Clinical pharmacology of AMG 181, a gut-specific human anti-$\alpha_4\beta_7$ monoclonal antibody, for treating inflammatory bowel diseases

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- The clinical pharmacology and safety of AMG 181 in humans were unknown before we conducted the first-in-human single ascending dose study.
- The pharmacokinetic (PK) characterization of AMG 181 in healthy subjects was required for AMG 181 dose selection for phase 2 trials in subjects with inflammatory bowel disease (IBD).

WHAT THIS STUDY ADDS

- AMG 181 is safe and well tolerated in healthy subjects.
- AMG 181 PK and target $\alpha_4\beta_7$ receptor occupancy are directly correlated in healthy humans, with no effects on $\alpha_4\beta_7$ receptor bearing target cell counts.
- AMG 181 has PK/PD and safety characteristics suitable for further testing in IBD subjects.

AIMS

AMG 181 pharmacokinetics/pharmacodynamics (PK/PD), safety, tolerability and effects after single subcutaneous (s.c.) or intravenous (i.v.) administration were evaluated in a randomized, double-blind, placebo-controlled study.

METHODS

Healthy male subjects ($n= 68$) received a single dose of AMG 181 or placebo at 0.7, 2.1, 7, 21, 70 mg s.c. (or i.v.), 210 mg s.c. (or i.v.), 420 mg i.v. or placebo. Four ulcerative colitis (UC) subjects ($n= 4$, male : female 2:2) received 210 mg AMG 181 or placebo s.c. (3:1). AMG 181 concentration, anti-AMG 181-antibody (ADA), $\alpha_4\beta_7$ receptor occupancy (RO), target cell counts, serum C-reactive protein, fecal biomarkers and Mayo score were measured. Subjects were followed 3–9 months after dose.

RESULTS

Following s.c. dosing, AMG 181 was absorbed with a median t_{max} ranging between 2–10 days and a bioavailability between 82% and 99%. C_{max} and AUC increased dose-proportionally and approximately dose-proportionally, respectively, within the 70–210 mg s.c. and 70–420 mg i.v. ranges. The linear β-phase $t_{1/2}$ was 31 (range 20–48) days. Target-mediated disposition occurred at serum AMG 181 concentrations of less than 1 μg ml^{-1}. The PD effect on $\alpha_4\beta_7$ RO showed an EC$_{50}$ of 0.01 μg ml^{-1}. Lymphocytes, eosinophils, CD4+ T cells and subset counts were unchanged. AMG 181-treated UC subjects were in remission with mucosal healing at weeks 6, 12 and/or 28. The placebo-treated UC subject experienced colitis flare at week 6. No ADA or AMG 181 treatment-related serious adverse events were observed.

CONCLUSIONS

AMG 181 has PK/PD, safety, and effect profiles suitable for further testing in subjects with inflammatory bowel diseases.

Introduction

Crohn's disease (CD) and ulcerative colitis (UC) are chronic, relapsing, inflammatory disorders of the gastrointestinal tract collectively termed inflammatory bowel diseases (IBDs). They affect approximately 1.4 million people in the United States (USA) and 2.2 million people in Europe with an incidence rate ranging between 10 and 30 per 100 000. Regions with traditionally lower incidence or prevalence of IBDs (e.g. Asia) have shown increasing trends in recent decades suggesting that IBDs are emerging as a global disease [1–3]. The reported mean age at diagnosis is generally similar between Western and Asian populations with peak incidence occurring between 20 and 40 years [1, 2, 4]. Although the pathophysiology is not fully understood, accumulating epidemiologic data suggest that IBDs may result from an inappropriate inflammatory response to alterations in intestinal microbiome in a genetically susceptible sub-group of the population [5–7].

Due to the autoimmune nature of IBDs, initial treatment typically includes conventional anti-inflammatory agents such as 5-aminosalicylic acids (5-ASA), corticosteroids and immunomodulators [8]. After failure with the aforementioned conventional agents, the mainstay of the current approach is the use of anti-tumour necrosis factor antibodies (anti-TNFs) including the chimeric immunoglobulin G_1 (IgG_1) infliximab [9–11] and the human IgG_1 adalimumab [12–14], both of which are indicated for treating CD and UC. In addition, the IgG_4-binding pegylated humanized Fab (fragment, antigen binding) certolizumab [15, 16] and the human IgG_1 golimumab [17] are indicated for treating CD and UC, respectively. Despite the proven therapeutic efficacy of the anti-TNFs in inducing and maintaining clinical remission in IBDs, meta-analyses of randomized clinical trials (RCT) have shown that the rate of subjects whose disease is not improved by treatment (non-responders) remains high [18]. For example, in RCTs for treating CD, the overall induction of remission rate was 18 to 48% at week 4, with 25 to 41% of those subjects remaining in remission after 1 year [19]. In RCTs for treating UC, the overall induction and maintenance of remission rates were 19 to 39% and 26 to 37%, respectively [18]. These data indicate that approximately 80 to 95% of the IBD patients were either non-responders to anti-TNFs or had lost anti-TNF treatment benefit within 1 year. From a safety perspective, anti-TNFs have been associated with an increased risk of serious infections and malignancy [20]. Therefore, an unmet medical need remains for developing safer and more efficacious therapies for IBD patients.

In recent years, studies have been focusing on agents that block the influx of pro-inflammatory cells into the intestinal mucosa (gut-homing specific) through integrin-mediated leukocyte tethering, rolling and arrest [21]. For example, blocking lymphocyte gut-homing integrin $\alpha_4\beta_7$ [22–24], integrin β_7 [25–27] or $\alpha_4\beta_7$ target ligand MAdCAM-1 (mucosal addressin cell adhesion molecule-1)

[28, 29] has demonstrated efficacy in various preclinical IBD models. In fact, some of these targets are being investigated in clinical trials to evaluate safety and efficacy of therapeutic candidates such as vedolizumab (anti-$\alpha_4\beta_7$), AMG 181 (anti-$\alpha_4\beta_7$), etrolizumab (anti-β_7) and PF-00547659 (anti-MAdCAM-1) [30].

Natalizumab, a pleiotropic pan-α_4 antagonist blocking both $\alpha_4\beta_1$ and $\alpha_4\beta_7$ integrins, has shown clinical efficacy in treating CD and is approved for this indication in the USA [31, 32]. Additionally, natalizumab has been approved in the USA and Europe for treating relapsing and remitting forms of multiple sclerosis (MS) [33]. Under certain conditions, and especially in the MS population, natalizumab has been found to increase the risk of developing progressive multifocal leukoencephalopathy (PML) due to re-activation of the latent human John Cunningham (JC) polyomavirus and subsequent infection of the central nervous system (CNS) [34]. It is hypothesized that inhibition of $\alpha_4\beta_1$-expressing cells by natalizumab may lead to broad impact on leukocyte trafficking to various tissues, including the brain and, by compromising local immune surveillance, may enable JC virus replication and ensuing PML [35–37].

Natalizumab and vedolizumab are humanized IgGs, and RCTs have shown that both antibodies have greater than 10% immunogenicity in IBD patients leading to reduced clinical efficacy [31, 38–40]. In order to reduce or eliminate the impact of immunogenicity and the safety implications associated with targeting $\alpha_4\beta_1$ expressing leukocytes, Amgen has developed AMG 181, a human monoclonal IgG_2 antibody that specifically binds to the $\alpha_4\beta_7$ integrin on gut-tropic lymphocytes, which blocks interaction with the $\alpha_4\beta_7$ target ligand MAdCAM-1 and thus prevents lymphocyte trafficking into mucosal tissue and alleviates inflammation. We have previously reported the *in vitro* pharmacology, as well as PK/PD and safety characteristics of AMG 181 in cynomolgus monkeys that support its testing in humans [41].

In this report, we present results from the first-in-human study conducted in healthy subjects and subjects with UC. Our specific aims for this phase 1 clinical trial were to evaluate single-dose PK/PD, safety, tolerability, and effects of AMG 181 after s.c. or i.v. administration [42]. The results from this study have suggested that, given its pharmacological potency, apparent lack of immunogenicity and gut-specificity, AMG 181 has the potential for providing IBD patients with another treatment option. AMG 181 is currently being tested in subjects with UC or CD in phase 2 clinical trials [43, 44].

Methods

Investigational product
AMG 181 (~144 kD) was manufactured at Amgen Inc., Thousand Oaks, California (CA), USA, by expression in

Chinese hamster ovary (CHO). The investigational AMG 181 substance was formulated at 70 mg ml⁻¹ with pharmaceutically acceptable excipients, pH 5.2, and stored at −20°C to −70°C in a non-frost freezer, until ready for s.c. or i.v. administration. AMG 181 placebo (matching vehicle control) was formulated with the same excipients and pH, filled, packaged and stored to match the active AMG 181. The placebo was also used as the active s.c. dose diluent, while 5% dextrose (US Pharmacopeia) was used for i.v. infusion bags.

Study design and ethical considerations

This was a randomized, double-blind, placebo-controlled single ascending dose (SAD) study to evaluate the safety, tolerability, PK/PD, and effects of AMG 181 after s.c. or i.v. dose in healthy volunteers and subjects with UC (http://www.clinicaltrials.gov, identifier NCT01164904). The study design and dose escalation schedule are summarized in Figure 1.

The study was carried out in accordance with the ethical principles set forth in the Declaration of Helsinki and its revisions (the most recent in 2008), the International Conference on Harmonization (ICH) E6 Guidance for Good Clinical Practice (CPMP/ICH/135/95) and all other applicable country-specific regulatory requirements and site-specific standard operating procedures (SOPs). The study portion in healthy volunteers was conducted at California Clinical Trials Medical Group, Inc. Glendale, CA, USA (approved by the Aspire Institutional Review Board), while the UC portion was conducted at the Royal Adelaide Hospital, Adelaide, South Australia (approved by the Royal Adelaide Hospital Research Ethics Committee) and at the Royal Brisbane and Women's Hospital, Herston, Queensland, Australia (approved by the Royal Brisbane and

Women's Hospital Human Research Ethics Committee). Before subjects entered into the study, Amgen obtained a copy of the sites' written institutional review board's (IRB's) or ethics committees' (ECs') approval of the protocol and informed consent form, and all other subject information and/or recruitment material. All subjects or legally acceptable representatives personally signed and dated the IRB/EC-approved consent form before initiation of any screening procedures.

Subject enrolment criteria

Inclusion criteria for enrolment followed the standard practice for first-in-human studies. Briefly, eligible healthy men and women were 18 to 45 years of age (inclusive), with a body mass index (BMI) of 18 to 34 kg m⁻², and in good health based on physical and neurological examinations and were sero-positive for Epstein-Barr virus. Subjects had normal laboratory values and normal limits for 12-lead electrocardiogram (ECG). All male subjects practiced a highly effective method of birth control during the study. No female subjects were enrolled in the healthy subject portion of the study.

