Intravenous administration of expanded allogeneic adipose-derived mesenchymal stem cells in refractory rheumatoid arthritis (Cx611): results of a multicentre, dose escalation, randomised, single-blind, placebo-controlled phase Ib/IIa clinical trial.

Authors:

Jose M Álvaro-Gracia[1], Juan A Jover[2], Rosario García-Vicuña[1], Luis Carreño[3], Alberto Alonso[4], Sara Marsal[5], Francisco Blanco[6], Victor M Martínez-Taboada[7,8], Peter Taylor[9], Cristina Martín-Martín[10], Olga DelaRosa[11], Ignacio Tagarro[11], Federico Díaz-González[12,13]

Affiliations:

1 H. de La Princesa, IIS Princesa, Madrid, Spain.
2 H. Clínico San Carlos de Madrid, Madrid, Spain.
3 H. Gregorio Marañón, Madrid, Spain.
4 H. de Cruces, Bilbao, Spain.
5 H. Vall d'Hebron, Barcelona, Spain.
6 INIBIC - Complejo Hospitalario Universitario, A Coruña, Spain.
7 H. Marqués de Valdecilla, Santander, Spain.
8 Facultad de Medicina, Universidad de Cantabria, Santander, Spain
9 Kennedy Institute of Rheumatology, University of Oxford, Oxford, UK.
10 IRYCIS.Hospital Universitario Ramón y Cajal, Madrid, Spain.
11 TiGenix, Madrid, Spain.
12 Department of Medicine. Universidad de La Laguna, La Laguna, Spain.
13 Complejo Hospitalario Universitario de Canarias Tenerife, Spain.

Author for correspondence:

José M Álvaro-Gracia
Hospital Universitario de La Princesa, Madrid
Unidad de Terapias Biológicas, Servicio de Reumatología, IIS Princesa
28006 Madrid - SPAIN
Telephone: +34 915202438
e-mail: jalvarogracia@gmail.com

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ABSTRACT

Objectives: To evaluate the safety and tolerability of the intravenous administration of Cx611, a preparation of allogeneic expanded adipose-derived stem cells (eASCs), in refractory rheumatoid arthritis (RA) patients, as well as to obtain preliminary clinical efficacy data in this population.

Methods: Multicentre, dose escalation, randomised, single-blind (double-blind for efficacy), placebo-controlled, phase Ib/IIa clinical trial. Patients with active refractory RA (failure to at least two biologicals) were randomised to receive three intravenous infusions (iv) of Cx611: 1 million/kg (cohort A), 2 million/kg (cohort B), 4 million/kg (cohort C), or placebo, on days 1, 8 and 15, and they were followed for therapy assessment for 24 weeks.

Results: Fifty-three patients were treated (20 in cohort A, 20 in cohort B, 6 in cohort C, and 7 in placebo group). A total of 141 adverse events (AEs) were reported. Seventeen patients from the group A (85%), 15 from the group B (75%), 6 from the group C (100%) and 4 from the placebo group (57%) experienced at least one AE.

Eight AEs from 6 patients were grade 3 in intensity (severe), 5 in cohort A (lacunar infarction, diarrhoea, tendon rupture, rheumatoid nodule and arthritis), 2 in cohort B (sciatica and rheumatoid arthritis) and 1 in the placebo group (asthenia). Only one of the grade 3 AEs was serious (the lacunar infarction).ACR 20 responses for cohorts A, B, C and placebo were 45, 20, 33 and 29% respectively at month 1, and 25, 15, 17 and 0% respectively at month 3.

Conclusion: The intravenous infusion of Cx611 was in general well tolerated, without evidence of dose-related toxicity at the dose range and time period studied. In addition, a trend for clinical efficacy was observed. These data, in our opinion, justify further investigation of this innovative therapy in RA patients.

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INTRODUCTION

Mesenchymal stem cells (MSCs) are multi-potent cells that can be isolated from a variety of adult tissues of mesodermal origin, such as bone marrow, adipose tissue, placenta, umbilical cord or synovium.[1-5] These cells possess immunosuppressive properties, as they inhibit the proliferation and function of major immune cell populations, including T cells, B cells and natural killer (NK) cells.[6-12] In addition, they modulate the activity of dendritic cells and induce regulatory T cells, both in vivo and in vitro.[11, 13, 14] This modulator role can be a consequence of both cell contact mechanisms and paracrine effects.[15, 16] Furthermore MSCs show low immunogenicity, as they display low levels of MHC I (HLA-A, B, C) and the absence of MHC II (HLA-DR, DQ, DP) and co-stimulatory molecules, such as CD40 (TNFR), CD80 (B7-1) and CD86 (B7-2).[17-19] Consequently, MSCs appear to be adequate candidates for allogeneic therapy targeting auto-immune and inflammatory diseases, without the requirement of suppression of host immunity.[20]

Despite the success of biologic agents in RA[21-23], there is still a significant number of patients who do not respond to these drugs, showing the need of new therapies. It has been described that MSCs diminish in vitro the inflammatory response of peripheral blood mononuclear cells from RA patients with active disease.[24] In particular, expanded adipose-derived stem cells (eASCs) suppress responses of CollagenII-reactive T cells from patients with RA, by inhibition of T cell proliferation, production of inflammatory cytokines, release of the anti-inflammatory/suppressive cytokine IL10, and generation of antigen-specific regulatory T cells. Moreover, eASCs inhibit the production of inflammatory factors by activated synovial cells involved in cartilage and bone damage.[24-26] Consistently, eASCs have demonstrated therapeutic effect in experimental arthritis animal models .[27] However, little is known about the effect of eASCs in humans with RA.

