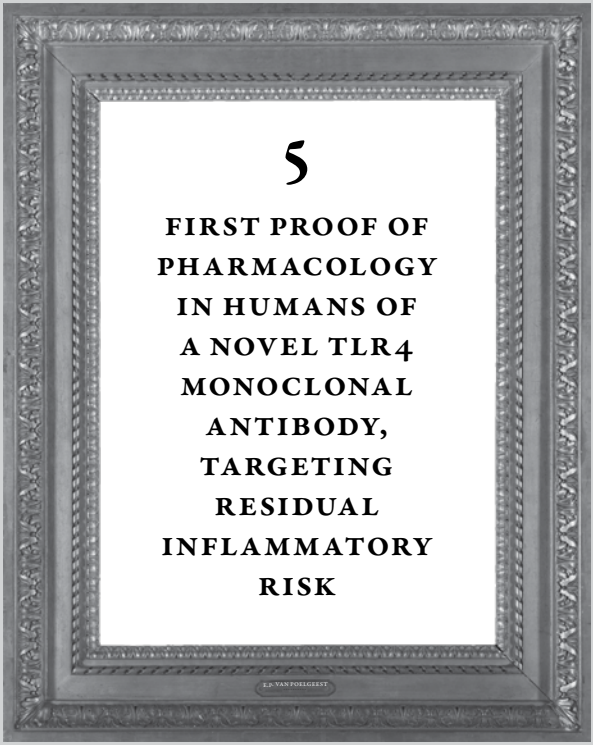
Future drugs in atherosclerotic cardiovascular disease

Poelgeest, E.P. van



5

FIRST PROOF OF PHARMACOLOGY IN HUMANS OF A NOVEL TLR4 MONOCLONAL ANTIBODY, TARGETING RESIDUAL INFLAMMATORY RISK

E Monnet, G Lapeyre*, EP van Poelgeest*, P Jacqmin, K de Graaf, J Reijers, M Moerland, J Burggraaf, C de Min. *Evidence of NI-0101 pharmacological activity, an anti-TLR4 antibody, in a randomized phase 1 dose escalation study in healthy volunteers receiving LPS.* Clinical Pharmacology and Therapeutics 2017 Feb;101(2):200-208. (* Equal contribution)

ABSTRACT

Toll-like receptor (TLR) 4 pathways are major contributors to pathological inflammatory responses induced by tissue damage. NI-0101 is the first monoclonal antibody blocking TLR4 signaling. This activity is independent of the ligand type and concentration, therefore blocking potentially any TLR4 ligands. A Phase 1 single ascending dose study was conducted in 73 healthy volunteers (HV) to evaluate NI-0101 tolerability, preliminary safety, pharmacokinetics and pharmacodynamics, in absence and in presence of a systemic challenge with lipopolysaccharide (LPS), a TLR4 ligand. NI-0101 was well tolerated without safety concern. The pharmacokinetic profile was characterized by a half-life of approximately 10 days at high concentrations and by a rapid elimination at low concentrations due to expected target mediated drug disposition. NI-0101 prevented cytokine release following *ex vivo* and *in vivo* LPS administration and also prevented the CRP increase and the occurrence of flu-like symptoms expected following the *in vivo* administration of LPS.

INTRODUCTION

NI-0101 is a humanized IgG1κ monoclonal antibody (mAb) engineered by Novimmune to bind to and block the activation and signaling of human TLR4 by interfering with its dimerization, independently of the ligand type and concentration. This characteristic confers NI-0101 its potential to block any TLR4 ligands present in pathological conditions and inducing inflammatory signals. NI-0101 binds with high affinity to human TLR4 (Kd = 139 pM). NI-0101 engineering destroyed the binding sites for interactions with FcγRIII and the complement component, C1q, while maintaining the binding to FcγRI and to FcγRIIa. This removed the ability of NI-0101 to provoke antibody dependent cellular cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC). TLR4 blockade is enhanced by NI-0101 binding to Fcγ receptors [1]. *In vitro* data with human whole blood have shown that a polymorphism at amino acid position 131 (R/H) of the FcγRIIa affects the potency of NI-0101 for blocking TLR4-mediated cytokine release (NI-0101 IC_{50} in LPS-induced IL6 release were 2191, 223 and 128 pM for 131HH, 131HR group and 131RR, respectively).

In vitro and *in vivo* preclinical pharmacology and toxicology studies performed with NI-0101 or a functionally equivalent anti-mouse TLR4 mAb, 5E3, serving as a surrogate molecule, showed no safety concerns (data on file). NI-0101 is being developed for the treatment of inflammatory disease, in particular Rheumatoid Arthritis (RA). Despite advances in the treatment of RA brought by biologic treatments, approximately 40% of patients require switching biological treatments because of inadequate response or loss of response, and only 30% achieved sustained remission [2].

RA is an immunologically driven condition characterized by persistent joint (synovitis) and systemic inflammation, and presence of autoantibodies, in particular against citrullinated proteins [3]. citrullinated proteins and anti-citrullinated protein antibodies (ACPA) can form immune complexes in inflamed joints. These immune complexes are considered to be inflammatory mediators and similarly to the so called Damage-Associated Molecular Pattern molecules (DAMPs) [4, 5, 6]. DAMPs are recognized by TLR family expressed in particular on monocytes and macrophages responsible for innate inflammatory response, and by osteoclasts and chondrocytes contributing to cartilage erosion and destabilizing repair mechanisms [7]. TLR is a family of at least 11 proteins that recognize structurally conserved molecules derived from microbes. The bacterial component, LPS, binds TLR4 [4]. In addition, literature reports that immune complexes formed between citrullinated proteins and their associated ACPA can engage TLR4 and Fcγ

receptors on immune cells [6, 8]. Engagement of immune complexes present in RA patients with FcγRIIa has been shown to enhance inflammation[9] and higher levels of FcγRIIb is prognostic of lower disease activity in RA and dampens TLR4 activation.[10] TLR4 activation leads to cytokine production including TNFα and IL6, which are established therapeutic targets in ra.[11] Investigations using RA human cells stimulated with RA synovial fluids have shown that cytokine release is inhibited by NI-0101 when specific ACPA are present in the RA synovial fluids (data submitted for publication). In addition, 5E3 in the mouse Collagen Induced Arthritis model and the IL1Rn-/- arthritis mouse model prevented disease progression. Based on these data, specific blockade of TLR4 activation by NI-0101 appears as a promising therapeutic approach in RA.

