Ariel Bourla, MD, PhD
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The role of CD28 expression in predicting responsiveness to abatacept treatment in patients with rheumatoid arthritis refractory to methotrexate

1) Study Purpose and Rationale

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by progressive joint damage. It affects approximately 1% of North Americans and Europeans. While a small percentage of affected patients have relatively mild disease with intermittent flares, the vast majority of patients have persistent symptoms accompanied by joint destruction. This can lead to significant functional limitation. For this reason, long-term therapy is required both for symptomatic control and to prevent the consequences of chronic inflammation.

While NSAIDs and corticosteroids can help control symptoms, they do not prevent disease progression. Thus, disease modifying antirheumatic drugs (DMARDs) are usually initiated within 3 months of disease onset. Methotrexate is the most commonly used DMARD; others include hydroxychloroquine, sulfasalazine, and leflunomide. They work by suppressing the immune system and take effect over weeks to months. Often, they are used in combination with NSAIDs or other DMARDs. Because of their mechanism of action, they have a significant side effect profile.

However, adequate disease control is not always achieved with oral DMARDs. More than a decade ago, the first biologic therapies, which inhibit inflammatory cytokines such as TNF, were approved for the treatment of RA[i]. Since then, a number of different agents with varying mechanisms of action have been discovered. Biologic therapy should be initiated in patients who are refractory to oral DMARDs. TNF inhibitors were the first agents approved for RA, and as a result have become the preferred first-line biologic therapy. There are three currently available agents: etanercept (TNFR2-IgG1 fusion protein), infliximab (monoclonal antibody) and adalimumab (monoclonal antibody). They have been shown to have similar efficacy despite different dosing regimens and administration methods. Patients who do not respond to one of the agents may still have a clinical response to a different one. Other biologics used for RA include anakinra (anti-IL-1), rituximab (anti-CD20), and abatacept.

Abatacept is a recombinant fusion protein comprising the extracellular domain of human CTLA4 and a fragment of the Fc domain of human IgG1, which has been modified to prevent complement fixation. The intravenous formulation was approved by the FDA in December 2005, and the subcutaneous formulation was approved in August 2011. The drug competes with CD28 for CD80 and CD86 binding and thus can be used to selectively modulate T cell activation[ii]. Normally, CD28 binds CD80 and CD86 on an antigen presenting cell, delivering the "second signal" and leading to T cell activation. Following this, the inhibitory molecule CTLA4 is induced on the surface of the T cell. Due to its greater affinity for CD80/ CD86 than CD28, it outcompetes CD28 and thus modulates the T cell response[iii].

A number of studies have been conducted to evaluate the efficacy and safety of this therapy in treatment of RA. In particular, the AIM (Abatacept in Inadequate responders to Methotrexate) study evaluated abatacept plus methotrexate in patients failing DMARD treatment. Abatacept demonstrated sustained clinical efficacy and consistent safety over 3 years of treatment, with increasing inhibition of radiographic progression over each year[iv]. The more recent ATTEST (Abatacept or infliximab versus placebo, a Trial for Tolerability, Efficacy and Safety in Treating RA) study performed a direct comparison of abatacept, infliximab, and placebo in patients with an inadequate response to methotrexate. Results showed that abatacept and infliximab had similar efficacy, though abatacept had a relatively more acceptable safety and tolerability profile[v]. The ATTAIN (Abatacept Trial in Treatment of Anti-TNF Inadequate responders) study differed in that enrolled participants had shown prior refractoriness to TNF inhibition. Participants were given abatacept or placebo in addition to background DMARD therapy. The initial study showed significantly greater ACR20, ACR50, and ACR70 response rates in the abatacept-treated group[vi]. In addition, the long-term extension of the ATTAIN study showed that abatacept safety and efficacy was sustained over 5 years[vii]. Overall, abatacept has proven to have similar to superior efficacy compared with TNF inhibition with a similar safety profile. Following FDA approval of subcutaneous abatacept in 2008, a study was conducted to compare the efficacy and safety of subcutaneous versus intravenous abatacept. The ACQUIRE (Abatacept Comparison of Subcutaneous versus Intravenous in Inadequate Responders to Methotrexate) study found that the subcutaneous formulation had similar efficacy and safety compared to IV abatacept, with similarly low immunogenicity rates and high retention rates[viii]. Thus, the newer formulation provides an alternative treatment option for those wishing to self-administer their therapy.

In healthy individuals, CD28 is constitutively expressed by nearly all CD4+ T cells and greater than 50% of CD8+ T cells[ix]. CD28 expression is downmodulated after engagement with its ligand[x] or prolonged stimulation with specific peptide antigens[xi] or = cytokines such as IL-4[xii] or IL-2[xiii]. Accordingly, the population of CD28-cells has been shown to expand in conditions characterized by chronic immune activation. Numerous studies have demonstrated an expansion of CD8+CD28- cells and sometimes of a small population of CD4+CD28- cells in HIV[xiv], lupus[xv], and, importantly, RA[xvi]. In addition, the expansion of CD28- T cells in RA has been associated with aggressive disease, extraarticular manifestations[xvii], and preclinical atherosclerotic changes[xviii]. Given these findings, it was hypothesized that the blockade of CD28 costimulation by abatacept might induce a reduction in the number of CD28- cells, leading to improvement in disease severity. A study by Scarsi et al in 2010 showed that, indeed, circulating CD28- T cells decreased after abatacept in patients with RA, which also correlated with clinical response[xix]. However, the study was limited by their small sample size (20 patients). In addition, although they did find that the proportion of CD8+CD28- cells at baseline was higher in the RA group than in healthy controls (38.7% vs 24.7%, $p = 0.048$), as well as the proportion of CD4+CD28- cells (4.8% vs 3.6%, $p = 0.029$), they failed to measure the efficacy of abatacept in individual patients with regard to their baseline CD28 profile.

