

RESEARCH PAPER

Preclinical development of AMG 139, a human antibody specifically targeting IL-23

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BACKGROUND AND PURPOSE

AMG 139 is a human anti-IL-23 antibody currently in a phase II trial for treating Crohn's disease. To support its clinical development in humans, *in vitro* assays and *in vivo* studies were conducted in cynomolgus monkeys to determine the pharmacology, preclinical characteristics and safety of this monoclonal antibody.

EXPERIMENTAL APPROACH

The *in vitro* pharmacology, pharmacokinetics (PK), pharmacodynamics and toxicology of AMG 139, after single or weekly i.v. or s.c. administration for up to 26 weeks, were evaluated in cynomolgus monkeys.

KEY RESULTS

AMG 139 bound with high affinity to both human and cynomolgus monkey IL-23 and specifically neutralized the biological activity of IL-23 without binding or blocking IL-12. After a single dose, linear PK with s.c. bioavailability of 81% and mean half-life of 8.4–13 days were observed. After weekly s.c. dosing for 3 or 6 months, AMG 139 exposure increased approximately dose-proportionally from 30 to 300 mg·kg⁻¹ and mean accumulation between the first and last dose ranged from 2- to 3.5-fold. Peripheral blood immunophenotyping, T-cell-dependent antigen responses and bone formation markers were not different between AMG 139 and vehicle treatment. No adverse clinical signs, effects on body weight, vital signs, ophthalmic parameters, clinical pathology, ECG, organ weights or histopathology were observed in the monkeys with the highest dose of AMG 139 tested (300 mg·kg⁻¹ s.c. or i.v.).

CONCLUSIONS AND IMPLICATIONS

The *in vitro* pharmacology, PK, immunogenicity and safety characteristics of AMG 139 in cynomolgus monkeys support its continued clinical development for the treatment of various inflammatory diseases.

Abbreviations

ADA, anti-drug antibodies; CI, confidence interval; C_{max}, maximum observed concentration; GLP, good laboratory practice; K_a, association constant; KLH, Keyhole limpet hemocyanin; moDCs, monocyte-derived dendritic cells; NK, natural killer cell; PK, pharmacokinetic

Tables of Links

TARGETS
Other protein targets^a
TNF- α
Catalytic receptors^b
IL-12 receptor
IL-23 receptor

LIGANDS	
CD40 ligand	IL-6
GM-CSF	IL-12
IFN- γ	IL-18
IL-2	IL-23
IL-4	Ustekinumab

These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Pawson *et al.*, 2014) and are permanently archived in the Concise Guide to PHARMACOLOGY 2013/14 (^{a,b}Alexander *et al.*, 2013a,b).

Introduction

IL-23 is a member of the IL-12 family of heterodimeric cytokines and is composed of the IL-23 specific p19 subunit and the common subunit p40, which it shares with IL-12 (Oppmann *et al.*, 2000; Hunter, 2005; Kastelein *et al.*, 2007). Similarly, the heterodimeric cell surface transmembrane IL-12 and IL-23 receptor complexes share a common subunit, IL-12 receptor β 1 subunit, that associates with IL-12 receptor β 2 subunit or the IL-23 receptor for IL-12 and IL-23 signalling, respectively, which allows for control of distinct biological pathways.

IL-12 and IL-23 are produced by antigen-presenting cells such as dendritic cells and macrophages in response to inflammation or infection. While IL-12 acts on naïve T-cells to induce their differentiation into T-helper 1 cells which produce IFN- γ and other pro-inflammatory cytokines, IL-23 acts on immune cells, including T-helper 17, $\gamma\delta$ T-cells, natural killer (NK) cells, dendritic cells, macrophages and innate lymphoid cells to induce production of cytokines such as IL-17, IL-6, TNF- α and GM-CSF (Langrish *et al.*, 2005; Bettelli *et al.*, 2007; Kastelein *et al.*, 2007; Buonocore *et al.*, 2010; Geremia *et al.*, 2011).

Increased expression of IL-23 is found in the target tissue of inflammatory/autoimmune diseases including Crohn's disease, ulcerative colitis, psoriasis, rheumatoid arthritis and multiple sclerosis (Lee *et al.*, 2004; Schmidt *et al.*, 2005; Vaknin-Dembinsky *et al.*, 2006; Li *et al.*, 2007; Guo *et al.*, 2013). Furthermore, genome-wide association studies and targeted single nucleotide polymorphism analyses have revealed an association between specific polymorphisms in genes encoding the unique IL-23 receptor subunit (IL-23 receptor) and IL-12/23 p40 subunit (IL-12B) and susceptibility to Crohn's disease, ulcerative colitis, psoriasis and psoriatic and rheumatoid arthritis (Duerr *et al.*, 2006; Cargill *et al.*, 2007; Ellinghaus *et al.*, 2012; Song *et al.*, 2012; Zhu *et al.*, 2012).

The involvement of IL-23 in inflammatory/autoimmune disease is strongly supported by animal models demonstrating that IL-23-deficient mice (IL-23p19^{-/-}) are resistant to experimentally induced autoimmune encephalitis and collagen-induced arthritis and that IL-23 neutralizing antibodies are efficacious in a number of inflammatory bowel disease and skin inflammation models (Cua *et al.*, 2003;

Murphy *et al.*, 2003; van der Fits *et al.*, 2009; Blumberg *et al.*, 2010; Cox *et al.*, 2012). Clinical trials with ustekinumab and briakinumab, which target the common p40 subunit shared by IL-12 and IL-23, in psoriasis and Crohn's disease, and tildrakizumab and guselkumab, which target the p19 subunit of IL-23, in psoriasis highlight the potential of IL-23 signalling blockade in human disease (Mannon *et al.*, 2004; Kimball *et al.*, 2012; Langley *et al.*, 2012; Sandborn *et al.*, 2012; Traczewski and Rudnicka, 2012; Reichert, 2013).

Studies using IL-12/23p40 and IL-12p35 null mice suggest a dominant role for IL-12 in host defence against intracellular pathogens and in tumour immune surveillance (Fieschi and Casanova, 2003; Bowman *et al.*, 2006; Langowski *et al.*, 2006; Meeran *et al.*, 2006). Consistent with the recognized role of IL-12 in cancer suppression (Meeran *et al.*, 2006; Teng *et al.*, 2012; Yuzhalin and Kutikhin, 2012), emerging data from clinical trials with briakinumab and ustekinumab suggest a possible association between dual inhibition of IL-12 and IL-23 and the development of certain malignancies (Reich *et al.*, 2011; Gordon *et al.*, 2012). While a meta-analysis of four phase II/III randomized ustekinumab trials with up to 4 years of follow-up did not demonstrate an increased risk of malignancies compared with the general or psoriasis populations (Reich *et al.*, 2012), there have been post-marketing reports of rapid appearance of cutaneous squamous cell carcinomas in patients with pre-existing risk factors receiving ustekinumab (Food and Drug Administration, 2014). In contrast to IL-12 inhibition, IL-23 inhibition is associated with a reduced tumour incidence and faster elimination of injected tumour cells (Langowski *et al.*, 2006; Teng *et al.*, 2010).

In addition, safety concerns have been raised for ustekinumab and/or briakinumab regarding the incidence of serious infections and major adverse cardiovascular events, although this is still controversially discussed (Ryan *et al.*, 2011; Langley *et al.*, 2012; Tzellos *et al.*, 2012; 2013; Papp *et al.*, 2013a).

Based on the mechanism of action, experimental data and clinical experience with the IL-12/23p40 antagonists, an IL-23-specific antagonist may provide similar or greater efficacy than the anti-IL-12/23p40 antibodies without the (potential) risks associated with blocking IL-12. Therefore, AMG 139, a human IgG₂ monoclonal antibody, was developed to specifically bind to IL-23p19 and block binding of IL-23 to

the IL-23 receptor. Currently, this antibody is in or has recently completed clinical trials in patients with Crohn's disease and moderate to severe psoriasis (Clinicaltrials.gov NCT01258205 and NCT01094093 respectively).