The primary aim of this study was to assess the safety of intravenous (iv) infusions of eASCs in refractory RA. Indeed, MSCs have generally been reported to be well tolerated,[28] but theoretical safety concerns on the therapeutic use of this cell type need further analysis.[29-38] Additionally, we obtained preliminary efficacy information from a study with a controlled design.

METHODS

Study design, cell therapies and other treatments

Multicentre, dose escalation, randomised, single-blind (double-blind for efficacy), placebo-controlled, phase Ib/IIa study, with a follow-up period of up to 6 months, conducted between April 2011 and January 2013. In order to safeguard patients' safety as much as possible, safety assessments were single-blinded (blinded patient and unblinded investigators) whereas efficacy assessments were double-blinded. The overall study scheme is shown in Figure 1, and the dose escalation scheme is detailed in Figure 2.

The MSCs chosen to run this trial (Cx611) were eASCs fulfilling ISCT criteria for MSCs.[39] Cells were obtained from adipose tissue, as this is an abundant and accessible source of adult stem cells.[40] Lipoaspirates were digested with collagenase.. Erythrocytes were removed by lysis, and the stromal vascular fraction was obtained through filtration. ASCs were expanded through successive expansion passages. At various stages during the process the eASCs were tested for viability, population doublings, morphology, potency, identity, purity, sterility, and genetic stability, amongst other quality controls. The product for clinical use was released after recovery from the cryopreserved cell banks, formulation in the corresponding vehicle and confirmation of compliance with quality specifications.

Three cohorts (A, B, C) received active treatment, with doses of 1, 2 and 4 million cells/kg, respectively, administered intravenously at days 1, 8 and 15. Placebo was Ringer's lactate solution.

Background non-biologic DMARDs were kept stable, as well as NSAIDs and glucocorticoids (≤10 mg per day of prednisone or equivalent). Rescue therapy with any DMARD, including biologics was allowed after the 3rd month. Follow-up visits were conducted at weeks 1, 2 and 3 (visits 1-3), and at months 1, 2, 3, 4, 5 and 6 (visits 4-9).

Ethics

This study was performed according to the applicable regulations, to GCP standards (CPMP/ICH/135/95) and to the amended Declaration of Helsinki, (Seoul, October 2008) after approval by the corresponding ethics committees. All patients provided written informed consent. An independent Safety Monitoring Board, was created to ensure and safeguard the welfare and safety of the patients.

Patients

Eligible patients were adults with a diagnosis of RA for ≥6 months, treated with at least one non-biologic agent, and with previous failure (inefficacy according to investigator judgement) to at least two biologics (any of the EULAR approved biologics for RA). The wash-out periods for biologics are detailed in the online supplementary Table S1. Patients' EULAR Disease Activity Score (DAS28-ESR) had to be >3.2; they had to have 4 tender joints to palpation and 4 swollen joints (based on a 68/66-joint count) and had to be receiving treatment on an outpatient basis.

Evaluation criteria

Primary endpoint

The primary endpoint was to determine the safety and tolerability of the iv administration of Cx611 through the identification of adverse events (AEs) and serious adverse events (SAEs).

It was also intended to identify, if possible, the dose limiting toxicity (DLT). Clinically relevant AEs (i.e. grades 3-5) related to Cx611 administration were considered DLTs. Intensity of AEs was assessed following the Common Terminology Criteria for AEs, version 4.0, of the National Cancer Institute, ranging from 1 to 5.[41]

Vital signs, physical examination, haematology, serum chemistry, urinalysis and coagulation parameters were collected at baseline and at each follow-up visit. Additional evaluations included arterial oxygen saturation and 12-lead ECG. EDTA-blood was collected from patients at baseline and at day 30; distribution of circulating T and regulatory T (CD3+CD4+CD25+foxp3+) cells in PBMCs was analyzed by flow cytometry. Plasma levels of anti-HLA antibodies were measured with Luminex LABScreen Mixed (One Lambda Inc., Canoga Park, CA, USA). Positive samples were confirmed by LABScreen Single Antigen (One Lambda)

Secondary endpoints

Secondary exploratory efficacy endpoints included the proportion of ACR20, ACR50 and ACR70 patients,[42]EULAR response (DAS28-ESR and DAS28-CRP)[43, 44] and the Short Form 36 Health Survey (SF-36) questionnaire.[45]

Statistical analysis

Given the lack of safety and efficacy data of iv administered eASCs or other mesenchymal stem cells in patients with RA at the time of study design, the sample size was based on other mesenchymal stem cells clinical trials for other indications.

A descriptive analysis, including anthropometric data, variables related to the medical history of patients, efficacy endpoints reported at baseline and baseline laboratory parameters, was conducted. Analysis of the primary safety endpoint was performed on the intent-to-treat (ITT) population. Number and percentages of patients who experienced AEs, SAEs, treatment-related AEs and treatment-related SAEs were described for the overall population and by treatment group, as well as those who reported grade 3-4 AEs. These values might be compared between the groups in the maintenance phase using a χ^2 test; otherwise, a Fisher's exact test was used. Laboratory parameters, particularly of the parameters included in the selection criteria, were described by visit.

