4.4 Bioanalytical Validation and Antibody Detection Methods Consult Review (CMC)

Review Memo

Ref:	**125289**
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To:	Lei Zhang, MD, Senior Staff Fellow, OTS/OCP/DCP2
Through:	David Frucht, M.D., Acting Chief, Laboratory of Cell Biology, DMA 2/23/09 Kathleen Clouse, Ph.D., Director, DMA 02/23/09
RPM:	Sharon Turner-Rinehardt, DAARP
Sponsor:	Centocor
Product:	Golimumab (CNTO148, SIMPONI)
Date of submission:	**6/24/08**
Date of Review:	**1/14/09**

Summary

CNTO 148 is a human $IgG_1\kappa$ monoclonal antibody specific for human tumor necrosis factor alpha (TNFα), thereby neutralizing the biological activity of TNFα. The BLA, submitted June 2008, contains extensive characterization of golimumab clinical pharmacology, *in vivo* biomarker activity and immunogenicity information. This is a consult review of the validation of analytical and immunogenicity assays used in the clinical pharmacology studies (section 5.3.1.4 of the BLA).

There were three generations of assays to measure CNTO 148 serum levels in PK studies. They were cross-validated when they were introduced into the development program:

- An Electrochemiluminescence-based Immunoassay (EIA) for the quantification of CNTO 148 in human serum. This assay is a sandwich immunoassay that uses ECL detection.
  - Validation report CP2006V-022, approved 12/2006
  - Test samples (serum) are incubated with an assay mixture consisting of streptavidin-coated magnetic beads, biotinylated anti-variable region antibody to CNTO 148, and a ruthenium-labeled anti-V region antibody to CNTO 148. CNTO 148 in the test sample binds to the biotinylated anti-variable region antibody and is then sandwiched by the ruthenium labeled anti-V region antibody. The bound complex is captured on streptavidin-coated magnetic beads

during a two-hour room temperature incubation. Plates are read on a [redacted] analyzer, which separates the material bound on the magnetic beads from unbound components in the assay mixture. CNTO 148 concentration in test samples is interpolated from a 4-parameter standard curve. b(4)

- The assay was validated for:
  - Accuracy (spike/recovery in normal and RA sera)
  - Linearity. Some high-zone interference is seen at 10,000 and 100,000 ng/ml, but when diluted, adequate recovery is seen.
  - LOQ (200 ng/mL)
  - Precision (intra- and inter-assay and inter-operator). Assay variability is <20%.
  - Ruggedness/robustness (7 day reagent stability, 48 hour plate storage, ± 15 minute incubation time, multiple instruments can be used)
  - Specificity (absence of cross-reaction with other Centocor antibodies)
  - Matrix effects by IR^+ sera and whole blood
  - Test article stability after extended storage and freeze/thaw. Test articles can be stored 24 hours at RT, 36 months at -70°C, 8 weeks at -20°C or freeze/thawed three times with no loss of activity.
  - Comparability to the competition format immunoassay (below).

- A one step competitive electrochemiluminescence (ECL) assay for the quantitative determination of CNTO 148 in human serum.
  - Validation report CP2003V-059, approved 12/2003
  - Test samples (serum) are incubated with an assay mixture consisting of streptavidin-coated magnetic beads, a biotinylated anti-idiotypic antibody to CNTO 148, and ruthenium (Ru)-labeled CNTO 148. CNTO 148 in the test sample competes with the ruthenium labeled CNTO 148 for binding to the biotinylated anti-idiotypic antibody. The bound complex is captured on the streptavidin-coated magnetic beads. A single two hour incubation at room temperature is used. ECL is read on an [redacted] analyzer, which separates the material bound on the magnetic beads from unbound components in the assay mixture. The CNTO 148 concentration in test samples is interpolated from a standard curve. b(4)
  - The assay was validated for the following:
    - Accuracy (spike/recovery in normal and RA sera)
    - Linearity (300 ng/mL to 50,000 ng/mL, which can be extended by dilution)
    - LOQ (300 ng/mL)
    - Precision (intra- and inter-assay and inter-operator). Assay variability is <20%.