Subjects who met any one of the following criteria were excluded from the study: history or evidence of clinically significant disorders, conditions or diseases that posed a risk to the subject's safety or interfered with study evaluation, procedures or completion; history of malignancy of any type, men who had pregnant partners, known history of drug or alcohol abuse within 1 year of screening or positive screen for alcohol and/or drugs with a high potential for abuse at screening or day −1, positive for HIV antibodies, hepatitis B surface antigen, hepatitis C antibodies, tuberculosis, or JC viraemia determined by PCR at screening, sero-negative for Epstein-Barr virus, use of
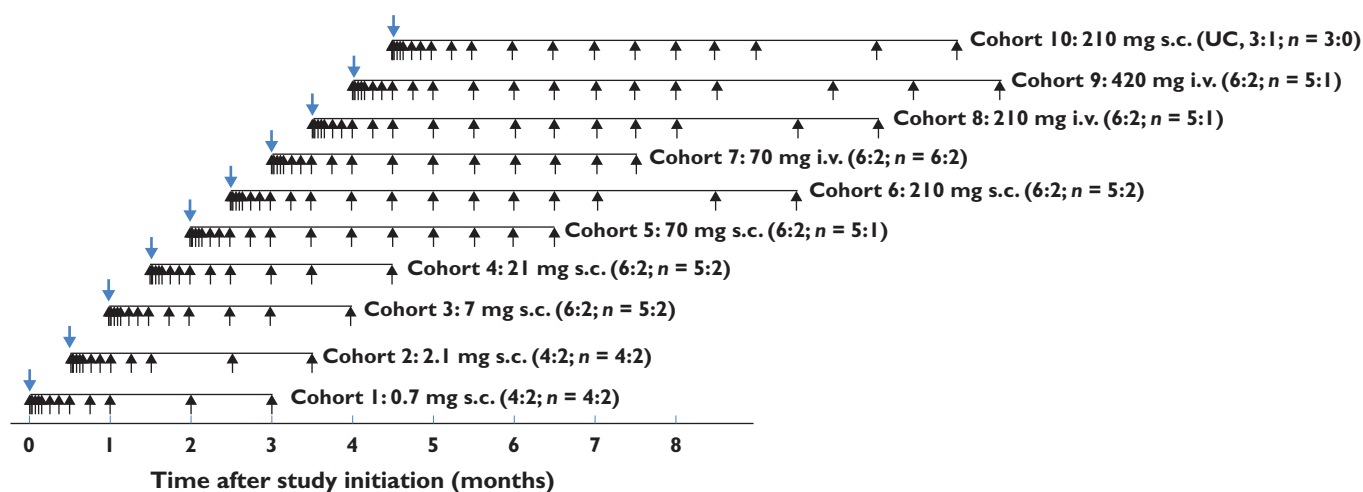
Figure 1

Study design and dose escalation schedule. Blue arrows represent dosing; black arrows represent pharmacokinetic, pharmacodynamic, immunogenicity, safety and/or effect assessment visits. Numbers in parenthesis indicate AMG 181: placebo randomization ratio per study protocol and the number of subjects (N) completing the study. s.c. = subcutaneous; i.v. = intravenous; UC = ulcerative colitis subjects else, healthy subjects

any investigational biologic ≤ 6 months or use of prescription or non-prescription drugs (with the exception of paracetamol/acetaminophen [up to 2 g day⁻¹]) within 14 days prior to investigational product administration.

The UC subjects met the above inclusion/exclusion criteria for healthy subjects but were allowed to be up to 65 years of age. Women were of either child bearing or non-child-bearing potential, with those of child-bearing potential having a negative pregnancy test on day −1 and agreeing to use highly effective methods of birth control for the duration of the study. Subjects with UC had a documented diagnosis of at least 2 months of active UC defined by an Ulcerative Colitis Disease Activity Index (UCDAI, or Mayo score) score [45] of 4 to 9 (inclusive), with a minimum sigmoidoscopy score of 1.

The main exclusion criteria for UC subjects included disease limited to the rectum, women planning to become pregnant during the study, current use of oral steroids in excess of 20 mg prednisone or equivalent per day, tuberculosis or fungal infection within 3 months of screening or during screening, immunosuppressive therapy with either azathioprine, methotrexate, or mercaptopurine, within 3 months of day 1, immunosuppressive therapy with either ciclosporin A, tacrolimus, mycophenolate mofetil within 2 months of day 1, exposure to anti-TNF agents within 2 months, or five times the respective elimination half-life (whichever was longer) prior to day 1, prior exposure to natalizumab, rituximab or efalizumab, change of dose of oral mesalamine within the past weeks of screening or use of topical mesalamine or steroids within 2 weeks prior to study day 1.

Dosing and dose escalation procedures

After informed consent and within a period of 21 days prior to dosing, all screening tests establishing subject eligibility were performed. Subjects were admitted to the study clinic on the day prior to dosing (day −1) to perform day −1 procedures. On day 1, after completion of all pre-dose procedures, subjects received a single dose of AMG 181 or placebo and remained in the clinic until completion of study procedures on day 3 (48 h after dosing). Subjects were discharged from the study clinic on day 3 and returned for safety follow-up and PK/PD assessments.

AMG 181 was administered to healthy volunteers as a single dose of 0.7, 2.1, 7, 21, 70 or 210 mg s.c. or 70, 210 or 420 mg i.v. (1 h infusion) and, to UC subjects, 210 mg s.c., corresponding to cohorts 1 to 10, respectively (Figure 1). Within each single dose cohort, subjects were to be randomized such that six received AMG 181 and two received matching placebo (except for cohorts 1 and 2, where four received AMG 181 and two received placebo). A sentinel dosing design was applied for the 0.7 and 2.1 mg s.c. and 70 mg i.v. cohorts, where the first two subjects received AMG 181 treatment or placebo in a blinded fashion. In cohort 10, four staggered UC subjects received a single 210 mg AMG 181 or placebo s.c. dose (3:1 ratio).

S.c. doses were administered to the anterior abdominal wall of the subject. For individual s.c. dose volumes that exceeded 1.5 ml, the dose was split into separate syringes and administered into different sites on the subject's anterior abdominal wall. The s.c. injections were administered in a consecutive fashion with the injections separated by no more than 30 s. The i.v. dosing was performed through infusion in 5% dextrose in a volume of 100 ml over a 1 h period using a volumetric infusion pump through an i.v. access.

With the previous regimen(s) being well tolerated, no stopping rules being met, and under an unanimous dose level review meeting recommendation, escalation to the next higher dose cohort (cohorts 2 through 10) proceeded based on a safety data review through day 15 for all subjects in the current cohort and all available safety data from all subjects in the previous cohort(s). Subjects who dropped out of the study for reasons other than adverse events after dosing were replaced at the discretion of Amgen in consultation with the investigator or their designee. Replacement subjects received the identical treatment as the assigned treatment for the subject to be replaced. Completion of the end of study (EOS) assessments occurred on day 169 for cohorts 1–5 and 7, day 197 for cohorts 6, 8 and 10 and day 225 for cohort 9.

Safety and laboratory assessments

A detailed summary of the safety and laboratory study procedures and schedules is presented in Supplementary Table S1.

Adverse events and concomitant medication were monitored throughout the study. All adverse events were reported according to the United States National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) v4.0 [46]. The study sites recorded use of concomitant medication and all adverse events (treatment-emergent and treatment-related as assessed by the investigator) that occurred during or after dosing through the end of the study, as observed by the investigator or reported by the subject. All treatment emergent adverse events or laboratory abnormalities were followed until the resolution of the abnormality or until the condition was considered clinically stable in the opinion of the investigator. Descriptive statistics were calculated per dose level and tabulated by the system organ class and preferred term. Due to scarcity of the adverse events in this study, data using the preferred term and the treatment-emergent adverse events occurring in > 1 of healthy subjects are presented.

Blood samples were collected for CD4+ T cell subset enumeration and $\alpha_4\beta_7$ RO/expression, and for JC viraemia determination. Other laboratory assessments included pregnancy test, follicle-stimulating hormone (FSH) measurement, human immune deficiency virus (HIV) and hepatitis B and C screening, and stool culture and stool ova and parasites, and drug and alcohol screening.

For UC subjects, the effects of AMG 181 treatment were assessed through the Mayo score, partial Mayo score, serum C-reactive protein, and fecal biomarkers including calprotectin and lactoferrin. The Mayo score has four components, including stool frequency, rectal bleeding, endoscopic findings and physician's global assessment. Each of the four components has an integer score range of 0 to 3, with the total score ranging from 0 to 12 [45]. When recto-sigmoidoscopy is not preformed, the partial Mayo score with a total score ranging from 0 to 9 can be assessed. Empirically and in most recent clinical trials, remission has been defined as Mayo score $\leq$ 2, with no individual subscore > 1 point, response as Mayo score decrease from baseline of $\geq$ 3 and $\geq$ 30%, plus rectal bleeding score decrease of $\geq$ 1 point or absolute reading of 0 or 1, mucosal healing as recto-sigmoidoscopy score of $\leq$ 1 [14, 47]. Up to two stools were collected on each of 3 consecutive days during 5 different weeks to support assessment of fecal calprotectin and lactoferrin levels. Subjects returned stool specimens to the clinical sites for processing and subsequent testing within 72 h of collection. The assay limits of detection for fecal calprotectin and lactoferrin were 15.4 μg g^{-1} and 31 μg ml^{-1}, respectively.

Serum AMG 181 concentration and immunogenicity assays

Serum AMG 181 concentrations were determined with a validated immunoassay utilizing the Meso Scale Discovery (Gaithersburg, MD, USA) electrochemiluminescence (ECL) platform with a lower limit of quantification (LLOQ) of 10.0 ng ml^{-1}.

Serum was analyzed for anti-AMG 181 antibodies (ADAs) with a validated immunoassay utilizing the Meso Scale Discovery ECL platform with a lower limit of reliable detection (LLRD) of 20 ng ml^{-1} of affinity purified rabbit ADAs in neat pooled normal human serum. Detection of ADAs at the LLRD may have been prevented, creating a false negative result, if a test sample AMG 181 concentration was > 25 μg ml^{-1}. Detailed information on the serum AMG 181 concentration and immunogenicity assays can be found in the supplementary material.

Pharmacokinetic analyses

Non-compartmental PK analysis was performed using WinNonlin® 5.02 (Pharsight® Corporation, Mountain View, CA, USA). Actual sampling times elapsed from dosing time for each individual subject were used for the calculation of AMG 181 PK parameters. These included maximum observed AMG 181 concentration in serum (C_{max} and dose-normalized value: C_{max}/Dose), time to C_{max} (t_{max}) and area under the concentration–time curve from time zero to the last observed concentration (AUC(0,t)) and extrapolated to infinity (AUC(0,∞) and dose-normalized value, AUC(0,∞)/ Dose). AUC values were calculated by the linear trapezoidal rule. AMG 181 bioavailability was calculated by

dividing the cohort AUC(0,∞) means between 70 mg s.c. and i.v. or 210 mg s.c. and i.v..

AMG 181 exhibited target-mediated disposition, where capacity-limited binding to the target $\alpha_4\beta_7$ integrin resulted in non-linear PK. This non-linear PK was obvious at low AMG 181 concentrations of < 1 μg ml^{-1}, where AMG 181 disposition was predominantly driven by high affinity low capacity binding between AMG 181 and the limited amount of $\alpha_4\beta_7$ receptors. At higher AMG 181 concentrations of > 1 μg ml^{-1}, the saturable AMG 181 disposition through binding to $\alpha_4\beta_7$ receptors was masked by the lower affinity, high capacity linear non-specific binding of AMG 181 to neonatal Fc receptors (FcRns).

