Nonclinical safety of mavrilimumab, an anti-GMCSF receptor alpha monoclonal antibody, in cynomolgus monkeys: Relevance for human safety

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ABSTRACT

Mavrilimumab (CAM-3001) is an investigational human IgG4 monoclonal antibody (MAb) targeting GM-CSF receptor alpha which is currently being developed for the treatment of RA. GM-CSF plays a central role in the pathogenesis of rheumatoid arthritis (RA) through the activation, differentiation, and survival of macrophages and neutrophils. To support clinical development, the nonclinical safety of mavrilimumab was evaluated in several studies with cynomolgus monkeys as the pharmacologically relevant species. Comprehensive toxicity parameters were assessed in each study, and treatment duration ranged from 4 to 26 weeks. Mavrilimumab has an acceptable safety profile in monkeys with no changes in any parameters other than microscopic findings in lung. In several studies, minimal accumulation of foamy alveolar macrophages was observed. This finding was only seen in studies of at least 11 weeks duration, was reversible following a dose-free recovery period and was considered non-adverse. At higher dose levels (≥30 mg/kg/week), in a 26-week repeat-IV dose study, the presence of lung foreign material, cholesterol clefts, and granulomatous inflammation was also observed in a few animals and was considered adverse. The dose- and time-related accumulation of foamy macrophages in lung following exposure to mavrilimumab observed in several NHP studies was expected based upon the known role of GM-CSFRα signaling in the function of alveolar macrophages. Overall, a clean no-observed-adverse-effect-level (NOAEL) without any effects in lung was established and provided adequate clinical safety margins. In clinical studies in RA patients, mavrilimumab has demonstrated good clinical activity with adequate safety to support further clinical development. A Phase 2b study of mavrilimumab in subjects with RA is in progress.

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Introduction

Mavrilimumab is a human IgG4 Mab which binds GM-CSFRα to neutralize GM-CSF activity. A recently completed Ph2a randomized, double-blind, placebo-controlled study showed mavrilimumab induced rapid clinically significant responses in RA subjects suggesting that suppressing neutrophil and macrophage activity by targeting the GMCSF receptor may provide an effective and novel therapeutic approach for RA (Burmeister et al., 2013; EARTH NCT00771420).

Given the central role of GM-CSF signaling in alveolar macrophages for the regulation of pulmonary surfactant homeostasis, lung toxicity is a potential concern for agents such as mavrilimumab which target the GM-CSF pathway (Trapnell and Whitsett, 2002). The role of GM-CSF in the regulation of lung surfactant homeostasis and alveolar macrophage innate immune function in the lung is based in part on evidence from mice in which GM-CSF signaling is ablated (Dranoff et al., 1994; Stanley et al., 1994). In these GM-CSF knockout (GM KO) mice, accumulation of phospholipids and proteins in lung is seen resulting from an inhibition of surfactant catabolism and reduced clearance by alveolar macrophages. In humans, pulmonary alveolar proteinosis (PAP) is a heterogeneous group of rare restrictive lung disorders characterized by the accumulation of surfactant lipids and proteins in pulmonary alveolar macrophages resulting in respiratory insufficiency, and in severe cases, respiratory failure. Some forms of hereditary PAP are associated with mutations in the genes encoding the GM-CSF receptor while autoimmune PAP is characterized by high levels of anti-GM-CSF autoantibodies (Carey and Trapnell, 2010). Because GM-CSF plays a role in the...
regulation of pulmonary surfactant homeostasis, lung toxicity is a potential concern for biologics such as mavrilimumab which inhibit GM-CSF receptor signaling. As a result, additional attention was given to the potential impact of lung toxicity during the development of mavrilimumab.

**Materials and methods**

**Test and control article.** Mavrilimumab is a fully human IgG4 monoclonal antibody targeting the GMCSF receptor alpha. It is expressed in CHO cells, purified by chromatography and formulated in 50 mM sodium acetate/acetate acid, 100 mM sodium chloride, 0.02% polysorbate 80, pH 5.5 allowing a final concentration of product of 100 mg/mL. Control article was formulation buffer, 50 mM sodium acetate/acetate acid, 100 mM sodium chloride, 0.02% polysorbate 80, pH 5.5.

**Nonhuman primate studies.** Nonhuman primate (NHP) toxicity studies were performed in male and female cynomolgus monkeys at AAALAC-accredited laboratories. All study protocols were approved by testing facility Institutional Animal Care and Use Committee. Two non-GLP and five GLP-compliant toxicity studies were performed ranging in treatment duration from 4 to 26 weeks using once weekly intravenous (IV) or subcutaneous (SC) injection of mavrilimumab or vehicle control. In some studies, a separate cohort of animals was necropsied after a dose-free recovery period ranging in duration from 4 to 17 weeks. Study designs, dose levels and numbers of animals per group for each study are summarized in Table 1.

Comprehensive toxicity parameters were assessed in each study including physical examinations, body weights, food consumption, behavior, ophthalmology, ECGs, clinical chemistry, urinalysis, hematology, organ weights, macroscopic and microscopic pathological observations. In some studies, bronchoalveolar lavage (BAL) samples were collected and evaluated microscopically to enumerate cell types present in BAL. Blood samples were collected at various timepoints and analyzed for determination of serum concentrations of mavrilimumab and the presence of anti-drug antibodies (ADA).

