CENTER FOR DRUG EVALUATION AND RESEARCH

APPROVAL PACKAGE FOR:

APPLICATION NUMBER

BLA 125118/000

Immunogenicity Review

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Food and Drug Administration
Center for Drug Evaluation and Research

Memorandum

Date: **August 12, 2005** 9/20/05

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To: **BLA 125118/000**

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SUMMARY:

In general the methods provided here for testing for the presence of antibodies to the whole CTLA4Ig and CTLA-4 T (tip) are adequate. Testing for antibodies to the whole molecule is confounded by the high level of preexisting antibodies to the Ig region, particularly in the setting of RA where RF is present. BMS has made great efforts to confront this problem. The assay to the CTLA4 region is much more sensitive and provides a reasonable technique to screen patients for relevant antibodies. In addition, the sponsor has developed a neutralizing antibody. The interpretation of the assay relies on highly manipulated data and appears relatively insensitive. Nonetheless, the assay did detect patients with neutralizing antibodies. There did not appear to be a correlation between neutralizing antibodies and AE. Importantly, trough levels of product can interfere with the assay results. Consequently, it is really only relevant to assess immunogenicity rates in patients that have a washout period long enough to clear the majority of the product. In total, immunogenicity rates appear to be low, around ~1-2% for all patients, and ~5-6% for patients that had undergone a significant washout period.

Importantly, it should be appreciated that antibodies to CTLA4 could have adverse impacts on patients. CTLA4 is a negative regulatory molecule expressed on normal T cells. Neutralizing antibodies to this molecule could prevent its activity and lead to uncontrolled immune responses. Indeed, anti-CTA4 is under development for enhancing immune responses in the setting of neoplasia. It will be essential to monitor development of autoimmune phenomenon and correlate with immunogenicity as this product gets released for treatment of RA in large populations. Moreover, such monitoring and testing is critical as Abatacept gets developed for treatment of other autoimmune diseases to ensure it does not exacerbate such conditions or cause new autoimmune conditions.

I. Background and Rationale for Testing Paradigm

Abatacept (CTLA4Ig) is a soluble molecule composed of the B7 binding domain of human CTLA-4 and the Fc domains of human IgG1. It binds to B7 molecules (B71/B7-2) and blocks interactions between CD28 on T cells and B7 molecules on antigen presenting cells. Consequently, it serves to block critical costimulatory signals required for T-cell activation in an immune response. In earlier literature, it was suggested that blocking these costimulatory interaction could lead to long lasting immune tolerance. However, studies in this application suggest that Abatacept works more to induce a non-permanent state of immune suppression. Consequently the molecule will need to be delivered regularly to patients. It is interesting to note that recent data suggests B7 can also directly transduce signals that result in immunosuppression by stimulating tolerizing dendritic cells to product the tryptophan inhibitor, IDO.

Due to immune-suppressive capabilities, CTLA4Ig (Abatacept) is being developed to treat the autoimmune disease, RA. Because of the fusion protein nature of the molecule, it may appear foreign to the immune system and monitoring immunogenicity to the molecule is essential to understanding its activity in patients. This review focuses on the assessment of antibody generation in patients. It also assesses the validation studies performed for the immunogenicity assays used to assess the immune status of patients.

The generation of antibodies to this molecule could have several implications for patients. CTLA4 when expressed on lymphocytes is associated with the generation of signals that inhibit T cell function, particularly if engaged by its B7 ligands. Consequently antibodies to CTLA4 could block these interactions and serve to inhibit an inhibitor, exacerbate T cell responses and make RA "worse". Such responses could also contribute to the development of other autoimmune conditions. Indeed, several monoclonal antibodies to CTLA4 are under development and treatment with these antibodies is associated with the development autoimmune phenomenon. Alternatively, antibodies could act as an agonist and work to further turn off T cell responses. Antibodies to the Ig portion of the molecule (HAHA, RF) might serve to cross link CTLA4Ig bound to B7 molecules. Recent data suggests that B7 can transduce signals that lead to the production of the tryptophan degrading enzyme, IDO. IDO can then serve to suppress T cells responses. It is interesting to speculate that RF could than be contributing to the efficacy of CTLA4Ig treatment in RA patients. If true, it may not be as effective in other autoimmune disease.