Because AMG 181 exhibited target-mediated disposition at the terminal elimination phase (γ-phase), individual linear β-phase rate constant (β), a relevant measure of effective half-life for repeated dosing, was determined in the linear portion of the individual concentration–time profiles by visual inspection. At least three data points were in the linear portion with the coefficient of correlation for the linear regression of $\geq$ 0.90. The linear β-phase half-life ($t_{1/2}$) was calculated as ln(2)/β. Other calculated linear PK parameters included: clearance (CL) and apparent clearance (CL/F) for i.v. and s.c., respectively, volume (V_z) and apparent volume of distribution (V_z/F) at the terminal elimination phase for i.v. and s.c., respectively, and volume of distribution at steady-state for i.v. (V_{ss}). Descriptive statistics were calculated per cohort: n, mean (median for t_{max}) and standard deviation (SD) (range for t_{max}).

To explore the effects of demography on the AMG 181 PK, dose-normalized C_{max} and AUC(0,∞) were plotted against body weight, BMI, age and race, with the latter being categorized into White, Black and others.

Pharmacodynamic assays and analyses

To characterize the direct PD effects of AMG 181, $\alpha_4\beta_7$ receptor occupancy (RO) and changes in target cell counts in the selected lymphocyte subset populations were investigated.

A semi-quantitative flow cytometric assay was developed to assess coverage of $\alpha_4\beta_7$ receptors by AMG 181 on CD4+ T cell subsets namely, naïve, central memory and effector memory T cells, in whole blood specimens. The principle of this assay was that exposure to AMG 181 was expected to reduce staining intensity of a fluorochrome-labelled anti-$\alpha_4\beta_7$ RO antibody 1 (anti-$\alpha_4\beta_7$-ROA1) which competes with AMG 181 for binding on target cell surface. To identify the floor of the assay, a separate aliquot of each blood specimen was pre-incubated with a saturating dose of AMG 181 (500 μg ml^{-1}) ex vivo. For each specimen, the number of anti-$\alpha_4\beta_7$-ROA1 molecules bound per CD4+ T cell subsets was calculated relative to that in the presence of saturating AMG 181, thereby providing an estimate of the fractional occupancy of $\alpha_4\beta_7$ molecules per cell, where percent fractional receptor occupancy (%RO) = 100 – free fraction*100. A second, non-competing, anti-β_7 RO

antibody 2 (anti-β_7-ROA2) was also used to quantify total $\alpha_4\beta_7$ receptor levels on CD4+ T cell subsets after exclusion of CD103 (α_E integrin, which binds to β_7 integrin and forms $\alpha_E\beta_7$ heterodimer) positive cells.

CD4+ T cell subsets were enumerated by flow cytometric assay in conjunction with absolute lymphocyte count from complete blood counts (CBCs). In addition, $\alpha_4\beta_7$ expressing CD4+ memory T cell subsets in blood were enumerated by multiplying the $\alpha_4\beta_7$ high or low CD4+ memory T cell event count as a fraction of total lymphocyte event count by the total lymphocyte count determined by CBC. CD4+ $\alpha_4\beta_7$ high or low memory T cell event count was measured by the anti-β_7-ROA2. Other AMG 181 target cells expressing $\alpha_4\beta_7$ receptors such as eosinophils and total lymphocytes were enumerated directly through CBCs.

The relationships between serum AMG 181 concentration and the subject/time-matched $\alpha_4\beta_7$ RO on total CD4+, naïve, central memory and effector memory T cells were quantitatively assessed using a simple E_{max} PD model:

$$E = E_0 + (E_{max} - E_0) \times C/(EC_{50} + C)$$

where E_0 stands for the baseline, E_{max} is the maximal RO, C is serum AMG 181 concentration and EC_{50} is AMG 181 concentration at which the RO is half-maximal. An E_{max} PD model with Hill coefficient was tested but did not result in a significantly improved model fit. The AMG 181 concentrations at which RO was 90% (EC_{90}) or 99% (EC_{99}) maximum were calculated using the above equation. RO data from all subjects pre-dose and from all placebo subjects were pooled across the regimens with the assigned AMG 181 concentration of zero. Because the assay for the AMG 181 concentration had a lower limit of quantification (LLOQ) of 0.01 µg ml^{-1}, RO data corresponding to AMG 181 concentrations that were below the assay LLOQ (BQL) and collected after AMG 181 treatment were not used for modelling (but were plotted with AMG 181 concentrations assigned a value at half of the LLOQ in Figure 5).

The relationship between serum AMG 181 concentration and the subject/time-matched $\alpha_4\beta_7$ RO on total CD4+ T cells (including its three subsets: CD4+ naïve, central memory, and effector memory T cells) was further explored by plotting AUEC$_{all}$ or area-under-the-effect (RO) – time curve vs. AMG 181 area under the concentration–time curve extrapolated to concentration of zero (AUC$_{all}$) using data from all healthy subjects who were dosed AMG 181 or received placebo and completed the study. A simple E_{max} PD model as described above was used to fit the data for quantifying EAUC$_{all,90}$ from EAUC$_{all,50}$ (the AMG 181 AUC$_{all}$ at which the RO AUEC$_{all}$ was half-maximum), to estimate an AUC$_{all}$ (or the associated dose) beyond which there would be no further meaningful $\alpha_4\beta_7$ RO AUEC$_{all}$ increases (e.g., ~90% AUEC$_{all,max}$).

Statistical analyses

This was a phase 1 clinical trial, with sample size chosen based on practical considerations and consistent with conventions and, therefore, data have been either presented using descriptive statistics or individually (mostly UC data). We have conducted *post hoc* linear regression analyses of the demographic effects on dose-normalized AMG 181 peak (C_{max}) and cumulative exposure (AUC) parameters. This will guide our future population analysis when more abundant data become available from the on-going phase 2 studies.

Results

Baseline demographics

Sixty-eight (68) healthy male subjects and four male and female UC subjects were enrolled in this single ascending dose study. Summary statistics of subject baseline demography by dosing regimen or cohort, as well as the overall summary across cohorts are provided in Table 1. The baseline characteristics were similar among dosing regimens regarding age, body weight, height and BMI, except for race, for which the percentage of White subjects ranged from 0 to 83%.

Of the 68 healthy subjects, 60 completed the study, two withdrew consent due to personal reasons and six were lost to follow-up. Of the four UC subjects, one subject who received placebo withdrew from the study after the day 43 visit due to colitis flare.

Safety, tolerability and immunogenicity

For healthy subjects, due to small cohort sizes, low subject incidences of adverse events, and absence of apparent trends in subject incidence of adverse events associated with dose or route of administration, data from all cohorts have been pooled by AMG 181 or placebo treatment (Table 2). The overall subject incidences for treatment-emergent adverse events were comparable between the AMG 181 group and the placebo group (60% vs. 50%). All adverse events were reported as grade 1 in severity in healthy subjects except for one adverse event of grade 2 hypertension (210 mg AMG 181 s.c. subject) and two adverse events of grade 3 syncope (70 mg AMG 181 i.v. and placebo s.c. subject). Mild injection site reactions were reported for two subjects who received AMG 181 and no infusion reactions were reported.

All four subjects with UC had treatment-emergent adverse events. Two cases each of upper respiratory tract infection and headache occurred in UC subjects who received AMG 181 and abdominal pain and injection site erythema were each reported for two subjects (one AMG 181, one placebo). Among the four subjects with UC, there were a total of 37 adverse events (34 AMG 181, three placebo). These adverse events were reported as grade 1

Table 1

Mean (SD) baseline demographics

Regimen	n	Age (years)	Weight (kg)	Height (cm)	BMI (kg m⁻²)	Race (%White)
0.7 mg s.c.	4	32.3 (9.1)	78.7 (8.82)	177.4 (4.1)	25.0 (2.64)	50
2.1 mg s.c.	4	30.5 (3.4)	80.5 (3.61)	180.7 (7.8)	24.8 (2.87)	0
7 mg s.c.	6	26.5 (6.3)	79.4 (10.7)	178.0 (8.0)	25.1 (3.58)	33
21 mg s.c.	6	24.2 (3.1)	76.4 (11.1)	174.5 (5.2)	25.2 (3.87)	50
70 mg s.c.	6	28.3 (6.2)	72.7 (10.0)	172.1 (3.8)	24.5 (3.11)	33
210 mg s.c.	6	29.5 (5.9)	80.3 (12.0)	174.6 (5.4)	26.4 (4.06)	50
70 mg i.v.	6	29.0 (4.9)	82.6 (6.27)	179.6 (3.7)	25.7 (2.39)	50
210 mg i.v.	6	28.5 (7.8)	84.4 (22.3)	181.4 (15.2)	25.4 (4.43)	83
420 mg i.v.	6	29.8 (5.7)	79.9 (11.3)	179.8 (7.5)	24.7 (2.76)	0
210 mg s.c. (UC)	3	37.3 (5.1)	66.5 (11.0)	168.0 (3.1)	23.5 (2.98)	67
Combined	53	29.0 (6.2)	78.7 (11.8)	176.9 (7.8)	25.1 (3.17)	42
Placebo	19	29.9 (6.5)	82.7 (12.1)	176.3 (6.0)	26.7 (4.24)	63
All	72	29.3 (6.2)	79.7 (12.0)	176.7 (7.3)	25.5 (3.52)	47

Healthy subjects: all male; UC subjects: two female and one male were AMG 181-treated, one male received placebo. Combined = all AMG 181-treated, including healthy and UC subjects; Placebo = all placebo treated, including healthy and one UC subjects. BMI = body mass index; SD = standard deviation; s.c. = subcutaneous; i.v. = intravenous; UC = ulcerative colitis.

Table 2

Treatment-emergent adverse events in healthy subjects (reported for more than subject)

Preferred term	AMG 181 n = 50 n (%)	Placebo n = 18 n (%)
Any adverse event	30 (60)	9 (50)
Upper respiratory tract infection	15 (30)	1 (6)
Headache	8 (16)	1 (6)
Oropharyngeal pain	3 (6)	1 (6)
Diarrhoea	1 (2)	2 (11)
Nausea	2 (4)	1 (6)
Dizziness	2 (4)	0 (0)
Abdominal pain	0 (0)	2 (11)
Fatigue	1 (2)	1 (6)
Syncope	1 (2)	1 (6)
Viral infection	1 (2)	1 (6)

in severity, except for a grade 2 malaise and a grade 3 ulcerative colitis flare, both of which occurred in one subject who received 210 mg s.c. and grade 2 arthralgia in another subject who received 210 mg s.c. In addition, a serious, grade 4 colitis flare was reported for the subject who received placebo leading to the subject's early discontinuation at the day 43. Aside from the serious adverse event of colitis, no other serious adverse events were reported.

For healthy subjects and subjects with UC, no trends indicative of clinically important treatment-related laboratory abnormalities, ECG changes or changes in vital signs were observed.

