SYNOPSIS

Study title:

A Phase I/II, multi-center, randomized, double-blind, placebo-controlled study, with a single ascending dose part followed by a multiple ascending dose part, evaluating the safety, pharmacokinetics, pharmacodynamics and efficacy of intravenous ALX-0061 in patients with rheumatoid arthritis.

Phase of development:

Phase I/II single ascending dose (SAD) and multiple ascending dose (MAD): first in-man/first-in-patient.

EudraCT number:

2010-022865-81

Protocol Code:

ALX-0061-1.1/10

Investigators and study centres:

Four countries (The Netherlands, Hungary, Czech Republic and Poland) participated in this study.

Study period:

- Start of Screening (SAD part): 21 March 2011
- End of SAD part: 06 September 2011
- Start of MAD part: 07 November 2011
- End of MAD part: 25 September 2012

Objectives:

- Primary objectives:

- To investigate the safety and tolerability of single and multiple doses of ALX-0061 given by intravenous (i.v.) injection/infusion to patients with rheumatoid arthritis (RA).
- To determine the maximum tolerated dose (MTD) and/or biologically effective dose (BED) of ALX-0061.

- Secondary objectives:

- To determine the efficacy of multiple dosing with ALX-0061 in patients with RA.

- To investigate the pharmacokinetics (PK) of ALX-0061 after single and multiple dosing in patients with RA.
- To investigate the pharmacodynamics (PD) of ALX-0061 after single and multiple dosing in patients with RA.
- To investigate the immunogenicity of ALX-0061 after single and multiple dosing in patients with RA.

Study design:

This was a multi-centre, randomized, double-blind, placebo-controlled, dose-escalation Phase I/II study in patients with active RA, which comprised a SAD and a MAD part.

- The planned SAD part consisted of up to five sequential treatment groups, at dose levels ranging from 0.3 mg/kg to 8 mg/kg. In each group, patients were assigned to receive a single i.v. dose of ALX-0061 or matching placebo (Group 1: ALX-0061 [N=2] or placebo [N=2]; Groups 2 to 5: ALX-0061 [N=6] or matching placebo [N=2]).

Dose escalation was to be stopped when the MTD or BED was reached, or as determined by the Sponsor and/or Investigator (based upon safety data from the lower dose level groups that would indicate a risk to patients treated at a higher dose level). At the end of the SAD part, an interim PK/PD analysis was performed to confirm the adequacy of the anticipated doses and dosing regimens to be used in the MAD part of the study.

- The MAD part consisted of three dose groups (Groups 6 to 8) of 12 planned patients each, who received multiple i.v. doses ranging from 1 mg/kg every 4 weeks (Q4W) to 6 mg/kg every 8 weeks (Q8W) of either ALX-0061 (N=10) or matching placebo (N=2).

An interim analysis of the MAD part data was performed after all patients in the MAD part had received 12 weeks of treatment (up to and including the Day 85 visit). The results of this interim analysis were not intended to have an impact on the further conduct of the trial, but were intended to help the Sponsor decide on further steps in the development program for ALX-0061.

The study drug administration was to be continued for an additional period of 12 weeks if no safety issues were identified during the first 12 weeks of treatment, with potential switch in study medication for placebo-treated patients, and potential dose escalation or increased frequency of administration for ALX-0061-treated patients, based on the European League Against Rheumatism (EULAR) response criteria (good, moderate or no response), assessed after 10 weeks at the Day 71 visit (see **Study treatment** below for additional details).