Analysis of the secondary efficacy endpoints was performed on the ITT and per-protocol (PP) populations. Statistical analysis was conducted with SAS package v9.2.

RESULTS

Patients

Eighteen investigational sites recruited 67 patients. Fourteen patients were screening failures; therefore 53 patients continued in the trial and received at least one dose of the study treatment (ITT population). Twenty patients were allocated to cohort A, 20 to cohort B, 6 to cohort C, and 7 received placebo.

Major protocol violations occurred in 5 patients (intake of prohibited medication in 4 patients and non-compliance with selection criteria in 1 patient), therefore the PP population consisted of 48 patients. Ten patients discontinued the study prematurely, 4 in cohort A, 1 in cohort B, 2 in cohort C and 3 in placebo group.

Details on demographic and clinical characteristics are provided in Table 1. Baseline demographic and clinical characteristics were typical of refractory RA and generally comparable among treatment groups (with the exception in the number of previous DMARDs, which was higher in cohort C compared to the other groups). The mean (SD) number of previous biologics was 2.98 (1. 38). The proportion of patients taking corticosteroids and NSAIDs were balanced in the different cohorts.

Safety

A total of 141 AEs were reported, of which 133 were of mild or moderate intensity (94%). Seventeen patients from the group A (85%), 15 from the group B (75%), 6 from the group C (100%) and 4 from the placebo group (57%) experienced at least one AE.

The most frequent AEs (≥5%) were fever (9; 17%), respiratory infections (8; 15%), headache (6; 11%), urinary tract infections (6; 11%), nausea (5; 9%), arthralgia (3; 6%), asthenia (3; 6%), malaise (3; 6%) and vomiting (3; 6%),. The split of the most frequent AEs per treatment group is shown in Table 2..

By body system, the most frequently reported AEs were infections. None was serious. No opportunistic infections were reported. A listing of the infections, antimicrobials and outcomes is included in the online supplementary Table S2.

Eight AEs from 6 patients were grade 3 in intensity (severe), 5 in cohort A (lacunar infarction, diarrhoea, tendon rupture, rheumatoid nodule and arthritis), 2 in cohort B (sciatica and rheumatoid arthritis) and 1 in the placebo group (asthenia).

Three SAEs were reported, all occurring in -Cx611- treated patients: 1 lacunar infarction (severe), 1 peroneal nerve palsy (moderate intensity), and 1 case of pyrexia (moderate intensity). The lacunar infarction was considered a DLT, according to the pre-established definition. This event encompassed 3 consecutive SAEs (2 events of generalised muscle weakness, and 1 event of left hemihypoesthesia and paretic ataxic gait, finally diagnosed as lacunar infarction). These episodes were transient and patient recovered with minimum sequelae. This was the only patient who discontinued the study due to AEs.

No abnormal laboratory values were reported and no relevant vital signs abnormalities occurred, other than those related to reported AEs. No malignancies or deaths were reported.

Clinical efficacy

This study was not powered to compare clinical efficacy between cohorts, so that it was evaluated only in an exploratory context. ACR 20 responses for cohorts A, B, C and placebo were 45, 20, 33 and 29% respectively at month 1, 25, 30, 17 and 14% respectively at month 2, and 25, 15, 17 and 0% respectively at month 3 (Figure 3). ACR 50 responses were 20, 0, 17 and 14% respectively at month 1, 10, 15, 17 and 0% respectively at month 2 and 15, 5, 17 and 0% at month 3. ACR 70 responses were very low . Table 3 also shows the results on EULAR good response, low disease activity (DAS28-ESR<3.2), DAS28-ESR and CRP. Of note, in contrast to Cx611-treated cohorts, no patients in the placebo group showed good EULAR response or low disease activity. DAS28-ESR evolution over time showed an overall decreasing trend in the cells-treated cohorts, more marked in the case of cohort C, whereas the placebo arm showed a fluctuating response, with a mean value at month 3 similar to that registered at baseline. . DAS28-CRP was similar to DAS28-ESR (not shown). CRP evolution over time showed a tendency to decrease from baseline in cohorts A and C but not in cohort B or placebo. Efficacy results did not differ in the PP and ITT populations.

After 3 months, we did not observe persistent clinical benefit in Cx611- treated patients, as treatment effect waned or fluctuated (data not shown). The physical and mental subscales (SF-36) improved in the different groups, without statistically differences among them (data not shown).

Immune readouts

We did not find significant changes in T cell populations, including Tregs, among cohorts. (not shown). In terms of immunogenicity, nine patients (19%) generated eASC specific anti-HLA-I antibodies without apparent clinical consequences. In fact, 43% of treated patients presented baseline anti-HLA-I antibodies (presensitized), presumably related to previous pregnancies or transfusions. Presensitized patients showed higher frequency of eASC donor-specific antibodies (30% vs 11 %). No dose-related impact was observed (supplementary table S3). Anti-HLA-II antibodies were not found.

