

Assessment of the anti-CD40 antibody iscalimab in patients with primary Sjögren's syndrome

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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

6

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59 Summary (277)

60 Background

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

67

68 Methods

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

78

79 Findings

80 AEs were similar between iscalimab treatment and placebo groups and two SAEs were reported
81 to be unrelated to treatment with iscalimab. Furthermore, IV treatment in Cohort 2 resulted in
82 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

86 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

91 Funding

92 Novartis Pharma, AG.

93 INTRODUCTION

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

102 Hallmark clinical features include the presence of anti-Sjögren's-syndrome-related antigen-A
103 (anti-Ro/SSA), with or without anti-Sjögren's-syndrome-related antigen-B (anti-La/SSB),² and
104 focal lymphocytic sialadenitis on labial salivary glands or parotid biopsies. Ectopic lymphoid
105 structures (ELS) resembling germinal-centers (GCs) have also been observed within salivary
106 glands from pSS patients,³ and have been reported to be colocalised with expression of the B
107 cell-attracting chemokine CXCL13.⁴ Recent data have indicated that the presence of salivary
108 gland ELS⁵ and increased systemic levels of CXCL13⁶ correlates with disease severity,
109 implicating ELS and accompanying B cell hyper-reactivity in pSS pathology.⁷

110 B cell activation, immunoglobulin class-switching, and GC formation are regulated by various
111 immunological costimulatory pathways, notably CD40-CD154 interactions.^{8,9} Reports on the
112 expression of CD154 and CD40 on salivary gland-infiltrating T and B cells respectively,^{10,11}
113 suggest that T cell-B cell interactions via this pathway may contribute to sialadenitis and ELS
114 formation and function. Furthermore, data suggest that activated CD154-expressing T cells
115 could facilitate the destruction of salivary gland epithelial cells expressing CD40, potentially
116 affecting salivary gland function.^{12,13} Finally, recent data show that inhibition/blocking of the
117 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 $2.0\text{--}15.0 \times 10^9/\text{L}$, platelets $<100 \times 10^9/\text{L}$, hemoglobin <9.0 g/dL,

165 lymphocyte count $<0.8 \times 10^9/\text{L}$, neutrophil count $<1.5 \times 10^9/\text{L}$, 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 standardise
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,

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

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

224

225 Assessment of safety and adverse events

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

235

236 Pharmacokinetic analysis in clinical study

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

240

241 Immunogenicity analysis in clinical study

242 Presence of anti-iscalimab antibodies in plasma was determined using a validated bridging
243 ELISA-based assay. In a pre-incubation step, biotinylated iscalimab was used for capture, and
244 digoxigenin labelled iscalimab and Fab fragments of a polyclonal anti-digoxigenin antibody
245 conjugated to horseradish peroxidase for detection. Drug interference testing: (i) 250 ng/mL of
246 rabbit polyclonal affinity purified anti-CFZ533 IgG (positive control) could be detected in the
247 presence of up to 20.2 µg/mL of iscalimab, and 500 ng/mL of the positive control could be
248 detected in the presence of up to 45.0 µg/mL of iscalimab. The immunogenicity testing adopted
249 a three-tiered strategy with; a screening assay, a confirmatory assay, and titer assay, as tier 1, 2
250 and 3, respectively.

251

252 Biomarker assessments in clinical study

253 The biomarker CXCL13 was measured in Cohort 2 using a validated bead-based sandwich
254 immunoassay (Cat# LXSAHM, R&D Systems, Inc., Minneapolis, MN, USA). The dynamic
255 range, defined by the LLOQ and ULOQ, was 32.6 – 2760 pg/mL in human serum.

256 Serum anti-SSB, anti-SSA 52, and anti-SSA 60 IgG were measured using validated individual
257 commercial in vitro diagnostic medical device (IVD) kits from Orgentec (Cat# ORG509,
258 ORG652, ORG660, ORGENTEC Diagnostika GmbH, Mainz, Germany). The dynamic ranges
259 defined by LLOQ-ULOQ were 1250 – 100000 U/mL in human serum, taking into account the
260 minimal dilution requirement (MRD) of 1:500 for anti-SSB and anti-SSA 52 IgG; and 2500 –
261 200000 U/mL in human serum, taking in account the MRD of 1:1000 for anti-SSA 60 IgG.