Study population and main criteria for inclusion:

The study population consisted of patients with active RA, who met the following main inclusion criteria:

- Age: 18 - 80 years, inclusive.
- Body Mass Index (BMI): < 35.0 kg/m^2.
- Gender: male or female.
- Diagnosed with RA according to the 2010 EULAR/American College of Rheumatology (ACR) criteria for at least 6 months prior to randomisation, and having composite disease activity score using 28 joint counts ($DAS28_{[CRP]}$) score ≥ 2.4 for the SAD part of the study and $DAS28_{[CRP]}$ score ≥ 3.2 and swollen joint count (SJC) ≥ 3 for the MAD part of the study.
- Treatment with methotrexate (MTX) for at least 12 weeks prior to screening, with a stable dose for at least 4 weeks before screening that was to remain stable throughout the study period (note: patients on any new/additional biologic disease-modifying anti-rheumatic drug (DMARD) therapy, cytotoxic drugs and immunosuppressants within 4 weeks prior to screening and between screening and Day 1 -with the exception of ALX-0061- were excluded from participation in the study).

Number of patients (planned and analysed):

It was anticipated to include up to 36 RA patients each in the SAD and MAD parts of the study. Patients from the SAD part could be re-randomised into the MAD part of the study based on the willingness of the patient, their eligibility according to the inclusion and exclusion criteria, and the discretion of the (blinded) Investigator. The number of patients that were planned, enrolled and analysed in the SAD and MAD parts of the study is given below.

SAD part of the study

Number of patients (N)	**Group 1** 0.3 mg/kg single i.v. dose	**Group 2** 1 mg/kg single i.v. dose	**Group 3** 3 mg/kg single i.v. dose	**Group 4** 6 mg/kg single i.v. dose	**Group 5** 8 mg/kg single i.v. dose	**Total**
Planned	4	8	8	8	8	36
Enrolled and analysed	4	8	8	8	0	28
• ALX-0061	2	6	6	6	0	20
• Placebo	2	2	2	2	0	8
Re-enrolled into the MAD part	2	4	4	4	0	14
• ALX-0061	1	3	2	4	0	10
• Placebo	1	1	2	0	0	4

As the Sponsor decided to immediately proceed to the MAD part of the study after completion of SAD Group 4 (6 mg/kg ALX-0061), no patients were enrolled in Group 5 and only 28 patients were finally included in the SAD part.

MAD part of the study

Number of patients (N)	Group 6 1 mg/kg Q4W	Group 7 3 mg/kg Q4W	Group 8 6 mg/kg Q8W	Total
Planned	12	12	12	36
Enrolled and analysed after roll-over from SAD part	6	4	4	14
• ALX-0061	5	4	32	12
• Placebo	1	0	1	2
Total enrolled and analysed	12	13*	12	37
• ALX-0061	10	11	10	31
• Placebo	2	2	2	6

Study medication:

The study medication (ALX-0061 or matching placebo) was given as a solution for injection/infusion, supplied in a 5.5 mL (extractable volume) glass vial. The route of administration was (slow and controlled) i.v. infusion, using a syringe pump at a planned constant infusion speed of 1.5 mL/min.

	Investigational Product	Placebo
Active substance	ALX-0061 Nanobody (10 mg/mL)	Visually matching inactive placebo/diluent with excipients.
Mechanism of action	Inhibition of the activity of both membrane-bound human Interleukin-6 receptor (IL-6R) and soluble IL-6R (sIL-6R)	

Study treatments:

In the SAD part of the study, eligible patients were to be sequentially assigned to the treatment groups and administered a single dose of ALX-0061 or placebo on Day 1 by slow i.v. infusion, according to the dose level for each treatment group:

- Group 1: single i.v. dose of 0.3 mg/kg ALX-0061 (N=2) or placebo (N=2).
- Group 2: single i.v. dose of 1 mg/kg ALX-0061 (N=6) or placebo (N=2).
- Group 3: single i.v. dose of 3 mg/kg ALX-0061 (N=6) or placebo (N=2).
- Group 4: single i.v. dose of 6 mg/kg ALX-0061 (N=6) or placebo (N=2).
- Group 5: single i.v. dose of 8 mg/kg ALX-0061 (N=6) or placebo (N=2).

