1 **TITLE**

2 Safety, tolerability, efficacy, and pharmacokinetics of the anti-CD40

3 antibody iscalimab in patients with primary Sjögren's syndrome: a

4 multi-center, randomised, double-blind, placebo-controlled, parallel

5 group proof-of-concept study

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Summary (277)

Background

Primary Sjögren's syndrome (pSS) is an autoimmune disease that presents as dryness of the mouth and eyes due to impairment of the exocrine glands, where currently there are no systemic therapies that have demonstrated efficiacy. CD40-CD154 mediated T cell-B cell interactions in pSS contribute to aberrant lymphocyte activation in inflamed tissue leading to sialadenitis and other tissue injury. Therefore, we investigated the role of this costimulatory immune pathway in pSS.

Methods

A multi-center, randomised, double-blind, placebo-controlled, proof-of-concept study including two sequential cohorts was conducted to assess the safety, tolerability, efficacy, and pharmacokinetics of iscalimab (CFZ533), a blocking anti-CD40 antibody in pSS patients, by measuring the change after 12 weeks of treatment in the EULAR Sjögren's syndrome disease activity index (ESSDAI) score compared with placebo (Clinicaltrials.gov identifier: NCT02291029). Cohorts 1 (subcutaneous[SC]) and 2 (intravenous[IV]) underwent a double-blind, placebo-controlled period, where patients were randomised (2:1) to receive either iscalimab or placebo, followed by an open-label period where all patients received iscalimab for an additional 12 weeks.

Findings

AEs were similar between iscalimab treatment and placebo groups and two SAEs were reported to be unrelated to treatment with iscalimab. Furthermore, IV treatment in Cohort 2 resulted in a mean reduction of 5·21 points (95% CI:0·96-9·46; one-sided p=0·009) in ESSDAI score

83 versus placebo with some improvements in other pSS relevant outcomes and reductions in

84 levels of the germinal-center biomarker CXCL13 after 12 weeks of treatment.

85

Interpretation

87 This is the first randomised, placebo-controlled trial of a new investigational drug for pSS that

88 has met its primary endpoint. Our data suggest a pivotal role of CD40-CD154 interactions in

89 pSS pathology and indicate a therapeutic potential for CD40 blockade in pSS.

90

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INTRODUCTION

Primary Sjögren's syndrome (pSS) is a systemic autoimmune disease that is characterised by progressive multiorgan immune-mediated dysfunction that mainly affects exocrine glands; notably lacrimal and salivary glands. This dysfunction leads to oral and ocular dryness, excessive fatigue, extraglandular manifestations, and an increased risk of lymphoma.[1] Treatment is limited to symptomatic care for dryness and muscosal symptoms. Disease modifying therapies are used for some extraglandular manifestations, but can lead to adverse effects on the lungs, kidneys, and joints. To date there is no evidence-based, systemic disease-modifying therapy approved for pSS.

Hallmark clinical features include the presence of anti-Sjögren's-syndrome-related antigen-A (anti-Ro/SSA), with or without anti-Sjögren's-syndrome-related antigen-B (anti-La/SSB),[2] and focal lymphocytic sialadenitis on labial salivary glands or parotid biopsies. Ectopic lymphoid structures (ELS) resembling germinal-centers (GCs) have also been observed within salivary glands from pSS patients,[3] and have been reported to be colocalised with expression of the B cell-attracting chemokine CXCL13.[4] Recent data have indicated that the presence of salivary gland ELS[5] and increased systemic levels of CXCL13[6] correlates with disease severity, implicating ELS and accompanying B cell hyper-reactivity in pSS pathology.[7]

B cell activation, immunoglobulin class-switching, and GC formation are regulated by various immunological costimulatory pathways, notably CD40-CD154 interactions.[8,9] Reports on the expression of CD154 and CD40 on salivary gland-infiltrating T and B cells respectively,[10,11] suggest that T cell-B cell interactions via this pathway may contribute to sialadenitis and ELS formation and function. Furthermore, data suggest that activated CD154-expressing T cells could facilitate the destruction of salivary gland epithelial cells expressing CD40, potentially affecting salivary gland function.[12,13] Finally, recent data show that inhibition/blocking of the CD40 pathway in mouse models of Sjögren's syndrome suppresses sialadenitis.[14,15]

118   Collectively, these data suggest CD40-CD154 interactions may be involved in pSS pathology;

119   however, to date, there is no clinical evidence that supports a pivotal role for this pathway in

120   pSS. If this costimulatory pathway were to be involved, blockade of this pathway in clinically

121   active pSS patients might provide therapeutic benefit. The aim of this double-blind, placebo-

122   controlled trial was therefore, to evaluate the ability of iscalimab (CFZ533),[16] a novel

123   antagonistic and non-depleting, anti-CD40 monoclonal antibody, to provide therapeutic benefit

124   to pSS patients.

125

126   ## METHODS

127   ### Study design

128   An exploratory multi-center, randomised, double-blind, placebo-controlled, parallel group,

129   proof-of-concept study (PoC), composed of three sequential cohorts, was conducted to assess

130   the safety, tolerability, efficacy, and pharmacokinetics (PK) of iscalimab in pSS patients

131   (Clinicaltrials.gov identifier: NCT02291029). After completion of the initially planned Cohorts

132   1 and 2, the study was amended to include an open-label Cohort 3 with the primary objective

133   of assessing iscalimab dosing regimens on the PK properties. The results of the first two cohorts

134   are presented in this paper. The study was sponsored by Novartis Pharma, AG.

135   The study including the first two cohorts took place at ten investigational sites from Oct 2014

136   till February 2017. Sites were University Hospitals Birmingham NHS Foundation Trust, UK;

137   University of Debrecen, Hungary; Newcastle upon Tyne Hospitals NHS Foundation Trust, UK;

138   William Harvey Research Institute, Queen Mary University of London, Barts Health NHS

139   Trust, UK; Charité Research Organisation GmbH, Berlin, Germany; Tufts University, USA;

140   University Hospital Basel, Switzerland; Altoona Center for Clinical Research, USA;

141   Rheumatology, Allergy and Immunology NYU Winthrop Hospital, USA; and University

142   College Hospital, London, UK.

143   Study site health authorities and review boards approved the study protocol.

144

145

146   Participants

147   After a four-week screening period, a total of 44 pSS patients were recruited to the two cohorts.

148   Eligible patients were aged 18–75 years, weighed 50–150 kg and fulfilled the 2002 American

149   European consensus group (AECG) diagnostic classification criteria for pSS.[19] Patients had at

150   least moderate systemic disease activity with an ESSDAI$\geq$6,[17,20] and a stimulated whole

151   salivary flow rate >0 mL/min. Only autoantibody seropositive patients were enrolled,

152   dependent on the presence of either anti-SSA/SSB antibodies or both elevated serum titers of

153   antinuclear antibody (ANA) ($\geq$1:160) and positive rheumatoid factor (RF).

154   Eligible patients could remain on a stable dose standard-of-care treatment such as

155   hydroxychloroquine, or low dose immunosuppressives (including methotrexate, azathioprine,

156   or corticosteroids up to 10 mg per day [equivalent to prednisolone]). Women of child-bearing

157   potential were required to use highly-effective contraception and were assessed by a pregnancy

158   test throughout the study.

159   Exclusion criteria included treatment with biologic therapies or strong immunosuppressives

160   (such as cyclophosphamide, rituximab) within six-months prior to enrollment; infection;

161   malignancy; secondary Sjögren`s syndrome; or any significant and uncontrolled concurrent

162   medical condition, e.g. presence of lupus anticoagulant factor, hypertension, or psychiatric

163   conditions. Patients with significant laboratory abnormalities, e.g. total white blood cell count

164   outside the range of 2·0-15·0 x 10^9/L, platelets <100 x 10^9/L, hemoglobin <9·0 g/dL,

165   lymphocyte count <0·8 x 10^9/L, neutrophil count <1·5 x 10^9/L, or liver abnormalities at

166   screening, were not eligible. Written informed consent was obtained from patients before any

167   assessment.