262

263 **Statistical analysis**

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

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

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

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

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

288 Clinicaltrials.gov identifier: NCT02291029.

289

290 Role of the funding source

291 The sponsor, Novartis, had a role in the study design, data analysis, and writing of the report in
292 addition to the authors and investigators. All authors had full access to the data and reviewed
293 and approved the final version of the manuscript. The corresponding author had the
294 responsibility to submit for publication.

295

296 RESULTS

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

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

308 Although, two serious adverse events (SAEs) were observed during the safety follow-up period
309 (approximately 12 weeks after the last dose of iscalimab) these were considered to be unrelated
310 to iscalimab by the investigator, namely, one case of bacterial conjunctivitis in Cohort 1 and
311 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 primates¹⁶ 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 more so 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

337 (95% CI 0.38 to 7.16; one-sided $p=0.015$) compared to the placebo group that switched to 10
338 mg/kg IV after week 12. Improvements were also observed in the placebo group after switching
339 to 10 mg/kg IV iscalimab at week 12 (figure 3a, lower panel).

340 In Cohort 2 the percentages of ESSDAI and ESSPRI responders at week 12 were higher in the
341 iscalimab group than in the placebo group. For ESSDAI, 76% of iscalimab patients achieved
342 ≥ 3 point reduction compared with 55% of placebo patients. Thirteen (62%) iscalimab patients
343 reduced their ESSDAI to below 5 points compared with four (36%) in the placebo group. For
344 ESSPRI, 62% of iscalimab patients achieved ≥ 1 point reduction compared with 45% for
345 placebo.

346 In the placebo-controlled period of Cohort 2, some improvements were observed in secondary
347 outcomes at week 12: mean decrease vs. placebo in ESSPRI was 0.95 (95% CI -0.50 to 2.41)
348 (figure 3b, lower panel), SF-36; mean increase vs placebo in physical and mental component
349 score of 3.83 (95% CI -1.81 to 9.48) and 2.52 (95% CI -4.50 to 9.53), respectively, total MFI;
350 mean reduction vs placebo of 9.83 (95% CI -1.01 to 20.66), and patient- and investigator-
351 assessments using VAS; a mean decrease of 8.14 (95% CI -10.39 to 26.67) and 12.16 (95% CI
352 2.38 to 21.94), respectively (supplementary figure 3).

353 Improvements were observed in the 10 mg/kg IV iscalimab group compared with placebo in
354 unstimulated and stimulated salivary flow and in Schirmer's test. A mean increase vs. placebo
355 in unstimulated salivary flow at week 12 of 0.04 mL/min (95% CI -0.03 to 0.10), in stimulated
356 flow of 0.16 mL/min (95% CI -0.15 to 0.46), and for Schirmer's test of 8.06 mm (95% CI -
357 1.37 to 17.50) and 9.07mm (95% CI -4.61 to 22.75) for the left and right eye, respectively
358 (supplementary figure 4).

359 Decreases in CXCL13 serum concentrations after 10 mg/kg IV iscalimab compared to placebo
360 in Cohort 2 (56% reduction in geometric mean vs placebo at week 12; 95% CI 27% to 70%
361 [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 lambda 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

388 **Research in context**

389 **Evidence before this study**

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

404 **Added value of this study**

405 We opted to run a randomised, placebo-controlled study with iscalimab in patients with
406 clinically active pSS, using ESSDAI to define disease activity (ESSDAI \geq 6, reflecting the
407 systemic manifestations of the disease) for both inclusion of patients and for the primary
408 endpoint. This established and validated composite outcome measure is regarded as an
409 acceptable regulatory endpoint for pivotal clinical trials, and has been previously used in other
410 pSS clinical trials. Iscalimab is a fully-human, pathway-blocking, non-depleting, anti-CD40
411 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