Note that no patients were enrolled in Group 5. Although no dose-limiting toxicity (DLT) occurred, the Sponsor decided not to proceed with dose escalation in Group 5 (and not to perform an interim dose cohort analysis)

In the initial 12 weeks of the MAD part of the study, the following treatments were to be administered:

- Group 6: multiple i.v. doses of 1 mg/kg ALX-0061 BED (N=10) or placebo (N=2) once every 4 weeks (Q4W) for 12 weeks; in total 3 doses.
- Group 7: multiple i.v. doses of 3 mg/kg ALX-0061 BED (N=10) or placebo (N=2) Q4W for 12 weeks; in total 3 doses.
- Group 8: multiple i.v. doses of 6 mg/kg ALX-0061 BED (N=10) or placebo (N=2) once every 8 weeks (Q8W) for 12 weeks; in total 2 doses.

Since no safety issues were identified during the first 12 weeks of treatment, study drug administration was continued for an additional MAD period of 12 more weeks of treatment (up to 24 weeks), based on the EULAR response criteria assessed after 10 weeks (at the Day 71 visit), which included a switch in study medication for non-responding patients who received placebo, and dose escalation or increased frequency of administration for non-responding ALX-0061-treated patients, as follows:

- If a patient showed a good or moderate response according to the EULAR classification criteria of improvement in $DAS28_{[CRP]}$ relative to baseline, then the treatment (ALX-0061 or placebo) was to be continued at the same dose level and frequency for the subsequent 12 weeks (Week 13 to 24).
  - Group 6: multiple i.v. doses of placebo or 1 mg/kg ALX-0061 Q4W for another 12 weeks, in total 3 more doses.
  - Group 7: multiple doses of placebo or 3 mg/kg ALX-0061 Q4W for another 12 weeks, in total 3 more doses.
  - Group 8: multiple doses of placebo or 6 mg/kg ALX-0061 Q8W for another 12 weeks, in total 1 more dose.
- If a patient showed no response according to the EULAR classification criteria of improvement in $DAS28_{[CRP]}$ relative to baseline, then the treatment of the patient during the extended period from Week 13 to 24 depended on whether the patient received ALX-0061 or placebo in the first 12 weeks.
  - Group 6: multiple i.v. doses of 3 mg/kg ALX-0061 Q4W for another 12 weeks, in total 3 more doses.
  - Group 7: multiple doses of 6 mg/kg ALX-0061 Q4W for another 12 weeks, in total 3 more doses.
  - Group 8: multiple doses of 6 mg/kg ALX-0061 Q4W for another 12 weeks, in total 3 more doses.
- All patients who received placebo and showed no response, irrespective of whether they were enrolled in Group 6, 7 or 8, were to be switched to ALX-0061 during the extended period from Week 13 to 24 at the following dose level: multiple i.v. doses of 3 mg/kg ALX-0061 Q4W for another 12 weeks, in total 3 more doses.

Study duration:

Patients were screened for participation in this study within 28 days prior to first dosing. Eligible patients were admitted to the site on Day -1 (before first dose administration), and stayed at the study site until Day 3 or Day 4, based on the judgement of the Investigator. Patients participating in the SAD part returned for 6 or 7 ambulatory visits, up to 57 ± 5 days after the first dosing. Patients participating in the MAD part, who received up to 24 weeks of treatment, returned for 10 or 11 ambulatory visits, up to 169 ± 5 days after the first dosing. A Follow-up visit was scheduled 27 to 33 days after the last ambulatory visit. The total duration of the SAD part of the study for each patient was about 28 + 57 (± 5) + 30 (± 3) = 115 (± 8) days; the total duration of the MAD part of the study for each patient was about 28 + 169 (± 5) + 30 (± 3) = 227 (± 8) days.

Criteria for evaluation:

Safety

The following parameters were assessed as part of the safety evaluation at the time points defined in the schedules of assessments:

- Adverse events (AEs) and concomitant medications
- Clinical laboratory (clinical chemistry, haematology and urinalysis).
- Physical examination
- Vital signs.
- 12-lead electrocardiogram (ECG).

