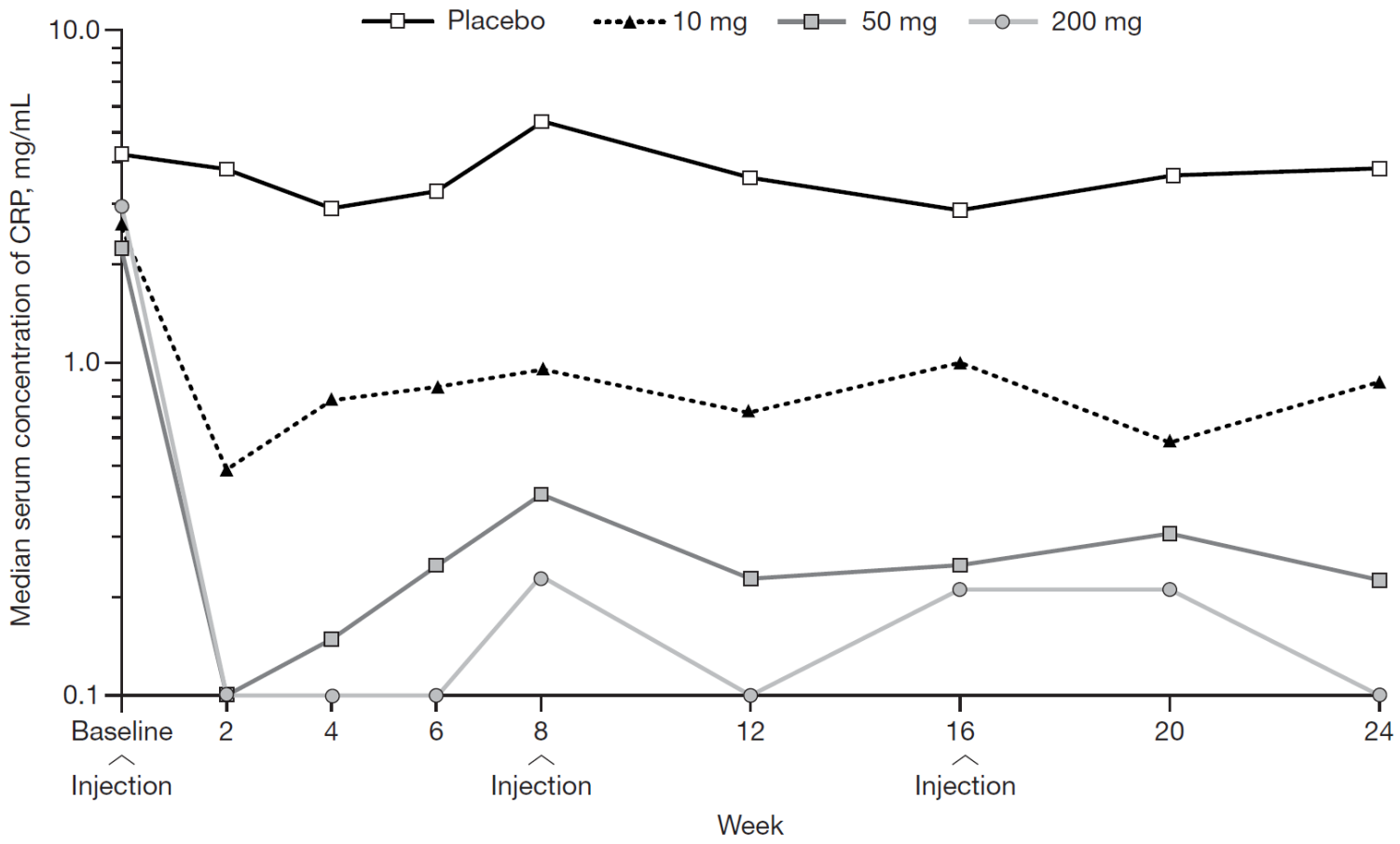
Positive test results for ADAbs were reported in two samples for one patient receiving 10 mg (Weeks 0 and 8) and another receiving 200 mg (Week 0). Neither had a positive test result for neutralising antibodies.

Supplementary figure S1 Median serum levels of PF-04236921 over time



10 mg, n=	42	37	39	39	39	32	31	30	31
50 mg, n=	45	41	42	40	38	33	36	34	34
200 mg, n=	43	40	38	37	30	28	23	17	18

Supplementary figure S2 Median serum concentrations of CRP over time



Placebo, n=	45	43	42	43	44	43	40	41	41
10 mg, n=	45	44	43	41	43	38	35	34	35
50 mg, n=	47	45	42	41	42	39	38	39	36
200 mg, n=	46	44	42	38	35	33	25	21	19

CRP, C-reactive protein.