We report the results of the Phase 1 study assessing the initial tolerability, safety, and PK/PD profiles of NI-0101 after infusion of single escalating iv doses in HV. The study aimed to establish evidence of human pharmacology by evaluating the capacity of NI-0101 to prevent the effects of the *ex vivo* and *in vivo* administration of a TLR4 ligand, LPS. In Part 1, NI-0101 was administered at single ascending doses with *ex vivo* LPS challenges. In Part 2, the administration of NI-0101 was followed by both *ex vivo* and *in vivo* LPS challenges. NI-0101 PD profile was evaluated by measuring the levels of IL6 and TNFα (MYD88 pathway) and the levels of IFNγ and CXCL10 (TRIF pathway) after the LPS challenges. *In vivo* LPS effects on CRP, hematological parameters and vital signs were assessed in the presence and absence of NI-0101 to further demonstrate its pharmacological effects. Finally, the anticipated impact of the FcγRIIa polymorphism on NI-0101 PD profile was assessed in the 3 FcγRIIa genotypes HH, RH and RR equally represented in the study.

METHODS

All study documentations were submitted to the Medical Ethics Committee of Academic Medical Center (AMC) in Amsterdam and the CCMO (Central Committee on Research involving Human Subjects; Competent Authority) for approval. The study was conducted in accordance with the principles set forth in the Declaration of Helsinki, the Guidelines of the International Conference on Harmonisation (ICH) on Good Clinical Practice (GCP) (CPMP/ICH/135/95), European Union (EU) Directive 95/46/EC, the Dutch Act on Medical Research involving Human Subjects (Wet Mensgebonden Onderzoek, WMO), and other applicable regulatory requirements.

STUDY DESIGN The study was a randomized, double-blind, placebo-controlled, PK/PD guided, Phase 1 study in HV adults. Subjects were administered for 2 hours iv infusion of either placebo (saline solution) or NI-0101 in saline solution. Part 1 objectives were to assess the tolerability, safety, PK and PD with *ex vivo* LPS challenge after single ascending dose of NI-0101, taking into account the FcγRIIa genotype. Fifty-seven subjects received escalating doses of NI-0101, from 0.001 mg/kg to 15 mg/kg, or placebo. Male subjects were included in all cohorts of both parts. Because of potential effect of menstrual cycle on TLR expression, female subjects were only included from the moment that a certain NI-0101 dose had achieved complete inhibition of *ex vivo* LPS-induced cytokine release in men; female subjects participated in cohorts 3 to 7 of Part 1. Each cohort included at least 2 representatives of each of the 3 FcγRIIa-131 genotypes (H/H, R/R, R/H) who received NI-0101. Genotype was not specified for placebo subjects. Nine subjects were enrolled in the first cohort (NI-0101:placebo, 6:3), and all subsequent cohorts enrolled 8 subjects (NI-0101:placebo, 7:1, including one subject receiving the same dose as the previous cohort). NI-0101 PD effects were assessed by whole blood *ex vivo* stimulation with LPS.

Part 2 objectives were to assess the tolerability, safety, PK and PD with *ex vivo* and *in vivo* LPS challenge after single selected doses of NI-0101. The first cohort (0.01 mg/kg) enrolled 4 subjects (NI-0101:placebo, 3:1) and the second cohort (0.25 mg/kg) enrolled 12 subjects divided into three subgroups (2a, 2b and 2c), each composed of 4 subjects (NI-0101:placebo, 3:1). Subjects also received an *in vivo* LPS challenge at 2 ng/kg, either immediately at the end of NI-0101/placebo infusion (cohort 1 and cohort 2a), or at a delayed time-point (22 or 40 days, for cohort 2b and 2c, respectively) after infusion. For all groups, the selected genotype of the subjects who received active treatment was 131R/R and 131R/H (ratio of 1:2 per group). The genotype of placebo subjects was not specified.

The assignment of subjects and treatments to the randomization schedule was done with SAS (SAS Institute, USA) by CHDR's statistician. All personnel involved in the execution and evaluation of the study (except statistician generating the study randomization schedule) was blinded.

NI-0101 drug product (manufactured by Nova Laboratories Ltd, UK) and matching placebo compositions were L-Histidine (1.88 mg/mL), L-Histidine monohydrochloride monohydrate (2.70 mg/mL), Sucrose (68.46 mg/mL), Polysorbate 80 (0.05 mg/mL), water for injection to 1 mL, pH 6.0. LPS lot#3 was manufactured in the United States of America by the National Institute of Health.

The first subject was enrolled on 29 October 2012 and last subject completed the study on 27 March 2014. All patients were followed until NI-0101 concentration could not be measured or close to the limit of quantification. The study was registered on the following databases: http://www.clinicaltrials.gov (NCT01808469) and on http://eudract.emea.eu.int (2012-003657-28).

DOSE SELECTION As NI-0101 does not bind to TLR4 in any animal species commonly used for preclinical safety assessment, a mouse surrogate antibody (5E3) was used for *in vivo* pharmacology, pharmacokinetics and toxicology studies. In a 4-week toxicity study in mice, no unintended effects of 5E3 were observed at the highest dose tested (240 mg/kg/week intravenously). The Minimal Anticipated Biological Effect Level (MABEL) was assessed by a modeling and simulation approach, using data from *in vitro* human whole blood LPS challenges and target occupancy data, and predicted PK based on other NovImmune antibodies and canakinumab. As consequence, the starting dose for Part 1 of the study was calculated to be 0.001 mg/kg, predicted to result in a 3-37% reduction in *ex vivo* LPS-induced cytokine release, dependent on FcγRIIa genotype. Dose selection for subsequent cohorts was guided by PK/PD data from preceding cohorts.

For Part 2, the starting dose was selected based on *ex vivo* cytokine release inhibition observed in Part 1, with an anticipated inhibition of *in vivo* LPS-induced cytokine production of at least 75% with duration of 3 days. The second dose to be administered was intended to induce prolonged (i.e. up to 28 days) and marked (≥90%) inhibition of *in vivo* LPS-induced cytokine production.

STATISTICAL METHODS All statistical analyses were exploratory; therefore, no formal sample size calculation was performed. As standardly conducted in phase 1 studies to allow initial PK/PD assessment, a minimum of 6 subjects per dose were included in part 1 (a minimum of 2 subjects with each FcγRIIa genotype) and 4 subjects per group were included in part 2 (RH or RR FcγRIIa genotypes only) in order to explore the *in vivo* LPS effect in presence of NI-0101 over time. No adjustments were made to compensate for multiple testing. Placebo subjects from each cohort were pooled into a single placebo group. Safety and PD data were pooled by defined analysis periods in Part 2: outside of the LPS challenge period and 72h post the LPS challenge. For all continuous variables, summary statistics included n, mean, median, standard deviation, minimum and maximum. No descriptive statistics were calculated in the event of very few observations ($n < 3$); if N=2, only the arithmetic mean was calculated; if N=1, the single value was reported. PK parameters were determined using serum concentrations with WinNonlin (Certara, USA) and SAS for dose proportionality assessment, and presented with 3 significant digits, except for T_{max}, which was presented to one decimal place. Change and percentage change from baseline was computed for selected parameters.

