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Research Article

Measurement of IL-17AA and IL-17FF as Pharmacodynamic Biomarkers to Demonstrate Target Engagement in the Phase I Study of MCAF5352A

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Abstract. The interleukin (IL)-17 pathway has been implicated in the pathophysiology of many autoimmune diseases. MCAF5352A is a humanized monoclonal antibody which targets both IL-17A and IL-17F, thereby inhibiting the activity of IL-17 dimers (IL-17AA, IL-17AF, and IL-17FF). The pharmacokinetic profile of MCAF5352A has been characterized in both a Phase Ia single ascending dose study and a Phase Ib multiple ascending dose study. Two qualified enzyme-linked immunosorbent assays were used to measure total IL-17AA and IL-17FF levels in serum as pharmacodynamic biomarkers in the Phase I studies. The two assays demonstrated specificity for IL-17AA or IL-17FF with sensitivity at low picogram/milliliter levels. The assay precision and accuracy also met acceptance criteria. Although total serum IL-17AA and IL-17FF levels were below the assay detection limits prior to administration of MCAF5352A, post-treatment levels in both the single and multiple dose cohorts became detectable and increased in a dose-dependent manner. These data are consistent with target engagement by MCAF5352A. Our work highlights bioanalytical challenges encountered while developing biomarker assays requiring high sensitivity and specificity. Data generated using these assays enabled the confirmation of target engagement during early clinical drug development.

KEY WORDS: anti-IL-17 monoclonal antibody; biomarker assay; IL-17AA; IL-17FF; pharmacodynamics.

INTRODUCTION

Interleukin (IL)-17A and IL-17F belong to the IL-17 cytokine family. IL-17A and IL-17F share approximately 50% sequence homology and can form both homodimers and a heterodimer, designated as IL-17AA and IL-17FF and IL-17AF, respectively (1). The heterodimer and homodimers bind to and signal through a heterodimeric receptor complex consisting of IL-17Ra and IL-17Rc chains, which are broadly expressed on various tissue cell types, including epithelial cells, fibroblasts, astrocytes, and oligodendrocytes (2). The IL-17AA, IL-17FF, and IL-17AF cytokines are produced by activated leukocytes, particularly T-helper 17 (Th17) cells, and induce similar downstream pro-inflammatory responses such as chemokine and cytokine production (3). In many autoimmune diseases, such as rheumatoid arthritis, psoriasis, inflammatory bowel disease, and multiple sclerosis, the expression of IL-17A and IL-17F in serum and tissues is elevated (4–7). IL-17A and IL-17F can amplify inflammation and trigger tissue damage in these autoimmune diseases (3). Since the IL-17 pathway is likely to play a role in numerous immune-mediated disorders, several antibodies targeting the IL-17 pathway are being developed. These molecules either target individual IL-17 family members (e.g., secukinumab and ixekizumab) or the IL-17 receptor (e.g., brodalumab) which leads to inhibition of multiple IL-17 family members (8–10). Several of these molecules have been tested in clinical studies across multiple indications (11–13).

MCAF5352A is a human IgG1 antibody that binds to IL-17AA, IL-17AF, and IL-17FF with high affinity and neutralizes their biological activities with similar affinity, assessed in cell-based assays (data not shown). MCAF5352A therefore represents a novel therapeutic approach distinguished by its ability to block not only IL-17AA but also IL-17AF and IL-17FF with high affinity, which may be more effective in reducing the inflammatory effects of the IL-17 pathway. The resulting complete

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suppression of IL-17-induced inflammation may address the unmet medical need in human inflammatory and immune-mediated diseases. Our pharmacodynamic biomarker strategy for the Phase I study focused on confirming binding of MCAF5352A to both IL-17A and IL-17F in healthies during early clinical development. Literature survey on circulating IL-17 levels, measured using various assay platforms and reagents, have reported substantial variability, ranging from sub- to hundred-picogram per milliliter (14–19). The reported IL-17 levels were primarily in disease samples and often with limited or no method characterization information. The work described in this manuscript was done before technologies (such as Quanterix and Singulex) capable of achieving sub-picogram per milliliter sensitivity were available. This work triggered our effort to evaluate Singulex technology, and the evaluation work was published elsewhere (20). As described in the paper (20), the Singulex assay was proven to be specific and sensitive. However, MCAF5352A interference was observed in the assay and could not be used for analysis of samples from subjects that received MCAF532A treatments. We developed two enzyme-linked immunosorbent assays (ELISAs) (Fig. 1) for specific measurement of total levels of IL-17AA and IL-17FF, respectively. An elevation in levels of these biomarkers was observed after administration of MCAF5352A in the Phase I study, confirming target engagement.

METHODS

Reagents and Antibodies

Pooled and individual serum samples from healthy volunteers were purchased from Bioreclamation IVT and BioChemed. MCAF5352A, recombinant human IL-17AA (rhuIL-17AA), IL-17FF (rhuIL-17FF), mouse monoclonal antibody (mAb) 11D5 specific to human IL-17AA, mouse mAbs 15B11 and 42G11 specific to human IL-17FF, and mouse mAb 1B9 specific to the complementary determining region (CDR) of MCAF5352A were produced at Genentech, Inc. Horseradish peroxidase-conjugated streptavidin (SA-HRP) was purchased from Amersham, GE Healthcare (Cat. no. RPN4401V). HRP-conjugated anti-mouse IgG (γ) (Cat. no. 074-1802) and 3,3′,5,5′-tetramethylbenzidine (TMB) (Cat. no. 50–76-00) were purchased from Kirkegaard & Perry Laboratories. Immunoglobulin Inhibiting Reagent (IIR), a blocker to heterophilic antibodies, was purchased from Bioreclamation IVT (Cat. no. 6LD1074).