        - Ruggedness/robustness (48 hour plate storage before reading)
        - Specificity (absence of cross-reaction with other Centocor antibodies)
        - Test articles can be stored 28 days at 4°C or freeze/thawed three times with no loss of activity.
        - Comparison with the ELISA method below.
- An ELISA for the quantitative determination of CNTO 148 in human serum. This assay was modified from an assay used to test cynomologous monkey serum for CNTO 148. For reasons outlined below, it was less than optimal for this purpose and replaced with the above methods.
    - Validation report CP2002V-015, approved 2/2002
    - Plates are coated with an anti-CNTO 148 mAb overnight and then blocked with BLA. Test samples (serum) are diluted with a 20% goat serum diluent and applied to the plate for one hour. After washing, a biotin-labeled anti-CNTO 148 mAb is incubated on the plate. This is followed by a wash and then incubation with HRP-streptavidin. The plate is then washed and a TMB substrate is allowed to react and the plate is read with a microplate reader. The CNTO 148 concentration in test samples is interpolated from a standard curve.
    - The assay was validated for:
        - Accuracy (spike/recovery in normal sera)
        - LOQ (8 ng/mL)
        - Precision (intra- and inter-assay and inter-operator). Assay variability is <20%.
        - Ruggedness/robustness (48 hour plate storage before reading)
        - Specificity (absence of cross-reaction with other Centocor antibodies).
        - There were considerable non-specific effects from human serum and a hook-effect at high CNTO 148 concentrations. They could be minimized by (1) incorporating 20% goat serum in the test article diluent or (2) starting the test article dilution series at 1:140.
        - Test articles can be freeze/thawed three times with no loss of activity.

- After phase 1 clinical trials, [redacted] and a biochemical equivalency study was performed at the time to demonstrate that product made by the two cell lines was equivalent. CNTO 148 produced by the two cell lines was spiked into normal and RA sera and compared using the above assays (ECL and ELISA); the two spiked products yielded comparable results.

b(4)

- A performance qualification exercise was performed on the [redacted] system used to automate testing of the PK serum samples. The performance qualification verified acceptable test article recovery and precision, as well as absence of carry-over and test article stability during processing. b(4)

There were two immunogenicity assays measuring total anti-product antibody and neutralizing antibodies:

- Anti-CNTO 148 immune response assay. This is a bridging EIA.
  - CNTO 148 is bound to a microtiter plate. Controls and patient serum samples are added to the plate to allow binding of anti-CNTO 148 immune response antibodies. Biotin-labeled CNTO 148 is then added and permitted to bind to immobilized anti- CNTO 148 IR antibodies. This is followed by incubation with horseradish peroxidase conjugated streptavidin (SA-HRP). Detection was visualized through the colorimetric conversion of TMB, a substrate for HRP. The positive control is affinity purified anti-CNTO148 immunized cynomologous monkey serum.
  - Validation consisted of:
    - Determination of cut-off using normal human serum: 0.106 OD units
    - Sample dilutability using Ab^+ patient serum. 1:10 was found to be an acceptable screening concentration.
    - Assay sensitivity was determined using an anti-CNTO148 immunized cynomologous monkey serum and a monoclonal antibody: 1.2 to 4.9 ng/mL. The low positive control was set at 2.5 ng/mL of the monkey antibodies.
    - Serum recovery was 85-90%.
    - Specificity was demonstrated using irrelevant antibody response sera and competition with irrelevant mAbs. Some interference was seen when Ab^+ serum samples also contained CNTO 148.
    - Sample stability at -70 °C (47 months), -20 °C (62 days), and room temperature (30 hours).
    - Precision (intra and inter-assay).
    - Robustness of incubation times (±5 minutes) and reagent lots.
    - Ruggedness (plate washers and incubators).
- Anti-CNTO 148 neutralizing antibody bioassay. This is a semi-quantitative cell-based neutralization assay.
  - TNF is neutralized by the drug (CNTO 148), which in turn can be neutralized by anti-CNTO 148 in a 96-well based bioassay. At a fixed concentration of TNF and CNTO 148, the presence of antibodies neutralizing CNTO 148 is detected by the killing of TNF-sensitive WEHI-C527 cells by anti-CNTO 148-antibody present in

patient serum test articles (diluted 1:20; TNF would benon-neutralized due to CNTO 148 neutralization) The viability and functionality of the cells is quantified by [redacted] . reader in relative luminescence unit (RLU) values, which are transformed to percent inhibition of the bioactivity of the CNTO 148 drug. The positive controls are two individual mouse anti-CNTO 148 (CNTO2064 and CNTO8370) monoclonal antibodies. b(4)