FCΓRIIA GENOTYPING DNA was extracted from whole blood using the QiaAmp DNA Blood Mini Kit (Qiagen) and analysis was performed at Eurofins Medigenomix GmbH (Ebersberg, Germany). 50 ng of DNA was used for PCR and sequencing using primers detecting the FcγRIIa-131 R/H mutation.

PK ASSAY The serum concentrations of NI-0101 were determined by Covance Laboratories (Harrogate, UK) using an ELISA on the Gyrolab™ xP immunoassay platform (Gyros, Sweden), using biotin labelled mouse IgG1 kappa anti-NI-0101 idiotype monoclonal antibody 4C4 (Novimmune SA) as capture reagent and Alexa Fluor® 647 labelled 4C4 as detection reagent.

EX VIVO LPS CHALLENGE Six mL of blood were collected in sodium heparin (Greiner). Blood was diluted (1:1) with RMPI 1640 medium with 25 mM Hepes and L-Glutamine in the presence of LPS (2.5 ng/mL). The samples were incubated for 24 hours at 37°C (5% CO_2), then were centrifuged (20 minutes 2000G at 20°C) prior to measure cytokines in the supernatant. IL6, TNFα and CXCL10 were measured with R&D Quantikine® ELISA and Multi-array Meso Scale Discovery® by Good Biomarker Sciences (Leiden, Netherlands). IFNγ was measured by ELISA Verikine HStm (PBL-Interferon source) by Novimmune (Plan-Les-Ouates, Switzerland).

ANTI-DRUG ANTIBODY (ADA) ASSAY Evaluation of Ada against NI-0101 was performed by Covance (Horrogate, UK) using bridging ELISA on the Gyrolab™ xP immunoassay platform, with biotin labelled NI-0101 as capture reagent and Alexa Fluor® 647 labelled NI-0101 as detection reagent, and followed the tiered approach design.

RESULTS

DEMOGRAPHICS Demographic data are summarized in Table 1a and Table 1b for the *ex vivo* (Part 1) and *in vivo* (Part 2) LPS challenges, respectively. Fifty seven subjects (9 received placebo and 48 received NI-0101) were recruited in Part 1 and 16 subjects in Part 2 (4 received placebo and 12 received NI-0101). No differences between cohorts were observed for ethnicity (primarily white), age (mean between 21.0 and 30.3 years) and BMI (mean between 21 and 25 kg/m^2).

SAFETY Overall, single infusions of NI-0101 were well tolerated and no safety concern was identified up to the highest dose of 15 mg/kg. In total 74% of the 73 HV reported 140 treatment-emergent adverse events (TEAEs), including 27 events reported by 8 subjects who received placebo. Overall, the type, intensity, duration,

and frequency of AEs were comparable across the placebo and all NI-0101 groups, as well as across all 3 FcγRIIa genotypes (data not shown). The majority of AEs were mild and resolved within hours to days. None of the AEs was severe or serious and there were no AE-related withdrawals. The most commonly reported AE was headache (35% in NI-0101-treated subjects, and 23% in placebo-treated subjects). The most frequently reported system organ class was infections and infestations with predominantly nasopharyngitis (21 events reported by 20 subjects, time of onset 2-62 days after NI-0101/placebo infusion; most being of mild intensity and not requiring treatment) and gastro-enteritis (3 events reported by subjects at 2 different doses and in placebo groups). Two Gram-negative bacterial infections (*E.coli* urinary tract infections) were reported in 2 female subjects with reported history of urinary tract infections, one following placebo and one following NI-0101 infusion. Nine events attributable to 'Influenza-like illness' were observed in different NI-0101 dose groups; none corresponding to an infusion-related reaction, as their onset were between 12 and 47 days post-infusion. Most of the other AEs occurred as single events across different system organ classes in individual subjects (see Table 2) and are events commonly reported in Phase 1 studies.

Low anti-NI-0101 antibody positive titers were observed post-infusion in 5 out of 60 subjects who received NI-0101 and only at a single time point. The presence of pre-infusion positive titers in 2 of these subjects, in one placebo and the low titers suggest a degree of non-specificity of the assay or cross-reactivity to pre-existing antibodies.

No clinically relevant changes were observed in clinical chemistry, hematology, coagulation tests, vital signs and physical examinations. No trend was noticed in electrocardiogram results and in particular there was no evidence of an effect on the QT_c interval. In few subjects receiving NI-0101 or placebo, sporadic mild elevations of IL6 were observed, as expected as part of the normal variability [12, 13] and without concomitant clinical effects. No elevation was observed for other cytokines following NI-0101 administration. Importantly, these data suggest the absence of TLR4 agonist activity of NI-0101.

PHARMACOKINETICS As shown in Figure 1, NI-0101 maximal mean serum concentrations were achieved between the end of infusion and 2h after end of infusion with the highest value achieved at the dose of 15 ng/mL (367,000 ng/mL) and comparable concentrations between Part 1 and Part 2 (118 and 99.5 ng/mL at 0.01 mg/kg and 5,850 and 5,270 ng/mL at 0.25 mg/kg for Part 1 and 2, respectively). As expected, at the dose of 0.001 mg/kg, NI-0101 serum concentrations were not quantifiable.

The higher the administered dose, the longer the NI-0101 concentrations were quantifiable in serum, i.e. 4 days after infusion of 0.01 mg/kg and up to 18 weeks after infusion of 15 mg/kg (the last sampling time), but close to the limit of quantification. The PK profile of NI-0101 was neither influenced by the administration of LPS nor by the different FcγRIIa genotypes (see supplementary Table S1 online).

The maximal NI-0101 concentration (C_{max}), the end-of-infusion NI-0101 concentration (CE01) and the exposure (AUC_{inf}) increased with the dose (see Table 3). However, a nonlinear profile was observed, particularly at lower doses, because of the expected target mediated drug disposition (TMDD). The PK profiles and the calculation of the clearance per dose group indicated that the terminal elimination of NI-0101 decreased with increasing dose to reach a plateau at the dose of 1 mg/kg. Different phases in the elimination of NI-0101 could be observed, especially at higher doses when the target-mediated elimination pathway was saturated. The half-life of the linear elimination phase, calculated based on the pooled observations between day 20 and 80 of the 15 mg/kg dose group, was estimated to be approximately 10 days (k_{el} = -0.0027 h-1; data not shown). The volume of distribution (V_{ss}) was larger than the total blood volume suggesting a distribution of NI-0101 in the tissues.