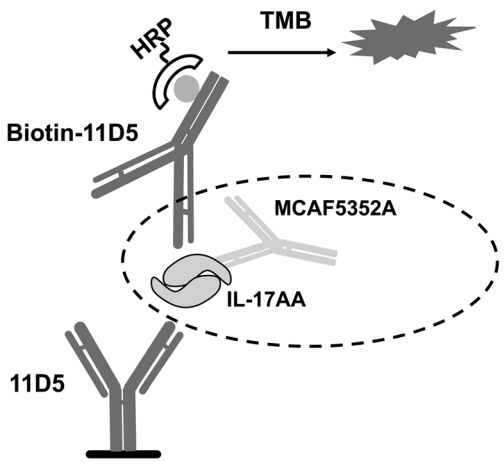

Fig. 1. Schema of the human IL-17AA assay

Assay diluent contained phosphate-buffered saline (PBS), 0.5% bovine serum albumin (BSA), 0.05% polysorbate 20, 0.05% proclin300, 0.25% CHAPS, 5 mM ethylenediaminetetraacetic acid (EDTA), and 350 mM sodium chloride at pH 7.4. HRP diluent contained PBS, 0.5% BSA, 0.05% polysorbate 20, 0.05% ProClin300, and 0.25% CHAPS at pH 7.4. Control and sample diluent was prepared in assay diluent plus 20 μg/mL MCAF5352A and 1 mg/mL IIR. Standard diluent was prepared in assay diluent plus 10 μg/mL MCAF5352A plus 0.5 mg/mL IIR. Washing buffer contained PBS, 0.5% BSA, and 0.05% polysorbate 20 at pH 7.4.

Human IL-17AA Assay

The total concentration of IL-17AA in human serum samples was determined using a qualified ELISA (Fig. 1). Microtiter plates were coated with 100 μL of IL-17AA-specific mAb 11D5 at 0.5 μg/mL in carbonate buffer for capture and incubated at room temperature (RT) for 5 h with agitation. An IL-17AA standard curve, ranging from 1.56 to 200 pg/mL (in-well concentrations) at 1:2 serial dilutions, was prepared in the standard diluent. Serum samples and quality controls were prepared at 1:2 dilutions in the sample diluent. One hundred microliters of samples, standards, and controls prepared in diluent containing 20 μg/mL (equivalent to 10 μg/mL in-well after 1:2 dilution) MCAF5352A were applied to the coated plate and incubated at RT overnight with agitation. After six washes in wash buffer, 100 μL of 500 ng/mL biotin-labeled mAb 11D5 and 100 μL of SA-HRP at 1 ng/mL were applied sequentially for detection, with three washes after each addition. Peroxidase substrate TMB was added for color development.

Human IL-17FF Assay

The total concentration of IL-17FF in human serum samples was determined by using a qualified ELISA. IL-17FF-specific mAb 15B11 was coated on 96-well microtiter plates at 0.5 μg/mL in carbonate buffer for capture and incubated at RT for 5 h with agitation. An IL-17FF standard curve, ranging from 1.56 to 200 pg/mL (in-well concentrations) at 1:2 serial dilutions, was prepared in the standard diluent. Serum samples and controls were prepared at 1:2 dilutions in the sample diluent. One hundred microliters of each sample, the standards, and quality controls prepared in diluent containing 20 μg/mL (equivalent to 10 μg/mL in-well after 1:2 dilution) MCAF5352A were applied to the coated plate and incubated at RT overnight with agitation. After six washes in wash buffer, 100 μL of 500 ng/mL biotin-labeled mAb 42G11 and 100 μL of SA-HRP at 1 ng/mL were applied sequentially for detection with three washes after each addition, incubating at RT for 1 h with agitation at each step. TMB substrate was added for color development.

Human IL-17AA and IL-17FF Assay Optimization

The specific binding of mouse mAb 11D5 to human IL-17AA was verified in an ELISA experiment (Fig. 2a). IL-17AA or IL-17FF at 0.1 μg/mL in PBS was coated on a 96-well microtiter plate with IL-17AA on the right half and IL-17FF on the left half of the plate and incubated overnight at 4 °C. Following overnight incubation, preparations of mAbs 11D5 at 50, 20, 5, and 0 ng/mL in assay diluent were added to the plate coated with IL-17AA or IL-17FF and incubated at RT for an hour with agitation. After wash three times, HRP-conjugated anti-mouse IgG at 50 ng/mL was applied for detection and incubated at RT for an hour. TMB substrate was applied for color development. Another similar experiment verified the specific binding of mouse mAbs 15B11 and 42G11 to human IL-17FF (Fig. 2b).

IL-17AA assay specificity was verified by testing assay performance in the presence and absence of IL-17FF and IL-17AF (Fig. 2c). The experiment was performed following human IL-17AA assay procedures described above. IL-17AA samples were first prepared at same levels as the assay standard curve, ranging from 1.56 to 200 pg/mL (in-well concentrations). Then, each IL-17AA sample was prepared with and without spiking in 1000 pg/mL IL-17FF or IL-17AF. All samples were tested side-by-side to verify if the IL-17AA measurements were similar with IL-17FF and IL-17AF spiked-in. Another similar experiment was performed to confirm the IL-17FF assay specificity (Fig. 2d).

Drug interference to the assay was evaluated by preparing IL-17AA assay standard curve in the assay diluent or in the assay diluent plus 1, 10, or 100 μg/mL of MCAF5352A (Fig. 2e). A similar experiment was performed for the IL-17FF assay (data not shown).