- Validation consisted of:
  - System suitability criteria for controls, consistency controls (CNTO2064 and CNTO8370) and normal serum cut-off values.
  - Specificity-unrelated antibodies are nonreactive in assay.
  - Linearity using CNTO2064 and CNTO8370
  - LOD- 150 ng/ml
  - Precision- intra- and inter-assay.
  - Robustness to 1 hour variations in incubation times.

There were numerous assays for different biomarkers:

- Collagen Type II Cleavage (C2C) ELISA
  - The assay is performed at [redacted] using an [redacted] commercial assay. b(4)
  - A synthetic C2C neoepitope: protein conjugate is pre-coated onto an ELISA plate. A specific anti-C2C neoepitope mouse IgG antibody is added and either binds to the C2C coating conjugate on the plate, the C2C standards, or the endogenous neoepitope in samples. After washing, goat anti-mouse horseradish peroxidase (GAM-HRP) conjugate is added as a secondary reagent. After a wash, the colorimetric substrate Tetra-methylbenzidine (TMB) is added and then GAM-HRP, if still on the plate, forms a blue product. The reaction is stopped and signal amplified with an acid, which converts the product from a blue to a yellow color that can be quantified at 450 nm.
  - The assay was validated for precision, linearity, range, recovery, and specimen stability. The validation met acceptance criteria
- P1NP (amino terminal propeptide of collagen type I) RIA
  - The assay is performed at [redacted] using an [redacted] commercial assay. It was cleared by CDRH under 510k, k043125. b(4)
  - A known amount of labeled P1NP and an unknown amount of unlabelled P1NP in the sample compete for the limited number of high affinity binding sites of anti-P1NP antibody in a tube. After separating the free antigen, the amount of labeled PINP in the sample tube is inversely proportional to the amount of PINP in the sample.

- The assay was validated for precision, linearity, recovery, sensitivity and equivalency to a kit available from [redacted] The validation met acceptance criteria

- N-MID osteocalcin ELISA
  - The assay is performed at [redacted], using a [redacted] commercial assay.
  - The assay was validated for precision, linearity, recovery, sensitivity and equivalency to a kit available from [redacted] The validation met acceptance criteria

b(4)

- Human S100 A12/EN-RAGE Enzyme Immunoassay
  - The assay is performed at [redacted] using a [redacted] commercial assay.
  - A microplate is pre-coated with a S100A12/EN-RAGE mAb. After wash and block, standards and samples are pipetted into the wells and the immobilized antibody binds any S100A12/EN-RAGE present. After washing, an HRP conjugated polyclonal antibody specific for S100A12/EN-RAGE is added to the wells. Following a wash, the colorimetric substrate H_20_2-tetramethylbenzidine is added and measured at 450 nm.
  - The assay was validated for precision, linearity, recovery, sensitivity and test article stability. The validation met acceptance criteria

- Hyaluronic Acid (HA) ELISA
  - The assay is performed at [redacted] using a [redacted] commercial assay.
  - A microplate is pre-coated with a naturally occurring hyaluronic acid binding protein (HABP) from bovine cartilage. After wash and block, properly diluted serum and HA reference solutions are incubated in HABP-coated microwells. After washing, HABP conjugated with horseradish peroxidase (HRP) as a secondary reagent. Following another washing step, a chromogenic substrate, TMB and H_2O_2, is added and measured at 450 nm.
  - The assay was validated for precision, linearity, recovery, sensitivity and test article stability. The validation met acceptance criteria

b(4)