**Bioanalysis.** Concentrations of mavrilimumab in cynomolgus monkey serum were determined using a validated ELISA on a Gyrolab immunoassay platform (Quotient BioResearch, Cambridge, England). Each of 112 microstructures on a Gyrolab Bioaffy CD contained a column prepacked with streptavidin-coated beads which were functionalized with a biotinylated human GM-CSFR-Fc fusion protein as the capture reagent. Upon mavrilimumab binding a fluorophor labeled detection reagent (sheep anti-human IgG4) was then added and fluorescence signals were determined. The lower limit of quantitation (LLOQ) of the validated assay was 50 ng/mL. For the 26 week SC study (Study 7 in Table 1), the method for the quantitation of mavrilimumab in cynomolgus monkey serum was developed and validated at ALTA Analytical Laboratory (San Diego, CA). This method utilized an electrochemiluminescent (ECL) assay format to measure the concentration of mavrilimumab in cynomolgus monkey serum. An MSD Streptavidin Coated Standard MA2400 96-Well Plate was first incubated with CAM-3020 Biotin (anti-mavrilimumab monoclonal antibody). After incubation, Standards/QCs and samples were added to the plate. Unbound material was then washed away and Sheep anti-Human IgG4 SulfoTAG was added to the plate. After incubation, mavrilimumab was detected with MSD Read Buffer T (1×) and read on the Meso Scale Discovery (MSD) SectorTM Imager 2400. The assay lower limit of quantification (LLOQ) was 0.375 ng/mL.

The presence of ADA in monkey serum was detected using an electrochemiluminescent immunoassay. Biotinylated mavrilimumab and ruthenylated mavrilimumab were allowed to form a complex with the ADA and these ADA-bridged complexes were subsequently captured by the streptavidin-coated MSD plate containing embedded carbon electrodes. Upon electrical excitation the ECL signals were detected by the MSD Sector™ Imager. Pooled normal monkey serum was used as the negative control and a monoclonal antibody against mavrilimumab served as the positive control. The assay cut point factor was 2.02, and the assay sensitivity was estimated to be approximately 800 ng/mL.

**Pharmacokinetic data analysis.** Noncompartmental toxicokinetic data analysis was performed using WinNonlin Professional (version 5.2, Pharsight Corp., San Louis, MO). The area under serum concentration-time curves (AUC) were estimated by the linear/log trapezoidal rule. After SC administration of mavrilimumab the maximum concentration (C_max) was recorded as observed. Nominal collection times were used for the TK data analyses.

The steady-state PK exposure in adult subjects with rheumatoid arthritis was projected using a mechanistic model previously developed to describe the disposition of mavrilimumab, GM-CSFRα binding, receptor internalization and subsequent intra-cellular degradation (Minter et al., 2012; Wang et al., 2012). Based on the simulated PK profiles, the projected steady-state C_max and AUC were calculated and compared with those observed in cynomolgus monkeys at NOAEL dose level. In toxicology studies cynomolgus monkeys received weekly mavrilimumab administrations. In human subjects with RA, mavrilimumab was administered once every other week. To account for the difference in dosing frequency, the observed steady-state weekly AUC in cynomolgus monkeys was doubled prior to the comparison (Table 6).

**Results**

The nonclinical safety of mavrilimumab was evaluated in several repeat-IV and SC dose toxicity studies in cynomolgus monkeys. Cynomolgus monkeys were selected as the pharmacologically relevant species based on the similar binding affinity of mavrilimumab to cynomolgus monkey GM-CSFRα compared to the human receptor and the lack of binding to rodent GM-CSFRα (Minter et al., 2012). The activity of mavrilimumab was further tested in a functional assay against cells expressing cynomolgus monkey GM-CSFRα by pA2 analysis using a granulocyte shape-change assay and shown to be active and equally potent on monkey cells compared to human cells (Minter et al., 2012).

Seven independent repeat-dose toxicity studies were performed in cynomolgus monkeys. The dosing phase of the studies ranged from 4 to 26 weeks duration and utilized once weekly IV or SC injections of mavrilimumab. Five of the studies were performed in compliance with Good Laboratory Practices (GLP). In some studies, a negative control group was administered formulation buffer. In some studies, a dose-free recovery phase was included in the study design to examine reversibility of effects. In each of these studies, there were no treatment-related changes in clinical observations, body weights, clinical pathology parameters, urinalysis, immunophenotyping, organ weights, and macroscopic or microscopic pathologic findings in any tissues other than lung.

All IV studies utilized mg/kg dosing and all SC studies utilized fixed dosing without correcting for body weight. The rationale for this was to be consistent with the human clinical program which initially used mg/kg IV dosing for the first-time-in-human study and then switched to SC fixed mg dosing for Phase 2. Compared with weight-based dosing (mg/kg), fixed dosing (mg) is more convenient for SC dose administration and is associated with a lower risk of dosing error. Furthermore, fixed dosing can lead to reduced variability for some monoclonal antibodies and is recommended for adult human clinical trials of monoclonal antibodies (Wang et al., 2009). For the sake of making comparisons between nonclinical studies that used mg/kg IV doses and mg fixed SC doses, the typical body weight per animal of 2.5 kg is used as a denominator for each fixed SC dose. This “correction factor” is not the actual mean body weight for each study but merely a rough approximation. Since the majority of PK variability is unexplainable by body weight, utilizing the typical instead of individually observed body weight as the “correction factor” is not expected to have any major impact on cross-study comparisons.
Table 1: Summary overview of repeat-dose toxicology studies of mavrilimumab in cynomolgus monkeys.