All subjects enrolled in this study tested negative for JC viraemia and ADAs at all pre-selected time points as outlined in Supplementary Table S1, with the exception of one subject who was mistakenly enrolled with a positive JC viraemia test at screening. This subject received 210 mg AMG 181 s.c. and tested negative for JC viraemia at all post-screening time points.

Based on review of the safety and immunogenicity data, the safety and tolerability profile of AMG 181 has been acceptable in healthy subjects receiving single s.c. dose of up to 210 mg or single i.v. dose of up to 420 mg and in UC subjects receiving single s.c. dose of up to 210 mg.

Pharmacokinetics

The mean (SD) serum AMG 181 concentration–time profiles from all 53 AMG 181-treated subjects (50 healthy and three UC) are presented in Figure 2. After a single 0.7 mg s.c. administration, a complete AMG 181 concentration–time profile was available only for one subject. The two other subjects each had only one measureable concentration.

AMG 181 exhibited non-linear PK with rapid concentration decline when concentrations fell below approximately 1 µg ml⁻¹. This is consistent with target-mediated disposition characteristics that have previously been reported for monoclonal antibodies. At low AMG 181 concentration, clearance is mainly driven by the low-capacity and highly specific saturable binding (and subsequent internalization) to the target cell surface receptor $\alpha_4\beta_7$. At higher concentration, high-capacity and non-specific clearance through FcRn is more predominant and thus is masking the target-mediated disposition. For doses of ≥ 21 mg s.c., AMG 181 PK was characterized by a rapid absorption phase, a slow linear disposition phase, and a target-mediated disposition phase. The AMG 181 PK profiles after 70 and 210 mg i.v. were generally comparable with those through the s.c. route at the same doses, except for the initial rapid distribution phase typically observed for i.v. administration.
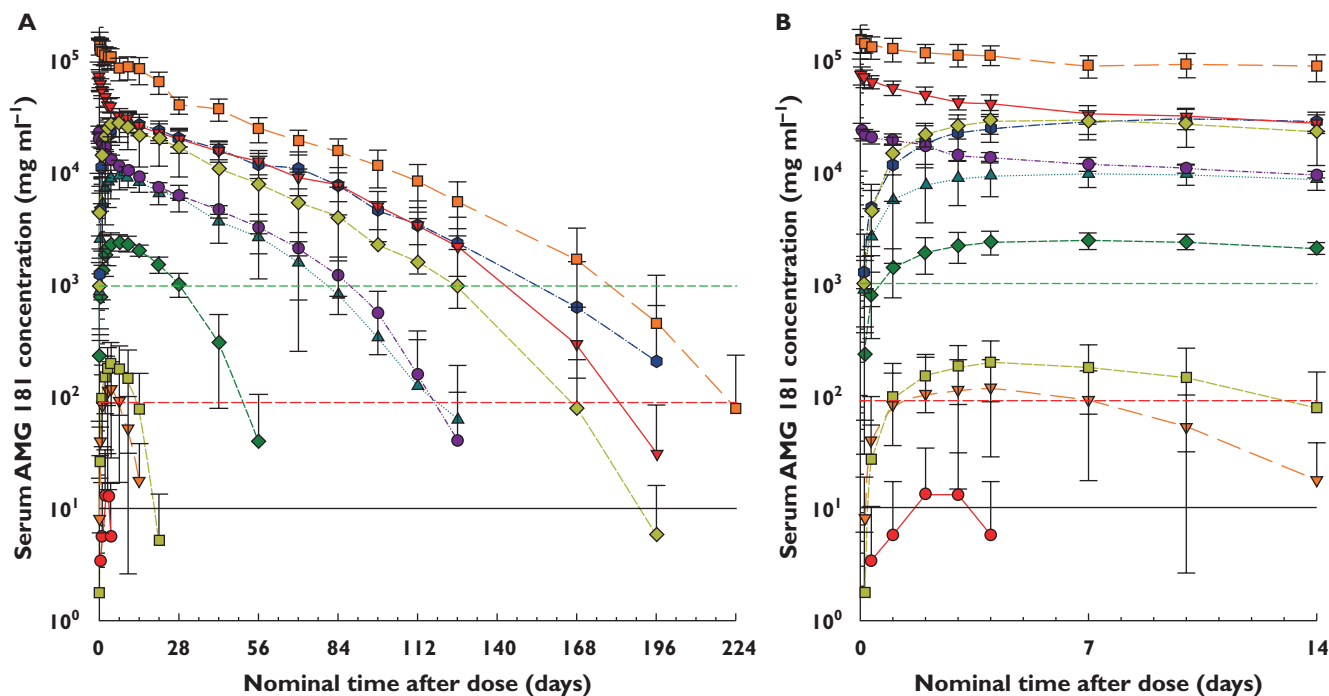
Figure 2

Mean (SD) serum AMG 181 concentration–time profiles after single subcutaneous or intravenous dose in healthy or ulcerative colitis subjects (UC). The full PK profile (A) and the absorption phase only (B) are shown. s.c. = subcutaneous; i.v. = intravenous; LLOQ = lower limit of quantification; RO = $\alpha_4\beta_7$ receptor occupancy on CD4+ naïve T cells. LLOQ and EC_{50} = 0.01 μg ml^{-1} (—), EC_{90} = 0.09 μg ml^{-1} (– –), and EC_{99} = 0.99 μg ml^{-1} (- - -). ▬●▬, Cohort 1: 0.7 mg s.c. (n = 3–4); ▬▼▬, Cohort 2: 2.1 mg s.c. (n = 4); ▬■▬, Cohort 3: 7 mg s.c. (n = 5–6); ▬◆▬, Cohort 4: 21 mg s.c. (n = 5–6); ▬▲▬, Cohort 5: 70 mg s.c. (n = 5–6); ▬●▬, Cohort 6: 210 mg s.c. (n = 5–6); ▬●▬, Cohort 7: 70 mg i.v. (n = 5–6); ▬▼▬, Cohort 8: 210 mg i.v. (n = 4–6); ▬■▬, Cohort 9: 420 mg i.v. (n = 4–6); ▬◆▬, Cohort 10: 210 mg s.c.UC (n = 3).

A summary of the AMG 181 PK parameters using non-compartmental analysis is provided in Table 3. Of the 50 healthy subjects who received AMG 181, two (one each for 0.7 and 7 mg s.c.) were excluded from non-compartmental analysis due to insufficient data. After s.c. administration, the t_{max} increased with dose and ranged between 2 to 10 days (2 to 14 days for individual subjects). A relative bioavailability of 82% and 99% was observed for 70 and 210 mg, respectively. As described above, AMG 181 PK profiles exhibited non-linearity due to target-mediated disposition at concentrations < 1 μg ml^{-1}, resulting in greater than dose proportional increases in C_{max}, AUC(0,∞) and β phase $t_{1/2}$ when doses were between 0.7 and 21 mg s.c. AMG 181 C_{max} was dose proportional across the 21 to 210 mg s.c. and 70 to 420 mg i.v. ranges. Across the 70 to 210 mg s.c. and 70 to 420 mg i.v. ranges, AMG 181 AUC(0,∞) was approximately dose proportional. The estimated mean AMG 181 linear β phase $t_{1/2}$ was identical for 70 to 210 mg s.c. (31 days; n = 14, including UC) and for 70 to 420 mg i.v. (31 days, n = 17) administrations. The grand mean (SD) for $t_{1/2}$ across all 70 to 420 mg s.c. and i.v. doses was 31 (7) days (n = 31, including UC; individual range 20 to 48) days. For 70 to 420 mg i.v. doses, the estimated AMG 181 CL and V_{ss} grand mean (SD) values (n = 17) were 0.120 (0.025) l day^{-1} and 4.30 (0.82) l, respectively.

Although this study was not prospectively designed to evaluate the potential effect of demographic parameters, plots of individual C_{max}/Dose and AUC(0,∞)/Dose values *vs*. body weight, BMI, age and race were examined. Figure 3 shows that the larger the body weight, the lower the AMG 181 C_{max}/Dose and AUC(0,∞)/Dose values, with a smaller effect observed for C_{max}/Dose for s.c. doses. The body weight effect was apparent within each cohort and among all cohorts combined. Except for C_{max}/Dose after dosing (Figure 3C), no other slopes of the linear regression lines were statistically significantly different from zero (P > 0.05). Results for BMI effect on AMG 181 C_{max}/Dose and AUC(0,∞)/Dose were consistent with those for body weight, with the effect of BMI being much less pronounced. Age had virtually no effect on AMG 181 C_{max}/Dose and AUC(0,∞)/Dose. Race, categorized by White, Black and others had no effect on AMG 181 C_{max}/Dose and AUC(0,∞)/Dose (analysis of variance or ANOVA for race effect: P > 0.05).

Pharmacodynamics

AMG 181 effects on free (measured by anti-$\alpha_4\beta_7$-ROA1 with competitive binding against AMG 181) and total (measured by anti-β_7-ROA2, binding non-competitively against AMG 181) $\alpha_4\beta_7$ receptors on CD4+ naïve T cells and central

Table 3

Mean (SD) pharmacokinetic parameters of AMG 181 after a single intravenous (i.v.) or subcutaneous (s.c.) administration in healthy subjects, or subjects with ulcerative colitis (UC)

Regimen	t_{max} (days, h)	C_{max} (µg ml⁻¹)	C_{max}/Dose (µg ml⁻¹ mg⁻¹)	AUC(0,t) (µg ml⁻¹ day)	AUC(0,∞) (µg ml⁻¹ day)	AUC(0,∞)/Dose (µg ml⁻¹ day mg⁻¹)	$t_{1/2}$ (days)	V_z, V_z/F (l)	CL, CL/F (l day⁻¹)	V_{ss} (l)
0.7 mg s.c.	2.0	0.0225	0.0322	0.0433	NC	NC	NC	NC	NC	NC
(n = 3)	(2.0, 3.0)	(0.0180)	(0.0256)	(0.0644)						
2.1 mg s.c.	3.5	0.126	0.0598	1.01	2.84*	1.35*	11.0*	11.7*	0.739*	NC
(n = 4)	(2.0, 7.1)	(0.100)	(0.0476)	(0.95)						
7 mg s.c.	4.0	0.245	0.0351	2.64	4.12*	0.589*	10.0*	24.5*	1.70*	NC
(n = 5)	(2.0, 4.0)	(0.046)	(0.0066)	(1.25)						
21 mg s.c.	7.1	2.51	0.119	63.5†	67.2†	3.20†	20.6†	9.53†	0.326†	NC
(n = 6)	(3.1, 10.0)	(0.51)	(0.024)	(13.6)	(15.0)	(0.71)	(9.0)	(3.92)	(0.078)	
70 mg s.c.	5.6	10.1	0.144	410†	414†	5.92†	25.2†	6.36†	0.185†	NC
(n = 6)	(3.0, 10.0)	(2.45)	(0.035)	(149)	(155)	(2.22)	(7.0)	(1.16)	(0.056)	
210 mg s.c.	10.0	29.8	0.142	1690†	1720	8.21	37.3	6.76	0.132	NC
(n = 6)	(7.0, 14.0)	(6.2)	(0.030)	(520)	(487)	(2.32)	(7.43)	(1.08)	(0.046)	
70 mg i.v.	1.4	23.0	0.329	501	503	7.19	27.3	5.50	0.140	4.12
(n = 6)	(1.4, 1.6)	(3.5)	(0.050)	(34)	(34)	(0.48)	(3.5)	(0.63)	(0.010)	(0.43)
210 mg i.v.	1.0	72.1	0.343	1720†	1730†	8.25†	34.5†	6.17†	0.124†	5.08†
(n = 6)	(1.0, 1.0)	(13.3)	(0.063)	(290)	(277)	(1.32)	(4.1)	(1.25)	(0.023)	(0.86)
420 mg i.v.	1.0	151	0.360	4380†	4480	10.7	32.2	4.40	0.0965	3.83
(n = 6)	(1.0, 94.8)	(35.3)	(0.084)	(860)	(802)	(1.91)	(6.6)	(0.78)	(0.0189)	(0.66)
210 mg s.c.	4.0	30.1	0.143	1270	1270	6.07	24.0	7.31	0.228	NC
(UC; n = 3)	(3.9, 7.2)	(8.47)	(0.040)	(682)	(687)	(3.27)	(5.1)	(4.60)	(0.176)	