PHARMACODYNAMICS *Ex vivo* LPS challenge. The PD profile of NI-0101 obtained after *ex vivo* LPS challenges is presented in Figure 2 for IL6 per FcγRIIa genotype.

A dose-dependent inhibition of IL6, TNFα, CXCL10 and IFNβ (see supplementary Table S2 online) release induced by an *ex vivo* LPS challenge was observed after a single administration of NI-0101 compared to placebo. The inhibitory effect was comparable for all cytokines measured, lasted longer when the dose of NI-0101 was increased and was influenced by the FcγRIIa genotype. For the FcγRIIa genotype H/H (Figure 2A), inhibition of IL6 release was 49.0% at the 0.05 mg/kg dose of NI-0101 and > 90% at the dose of 0.25 mg/kg and at higher doses. For the FcγRIIa genotype R/H (Figure 2B) and R/R (Figure 2C), the average inhibition of IL6 release at the end of the NI-0101 infusion was already > 70% and > 80%, respectively, at the 0.01 mg/kg dose of NI-0101 and > 90% at the dose of 0.05 mg/kg and at higher doses. The average duration of a significant inhibition (>80%) of cytokine release increased with the increase of the dose to reach between 8 weeks and 14 weeks at the 15 mg/kg dose, depending on the FcγRIIa genotype (H/H<<R/H<R/R).

IN VIVO LPS CHALLENGE NI-0101 pharmacological effects were studied in Part 2 of the study by the administration of an *in vivo* LPS challenge at three different

time-points after the end of NI-0101 infusion in three separated subgroups of HV (end of NI-0101 infusion, 22 days later and 40 days later).

In subjects administered placebo, an increase in serum IL6 concentration was observed with a maximal measured concentration achieved at 2h after the LPS challenge (Figure 3A). In subjects administered NI-0101 at the 0.01 or 0.25 mg/kg dose and who received the LPS challenge immediately after the infusion, no increase in serum cytokine concentration was observed. This absence of IL6 induction was also observed in subjects exposed to the LPS challenge 22 days after NI-0101 infusion. However, in subjects administered NI-0101 at a dose of 0.25 mg/kg and exposed to the LPS challenge 40 days after the NI-0101 infusion, an increase in serum IL6 concentration was observed with a maximal measured concentration achieved post LPS challenge approximately after 2h. Similar increase in serum concentration was also observed for TNFα, CXCL10 and IFNγ (data not shown).
Consistent with the observation on cytokines, LPS induced CRP release was prevented by NI-0101 up to 22 days after iv infusion at a dose of 0.25 mg/kg (Figure 3B). Similarly, prevention of LPS induced changes in neutrophils and leucocytes counts, coagulation factor (aPTT), heart rate (data not shown) and temperature (Figure 3C) was observed in presence of NI-0101.

Overall, duration of NI-0101 effect after *in vivo* and *ex vivo* LPS administration was comparable.

DISCUSSION

We report on the first-in-human administration of NI-0101, an antibody binding and blocking TLR4, while ENGAGING FcγRI and FcγRII. In this study, single ascending doses of NI-0101 were administered to HV. *Ex vivo* and *in vivo* LPS challenges allowed assessing the pharmacological activity of NI-0101. Dose escalation decisions and follow-up duration were guided by interim analysis and modeling of PK/PD data.

Similarly to previous clinical experiences with other molecules blocking TLR4[14,15], as well as with an anti-TLR2 antibody16, NI-0101 was well-tolerated in HV at doses up to 15 mg/kg with no apparent dose-event or genotype-event relationship. No infusion-related adverse events were reported. No clinically relevant increases of cytokines were observed. The absence of indications for increased susceptibility to gram negative bacteria for the duration of TLR4 blockade (up to 14 weeks following the administration of NI-0101 at 15 mg/kg) is in line with published data on TLR4-independent responses to LPS, such as autophagy, endocytosis, phagocytosis, oxidative burst and inflammasome activation[17,18] demonstrating that the immune system is endowed with alternative pathways for a single offensive stimulus.

The observed PK profile of NI-0101 was non-linear due to the expected TMDD at low NI-0101 concentrations and was characterized by a long half-life of approximately 10 days as reported by most mAb-targeting cell surface receptors.[16,19,20,21]The elimination of NI-0101 decreased with increasing doses, reaching a plateau at 1 mg/kg, likely reflecting the expected saturation of the targeted receptor, as previously described for an antibody targeting TLR2.[16] The presence of a TLR4 ligand (i.e. LPS) *in vivo* did not influence the PK of NI-0101. Considering this observation and the known ligand independent effect of NI-0101 *in vitro*, it can be speculated that the presence of TLR4 ligands in pathological conditions will not influence NI-0101 PK. Finally, NI-0101 elimination was not affected by the FcγRIIa-131 R/H polymorphism.

Consistent with NI-0101 PK, MYD88 (CXCL10 and IFNγ) and TRIF (IL6 and TNFα pathways were completely blocked in blood upon the administration of NI-0101 from the dose of 1 mg/kg onwards. At lower doses, NI-0101 achieved incomplete blockade of cytokine release induced by LPS. The maximum duration of complete inhibition also increased with the dose, up to 14 weeks for TNFα (supplementary Table S3) at the highest dose tested (15 mg/kg) depending on the genotype. The long lasting effect observed for the higher doses of NI-0101 is associated with the well-known long half-life of monoclonal antibodies. This PK/PD characteristic gave the opportunity to perform an initial safety evaluation of the consequences of a prolonged inhibition of the TLR4 pathway, up to 14 weeks. It also offers flexibility in the choice of dosing regimen which can be adapted to satisfy the requirements to study NI-0101 at different development stages or in different indications.

As predicted by the PK/PD simulations, subjects with an FcγRIIa HH genotype showed a shorter duration of inhibition compared to RR or RH genotypes. This observation was consistent with previous IC_{50} data reported from *in vitro* experiments (internal report), which was considered in the design of the study where FcγRIIa genotype (HH) was selected to be enrolled in the sentinel group of the first cohort as a safety precaution. Future studies will address the effect of the FcγRIIa genotype on clinical response.
In Part 2 of the study, the *in vivo* effects of LPS on clinical parameters (such as body temperature and heart rate) and laboratory parameters (CRP, aPTT, platelets and leucocytes) were completely prevented by the administration of NI-0101. These *in vivo* observations in humans provide a confirmation of the hypothesized mechanism of action of NI-0101.