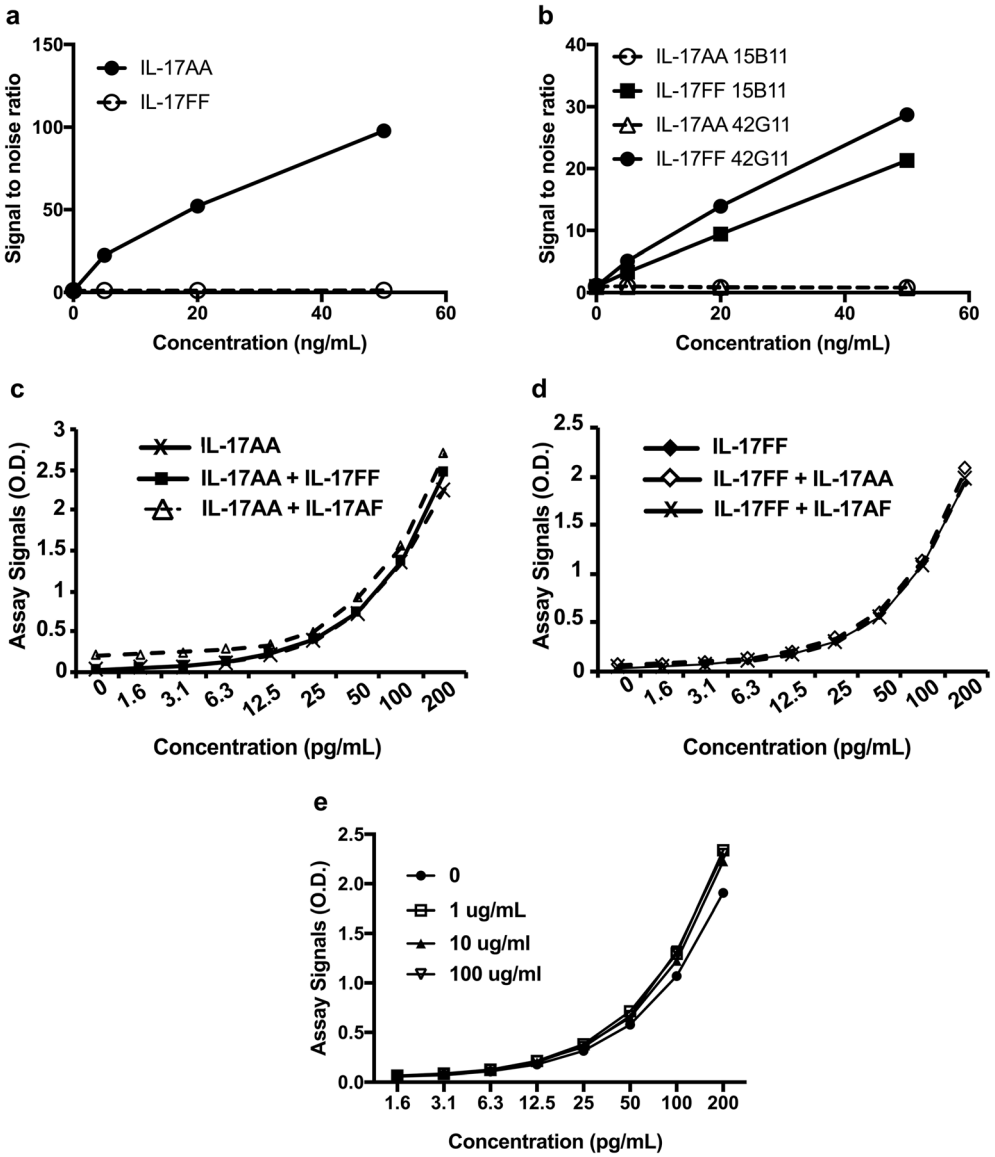

Fig. 2. Characterization of antibodies for the development of IL-17AA- and IL-17FF-specific ELISAs. **a** mAb 11D5 selectively binds to IL-17AA. **b** mAbs 15B11 and 42G11 selectively bind to IL-17FF. **c** The IL-17AA assay specifically detects IL-17AA and does not recognize IL-17FF and IL-17AF. **d** The IL-17FF assay specifically detects IL-17FF and does not recognize IL-17AA and IL-17AF. **e** Presence of MCAF5352A led to a left shift of the IL-17AA standard curve

Human PK Assay

Serum levels of MCAF5352A were determined using a validated ELISA, which was designed to measure free or partially bound drug. In the assay, microtiter plates were coated with 100 μL of 0.5 μg/mL rhuIL-17AA in PBS for capture. An MCAF5352A standard curve, ranging from 0.938 to 60 ng/mL (in-well concentrations) at 1:2 serial dilutions, was prepared in the standard diluent (1% serum in assay diluent). One hundred microliters of the serum samples and quality controls, prepared at a minimum required dilution of 1:100 in the assay diluent, were added to the coated plate and incubated for 2 h at RT with agitation. After three washes in wash buffer, MCAF5352A CDR-specific mouse mAb 1B9 and HRP-conjugated anti-mouse IgG (γ) were applied sequentially for detection, with three washes after each addition. TMB was added for color development.

PK Assay Validation and Biomarker Assay Qualifications

PK assay validation and acceptance criteria followed Food and Drug Administration guidance (21). Key assay parameters, such as precision and accuracy, lower limit of quantification (LLOQ), minimum required dilution, hook effect, spike recovery, selectivity, assay repeatability, and robustness, were evaluated during PK assay validation. Acceptance criteria are within ± 20% between the observed mean concentration and the nominal for most of the experiments listed above, except using coefficient of variation (CV) ≤ 20% for inter- and intra-assay precision.