*n = 1; †n = 5. Smaller ns indicated insufficient data for estimation due to BQL values or subjects withdrew consent early. All values are reported to three significant figures except for t_{max}, where values are reported to one decimal place. t_{max}: time to C_{max}, presented as median (min–max); units: days for s.c., h for i.v.. C_{max}: maximum observed AMG 181 concentration in serum; dose-normalized value: C_{max}/Dose. AUC(0,t): area under the concentration–time curve from time zero to the last observed concentration. AUC(0,∞): area under the concentration–time curve from time zero extrapolated to infinity; dose-normalized value: AUC(0,∞)/Dose. $t_{1/2}$: linear β-phase half-life. V_z and V_z/F: linear β-phase volume and apparent volume of distribution for i.v. and s.c., respectively; V_{ss}: volume of distribution at steady-state for i.v.. F: bioavailability after s.c. administration. CL and CL/F: clearance and apparent clearance for i.v. and s.c., respectively. NC, not calculated.
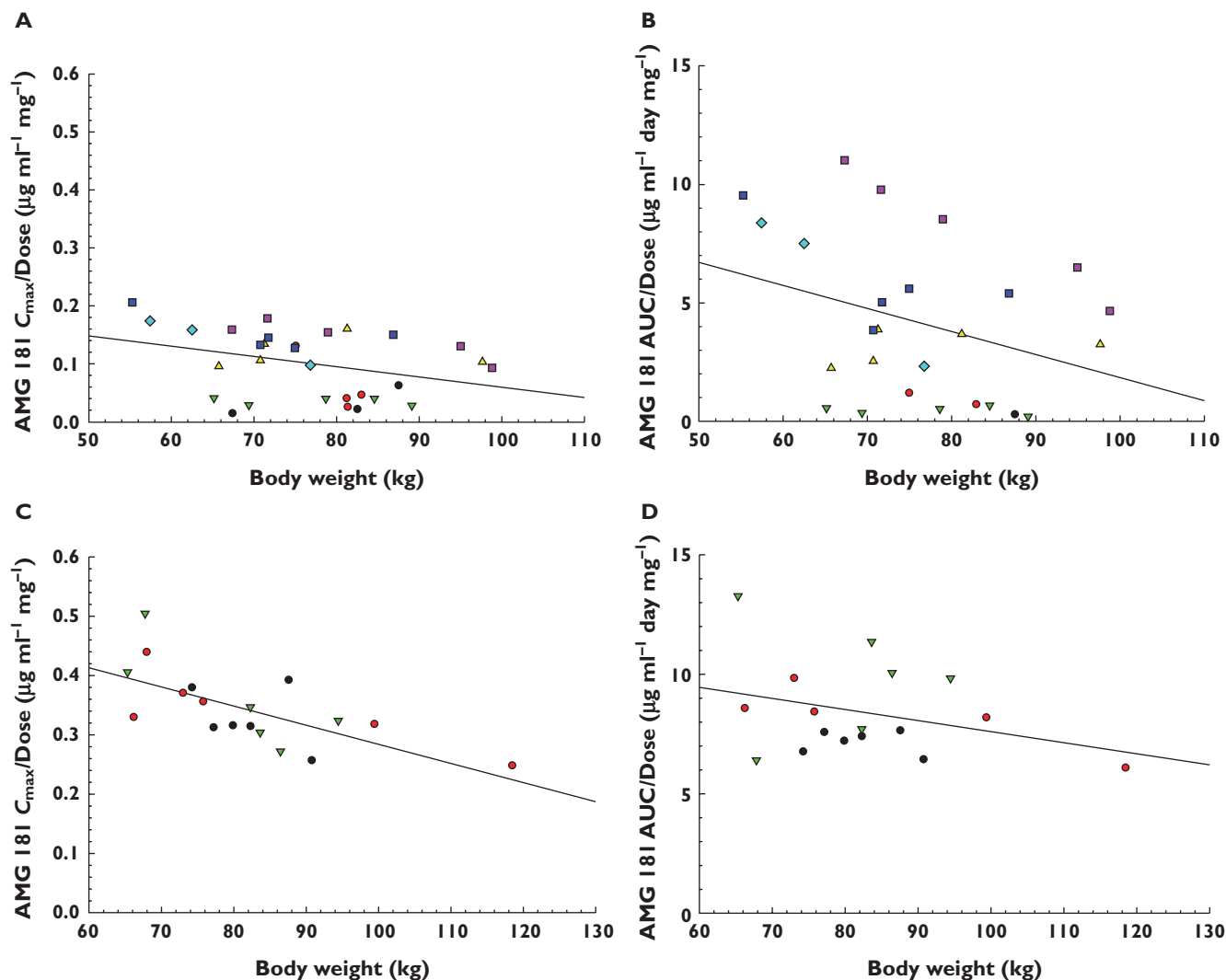
Figure 3

Body weight effects on dose-normalized AMG 181 C_{max} (C_{max}/Dose) and AUC(0,∞) (AUC(0,∞)/Dose) for subcutaneous (A and B) or intravenous (C and D) administration. Symbols are observations and lines represent linear regressions. s.c. = subcutaneous; i.v. = intravenous. (A and B) ●, Cohort 1: 0.7 mg s.c. (n = 1–3); ●, Cohort 2: 2.1 mg s.c. (n = 2–4); ▼, Cohort 3: 7 mg s.c. (n = 5); △, Cohort 4: 21 mg s.c. (n = 5); ■, Cohort 5: 70 mg s.c. (n = 5); ■, Cohort 6: 210 mg s.c. (n = 5); ◆, Cohort UC (10): 210 mg s.c. (n = 3); —, linear regression line. (C and D) ●, Cohort 7: 70 mg i.v. (n = 6); ●, Cohort 8: 210 mg i.v. (n = 5–6); ▼, Cohort 9: 420 mg i.v. (n = 5–6); —, linear regression line.

memory T cells are presented in Figure 4A–D. The pre-dose normalized percent free $\alpha_4\beta_7$ receptor profiles on circulating CD4+ naïve T cells were directly correlated with time-matched AMG 181 concentrations. The duration of this PD effect was dose-related and reversible upon elimination of AMG 181. For example, > 90% saturation of $\alpha_4\beta_7$ receptors was achieved for ≥ 43 days, ≥ 71 days, and ≥ 127 days following a single dose of 21 mg, 70 mg (i.v. or s.c.), and 210 mg (i.v. or s.c.) AMG 181, respectively, reaching or approaching pre-dose baseline at the end of study (Figure 4A).

Similar dose-associated and concentration-correlated profiles were observed for circulating CD4+ central memory T cells (Figure 4C) and CD4+ effector memory and

total memory T cells (data on file at Amgen Inc.). Total $\alpha_4\beta_7$ levels on both CD4+ naïve and central memory T cells were decreased by approximately 40 to 60% at nadir but were reversible upon elimination of AMG 181 (Figure 4B and D). Data for other CD4+ T cell subsets showed similar profiles (on file at Amgen Inc.).

Individual healthy subject pre-dose baseline-normalized $\alpha_4\beta_7$ RO on CD4+ naïve T cells $vs.$ serum AMG 181 concentration profile and the E_{max} PD model fitted curve are presented in Figure 5. As with AMG 181 PK, PD was apparently not affected by race (data on file at Amgen Inc.). The majority of the data points between AMG 181 concentrations of 0.01 to 100 µg ml^{-1} corresponded to > 90% $\alpha_4\beta_7$ RO, indicating a very potent PD effect of AMG
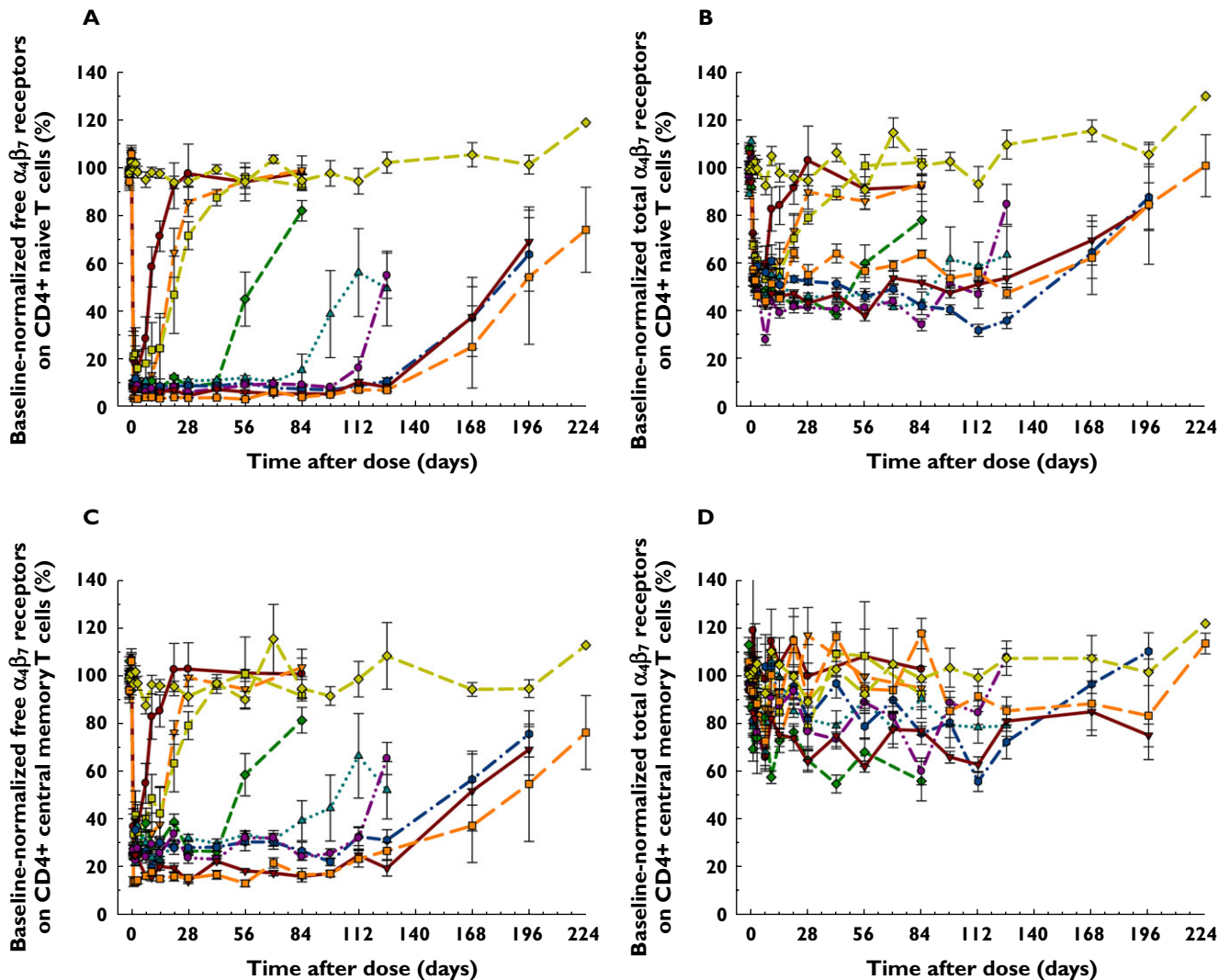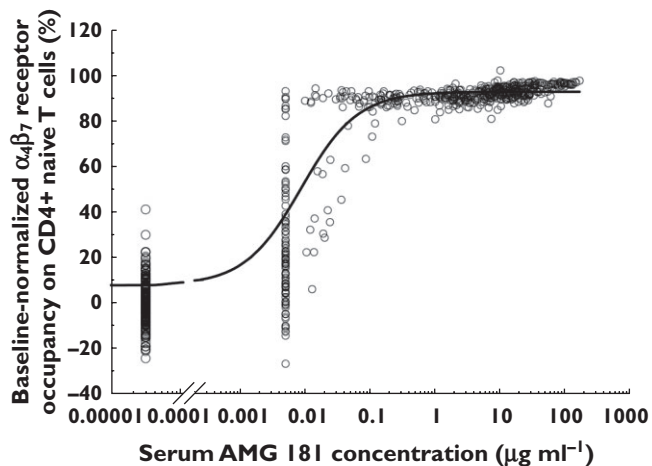
Figure 4