The magnitude and duration of the effects of NI-0101 were well comparable after *ex vivo* and *in vivo* LPS challenges, allowing using *ex vivo* data to predict *in vivo* pharmacological effects.

Taken together, the PK/PD data generated in HV in the presence and absence of a TLR4 ligand can be used to generate a robust PK/PD model for the prediction of the PK/PD profile in patients, therefore likely reducing the risk for the selection of the expected effective dose. Based on the data from this study and assumptions specific to the disease of interest (target tissue, chronic versus acute disease…), expected NI-0101 pharmacological effects can be simulated in relation to its concentration, providing a sound rational to select the dose and frequency of administrations for Phase 2 studies.

In conclusion, this study has demonstrated NI-0101 good tolerability, a favorable safety and PK profile and a robust and durable anti-inflammatory effect in HV. The *ex vivo* LPS challenge appeared to be a reliable surrogate for the *in vivo* pharmacodynamics profile of NI-0101. The prevention of *in vivo* LPS effects provides evidence of NI-0101 pharmacological activity. These data enable the further development of NI-0101 in a wide range of diseases that are mediated through the activation of TLR4 and provide flexibility in the choice of dosing regimen. In particular, NI-0101 appears a promising treatment to block efficiently major inflammatory mediators signaling through TLR4 which are present in the synovial fluid and blood of RA patients.

Table 1A. Baseline characteristics Part 1.

		Placebo	NI-0101						
			0.001	0.01	0.05	0.25	1	5	15
		N=9	N=7	N=7	N=7	N=7	N=7	N=7	N=6
FcγRIIa	H/H	4	3	2	2	2	2	2	2
	R/H	5	2	3	3	3	3	3	2
	R/R	0	2	2	2	2	2	2	2
Sex	Female	2	0	0	1	1	1	1	2
	Male	7	7	7	6	6	6	6	4
Age	Mean	26.8	23.4	22.6	24.7	21.1	24.1	24.0	21.0
	SD	7.84	5.38	2.15	5.41	1.86	5.93	3.51	0.00
BMI	Mean	23.58	21.16	21.39	23.41	21.46	24.99	22.44	23.72
	SD	3.063	1.045	1.419	3.456	1.760	3.248	1.69	33.276

Abbreviations: BMI: body mass index; H/H, H/R, R/R: FcγRIIa-131 polymorphisms.

Table 1B. Baseline characteristics Part 2.

		Placebo	NI-0101				
			0.01 mg/kg		0.25 mg/kg		All
			Immediate*	Immediate*	Day 22*	Day 40*	
		(N=4)	(N=3)	(N=3)	(N=3)	(N=3)	(N=9)
FcγRIIa	H/H	2	0	0	0	0	0
	R/H	2	2	2	2	2	6
	R/R	0	1	1	1	1	3
Sex	Female	0	0	0	0	0	0
	Male	4	3	3	3	3	9
Age	Mean	27.5	24.0	25.0	30.3	22.3	25.9
	SD	3.11	4.00	4.36	8.62	3.51	6.23
BMI	Mean	22.45	21.37	23.57	23.47	23.17	23.40
	SD	2.740	1.266	1.762	4.539	4.203	3.221

Abbreviations: BMI: body mass index; H/H, H/R, R/R: FcγRIIa-131 polymorphism; SD: standard deviation.

** Immediate: lipopolysaccharide administration immediately after NI-0101 administration (study day 1), at study day 22 or 40, respectively.*

Table 2. Adverse events reported in at least 2 subjects by preferred term by treatment group (excluding AEs reported during 72h after in vivo endotoxin administration).

	Placebo	NI-0101							All
		0.001	0.01	0.05	0.25	1	5	15	
	(N=13)	(N=7)	(N=10)	(N=7)	(N=16)	(N=7)	(N=7)	(N=6)	(N=73)
	N (E)	N (E)	N (E)	N (E)	N (E)	N (E)	N (E)	N (E)	N (E)
Headache	3 (3)	1 (1)	1 (1)	3 (5)	5 (7)	2 (4)	4 (4)	5 (5)	**24 (30)**
Nasopharyngitis	4 (5)	2 (2)	2 (2)	2 (2)	0	2 (2)	4 (4)	4 (4)	**20 (21)**
Influenza like illness	0	0	2 (2)	0	1 (1)	2 (2)	2 (2)	2 (2)	**9 (9)**
Myalgia	2 (2)	0	2 (2)	0	2 (3)	0	0	1 (1)	**7 (8)**
Dizziness	0	0	3 (3)	0	0	1 (1)	0	0	**4 (4)**
Oropharyngeal pain	0	0	3 (3)	1 (1)	0	0	0	0	**4 (4)**
Nausea	2 (2)	0	0	1 (1)	1 (1)	0	0	0	**4 (4)**
Gastroenteritis	1 (1)	0	0	0	1 (1)	0	0	1 (1)	**3 (3)**
Catheter site hematoma	1 (1)	0	0	0	0	0	0	1 (1)	**2 (2)**
Back pain	0	2 (2)	0	0	0	0	0	0	**2 (2)**
Musculoskeletal chest pain	0	0	1 (1)	0	0	0	1 (1)	0	**2 (2)**
Cough	1 (1)	0	1 (1)	0	0	0	0	0	**2 (2)**
Rhinorrhoea	0	0	1 (1)	0	1 (1)	0	0	0	**2 (2)**
Feeling hot	0	0	0	0	1 (1)	0	1 (1)	0	**2 (2)**
Bacteriuria/UTI	1 (1)	0	0	0	0	1 (1)	0	0	**2 (2)**
Catheter site pain	2 (2)	0	0	0	0	0	0	0	**2 (2)**

N= number of subjects
E= number of events

Table 3. Summary of NI-0101 Pharmacokinetic Parameters and Dose Proportionality Assessment