Similar key assay parameters, such as precision and accuracy, LLOQ, minimum required dilution, hook effect, spike recovery, and selectivity, were evaluated in IL-17AA and IL-17FF assay qualifications. Assay repeatability and robustness were evaluated, however not as extensive as in the PK assay validation. For example, long-term stability was not evaluated in the qualifications. Parallelism was not performed due to low levels of endogenous analytes. Acceptance criteria are within ± 30% between the observed mean concentration and the nominal for most of the experiments listed above, except using CV ≤ 20% for inter- and intra-assay precision.

PK assay validation, biomarker assay qualifications, as well as PK and PD sample analysis were conducted at Genentech.

Ethics

The MCAF5352A Phase I study was conducted in accordance with the European Union Clinical Trial Directive 2001/20/EC, the International Council on Harmonisation E6 Guideline for Good Clinical Practice, the Declaration of Helsinki (October 1996), and applicable local, state, and federal laws, as well as other applicable country laws (including the United States Food and Drug Administration regulations). All patients provided written, informed consent. The protocol, informed consent forms, and any relevant supporting information were reviewed and approved by an institutional review board.

RESULTS

Development of Assays for Specific Measurement of IL-17AA and IL-17FF

As IL-17AA and IL-17FF share approximately 50% sequence homology, it was critical to have antibodies specific to IL-17A or IL-17F in order to develop assays that could distinctly recognize IL-17AA or IL-17FF. Both commercially available and in-house generated antibodies were evaluated to identify antibodies specific to IL-17AA or IL-17FF. Three in-house mAbs (one for IL-17AA and two for IL-17FF) were determined suitable and were used for subsequent development of the IL-17AA and IL-17FF assays. Using microtiter plates coated with rhuIL-17AA or rhuIL-17FF proteins, mAb 11D5 was shown to selectively bind to IL-17AA (Fig. 2a); mAbs 15B11 and 42G11 were shown to selectively bind to IL-17FF (Fig. 2b). In addition to specifically recognizing IL-17AA or IL-17FF, it was important to show that both assays did not have cross-reactivity to the heterodimer IL-17AF. One effective strategy would be to use the same mAb for both capture and detection so that only the intended homodimer could be detected. This strategy was implemented in the development of the IL-17AA assay, using the IL-17AA-specific mAb 11D5 for capture and biotin-labeled 11D5 for detection. The assay proved to be specific for IL-17AA detection and did not cross-react with IL-17FF and IL-17AF (Fig. 2c). However, the strategy of using the same mAb for capture and detection did not work for the IL-17FF assay development. In this case, an IL-17F-specific mAb 15B11 was used for capture and biotin-labeled 42G11, another IL-17F specific mAb, was used for detection. The reason neither of the two antibodies could be paired with their own for both capture and detection could be that the binding epitopes of both 15B11 and 42G11 were proximate to the junction site of the FF dimers, which prevented the same mAb from accessing the site for both capture and detection. Although the IL-17FF assay was developed using two antibodies, the assay preserved specificity to IL-17FF and did not cross-react with either IL-17AA or with IL-17AF (Fig. 2d).

Both assays were developed to measure samples collected at baseline and post-dose. Therefore, drug interference in the assay was also evaluated during development work. Assay conditions were evaluated at 0 and up to 100 μg/mL of MCAF5352A (in-well concentration), which covered the expected maximum circulating drug levels in the Phase I studies. The presence of MCAF5352A led to a left shift of the standard curve; however, presence of drug at the three testing levels (1, 10, and 100 μg/mL) had similar impact on IL-17AA measurement in the assay (Fig. 2e). Similar results of drug interference were observed in the IL-17FF assay (data not shown). Since study samples contained various levels of the drug, a fixed level of MCAF5352A was incorporated in the assay diluent to overcome the drug effect in the assay. Because the assay performance was similar at three testing drug levels, 10 μg/mL MCAF5352A was chosen due to operational convenience. The addition of MCAF5352A led to the formation of drug complexed IL-17AA and IL-17FF. Therefore, both assays were designed to measure total levels of IL-17AA and IL-17FF.

Human IL-17AA and IL-17FF Assays

The IL-17AA-specific assay had a standard curve ranging from 1.56 to 200 pg/mL (in-well concentrations) at 1:2 serial dilutions. The LLOQ of the assay was 8 pg/mL in neat serum. Samples with concentrations below the level of detection of the assay were plotted as half of the LLOQ. The accuracy of the assay was assessed using controls prepared by spiking five levels of rhuIL-17AA in standard diluent and found to be acceptable, with percent difference ranging from 3 to 7%. The CV for the intra-assay precision ranged from 2 to 5%, and the CV for inter-assay precision ranged from 6 to 11%.

The final IL-17FF-specific assay had a standard curve ranging from 1.56 to 200 pg/mL (in-well concentrations) at 1:2 serial dilutions. The LLOQ of the assay was 6 pg/mL in neat serum. Samples with very low concentrations, below the level of detection of the assay, were plotted as half of LLOQ. The accuracy of the assay was assessed using controls prepared by spiking five levels of rhuIL-17FF in standard diluent and found to be acceptable with percent difference ranging from 6 to 15%. The CV for intra-assay precision ranged from 1 to 3%, and the CV for inter-assay precision ranged from 3 to 5%.

Human PK Assay

The validated MCAF5352A PK assay standard curve ranged from 0.938 to 60 ng/mL (in-well concentrations) at 1:2 serial dilutions. The LLOQ of the assay was 200 ng/mL. Accuracy of the assay was assessed using controls prepared by spiking MCAF5352A in pooled normal human serum at five levels, representing the low, mid, and high portions of the standard curve. Accuracy of the assay was found to be acceptable with percent difference ranging from 2 to 11%. The CV for the intra-assay precision ranged from 2 to 6%, and the CV for the inter-assay precision ranged from 2 to 6%. Up to 2 ng/mL of IL-17AA, IL-17AF, and IL-17FF were tested and no interference to the assay was observed.