<table>
<thead>
<tr>
<th>Study no./type</th>
<th>Objective</th>
<th>Study design and dose levels</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1: Non-GLP 11-week IV study.</td>
<td>To explore potential lung toxicity following repeat-IV dosing up to 11 weeks.</td>
<td>Doses 0, 10, 30 and 100 mg/kg/week; N = 3 females/group; 4 females in 100 mg/kg group</td>
<td>No lung findings; NOAEL 100 mg/kg/week</td>
</tr>
<tr>
<td>2: GLP 4-week IV study with 4-week recovery.</td>
<td>To support first-in-human single ascending dose IV dose study of mavrilimumab in RA subjects.</td>
<td>Doses 0, 10, 30 and 100 mg/kg/week; N = 5/sex/group; 3/sex/group for 10 mg group</td>
<td>No lung findings; NOAEL 100 mg/kg/week</td>
</tr>
<tr>
<td>3: Non-GLP 26-week IV study.</td>
<td>To explore potential lung toxicity following repeat-IV dosing in a chronic setting up to 26 weeks.</td>
<td>Doses 35 and 100 mg/kg/week (no control); N = 4 females/group</td>
<td>Foamy macrophages in lung at high doses only; NOAEL 100 mg/kg/week</td>
</tr>
<tr>
<td>4: GLP SC Bridging 4-week study with 6-week recovery</td>
<td>To support multiple ascending dose SC study in RA subjects.</td>
<td>Doses 50, 100, 200 mg/kg (no control); N = 4 males/group</td>
<td>No lung findings; NOAEL 200 mg/kg/week</td>
</tr>
<tr>
<td>5: GLP 26-week IV study with 17-week recovery</td>
<td>To support chronic administration in RA subjects with high margins of safety.</td>
<td>Doses 0, 15, 30, 100 mg/kg/week; N = 8/sex/group</td>
<td>Foamy macrophages in lung at all dose levels; Adverse findings in lung at 30 and 100 mg/kg/week; NOAEL 15 mg/kg/week</td>
</tr>
<tr>
<td>6: GLP 13 week SC study with 17-wk recovery</td>
<td>To characterize toxicity of SC administration in well-controlled GLP-compliant study in both sexes.</td>
<td>Doses 0, 50, 150, 400 mg/kg/week; N = 6/sex/group</td>
<td>Foamy macrophages at all doses; Reduced incidence at end of recovery; NOAEL = 400 mg/kg/week</td>
</tr>
<tr>
<td>7: GLP SC low dose 26-week study with 9-week recovery</td>
<td>To establish a no-effect-level dose for lung effects in a chronic study.</td>
<td>Doses 0, 3, 10 mg/kg/week; N = 10/sex/group; 6/sex in control</td>
<td>No lung findings; NOEL = 10 mg/kg/week</td>
</tr>
</tbody>
</table>

The studies in the nonclinical program are listed in chronological order in Table 1 to reflect how the nonclinical program progressed to support clinical development of mavrilimumab. The first study (Study 1) was an exploratory study to examine the potential lung effects of mavrilimumab given the available literature on the role of the target pathway in maintaining lung homeostasis in knockout mice (Robb et al., 1995; Stanley et al., 1994). The second study (Study 2) was a GLP-compliant 4 week repeat-IV dose study designed to support the first-in-human study of mavrilimumab, a single ascending IV dose study in RA subjects (Burmester et al., 2011). Males and females and a vehicle control group were included in this pivotal NHP study to satisfy regulatory requirements. The third study (Study 3) was a further exploratory study to examine the potential for lung toxicity following a 26-week dosing phase given the intended chronic use of mavrilimumab in the clinical setting. The rationale for including only females in the two early exploratory non-GLP studies was to simplify the study design, to reduce the total number of animals and because sex differences in the toxicity profile were not anticipated. The fourth study (Study 4) was a GLP-compliant study in males to support the transition from IV to SC dosing in the clinical program. The reason this study included only males and no vehicle control group is because this study was designed as a streamlined bridging study to focus on local effects following SC administration. In parallel to this SC bridging study, the fifth study (Study 5), a 26-week GLP repeat-IV dose study in males and females, was performed. This study was intended to be the pivotal study to fully characterize systemic toxicity and to support chronic dosing in the clinic. Later in development, because of the minimal design of the 4 week SC bridging study in males, and because the clinical program transitioned exclusively to SC dosing, the sixth study (Study 6) was conducted to satisfy regulatory requirements with a more robust design, including males and females, three dose levels and a vehicle control group. Finally, the seventh study (Study 7) was conducted using very low SC doses to satisfy a regulatory requirement to establish a no-effect-level dose for lung toxicity.

It is important to explain the impact of ADAs on the interpretation of toxicity findings for mavrilimumab. Mavrilimumab is a human IgG that cross-reacts with cynomolgus GM-CSFRα. The immunogenicity assay to measure ADAs utilized mavrilimumab as both the capture and detection reagent (double-sandwich assay), and theoretically detected the presence of both binding- and neutralizing ADAs. The TK assay utilized a fusion protein IgM-CSFR-Fc as the capture reagent and sheep anti-human IgG as the detection reagent. As such, the TK assay measured the unbound, biologically active mavrilimumab molecule. The reduced TK exposure observed in the presence of ADA suggests that the majority, if not all, of these detected ADAs were neutralizing antibodies, blocking the interaction of mavrilimumab with the target antigen receptor. As such the TK exposure–response relationship in nonhuman primates and the safety margin assessments were based on the measured unbound (free), biologically active mavrilimumab. All ADA positive animals are excluded from determination of mean TK parameters.