Mean (SEM) percent of pre-dose baseline-normalized $\alpha_4\beta_7$ receptor profiles on CD4+ naïve (A free, B total) and central memory (C free, D total) T cells after AMG 181 or placebo administration in healthy subjects. s.c. = subcutaneous; i.v. = intravenous. HV = healthy volunteers. ━●━, Cohort 1: 0.7 mg s.c. ($n = 3–4$); ┄▼┄, Cohort 2: 2.1 mg s.c. ($n = 4$); ┄□┄, Cohort 3: 7.0 mg s.c. ($n = 5–6$); ━◆━, Cohort 4: 21 mg s.c. ($n = 5–6$); ┅▲┅, Cohort 5: 70 mg s.c. ($n = 4–6$); ┄●┄, Cohort 6: 210 mg s.c. ($n = 4–6$); ┈●┈, Cohort 7: 70 mg i.v. ($n = 5–6$); ━▼━, Cohort 8: 210 mg i.v. ($n = 4–6$); ━□━, Cohort 9: 420 mg i.v. ($n = 4–6$); ━◇━, placebo ($n = 1–18$)

181. The PD model estimated EC_{50} value for $\alpha_4\beta_7$ RO on CD4+ naïve T cells was 0.00884 µg ml^{-1} (%CV 13.7%) with the estimated E_{max} and E_0 values of 93.1% and 7.73%, respectively. For the $\alpha_4\beta_7$ RO on CD4+ central memory and effector memory T cells, the estimated EC_{50} values were 0.0140 (%CV 20.8%) and 0.0102 µg ml^{-1} (%CV: 29.2%), respectively. Because all three estimated RO EC_{50} values were close to the assay LLOQ of 0.01 µg ml^{-1}, the EC_{50} value was set at 0.01 µg ml^{-1} and plotted, together with the estimated EC_{90} and EC_{99} lines, in Figure 2.

Individual healthy subject $AUEC_{all}$ for pre-dose baseline-normalized $\alpha_4\beta_7$ receptor occupancy on CD4+ naïve T cells *vs.* AMG 181 AUC_{all} profile and the E_{max} PD

model fitted curve are presented in Figure 6. The PD model estimated $EAUC_{all,50}$ value for CD4+ naïve T cells was 411 µg ml^{-1} day (%CV 15%; E_{max} 19900 day·%), coinciding with a 70 mg dose. The estimated $EAUC_{all,90}$ of 3460 µg ml^{-1} day corresponds to a 324 mg dose. For CD4+ central memory and effector memory T cells, the estimated $EAUC_{all,50}$ values were 538 and 530 µg ml^{-1} day, respectively, neither of which was substantially different from that of CD4+ naïve T cells.

Figure 7 shows that the absolute mean (SEM) count profiles of lymphocytes, eosinophils, CD4+ T cells and their subsets were virtually identical between AMG 181 and placebo treatments in all subjects.

Figure 5

Individual healthy subject pre-dose baseline-normalized $\alpha_4\beta_7$ receptor occupancy on CD4+ naïve T cell *vs.* serum AMG 181 concentrations. All data from pre-dose and placebo treated subjects are plotted at AMG 181 = 0 μg ml^{-1}. The line represents the E$_{max}$ pharmacodynamic model fit of the observed data. RO data with AMG 181 concentrations measured to be < 0.01 μg ml^{-1} (assay LLOQ) were plotted against 0.005 μg ml^{-1}, corresponding to LLOQ/2. These data were not used for PD modelling because the exact AMG 181 concentrations for these samples could not be quantified
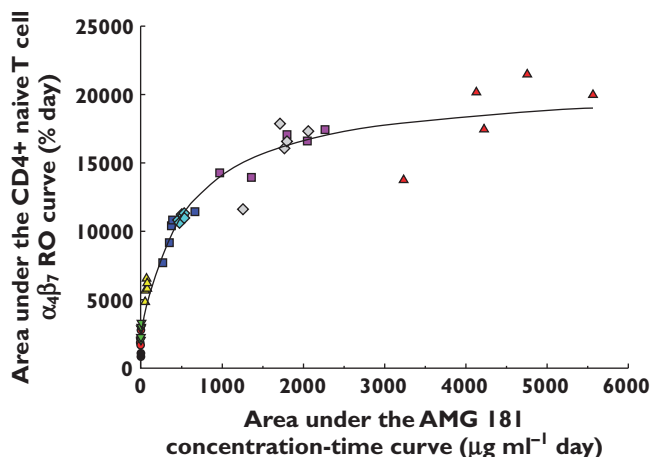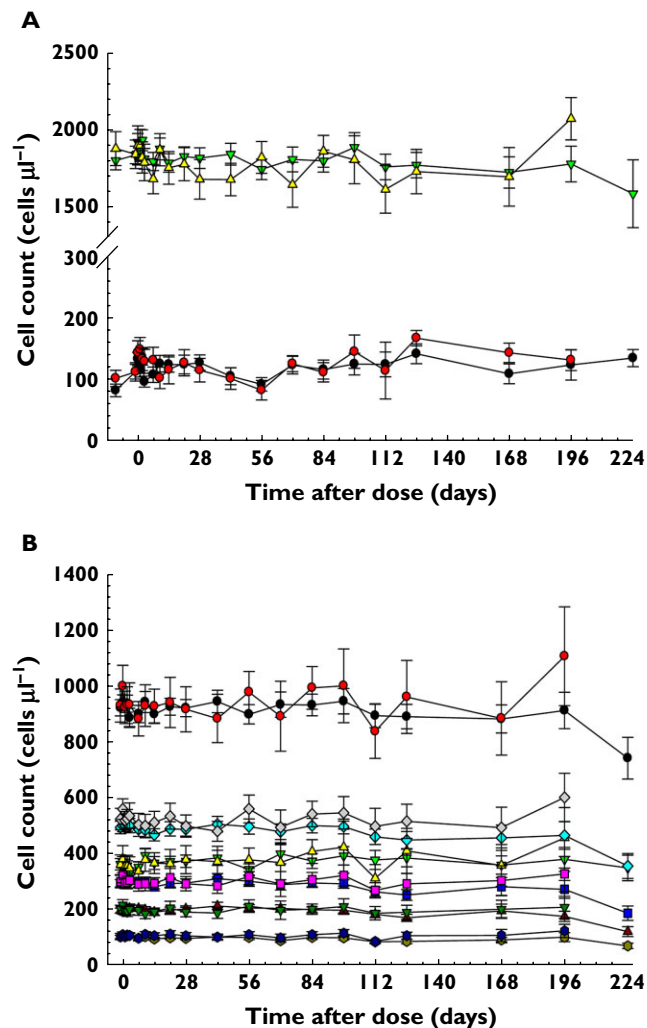

Figure 6

Individual healthy subject area under the effect–time curve for pre-dose baseline-normalized $\alpha_4\beta_7$ receptor occupancy (RO) on CD4+ naïve T cells (AUEC$_{all}$) *vs.* serum AMG 181 area under the concentration–time curve (AUC$_{all}$) profile (symbols) and the E$_{max}$ PD model fitting of the observed data (line). s.c. = subcutaneous; i.v. = intravenous. ●, Cohort 1: 0.7 mg s.c. (n = 3); ⬤, Cohort 2: 2.1 mg s.c. (n = 4); ▽, Cohort 3: 7 mg s.c. (n = 5); △, Cohort 4: 21 mg s.c. (n = 5); ■, Cohort 5: 70 mg s.c. (n = 5); ■, Cohort 6: 210 mg s.c. (n = 5); ◆, Cohort 7: 70 mg i.v. (n = 6); ◇, Cohort 8: 210 mg i.v. (n = 5); ▲, Cohort 9: 420 mg i.v. (n = 5)