Phase I Study of MCAF5352A in Healthy Volunteers

Single and multiple doses of MCAF5352A were evaluated in two completed Phase I clinical trials in healthy volunteers: (i) Phase Ia study with 18 subjects who received a single dose of MCAF5352A of up to 450 mg via subcutaneous (SC) administration and (ii) Phase Ib study with 19 subjects who were exposed to biweekly (Q2W) SC doses of MCAF5352A, given three times, of up to 300 mg.

The Phase Ia single ascending dose (SAD) study (NCT01480310) was a randomized, double-blinded, placebo-controlled study in male and female healthy volunteers, aged 18–55 years, enrolled at a single study site in Canada. This study tested multiple dose levels of 5, 15, 50, 150, and 450 mg concentration with approximately 4:1 MCAF5352A/placebo randomization ratio (except for the lowest dose of 5 mg, in which the allocation was 2:1 MCAF5352A:placebo). A total of 23 subjects were enrolled, of which 18 subjects received active treatment with MCAF5352A (Table I).

The Phase Ib, multiple ascending dose (MAD), study (NCT01540760) was a randomized, double-blinded, placebo-controlled study in male and female healthy volunteers, within the age range of 18–55 years, enrolled at the same clinical site as SAD study in Canada. Each subject was randomly assigned to receive SC doses of MCAF5352A or matching placebo (treatment allocation is 6:1, MCAF5352A: placebo) Q2W for a total of three doses with dose levels of 30, 100, and 300 mg. A total of 23 subjects were enrolled and of which 19 subjects received active treatment with MCAF5352A (Table II).

Table I. Phase Ia single ascending dose study in healthy volunteers

Cohort	Dose (mg)	Route	Number of subjects	
			MCAF5352A	Placebo
A	5	SC	2	1
B	15	SC	4	1
C	50	SC	4	1
D	150	SC	4	1
E	450	SC	4	1

SC subcutaneous

Pharmacokinetics

After a single SC administration, the maximal concentration (C_{max}) and area under the concentration-time curve from time 0 to infinity (AUC_{inf}) were approximately dose proportional within the dose range tested from 15 to 450 mg, and the time to achieve C_{max} (T_{max}) was around 3 to 5.5 days. The terminal half-life was estimated to be 16 to 25 days. Following three SC Q2W doses of MCAF5352A, there was a moderate accumulation of MCAF5352A in the circulation with the accumulation ratio averaging from 1.84- to 2.57-fold based on non-compartmental analysis. Immunogenicity was assessed using a validated bridging ELISA and similar assay format described previously (22). Antibodies to MCAF5352A were detected in 2 of the 18 MCAF5352A-treated subjects in the Phase Ia SAD study, including one subject in the Cohort D (150 mg, PK profile of open circle symbol in Fig. 3h) and one subject in the Cohort E (450 mg, PK profile of open diamond symbol in Fig. 3i). Antibodies to MCAF5352A were detected in 2 of the 19 MCAF5352A-treated subjects in the Phase Ib MAD study, and both subjects were from Cohort G (100 mg, PK profiles of open triangle and open circle symbols in Fig. 3i). The overall ADA incidence in MCAF5352A-treated subjects was 10.8% (4 of 37 subjects) in the Phase I

Table II. Phase Ib multiple ascending dose study in healthy volunteers

Cohort	Dose (mg)/regimen	Route	Number of subjects	
			MCAF5352A	Placebo
F	30/Q2W	SC	6	2
G	100/Q2W	SC	7	1
H	300/Q2W	SC	6	1