168   Baseline characteristics of patients are shown in Table 1.

169

170   ## Randomisation and masking

171   From 82 patients assessed for eligibility, 44 were assigned computer-generated unique

172   randomisation numbers in ascending, sequential order within cohorts i.e. Cohort 1 (October

173   2014 to June 2016), then Cohort 2 (December 2015 to February 2017) to ensure treatment

174   assignment was unbiased and concealed from patients and investigator staff. A randomisation

175   list per cohort automated the assignment of treatment arms to randomisation numbers in the

176   specified ratio. An unblinded pharmacist's sole role ensured patients received the correct

177   medication.

178   Patients were randomised 2:1 to receive either active drug treatment or placebo to assess the

179   safety, tolerability, PK, and preliminary clinical efficacy of multiple subcutaneous (SC, Cohort

180   1) or intravenous (IV, Cohort 2) doses of iscalimab. Randomisation was stratified according to

181   baseline intake of oral corticosteroids. Both cohorts comprised a 12-week double-blind,

182   placebo-controlled period which was followed by a 12-week open-label period where all

183   patients received iscalimab, and then follow-up (figure 1). All treatment drugs were identical

184   to ensure treatment identities were concealed.

185

186   ## Procedures

187   Iscalimab (150 mg lyophilisate) and matching placebo were prepared by Novartis, Switzerland,

188   and supplied to the investigator site as open-labeled bulk medication.

189   In the double-blind period of Cohort 1, 12 patients were assigned to receive either 3 mg/kg

190   doses of iscalimab or placebo via SC injections at a 2:1 ratio at weeks 0, 2, 4, and 8.

191 In the double-blind period of Cohort 2, 32 patients were randomised to receive either IV 10

192 mg/kg doses of iscalimab or placebo at a 2:1 ratio. Cohort 2 study design was identical to Cohort

193 1.

194 After the double-blind phase, patients entered an open-label period, in which all patients

195 received their respective active treatment doses at weeks 12, 14, 16, and 20, i.e. 3 mg/kg SC for

196 Cohort 1 and 10 mg/kg IV for Cohort 2. All patients were assessed at the end of the open-label

197 period at week 24 and then entered an eight-week follow-up period assessed at weeks 28 and

198 32.

199 During treatment and follow-up periods, PK data were collected to characterise the disposition

200 of iscalimab under saturating or non-saturating conditions, and the contribution of CD40

201 receptors to the elimination of iscalimab (iscalimab is subject to target mediated disposition).

202

203 Outcomes

204 To evaluate the efficacy of iscalimab several disease-relevant outcome-measures recorded

205 changes in physician and patient clinical assessments, including EULAR Sjögren's syndrome

206 disease activity index (ESSDAI), a validated systemic disease activity index used to standarise

207 assessments of systemic complications in clinical trials,[17,18] and EULAR Sjögren's syndrome

208 patient reported index (ESSPRI). ESSDAI score is derived from the total weighted scores of 12

209 organ-specific domains that contribute to disease activity, with each being scored in four levels

210 according to severity, and ESSPRI score is derived from the mean scores of three symptoms;

211 dryness, limb-pain, and fatigue.

212 The two primary objectives of the study were to: i) assess safety and tolerability of multiple SC

213 injections and IV infusions of iscalimab in pSS patients as measured by adverse events (AEs),

214 and ii) compare the effect of multiple SC injections or IV infusions of iscalimab versus placebo,

on the clinical disease activity of pSS patients by the change of ESSDAI score after 12 weeks of treatment.

Secondary outcome measures included the PK assessment of multiple SC and IV doses of iscalimab. Self-reported outcomes in patients were evaluated after 12 weeks , as measured by ESSPRI, the short form health survey (SF-36); a 36-item patient-reported survey of QoL measures, the multidimensional fatigue inventory (MFI); a 20-item patient-reported questionnaire designed to measure forms of fatigue, and a visual analog scale (VAS) that recorded changes in the physician's and patient's global assessment of the patient's overall disease activity.

Assessment of safety and adverse events

Safety, PK, and pharmacodynamic (PD) assessments within 6 and 2 hours following each dose administration, respectively, were conducted and any AEs were documented throughout the study at each treatment visit. Safety assessments included physical examinations, electrocardiograms (ECGs), and standard clinical laboratory parameters such as hematology, blood chemistry (blood alkaline phosphate level, liver function tests, amylase, and lipase levels), complement and B cell hyperactivity markers (levels of C3, C4, cryoglobulin, free lamba and lambda immunoglobulin light chains, and beta2 microglobulin), urinalysis, and coagulation tests. Immunogenicity testing (anti-drug antibodies [ADA]) was performed at pre-dose, during treatment, and follow-up periods.

Pharmacokinetic analysis in clinical study

Free-iscalimab plasma concentrations were determined by a standard validated enzyme-linked immunosorbent assay using biotinylated recombinant CD40 bound to streptavidin-coated microtiter plates and detected by a peroxidase-conjugated goat anti-human IgG antibody.

Immunogenicity analysis in clinical study

Presence of anti-iscalimab antibodies in plasma was determined using a validated bridging ELISA-based assay. In a pre-incubation step, biotinylated iscalimab was used for capture, and digoxigenin labelled iscalimab and Fab fragments of a polyclonal anti-digoxigenin antibody conjugated to horseradish peroxidase for detection. Drug interference testing: (i) 250 ng/mL of rabbit polyclonal affinity purified anti-CFZ533 IgG (positive control) could be detected in the presence of up to 20·2 µg/mL of iscalimab, and 500 ng/mL of the positive control could be detected in the presence of up to 45·0 µg/mL of iscalimab. The immunogenicity testing adopted a three-tiered strategy with; a screening assay, a confirmatory assay, and titer assay, as tier 1, 2 and 3, respectively.

Biomarker assessments in clinical study

The biomarker CXCL13 was measured in Cohort 2 using a validated bead-based sandwich immunoassay (Cat# LXSAHM, R&D Systems, Inc., Minneapolis, MN, USA). The dynamic range, defined by the LLOQ and ULOQ, was 32·6 – 2760 pg/mL in human serum.

Serum anti-SSB, anti-SSA 52, and anti-SSA 60 IgG were measured using validated individual commercial in vitro diagnostic medical device (IVD) kits from Orgentec (Cat# ORG509, ORG652, ORG660, ORGENTEC Diagnostika GmbH, Mainz, Germany). The dynamic ranges defined by LLOQ-ULOQ were 1250 – 100000 U/mL in human serum, taking into account the minimal dilution requirement (MRD) of 1:500 for anti-SSB and anti-SSA 52 IgG; and 2500 – 200000 U/mL in human serum, taking in account the MRD of 1:1000 for anti-SSA 60 IgG.

Statistical analysis

For clinical outcomes, mixed models for repeated measures were fitted with factors for treatment group, visit, baseline, and interaction between visit and treatment group. Where possible, an unstructured correlation matrix was assumed to account for correlation within patients. Separate models were fitted for each of the cohorts. At each visit, the mean difference in outcome measure between iscalimab and placebo was calculated together with its 95% confidence interval. The same model was fitted to the log-transformed CXCL13 data. Analysis of covariance adjusting for baseline levels was performed on week 12 salivary flow rates, Schirmer's test, and auto-antibody measurements. The auto-antibody levels were log-transformed prior to analysis.

The planned sample size for Cohort 1 was 12 patients based on safety considerations. The planned sample size for Cohort 2 was 30 patients based on the primary analysis of ESSDAI. It was pre-specified in the protocol that the primary endpoint would be considered to be achieved only if both of the following two conditions held for the reduction in ESSDAI after 12 weeks treatment in the iscalimab 10 mg/kg IV group (compared to placebo):

1.   The reduction vs. placebo is statistically significant at the one-sided 10% significance level and,

2.   The reduction vs. placebo is estimated to be at least 5-points in magnitude.

The one-sided 10% alpha level in the first condition was considered an appropriate upper-bound for the false-positive risk for an exploratory study. The second condition was included to ensure that the primary endpoint would only be considered to be achieved if the reduction was highly clinically relevant. The sample size of 30 patients in Cohort 2 was chosen such that there was an 83% chance of achieving the primary endpoint (i.e. meeting both these conditions), assuming a true reduction of seven ESSDAI points compared with placebo, a standard deviation (SD) of five and a drop-out rate of 20%. Eventually, 32 patients in total were recruited in Cohort 2.