Overall, lung was identified as the target organ of toxicity following chronic dosing with mavrilimumab. An increase in foamy macrophages in lung tissues was observed in studies of longer duration (i.e., all studies of 11 weeks or longer) but not in two independent studies of only 4 weeks duration. Where this finding occurred in the absence of any changes in lung structure or function, or any other changes in toxicology parameters, a NOAEL was identified. In a single study, at higher IV doses after 26 weeks of chronic dosing, adverse findings in lung were observed in some animals in addition to the accumulation of foamy macrophages (Study 5). These adverse changes included the presence of lung foreign material, cholesterol clefts, and granulomatous inflammation.

In both a GLP 4-week repeat-IV dose study (Study 2) and a GLP 4-week repeat-SC dose bridging study (Study 4), no findings were observed in the lungs up to the highest dose tested (100 mg/kg/week IV or 200 mg/kg/week SC; corresponding to body weight scaled dose level of approximately 80 mg/kg) providing evidence that treatment-related changes in lung requires dosing longer than 4 weeks. In studies of longer duration, the incidence and severity of the findings in lung were demonstrated to be mavrilimumab dose-related. In a non-GLP 11-week IV study in female cynomolgus monkeys (Study 1), dose-related increased numbers of foamy macrophages was seen microscopically in 1 of 3 animals at 30 mg/kg/week and in 4 of 4 animals at 100 mg/kg/week. This finding was only seen following microscopic examination of hematoxylin and eosin (H&E) sections of lung tissue and was given a severity grade of very slight to slight. Foamy macrophage changes were not observed following microscopic examination of BAL samples from this study suggesting analysis of BAL is a less sensitive measure for this finding compared to H&E. In a longer study (a non-GLP 26-week IV study in female cynomolgus monkeys; Study 3), this dose-related finding of increased lung foamy macrophages was evident in both lung H&E sections and in BAL samples. In this study, histopathological findings showed the accumulation of foamy macrophages was slight to minimal in 4 of 4 animals at 35 mg/kg/week and in 4 of 4 animals at 100 mg/kg/week. Evaluation of BAL samples after 26 weeks of treatment in ADA-negative animals showed that the percent of foamy macrophages was higher at the 100 mg/kg/week dose compared to 35 mg/kg/week (Table 2).

In a 26-week GLP repeat-IV dose study (Study 5), the severity of the finding of increased foamy macrophages was likewise dose-related. In this study, the finding was minimal to moderate in severity in animals dosed at 15 and 30 mg/kg/week and slight to marked in severity at 100 mg/kg/week. Adverse findings in lung were observed in some...
animals in this study and included the presence of foreign material or endogenous cell debris (crystalline, needle shaped) in the lung of one 30 mg/kg/week male (13755), one 100 mg/kg/week male (14071), and one 100 mg/kg/week female (14081) at the end of the dosing phase (terminal necropsy) and in one 30 mg/kg/week male (14053) at the end of the recovery period (recovery necropsy). Bronchioalveolar hyperplasia was also observed in some of these animals: in one 100 mg/kg/week male (14071) at the terminal necropsy and in one 30 mg/kg/week (14053) and one 100 mg/kg/week male (14059) at the recovery necropsy. Of these animals, the 30 mg/kg/week male in the recovery group had the most affected lung, with moderate foamy macrophages, severe fibrosis, marked areas with needle shaped crystalline deposits consistent with cholesterol crystals/clefts in the lung with associated granulomatous inflammation, marked bronchioalveolar hyperplasia, and slight inflammation of the bronchioles (Fig. 1C). The lung lesion in this animal was considered to be severe granulomatous inflammation associated with lipoproteinic material (cholesterol) from degradation of lung surfactant. These findings represent an exaggerated pharmacological effect arising from prolonged mavrilimumab blockade of GM-CSF signaling and inhibitory effects on lung macrophage function. The lung lesions observed in these NHP studies are consistent with lung lesions observed in human PAP including the presence of cholesterol clefts and increased foamy macrophages (http://www.google.com/imgres?imgurl=http%3A%2F%2Fpathhsw5m54.ucsf.edu%2Foverview%2Fimagesover%2Fyeast%252520fungi%2Fpap1.jpg&imgrefurl=http%3A%2F%2Fpathhsw5m54.ucsf.edu%2Foverview%2Ffungi1a.html&h=252&w=378&tbnid=17mhY74TC-bCLM%3A&zoom=1&docid=O-lcpWdm5HJlWM&ei=qpa9U-i9EsaiyASH7IDgBw&ved=0CDcQMygEMAQ&iact=rc&uact=3&dur=823&page=1&start=0&ndsp=28).

Milder effects were seen in the 13-week repeat-SC dose study (Study 6) and included only minimal accumulation of foamy macrophages at the terminal necropsy in some animals at all dose levels without evidence of granulomatous inflammation, cholesterol clefts or any other adverse changes. Foamy macrophages are a common background finding in cynomolgus monkeys (Drevon-Gaillot et al., 2006) and at the lowest dose in this study (50 mg/week or approximately 20 mg/kg/week), foamy macrophages were only marginally increased compared to the background incidence seen in control animals. At the highest dose (400 mg/week or approximately 160 mg/kg/week),
while the severity of the finding was still graded “minimal,” there appeared to be more clusters of foamy macrophages at the highest dose compared with only occasional individual foamy cells at the lowest dose. Representative photomicrographs of lungs from a low dose (50 mg/week) and a high dose (400 mg/week) animal at the terminal necropsy are shown in Fig. 2A and B and illustrates the increased numbers of these cells at the higher dose compared to the lower dose. These results confirm the dose-dependency of this effect.

