SUPPLEMENTAL MATERIAL

SUPPLEMENTAL METHODS

Pharmacokinetic (PK) and Immunogenicity (ADA) Assessments

PK Assay

For PK analyses, blood samples for serum MOXR0916 concentration were collected from all patients treated with MOXR0916 at the following time points: predose, end of infusion, day 2, day 4 or 5, day 8, and day 15 of cycle 1; predose and end of infusion cycles 2-7. A validated antigen bridging ELISA was used to quantify MOXR0916 in human serum. Diluted serum samples were incubated at room temperature overnight with two reagents, OX40 conjugated with biotin (OX40-BIO) and OX40 conjugated with digoxigenin (OX40-DIG), to capture the MOXR0916 drug present in patient samples. The sample/conjugate mixtures containing complexes with MOXR0916 bridging OX40-BIO and OX40-DIG were transferred to a prewashed 96-well streptavidin-coated microtiter plates (Roche, Indianapolis, IN) and incubated for 2 hours at room temperature to capture the bridge complexes by their biotin label. Plates were washed 6 times with phosphate buffered saline buffer (PBS) with 0.05% Tween 20. Subsequently, mouse monoclonal horseradish peroxidase- (HRP)-conjugated anti-DIG antibody (Jackson ImmunoResearch, West Grove, PA) was added and incubated for 1 hour at room temperature for detection. Plates were washed four times with PBS buffer with 0.05% Tween 20. A peroxidase substrate (tetramethyl benzidine) (Kirkegaard and Perry, Gaithersburg, MD) for the HRP enzyme was added for color development, and the reaction was subsequently stopped by adding 1 M phosphoric acid. Absorbance was read

using a plate reader using 450 nm read and 630 nm reference wavelengths. The lower limit of quantitation of this assay was 0.080 g/mL MOXR0916. PK parameters for MOXR0916 were determined using non-compartmental approach based on concentration-time profile in cycle 1.

Immunogenicity Assay

A validated antibody bridging ELISA was used to detect antibodies to MOXR0916 (anti-drug antibody [ADA]) in human serum. The assay used two conjugated reagents: biotin conjugated to drug (MOXR0916-BIO) and digoxigenin conjugated to drug (MOXR0916-DIG) to capture ADA in patient serum samples directed against MOXR0916. The two conjugated reagents were co-incubated overnight with diluted serum samples and controls in 96-well polypropylene plates to generate complexes with ADA bridging MOXR0916-BIO and MOXR0916-DIG molecules. The samples were then transferred to a prewashed streptavidin-coated 96-well plate and incubated at room temperature for 2 hours to capture complexes by their biotin label. Next, plates were washed three times with PBS with 0.05% Tween 20. Subsequently, mouse anti-digoxin-horseradish peroxidase (HRP) antibody (Jackson ImmunoResearch, West Grove, PA) was added and incubated for detection of complexes by their digoxin label. A peroxidase substrate (tetramethyl benzidine) (Kirkegaard and Perry, Gaithersburg, MD) for the HRP enzyme was added for color development, and the reaction was subsequently stopped by adding 1 molar phosphoric acid. The plates were read at 450 nm for detection absorbance and 620 or 630 nm for reference absorbance. Antibody titer values were determined by a log titer reduction program.