Clinicaltrials.gov identifier: NCT02291029.

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295

RESULTS

Between October, 2014 - February, 2017, a randomised, placebo-controlled PoC study of the blocking and non-depleting anti-human CD40 monoclonal antibody iscalimab was conducted, in pSS patients (figure 1). A total of 82 patients were assessed for eligibility, of which 25 were assessed for Cohort 1 and 57 for Cohort 2. Two patients left the study due to an allergic reaction and skin rash (after completing treatment in the double-blind period of Cohort 1) and one other withdrew consent (after two doses of study treatment in Cohort 2) (figure 2).

In Cohort 1, all patients experienced at least one mild or moderate AE in both the iscalimab treatment group and placebo group. In Cohort 2, however, AE incidence was lower in iscalimab treated patients than placebo with 53% compared to 64%, respectively. These results suggest that iscalimab was well-tolerated (Table 2). Furthermore, no major safety signals were observed during the iscalimab treatment open-label periods of both cohorts.

Although, two serious adverse events (SAEs) were observed during the safety follow-up period (approximately 12 weeks after the last dose of iscalimab) these were considered to be unrelated to iscalimab by the investigator, namely, one case of bacterial conjunctivitis in Cohort 1 and one case of atrial fibrillation in Cohort 2. There were no clinically significant abnormalities in

312   the laboratory assessments or ECG evaluations, and no B cell or other cytopenia was noted

313   which was consistent with the non-depleting nature of iscalimab.[16,21]

314   In Cohort 1, 3 mg/kg SC resulted in mean trough plasma concentrations less than 10 µg/mL

315   (supplementary figure 1, upper panel), significantly lower than expected based on PK data of

316   healthy volunteers in the first-in-human study.[22]

317   In Cohort 2 (10 mg/kg IV), mean trough plasma concentrations were above levels previously

318   reported to be sufficient for the suppression of GC development and inhibition of T cell-

319   dependent antigen responses in non-human primates[16] at approximately 100-200 µg/mL

320   (supplementary figure 1, lower panel).

321   Consequently, no improvement in ESSDAI was seen in Cohort 1, where the baseline-adjusted

322   reduction from placebo at week 12 was only 0·41 points (95% CI -2·89 to 3·70) (figure 3a,

323   upper panel). However, a reduction in disease activity relative to placebo did meet the pre-

324   specified criterion after four infusions of 10 mg/kg IV iscalimab in Cohort 2 with a baseline-

325   adjusted mean reduction in ESSDAI at week 12 of 5·21 points (95% CI 0·96 to 9·46; one-sided

326   p=0·009) (figure 3a, lower panel) (supplementary table 1). Improvements were also seen in

327   clinESSDAI at week 12 for Cohort 2 compared to placebo, where a reduction of 6·10 points

328   (95% CI 1.08 to 11.11) was recorded.

329   ESSDAI domain results showed the most severely affected domain at baseline - the articular

330   domain, achieved the greatest reduction in ESSDAI score at week 12 (supplementary figure 2)

331   suggesting the articular domain may be associated with disease activity moreso than any other

332   domain.

333   ESSDAI improvements in the double-blind period of Cohort 2 were sustained in the open-label

334   period in patients who started on iscalimab (figure 3a, lower panel). For example, the reduction

335   in ESSDAI at week 14 was 3·85 points (95% CI -0·43 to 8.12; one-sided p=0·038); at week 16

336   it was 2·57 points (95% CI -0·51 to 5.66; one-sided p=0·049); and at week 20 it was 3·77 points

(95% CI 0·38 to 7·16; one-sided p=0·015) compared to the placebo group that switched to 10 mg/kg IV after week 12. Improvements were also observed in the placebo group after switching to 10 mg/kg IV iscalimab at week 12 (figure 3a, lower panel).

In Cohort 2 the percentages of ESSDAI and ESSPRI responders at week 12 were higher in the iscalimab group than in the placebo group. For ESSDAI, 76% of iscalimab patients achieved ≥3 point reduction compared with 55% of placebo patients. Thirteen (62%) iscalimab patients reduced their ESSDAI to below 5 points compared with four (36%) in the placebo group. For ESSPRI, 62% of iscalimab patients achieved ≥1 point reduction compared with 45% for placebo.

In the placebo-controlled period of Cohort 2, some improvements were observed in secondary outcomes at week 12: mean decrease vs. placebo in ESSPRI was 0·95 (95% CI -0·50 to 2·41) (figure 3b, lower panel), SF-36; mean increase vs placebo in physical and mental component score of 3·83 (95% CI -1·81 to 9·48) and 2·52 (95% CI -4·50 to 9·53), respectively, total MFI; mean reduction vs placebo of 9·83 (95% CI -1·01 to 20·66), and patient- and investigator-assesments using VAS; a mean decrease of 8·14 (95% CI -10·39 to 26·67) and 12·16 (95% CI 2·38 to 21·94), respectively (supplementary figure 3).

Improvements were observed in the 10 mg/kg IV iscalimab group compared with placebo in unstimulated and stimulated salivary flow and in Schirmer's test. A mean increase vs. placebo in unstimulated salivary flow at week 12 of 0·04 mL/min (95% CI -0·03 to 0·10), in stimulated flow of 0·16 mL/min (95% CI -0·15 to 0·46), and for Schirmer's test of 8·06 mm (95% CI -1·37 to 17·50) and 9·07mm (95% CI -4·61 to 22·75) for the left and right eye, respectively (supplementary figure 4).

Decreases in CXCL13 serum concentrations after 10 mg/kg IV iscalimab compared to placebo in Cohort 2 (56% reduction in geometric mean vs placebo at week 12; 95% CI 27% to 70% [figure 3c]), followed a similar pattern as the changes in ESSDAI (figure 3a, lower panel).

362   Maximal decreases in CXCL13 were reached at week 8 (61% reduction vs. placebo) and were

363   sustained to week 24 (31% reduction vs. placebo). In patients who were switched to iscalimab

364   from placebo at week 12, a significant decrease in CXCL13 serum concentrations was seen at

365   week 16 with a slight increase towards the end of the study up to week 24 (figure 3c).

366   Levels of anti-SSA 52, anti-SSA 60, and anti-SSB IgG levels were increased at baseline in pSS

367   patients compared to age- and gender-matched healthy donors (Table 1). A modest decrease

368   from baseline in anti-SSA 52 IgG levels was observed after four doses of 10 mg/kg IV iscalimab

369   at week 12, but this was similar to placebo. Although decreases from baseline in serum levels

370   of anti-SSA 60 and anti-SSB IgG after 12 weeks of treatment with iscalimab (10 mg/kg IV)

371   were more pronounced compared with placebo, they were not statistically significant (anti-

372   SSA: 20% reduction vs placebo, 95% CI 17% increase to 45% reduction; anti-SSB: 38%

373   reduction vs placebo, 95% CI 19% increase to 68% reduction) (supplementary figure 4).

374   In Cohort 1, there was no consistent change from screening in the blood levels of RF in

375   iscalimab and placebo arms. In iscalimab treated patients in Cohort 2, RF decreased from a

376   median of 22.7 U/mL at screening to 15.3 U/mL at week 12. In the placebo group, the median

377   decreased from 27.1 to 20.6 U/mL. For ANA, the titers decreased from a median of 640 at

378   screening to 320 at week 12 in the iscalimab group. Median titer levels in the placebo group

379   also decreased from 640 at screening to 240 at week 12.

380   In the standard assays reflecting complement and B cell hyperactivity including levels of C3,

381   C4, cryoglobulin, free lamba and lambda immunoglobulin light chains, and beta2

382   microglobulin, no clinically meaningful changes were observed after 12 weeks treatment

383   compared to placebo (data not shown).

384   During immunogenicity testing, one sample (at Day 225; patient in Cohort 1 treated with

385   iscalimab in the open-label period) was confirmed positive. The presence of ADAs was not

386   associated with any immune-related safety signals.