An assay to detect antibodies to the whole CTLA4Ig molecule was developed in early clinical trial phases such that antibody responses would detect binding to both the CTLA4 and immunoglobulin (Ig) portion. However predose titers were high and ranged widely (270-32000). A series of studies with various Ig fusion proteins (CTLA4Ig, B7Ig, CD40Ig) and truncated proteins (CTLA4-T) were initiated in order to understand the specificity of potential immune responses. The reactivity of human serum to CTLA4Ig, B7Ig, or CD40Ig was very similar, whereas little reactivity was observed to CTLA4-T (truncated protein formed by cleavage with activated thrombin, also called "Tip Assay"). Moreover, reactivity to the whole molecule correlated with levels of rheumatoid factor. The mean level of abatacept-reactive antibodies in RA subject which were RF+ was significantly higher than the mean level in normal donors ($p<0.001$), and these levels were approximately 700 and 1400 times higher than the mean levels of anti-CTLA4-T

antibody. This finding suggests that the increased reactivity detected in the whole-molecule assay was largely directed against the Ig portion of the molecule. Therefore, the sponsor developed an assay that measures serum antibody responses to the CTLA4 domain (CTLA4-T) alone.

However, FDA thought that an assay to the whole molecule was also important to detect antibody responses to the neo-determinants that arise from the nature of the fusion protein. Moreover, the immunoglobulin region is altered from the endogenous sequence (to prevent complement fixation and extensive di-sulfide bond formation) so issues of immunogenicity to the non-CTLA4 domain were also considered important to investigate. Validation and patient results using CTLA4-T and the whole CTLA4Ig molecule are discussed below.

In summary, while the approach and the assay are passable, it is still difficult to comprehend why BMS has such enormous problems with background. RF is a confounding issue, but is not the whole problem. FDA worked extensively with t he sponsor to help with assay development issues and BMS made a good faith effort to incorporate FDA suggestions. Nonetheless problems with background and interference from on-board product still remain an issue.

II. Patient Immunogenicity Data

A. Studies Performed:

Serum samples were monitored for the development of an anti-abatacept or anti-CTLA4-T binding antibodies. Neutralizing antibodies were also assessed in patients confirmed for the presence of antibodies to CTLA4-T. Samples were collected from healthy subjects in 1 open-label, uncontrolled study; from subjects with RA in the 6 double-blind and 2 open-label study periods; and from subjects with psoriasis in 4 clinical Phase I and II studies. The duration of the double-blind study periods were from 6 months to 1 year and the open-label periods were up to 720 days. The Phase II open-label studies continued for another year beyond the 720 days; however, immunogenicity data are not available from that additional period. Doses of abatacept from 0.5 to 10 mg/kg have been studied across different trials.

Table S4: Frequency of Anti-Abatacept or Anti-CTLA4-T Responses by Study

Study Number	Abatacept Dosage (Dosing/Subjects)	Anti-Abatacept	Anti-CTLA4-T	Total
		No. Positive/No. Evaluated (%)		
Phase I - Assay A				
IM101017	10 mg/kg IV (Single/Healthy)	0/30	0/30	0/30
IM101001	0.5–50 mg/kg IV (Multiple/Psoriasis)	0/43	ND	0/43
IM101003	1–8 mg/kg IV (Single/Psoriasis)	0/25	ND	0/25
IM101004	1–8 mg/kg SC (Single/Psoriasis)	0/30	ND	0/30
Phase II - Assay A				
IM101100 DB	2 or 10 mg/kg IV (Multiple/RA)	0/218	1/219 (0.5%)	1/219 (0.5%)[a]
IM101100 OL	Fixed IV (Multiple/RA)	2/211 (1%)	0/211	2/211 (1%)[a]
IM101101 DB	2 mg/kg IV (Multiple/RA)	0/80	0/80	0/80[a]
IM101101 OL	Fixed IV (Multiple/RA)	0/76	0/76	0/76[a]
IM103002	0.5, 2, 10 mg/kg IV (Multiple/RA)	0/87	0/87	0/87
IM101005	8–25 mg/kg x 8 or 12 IV (Multiple/Psoriasis)	0/52	ND	0/52
Subtotal Phase II - Assay A				3/525 (0.6%)[a]
Phase III - Assay B				
IM101029	Fixed IV (Multiple/RA)	1/234 (0.4%)	2/234 (0.9%)	3/234 (1.3%)
IM101102	Fixed IV (Multiple/RA)	3/406 (0.7%)	3/406 (0.7%)	6/406 (1.5%)
IM101031	Fixed IV (Multiple/RA)	13/880 (1.5%)	9/880 (1.0%)	22/880 (2.5%)
Subtotal Phase III - Assay B				31/1520 (2.0%)

[a] Some subjects were evaluated in both the DB and OL periods; there are 282 subjects in IM101100 (DB and OL) and 104 subjects in IM101101 (DB and OL).

DB = double-blind; OL = open-label; Fixed = dose of abatacept approximating 10 mg/kg; ND = not done.

Source: Appendices 1.0A - 1.0F and CSRs for IM101001, IM101003, IM101004, IM101005, IM101017, IM101100 (DB & OL), IM101101 (DB & OL).