	Part1						Part 2		Dose
	NI-0101dose								prop*
	0.01 (N=7)	0.05 (N=7)	0.25 (N=7)	1 (N=7)	5 (N=7)	15 (N=6)	0.01 (N=3)	0.25 (N=9)	Rdnm#
C_{max} (ng/mL)	118 (24.2)	918 (18.0)	5850 (12.6)	30.6*103 (31.4)	118*103 (11.4)	367*103 (15.5)	99.5 (16.2)	5270 (16.9)	1.93 (1.56-2.4)
T_{max} (h)	2.0 (2.0-2.0)	4.0 (2.0-6.0)	2.2 (2.0-6.0)	4.0 (2.0-6.1)	4.0 (4.0-6.1)	3.0 (2.0-4.1)	2.1 (2.0-2.1)	2.1 (2.0-4.2)	NA
AUC_{inf} (h.ng/mL)	3490 (49.2)	67.7*103 (28.0)	809*103 (12.3)	559*103 (25.4)	241*105 (30.3)	811*105 (23.3)	2740 (29.1)	745*103 (16.5)	4.8 (9.5-22.9)
$t_{1/2}$ (h)	21.9 (21.3)	44.5 (10.4)	73.8 (22.5)	104 (19.1)	131 (20.7)	132 (31.0)	21.5 (15.7)	70.7 (27.7)	NA
CL (L/h)	0.251 (45.0)	0.0593 (23.7)	0.022 (10.6)	0.0161 (25.7)	0.0162 (25.5)	0.0146 (22.2)	0.288 (30.1)	0.0260 (18.9)	NA
V_{ss} (L)	6.87 (32.5)	4.06 (18.6)	3.11 (11.4)	3.62 (17.0)	4.55 (9.21)	4.71 (18.7)	8.12 (22.1)	3.83 (16.8)	NA

N: number of subjects in specified group; NA: not applicable; C_{max}: maximum serum concentration; t_{max}: time to maximum serum concentration; AUC_{inf}: area under the concentration-time curve; $t_{1/2}$: half-life; CL: Clearance; Vss: volume of distribution at steady state.
Note: PK parameters for NI-0101 dose group 0.001 mg/kg are not presented, as all except 1 concentration were not quantifiable.
Values are arithmetic mean (CV%) except median (min-max) for t_{max}.
* Dose proportionality for Part 1 assessed by the Power Model Statistical analysis.
# Rdnm: model-predicted geometric means ratio for high and low dose, for dose normalized PK parameters.

Supplementary table 1. Mean NI-0101 serum concentrations (ng/mL) per genotype

	0.01 mg/kg			0.1 mg/kg			0.25 mg/kg			1 mg/kg			5 mg/kg			15 mg/kg		
	H/H	R/H	R/R	H/H	R/H	R/R	H/H	R/H	R/R	H/H	R/H	R/R	H/H	R/H	R/R	H/H	R/H	R/R
2 hrs	115	121	115	939	919	809	5320	5970	5810	24100	30100	24900	108000	123000	96300	389000	384000	306000
4 hrs	94.4	110	101	900	943	782	5400	5650	5740	24500	35000	25900	105000	120000	112000	365000	384000	320000
6hrs	87.4	102	96.3	754	932	756	5280	5460	5790	24100	36500	24400	105000	125000	105000	357000	353000	310000
12 hrs	68.1	80.7	65.3	701	868	723	5060	5140	4440	22800	25700	17500	88800	110000	95000	333000	352000	297000
1 day	46.3	54.7	41.1	483	680	573	3930	4480	4210	17400	24800	20700	81500	95500	83400	300000	310000	219000
2 days	22.8	28.8	17	424	537	428	3390	3750	3700	19600	20400	17700	70400	80400	69800	251000	273000	211000
3 days	12.1	13.6	6.64	276	403	296	2640	2950	3110	14200	17300	15800	62100	65300	52500	206000	232000	164000
4 days	5.59	6.77	0	218	283	235	2390	2500	2760	14200	15900	11600	51300	56800	47800	174000	201000	144000
1 wk	0	0	0	60.4	133	84.5	1690	1700	2070	8320	14400	9180	37600	41900	34300	122000	135000	119000
2 wks	6.15	0	0	0	4.97	0	531	584	883	5350	5850	5040	19600	25700	19700	73700	87900	70200
3 wks	0	0	0	0	0	0	143	132	315	3210	3490	2850	10700	17000	12500	43000	54300	43500
4 wks	0	0	0	0	0	0	16.3	12	68	1480	1890	1520	6600	12400	7320	29200	37500	28100
6 wks	0	0	0	0	0	0	0	0	0	187	434	321	2320	5230	2600	11000	15700	11400
8 wks	0	0	0	0	0	0	0	0	0	4.33	39.2	15.7	625	2300	653	4720	7820	5930
10 wks													125	1020	58.6	1820	3050	2470
12 wks													8.92	515	0	780	1220	387
14 wks																319	405	153
16 wks																72.3	67.4	NC
18 wks																8.11	8.62	16.9

NC : Not calculated (N=1).

Supplementary table 2. Percentage of inhibition from baseline of ex vivo LPS-induced cytokine release, per genotype, for cohorts receiving 1 and 15 mg/kg NI-0101.

	TNFα							CXCL10							IFNβ					
	Placebo	1 mg/kg			15 mg/kg			1 mg/kg			15 mg/kg			1 mg/kg			15 mg/kg		
		H/H	R/H	R/R	H/H	R/H	R/R	H/H	R/H	R/R	H/H	R/H	R/R	H/H	R/H	R/R	H/H	R/H	R/R
2hrs	4.72	**-100**	**-100**	**-99.7**	**-100**	**-100**	**-100**	**-93.5**	**-98.5**	**-94.7**	-71.6	**-100**	**-100**	**-100**	**-100**	**-100**	**-100**	**-100**	**-100**
12hrs	36.9	**-100**	**-100**	**-100**	**-99.8**	**-100**	**-100**	**-94.1**	**-97.9**	**-97.3**	-78.9	**-95.2**	**-95.1**	**-100**	**-100**	**-100**	**-100**	**-100**	**-100**
24hrs	-17.7	**-100**	**-100**	**-100**	**-100**	**-100**	**-100**	**-94.4**	-100	**-97.8**	-79.7	**-100**	**-100**	**-100**	**-100**	**-100**	**-100**	**-100**	**-100**
2days	-22.3	**-100**	**-100**	**-100**	**-100**	**-100**	**-100**	**-98.1**	-100	**-97.8**	**-94.3**	**-100**	**-100**	**-100**	**-100**	**-100**	**-100**	**-98.9**	**-100**
3days	-8.68	**-100**	**-100**	**-100**	**-100**	**-100**	**-100**	**-97.0**	-100	**-97.6**	**-84.3**	**-100**	**-100**	**-100**	**-100**	**-100**	**-99.5**	**-100**	**-100**
7days	-12.9	**-98.8**	**-100**	**-100**	**-100**	**-100**	**-100**	**-92.1**	-100	**-97.8**	**-95.1**	**-100**	**-100**	**-100**	**-100**	**-100**	**-100**	**-100**	**-100**
2wks	-14.2	**-100**	**-100**	**-100**	**-100**	**-100**	**-100**	**-93.9**	-100	**-98.4**	**-89.1**	**-98.8**	**-100**	**-100**	**-100**	**-100**	**-100**	**-100**	**-100**
3wks	9.29	**-91.8**	**-99.0**	**-100**	**-99.8**	**-100**	**-100**	-5.78	**-96.1**	**-97.3**	**-86.6**	**-100**	**-100**	**-92.1**	**-100**	**-100**	**-100**	**-100**	**-100**
4wks	-24.9	**-85.9**	**-98.7**	**-99.7**	**-99.1**	**-100**	**-100**	1.25	**-93.7**	**-97.8**	-78.5	**-100**	**-100**	**-82.2**	**-100**	**-100**	**-100**	**-100**	**-100**
6wks	-21.7	**-6.11**	**-76.9**	**-97.9**	**-100**	**-100**	**-36.7**	17.5	**-68.5**	**-75.6**	-100	**-100**	**-60.9**	-55.4	-82.6	**-95.1**	**-100**	**-100**	**-100**
8wks	-28.1	-48.0	31.8	9.16	**-85.1**	**-100**	**-100**	13.4	175	9.22	8.26	**-100**	**-100**	47.7	32.5	10.4	-58.1	**-100**	**-100**
10wks	-31.9	484			**-48.6**	**-100**	**-99.4**				53.6	**-100**	**-100**				-48.9	**-100**	**-100**
12wks	-49.5				-20.3	**-98.3**	**-86.3**				71.6	**-93.2**	-22.0				-13.3	**-97.7**	**-89.5**
14wks	-25.5				14.9	**-87.4**	**-31.8**				-19.2	**-86.9**	-48.8				-18.6	**-94.6**	-67.8
16wks	-29.3				1.24	-25.1	-4.60				-21.8	-44.4	1.34				-23.8	-59.9	-45.2
18wks	-0.578				36.1	-10.8	148				16.3	-22.6	-8.04				33.0	-27.7	126