Q2W every 2 weeks, *SC* subcutaneous

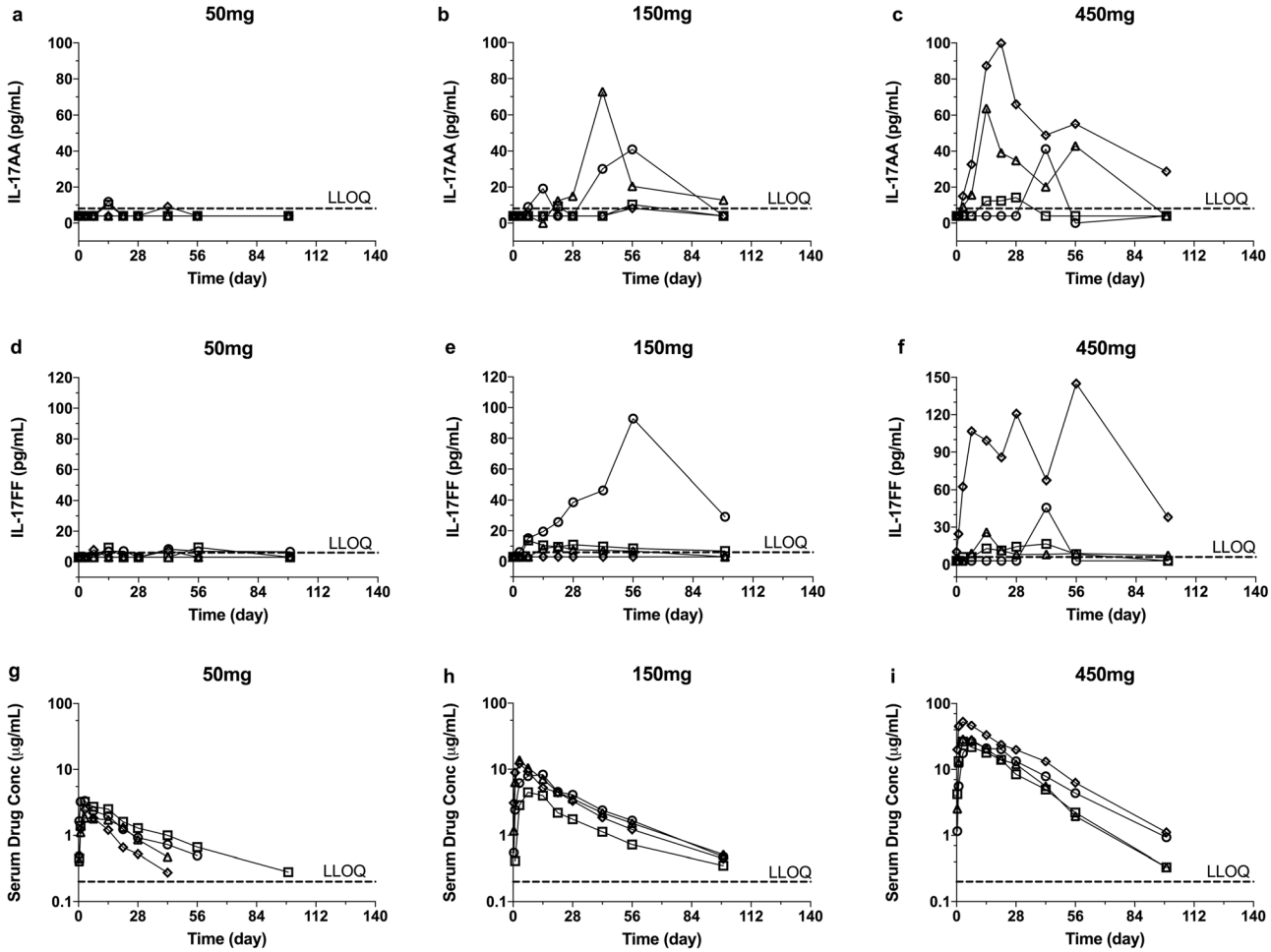

Fig. 3. Total serum IL-17AA, IL-17FF, and PK concentrations for each subject in cohorts C, D, and E following a single dose of MCAF5352A. Serum levels of IL-17AA (**a**–**c**), IL-17FF (**d**–**f**), and drug (**g**–**i**) in the single ascending dose study (cohorts C–E) after a single dose of 50 mg (**a**, **d**, **g**), 150 mg (**b**, **e**, **h**), and 450 mg (**c**, **f**, **i**). In each panel, individual patient was plotted separately (represented by a unique symbol). Delayed increases in total serum levels of IL-17AA and IL-17FF were observed after PK increase with a dose-dependent effect. The dotted line reflects the lower limit of quantification of IL-17AA and IL-17FF assays

studies. ADA signals were low, only slightly above the assay detection threshold. The presence of ADAs did not seem to impact the serum exposures of MCAF5352A significantly and it was not associated with any safety findings. There was low variability in the PK and no covariates were identified to impact PK variability.

Elevation of Total Serum IL-17AA and IL-17FF Post-Dose

The levels of total IL-17AA and IL-17FF in serum were measured as PD biomarkers in serum samples taken at baseline and several post-dose time points. Levels of the two analytes were below the assay detection limit prior to administration of MCAF5352A and in Phase Ia cohorts A, B post-dose, but increased after dosing in both the single dose cohorts C, D, and E as well as the multiple dose cohorts in a dose-dependent manner (Figs. 3 and 4). The observed increase in total IL-17AA and IL-17FF was likely due to the increase in half-life of IL-17- MCAF5352A complex, which has been observed with other therapeutics as well (23,24). The detection of serum IL-17 levels at post-dose suggested target engagement. With the high affinity of MCAF5352A for IL-17AA and IL-17FF as well as the vast molar excess of MCAF5352A relative to IL-17AA and IL-17 FF, it is reasonable to assume that virtually all IL-17AA and IL-17FF detected was bound in a complex with MCAF5352A and, therefore, inactive.

DISCUSSION

In clinical development, PD biomarkers can be helpful tools to provide information on optimal dosing, target engagement, and proof of mechanism. For many molecules in development for autoimmune diseases, the first in human studies are usually run in healthy volunteers, and thus, it can be a challenge to find appropriate PD biomarkers relevant to the pathway being targeted. Frequently, the targets of interest are upregulated in the disease state but not in healthy subjects where pathway biomarkers are only present in very low levels. Additionally, invasive sampling is typically not feasible in these healthy volunteer studies, and only circulating samples (such as blood, serum, and plasma) are available for biomarker analysis. These samples are distal from the site of action (where target is expressed) and levels of biomarkers