419 DISCUSSION

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 treatment 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 internalization¹⁶ 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 \geq 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 pathogenic 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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508 Contributors

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

516

517 Declaration of interests

518 Disclosures for consultancy fees (none of which exceeded \$10,000) are made for the following
519 authors: BAF (Novartis, Roche, MedImmune, BMS and Virtualscopics); W-FN (Novartis,
520 GlaxoSmithKline, Abbvie, MedImmune, BMS, Atheneum Partners); MB (GSK, Amgen,
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525 Novartis at the time of conducting and analysing the studies. All other authors declare no
526 competing financial interests.

527

528 Data sharing

529 Novartis Pharma, AG is committed to sharing with qualified external researchers, access to
530 patient-level data and supporting clinical documents from eligible studies. These requests are
531 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

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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

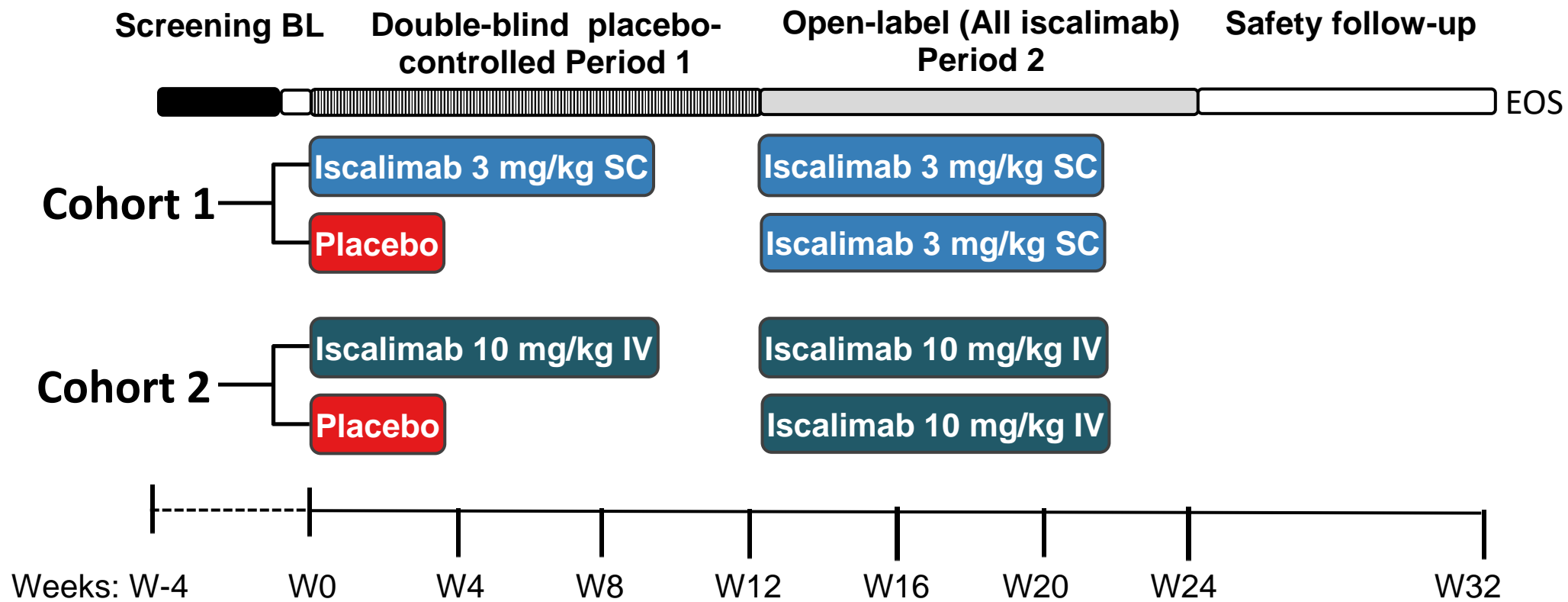
Supplementary Table 1. Results from statistical analysis of ESSDAI