Pharmacokinetics, pharmacodynamics, and effects in ulcerative colitis subjects

AMG 181 PK profiles and the estimated PK parameters in two UC subjects were similar to healthy subjects receiving
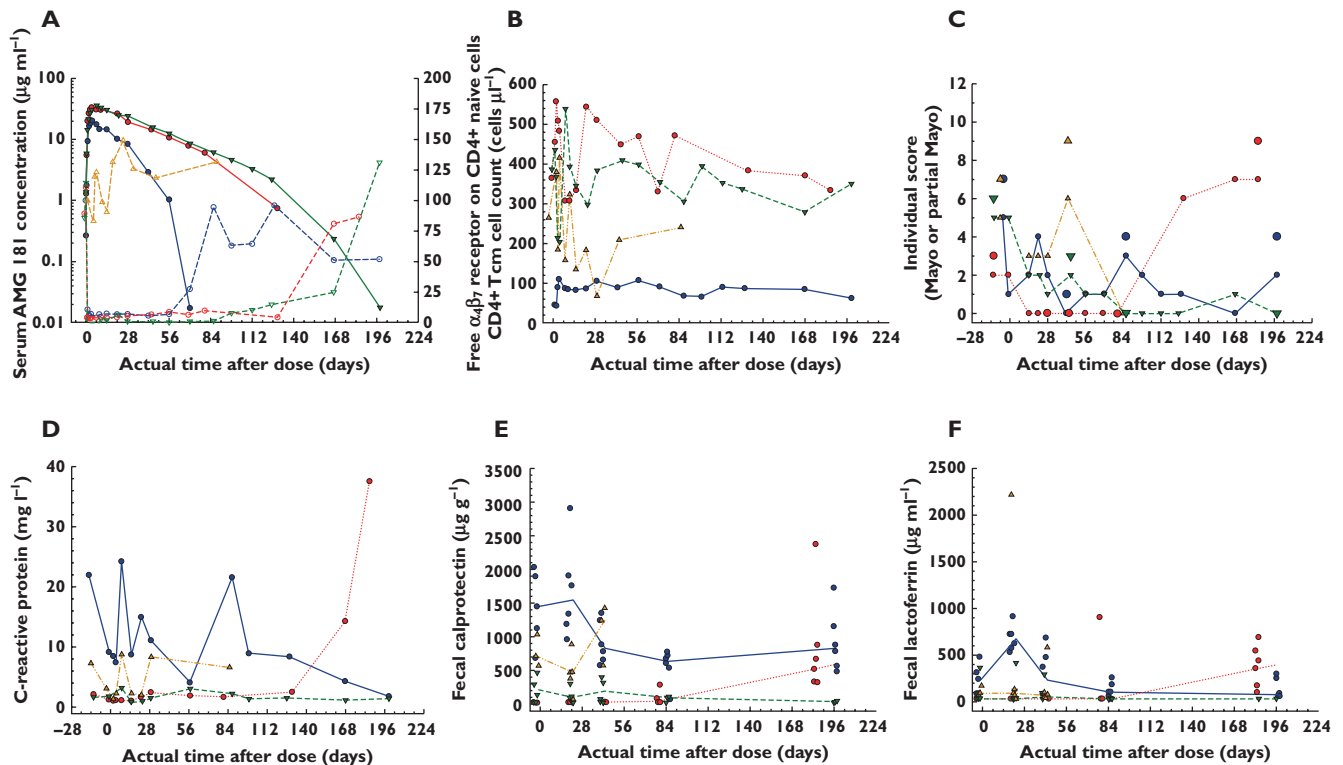

Figure 7

Mean count (SEM) profiles of lymphocytes and eosinophils (A) and CD4+ total, naïve, central memory (Tcm), and memory (mem) T cells, and Tcm cells expressing low or high levels of $\alpha_4\beta_7$ receptors (B) in all subjects treated with AMG 181 or placebo. (A) ●, Eosinophil, active; ⬤, Eosinophil, placebo; ▽, Lymphocyte, active; △, Lymphocyte, placebo; Active (n = 5–50); Placebo (n = 6–19). (B) ●, CD4 + total, active; ⬤, CD4 + total, placebo; ▽, CD4 + naive, active; △, CD4 + naive, placebo; ■, CD4 + Tcm, active; ■, CD4 + Tcm, placebo; ◆, CD4 + mem, active; ◇, CD4 + mem, placebo; ▲, $\alpha_4\beta_7$ low CD4 + Tcm, active; ▼, $\alpha_4\beta_7$ low CD4 + Tcm, placebo; ⬤, $\alpha_4\beta_7$ high CD4 + Tcm, active; ●, $\alpha_4\beta_7$ high CD4 + Tcm, placebo; Active (n = 5–52); Placebo (n = 5–18)

the same 210 mg s.c. dose. In the remaining actively treated UC subject, the AMG 181 PK profile was initially (up to day 8) similar to that of healthy subjects', but displayed a subsequent rapid decline and reached BQL on day 85 (Figure 8A). No ADAs were detected in any of this subject's serum samples. The PD profiles (free and total $\alpha_4\beta_7$ levels) mirrored the PK profiles: The two UC subjects with PK profiles comparable with healthy subjects also had PD profiles comparable with healthy subjects. The subject with the rapid decline in AMG 181 PK also had a rapid reversal of the

Figure 8

Individual profiles in ulcerative colitis subjects: AMG 181 concentration (left y-axis; PK) and pre-dose baseline-normalized rate of free $\alpha_4\beta_7$ receptor on CD4+ naïve T cells (right y -axis; PD) (A), CD4 + central memory T cell (Tcm) count (B), total (large symbols only) and partial (small symbols with lines connected) Mayo score (C), serum C-reactive protein (D), fecal calprotectin (E) and lactoferrin (F) presented as observed (symbols) and median (lines). Note: Subject 4 was on placebo; others were on 210 mg AMG 181s.c. (A) PK: —●—, Subject 1; —●—, Subject 2; —▼—, Subject 3; —▲—, Subject 4 PD: --○--, Subject 1; --○--, Subject 2; --▽--, Subject 3; --△--, Subject 4. (B and D) —●—, Subject 1; ····●····, Subject 2; —▼—, Subject 3; —▲—, Subject 4. (C) Mayo: ●, Subject 1; ●, Subject 2; ▼, Subject 3; ▲, Subject 4 Partial Mayo: —●—, Subject 1; ····●····, Subject 2 ; —▼—, Subject 3; —▲—, Subject 4 —●—, ····●····, —▼—, —▲—. (E and F) ●, Subject 1; ●, Subject 2; ▼, Subject 3; ▲, Subject 4; ●, ●, ▼, ▲

initial RO response (Figure 8A). Consistent with the findings in healthy subjects, AMG 181 concentration and the pre-dose baseline adjusted rate of free $\alpha_4\beta_7$ receptor on CD4+ naïve T cells were directly correlated (Figure 8A). In addition, similar to healthy subjects, the CD4+ central memory T cell counts did not change in UC subjects after 210 mg AMG 181 or placebo s.c. dosing (Figure 8B).

At screening, the four UC subjects had a median Mayo score of 6.5 (range 3 to 7), with median score of 6 for the three subjects who received AMG 181 and a score of 7 for the subject who received placebo (Table 4). One UC subject with a Mayo score of 3 was enrolled after discussion between the investigator and Amgen and approval by the Amgen medical monitor. The screening recto-sigmoidoscopy scores for the three AMG 181-treated subjects were 2, 2 and 1, and 2 for the placebo-treated subject.

After dosing, all three AMG 181-treated subjects completed the study, while the placebo-treated subject withdrew from the study after day 43 visit due to colitis flare and subsequently received rescue therapy (recovered from colitis on days 85 and 197 visits; Table 4). The three

AMG 181-treated subjects achieved remission, response and mucosal healing at multiple time points of the study. In all, the eight instances of responses were matched with five instances of remissions and seven instances of mucosal healing (Table 4).

Figure 8C shows that the Mayo and partial Mayo score profiles were consistent throughout the study for each of the subjects. By day 43, subjects 1 and 2, which received AMG 181 had reached the lowest Mayo and partial Mayo scores and were in remission. Subject 3 (also received AMG 181) reached remission beginning on day 85 and sustained remission until the end of the 28 week (day 197) study period. Based on the partial Mayo score alone, and by defining clinical response as a reduction of ≥ 2 points and $\geq 25\%$ change from baseline [48], sustained response was achieved for 10 (from week 2 to 12), 24 (from week 4 to 28), and 26 (from week 2 to 28) weeks for subjects 1, 2 and 3, respectively.

The two subjects who had the last measurable AMG 181 concentration on days 127 and 71 experienced Mayo and partial Mayo scores rebounding from nadir afterwards, while the third subject having an AMG 181 concentration

Table 4

Effects of a single subcutaneous 210 mg AMG 181 or placebo administration in individual subjects with ulcerative colitis

Subject	Regimen	Screening	Mayo score (Remission*, Response†, Mucosal Healing‡)§			Concomitant therapy for UC treatment
			Day 43	Day 85	Day 197	
1	AMG 181	7	1 (+, +, +)	4 (−, +, +)	4 (−, +, −)	None
2	AMG 181	3	0 (+, +, +)	0 (+, +, +)	9 (−, −, −)¶	Mesalazine
3	AMG 181	6	3 (−, +, +)	0 (+, +, +)	0 (+, +, +)	Balsalazide sodium
4	Placebo	7	9 (−, −, −)**	NA	NA	Aminosalicylic acid, prednisolone

Note: Full Mayo score assessed on days 43, 85 and 197, corresponding to weeks 6, 12 and 28 visits. *Remission: Mayo Score ≤ 2, with no individual subscore > 1 point. †Response: Mayo Score ↓ from baseline ≥ 3 and ≥ 30% + rectal bleeding score ↓ ≥ 1 point or absolute reading of 0 or 1. ‡Mucosal healing: rectosigmoidoscopy score of 0 or 1. §'+' or '−' = 'Yes' or 'No', respectively, for remission, response or mucosal healing. ¶Grade 3 ulcerative colitis flare. **Grade 4 ulcerative colitis flare. Subject experienced serious adverse event of colitis flare and withdrew from the study after day 43 visit. This subject received prednisolone, hydrocortisone and mesalazine as rescue therapies and recovered at days 85 and 197 visits. NA, Not Applicable.

measurable until day 197 had sustained response and remission to the end of the study on day 197 (Figure 8C). This indicates that maintaining AMG 181 exposure may be required for sustaining clinical effects.

Figure 8D to F presents serum C-reactive protein, fecal calprotectin and lactoferrin profiles for all four UC subjects. Compared with pre-dose baselines, the three biomarkers remained flat, trended lower or rebounded at different time points, generally matching the PK/PD profiles individually. Note that for subject 2, AMG 181 concentration reached LLOQ after week 20, with corresponding reduced $\alpha_4\beta_7$ receptor coverage on CD4+ naïve T cells (Figure 8A), return of CD4+ central memory T cell count toward baseline (Figure 8B), rebound in Mayo and partial Mayo scores (Figure 8C) and elevation of CRP and fecal biomarkers (Figure 8D–F). Overall, due to small sample size and data variability, the effects of AMG 181 treatment on these biomarkers could not be definitively or quantitatively assessed.

Discussion

In this study we evaluated single dose PK/PD, safety, tolerability and effects of the gut-specific fully human monoclonal anti-$\alpha_4\beta_7$ antibody AMG 181 after s.c. or i.v. administration in healthy subjects and subjects with mild to moderate UC. AMG 181 blocks the interaction of the $\alpha_4\beta_7$ integrin with its target ligand MAdCAM-1 and thus prevents lymphocyte adhesion, extravasation and homing to the gut mucosal tissue.