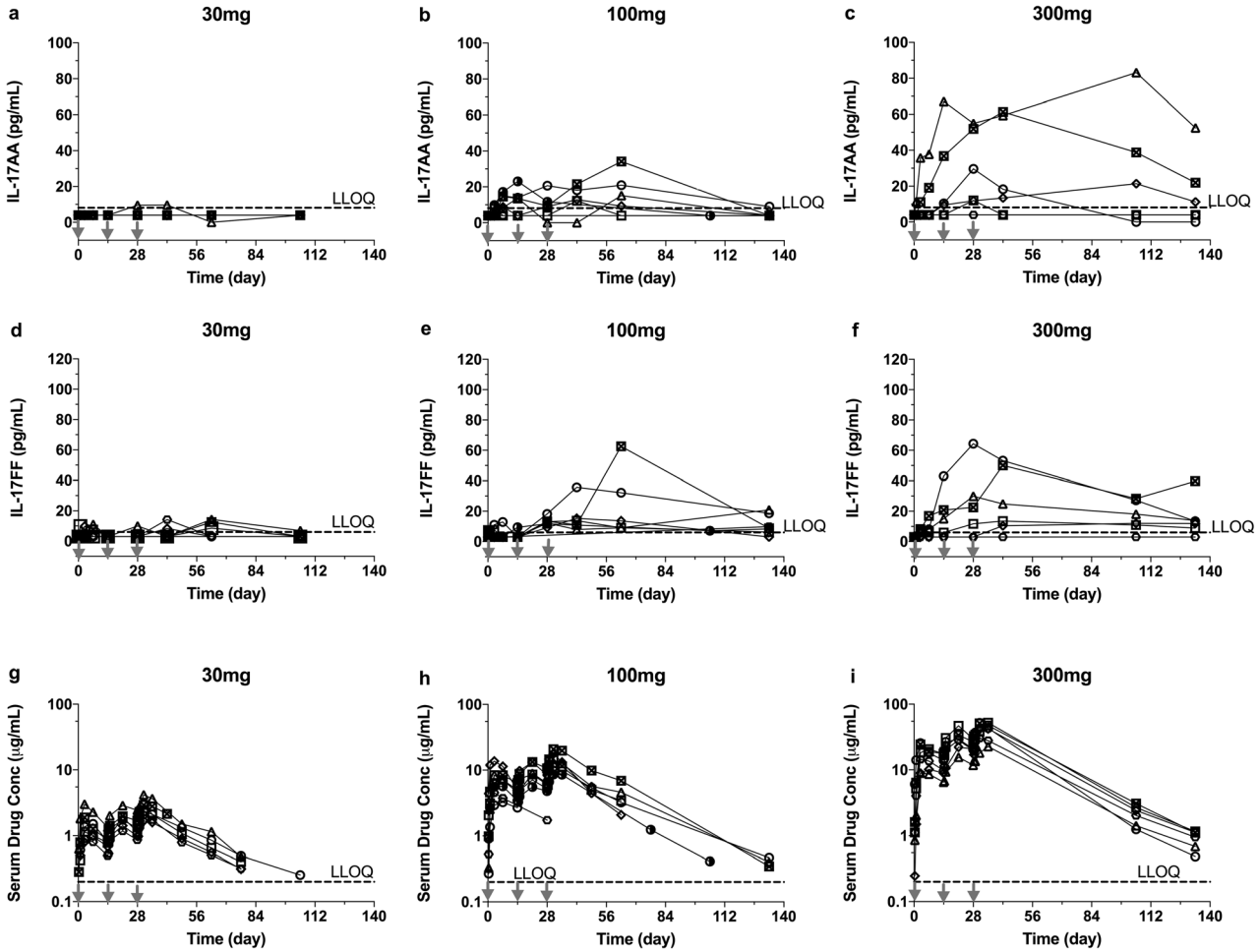

Fig. 4. Total serum IL-17AA and IL-17FF and PK concentrations for each subject following multiple doses of MCAF5352A. Serum levels of IL-17AA (**a**–**c**), IL-17FF (**d**–**f**), and drug (**g**–**i**) in the multiple ascending dose study (cohorts F–H) after three doses of 30 mg (**a**, **d**, **g**), 100 mg (**b**, **e**, **h**), and 300 mg (**c**, **f**, **i**) on days 0, 14, and 28. In each panel, individual patient was plotted separately (represented by a unique symbol). Delayed increases in total serum levels of IL-17AA and IL-17FF were observed after PK increase with a dose-dependent effect. The dotted line reflects the lower limit of quantification of IL-17AA and IL-17FF assays. The downward arrows on the *x*-axis of panels **g**–**i** indicate the time points of MCAF5352A administrations

are typically low in these samples. In other words, low levels of circulating biomarker in healthy individuals require assays of high sensitivity and specificity for accurate measurements.

MCAF5352A is capable of binding as well as blocking both IL-17AA and IL-17FF. Therefore, 17AA and IL-17FF were proposed as PD biomarkers to demonstrate target engagement in our clinical studies. Here, we have developed IL-17AA and IL-17FF assays using antibodies developed in-house that are specific to IL-17AA or IL-17FF. Both IL-17AA and IL-17FF assays demonstrated specificity to each of the targeted analyte, despite the 50% sequence homology between IL-17A and IL-17F. The presence of MCAF5352A led to a small shift of the calibrator curve, which can lead to an assay discrepancy when measuring baseline and post-dose samples. Therefore, a fixed level of drug was incorporated into the assay diluent to eliminate drug interference in the assay. Both assays were developed before technologies (such as Quanterix and Singulex) capable of achieving sub-picogram per milliliter sensitivity were available. The ELISA assays achieved low picogram per milliliter level sensitivity, which is mainly credited to using analyte-specific capture and detection antibodies. Additionally, we extended the sample incubation step to overnight and kept minimum dilution of sample at 1:2. Both conditions helped to improve assay sensitivity. Although the two ELISAs were not adequate to detect IL-17AA or IL-17FF at baseline levels (20,25), they were sufficient to detect robust PD signals after treatment in higher dose cohorts and served the purpose to demonstrate target engagement.