In bold: data points with percentage change from baseline ≥ -80%. Values are arithmetic mean. H/H, R/H, R/R: FcγRIIa genotypes; LPS: lipopolysaccharide.

Figure 1. Mean NI-0101 serum concentrations (ng/mL) after single ascending dose of NI-0101 Part 1 (black); Part 2 (grey).

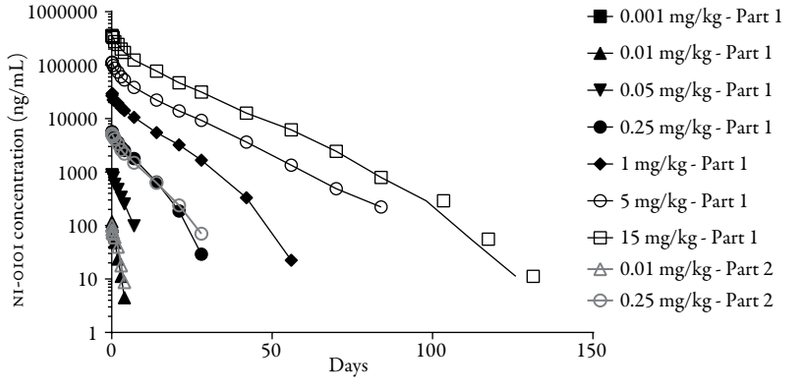


Figure 2. Mean IL6 percentage change from baseline per genotype (*ex vivo* LPS Challenge) in Part 1 FcγRIIa genotype H/H (A), R/H (B), R/R (C).

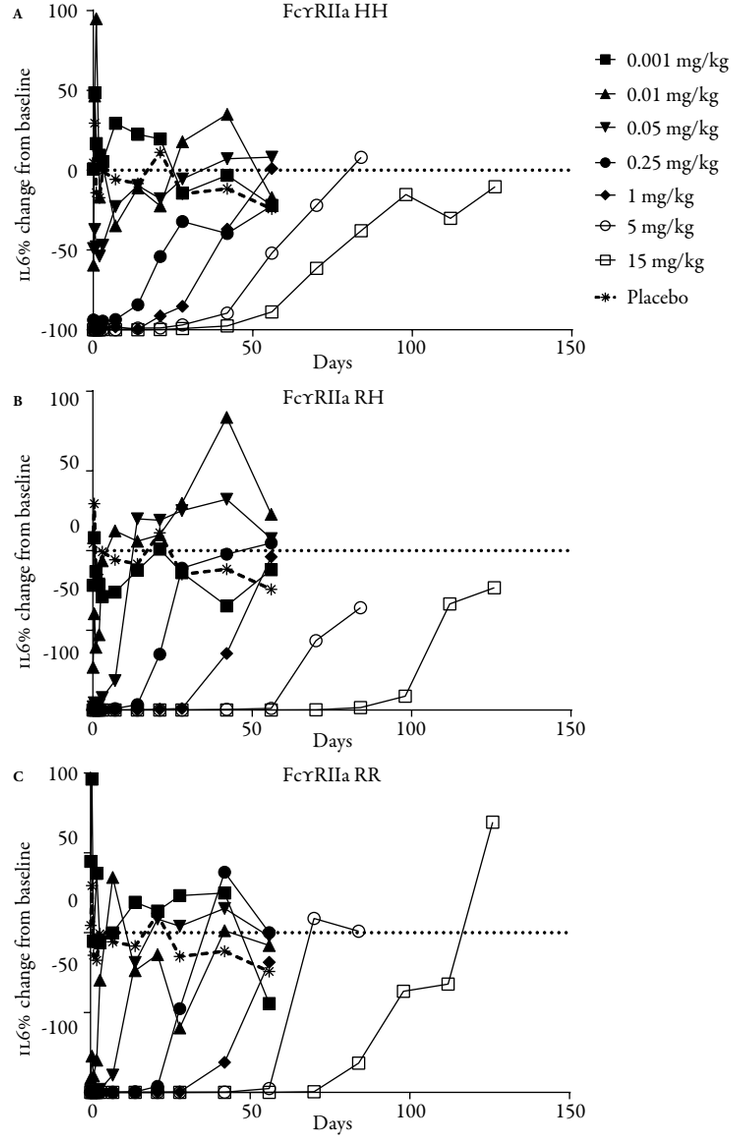
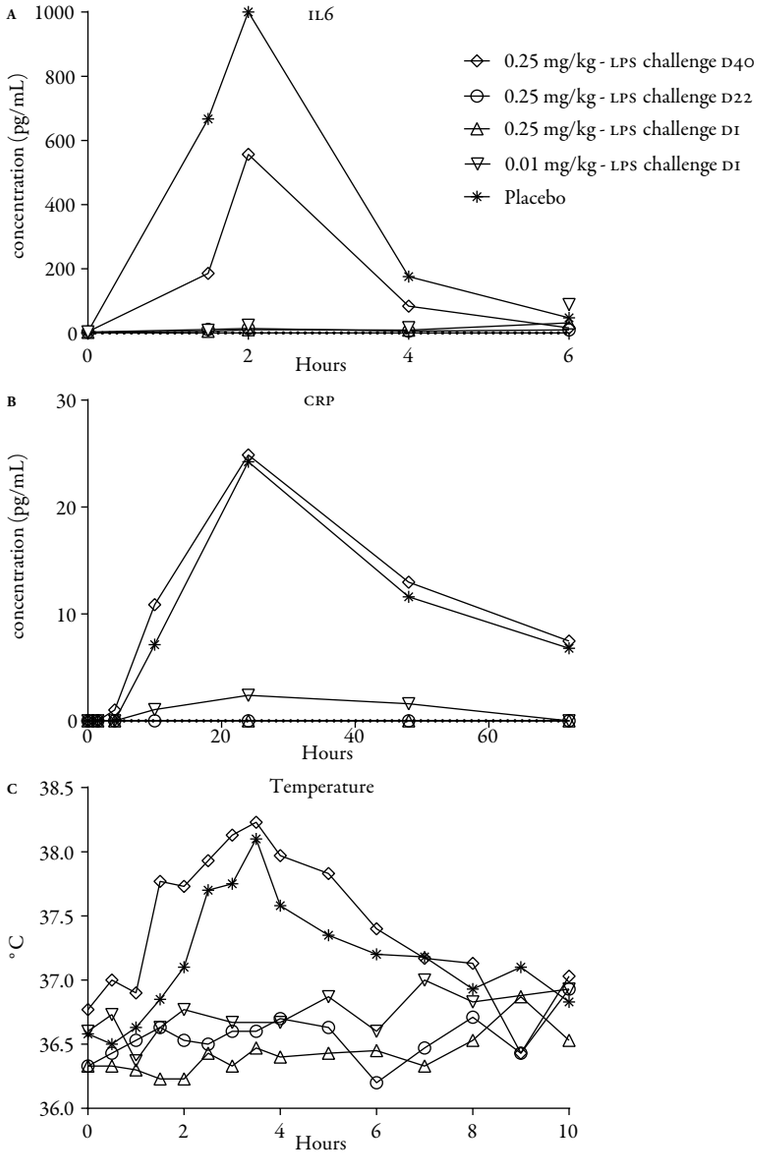