Note: Only Assay B has been fully validated so the most relevant data were collected during the phase III trials.

Note: In addition, neutralizing activity was assessed in subjects who had a positive anti-CTLA-4 response in the phase III DB trials.

B. Summary of findings and caveats to interpretation:

- Only a small percentage of patients developed an antibody response to the product (above). Of the 1520 RA subjects evaluated in the Phase III studies, 1336 subjects completed the DB period and continued into OL treatment. 22/1336 subjects (1.7%) had a positive antibody response during the DB or OL period; 17 subjects (1.3%) to abatacept and 5 subjects (0.4%) to CTLA4-T.

CAVEAT: On-board product, even at trough levels, can interfere with the assay (see below). Consequently, these numbers may underestimate the true incidence of antibodies.

- 154 subjects in the phase III trials discontinued therapy during the DB period, or did not enter into OL study period, and had sera collected 56 and/or 85 days after discontinuation of therapy. 9/154 subjects (5.8%) had positive responses to either abatacept (whole molecule; n = 1, 0.65%) or CTLA4-T (n = 8, 5.2%). This relatively higher percentage of positives in discontinued patients (5.8% vs 1.6%) could indicate assay sensitivity was compromised by on-board product as samples from discontinued patients were obtained after a longer washout period.
- Samples from 9 patients met criteria for evaluation of neutralizing antibodies. Samples from 6 subjects had neutralizing activity. 1 subject discontinued from abatacept therapy due to AEs (antibody unrelated); 2 subjects discontinued from therapy due to lack of

efficacy; 2 subjects discontinued from therapy due to withdrawn consent; and 1 subject discontinued for no longer meeting study criteria. Except for septicemia in subject IM101029-66-1, there were no other medically significant AEs reported in these 6 subjects.

CAVEAT: Product at levels ≥ 1 μg/ml can interfere with the assay. Consequently, results reported may underestimate the number of patients with neutralizing antibodies.

- Early studies (Phase II) used relatively insensitive assays, so failure to detect immune responses may be due to poor sensitivity
- There did not appear to be an increased incidence in subjects with interrupted dosing.
- The incidence seems unrelated to dose.
- Incidence unrelated to efficacy (ACR 20). Data is shown correlating patients with positive antibody response and ACR 20/HAQ response. However, the number of subject is low, making it difficult to draw conclusions.
- Use of DMARD seemed to have not impacted on immunogenicity as compared across studies but it is somewhat unclear.
- Rare (2) hypersensitivity responses were reported and did not correlate with antibody positivity.
- One subject who had an antibody response against the CTLA4-T portion 56 and 85 days after dosing developed a non-specific autoimmune disorder with an onset on Day 367.
- Because the incidence is rare and impacted by the presence of on-board product it is difficult to fully interpret the data. The most relevant data comes from those patients whose serum samples have little interference potential from on-board product either because they were off product for extended time periods or discontinued treatment.
- Because antibodies to CTLA4 have the potential to cause or exacerbate auto-reactivity, patients with positive responses, particularly neutralizing responses should be followed. In addition, this potential should be presented to AERs folks so when this product is introduced into larger populations or new populations, they can track autoimmune phenomenon as a signal or other related AEs.

C. Testing Paradigms: Assay A and Assay B

1. Assay A: Phase II Trials.

(Briefly mentioned here as results are used to support immunogenicity profiles). For Phase II trials, endpoint titer (EPT) assays were employed for both the CTLA4-T and whole molecule (Abatacept) assay. Sera samples were studied over 11, 3-fold dilutions starting at 1:10. Detection employed [redacted] cocktail. Monkey sera (anti-CTLA4Ig and anti-CTLA4-T) are used to QC the assay. Results are expressed as EPT. During Phase II RA studies, EPT was defined as the reciprocal of the highest dilution that gave an OD greater than or equal to fivefold of the mean plate background or the dilution which gave an OD reading that was equivalent to 5X mean background based on interpolation. An individual was considered to have seroconverted when the EPT increased by 2 or more serial dilutions (≥ nine-fold) relative to pre-dosing EPT. Positive samples were then subsequently evaluated for confirmation of positivity by immunodepletion and to determine the specificity of the response.

2. Assay B: Phase III trials

Based on FDA guidance, the assays for the Phase III trials were modified to improve sensitivity and specificity (different coating concentration, different buffer, different detecting agents, etc stemming from FDA input). The method of reporting was also altered such that positivity was based on a ratio between pre and post treatment values for a single dilution. Positivity was confirmed with an immunodepletion assay.