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

Figure 1: Schematic clinical study design*

Figure 2. Trial profile

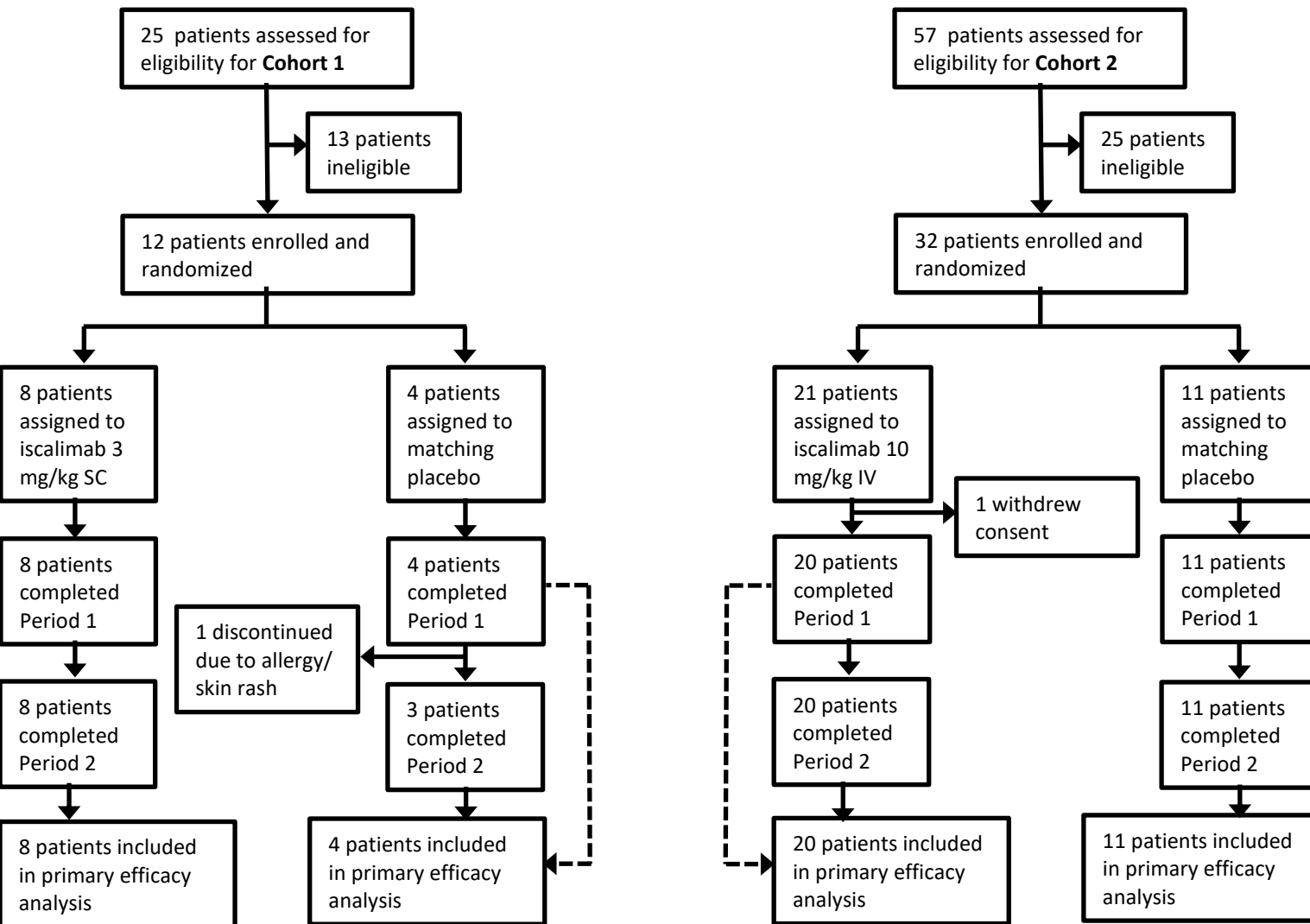


Figure 3a

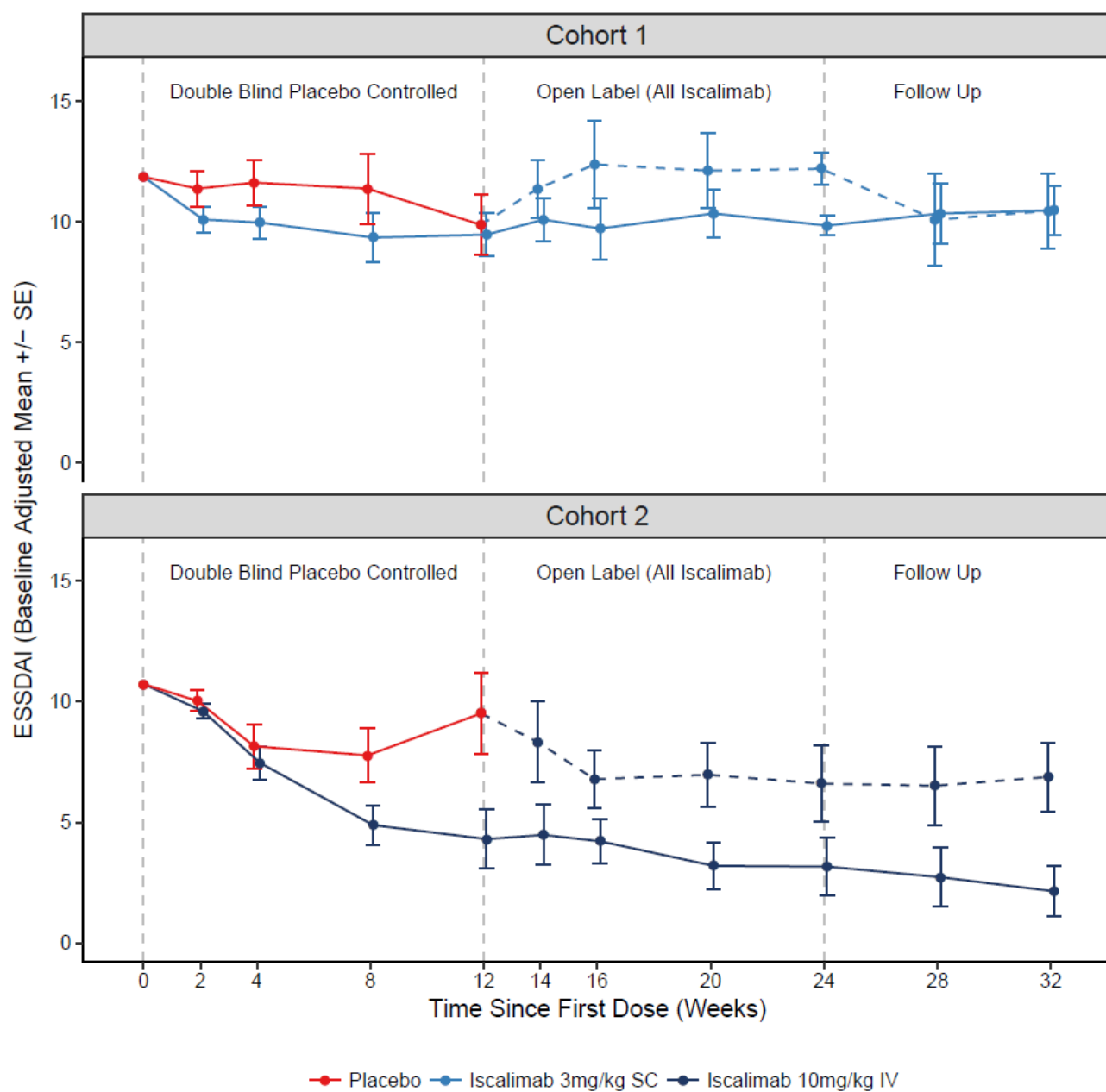


Figure 3b