- Serum Deoxypyridinoline (DPD) stripwell enzyme immunoassay
  - The assay is performed at [redacted] using an [redacted] commercial assay. DPD is a collagen breakdown product found in circulation and urine, and elevated in RA patients.
  - DPD in the samples or standards competes with alkaline phosphatase conjugated DPD for binding to monoclonal anti-DPD antibody coated on the strip. The reaction is detected with pNPP substrate.
  - The assay was validated for precision, linearity, recovery, and sensitivity. The validation met acceptance criteria

b(4)

- Serum Pyridinoline (sPYD) EIA

- The assay is performed at [redacted] using a [redacted] commercial assay. PYD is a collagen breakdown product found in circulation and urine, and elevated in RA patients. b(4)
- PYD in the samples or standards competes with PYD immobilized on the plate for a polyclonal rabbit anti-PYD antibody. Bound antibody is detected by goat anti-rabbit antibody conjugated to alkaline phosphatase, and the reaction is detected with p-Nitrophenyl phosphate substrate. Absorbance is read at 405 nm.
- The assay was validated for precision, linearity, recovery, and sensitivity. The validation met acceptance criteria.

- Bone-specific Alkaline Phosphatase (BAP) EIA
  - The assay is performed at [redacted] using a [redacted] commercial assay. BAP is a marker of bone turnover. b(4)
  - The [redacted] BAP is an immunoassay in a microtiter strip format utilizing a monoclonal anti-BAP antibody coated on the strip to capture BAP in the sample. The enzyme activity of the captured BAP is detected with a pNPP substrate.
  - The assay was validated for precision, linearity, recovery, sensitivity, and equivalency to IEF methods. The validation met acceptance criteria.

Reports for several assays (VEGF, IL-6, MMP-3, ICAM-1) were in the form of abbreviated tables with minimal description of the actual assay. **Note:** *Claims regarding these biomarkers* [redacted] *and the activity of these biomarkers is not critical for understanding the mechanism of action of CNTO 148. Thus, it is not critical for Centocor to submit validation reports for these assays.* b(4)

Recommendation. The pharmacology, *in vivo* biomarker activity and immunogenicity assays have been adequately validated.

Appears This Way
On Original

4.5 OCP Filing Memo

Office of Clinical Pharmacology *New Drug Application/Biologics License Application Filing and Review Form*			
General Information About the Submission			
	Information		Information
NDA/BLA Number	125289	Brand Name	SIMPONI
OCP Division	DCP2	Generic Name	Golimumab
Medical Division	DAARP	Drug Class	TNFα inhibitor
OCP Reviewers	Lei Zhang, Ph.D. Venkatesh Atul Bhattaram, Ph.D. (PM Secondary Review)	Indication(s)	For the treatment of adult patients (18 years or older) with active rheumatoid arthritis (RA), psoriatic arthritis (PsA), and ankylosing spondylitis (AS).
OCP Team Leaders	Suresh Doddapaneni, Ph.D Yaning Wang, Ph.D. (PM)	Dosage Form	• 50 mg/0.5 mL sterile solution in a single-use autoinjector • 50 mg/0.5 mL sterile solution in a single-use pre-filled syringe
		Dosing Regimen	50 mg once a month
Date of Submission	6/24/2008	Route of Administration	SC injection
Estimated Due Date of OCP Review	2/15/2009	Sponsor	Centocor
PDUFA Due Date	4/24/2009	Priority Classification	1S
Division Due Date	2/24/2009		IND 9,925, 12723 and 12729

Clin. Pharm. and Biopharm. Information

	"X" if included at filing	Number of studies submitted	Number of studies reviewed	Critical Comments if any
STUDY TYPE				
Table of Contents present and sufficient to locate reports, tables, data, etc.	X			
Tabular Listing of All Human Studies	X			
Human PK Summary	X			
Labeling	X			
Reference Bioanalytical and Analytical and Immunogenicity Methods	X	8	8	
I. Clinical Pharmacology				
Mass balance:				
Isozyme characterization:				
Blood/plasma ratio:				
Plasma protein binding:				
Pharmacokinetics (e.g., Phase I) -				
Healthy Volunteers-				
single dose:	X	1	1	C0524T13 (100 mg liquid formulation)-SC
multiple dose:				
Patients-				
single dose:	X	1	1	C0466T01 (0.1 mg/kg – 10 mg/kg)-IV infusion
multiple dose:	X	1	1	C0466T02 (SD + MD) 3 biweekly dose (0.3 and 1 mg/kg)-SC injection
Dose proportionality -				