1. Abatacept Assay: ELISA plates were coated with abatacept followed by incubation with either 1) a pooled RA subject sample (negative control); 2) a CTLA4Ig affinity-purified, polyclonal monkey antisera standard (positive control); or 3) samples from RA patients. All samples were initially diluted 1:25 in binding buffer and incubated with shaking overnight at room temperature (This step was used to dissociate RF-dependent interactions). Samples were then further diluted 1:16 (final dilution 1:400) prior to plating, or serially diluted across a range in a follow-up assay, to determine EPT. Detection employed [ ] specific antibody cocktail followed by TMB substrate. As results are reported as post-/pre-dose ratios, the sensitivity of the assay is dependent on the level of pre-existing reactivity of the sample assessed. If the ratio value was less than the validation-specified cutoff, the sample was considered negative and reported as a titer value of less than 400. If the ratio value was greater than or equal to the cutoff, the sample was considered positive and was further evaluated in a confirmation assay. If positive, EPT were determined by an 11-point, 3-fold dilution series starting at 1:10. The mean background OD for each plate was calculated from wells to which binding buffer was added instead of serum. A linear interpolation template in EXCEL was used to calculate EPT defined as the post-/pre-dose ratio value equal to the established cutoff value. The OD value for each of the Day 1 samples was provided and underscores the high background and variability for some RA samples. If a positive screening result was repeated, and the specificity of the reactivity was confirmed by immunodepletion with abatacept, the sample was considered positive for seroconversion and reported as a titer value corresponding to the reciprocal of the interpolated serum dilution that would result in a post-/pre-dose ratio value equal to the established cutoff value.

2. CTLA4-T Assay. Plates were coated with CTLA4-T followed by incubation with a pooled, RA sera (negative control); the pooled human sera sample spiked with approximately 500 ng/ml of an CTLA4-T affinity-purified, polyclonal, anti-CTLA4-T, monkey antibody standard (positive control); or patient samples. Samples were diluted 1:25 and detected as described in the whole-molecule assay above. Results in the CTLA4-T–specific antibody screening assay were expressed as a Ratio 1 value. This was calculated by dividing the mean sample OD by the mean OD of the negative control on the same plate. This method allows for evaluation of positivity and titer for BOTH the pre- and post-dose samples. If the ratio 1 value was less than the validation-specified cutoff, the sample was considered negative and reported as a titer value of less than 25. If the ratio value was greater than or equal to the cutoff, the sample was considered

positive and was further evaluated in a confirmation assay. If a positive screening result was repeated and the specificity of the\ reactivity was confirmed by immunodepletion with both CTLA4-T and abatacept, the sample was considered positive for seroconversion and reported as an EPT value corresponding to the reciprocal of the interpolated serum dilution that would result in a Ratio 1 value equal to the established cutoff value. To determine this titer, the subject's serum was evaluated in the screening ELISA assay using a twofold serial dilution. If a sample was still positive following confirmatory immunodepletion, but did not repeat a Ratio 1 value greater than or equal to the cutoff to identify the titer, a titer value of 25 was assigned, and the sample was considered positive.

Note: Due to the high reactivity to the Ig region (which is why the CTLA4-T assay was developed), the sensitivity of the whole-molecule (anti-abatacept) assay is in the µg/mL range, whereas the CTLA4-T assay is in the ng/mL range. Therefore, it is possible to have a positive anti-CTLA4-T response in the absence of a positive response in the whole-molecule assay. However, a positive anti-CTL4-T response should be inhibited by whole abatacept.

Note: To understand what part of the molecule the reactivity in the whole-molecule assay is directed, immunodepletion is conducted with abatacept and CD40Ig as well as CTLA4-T. The junction regions of abatacept and CD40Ig are different, whereas the Ig regions are the same. If immunodepletion to abatacept is seen but not to CD40Ig, it would suggest that the reactivity would be to that region. In all cases, reactivity was observed to both and at generally equal magnitudes, supporting that the reactivity is to the Ig region, not to the junction region.

Comparison Assays for Patient Immunogenicity Analysis

	Phase II	Phase III
Abatacept Assay Reporting Sensitivity Start Dilution	 EPT µg/ml 1:10	 Ratio: Mean Pre/Mean post µg/ml 1:400
CTLA4-T Assay Reporting Sensitivity Start Dilution	 EPT µg/m 1:10l	 Ratio 1: Mean Sample/Mean Neg. Control ng/ml 1:25

NOTE: While these values are sensitivities under ideal conditions, that presence of product at trough levels will significantly reduce the sensitivity of the assays. Indeed, the sponsor states in an IND Amendment that the sensitivity of the CTLA4-T assay with trough levels of product present (10-25 µg/ml) reduced sensitivity to 2.5 µg/ml.

17 Page(s) Withheld

✓ § 552(b)(4) Trade Secret / Confidential

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