Pharmacokinetics

Serum concentrations of ALX-0061 were measured at the time points defined in the schedules of assessments.

Pharmacodynamics

The following biomarkers were measured as an assessment of PD at the time points defined in the schedules of assessments:

- C-reactive protein (CRP).
- Erythrocyte sedimentation rate (ESR).
- Serum amyloid A (SAA).
- Fibrinogen.
- Interleukin-6 (IL-6).
- Soluble IL-6 receptor (sIL-6R).
- Tumour necrosis factor-α (TNF-α).
- Interleukin-1 beta (IL-1β).
- Interferon-γ (IFN-γ).

Efficacy (Assessments Related to Clinical Activity)

The following assessments related to clinical activity were performed at the time points defined in the schedules of assessments, to determine the clinical response to treatment with ALX-0061 or placebo:

- ACR classification of functional capacity (performed at screening and baseline only).
- ACR assessment of criteria for ACR response.
- $DAS28_{[CRP]}$ score.
- EULAR response (determined after treatment only).
- RA MRI score (RAMRIS) (MAD part only).
- In addition, the Clinical Disease Activity Index (CDAI) and Boolean remission were further calculated for the MAD part only.

Immunogenicity

Immunogenicity of ALX-0061 was assessed by the detection of anti-drug antibodies (ADA) before and after treatment.

Statistical methods:

- PK parameters: descriptive statistics.
- PD parameters: descriptive statistics.
- Assessments related to clinical activity: descriptive statistics.
- Immunogenicity: descriptive statistics.
- Safety parameters: descriptive statistics.

Results:

SAD part of the study

Safety:

- A serious adverse event (SAE) of hypersensitivity was reported in one patient. No DLT occurred and the MTD was not reached in the SAD part.
- There was no clear increase in frequency or severity of AEs with increased doses of ALX-0061. The observed hypersensitivity is a documented risk for most biological agents used in humans, and no unexpected safety concerns emerged from the SAD part of the study.
- The most frequently reported AEs in patients treated with ALX-0061 were headache, arthralgia and infection. Of the total of 51 AEs reported in 14 of the 28 patients enrolled in the study (48 of which were considered to be related to treatment), 13 were mild, 25 were moderate and 13 were severe in intensity.

- In most patients who received ALX 0061, a decrease in the fibrinogen level was observed, as expected based on the mechanism of action of ALX-0061. However, no decreases in fibrinogen levels below 3 µmol/L were seen, and none of the low fibrinogen values were considered clinically relevant, with no AEs related to decreased fibrinogen levels reported. Increased aspartate aminotransferase (AST) and alanine transaminase (ALT) levels were reported as mild AEs in one patient in the 3 mg/kg ALX-0061 group who also had elevated ALT and gamma-glutamyl transferase (GGT) values at Screening. No other clinically relevant changes from baseline in safety laboratory test results, vital signs, ECG or physical examination were noted.
- No immunogenicity was noted throughout the SAD part of the study: a treatment-emergent ADA response was not observed for any patient.

Pharmacokinetics:

- Following single i.v. administration of ALX-0061 at escalating doses (0.3 mg/kg to 6 mg/kg) to patients with active RA, serum ALX-0061 concentrations increased with increasing dose. The highest mean serum ALX-0061 concentration was observed at the 6 mg/kg dose level.