387

Research in context

Evidence before this study

We searched Pubmed with no language restrictions, first from Oct 15-21, 2018 and again from May 15-30, 2019 using key words Sjögren`s syndrome, clinical trial, CD40, pathology, germinal center, and CXCL13, to find studies on the pathophysiology and treatment of pSS. To date, a small number of randomised, controlled trials have tested various treatment modalities in pSS, with none of these demonstrating convincing efficacy. A published meta-analysis of 32 trials that evaluated 19 different medications of pSS revealed that none of these demonstrated consistent benefit in xerostomia and keratoconjunctivitis sicca. Previously published results have suggested an association between pSS and CD40-CD154 interactions; for example, ELS formation alongside CD40 and CD154 expression in salivary gland biopsies from pSS patients has been demonstrated. Furthermore, amelioration of sialadenitis and reductions in anti-SSA and SSB autoantibodies in the NOD model of Sjögren`s syndrome following CD40-CD154 blockade have been observed. While an association between the CD40-CD154 pathway and autoimmune diseases such as pSS has been suggested, no clinical trial has assessed the efficacy and safety of any modality that blocks this pathway in pSS patients.

Added value of this study

We opted to run a randomised, placebo-controlled study with iscalimab in patients with clinically active pSS, using ESSDAI to define disease activity (ESSDAI ≥6, reflecting the systemic manifestations of the disease) for both inclusion of patients and for the primary endpoint. This established and validated composite outcome measure is regarded as an acceptable regulatory endpoint for pivotal clinical trials, and has been previously used in other pSS clinical trials. Iscalimab is a fully-human, pathway-blocking, non-depleting, anti-CD40 antibody shown to prolong allograft survival when dosed as a monotherapy in a non-human

412 primate model of kidney transplantation, and is currently under clinical evaluation in several

413 indications.

414 **Implications of all the available evidence**

415 To date, no drug treatment has resulted in a clinically-meaningful change in systemic

416 complications as measured by ESSDAI. The data here suggest that the novel anti-CD40

417 antibody iscalimab shows clinically meaningful benefit in patients with pSS.

418

DISCUSSION

419

420 To date, a small number of randomised controlled trials have examined the effects of different

421 treatments in pSS, with none of these demonstrating convincing clinical efficacy.[23,24]

422 In the double-blind period of Cohort 2 (10 mg/kg IV), a clinically meaningful reduction in

423 disease activity (measured by ESSDAI) after 12 weeks in patients compared with placebo was

424 observed. The continued reductions observed in ESSDAI after week 12 through to week 32 and

425 the reduction in ESSDAI observed in the placebo group once switched to active treatement at

426 week 12 as shown in figure 3a, suggest iscalimab efficacy in the treatment of patients with pSS.

427 The articular domain of ESSDAI showed the greatest improvement whilst also being the most

428 severely affected at baseline (supplementary figure 2). This finding warrants further studies on

429 tender and swollen joint counts and DAS-28, to understand the importance of this domain in

430 driving ESSDAI improvements.

431 Iscalimab plasma concentrations in Cohort 1 are likely the consequence of an efficient target-

432 mediated drug disposition (TMDD) of iscalimab and efficient first-past effect. TMDD is a

433 process in which a substantial proportion of the drug (relative to dose) is bound to its target,

434 affecting the disposition of the drug. TMDD involves the binding of iscalimab to CD40

435 receptors (CD40R) on the cell surface, which triggers internalization[16] and subsequent

436 lysosomal degradation of the complex.

437 The disposition of iscalimab depends on its plasma concentration, interstitial fluid, and lymph

438 circulation, along with CD40R expression, internalization, and turnover rates. When CD40R's

439 are fully saturated TMDD becomes saturated and the contribution of CD40R's to the overall

440 clearance of iscalimab is minimal. In Cohort 1, the dosing regimen generated non-saturating

441 conditions, and CD40-mediated clearance was efficient and associated with rapid elimination

442 of iscalimab.

443 TMDD of iscalimab is illustrated in the PK profiles from Cohort 2 (supplementary figure 1,

444 lower panel). During the follow-up period, the inflection point in the PK profiles (at about 10-

445 20 µg/mL) is a marker of target engagement, and is associated with an increased contribution

446 of CD40R's to the overall clearance of iscalimab and a faster elimination. This inflection point

447 was associated with a loss of target engagement (total soluble CD40 profiles in plasma – not

448 shown). In Cohort 1 also, total soluble CD40 profiles in plasma (not shown) demonstrated lack

449 of sustained target engagement by iscalimab.

450 As CD40R's have been reported to be upregulated on parenchyma in inflamed tissues, the

451 elevated expression of CD40R's is likely responsible of the rapid elimination of iscalimab and

452 efficient first-pass effect after 3 mg/kg SC.

453 This is further suggested by improvements in ESSDAI in Cohort 2 where trough plasma

454 concentrations were above 100 µg/ml, and suggests a dose-response relationship for iscalimab

455 which is in agreement with the dose-dependent pharmacology seen in non-human primates.[16,25]

456 Importantly, iscalimab was well-tolerated and the number of AEs for both cohorts was similar

457 to placebo. Furthermore, there was no evidence of cytopenias, injection site reactions,

458 thromboembolic events, or increased risk for infections, which have previously been observed

459 with biologics targeting CD154.[26]

460   The clinical efficacy observed with iscalimab suggests that CD40-CD154 signaling is a key

461   pathway associated with underlying pSS pathology. Our recent preclinical data,[15] assumes that

462   the antibody acts by several different mechanisms, including the suppression of ELS formation

463   and function by inhibition of CD40-CD154 interactions in salivary glands; the inhibition of

464   sialadenitis; and reduction of B cell hyper-reactivity. The GC-associated chemokine CXCL13

465   has previously been shown to be elevated in the sera of some pSS patients,[6,27] and is expressed

466   by infiltrating cells in salivary gland tissue.[28] When patients in this study were compared with

467   CXCL13 levels obtained from healthy volunteers, CXCL13 levels in some patients were

468   elevated at baseline, but returned to a range similar to untreated healthy volunteers after

469   iscalimab treatment with 10 mg/kg IV.[29]

470   Published data on CXCL13 suggest that it may be a biomarker that reflects GC activity and

471   correlates with the extent of salivary gland pathology.[7] Therefore, the iscalimab-dependent

472   reductions in CXCL13 that preceded clinical improvement observed here, might reflect

473   suppression of the structure and function of GCs, and possibly salivary gland ELS. Given that

474   the individuals in this study also had extraglandular involvement based on their ESSDAI≥6

475   scores, the clinical benefit observed with iscalimab may also extend to extraglandular tissues.

476   In contrast to the significant reductions in CXCL13 levels, we observed no or modest reductions

477   in pSS-associated IgG autoantibodies in iscalimab treated patients. This suggests that CD40-

478   CD154 blockade may be less effective in reducing antibody productions from long-lived plasma

479   cells, a notion consistent with the observation of unaffected serum immunoglobulin levels in

480   preclinical and clinical studies with iscalimab.[16,19] However, SSA and SSB antibodies are rather

481   diagnostic markers of the disease than pathogeneic factors.

482   While the B cell depleting agent rituximab has been used in selected cases of pSS, previous

483   clinical studies have reported variable therapeutic benefit.[30] It is therefore possible that CD40

484   blockade with iscalimab has additional effects beyond targeting B cell hyper-reactivity in pSS.

485   This is likely due to involvement of CD40 expressing non-B cell types in pSS pathology,

486   including macrophages and other antigen-presenting cells like dendritic cells. This notion was

487   also supported by recent data showing a reduction in macrophages following therapeutic

488   blockade of CD40-CD154 in the non-obese diabetic mouse-model of Sjögren's syndrome.[15]

489   Additionally, iscalimab may be more effective at suppressing the effector functions of

490   pathogenic B cells in tissue than B cell depleting strategies.

491   Our study was designed as an exploratory Phase 2a trial to provide initial safety and Proof-of-

492   Concept for iscalimab in pSS. However, the study has limitations including the small sample

493   size and many of the improvements seen in the clinical outcomes did not reach statistically

494   significance. Furthermore, our results on the ESSDAI domain level do not allow firm

495   conclusions due to the lack of baseline activity for many domains.