DISCUSSION

This is, to our knowledge, the first randomized, placebo-controlled study with MSC in RA. The most important findings of our work are: 1) in RA patients, the i.v. treatment with Cx611 was not associated to significant toxicity and 2) signs of possible efficacy of this therapy in RA patients could be envisioned.

An overall favourable safety profile of Cx611 is suggested by the fact that most of the reported AEs were of mild to moderate intensity , consistent with the patient profile and considered unrelated to the study treatments by investigators. No life-threatening events (grade 4) or deaths occurred. There was no apparent relationship between dose and tolerability. In fact, the rate of treatment-related AEs was lower in cohort C (higher dose) than in both cohorts A and B, although this is probably due to the small size of the cohort C. Transient fever was the most frequent treatment-related AE observed in patients treated with Cx611. The mechanisms for fever are not clear but could be considered as some form of infusion reaction. [28]

There was one DLT, a lacunar infarction (left hemihypoesthesia and paretic ataxic gait), which occurred in a patient of cohort A eight days after the second treatment administration. It was deemed as likely related because there were no other apparent causes, even though the pathophysiology of this event is unclear. Therefore, a conservative position was adopted in the causality assessment. This SAE encompassed 3 consecutive SAEs (see Results), in which muscle weakness, the most consistent manifestation, was probably a form of infusion reaction, because, after discontinuation from study, this patient suffered a similar episode subsequent to the administration of iv tocilizumab.

Infections were the most frequently reported AEs by body system, none of them being severe or serious. It is difficult to know their relationship to Cx611, since RA patients are at a higher risk for serious infections than the general population, and prednisone and biologic agents have been shown to increase this risk.[46-48]

No venous thrombotic events, nor signs of pulmonary thromboembolism were detected. This is reassuring giving that thrombotic events have been described in animal models in which very high doses of iv MSCs were administered.[36] In addition, no acute or delayed hypersensitivity reactions or haematological AEs, with the exception of anaemia (3 cases), were observed in our patients. No malignancies were found although the size and duration of the trial limit the relevance of this finding. Additionally, clinical experience does not indicate that tumorigenicity associated to MSC-based therapies represents a significant risk.[35]

We detected sensitization against allogeneic ASCs in some patients, without apparent clinical consequences. This has been previously shown in allogeneic stem cell treatment (X) and in patients with previous pregnancies and transfusions . [xxx. The duration and impact of these allo-antibodies needs to be further analyzed.As this study was not designed nor powered for efficacy evaluation, clinical efficacy outcomes should be interpreted cautiously and within an exploratory context. The very refractory profile of the included population increases the difficulty for detecting efficacy. However, a tendency to better response was observed in patients treated with Cx611 than in placebo treated patients. No conclusion can be made on dose-responses due to the limited number of patients. The clinical benefit achieved in RA patients treated with i.v. Cx611 tends either to wane or fluctuate after 3 months of cell administration. This suggests that RA therapy with these cells would require repeated administrations.

Apart from anecdotal experiences from isolated cases, [49-51] only one study has been published so far on MSC for RA treatment.[52] This corresponds to an ongoing cohort that included 136 heterogeneous RA patients with inadequate response to various medications, who received DMARDs plus intravenously umbilical cord MSCs (UC-MSCs). A non-randomized control group comprised by 36 patients that started treatment with DMARDs plus medium two years later was used as comparator. UC-MSC treatment seemed to induce a significant clinical benefit that was maintained for 3-6 months and it was well tolerated. Therefore, our findings of a lack of short-term serious toxicity associated with the therapeutic administration of Cx611 to RA patients, and preliminary evidence of clinical efficacy, are in line with the results of this study.

One area of debate can be the choice of allogeneic cells instead of autologous. The main reason for it is that the allogeneic product, being stored in a cell bank, can be readily made available to the patients when required. Using autologous cells would complicate substantially the therapy, since liposuction (sometimes difficult) is required, and cell expansion would need a much longer time to obtain enough cells.

The limitations of this study include the single-blind design for safety (considered adequate in the best interest of patients) and the limited number of patients in some cohorts. In contrast, the randomized placebo-controlled design and the double-blind assessment of efficacy, performed for the first time to our knowledge with i.v. eASC are its main strengths. Our results suggest that the i.v. administration of Cx611 is in general well tolerated and without dose-related toxicity at the dose range and time period studied. In addition, signs for a potential therapeutic effect of these cells were observed in a highly challenging population of refractory RA patients. This opens the possibility of further research to investigate the duration of the therapeutic effects, the optimal dosing strategy, and the most suitable patient profile for this treatment.