In this manuscript, we describe the *in vitro* pharmacology as well as the pharmacokinetic (PK) and safety results from four cynomolgus monkey studies. The data obtained demonstrate that the *in vitro* pharmacology, PK and safety profile of AMG 139 in cynomolgus monkeys support its clinical development.

Methods

Test article

AMG 139 is a human monoclonal IgG₂ antibody. It was manufactured at Amgen Inc. (Thousand Oaks, CA, USA) by expression in a CHO cell line in accordance with Good Manufacturing Practices. For studies in cynomolgus monkeys, the test sample was supplied at a nominal concentration of 70 mg·mL⁻¹ with pharmaceutically acceptable excipients, pH 5.2 and stored at -60 to -80°C. The vehicle control sample was formulated with the same excipients and packaged and stored like the active test sample.

Material

Recombinant human and cynomolgus monkey IL-23 and GM-CSF were generated by the Protein Sciences Department (Amgen Inc., Seattle, WA, USA). Native human IL-23 (huIL-23) was generated by culturing human monocytes in the presence of GM-CSF and IL-4 for 7 days to generate monocyte-derived dendritic cells (MoDCs). MoDCs were then stimulated with CD40 ligand for 48 h, which induced IL-23 but not IFN- γ production. Similarly, native murine IL-23 was generated by culturing murine bone marrow-derived dendritic cells as described for native huIL-23.

IL-2, IL-4, IL-12, IL-18 (human and/or murine), and the IFN- γ (human, mouse and primate) and mouse IL-17 ELISA kits were purchased from R&D systems (Minneapolis, MN, USA). Cell culture media and supplements were from Invitrogen (Carlsbad, CA, USA). The luciferase assay system was from Promega (Madison, WI, USA). Fc γ fragment-specific Cy5-conjugated goat anti-human IgG was purchased from Jackson ImmunoResearch (West Grove, PA, USA).

In vitro pharmacology studies

Kinetic exclusion assay (KinExA). To assess the binding interaction of AMG 139 with human and cynomolgus monkey IL-23, the equilibrium K_D was determined using the KinExA method as described previously (Rathanaswami *et al.*, 2008). Briefly, 50 pM AMG 139 was incubated with IL-23 (concentrations ranging from 0.39 to 800 pM) and allowed to equilibrate for 72 h at room temperature. The pre-incubated mixtures were passed over human or cynomolgus monkey IL-23-coated beads mounted in the flow cell of a KinExA machine (Sapidyne Instruments Inc., Boise, ID, USA) to allow capturing of unbound AMG 139 to the solid support. To determine the association rate (K_a), 200 pM AMG 139 was mixed with 300 pM human or cynomolgus monkey IL-23 and the unbound AMG 139 concentrations were measured at

fixed time intervals (46.5 min) over 13.3 h using human or cynomolgus monkey IL-23-coated beads, respectively, as described earlier. For both K_D and K_a , bead-bound antibodies were detected using Fc γ fragment-specific Cy5-conjugated goat anti-human IgG. The fluorescence signals were measured and the KinExA software was used for curve fitting to obtain K_D and K_a values and their 95% confidence intervals (CIs).

COS reporter assay. To determine the neutralizing capacity and species specificity of AMG 139 for IL-23, COS cells stably transfected with huIL-12 receptor $\beta 1$ subunit and huIL-23 receptor (COS-pool 12) were transiently transfected with a STAT1/4-luciferase reporter construct using FUGENE 6 from Roche (Indianapolis, IN, USA) according to the manufacturer's instructions. After 18 h of transfection, cells were detached using Versene from Invitrogen and seeded in 96-well plates at a density of ~10 000 cells per well. AMG 139 with serial diluted concentrations was pre-incubated with human native and recombinant, cynomolgus monkey or rat IL-23 at room temperature for 20 min, added to the COS pool-12 cells, at a final concentration of 2 ng·mL⁻¹ for human native and recombinant and cynomolgus monkey IL-23 (the predetermined EC₉₀) or 100 ng·mL⁻¹ rat IL-23 (the predetermined EC₆₅) and 0.02 pM – 84 nM for AMG 139. Then after being incubated at 37°C for 5 h, the culture medium was removed and cells were lysed with Glo Lysis buffer (100 μ L per well) at room temperature for 5 min. Fifty microlitres of the lysate from each well were transferred to an assay plate and 50 μ L Bright-Glo luciferase substrate were added into each well and luminescence was measured for 5 s on a Microlumat Plus LB luminometer (Berthold, Bad Wildbad, Germany). Medium alone served as a negative control.

NK cell assay. To assess the potency of AMG 139 at inhibiting IL-23-induced bioactivity, IL-2-activated NK cells were stimulated with IL-23 (10 ng·mL⁻¹ recombinant human or cynomolgus monkey, or 20 ng·mL⁻¹ native human; the predetermined EC₅₀ to EC₉₀) and IL-18 (20 ng·mL⁻¹) in the presence of serially diluted AMG 139. NK cells were isolated from human peripheral blood of healthy donors by negative selection (Miltenyi Biotech, Auburn, CA, USA) and cultured for 7 days in RPMI 1640 and 10% FBS supplemented with 10 ng·mL⁻¹ IL-2. After 7 days, cells were centrifuged and resuspended in medium at a concentration of 1 \times 10⁶ cells·mL⁻¹. IL-23 and IL-18 were incubated with AMG 139 for 30 min at room temperature, before the addition of 1 \times 10⁵ NK cells per well for final concentrations of 10 ng·mL⁻¹ IL-23, 20 ng·mL⁻¹ IL-18 and 0 to 300 ng·mL⁻¹ AMG 139. After 24 h, IFN- γ in the supernatant was measured using an ELISA according to the manufacturer's protocol.

Whole blood assay. To assess AMG 139-mediated inhibition of IL-23-induced activity in whole blood, blood samples from healthy human volunteers and cynomolgus monkeys were collected using Repludan (Bayer, Pittsburgh, PA, USA) as an anticoagulant. The assay was performed as described for the NK cell assay with pre-incubation of recombinant human IL-23 (1 ng·mL⁻¹; the predetermined EC₉₀ value), IL-18 (20 ng·mL⁻¹) and IL-2 (5 ng·mL⁻¹) with 0 to 300 ng·mL⁻¹ AMG 139. The final blood was 60% in volume. For the cynomolgus monkey whole blood assay, recombinant human IL-23 was

substituted by recombinant cynomolgus monkey IL-23 (1 ng·mL⁻¹). Human and cynomolgus monkey IFN-γ was measured in the supernatant by ELISAs according to the manufacturer's protocol.

In vivo studies

Animals. Studies in naïve male and female cynomolgus monkeys (*Macaca fascicularis*; 3 to 8.8 years old; 2.6 to 9.1 kg for male and 2.1 to 5.4 kg for female) were conducted at facilities accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (Frederick, MD, USA) based on regulations outlined in the United States Department of Agriculture Animal Welfare Act (Title 9, Code of Federal Regulations Parts 1, 2 and 3) (Animal and Plant Health Inspection Service USA, 2005), the conditions specified in the Guide for the Care and Use of Laboratory Animals (National Research Council (U.S.). Committee for the Update of the Guide for the Care and Use of Laboratory Animals *et al.*, 2011) and in compliance with the Testing Facilities' Animal Welfare Assurance (A4112-01) on file at the National Institute of Health. The protocols were reviewed and approved by the Testing Facilities' Institutional Animal Care and Use Committee. The multiple dose studies were conducted under current Good Laboratory Practice (GLP) standards (FDA, 1979; MHLW, 1997; OECD, 1997). A total of 130 monkeys were used in this studies. Animals were housed in stainless steel cages in a controlled environment (18–29°C;

30–70% relative humidity) and offered Certified Primate Diet #2055C (Harlan Laboratories, Indianapolis, IN, USA) daily and tap water *ad libitum*. As part of the Testing Facilities' environmental enrichment programme, small bits of primate treats and cage-enrichment devices were supplied. All studies involving animals are reported in accordance with the ARRIVE guidelines for reporting experiments involving animals (Kilkenny *et al.*, 2010; McGrath *et al.*, 2010).