496   In conclusion, the results of this exploratory clinical trial suggest a pivotal role of CD40-CD154

497   interactions in pSS pathology. Further studies are warranted that are larger in sample size and

498   longer in duration to confirm that CD40-blockade with iscalimab might show clinically

499   meaningful benefit in patients suffering from pSS.

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Contributors

BAF, PE, AMW, XR, PMF, JSR and PG conceived and designed the CFZ533 clinical study.

BAF, AS, W-FN, MB, MGP, ASP, AMF, DK,TD, BB, AJK, SEC, DAI, FB and SJB recruited cohort individuals and performed the CFZ533 clinical study. AMW and H-UH conducted the statistical analysis for the CFZ533 clinical study. AA, MR, JM and M-AV performed the biomarker assessments and analyses for the CFZ533 clinical study. PE, DF, and CD performed the PK and ADA analysis for the CFZ533 clinical study. BAF, JSR and PG wrote the manuscript with input and comments from all co-authors.

Declaration of interests

Disclosures for consultancy fees (none of which exceeded $10,000) are made for the following authors: BAF (Novartis, Roche, MedImmune, BMS and Virtualscopics); W-FN (Novartis, GlaxoSmithKline, Abbvie, MedImmune, BMS, Atheneum Partners); MB (GSK, Amgen, MedImmune and UCB); AJK (Sanofi, Pfizer, Roche, and UCB); DAI (EMD and Serono); SJB (AstraZeneca/MedImmune, BMS, Celgene, Eli Lilly, Glenmark, GSK, MTPharma, Novartis, Ono, Takeda, UCB, and XLT Bio); DK (Novartis, Roche, Abbvie, Pfizer, BMS, UCB); PE, DF, CD, XR, PMF, AMW, H-UH, MR, M-AV, JSR, JM, AA, and PG were employees of Novartis at the time of conducting and analysing the studies. All other authors declare no competing financial interests.

Data sharing

Novartis Pharma, AG is committed to sharing with qualified external researchers, access to patient-level data and supporting clinical documents from eligible studies. These requests are reviewed and approved by an independent review panel on the basis of scientific merit. All data

532 provided is anonymised to respect the privacy of patients who have participated in the trial in

533 line with applicable laws and regulations. This trial data availability is according to the criteria

534 and process described on www.clinicalstudydatarequest.com.

535

Acknowledgements

Figure headings and legends:

Figure 1. Schematic clinical study design

Treatment regimens:

Cohort 1: 3 mg/kg SC iscalimab (or placebo) at Weeks 0, 2, 4 and 8 followed by 3 mg/kg SC iscalimab at Weeks 12, 14, 16 and 20.

Cohort 2:10 mg/kg IV iscalimab (or placebo) at Weeks 0, 2, 4 and 8 followed by 10 mg/kg IV iscalimab at Weeks 12, 14, 16 and 20.

Figure 2. Trial profile

* The patient who withdrew consent was included in the primary analyses model but, due to discontinuation, only contributed data at the week 2 visit.

Figure 3. Baseline adjusted mean a. ESSDAI, b. ESSPRI and c. CXCL13 levels after administration of different doses of iscalimab or placebo

Mean estimates from the mixed models for repeated measures adjusting for baseline levels (i.e. 'baseline adjusted means') are presented along with their associated standard errors. The dotted line indicates the mean estimate for patients who were randomised to the placebo group but, in the open label part of the study, received iscalimab 3 mg/kg SC in Cohort 1 (upper panel) or, 10 mg/kg IV in Cohort 2 (lower panel).

Supplementary Figure 1. Plasma concentrations after administration of different doses of iscalimab

In each cohort, the dark thick lines indicate arithmetic mean profiles in the given treatment group and the light lines indicate the individual profiles. For Cohort 1 and 2, the solid line indicates the mean profile of the patients randomised to iscalimab. The dotted line indicates the mean profile for patients who received iscalimab in the open label part of the trial after being first randomised to the placebo group. Concentrations below the limit of quantification were set to zero and included in the calculation of the arithmetic mean. However, since a logarithmic scale is used, these values are excluded from the plot. Mean values of zero are also omitted from the plot for a similar reason.

Supplementary Figure 2. Involvement of various ESSDAI domains after administration of multiple doses of 10 mg/kg of iscalimab or placebo for 12 weeks

The percentage of patients in each severity category (None, Low, Moderate or High) in each ESSDAI domain is presented by treatment group at baseline ('B') and week 12 ('12'). Note that the patient in the iscalimab 10mg/kg IV group who discontinued early from the study is not included in the calculation of the week 12 percentages. The abbreviations for the ESSDAI domains are: Con = Constitutional; Lym = Lymphadenopathy; Gla = Glandular; Art = Articular; Cut = Cutaneous; Pul = Pulmonary; Ren = Renal; Mus = Muscular; PNS = Peripheral nervous system; CNS = Central nervous system; Hae = Haematological; Bio = Biological.

Supplementary figure 3. Forest plot of baseline adjusted treatment effects of 10 mg/kg iscalimab at Week 12 on clinical endpoints

Treatment differences (iscalimab vs placebo) from the MMRM adjusting for baseline levels (i.e. 'baseline adjusted means') are presented along with their associated 95% confidence intervals. Each treatment difference is scaled such that an improvement is plotted towards the positive direction (right hand side). For each endpoint, the vertical dotted line indicates a clinically relevant treatment difference

Supplementary figure 4. Changes in salivary flow, Schirmer's test, and auto-antibody levels after 12 weeks of treatment with iscalimab

For salivary flow (unstim = unstimulated; stim = stimulated) and Schirmer's test, the mean differences between iscalimab and placebo is presented along with the 95% confidence intervals. For the auto antibodies, the ratio between iscalimab and placebo is presented along with the 95% confidence intervals. Values below the lower limit of quantification (LLOQ) are imputed in the analyses as LLOQ/2.

Table headings

Table 1. Baseline characteristics of patients with pSS in the clinical study

Table 2. Summary of overall incidence and severity in the placebo-controlled first treatment period, and the most frequent adverse events during the clinical trial, for Cohorts 1 and 2

Supplementary Table 1. Results from statistical analysis of ESSDAI

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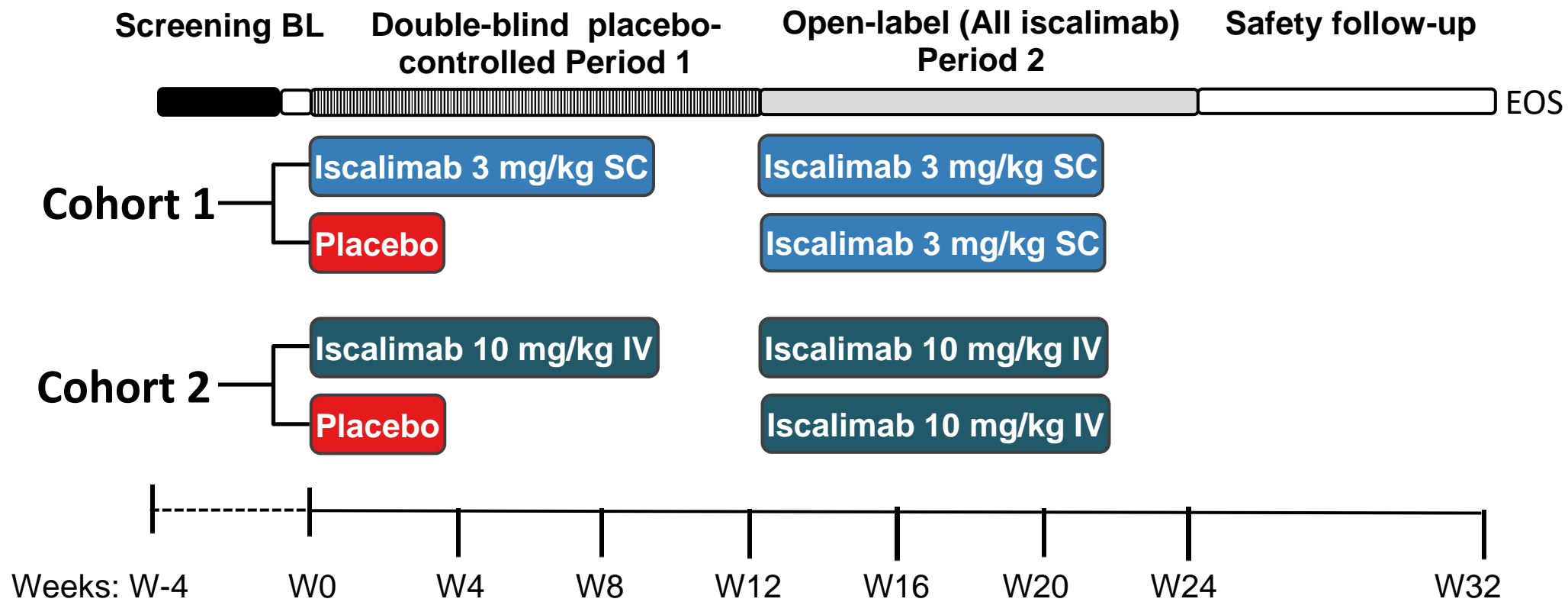
BL=Baseline. EOS=End of Study. SC=Subcutanous. IV=intravenous. *Each cohort was carried out sequentially