Pharmacodynamics:

- Following the administration of a single dose of ALX-0061 (0.3, 1, 3 or 6 mg/kg), a rapid and marked decrease from baseline in CRP, ESR, fibrinogen and SAA (disease activity markers used to demonstrate PD effects) was observed, with a tendency for a dose-related increase in the duration of inhibition.
- After ALX-0061 administration, mean serum IL-6 and plasma sIL-6R concentrations increased. ALX-0061 showed a dose-related effect on the magnitude and the duration of the increased sIL-6R concentrations, confirming the hypothesis that sIL-6R can be used as a potential biomarker to determine engagement of ALX-0061 on its target receptor.
- The DAS28 score and EULAR response showed an improvement for all doses of ALX-0061. Combined with the other results of the SAD part of the study, the assessments related to clinical activity indicated that the 1, 3 and 6 mg/kg single i.v. doses warranted further clinical investigation. The effects of the 1, 3 and 6 mg/kg doses appeared similar in magnitude, however, upon visual inspection of the data, the duration of effect seemed longer for the highest dose(s).

MAD part of the study

The results from the 24-week MAD part of the study were in line with the SAD part results; conclusions from the MAD part of the study are as follows:

Safety:

- Repeated doses of ALX-0061 at doses up to 6 mg/kg Q4W were well tolerated in all treatment groups, with no unexpected safety concerns. There was no evidence to suggest that an MTD was reached, and no apparent dose relationship with treatment-related TEAEs or withdrawals. No accumulation of effects or worsening over time, and no DLTs were observed after treatment for 24 weeks.
- In the combined ALX-0061 group [ALX-unmodified (24w) + ALX-modified (24w) + PLC Switchers], a total of 113 TEAEs were reported in 24 of the 34 ALX-0061-treated patients.
    - The most common TEAEs (occurring in > 2 patients) in the combined ALX-0061 group included headache, increased ALT and AST, arthralgia, back pain and joint swelling.
    - The most frequent TEAEs (> 2 events) in the combined ALX-0061 group included headache, increased ALT and AST, arthralgia, back pain, joint swelling, dizziness, rash, muscle spasm, abdominal pain upper, nasopharyngitis and fatigue.

  Nearly all TEAEs resolved during the treatment period, without sequelae, and most were mild or moderate in intensity.
- There were no meaningful differences in safety profile among the different treatment groups, including patients switching from placebo to ALX-0061, or changing to a higher ALX-0061 dose level or to a more frequent ALX-0061 dosing regimen after 12 weeks of treatment.
- Two patients reported SAEs (2 each, 4 SAEs in total), assessed as not or remotely related to study drug (haemorrhagic gastritis and upper gastrointestinal haemorrhage, and 2 events of cerebrovascular accident). One patient died due to an SAE of cerebrovascular accident, which was assessed as remotely related to study drug.
- In line with the mechanism of action of ALX-0061, dose-related decreases in neutrophil and platelet count were reported, however, these did not reach clinically relevant levels, and no serious infections or associated TEAEs were reported.
- Fluctuations in transaminases were observed, but these were generally transient and were reported as TEAEs in few patients. One patient had moderate elevation in liver enzyme parameters reported as TEAEs, which resolved spontaneously and without study drug interruption. There were no signs of dose-related increases in liver enzymes (ALT/AST) or lipid (cholesterol/LDL) levels beyond the normal ranges, and no meaningful trends across dose levels.
- A treatment-emergent ADA response was not observed for any patient.

Pharmacokinetics:

- Following multiple i.v. administration of ALX-0061 at escalating doses (1 mg/kg to 6 mg/kg) to patients with active RA, serum ALX-0061 concentrations increased with increasing dose. The highest mean serum ALX-0061 concentration was observed at the 6 mg/kg dose level.
- Mean serum concentrations and PK parameters were similar after the first dose during the SAD and MAD part of the study at the same dose level.
- Steady-state was reached after the second administration.

Pharmacodynamics:

- In line with the SAD part results, rapid and long-lasting decreases in mean CRP, ESR, fibrinogen and SAA concentration were observed following multiple i.v. administration of ALX-0061, with a tendency for a dose-related increase in the duration of inhibition. These results confirmed the SAD PK/PD modelling predictions that all ALX-0061 groups received biologically effective doses.
- As expected from the mechanism of action of ALX-0061, mean serum IL-6 and plasma sIL-6R concentrations increased rapidly after first ALX-0061 administration, with a tendency for a dose-related increase in the duration of the effect. These results confirmed that both IL-6 and IL-6R concentrations can be used as biomarkers for target engagement by ALX-0061.