Partial reversibility of the finding of lung foamy macrophages was demonstrated in several studies. In the 26 week GLP repeat-IV dose study (Study 5), reduced frequency of foamy macrophages was observed at the end of a 17-week dose-free period compared to end of dosing although foamy macrophages were still present. At recovery, the foamy macrophages tended to be found clustered in hilar and subpleural locations, rather than diffuse throughout the lung. The distribution of these cells in the periarteriolar and peribronchiolar locations at recovery is viewed as evidence of a recovery process, as this is the location where alveolar macrophages enter lymphatics and exit the lung (Courtice, 1963).

Partial reversibility of foamy macrophages was also observed in a 13 week repeat-SC dose study (Study 6) following weekly SC injections of 0, 50, 150 or 400 mg/week (approximately 0, 20, 60 and 160 mg/kg/week). In this study, minimal accumulation of foamy macrophages was seen at all dose levels at the end of the dosing phase. At the end of recovery, this minimal finding was primarily observed in the periarteriolar and peribronchiolar regions of the lung (lung hilus) suggesting that these cells were being cleared from the lung. Further evidence of the reversibility of foamy macrophages was the reduced number of animals with this finding at the end of the recovery period. Overall, minimal accumulation of foamy alveolar macrophages was observed in 13 of 18 mavrilimumab-treated animals at the end of the dosing period, while this finding was observed in only 9 of 18 animals at the end of a 17 week dose-free recovery phase (Table 3). In this study, the presence of ADA did not impact the lung findings and all animals were similarly exposed to active drug as measured in the TK assay. For that reason, both ADA positive and negative animals are included in Table 3. Notably, at the lowest dose, the fewest recovery animals were affected, suggesting that reversibility of this finding may take longer at higher doses.

Toxicokinetics

The TK properties of mavrilimumab in cynomolgus monkeys indicate a potential antigen sink effect related to GM-CSFRα-mediated clearance of mavrilimumab at low serum concentration levels. At higher serum concentrations, the PK of mavrilimumab was apparently linear with dose. No gender difference in PK was observed. The elimination t1/2 of mavrilimumab was 1–2 weeks, typical for a human IgG in cynomolgus monkeys. The absorption of SC administered mavrilimumab from the dosing site was slow, the peak serum concentration was observed 2–4 days postdose (Tmax). A summary of mean noncompartmental TK parameters in ADA-negative animals from a GLP 26 week repeat-IV dose study (Study 5) is shown in Table 4 and that in ADA-negative animals from a GLP 13 week repeat-SC dose study (Study 6) is shown in Table 5. The absolute SC bioavailability of mavrilimumab in cynomolgus monkeys was not formally determined in toxicology studies. However, by comparing the TK exposure across different IV and SC toxicology studies, the SC bioavailability of mavrilimumab in cynomolgus monkeys was predicted to be in the range of 50% to 100%.

Mean time versus concentration profiles for the GLP 13 week repeat-SC dose study (Study 6) for ADA-negative animals are shown in Fig. 2. At these dose levels, dose proportional PK was observed with no evidence of receptor mediated clearance. Notably, given the long elimination
half-life and the high exposures achieved during the dosing phase, substantial exposure to mavrilimumab persisted throughout the recovery phase (Fig. 3).

Immunogenicity and establishment of no observed effect level

Mavrilimumab is immunogenic in cynomolgus monkeys, and the immunogenicity response upon repeated dosing had a profound impact on clearance of mavrilimumab in repeat-dose studies. Thus, immunogenicity severely confounded the ability to investigate the effects of repeated doses of mavrilimumab to establish a no observed effect level (NOEL). Nevertheless, a low repeat-SC dose study (Study 7) was conducted to formally identify a dose level that did not lead to accumulation of foamy macrophages in lung following chronic dosing. To account for the predicted high rate of ADA at these low doses, large numbers of animals per group were used (N = 10/sex/group). Animals got once-weekly SC injections at 3 or 10 mg/week (corresponding to body weight scaled dose levels of approximately 1.2 and 4 mg/kg/week, respectively) for 26 weeks. As predicted, the majority of animals developed ADA which led to rapid clearance. TK exposure was maintained in 4 ADA-negative animals at 10 mg/week and in 1 ADA-negative animal at 3 mg/week. No findings were observed in any animals in this study and the NOEL is considered 10 mg/week (approximately 4 mg/kg/week).

Impact of ADA on clearance is illustrated in the time–concentration profiles for individual animals in this 26-week low repeat-SC dose study (Study 7) in which the majority of animals developed anti-drug antibodies (Figs. 4 and 5). These TK profiles demonstrate that serum concentrations of mavrilimumab decreased rapidly to below the lower limit of quantitation in all ADA-positive animals while animals that were ADA negative maintained exposure to mavrilimumab throughout the dosing phase. Prevalence of ADAs across all studies as a function of mavrilimumab exposure (AUC) in ADA-negative animals in three GLP studies (26 week repeat-IV dose, 13 week repeat-SC dose and 26 week repeat-low SC dose; Studies 5, 6, and 7) was analyzed in order to characterize the exposure–response relationship. By comparing the incidence of foamy macrophages versus AUC (0 – t) in ADA-negative animals across multiple NHP studies, it is clear that the probability of observing foamy macrophages in lung increases with dose (Fig. 7). There is a biphasic relationship between the probability of observing this finding as a function of log_{10}AUC suggesting that exposure to mavrilimumab must be above a critical threshold level before pharmacologically-mediated lung findings are observed. The safety margin for the accumulation of foamy macrophages was calculated by comparing predicted human exposures and observed TK exposure in the low SC repeat-dose study in cynomolgus monkeys (Study 7) at the NOEL of 10 mg/week mavrilimumab (Table 6). Because minimal accumulation of foamy macrophages in lung could be considered an unacceptable toxicity by certain health authorities, this was intended to be the definitive study to establish the mavrilimumab exposure safety margin. Human exposure to mavrilimumab was modeled based on available data from the completed EARTH study (Burmester et al., 2013) to predict the human exposures at the dose levels of 30, 100, and 150 mg mavrilimumab SC every other week (Q2W) under evaluation in the ongoing Phase 2b clinical study (EARTH Explorer 1; NCT01706926). At the NOEL of 10 mg/week, the mean values of C_{max} and AUC_{f} at steady-state were 83.3 μg/mL and 338 μg · d/mL, respectively. By comparing human and cynomolgus monkey exposures, the calculated safety margins for the ongoing Ph2b study are 38.6, 5.54, and 3.69, respectively (Table 6).