fasting / non-fasting single dose:	X	(2)	(2)	C0466T01 (0.1 mg/kg – 10 mg/kg) C0466T02 (SD 0.3 mg/kg – 3 mg/kg)
fasting / non-fasting multiple dose:				
Drug-drug interaction studies -				
In-vivo effects on primary drug:				
In-vivo effects of primary drug:				
In-vitro:				
Subpopulation studies -				The effects of intrinsic factors on pharmacokinetics of golimumab were not studied in separate clinical studies but were investigated as part of a population PK analysis using data from 4 of the Phase 3 studies.
ethnicity:	X	1	1	C0524T23 (50 and 100 mg liquid formulation)-Japanese vs. Caucasian single SC
gender:				
pediatrics:				
geriatrics:				
renal impairment:				
hepatic impairment:				
PD:				
Phase 2:	X	1	1	C0524T02 (50 and 100 mg, biweekly or every 4 weeks)
Phase 3:				
PK/PD:				
Phase 1 and/or 2, proof of concept:				
Phase 3 clinical trial:	X	5	4	C0524T05 (RA) C0524T06 (RA) C0524T11 (RA) C0524T08 (PsA) C0524T09 (AS)
Population Analyses -				
Data rich:				
Data sparse:	X	3	3	C0524T05 (RA) C0524T06 (RA) C0524T08 (PsA) C0524T09 (AS) 3 POP PK reports for 3 inidications
Biopharmaceutics				
Absolute bioavailability:				The bioavailability of golimumab was estimated by cross-study comparisons of mean AUCinf values following an IV or SC administration of C0466T01 (IV) and C0466T02 (SC) (44-58%)
Relative bioavailability -				
solution as reference:				
alternate formulation as reference:				
Bioequivalence studies -				
traditional design; single / multi dose:	X	1	1	C0524T24 (100 mg SD, compare 2 drug injection methods: Centocor autoinjector vs. a needle and syringe)-Cmax of autoinjector slightly exceed 125% upper limit

replicate design; single / multi dose:				
Food-drug interaction studies:				
Dissolution:				
(IVIVC):				
Bio-wavier request based on BCS				
BCS class				
Other CPB Studies				
QT/QTc Evaluation				
Genotype/phenotype studies:				
Immunogenicity study reports				
Studies for other indications	X	2		[redacted] C05241103 (Asthma)
Chronopharmacokinetics				
Pediatric development plan	X	1	1	Requesting a deferral for the conduct of a study of golimumab in juvenile idiopathic arthritis (JIA), and a full waiver for juvenile Ankylosing Spondylitis (JAS) and for all patients without the polyarticular form of juvenile psoriatic arthritis (JPsA).
Literature References	X			
Total Number of Studies		25	22	

b(4)

Filability and QBR comments		
	"X" if yes	*Comments*
Application filable?	X	
Comments sent to firm?		
QBR questions (key issues to be considered)	• Have the single and multiple dose PK of golimumab been adequately characterized in healthy subjects and RA patients? • Is PK dose proportional? • What is the to-be-marketed formulation of golimumab? • Are various formulations of golimumab used throughout the clinical development adequately linked? • Have the analytical methods been adequately validated? • Do different analytical assays affect PK assessment? • What is the immunogencity of the product? • Have the antibody assays been adequately validated? • Does immunogenecity affect PK, PD, and efficacy/safety? • Is POP-PK analysis acceptable? • What are main covariates for PK? o Is there a need for dose adjustment? • Does exposure-response support the dose recommendation?	
Other Comments or information not included above		
Primary reviewer Signature and Date	Lei Zhang	
Secondary reviewer Signature and Date	Suresh Doddapaneni	

CC: BLA 125289, DAARP (Turner-Rinehardt), DCP2 (Zhang, Doddapaneni, Sahajwalla), CDR