Cynomolgus monkeys were randomly assigned to treatment groups for the single-dose pharmacokinetics and the three GLP toxicology studies. Blood samples were collected as outlined in Table 1.

Measurement of serum AMG 139 concentrations. Serum samples were analysed for AMG 139 using a validated assay employing the electrochemiluminescent technology from MSD® (Meso Scale Discovery, Rockville, MD, USA). Briefly, murine anti-AMG 139, 1F2 Mab antibody (Amgen Inc., CA), was passively adsorbed to Multi-Array® 96-well high-bind microplates (MSD). After removing excess antibody, wells were blocked with StartingBlock™ T20 buffer (Thermo Scientific, Rockford, IL, USA). Standards, quality controls and dilutional quality controls, if applicable, were performed by spiking AMG 139 into 100% cynomolgus monkey serum, samples and blanks were loaded into wells after the pretreatment with StartingBlock T20 buffer. After a wash step, a murine anti-AMG 139, 1A4.1 Mab ruthenium-conjugated detection antibody (Amgen Inc., CA), was added to the wells.

Table 1

AMG 139 treatments and procedures for studies in cynomolgus monkeys

Study	n	Groups	Treatment	Sample collection for PK
Single dose	6 males	2 animals per group	3 mg·kg ⁻¹ i.v. 3 mg·kg ⁻¹ s.c. 8.63 mg·kg ⁻¹ s.c. Single dose	Samples were collected pre-dose and at 1, 4, 8, 24, 72, 96, 120, 168, 240, 336, 504, 840 and 1176 h post-dose; an additional sample at 0.25 h was collected for the i.v. group
14-day GLP	16 males 16 females	3–5 animals per group per sex	Vehicle control s.c. 30 mg·kg ⁻¹ s.c. 100 mg·kg ⁻¹ s.c. 300 mg·kg ⁻¹ s.c. QW for 2 weeks	Blood samples were collected pre-dose and at 4, 24, 48, 72 and 168 h post-dose on study days 1 and 8 of the dosing phase; the pre-dose sample collected prior to the second dose on day 8 is equivalent to 168 h post-dose for day 1. Blood samples from recovery monkeys (two monkeys from vehicle control and 300 mg·kg ⁻¹ group) were collected on study days 28, 56, 84 and 98 days
3-month GLP	26 males 26 females	4–6 animals per group per sex	Vehicle control s.c. and i.v. 30 mg·kg ⁻¹ s.c. 100 mg·kg ⁻¹ s.c. 300 mg·kg ⁻¹ s.c. 300 mg·kg ⁻¹ i.v. QW for 13 weeks	Serum samples were collected pre-dose and 0.5, 4, 24, 48, 72 and 168 h post-dose on study days 1 and 92. In addition, samples were collected from animals pre-dose on study days 15, 29 and 57. Blood samples from recovery monkeys (two monkeys per sex from vehicle control and the 300 mg·kg ⁻¹ dose groups) were collected on study days 126, 154, 182, 210, 238, 266, 295, 322, 350, 378, 406, 434 and 462.
6-month GLP	20 males 20 females	4–6 animals per group per sex	Vehicle control s.c. 30 mg·kg ⁻¹ s.c. 100 mg·kg ⁻¹ s.c. 300 mg·kg ⁻¹ s.c. QW for 26 weeks	Serum samples were collected pre-dose and at approximately 0.5, 4, 24, 48, 72 and 168 h post-dose on study days 1 and 176. In addition, blood samples were collected pre-dose and at 48 h post-dose on study days 29, 57, 92 and 134. Blood samples from recovery animals (2/sex/group) were collected on study day 211.

PK, pharmacokinetics; QW, once-weekly.

After a final wash step, a quarter of the diluted tripropylamine read buffer (4X MSD Read Buffer T, MSD) was added to the wells before taking a reading on the Sector Imager 6000 (MSD). The ruthenium label emitted light at 620 nm, when electrically stimulated by electrodes located in the bottom of the microplate and co-reacted with the tripropylamine buffer to enhance the electrochemiluminescent signals. The lower limit of quantification was 25 ng·mL⁻¹ and the nominal dynamic assay range was 25 to 16 000 ng·mL⁻¹.

Immunogenicity assay. Serum samples were assessed for anti-AMG 139 antibodies using a validated immunoassay (MSD; electrochemiluminescence platform). The lower limit of reliable detection (LLRD) was 40 ng·mL⁻¹ and tolerated up to 100 µg·mL⁻¹ of AMG 139 at the LLRD. Samples that tested positive for binding antibodies were further assessed for anti-AMG 139 neutralizing antibodies using a cell-based competitive reporter gene bioassay (similar to the COS reporter assay described earlier). The assay sensitivity of neat-pooled naïve cynomolgus monkey serum was 5 µg·mL⁻¹ AMG 139, but the presence of AMG 139 in concentrations greater than 330 ng·mL⁻¹ may compromise assay performance.

Safety evaluations and T-cell-dependent antibody responses assay. General observations of animals were performed twice daily. In addition, cage side observations were conducted 2 to 3 h post-dose to assess acute toxicity. Other observations included daily assessment of food consumption, weekly assessment of body weight, ophthalmic examinations, vital sign measurements, ECGs and clinical and anatomic pathology evaluations.

To assess potential drug effects on immune function, the T-cell-dependent antigen response was evaluated. Briefly, animals received a dose of Keyhole limpet hemocyanin (KLH; 1 mg) on study days 30 and 60 in the 3-month GLP study. Blood was collected before the first KLH immunization on study day 30 and on study days 37, 40 and 45 before the second KLH dose on study day 60 and on study days 67, 70, 75, 81 and 88. Samples were analysed for anti-KLH-IgM (up to study day 45, data on file at Amgen Inc., CA) and anti-KLH-IgG (all samples) using a validated ELISA.

PK data analyses. Non-compartmental analyses were performed using WinNonlin® (Enterprise version 5.1.1, 2006, Pharsight®, a Certara™ Company, Sunnyvale, CA, USA). The following PK parameters after single or multiple i.v. or s.c. administrations were determined: observed maximum concentration in serum (C_{max}), area under the serum AMG 139 concentration-time curve within the dosing interval (AUC_{τ} for multiple dosing) or extrapolated to infinity (AUC_{∞} for single dosing), clearance (CL for i.v. dosing; CL/F for s.c. dosing). The rate constant for the terminal log-linear phase of the concentration-time curve (λ_z) was estimated using linear regression. The terminal phase elimination half-life ($t_{1/2,z}$) was calculated as $\ln 2/\lambda_z$. For the single-dose study, the volume of distribution at steady state (V_{ss}) was calculated as $V_{ss} = CL \times MRT$ with MRT being the mean residence time calculated as $MRT = AUMC_{\infty}/AUC_{\infty}$, where $AUMC_{\infty}$ is the area under the first-moment-time curve extrapolated to infinity. Descriptive statistics [n , mean (median for t_{max}) and SD (range for t_{max})] were calculated for each cohort or dose level.

Results

In vitro pharmacology

Binding affinity of AMG 139 to human and cynomolgus monkey IL-23. AMG 139 bound to human and cynomolgus monkey IL-23 with high affinity; K_D values were 0.138 and 6.0 pM respectively. The association rates of AMG 139 with human and cynomolgus monkey IL-23 were $7.27 \times 10^5 \text{ M}^{-1}\cdot\text{s}^{-1}$ (95% CI 6.12×10^5 – $8.55 \times 10^5 \text{ M}^{-1}\cdot\text{s}^{-1}$) and $5.34 \times 10^5 \text{ M}^{-1}\cdot\text{s}^{-1}$ (95% CI 4.35×10^5 – $6.46 \times 10^5 \text{ M}^{-1}\cdot\text{s}^{-1}$) (data on file at Amgen Inc., CA).