In order to graphically depict the therapeutic window or safety margin, the incidence of foamy macrophages (percent of animals with the finding) versus exposure (AUC_{f-7d}) across all completed chronic GLP toxicology studies in ADA-negative monkeys is shown in Fig. 8. The three vertical colored lines represent the predicted correspondent human AUC_{f}, at each human dose level (30, 100, 150 mg Q2W). The human exposures are at least 10-fold below the monkey exposure levels associated with the finding of foamy macrophages in lung demonstrating an acceptable safety margin for continued mavrilimumab clinical development.

Discussion

Mavrilimumab demonstrated an acceptable safety profile in several repeat-IV and SC dose studies in cynomolgus monkeys with no changes in any of the evaluated parameters and no microscopic findings in any

Table 4
Noncompartmental TK parameters in ADA negative cynomolgus monkeys in 26 week GLP repeat IV dose study (Study 5).

<table>
<thead>
<tr>
<th>TK parametersa</th>
<th>Dose (mg/kg once per week)</th>
<th>15</th>
<th>30</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Week 1</td>
<td>Week 26</td>
<td>Week 1</td>
</tr>
<tr>
<td>AUC (μg · d/mL)</td>
<td></td>
<td>1110 (269)</td>
<td>6450 (1020)</td>
<td>1850 (410)</td>
</tr>
<tr>
<td>C_{max} (μg/mL)</td>
<td></td>
<td>357 (96.6)</td>
<td>1280 (436)</td>
<td>540 (111)</td>
</tr>
<tr>
<td>t_{1/2} (d)</td>
<td></td>
<td>6.27 (0.938)</td>
<td>8.68 (3.57)</td>
<td>8.26 (2.27)</td>
</tr>
</tbody>
</table>

a Parameters are rounded to 3 significant figures, and shown as mean (standard deviation). AUC_{f} = area under the concentration–time curve at steady-state in the 7-day dosing interval; C_{max} = maximal observed concentration; SC = subcutaneous; TK = toxicokinetics.

Table 5
Noncompartmental TK parameters in ADA negative cynomolgus monkeys in 13 week GLP repeat SC dose study (Study 6).

<table>
<thead>
<tr>
<th>TK parametersa</th>
<th>Dose (mg once per week; fixed dose)</th>
<th>50</th>
<th>150</th>
<th>400</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Week 1</td>
<td>Week 13</td>
<td>Week 1</td>
</tr>
<tr>
<td>AUC (μg · d/mL)</td>
<td></td>
<td>1000 (243)</td>
<td>3920 (1140)</td>
<td>3130 (644)</td>
</tr>
<tr>
<td>C_{max} (μg/mL)</td>
<td></td>
<td>175 (50.8)</td>
<td>691 (238)</td>
<td>560 (130)</td>
</tr>
<tr>
<td>t_{1/2} (d)</td>
<td></td>
<td>ND</td>
<td>9.64 (3.77)</td>
<td>ND</td>
</tr>
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</table>

a Parameters are rounded to 3 significant figures, and shown as mean (standard deviation). AUC_{f} = area under the concentration–time curve at steady-state in the 7-day dosing interval; C_{max} = maximal observed concentration; SC = subcutaneous; TK = toxicokinetics; ND = not determined.
tissues other than lung. The development of increased foamy macrophages in lung tissues was observed in several studies following long-term dosing (at least 11 weeks) and is predictable based on the pharmacology of inhibiting normal GM-CSF function (Trapnell and Whitsett, 2002; Uchida et al., 2009; Yoshida et al., 2001).

Catabolism of surfactant by alveolar macrophages requires GM-CSF signaling (Reed and Whitsett, 1998; Reed et al., 2000; Yoshida et al., 2001). The foamy appearance of alveolar macrophages, therefore, reflects the intracellular accumulation of neutral lipids within these cells resulting from target-mediated functional inhibition as opposed to an inflammatory response consisting of mixed cellular infiltration, or an increase in total macrophages in the lung. Where this occurred in the absence of any other changes in the pulmonary anatomic pathology or any other toxicology parameters, a NOAEL was identified. In a GLP 26-week repeat-IV dose study (Study 5), the presence of lung foreign material, cholesterol clefts, and granulomatous inflammation was observed in some animals at higher doses and was considered adverse. This exaggerated response to lung foreign material and cholesterol clefts is consistent with mavrilimumab inhibitory effects on lung macrophage function.