A delayed PD response was observed after the PK C_{max} was reached. Post-dose serum IL-17AA and IL-17FF levels became detectable starting from cohort C. This observed post-dose elevation in serum IL-17AA and IL-17FF levels is thought to be mainly due to the formation of IL-17: MCAF5352A complexes and not a reflection of any increase in active IL-17. At low doses (less than 100 mg), there was no or minimal detectable IL-17 levels even after dosing, which reflects the extreme low baseline IL-17 levels in healthy volunteers. At higher doses (above 100 mg), a more pronounced increase in IL-17 levels was detected, suggesting that there could be an equilibrium shift where additional IL-17 was pulled to the systemic circulation from the tissue. In addition, both MCAF5352A and IL-17: MCAF5352A complexes could potentially influx into tissues

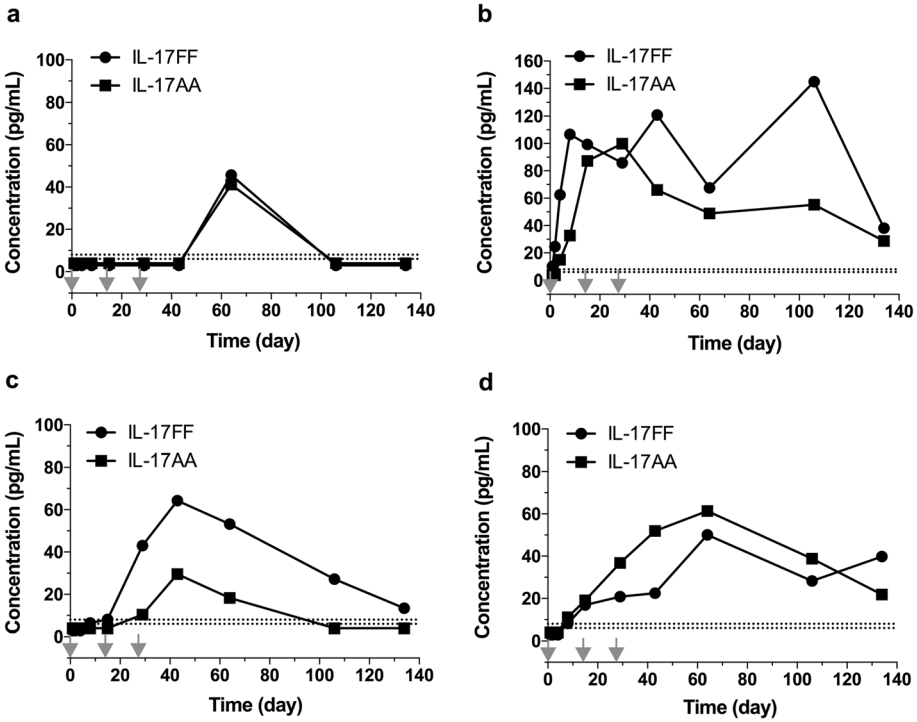

Fig. 5. Total serum IL-17AA and IL-17FF concentrations in selected subjects from cohorts E and H. Similar pattern for increases in serum levels of IL-17AA and IL-17FF levels. Representative plots shown for two subjects in cohort E (**a**, **b**) and two subjects in cohort H (**c**, **d**). The dotted line reflects the lower limit of quantification of IL-17AA and IL-17FF assays. The downward arrows on the *x*-axis indicate the time points of MCAF5352A administrations

and establish equilibrium. However, the response did not seem to plateau in highest dose cohorts (300 mg single dose and 450 mg multiple dose), suggesting that equilibrium has not been reached.

Furthermore, the relationship between MCAF5352A PK and PD response (IL-17 levels) was explored. In general, a dose-dependent PD response was observed. However, both the time to maximal PD response and the magnitude of the PD response varied dramatically within each cohort despite having similar drug exposure among individuals. Such large variability could not be attributed to PK variability. Individual physiological factors could contribute greatly to the PD variabilities, such as difference in tissue IL-17 levels, rate of drug influx into tissues, kinetics of drug-target binding, IL-17 turnover rate, as well as baseline IL-17 levels in circulation across individuals. Although we observed variability in magnitude of peak post-dose serum IL-17AA and IL-17FF levels across individuals in the same cohort, we found that there was a similar pattern in the timing and magnitude of increase within same individuals for both biomarkers (overlay of selected subjects from high dose cohorts E and H are shown in Fig. 5). Longitudinal PK/PD model was not developed due to large inter-subject variability and relatively small sample size. Considering the good assay precision and accuracy during sample analysis, inter-subject variability in PD response is unlikely due to assay variation rather a result of potential impacts by individual intrinsic physiological factors listed above.

Our data suggest that MCAF5352A could bind to IL-17AA and IL-17FF in vivo, consistent with our therapeutic hypothesis. Biacore data demonstrated that MCAF5352A bound to IL-17AF with similar affinity as those binding to IL-17AA and IL-17FF (data not shown). IL-17AF levels were not measured in this study; however, the observation that MCAF5352A can bind to IL-17AA and IL-17FF in vivo suggests that MCAF5352A is capable of also binding to IL-17AF dimer in vivo.

The dual ability of MCAF5352A to bind as well as block both IL-17AA and IL-17FF may make it a more effective therapeutic in reducing the inflammatory effects of the IL-17 pathway than blocking IL-17AA or IL-17FF alone. The PK profile of MCAF5352A and two PD biomarkers, IL-17AA and IL-17FF, have been characterized in Phase I studies. Healthy volunteers are normally evaluated in Phase I clinical studies for autoimmune diseases; therefore, pathway and PD biomarkers are often not tested until Phase II studies and beyond. Here we shared our experiences of using two PD biomarkers to demonstrate target engagement during early clinical development. Two ELISAs with desirable specificity and adequate sensitivities were developed for the measurement of total IL-17AA and IL-17FF levels. Post-treatment levels of total serum IL-17AA and IL-17FF increased in a dose-dependent manner, in both the single and multiple dose cohorts. PD data from the Phase I study further helped to confirm the planned Phase II target concentration.

CONCLUSION

To summarize, we successfully measured robust PD responses and demonstrated target engagement during early clinical development in healthy subjects. The PK and PD results from Phase I study were used to inform Phase II study planning.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest All authors are Genentech employees and Roche shareholders.

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