Efficacy (Assessments Related to Clinical Activity):

- In line with the PD changes, an improvement in ACR, EULAR, DAS28$_{[CRP]}$, CDAI and Boolean remission responses was seen for all ALX-0061 groups.
  - ACR:
    - The ACR results showed that all ALX-0061 doses were clinically active, with ACR20 response rates of up to 75% (1 mg/kg and 3 mg/kg Q4W (24 weeks) groups; n=8 each) seen after 12 weeks of treatment.
    - Of those who received [ALX-unmodified (24w) + ALX-modified (24w) + PLC Switchers], 26/31 (84%) achieved ACR20, 21/31 (68%) achieved ACR50 and 16/31 (52%) achieved ACR70.
  - DAS28$_{[CRP]}$:
    - The DAS28[CRP] scores showed rapid and long-lasting improvements from Day 8 onwards at all ALX-0061 dose levels and treatment combinations tested.
    - In the combined ALX-0061-treated group [ALX-unmodified (24w) + ALX-modified (24w) + PLC Switchers], 18/31 patients (58%) achieved remission, 5/31 (16%) showed low disease activity, 8/31 (26%) showed moderate disease activity and no patients reported high disease activity after 24 weeks of treatment.

- EULAR:
  - Similar to ACR scores, ALX-0061 showed a strong effect on EULAR response at all dose groups and treatment combinations, with 71% of the ALX-0061-treated patients (including ALX-unmodified (24w) + ALX-modified (24w) + PLC Switchers) reaching a good EULAR response at the end of the 24 weeks of treatment.
  - In the combined ALX-0061-treated group [ALX-unmodified (24w) + ALX-modified (24w) + PLC Switchers], 22/31 patients (71%) showed a good EULAR response, 8/31 (26%) showed a moderate EULAR response, and 1/31 patient (3%) showed no EULAR response.
- CDAI:
  - Similar to the other clinical activity scores, CDAI scores improved throughout the entire 24-week MAD part of the study.
  - In the combined ALX-0061-treated group [ALX-unmodified (24w) + ALX-modified (24w) + PLC Switchers], after 24 weeks of treatment, 11/31 (36%) of patients achieved remission, 12/31 (39%) showed low disease activity, 7/31 (23%) showed moderate disease activity and 1/31 patient (3%) showed high disease activity.
- RAMRIS:
  - Minor changes were noted in several RAMRIS parameters, but did not show any clear trends among the different ALX-0061 treatment groups.
  - Considering the limited disease activity at the start of the MAD part, the extent of bone marrow oedema was the only RAMRIS parameter that allowed meaningful assessment. A small but consistent reduction in the extent of bone marrow oedema, an important radiographic indicator of disease activity and a precursor for bone erosion, was noted in the pooled ALX-0061 (24 weeks) group, with an overall decrease in the bone marrow oedema score from baseline of 25.2% (and maximum decrease of 83.3%) at Week 24. The RAMRIS scores showed no worsening in the ALX-0061-treated groups after 24 weeks of treatment.
- Boolean remission:
  - For all ALX-0061-treated patients pooled together [ALX-unmodified (24w) + ALX-modified (24w) + PLC Switchers], a total of 8/31 (25.8%) patients achieved Boolean remission at Week 24. In the ALX-unmodified (24w) group, Boolean remission was achieved by a total of 7/24 (29.2%) patients at Week 24. In the ALX-modified (24w) group, 1 patient (in the 3 mg/kg Q4W (12 weeks) to 6 mg/kg Q4W (12 weeks) group) achieved remission at the end of the 24-week treatment period. None of the placebo-treated patients (including PLC Switchers and PLC Non-Switchers) achieved Boolean remission during the MAD part of the study.