Significant work has been done to characterize alveolar macrophage responses to inhaled small molecule therapeutics and these effects can pose regulatory challenges for development of inhaled medicines (Forbes et al., 2014; Lewis et al., 2014). The mavrilimumab effects in nonhuman primates described here can be compared and contrasted with the effects described for inhaled small molecules. While there is similarity in the phenotype of many of the findings, there are considerable differences in the underlying biology leading to these lung effects for inhaled small molecules compared to a systemically administered GM-CSF inhibitor such that designation of a “class effect” is not straightforward. In many cases, poorly soluble inhaled small molecules can induce macrophage responses such as the accumulation of foamy macrophages. These lesions may or may not be associated with neutrophilic inflammation (Lewis et al., 2014). The challenge to inhaled small molecule development has been distinguishing non-adverse phagocytic adaptive responses from adverse responses (Forbes et al., 2014). While these findings may be caused by deposition of particulates, they may also be modulated by the pharmacological action of these agents. In the case of mavrilimumab, which is a parenterally administered large molecule (i.e. not directly instilled into the lung), the best interpretation of the observed lung findings is that blockade of GM-CSFRα signaling in alveolar macrophages inhibits their ability to catabolize the lipoproteinic material that comprises surfactant. The alveolar macrophage response in inhaled small molecule drug development has been described as a common but a poorly understood phenomenon that requires development of optimized models and batteries of tests to better characterize the alveolar macrophage response (Forbes et al., 2014). As a result, the significance of the accumulation of foamy macrophages differs. That is, for small molecules deposited directly into the lung, the finding largely reflects impaired clearance of particulate matter, while for mavrilimumab it reflects blockade of a critical cytokine receptor signaling pathway. Despite these differences, we share the opinion of the authors in Lewis et al. that foamy macrophage accumulation without associated inflammatory changes should be considered a non-adverse effect. Furthermore, the wealth of scientific evidence available in the literature supports the pharmacological basis for the
Mavrilimumab induced lung effects while for the broad range of inhaled small molecule drugs, improved understanding of alveolar macrophage biology is important to advancing the development of drugs that cause these effects.

Mavrilimumab is immunogenic in cynomolgus monkeys, and the development of ADA is associated with substantially reduced exposures. As ADA greatly facilitates the systemic clearance of mavrilimumab, at lower dose levels the potentially high prevalence of ADA in cynomolgus monkeys predictably would preclude the ability to achieve and maintain meaningful TK exposure in these animals. Nevertheless, to formally demonstrate the NOEL dose of mavrilimumab in a chronic setting, a low SC 26-week repeat-dose study was performed in cynomolgus monkeys (Study 7). In this study, animals got once weekly SC injections at 3 or 10 mg for 26 weeks. TK exposure was maintained in 5 ADA-negative animals. No mavrilimumab-related effects were observed in the lung or any other parameter assessed and the NOEL was determined to be 10 mg/week. As predicted, the majority of animals developed ADA, which led to rapid clearance of mavrilimumab.

Homeostasis of surfactant is critical for lung function and is maintained by the balanced production and clearance of surfactant by cells in the lung. The ability of pulmonary alveolar macrophages to clear surfactant lipids and proteins from the lung surface is regulated by GM-CSF via the transcription factor PU.1 (Trapnell and Whitsett, 2002). Blockade of GM-CSF signaling would therefore be expected to lead to inhibition of alveolar macrophage function. The critical role of GM-CSF in surfactant homeostasis was identified in GM-CSF−/− and in GM-Rβ chain-deficient CSF2RB−/− mice (Dranoff et al., 1994; Robb et al., 1995; Stanley et al., 1994). In these mice, impaired clearance of surfactant by alveolar macrophages causes surfactant accumulation and a lung phenotype identical to PAP in humans.

Fig. 5. Individual TK profiles in GLP 26 week repeat-SC dose study at 10 mg per week (Study 7). Mavrilimumab serum concentrations in μg/mL versus time for individual animals treated with once weekly doses of 10 mg mavrilimumab. At week 5 and later, ADA-positive animals had serum levels below lower limit of quantitation (LLOQ).

Fig. 6. Prevalence of anti-drug antibodies in mavrilimumab nonhuman primate studies. The fraction of ADA positive animals (number of ADA + animals/total number of animals per dose group) for three mavrilimumab repeat-IV dose (Studies 2, 3 and 5) and three repeat-SC dose studies (Studies 4, 6 and 7) versus the administered dose. (Doses administered as fixed SC doses were converted to mg/kg dose assuming a typical animal body weight of 2.5 kg).
In humans, PAP is a rare lung disease that develops and progresses very slowly. Broadly, PAP can be classified in 3 forms: (1) primary or idiopathic (autoimmune), which is the most common clinical form and strongly associated with high levels of autoantibodies against GM-CSF; (2) hereditary PAP which occurs in patients with defects in genes encoding GM-CSF receptor (CSF2RA or CSF2RB) and (3) secondary, which can be due to lung infections, hematologic malignancies and inhalation of mineral dusts such as silica, titanium oxide, aluminium, and insecticides. Clinically, PAP is characterized by dyspnea when there is a substantial accumulation of surfactant proteins in alveolar spaces (Carey and Trapnell, 2010).

Thus, PAP is a theoretical risk factor associated with GM-CSF inhibition. Although high levels of circulating autoantibodies to GM-CSF are associated with PAP, data has shown that these GM-CSF autoantibodies can likewise be detected in healthy individuals, albeit at much reduced concentrations (Uchida et al., 2009). On average, PAP patients were found to have 59.8 μg/mL serum GM-CSF autoantibody levels compared to mean levels of 1.04 μg/mL in healthy controls (Uchida et al., 2009). The authors propose that there is a critical threshold level of autoantibodies, between about 10.4 and 19 μg/mL, above which development of PAP is more likely (Carey and Trapnell, 2010; Uchida et al., 2009). This “critical threshold” concept is consistent with mavrilimumab toxicity findings observed in NHP which identified an exposure level of mavrilimumab in serum which was not associated with development of foamy macrophages in lung (i.e. NOEL). The pulmonary alveolar macrophage response observed in cynomolgus monkeys following treatment with mavrilimumab was a pharmacologically-mediated finding with a biphasic dose response (Fig. 7). Taken all together, this evidence suggests there is a therapeutic window for the clinical use of antibody therapies to the GM-CSF pathway in inflammatory diseases like RA.