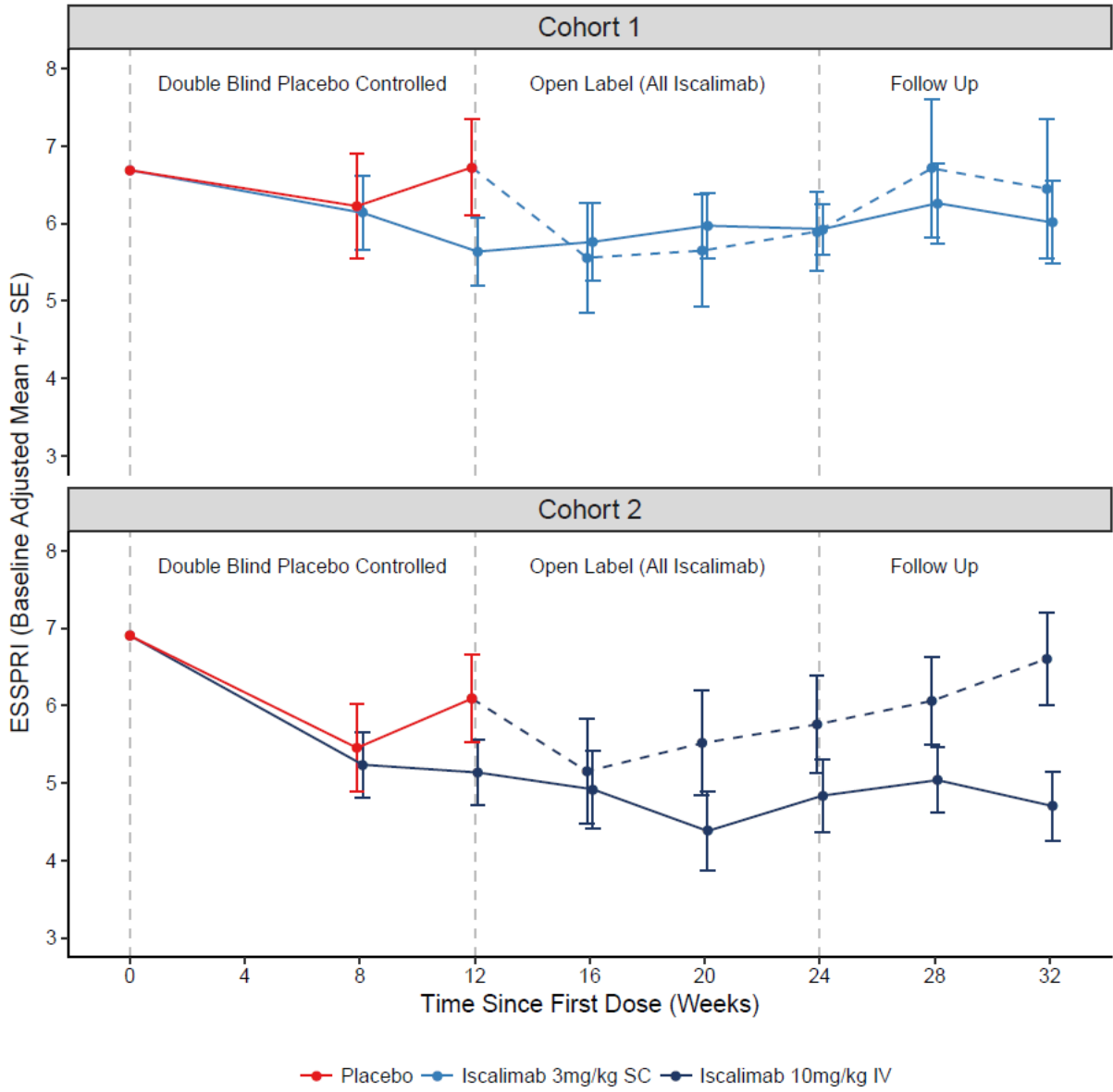
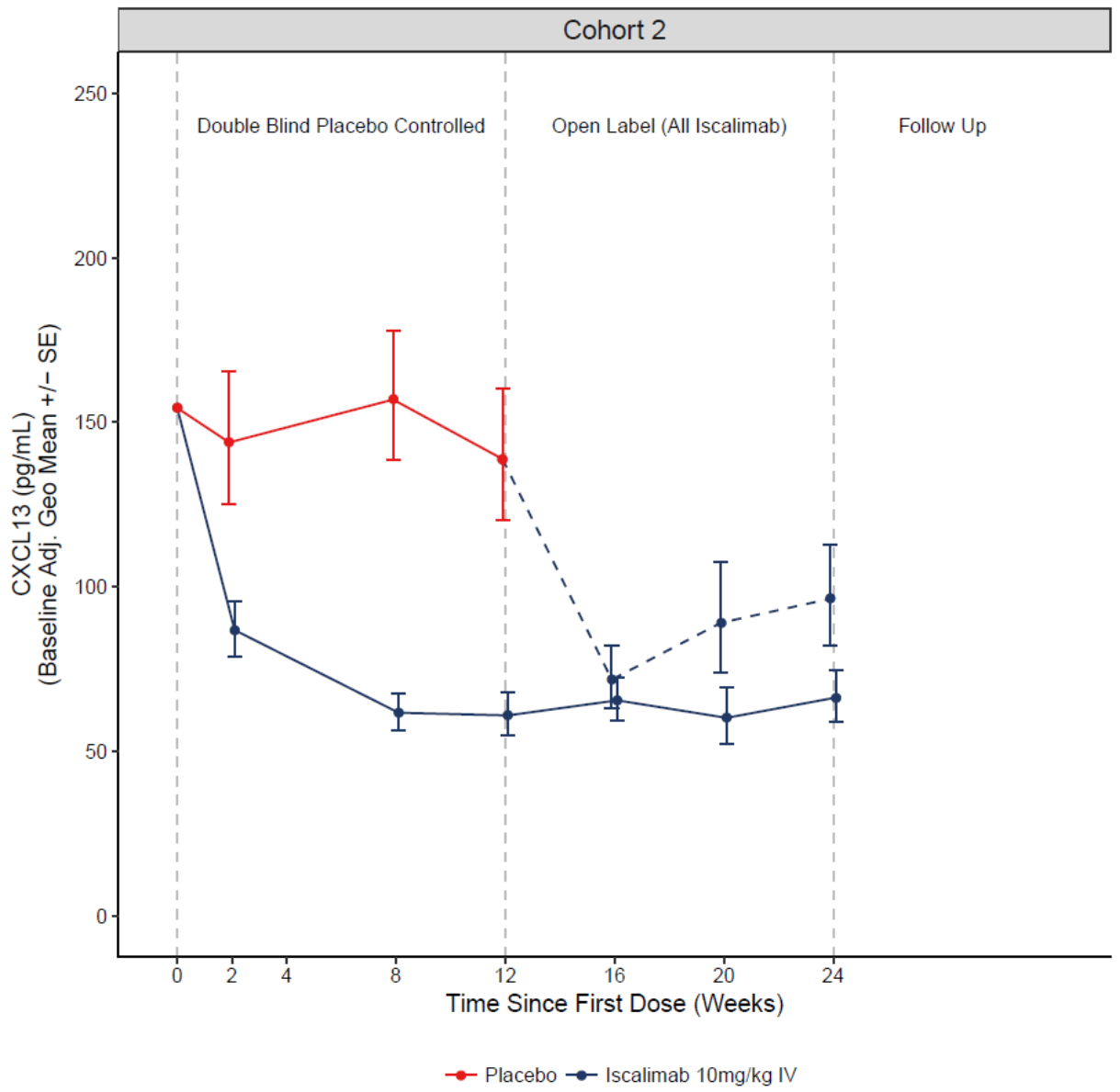


Figure 3c



		Cohort 1			Cohort 2		
		Iscalimab 3 mg/kg SC 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
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