Figure 3. Change in pharmacological parameters after *in vivo* LPS Challenge in Part 2 mean IL6 serum concentration (pg/mL; panel A), (B) mean CRP serum concentration (mg/L; panel B), and temperature (panel C).



REFERENCES

1 Dunn-Seigrist et al. Pivotal involvement of Fcg receptor IIA in the neutralization of lipopolysaccharide signaling via a potent novel anti-TLR4 monoclonal antibody 15C1. JBC. 282(48):34817-34827 (2007)
2 Curtis et al. The use of biologics in Rheumatoid Arthritis: current and emerging paradigms of care. Clinical therapeutics. 33(6):679-707 (2011)
3 Klareskog et al. Rheumatoid Arthritis. Lancet. 373(9664):659-72 (2009)
4 O´Neill et al. Therapeutic targeting of toll-like receptors for infectious and inflammatory diseases and cancer. Pharmacol. Rev. 61:177-197 (2009)
5 Shotorbani et al. Toll-like receptors are potential therapeutic targets in rheumatoid arthritis. World J. Biol. Chem. 2(7):167-72 (2011).
6 Sokolove et al. Immune Complexes Containing citrullinated Fibrinogen Costimulate Macrophages via Toll-like Receptor 4 and FcγReceptor. Arthritis & rheumatism. 63(1):53-62 (2011)
7 Bobacz et al. Toll-like receptors and chondrocytes. Arthritis & rheumatism. 56(6):1880-93 (2007)
8 Zhao et al. circulating immune complexes contain citrullinated fibrinogen in rheumatoid arthritis Arthritis Research & Therapy. 10:R94 (2008)
9 Clavel et al. Induction of macrophage secretion of tumor necrosis factor alpha through Fcgamma receptor IIA engagement by rheumatoid arthritis-specific autoantibodies to citrullinated proteins complexed with fibrinogen. Arthritis Rheum. 58(3):678-88 (2008)
10 Wenink et al. The inhibitory Fc gamma IIb receptor DAMPens TLR4-mediated immune responses and is selectively up-regulated on dendritic cells from rheumatoid arthritis patients with quiescent disease. J Immunol. 183(7):4509-20 (2009)
11 Govoni et al. Therapeutic options after treatment failure in rheumatoid arthritis or Spondyloarthritides. Adv. Ther. 31:780-802 (2014)
12 Haack et al. Diurnal variations of interleukin-6 plasma levels are confounded by blood drawing procedures. Psychoneuroendocrinology. 27:921-31 (2002)
13 Vgontzas et al. IL6 and its circadian secretion in humans. Neuroimmunomodulation. 12:131-40 (2005)
14 Rice et al. A randomized, double-blind, placebo-controlled trial of TAK-242 for the treatment of severe sepsis. Crit Care Med. 38: 1685-1694 (2010)
15 Barochia et al. Eritoran tetrasodium (E5564) treatment for sepsis: review of preclinical and clinical studies. Expert. Opin. Drug Metab Toxicol. 7: 479-494 (2011)
16 Reilly M, et al. randomized, double-blind, placebo-controlled, dose-escalating phase I, healthy subjects study of intravenous OPN-305, a humanized anti-TLR2 antibody. Clin. Pharmacol. Ther. 94(5):593-600 (2013).
17 Hagar et al. Cytoplasmic LPS activates caspase-11: implications in TLR4-independent endotoxic shock. Science. 341(6151):1250-3 (2013).
18 Kayagaki et al. Noncanonical inflammasome activation by intracellular LPS independent of TLR4. Science. 341(6151):1246-9 (2013).
19 Burmester GR, et al. Mavrilimumab, a human monoclonal antibody targeting GM-CSF receptor-alpha, in subjects with rheumatoid arthritis: a randomised, double-blind, placebo-controlled, phase I, first-in-human study. Ann. Rheum. Dis. 70(9):1542-9 (2011).
20 Hale G, et al. Pharmacokinetics and antibody responses to the CD3 antibody otelixizumab used in the treatment of type 1 diabetes. J. Clin. Pharmacol. 50(11):1238-48 (2010).
21 Yin D, Barker KB, Li R, Meng X, Reich SD, Ricart AD, et al. A randomised phase 1 pharmacokinetic trial comparing the potential biosimilar PF-05280014 with trastuzumab in healthy volunteers (REFLECTIONS B327-01). Br. J. Clin. Pharmacol. (2014).