Blocking activity and specificity of AMG 139 for IL-23. AMG 139 potently and completely inhibited recombinant human and cynomolgus monkey IL-23 but not rat IL-23 or mouse IL-23 (data on file at Amgen Inc., CA) in COS cells stably transfected with the IL-23 receptor and IL-12 receptor $\beta 1$ subunit and transiently transfected with a STAT-luciferase reporter (Figure 1), demonstrating strong cross-reactivity between human and cynomolgus monkey but not rat or mouse IL-23. Native human ligand was produced from MoDCs following stimulation with CD40 ligand for 48 h. The supernatant was then added to transfected COS cells in the presence or absence of AMG 139. In screening for antibodies to IL-23, some antibodies bound and potently inhibited recombinant IL-23 but not native IL-23. Therefore, demonstrating comparable inhibition of native ligand was a crucial step in identifying the antibodies most likely to inhibit IL-23 *in vivo*. Comparable inhibition for AMG 139 was observed with human native and human recombinant IL-23 with IC_{50} values of 284 ± 48 and 188 ± 25 pM (mean \pm SD, $n = 3$ independent experiments) respectively. For cynomolgus monkey IL-23, IC_{50} values were 250 ± 6 pM (mean \pm SD, $n = 3$ independent experiments).

Blocking activity of AMG 139 in IL-23-induced IFN- γ production in human NK cells. IL-23 in combination with IL-18 induces IFN- γ production in primary human NK cells. AMG 139 potently inhibited this induction by human native and recombinant IL-23 and recombinant cynomolgus monkey IL-23 with IC_{50} values of 238 ± 248 , 93 ± 64 and 71 ± 66 pM (mean \pm SD, $n = 3$ – 6 donors) respectively. In addition, IFN- γ production by NK cells can also be induced by IL-12; AMG 139 did not inhibit this activity even at a concentration of 0.5 µM. These data demonstrate that AMG 139 is specific for IL-23 and does not inhibit IL-12 signalling. Conversely, the positive control IL-12p35 antibody, mAB219, potently inhibited IL-12-mediated IFN- γ production (Figure 2).

Blocking activity of AMG 139 on IL-23-induced IFN- γ production in human and cynomolgus monkey whole blood. IL-23 will also induce IFN- γ production in human and cynomolgus monkey whole blood. AMG 139 inhibited IL-23-induced IFN- γ production with IC_{50} values of 28 ± 20 and 125 ± 256 pM (mean \pm SD, $n = 8$ – 9 donors) for recombinant human and cynomolgus monkey IL-23 in human whole blood, respectively, demonstrating that the presence of serum does not affect the ability of AMG 139 to bind its target and block the signalling pathway. AMG 139 also potently inhibited recombinant cynomolgus IL-23-induced IFN- γ production in cynomolgus monkey whole blood with an IC_{50} value of 91 ± 95 pM (mean \pm SD, $n = 4$ donors) (Figure 3).

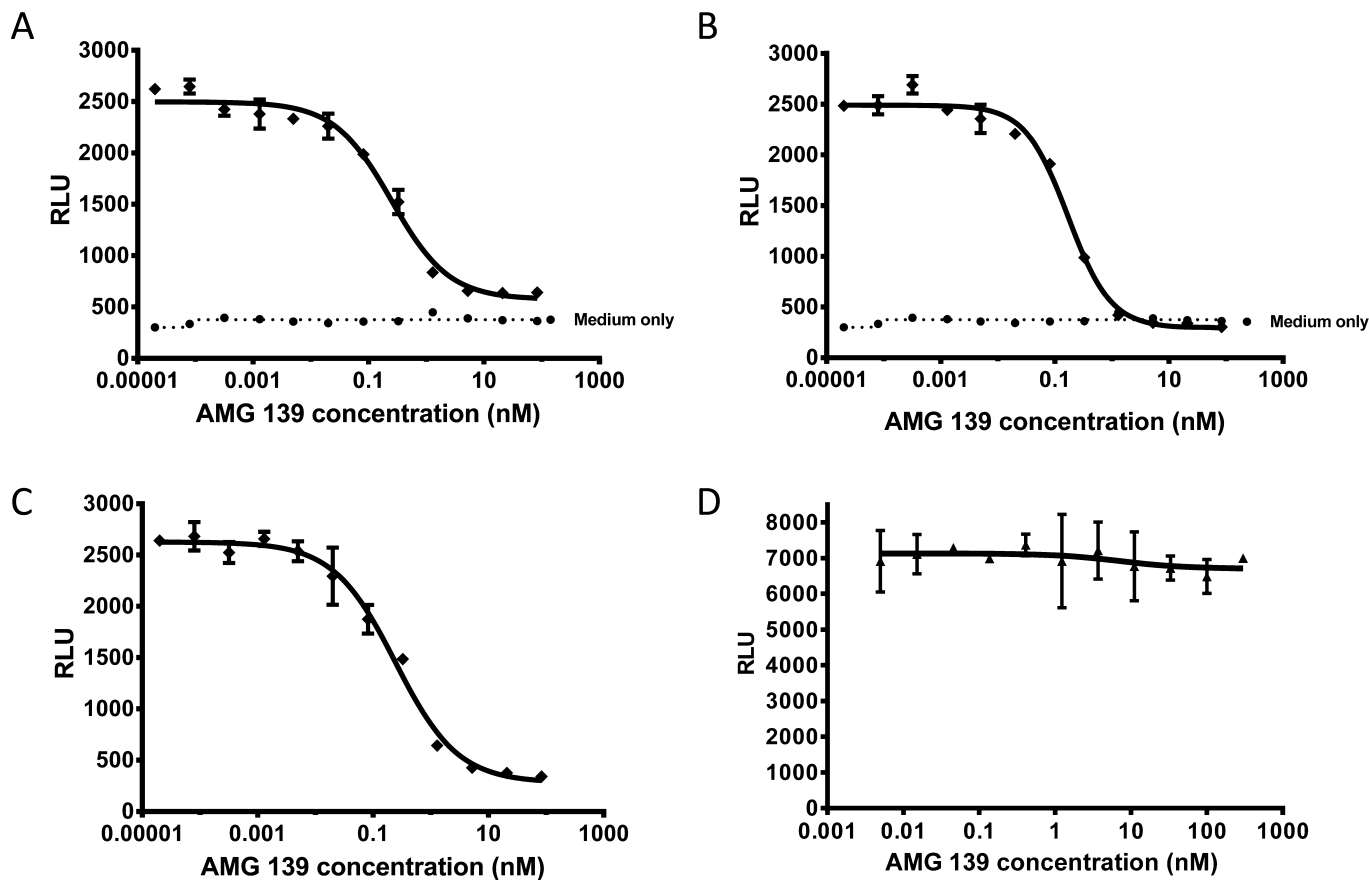


Figure 1

AMG 139 inhibits IL-23 activity in a COS cell reporter gene assay. COS-pool 12 cells (expressing huIL-12 receptors $\beta 1$ subunit and huIL-23 receptors) were transiently transfected with a STAT1/4-luciferase reporter for 48 h. At the day of the experiment, COS-pool 12 cells were incubated with native human IL-23 (A), recombinant human IL-23 (B), recombinant cynomolgus monkey IL-23 (C) or recombinant rat IL-23 (D) in the presence of various concentrations of AMG 139. AMG 139 was pre-incubated with IL-23 for 20 min prior to addition to the cells. After 5 h, relative light units (RLU) were measured as an indicator of the STAT1/4-luciferase reporter using the Bright-Glo luciferase as described in the Methods section. Data are representative of $n = 3$ (A–C) or $n = 2$ (D) independent experiments. Values are expressed as mean \pm SEM for duplicated measures.