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ADDITIONAL LIST OF INVESTIGATORS

Javier Ballina, Hospital Central Asturias, Oviedo. Ricardo Blanco Alonso, Hospital Marqués de Valdecilla, Santander. Sagrario Bustabad, Complejo Hospitalario Universitario de Canarias, Tenerife. Eugenio Chamizo, Hospital General de Mérida. Antonio Fernández-Nebro, Hospital

Carlos Haya de Málaga. José Luis Marenco, Hospital Nuestra Sra. Valme, Sevilla. Emilio Martín-Mola, Hospital La Paz, Madrid. Federico Navarro, Hospital Virgen Macarena, Sevilla. Arturo Rodríguez, Hospital Santa Creu i Sant Pau, Barcelona. José Andrés Román-Ivorra, Hospital La Fe, Valencia. Raimon Sanmartí, Hospital Clinic i Provincial, Barcelona. Jesús Tornero, Hospital de Guadalajara

CONTRIBUTORSHIP

JMAG, VMMT, PT, FDG and OR were involved in study design, data analysis, and in drafting and reviewing the manuscript. IT was involved in data analysis, and in drafting and reviewing the manuscript., JAJ, RGV, LC, AA, SM and FN were involved in patient recruitment, treatment, and follow-up, and in reviewing the manuscript. CMM was involved in anti-HLA antibodies detection and manuscript review

DISCLOSURES

JMAG, VMMT, PT and FDG received advisory fees from Tigenix. OD anf IT are employees of Tigenix. The rest of coauthors declared no conflicts of interest with this manuscript

DATA SHARING

Data are available upon request to the corresponding author.

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Table 1. Summary of baseline demographic and clinical characteristics of patients randomised to the four study cohorts (ITT population); SD: standard deviation.

Patient characteristics	**Cx611 1 million/kg (Cohort A) n=20**	**Cx611 2 million/kg (Cohort B) *n*=20**	**Cx611 4 million/kg (Cohort C) n=6**	**placebo *n*=7**	***p*-value**
Age (years)					
Mean (SD)	54.15 (7.79)	57.40 (11.01)	50.33 (15.62)	58.43 (14.25)	0.4424
Sex					
Female, n (%)	18 (90.00)	18 (90.00)	6 (100.00)	6 (85.71)	1.0000
Disease duration (years)					
Mean (SD)	14.36 (6.60)	13.07 (9.36)	20.25 (8.12)	22.73 (22.65)	0.2283
Tender joint count (68 joints)					
Mean (SD)	29.15 (18.17)	21.05 (16.47)	24.67 (11.60)	14.43 (11.37)	0.1253
Swollen joint count (66 joints)					
Mean (SD)	13.85 (9.24)	11.00 (8.01)	11.50 (4.72)	7.14 (4.30)	0.1686
Erythrocyte sedimentation rate, mm/h					
Mean (SD)	37.80 (26.66)	34.05 (19.92)	48.00 (40.08)	41.71 (19.53)	0.8610
C-reactive protein mg/dl					
Mean (SD)	1.72 (2.27)	1.33 (1.41)	1.86 (1.75)	0.88 (0.95)	0.8657
DAS28-ESR					
Mean (SD)	6.24 (1.21)	5.78 (1.18)	6.16 (1.35)	5.77 (0.75)	0.5859
Patient global assessment					
Mean (SD)	69.45 (20.96)	61.20 (22.34)	58.67 (28.34)	61.14 (22.38)	0.5909
Physician global assessment					
Mean (SD)	63.00 (16.77)	61.50 (14.52)	67.17 (18.77)	58.71 (20.84)	0.8229
Health Assessment Questionnaire (HAQ)					
Mean (SD)	1.84 (0.66)	1.56 (0.53)	1.88 (0.51)	1.73 (0.74)	0.4569
No of previous biologics					
2, n (%)	11 (55.00)	12 (60.00)	2 (33.33)	4 (57.14)	0.7692
≥3, n (%)	9 (45.00)	8 (40.00)	4 (66.66)	3 (42.86)	
No of previous DMARDs					
Mean (SD)	3.65 (1.50)	3.05 (1.23)	4.83 (1.47)	2.29 (1.38)	0.0221
Corticosteroids at baseline, n (%)	17 (85.00)	13 (65.00)	5 (83.33)	5 (71.43)	0.5619

Patient characteristics	Cx611 1 million/kg (Cohort A) n=20	Cx611 2 million/kg (Cohort B) *n*=20	Cx611 4 million/kg (Cohort C) n=6	placebo *n*=7	*p*-value
NSAIDs at baseline, n (%)	12 (60.00)	14 (70.00)	5 (83.33)	6 (85.71)	0.6275

Table 2. Patients with adverse events per system organ class and preferred term (>1 event in any cohort; ITT population), according to MedDRA coding (Medical Dictionary for Regulatory Activities).

System Organ Class (MedDRA)	Preferred Term (MedDRA)	Cohort A n=20	Cohort B n=20	Cohort C n=6	Placebo n=7
General disorders and administration site conditions	Pyrexia, n (%)	2 (10.00)	6 (30.00)	1 (16.67)	0 (0.00)
	Malaise, n (%)	1 (5.00)	2 (10.00)	0 (0.00)	0 (0.00)
	Influenza like illness, n (%)	0 (0.00)	2 (10.00)	0 (0.00)	0 (0.00)
Infections and infestations	Urinary tract infection, n (%)	3 (15.00)	1 (5.00)	2 (33.33)	0 (0.00)
	Respiratory tract infection, n (%)	6 (30.00)	1 (5.00)	1 (16.67)	0 (0.00)
	Ear infection, n (%)	2 (10.00)	0 (0.00)	0 (0.00)	0 (0.00)
	Gastroenteritis, n (%)	2 (10.00)	0 (0.00)	0 (0.00)	0 (0.00)
Skin and subcutaneous tissue disorders	Rash, n (%)	2 (10.00)	0 (0.00)	0 (0.00)	0 (0.00)
Musculoskeletal and connective tissue disorders	Muscular weakness, n (%)	2 (10.0)	0 (0.00)	0 (0.00)	0 (0.00)
Nervous system disorders	Headache, n (%)	2 (10.00)	3 (15.00)	1 (16.67)	0 (0.00)
Gastrointestinal disorders	Nausea, n (%)	4 (20.00)	1 (5.00)	0 (0.00)	0 (0.00)
	Vomting, n (%)	2 (10.00)	1 (5.00)	0 (0.00)	0 (0.00)
	Diarrhoea, n (%)	2 (10.00)	0 (0.00)	0 (0.00)	0 (0.00)
	Dental caries, n (%)	2 (10.00)	0 (0.00)	0 (0.00)	0 (0.00)
Blood and lymphatic system disorders	Anaemia, n (%)	1 (5.00)	2 (10.00)	0 (0.00)	0 (0.00)