After s.c. dosing, AMG 181 was extensively absorbed with relatively high bioavailability of 82 to 99% and a long half-life of ~31 days. AMG 181 displayed non-linear PK with a rapid decline when concentrations fell below 1 μg ml^{-1}. This is consistent with target-mediated disposition and is especially important for AMG 181 doses of ≤ 21 mg. AMG 181 doses of ≥ 70 mg resulted in saturation of this target-specific mechanism and the non-specific linear processes masked the target-mediated disposition. Consequently,

approximately linear PK profiles were observed at higher AMG 181 doses, where the terminal non-linear phase had limited contribution to the AUC. The estimated AMG 181 linear CL and V_{ss} of 0.120 (0.025) l day^{-1} and 4.30 (0.82) l indicate that AMG 181 was cleared slowly during the linear elimination phase, with distribution mostly contained within the central circulation. Because the 21, 70 and 210 mg s.c. cohorts maintained AMG 181 concentrations above the RO EC$_{99}$ level of 0.9 μg ml^{-1} for approximately 4, 12 and 22 weeks, it may be possible to propose therapeutic regimens by dosing AMG 181 once every 1, 3 or 6 months, respectively, taking moderate accumulations after repeated dosing into consideration.

Based on non-compartmental analysis, the mean predicted extrapolation from AUC(0,t) for calculating AUC(0,∞) was ~5% for cohorts ≥ 70 mg s.c. or i.v., which was virtually identical to the mean observed percent extrapolation, indicating that the terminal non-linear target-mediated disposition phase was negligible and did not influence the calculation of linear β-phase PK parameters such as $t_{1/2}$, CL and V_{ss}. Because the influence of target-mediated disposition was minor after a single dose of ≥ 70 mg, and because of the expected moderate accumulation after repeated dosing which would result in trough AMG 181 concentrations of ≥ 1 μg ml^{-1} at doses larger than 21 mg s.c., we expect that repeated dosing of 21, 70 and 210 mg s.c. once every 1, 3, and 6 months, respectively, would show linear PK characteristics at steady-state with an estimated effective $t_{1/2}$ similar to the linear elimination $t_{1/2}$ of 31 days. In a future report, we will examine this prediction by correlating the linear beta phase $t_{1/2}$ estimated in this single dose study with the estimated effective $t_{1/2}$ from the multiple ascending dose study in healthy and UC subjects [43].

Potential demographic effects on AMG 181 PK were explored by plotting and regressing individual C_{max}/Dose and AUC(0,∞)/Dose values vs. body weight, BMI, age and race. While the latter three showed no statistically significant effects on AMG 181 exposures, the body weight had statistically significant effects on the C_{max}/Dose after i.v.

administration. The clinical relevance of this finding will need to be assessed in a larger sample of IBD subjects. Note that in the current study, heavier body weight, especially the one healthy subject weighing ~120 kg in the i.v. cohort had contributed to the statistical significance of the linear regression for C_{max}/Dose. Using target $\alpha_4\beta_7$ RO as the guide post for selecting a therapeutic regimen, one might argue that with repeated dosing, the expected PK accumulation at steady-state should ensure adequate target $\alpha_4\beta_7$ receptor coverage at the trough. Furthermore, the hysteresis phenomenon reported as prolonged therapeutic effects lasting longer than drug concentration and target coverage previously reported with natalizumab and vedolizumab suggests the potential for accommodating, at a population level, lower trough AMG 181 concentration due to heavier body weight [39, 40, 49].

No ADAs were detected in this study, so the potential effects of immunogenicity on AMG 181 PK/PD, safety and treatment effects could not be examined. Circulating serum AMG 181 concentrations in some subjects at certain time points were higher than the immunogenicity assay drug tolerance level of 25 µg ml^{-1}. For example, on days 15 and 29 for subjects dosed 210 mg s.c. or i.v., and on days 15, 29, and 57 for subjects dosed 420 mg i.v., determination of ADAs may have been prevented, providing false negative results at those time points. However, the five to six sampling time points after day 29 or 57, where AMG 181 concentrations were < 25 µg ml^{-1}, had provided a valid ADA negative result, suggesting that these subjects at least did not develop persistent ADAs.

Two of the three UC subjects exhibited AMG 181 PK similar to that of the healthy subjects given the same dose of 210 mg s.c. The one UC subject (subject 1) who had lower AMG 181 PK did not display unusually high target $\alpha_4\beta_7$ receptor expression (data on file at Amgen Inc.) or develop immunogenicity. One might speculate that high disease activity, and/or gut-leaking protein losing enteropathy as suggested by low serum albumin level at screening (< 3.5 g dl^{-1}) might have contributed to the faster AMG 181 clearance as has been previously reported for infliximab in IBD subjects [50, 51]. Also, AMG 181 PD and treatment effects in this subject were suboptimal *vs.* the other two AMG 181-treated UC subjects. Due to the small sample size, we could not reach a conclusion for possible impact of disease status on AMG 181 PK/PD in UC, even though the correlation between PK and PD appeared to be consistent between healthy and UC subjects.

AMG 181 showed potent yet reversible effects on saturating $\alpha_4\beta_7$ receptors on target T cells. One subset, the CD4+ naïve T cells, homogenously expressed $\alpha_4\beta_7$ receptors, while the other subset, CD4+ central memory T cells, was further divided into two subsets, one of which expressed low while the other expressed very high levels of $\alpha_4\beta_7$ receptors. The median flow cytometry measurements of $\alpha_4\beta_7$ receptors on CD4+ central memory T cells

included data from cells expressing low and high $\alpha_4\beta_7$ receptors and were thus less homogenous than those from CD4+ naïve T cells. Therefore, we presented both free and total $\alpha_4\beta_7$ receptors on these two cell types (Figure 4) and modelled their relationships with AMG 181 concentrations. The PD modelling results show that the EC_{50} values for the CD4+ naïve, central and effector memory T cells were similar, indicating similar potency of about 0.01 µg ml^{-1}, the PK assay LLOQ. Thus using $\alpha_4\beta_7$ RO on CD4+ naïve T cells as a surrogate representing RO values for central memory T cells was adequate.

Excluding data from all placebo-treated healthy subjects and all pre-dose PK samples from AMG 181-treated healthy subjects, a total of 111 out of 658 (16.9%) PK samples had AMG 181 concentrations < 0.01 µg ml^{-1}, the assay LLOQ. These values were excluded from analysis but were plotted at half of the LLOQ (0.005 µg ml^{-1}) in Figure 5, to provide information about the spread of RO. The calculated EC_{90} value of 0.09 µg ml^{-1} was a reasonable estimate, as the observations (symbols) in Figure 5 showed that RO was mostly > 90% when AMG 181 concentrations were > 0.1 µg ml^{-1}. This consistency can also be observed by comparing the $\alpha_4\beta_7$ RO of over 99% based on the duration of the PK profiles over the EC_{99} line (Figure 2) and those observed as presented in Figure 4A and C for doses ≥ 21 mg.

Two complimentary approaches were used to assess quantitatively the AMG 181 PK/PD relationship. One was the direct, time-matched, instant exposure (concentration) approach (Figure 5) and the other was the accumulative exposure (AUC) approach (Figure 6). The E_{max} model describing the relationship between serum AMG 181 concentration and the subject/time-matched $\alpha_4\beta_7$ RO on total CD4+ T cells (including the subsets) was a simple and direct assessment of AMG 181 *ex vivo* potency through EC_{50}. The disadvantages of this approach are that some PK/PD sampling time points did not match (Table 1), and that those AMG 181 concentrations that were BQL (below quantitative limit) were excluded from PD modelling as discussed above, even though RO were measurable. By assessing AMG 181 AUC_{all} *vs.* RO $AUEC_{all}$, mismatched PK/PD observations and BQL measures were no longer an issue and $AUEC_{all}$ was a useful guide for measuring the cumulative extent of RO. Figure 6 shows that data points associated with 70 mg i.v. or s.c. doses were concentrated at the inflection point of the curve fitting. To increase $AUEC_{all}$ from ~10000 to 20000 day % (~two-fold increase), AMG 181 dose would have to increase from 70 to 420 mg i.v., a six-fold increase. Both 210 mg s.c. and i.v. were estimated to achieve 82% of the E_{max} value of 19900 day %, suggesting limited cumulative RO improvement beyond the 210 mg dose, which is also apparent from visual inspection of Figure 6. The model estimated AMG 181 AUC_{all} leading to 90% maximal effect ($EAUC_{all,90}$) was approximately 3460·µg ml^{-1} day, corresponding to a 324 mg dose. Therefore, a ~1.5-fold dose increase from

210 to 324 mg, would only yield ~8% increase in cumulative RO. These results imply that the maximum practically useful single dose would be 210 mg i.v. or s.c., with no anticipated gain in PD and efficacy from further dose escalation.

Previous reports showed that natalizumab has led to increases in lymphocyte, eosinophil, basophil, monocyte and detectible nucleated red blood cells [31]. In the current study, we did not observe cell count changes in any of these cell types, consistent with the pharmacology of AMG 181, which specifically targets a small population of gut-homing T cells. There were no differences between cohorts, nor pooled AMG 181 *vs.* placebo data from all healthy and UC subjects. Both lymphocyte and eosinophil counts were well contained within the normal ranges of 1000 to 3500 and 30 to 600 cells μl^{-1}, respectively. Even among CD4+ T cells and the subsets targeted by AMG 181, there were no apparent differences between cohorts or between pooled AMG 181 vs. placebo (Figure 7). Compared with natalizumab, the implication for lack of AMG 181 effects on various immune cells warrants further examination in large confirmatory clinical trials. It remains to be more carefully investigated whether cell counts might change in the IBD population given that circulation dynamics of gut-homing lymphocytes in that population might be different from that in healthy subjects.

Consistent with previous reports [52, 53], our data have shown that the Mayo and partial Mayo score profiles were correlated throughout the study for each of the four UC subjects. The three AMG 181-treated UC subjects reached remission on study indicating favourable treatment effects of AMG 181. The onset of remission occurred as early as week 4 for subject 2 and as late as week 12 for subject 3. The lowest Mayo score was achieved within the week 4 to 12 window post-dose for the three AMG 181-treated subjects, suggesting a delayed onset of treatment effects relative to the median AMG 181 PK t_{max} of 4 days. Subject 1 did not take any concomitant UC therapies. Subjects 2 to 4 were taking aminosalicylic acid while on the study and subject 3 was also taking a steroid. Due to the small sample size, the potential contributions of these concomitant medications could not be assessed.

Greater inflammatory activities are expected in autoimmune diseases such as IBD when serum C-reactive protein, fecal calprotectin and lactoferrin are higher than 10 mg ml^{-1}, 250 $\mu g\ g^{-1}$, and 7 $\mu g\ ml^{-1}$, respectively [54–56]. Even though subjects with mild to moderate rather than moderate to severe UC were enrolled, both biological signs of active gut inflammation and clinical measures of disease activities were observed. Overall, serum C-reactive protein and fecal biomarker data were consistent with UC subjects' disease severity and AMG 181 treatment outcomes, even though small sample size and data variability precluded definitive or quantitative assessments.

In conclusion, this study has demonstrated that AMG 181 has an acceptable safety and tolerability profile at single doses of up to 210 mg s.c. or 420 mg i.v., and has the potential for convenient dosing intervals of every 1, 3, and 6 months at 21, 70 and 210 mg, respectively. Results from this study together with those from a repeated dosing study (to be reported separately) have supported advancement of AMG 181 into phase 2 clinical trials in UC and CD [43, 44].

Competing Interests

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