Cynomolgus monkey studies

PK and toxicokinetics. In a single-dose study in cynomolgus monkeys, serum AMG 139 concentrations declined in a biphasic manner after administration of $3 \text{ mg}\cdot\text{kg}^{-1}$ i.v. After s.c. administration, AMG 139 was rapidly absorbed with a median t_{max} of 3.5–4 days. The mean bioavailability after $3 \text{ mg}\cdot\text{kg}^{-1}$ s.c. was approximately 81%. AMG 139 exhibited approximately dose-proportional increases in exposure (C_{max} and AUC_{∞}) across the investigated dose range of 3 to $8.63 \text{ mg}\cdot\text{kg}^{-1}$. The mean terminal half-life ranged from 8.4 to 13 days (Figure 4A, Table 2). In one animal, after the $3 \text{ mg}\cdot\text{kg}^{-1}$ s.c. dose, a rapid decline in serum AMG 139 concentrations was observed >504 h post-dose. This increased elimination rate might be due to the formation of anti-drug antibodies (ADAs); however, ADA measurements were not performed in this single-dose study.

Multiple dose toxicokinetics were assessed in 2-week, 3-month and 6-month GLP toxicology studies in cynomolgus monkeys. In the 2-week study, AMG 139 was administered once weekly for 2 weeks followed by a 3-month treatment-free phase. The exposure to AMG 139, as assessed by C_{max} and

AUC_{τ} , increased approximately dose-proportionally for doses between 30 and $300 \text{ mg}\cdot\text{kg}^{-1}$. The median t_{max} ranged from 2.0 to 3.0 days (Figure 4B, Table 2). In the 3-month study, AMG 139 was administered to cynomolgus monkeys once weekly for 14 weeks followed by a 12-month treatment-free phase. On study days 1 and 92, the exposure to AMG 139 increased dose-proportionally across the investigated dose range. The mean accumulation ratio (based on AUC) after 14 weekly doses, ranged from 2.37 to 3.10 for s.c. and 1.91 for i.v. administration (Figure 5A, Table 2). The estimated bioavailability after $300 \text{ mg}\cdot\text{kg}^{-1}$ s.c. administration was 63% on study day 1 and 78% on study day 92. In the 6-month study, AMG 139 was administered once weekly via s.c. injection for 6 months followed by an 11-week treatment-free phase. Exposure increased approximately dose-proportionally over the 30 to $300 \text{ mg}\cdot\text{kg}^{-1}$ dose range. The mean accumulation ratios ranged from 2.0 to 3.5-fold after 6 months of weekly AMG 139 injections (Figure 5B, Table 2). For all studies, no or only minor, non-statistically significant gender differences were observed for the AMG 139 exposure parameters C_{max} and AUC.

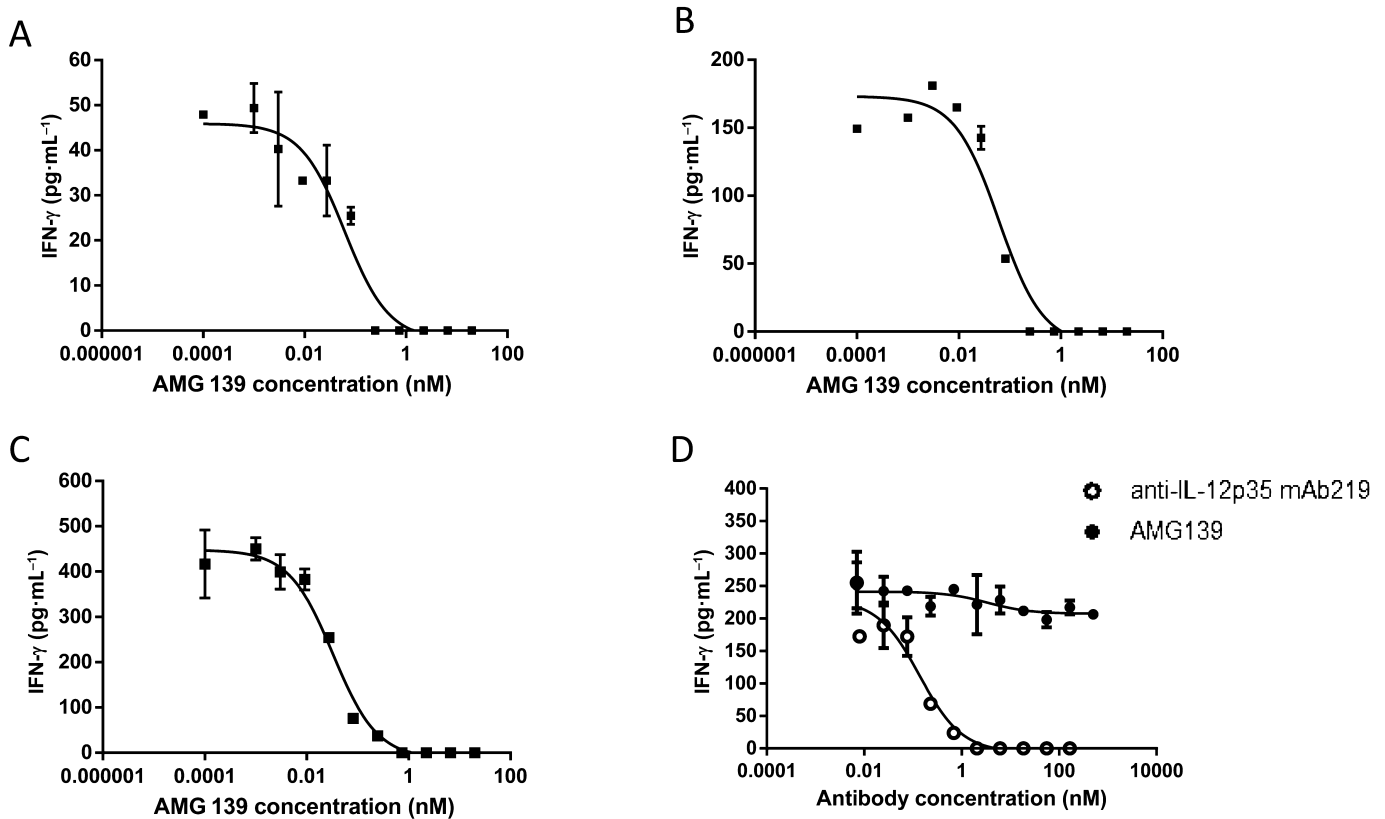


Figure 2

AMG 139 inhibits IL-23 activity in NK cells. Human NK cells were incubated with native human IL-23 (A), recombinant human IL-23 (B), recombinant cynomolgus monkey IL-23 (C) or recombinant human IL-12 (D) in the presence of various concentrations of AMG 139 (A–D) or the positive control IL-12p35 antibody mAb219 (D). AMG 139 or mAb219 was incubated with IL-23 or 0.3 ng·mL⁻¹ IL-12, for 30 min prior to addition to the cells. After 24 h in culture, the supernatant was collected and IFN- γ was measured via ELISA. Results are representative of at least three experiments, except (A) which is $n = 2$. Data are presented as mean \pm SD.