Table 3. Efficacy results throughout the study until month 3 (ITT population).

Patients reaching ACR20 n (%)				
	Cohort A (n=20)	Cohort B (n=20)	Cohort C (n=6)	Placebo (n=7)
Month 1	9 (45.0)	4 (20.0)	2 (33.3)	2 (28.6)
Month 2	5 (25.0)	6 (30.0)	1 (16.7)	1 (14.3)
Month 3	5 (25.0)	3 (15.0)	1 (16.7)	0 (0.0)
Patients reaching ACR50 n (%)				
	Cohort A (n=20)	Cohort B (n=20)	Cohort C (n=6)	Placebo (n=7)
Month 1	4 (20.0)	0 (0.0)	1 (16.7)	1 (14.3)
Month 2	2 (10.0)	3 (15.0)	1 (16.7)	0 (0.0)
Month 3	3 (15.0)	1 (5.0)	1 (16.7)	0 (0.0)
Patients reaching ACR70 n (%)				
	Cohort A (n=20)	Cohort B (n=20)	Cohort C (n=6)	Placebo (n=7)
Month 1	1 (5.0)	0 (0.0)	1 (16.7)	0 (0.0)
Month 2	0 (0.0)	1 (5.0)	1 (16.7)	0 (0.0)
Month 3	1 (5.0)	0 (0.0)	1 (16.7)	0 (0.0)
Patients with EULAR Good Response (DAS28-ESR) n (%)				
	Cohort A (n=20)	Cohort B (n=20)	Cohort C (n=6)	Placebo (n=7)
Month 1	2 (10.0)	1 (5.0)	2 (33.3)	0 (0.0)
Month 2	1 (5.0)	1 (5.0)	2 (33.3)	0 (0.0)
Month 3	4 (20.0)	0 (0.0)	2 (33.3)	0 (0.0)
Patients with DAS28-ESR < 3.2 n (%)				
	Cohort A (n=20)	Cohort B (n=20)	Cohort C (n=6)	Placebo (n=7)
Month 1	3 (15.0)	1 (5.0)	2 (33.3)	0 (0.0)
Month 2	1 (5.0)	1 (5.0)	2 (33.3)	0 (0.0)
Month 3	4 (20.0)	0 (0.0)	2 (33.3)	0 (0.0)
DAS28-ESR mean (SD)				
	Cohort A	Cohort B	Cohort C	Placebo

	(n=20)	(n=20)	(n=6)	(n=7)
Baseline	6.2 (1.1)	5.8 (1.2)	6.2 (1.7)	6.0 (0.6)
Month 1	5.2 (1.6)	5.4 (1.2)	3.2 (3.0)	4.8 (1.0)
Month 2	5.3 (1.5)	4.7 (1,1)	3.6 (2.5)	6.0 (2.2)
Month 3	4.9 (1.7)	5.1 (1.1)	2.0 (0.7)	5.8 (0.4)

CRP, md/dl mean (SD)

	Cohort A (n=20)	Cohort B (n=20)	Cohort C (n=6)	Placebo (n=7)
Baseline	1.8 (2.3)	1.4 (1.4)	1.9 (1.8)	0.9 (1.0)
Month 1	1.0 (0.9)	1.7 (1.7)	1.1 (1.2)	1.0 (1.1)
Month 2	1.2 (1.2)	1.4 (1.5)	0.7 (0.5)	1.7 (2.7)
Month 3	1.3 (1.2)	1.7 (1.8)	0.7 (0.5)	1.3 (0.9)

Figure 1.

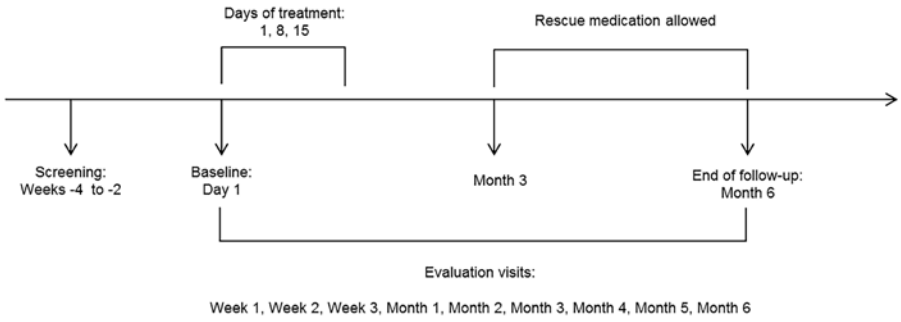

Days of treatment:
1, 8, 15
Rescue medication allowed
Screening:
Weeks -4 to -2
Baseline:
Day 1
Month 3
End of follow-up:
Month 6
Evaluation visits:
Week 1, Week 2, Week 3, Month 1, Month 2, Month 3, Month 4, Month 5, Month 6

Figure 2.