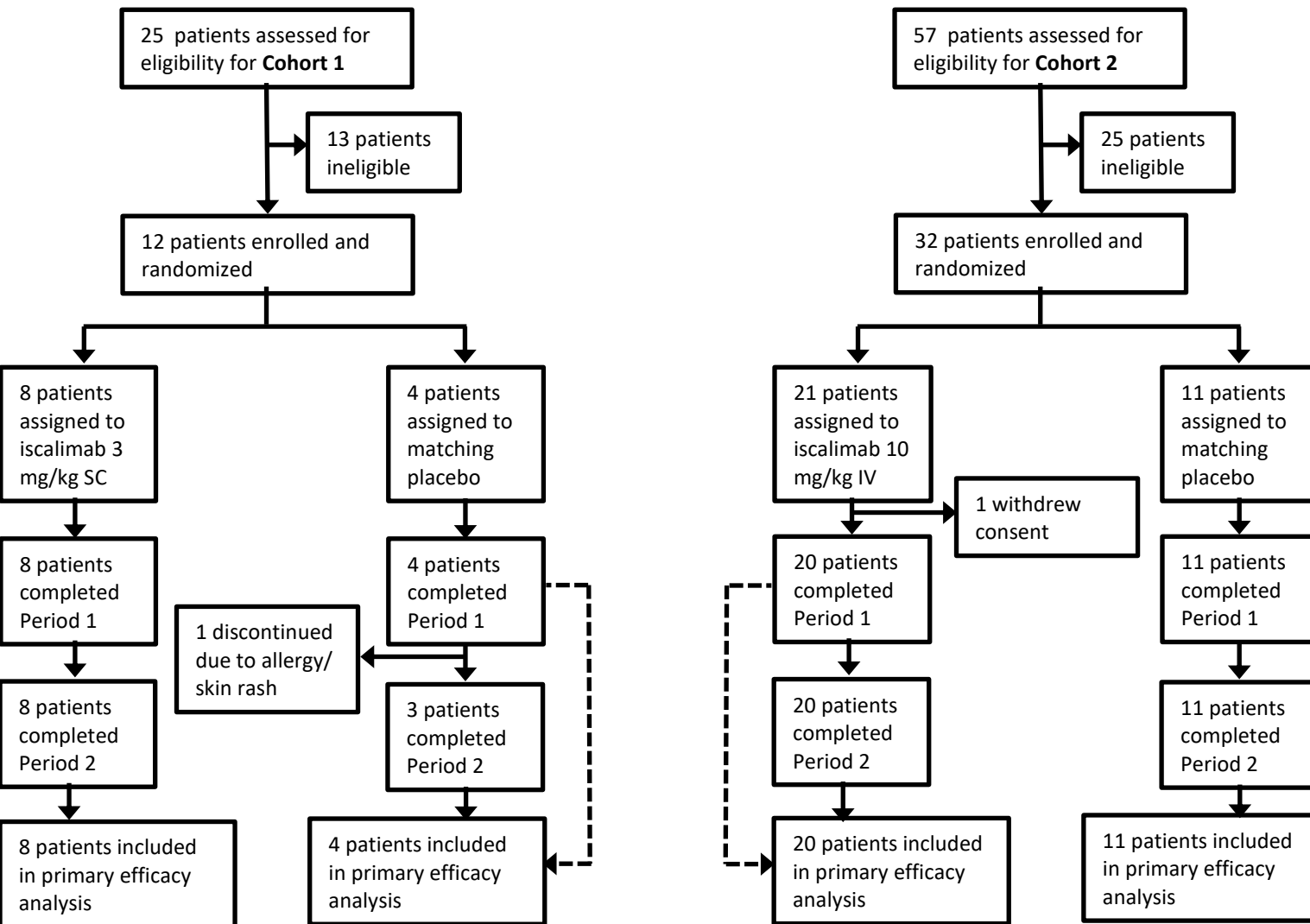**Figure 1: Schematic clinical study design***
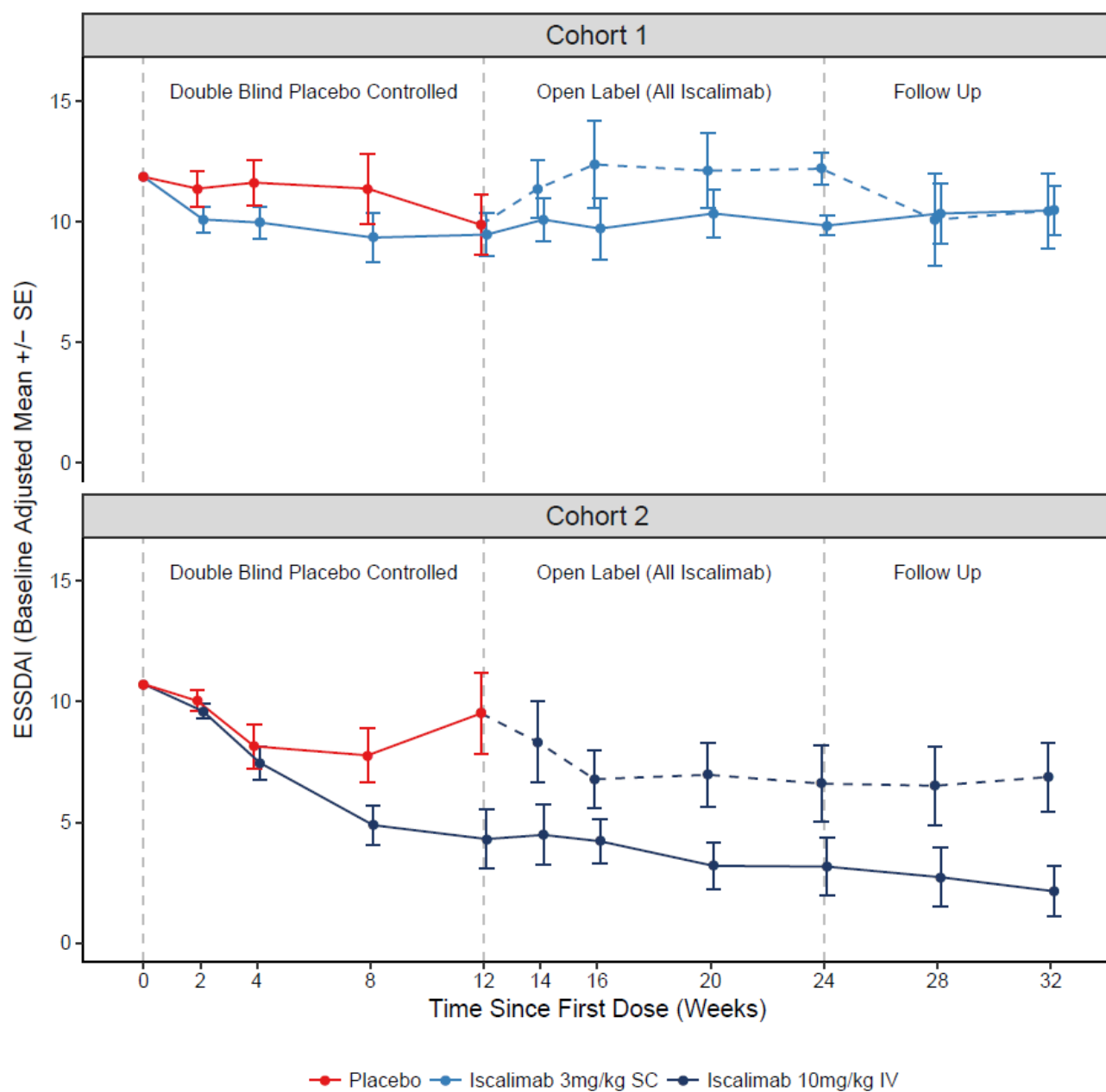