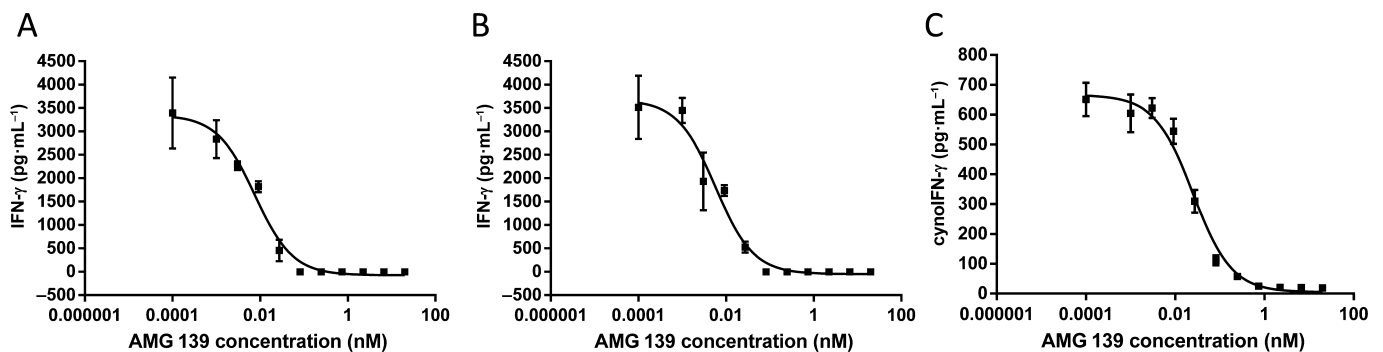


Figure 3

AMG 139 inhibits IL-23 activity in human (A and B) and cynomolgus monkey (C) whole blood. Whole blood was incubated with recombinant human IL-23 (A) or recombinant cynomolgus monkey IL-23 (B and C) in the presence of various concentrations of AMG 139; AMG 139 was pre-incubated with IL-23 for 30 min prior to addition to the cells. After 24 h in culture, the supernatant was collected and IFN- γ was measured via ELISA. Results are representative of experiments conducted in blood from four to eight donors; results are represented as mean \pm SD.

Immunogenicity. In the 2-week study, all animals tested negative for ADAs during the dosing phase. During the post-treatment phase, two of four animals in the 300 mg·kg⁻¹ s.c. dose group tested positive for anti-AMG 139 neutralizing

antibodies (on day 82 and 96 post-first dose), which appeared to have decreased the exposure to AMG 139. Anti-AMG 139 antibodies during the treatment period were detected in four of 28 animals (two of eight, one of eight, one of 12 and zero

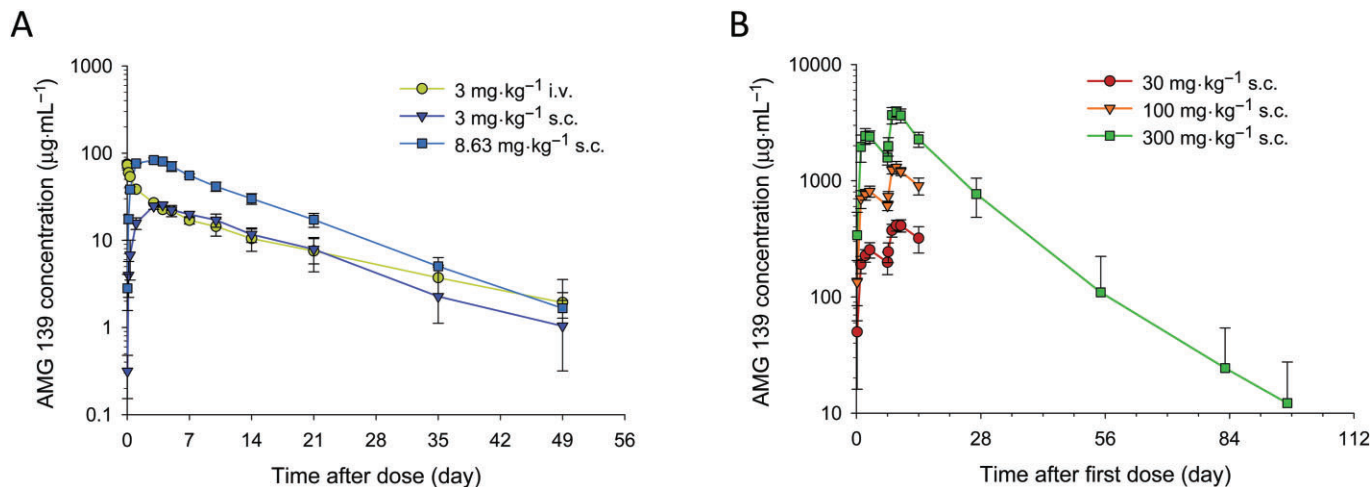


Figure 4

Mean (\pm SD) serum concentration-time profiles of AMG 139. Cynomolgus monkeys were administered a single i.v. or s.c. (A) or two-dose s.c. (B) of AMG 139.

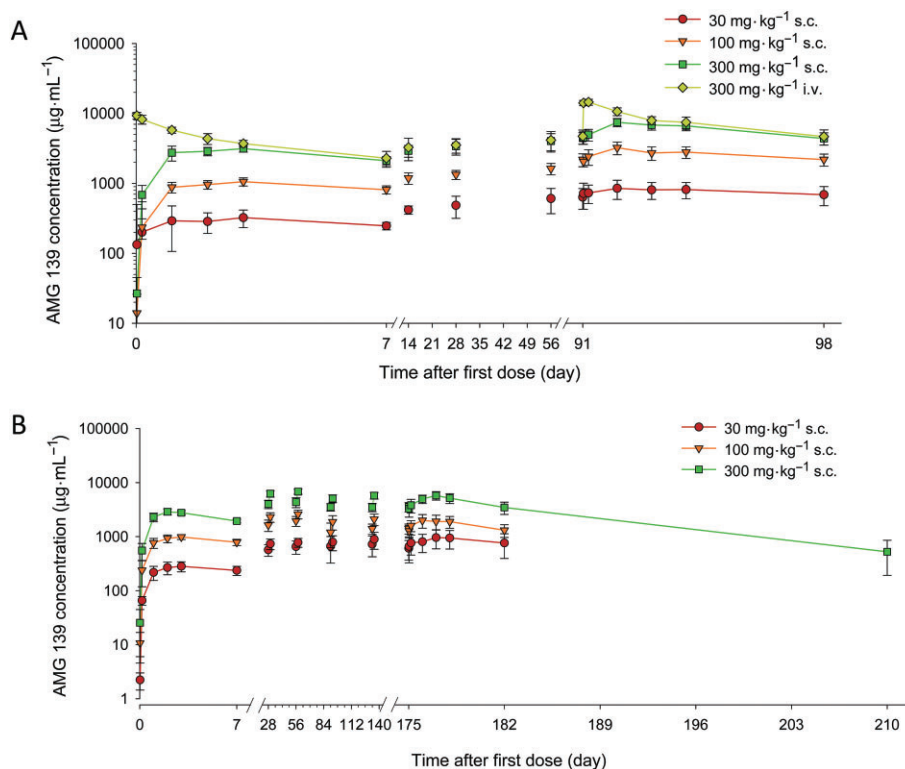


Figure 5

Mean (\pm SD) serum concentration-time profiles in cynomolgus monkeys after multiple i.v. or s.c. doses of AMG 139. Cynomolgus monkeys received 13 i.v. or s.c. (A) or 26 s.c. doses of AMG 139 (B) or vehicle every week.

of 12 in the 30 , 100 and $300\text{ mg}\cdot\text{kg}^{-1}$ s.c. and the $300\text{ mg}\cdot\text{kg}^{-1}$ i.v. dose groups, respectively) and in four of 28 animals (one of eight, two of eight and one of 12 in the 30 , 100 and $300\text{ mg}\cdot\text{kg}^{-1}$ s.c. dose group, respectively) in the 3- and 6-month studies respectively. In the latter study, one of four

monkeys ($300\text{ mg}\cdot\text{kg}^{-1}$ s.c. dose group) developed anti-AMG 139 binding antibodies in the treatment-free phase. No neutralizing antibodies were detected in either of these latter studies and the anti-AMG 139 binding antibodies did not appear to decrease the exposure of AMG 139 in these animals.