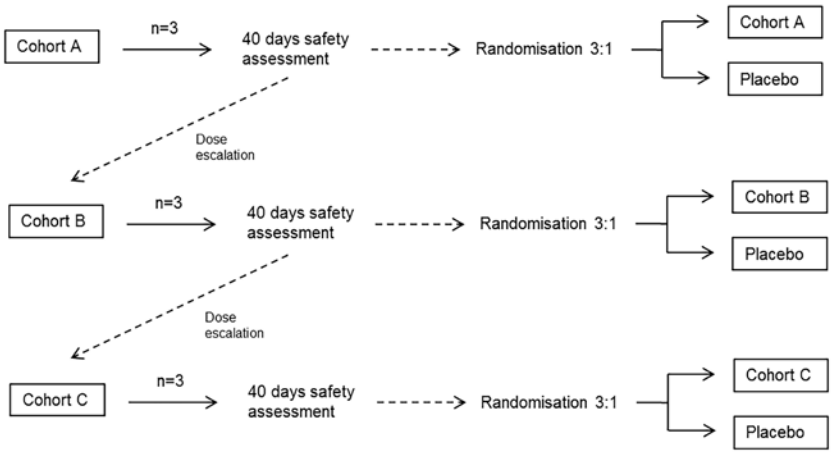
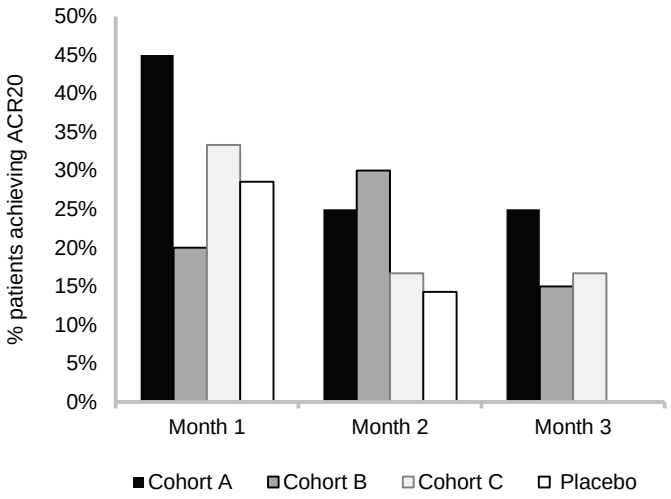
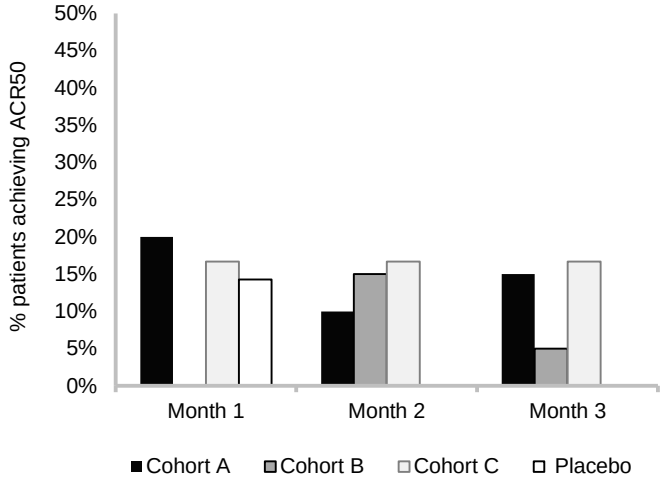

Cohort A
n=3
40 days safety assessment
Randomisation 3:1
Cohort A
Placebo
Dose escalation
Cohort B
n=3
40 days safety assessment
Randomisation 3:1
Cohort B
Placebo
Dose escalation
Cohort C
n=3
40 days safety assessment
Randomisation 3:1
Cohort C
Placebo

Figure 3.

a.



b.



c.

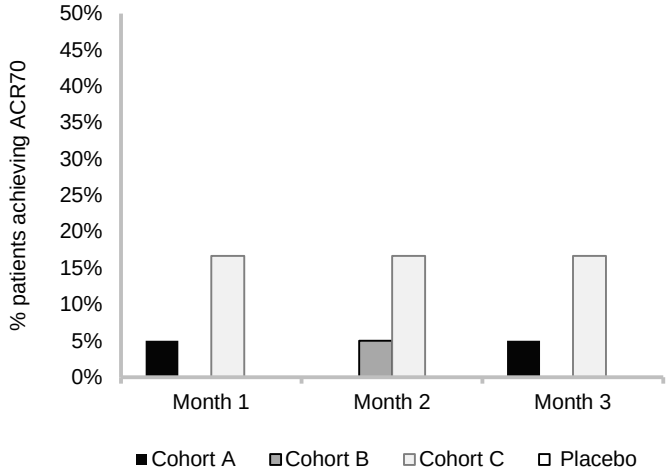


Figure 1. Study scheme. After a screening visit 2-4 weeks prior to study start, patients were randomised to one of the four study cohorts. Treatments were administered as three intravenous

doses one week apart (days 1, 8 and 15). Evaluations were conducted weekly for the first month, and then monthly till the end of follow-up at month 6. From month 3 onwards rescue medication with any DMARD was allowed, so that the relevant time-window for effectiveness assessment was considered till month 3.