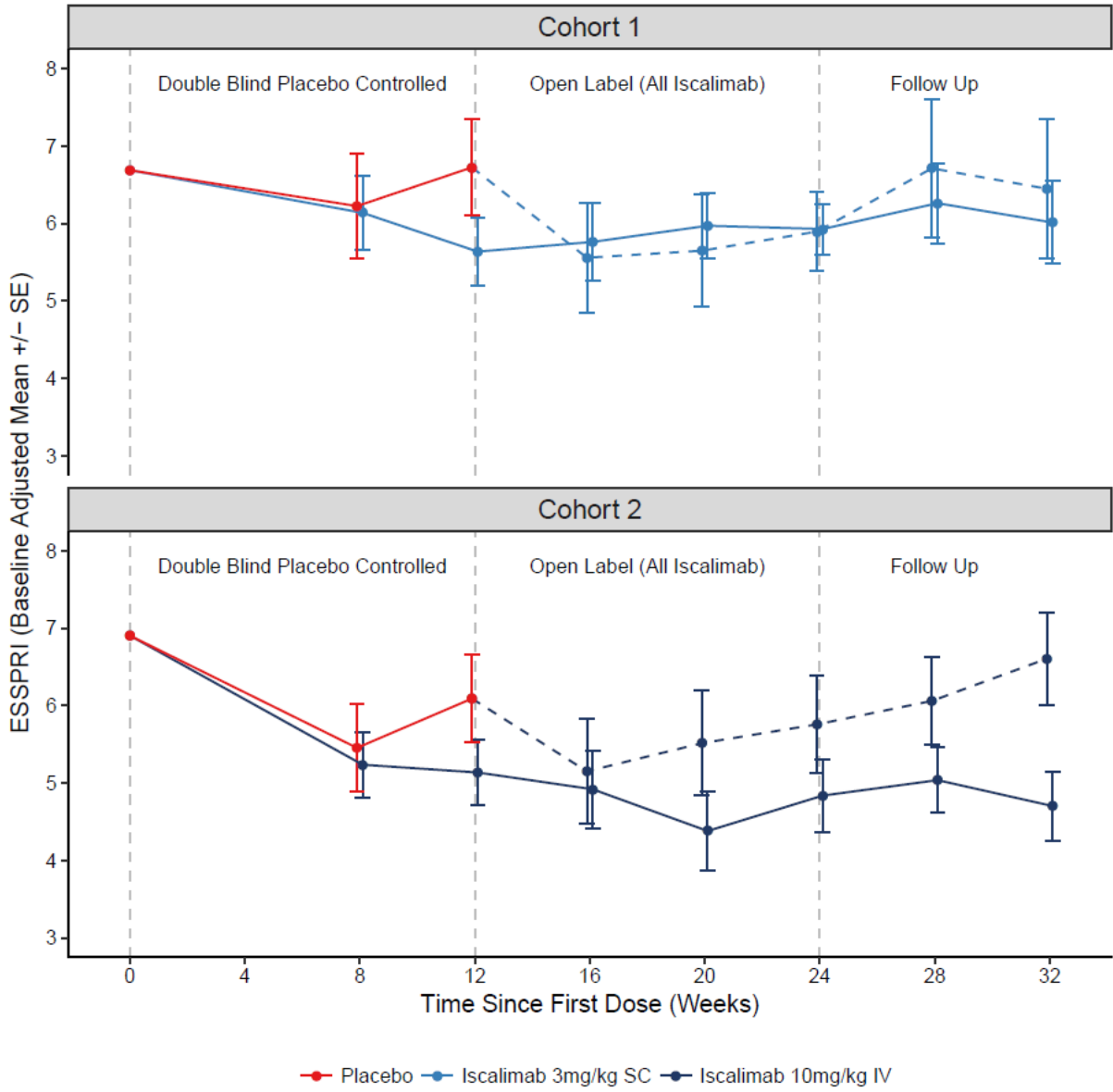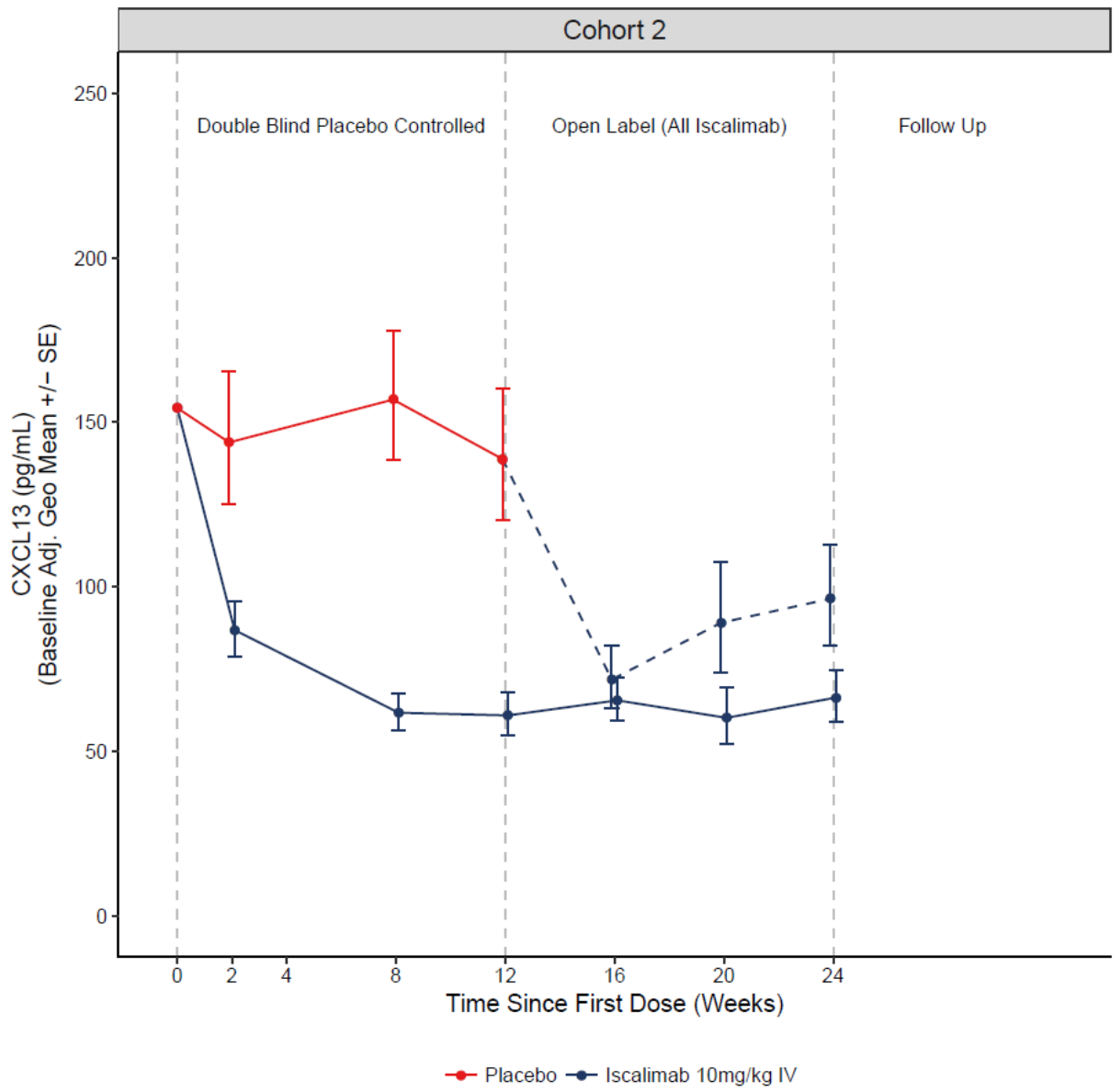
Figure 2. Trial profile