Table 2

Non-compartmental pharmacokinetic parameters for AMG 139

Single dose	n = 2 per group	3 mg·kg ⁻¹ i.v.	3 mg·kg ⁻¹ s.c.	8.63 mg·kg ⁻¹ s.c.	
	C ₀ /C _{max} (µg·mL ⁻¹)	75.1	25.5	83.5	
	t _{max} (day)	0.0104	4.0	3.5	
	AUC _∞ (µg·day·mL ⁻¹)	517	435	1160	
	CL (mL·day ⁻¹ ·kg ⁻¹) ^a	6.12	7.63	7.63	
	V (mL·kg ⁻¹) ^b	97.5	84.4	93.0	
	t _{1/2} (day)	13.5	8.65	8.41	
14-day GLP	n = 6–10 per group	30 mg·kg ⁻¹ s.c.	100 mg·kg ⁻¹ s.c.	300 mg·kg ⁻¹ s.c.	
Day 1	t _{max} (day)	3.0 (2.0–3.0)	3.0 (1.0–3.0)	2.0 (2.0–3.0)	
	C _{max} (µg·mL ⁻¹)	255 (37.8)	813 (89.5)	2 480 (323)	
	AUC _τ (µg·day·mL ⁻¹)	1 450 (208)	4 700 (486)	13 400 (1880)	
Day 8	t _{max} (day)	2.5 (2.0–3.0)	2.0 (2.0–3.0)	2.0 (1.0–2.0)	
	C _{max} (µg·mL ⁻¹)	417 (46.5)	1 330 (135)	3 940 (407)	
	AUC _τ (µg·day·mL ⁻¹)	2 550 (397)	7 630 (698)	21 800 (2730)	
	AR	1.76 (0.173)	1.63 (0.141)	1.64 (0.131)	
3-month GLP	n = 7–12 per group	30 mg·kg ⁻¹ s.c.	100 mg·kg ⁻¹ s.c.	300 mg·kg ⁻¹ s.c.	300 mg·kg ⁻¹ i.v.
Day 1	t _{max} (day)	3.0 (3.0–3.0)	3.0 (2.0–3.0)	3.0 (1.0–3.0)	0.02 (0.02–0.167)
Day 92	C ₀ /C _{max} (µg·mL ⁻¹)	293 (26.5)	1 060 (136)	3 230 (334)	9 370 (970)
	AUC _τ (µg·day·mL ⁻¹)	1 710 (139)	6 120 (822)	17 600 (2060)	28 000 (3130)
	t _{max} (day)	1.5 (0.02–3.0)	1.0 (1.0–3.0)	1.0 (1.0–3.0)	0.167 (0.02–0.167)
	C ₀ /C _{max} (µg·mL ⁻¹)	929 (283)	3 250 (652)	7 500 (987)	14 800 (1300)
	AUC _τ (µg·day·mL ⁻¹)	5 430 (1510)	18 400 (3520)	41 500 (6220)	53 500 (8210)
	AR	3.10 (0.831)	3.01 (0.491)	2.37 (0.393)	1.91 (0.250)
6-month GLP	n = 8–12 per group	30 mg·kg ⁻¹ s.c.	100 mg·kg ⁻¹ s.c.	300 mg·kg ⁻¹ s.c.	
Day 1	t _{max} (day)	3.0 (2.0–7.0)	3.0 (2.0–3.0)	2.0 (2.0–3.0)	
	C _{max} (µg·mL ⁻¹)	285 (61.3)	993 (99.5)	2 910 (363)	
	AUC _τ (µg·day·mL ⁻¹)	1 670 (342)	5 760 (748)	16 000 (1310)	
Day 176	t _{max} (day)	2.0 (0.167–3.0)	1.5 (0.167–3.0)	2.0 (1.0–3.0)	
	C _{max} (µg·mL ⁻¹)	1 010 (370)	2 090 (507)	5 900 (1030)	
	AUC _τ (µg·day·mL ⁻¹)	5 980 (2350)	11 900 (2710)	32 100 (5600)	
	AR	3.52 (1.12)	2.07 (0.390)	2.03 (0.410)	

Results are presented as means with SD in parentheses. ^aCL is reported for i.v. dosing, CL/F for s.c. dosing.

^bV_{ss} is reported for i.v. dosing, V_z/F for s.c. dosing.

AR, accumulation ratio for AUC; AUC_τ, area under the concentration-time curve from time zero to the last observed concentration;

C₀, extrapolated concentration at time zero for i.v. dosing; C_{max}, maximum observed concentration for s.c. dosing; t_{max}, time to C_{max}, expressed as median (minimum–maximum).

It should be noted that high circulating levels of the drug could have prevented the detection of anti-AMG 139 antibodies, as the assays testing for binding and neutralizing antibodies were only accurate for up to 100 µg·mL⁻¹ and 330 ng·mL⁻¹ AMG 139 respectively.

Safety evaluation. In all studies, AMG 139 was well-tolerated; no AMG 139-related clinical signs or changes in food con-

sumption, body weight, physical parameters, ECGs, ophthalmic tests, serum chemistry, haematology, peripheral blood phenotyping, bone formation markers or coagulation parameters were observed after up to and including 300 mg·kg⁻¹ AMG 139, administered s.c. or i.v. AMG 139-related microscopic changes were limited to a minor increase in intensity of cellular infiltrates at the s.c. injection site relative to controls. These findings were reversed in the 2-week study. Based

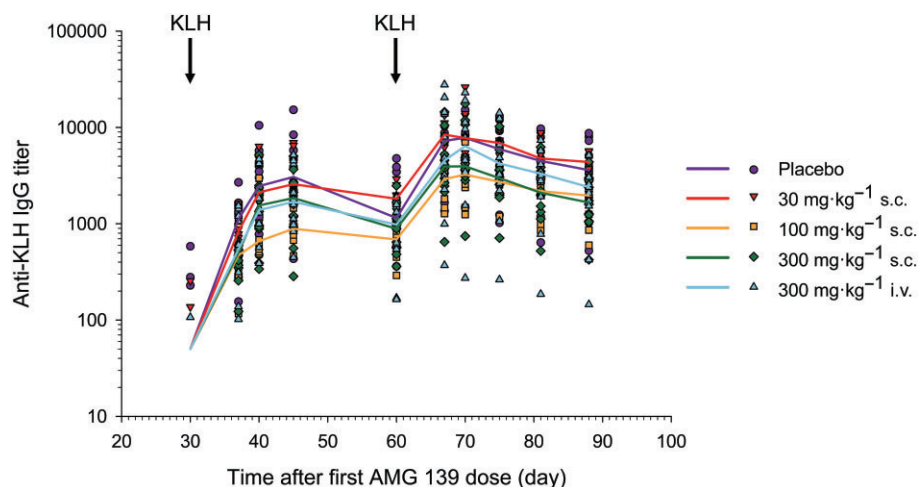


Figure 6

Kinetics of the anti-KLH IgG response in monkeys following immunization with KLH. Male and female cynomolgus monkeys were given a dose of KLH at 1 mg per animal on day 30 and 60. Serum anti-KLH IgG titres were measured by ELISA. Symbols represent individual results. Lines represent the group median. Responses in the AMG 139-treated animals (combined sexes) were comparable with the responses of the controls after both the primary and secondary immunization with KLH when animals with pre-existing anti-KLH titres were excluded. Median values below the assay cut-off point were set to one-half of the assay cut-point.

on these observations, the no-observed-adverse-effect level of AMG 139 in cynomolgus monkeys was determined to be $300 \text{ mg}\cdot\text{kg}^{-1}$ s.c. or i.v., the highest dose tested. In addition, no changes in the T-cell-dependent antigen response assay against KLH compared with the placebo control were observed (Figure 6).

Discussion and conclusions

AMG 139, a human monoclonal IgG₂ antibody that targets IL-23, has been developed as a therapeutic agent for treatment of autoimmune/inflammatory diseases. AMG 139 is currently in a phase II clinical trial in patients with Crohn's disease and recently completed a phase I clinical trial in patients with psoriasis. As part of the preclinical development of AMG 139, *in vitro* assays and *in vivo* studies were conducted in cynomolgus monkeys to determine the pharmacology, PK and safety of this human monoclonal antibody.