Figure 2. Dose escalation scheme. Three patients were initially included in cohort A. After the evaluation of acute toxicity 40 days later, the dose escalation to cohort B was started (another 3 patients), and the expansion of the cohort A began (randomisation 3:1 to cohort A or placebo). Similarly, after the evaluation of acute toxicity in the three first patients in cohort B 40 days later, the dose escalation to cohort C was started (another 3 patients), and the expansion of the cohort B was initiated (randomisation 3:1 to cohort B or placebo). Finally, after the evaluation of acute toxicity at cohort C in the three treated patients 40 days after, the cohort C was expanded (randomisation 3:1 to cohort B or placebo).

Figure 3. Percent patients achieving ACR20, 50 and 70 responses till month 3 (ITT population); Patients were treated with Cx611 or placebo, administered intravenously at days 1, 8 and 15. Cohort A: 1 million cells/kg (n=20), Cohort B: 2 million cells/kg (n=20), Cohort C: 4 million cells/kg (n=6) and Placebo (n=7).

ON-LINE SUPPLEMENTARY MATERIAL

Table S1. Wash-out periods for biologics.

Biologic	**Minimum wash-out period duration**
Rituximab	6 months
Infliximab	12 weeks
Abatacept	10 weeks
Tocilizumab	10 weeks
Etanercept	8 weeks
Adalimumab	8 weeks
Certolizumab	8 weeks
Anakinra	3 days

Table S2. Infections reported, seriousness, outcome, and anti-infectives administered.

Infection	Seriousness	Outcome	Anti-infective(s) administered
Gastroenteritis	No	Recovered	None
Urinary tract infection	No	Recovered	Cefuroxime
Upper respiratory tract infection	No	Recovered	None
Upper respiratory tract infection	No	Recovered	None
Respiratory tract infection	No	Recovered	Amoxicillin / clavulanic acid
Herpes simplex	No	Recovered	Acyclovir, fusidic acid
Urinary tract infection	No	Recovered	Norfloxacin
Gastroenteritis	No	Recovered	None
Otitis media	No	Improvement	Amoxicillin / clavulanic acid
Upper respiratory tract infection	No	Recovered	Amoxicillin
Respiratory tract infection	No	Recovered	Azithromycin
Influenza	No	Recovered	None
Ear infection	No	Recovered	Amoxicillin / clavulanic acid, ciprofloxacin
Nasopharyngitis	No	Recovered	None
Urinary tract infection	No	Recovered	Ciprofloxacin
Respiratory tract infection	No	No changes	Azithromycin, levofloxacin
Pericoronitis	No	Recovered	Amoxicillin / clavulanic acid
Oral herpes	No	Recovered	None
Vulvovaginal candidiasis	No	Improvement	Fluconazole
Acute tonsillitis	No	Recovered	Amoxicillin / clavulanic acid
Upper respiratory tract infection	No	Recovered	None
Nasopharyngitis	No	Recovered	Unknown
Urinary tract infection	No	Recovered	Cefditoren
Urinary tract infection	No	Recovered	Fosfomycin

Infection	Seriousness	Outcome	Anti-infective(s) administered
Helicobacter infection	No	Recovered	Azithromycin, clarithromycin
Pharyngitis	No	Improvement	None
Upper respiratory tract infection	No	Unknown	None
Urinary tract infection	No	Recovered	Fosfomycin
Rash pustular	No	Recovered	None
Hordeolum	No	No changes	None

Table S3.Patient distribution of anti-HLA class I antibody responses against administered eASCs.
Presensitized: Patients with baseline anti-HLA class I antibodies not related to administered eASC.
Naïve: Patients without baseline anti-HLA class I antibodies

eASC arm (n=47)			
Presensitized		Naive	
20/47(43%)		27/47 (57%)	
anti-eASC	no antibodies	anti-eASC	no antibodies
6/20 (30%)	14/20 (70%)	3/27 (11%)	24/27 (89%)

Cohort A (n=21)				Cohort B(n=20)				Cohort C (n=6)			
Presensitized		Naive		Presensitized		Naive		Presensitized		Naive	
9/21 (43%)		12/21 (57%)		9/20 (45%)		11/20 (55%)		2/6 (33%)		4/6 (67%)	
anti-eASC	no antibodies	anti-eASC	no antibodies	anti-eASC	no antibodies	anti-eASC	no antibodies	anti-eASC	no antibodies	anti-eASC	no antibodies
3/9 (33%)	6/9 (67%)	1/12 (8%)	11/12 (92%)	3/9 ((33%)	6 (67%)	1/11 (9%)	10/11 (91%)	0	2/2 (100%)	1/4 (25%)	3/4 (75%)