Figure 3a

Figure 3b

Figure 3c

		Cohort 1			Cohort 2		
		Iscalimab 3 mg/kg SC	Placebo→ Iscalimab 3 mg/kg SC	Total	Iscalimab 10 mg/kg IV	Placebo→ Iscalimab 10 mg/kg IV	Total
		n=8	n=4	n=12	n=21	n=11	n=32
Age (years)	Mean (SD)	56.4 (12.2)	48.8 (3.3)	53.8 (10.6)	51.7 (14.3)	50.6 (12.4)	51.3 (13.5)
	Range	34, 72	45, 52	34, 72	24, 72	25, 69	24, 72
Sex – n (%)	Male	0 (0%)	0 (0%)	0 (0%)	2 (10%)	0 (0%)	2 (6%)
	Female	8 (100%)	4 (100%)	12 (100%)	19 (90%)	11 (100%)	30 (94%)
Weight (kg)	Mean (SD)	66.7 (16.6)	78.7 (11.0)	70.7 (15.6)	72.9 (15.6)	71.9 (11.1)	72.6 (14.1)
	Range	50.0, 91.1	64.1, 89.1	50.0, 91.1	50.0, 107.2	52.8, 92.0	50.0, 107.2
Baseline ESSDAI	Mean (SD)	12.0 (3.8)	11.8 (3.9)	11.9 (3.6)	10.6 (4.4)	11.0 (5.2)	10.7 (4.6)
	Range	7, 17	6, 14	6, 17	6, 25	6, 23	6, 25
Baseline ESSPRI	Mean (SD)	6.8 (1.9)	7.0 (1.8)	6.8 (1.8)	6.7 (1.7)	7.2 (1.5)	6.9 (1.6)
	Range	4.0, 10.0	5.0, 9.0	4.0, 10.0	2.3, 9.7	3.7, 9.0	2.3, 9.7
Ant Nuclear Antibodies	n (%)	8 (100.0)	3 (75)	11 (91.7)	19 (90)	9 (82)	28 (87.5)
Anti SSA	n (%)	7 (88)	4 (100)	11 (92)	21 (100)	11 (100)	32 (100)
Anti SSB	n (%)	7 (88)	3 (75)	10 (83)	13 (62)	8 (73)	21 (66)
Rheumatoid factor[*]	n (%)	7 (88)	2 (50)	9 (75)	16 (76)	10 (91)	26 (81)
Concomitant medications	n (%)						
Antimalarials		1 (13)	0	1(13)	7 (33)	3 (27)	10 (31)
Azathioprine		0	0	0	1	0	1 (3)
Corticosteroids		0	0	0	8 (38)	3 (27)	11 (34)
Methotrexate		0	0	0	2 (10)	0	2 (6)

n=number of participants. SC=Subcutaneous. IV=Intravenous. ESSDAI=EULAR Sjögren's Syndrome Disease Activity Index. ESSPRI=EULAR Sjögren's syndrome patient reported intensity. [*]Patients with rheumatoid factor above normal are recorded.

Table 1. Baseline characteristics of patients with pSS in the clinical study

		Cohort 1			Cohort 2		
		Iscalimab (3 mg/kg SC) n=8	Placebo → Iscalimab (3 mg/kg SC) n=4	Total n=12	Iscalimab (10 mg/kg IV) n=21	Placebo → Iscalimab (10 mg/kg IV) n=11	Total n=32
		nE, nS (%)	nE, nS (%)	nE, nS (%)	nE, nS (%)	nE, nS (%)	nE, nS (%)
Placebo-controlled first treatment period	**Patients with AEs**	34, 7 (88)	17, 4 (100)	51, 11 (92)	23, 7 (33)	14, 6 (55)	37, 13 (41)
	AEs of Mild intensity	29, 6 (75)	14, 3 (75)	43, 9 (75)	16, 7 (33)	10, 4 (36)	26, 11 (34)
	AEs of Moderate intensity	5, 3 (38)	2, 1 (25)	7, 4 (33)	7, 4 (19)	4, 4 (36)	11, 8 (25)
	AEs of Severe intensity	0, 0 (0)	1, 1 (25)	1, 1 (8)	0, 0 (0)	0, 0 (0)	0, 0 (0)
	Study drug-related AEs	11, 3 (38)	0, 0 (0)	11, 3 (25)	0, 0 (0)	2, 1 (9)	2, 1 (3)
	Serious AEs	0, 0 (0)	0, 0 (0)	0, 0 (0)	0, 0 (0)	0, 0 (0)	0, 0 (0)
	AEs leading to discontinuation of study treatment	0, 0 (0)	0, 0 (0)	0, 0 (0)	0, 0 (0)	0, 0 (0)	0, 0 (0)
	Study-drug related AEs leading to discontinuation of study treatment	0, 0 (0)	0, 0 (0)	0, 0 (0)	0, 0 (0)	0, 0 (0)	0, 0 (0)
Most frequent adverse events during the clinical trial	**Patients with AE(s)**	8 (100%)	4 (100%)	12 (100%)	11 (52%)	7 (64%)	18 (56%)
	Preferred term						
	Upper respiratory tract infection, nS (%)	2 (25%)	2 (50%)	4 (33%)	2 (10%)	2 (18%)	4 (13%)
	Headache, nS (%)	0 (0)	1 (25)	1 (8)	2 (10)	1 (9)	3 (9)
	Nasopharyngitis, nS (%)	0 (0)	0 (0)	0 (0)	0 (0)	2 (18)	2 (6)
	Arthralgia, nS (%)	2 (25)	1 (25)	3 (25)	0 (0)	1 (9)	1 (3)
	Diarrhoea, nS (%)	1 (13)	1 (25)	2 (17)	2 (10)	1 (9)	3 (9)
	Rash, nS (%)	2 (25)	1 (25)	3 (25)	1 (5)	1 (9)	2 (6)
	Dizziness, nS (%)	2 (25)	1 (25)	3 (25)	1 (5)	0 (0)	1 (3)
	Lower respiratory tract infection, nS (%)	2 (25)	1 (25)	3 (25)	0 (0)	0 (0)	0 (0)
	Urinary tract infection, nS (%)	2 (25)	0 (0)	2 (17)	0 (0)	0 (0)	0 (0)
	Nausea, nS (%)	1 (13)	1 (25)	2 (17)	1 (5)	0 (0)	1 (3)
	Toothache, nS (%)	1 (13)	1 (25)	2 (17)	1 (5)	0 (0)	1 (3)
	Vomiting, nS (%)	2 (25)	0 (0)	2 (17)	1 (5)	0 (0)	1 (3)
	Contusion, nS (%)	0 (0)	0 (0)	0 (0)	2 (10)	1 (9)	3 (9)
	Bronchitis, nS (%)	0 (0)	0 (0)	0 (0)	0 (0)	1 (9)	1 (3)

Constipation, nS (%)	0 (0)	0 (0)	0 (0)	0 (0)	1 (9)	1 (3)
Iron deficiency anaemia, nS (%)	0 (0)	0 (0)	0 (0)	1 (5)	1 (9)	2 (6)
Lipase increased, nS (%)	0 (0)	0 (0)	0 (0)	0 (0)	2 (18)	2 (6)
Photosensitivity reaction, nS (%)	0 (0)	0 (0)	0 (0)	1 (5)	1 (9)	2 (6)
Dehydration, nS (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Dyspepsia, nS (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Injection site haematoma, nS (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Oral herpes nS (%),	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Peripheral swelling, nS (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

In the grouping by MedDRA preferred terms, only adverse events that occurred in more than 1 patient within at least one cohort are presented. Preferred terms are sorted by total number of AEs across all cohorts. n=number of patients studied. nE=number of AE events in the category. nS=number of patients with at least one AE in the category. % is based on the number of patients. SC=Subcutaneous. IV=Intravenous.

Table 2 Summary of overall incidence and severity in the placebo-controlled first treatment period, and the most frequent adverse events during the clinical trial, for Cohorts 1 and 2