In vitro experiments demonstrated that AMG 139 binds human IL-23 and cynomolgus monkey IL-23 with high potencies; affinities (dissociation constant) of 0.138 and 6 pM, respectively, were estimated. Furthermore, AMG 139 inhibited human (recombinant and native) and recombinant cynomolgus monkey IL-23 with similar IC₅₀ values in a COS cell-based luciferase reporter assay and suppressed IL-23-induced IFN- γ production in primary human NK cells and in human and cynomolgus monkey whole blood. The comparable results between whole blood cell assays and the other cellular assays demonstrate that the presence of serum does not affect AMG 139's ability to bind its target and inhibit signalling. Most importantly, the similar inhibitory potencies for human and cynomolgus monkey IL-23 suggest that AMG 139 has comparable *in vitro* pharmacodynamic properties in

both species, which supports the use of cynomolgus monkeys as a relevant toxicology model for this antibody.

Our data further demonstrated that AMG 139 is specific for IL-23 and does not interact with IL-12 signalling. This is especially important because of the predominant role of IL-12 in defence against intracellular pathogens and tumour immune surveillance (Fieschi and Casanova, 2003; Bowman *et al.*, 2006; Langowski *et al.*, 2006; Meeran *et al.*, 2006; Schulz *et al.*, 2008) and the controversially debated safety concerns (increased risk for malignancies and major adverse cardiovascular events) raised for the dual IL-12/23 inhibitors briakinumab and ustekinumab in recent clinical trials (Lee *et al.*, 1999; Reddy *et al.*, 2010; Gordon *et al.*, 2012; Lin *et al.*, 2012; Tzellos *et al.*, 2012; Papp *et al.*, 2013b). Targeting IL-23 alone may offer a better benefit : risk profile compared with dual inhibition of IL-12 and IL-23.

The PK of AMG 139 in cynomolgus monkeys were characterized by a biphasic decline in serum concentration after administration of a single i.v. dose and rapid absorption after s.c. administrations. The observed clearance of 6.1 to $7.6 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ is in the range of what has been previously reported for monoclonal antibodies in cynomolgus monkeys (Hotzel *et al.*, 2012). With 84 to $98 \text{ mL}\cdot\text{kg}^{-1}$, the volume of distribution was slightly higher than the reported plasma volume of $45 \text{ mL}\cdot\text{kg}^{-1}$ in cynomolgus monkeys suggesting that this antibody also distributes to extracellular fluid (Davies and Morris, 1993).

The PK of monoclonal antibodies often appear non-linear because of binding to the specific target; at high antibody concentrations, this target binding will be saturated and linear elimination predominates (Dong *et al.*, 2011). In contrast to many other monoclonal antibodies, no non-linear kinetics as a result of target-mediated disposition were observed for AMG 139, which is not surprising as the levels of

IL-23 are expected to be low in cynomolgus monkeys and healthy human subjects (Nogueira *et al.*, 2010; Gagliardi *et al.*, 2011). Further research is needed to determine whether target-mediated disposition can affect AMG 139's PK under pathophysiological conditions with increased concentrations of the target IL-23p19 in plasma and/or tissue. Insights into the effect of disease on target-mediated disposition has been addressed in a single ascending dose in healthy volunteers and patients with psoriasis (Clinicaltrials.gov NCT01094093) and a multiple ascending dose study in healthy volunteers and subjects with Crohn's disease (Clinicaltrials.gov NCT01258205, ongoing).

Of note, the AMG 139 exposure parameters C_{max} and AUC were lower after 6-month dosing than after 3-month dosing. Although the exact mechanism is not clear, this discrepancy might be due to high between-study and/or between-animal variability often observed for monoclonal antibodies (Dirks and Meibohm, 2010) or other non-observed or non-observable covariates (e.g. changes in target expression or physiological changes). In addition, the longer duration of weekly AMG 139 treatment in the 6-month study may have resulted in a higher rate of immunogenic response and thus lower AMG 139 exposure at the last week of the dosing phase compared with the 3-month study. Although a similar rate of immunogenicity was detected in both studies, high levels of circulating AMG 139 might have interfered with the immunogenicity assay leading to under-detection of the anti-AMG 139 antibodies and hence an inability to more definitively assess its potential effect on the AMG 139 exposure. Across all the multiple dose studies, 10 out of 90 actively treated monkeys (11.1%) developed anti-AMG 139 antibodies. Immunogenicity in monkeys was anticipated because AMG 139 is a human antibody and would be expected to be immunogenic in non-human species. Interestingly, except for two animals in the 2-week toxicology study, no neutralizing anti-AMG 139 antibodies were detected and no effect on AMG 139 exposure was observed. The incidence of anti-AMG 139 antibodies appeared to decrease with increasing dose of AMG 139. Such an inverse relationship between dose and the incidence of immunogenicity has previously been observed for other antibody therapies (e.g. for AMG 181 or infliximab) (Wagner *et al.*, 2003; Pan *et al.*, 2013a).

The multiple dose toxicology studies in cynomolgus monkeys indicate that once-weekly i.v. or s.c. administration for up to 6 months was well tolerated at doses up to 300 mg·kg⁻¹; only injection site reactions were observed, which is a common side effect after s.c. administration of biological agents.

The data obtained in the present study were used to inform further clinical development of AMG 139. Based on the observed linear elimination and dose-proportionality of this antibody in cynomolgus monkeys and the exposure parameters (AUC and C_{max}) at the no-observed-adverse-effect level of 300 mg·kg⁻¹ s.c. (the highest dose tested), monkey PK parameters were allometrically scaled to predict human exposure and safety margins (Dong *et al.*, 2011). Based on these calculations, doses up to 700 mg i.v. are predicted to be safe in humans. Indeed, preliminary analysis of the first-in-human study in healthy volunteers indicates that AMG 139 is well-tolerated and that the observed exposure parameters

were well predicted using the allometric scaling approach (within twofold).

Due to the low expression levels of the target (IL-23) in the central circulation, an effect of AMG 139 on the biomarkers evaluated in healthy cynomolgus monkeys, the only non-human species to which AMG 139 cross-reacts, could not be demonstrated. However, proof-of-concept clinical studies in patients with psoriasis and Crohn's disease are currently being performed (clinicaltrials.gov NCT01094093 and NCT01258205); results from these studies will be reported separately.

To infer efficacious concentration for clinical trials in psoriasis patients, the *in vitro* IC₅₀ values were used: AMG 139 concentrations higher than the *in vitro* IC₉₀ values (between approximately 0.038 and 0.38 µg·mL⁻¹) were targeted. The preliminary results indicated that after a single dose of 21 mg in patients with moderate to severe psoriasis, the AMG 139 concentrations were sustained above 0.1 µg·mL⁻¹ for approximately 16 weeks and this was associated with a reduction in psoriasis area severity index score and an improvement in psoriasis disease markers within the 16-week post-dosing period [(Pan *et al.*, 2013b) and data on file at Amgen Inc., CA]. Detailed results from the first-in-human study of AMG 139 will be reported separately.

Conclusions

Given the role of IL-23 in the pathogenesis of autoimmune/inflammatory diseases, our pharmacology, PK and safety data suggest that AMG 139-mediated IL-23 blockade is a promising therapeutic approach and support further clinical development.

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Author contributions

W. J. P., M. J. H., J. P. G., T. J. G., K. J. N., M. W. T., W. H. T., J. E. T. and W. A. R. participated in the research design. Y. S., Y. Z., K. J. N., A. N. U-R., S. P. P., C. D. K., A. C., L. S., S. A. B., M. W. T. and J. E. T. conducted the experiments. A. C., T. J. G., J. C. O. and J. E. T. contributed new reagents or analytic tools.

K. K., W.J. P., M. J. H., J. P. G., A. C., W. A. R., S. A. B., Y. S. and Y. Z. performed the data analysis. K. K., W. J. P., J. M. G., W. H. T. and J. E. T. wrote or contributed to the writing of the manuscript.

Conflict of interest

This work and the studies presented herein were financially funded by Amgen Inc., Thousand Oaks, CA, USA. The authors are current or former employees or contractors and shareholders of Amgen Inc. AMG 139 is being co-developed by MedImmune, LLC (a wholly owned subsidiary of AstraZeneca plc.) and Amgen Inc., and may be referred to as AMG 139 or MEDI2070.

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