In the recently completed Ph2a clinical study EARTH (Burmester et al., 2013) mavrilimumab (10, 30, 50 or 100 mg Q2W) or placebo was administered SC to subjects with moderate/severe RA (DA52 > 3.2) on stable methotrexate for 12 weeks followed by a 12 week drug free follow up period. Intensive respiratory monitoring including chest X-rays, forced expiratory volume (FEV1), forced vital capacity (FVC), diffusing capacity of the lung for carbon monoxide (DLCO) and dyspnea scores were incorporated into the study objectives. In addition, serum biomarkers of lung damage, surfactant protein D (SP-D) and Krebs von den Lungen-6 (KL-6) were measured using commercially available immunoassays.

The biomarker SP-D is a member of the collagensubfamily of glycoproteins and calcium-dependent lectins. In the lungs, SP-D participates in the innate response to inhaled microorganisms and organic antigens. Studies have shown that expression of SP-D may be related to a number of human diseases, including cystic fibrosis (Olesen et al., 2010), asthma (Atschoina-Vasserman et al., 2011), acute interstitial pneumonias (Kucejko et al., 2009), acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) (Ware et al., 2010), and PAP (Sakagami et al., 2010).

The biomarker KL-6 is a member of the collagensubfamily of glycoproteins secreted by type II pneumocytes and has emerged as a potential surrogate biomarker of alveolitis (Hant et al., 2009). Studies have shown that expression of KL-6 may be directly related to alveolar damage and inflammation caused by lung disease, including cystic fibrosis (Olishimo et al., 2009), asthma (Imai et al., 2002), ARDS (Kondo et al., 2011), and PAP (Carey and Trapnell, 2010). For this reason, SP-D and KL-6 were evaluated across the treatment (Day 85) and safety follow-up phase of the EARTH study (Day 169) to investigate a potential effect of treatment with mavrilimumab on these parameters and corroborate the clinical findings.

In EARTH, mavrilimumab demonstrated good clinical activity with no clinically significant or persistent changes in the lung function tests performed. Likewise, the serum biomarkers of lung damage, SP-D and KL-6, showed no clinically significant changes following mavrilimumab treatment. A phase 2b study in RA patients to assess safety and efficacy of mavrilimumab administered SC at 30, 100 or 150 mg Q2W over a 24 week treatment period is in progress (EARTH Explorer 1). In this study, further pulmonary monitoring to assess potential impact on lung safety will be evaluated.

**Conclusions**

The dose-and time-related accumulation of foamy macrophages in lung following exposure to mavrilimumab was observed in several NHP studies and was expected based upon the known role of GM-CSFRα signaling in the function of alveolar macrophages (Trapnell and Whitsett, 2002; Uchida et al., 2009; Yoshida et al., 2001). After a dose-free recovery period, there was evidence of reversibility for the accumulation of foamy macrophages although full recovery was not achieved. The foamy appearance of alveolar macrophages reflects the intracellular accumulation of neutral lipids. Adverse chronic changes observed in lung of some animals exposed to high doses of mavrilimumab were

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**Table 6**

<table>
<thead>
<tr>
<th>Observed human exposure (Q2W)</th>
<th>Observed TK exposure at 10 mg/week SC (Study 7)</th>
<th>Safety margin for proposed P2b study</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>30 mg</td>
<td>100 mg</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;max&lt;/sub&gt; (μg·d·mL&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>17.5</td>
<td>122</td>
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<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (μg/mL)</td>
<td>2.16</td>
<td>11.1</td>
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</table>

<sup>a</sup> AUC<sub>max</sub> = area under the concentration–time curve at steady-state in the 14-day dosing interval; C<sub>max</sub> = maximal observed concentration; NOEL = no-observed-effect level; P2b = - Phase 2b; SC = subcutaneous; TK = toxicokinetics

<sup>b</sup> The safety margin is estimated based on projected 150 mg Q2W human exposure assuming linear pharmacokinetics upon saturation of the GM-CSF receptor sink.  

<sup>a</sup> Steady-state AUC in a 2-week interval.
characterized as an inflammatory reaction (granulomatous) to foreign material and may reflect impaired ability of macrophages to clear foreign material or endogenous cell debris. Despite these findings, there were high clinical exposure safety margins compared to the doses that caused these adverse changes in lung. To formally identify a dose that did not cause accumulation of foamy macrophages in lung, a low-dose SC study was performed (Study 7). As expected, immunogenicity of mavrilimumab led to a high rate of ADA with insufficient toxicokinetic exposures in a majority of animals. Nevertheless, exposure was maintained in 4 animals at 10 mg/week (approximately 4 mg/kg/week) which represented the NOEL. The human exposures planned in Phase 2b are below the exposure levels expected to induce foamy macrophages in lung and so are anticipated to confirm the adequacy of the safety margin (3.7-fold AUC-based and 5-fold Cmax-based) to support the continued clinical development of mavrilimumab.

Conflict of interest

This work was sponsored by MedImmune. All authors were employed by MedImmune at the time the work was performed.

References