The purpose of this study will be to determine if CD28 expression on CD4+ and CD8+ T cells can serve as a predictor of responsiveness to abatacept treatment in RA patients who are refractory to methotrexate.

2) Study Design and Statistical Analysis

The study will be a prospective open-label treatment trial to determine the relative efficacy of abatacept based on participants' percentage of CD28- T cells. 330 participants will be recruited from the outpatient rheumatology clinic at Columbia/New York Presbyterian Hospital.

Inclusion criteria: 1) Patients ≥ 18 yrs with diagnosis of RA. 2) Active RA (despite MTX) defined by ≥ 6 swollen joints and ≥ 8 tender joints and ESR ≥ 28 or CRP ≥ 15mg/L. These parameters will yield a minimum DAS 28 score of approximately 5.1 (high disease activity). 3) Patients must have tried and failed methotrexate therapy, and been on a stable dose of methotrexate for at least 2 months.

Exclusion criteria: 1) Current or past treatment with any biologic agent. 2) Oral corticosteroid use within the past 30 days. 3) A change in methotrexate dose over the past 2 months. 4) A prior diagnosis of COPD.

After enrollment into the study, participants will be evaluated for their percentage of CD28- T cells using flow cytometry, and then separated into tertiles based on these results. Prior to the first administration of study drug, participants will undergo initial testing of disease burden including joint examination and determination of patient pain score, patient global assessment, physician global assessment, and patient disability assessment/HAQ (Stanford Health Assessment Questionnaire).

Study participants will begin subcutaneous abatacept on Day 1 of the study, consisting of a 1 mL solution containing 125 mg of abatacept. Patients will receive SC injections every 7 days thereafter until the end of the study period. Patients will also receive a loading dose of abatacept approximating 10mg per kilogram of body weight on Day 1 of the study.

At the end of the study period, patients will again undergo testing of disease burden including joint examination and determination of patient pain score, patient global assessment, physician global assessment, and patient disability assessment/HAQ (Stanford Health Assessment Questionnaire). By comparing this information with the data from before the treatment period, we will be able to calculate ACR response.

Patients will be free to withdraw from the study at any time, for any reason. Based on previous studies using abatacept for treatment of RA[xx], we do not expect many significant adverse effects but some patients do get serious infections. We will monitor patients closely for any side effects that would prompt withdrawal from the study. Subjects will be analyzed in an intention to treat analysis.

The primary outcome will be ACR20 response, and we will compare the frequency of this outcome in the top and bottom tertiles. Statistical analysis will be

performed by means of a chi-square test for primary outcome, in which the categorical outcome of ≥20% improvement vs. <20% improvement will be assessed at a significance level of p = 0.05. The secondary outcomes will be ACR50 and ACR70 responses, and a similar statistical method will be used to detect a 50% or 70% improvement.

Power calculation for a chi-square test:
n (in each group) = 8 $(p_1q_1 + p_2q_2)/(\text{effect})^2 + 2/\text{effect} + 2$
In a prior study evaluating the efficacy of abatacept in patients refractory to TNFα inhibition, 50.4% of patients achieved ACR20 responses[xxi]. Thus, the null hypothesis would state that there is no difference between the top and bottom tertiles (50% in each cohort). In order to be able to detect a difference as small as 60%/40% using a chi-square test with 80% power, we will need a sample size of 108 in each group. In total, we will recruit 330 study participants.

3) Study Procedures

Patients with a diagnosis of rheumatoid arthritis who are seen at the Columbia/NY Presbyterian rheumatology clinic will be screened for eligibility. As discussed in the study design, those meeting criteria will be evaluated for baseline disease scores including joint examination and determination of patient pain score, patient global assessment, physician global assessment, and patient disability assessment/HAQ (Stanford Health Assessment Questionnaire). Study participants will begin subcutaneous abatacept on Day 1 of the treatment period, consisting of a 1 mL solution containing 125 mg of abatacept. Patients will receive SC injections every 7 days thereafter until the end of the treatment period. Patients will also receive a loading dose of abatacept approximating 10mg per kilogram of body weight on Day 1 of the study. They will continue treatment through Day 180. At the end of the treatment period, they will again be evaluated by joint examination and determination of patient pain score, patient global assessment, physician global assessment, and patient disability assessment/HAQ (Stanford Health Assessment Questionnaire). During the 6 months, their side effect profile will be monitored in case they require withdrawal from the study.

4) Study Drugs or Devices

The study drug will be Abatacept, a drug approved by the FDA in December 2005 for rheumatoid arthritis. The subcutaneous formulation was approved in August 2011. Abatacept is a recombinant fusion protein comprising the extracellular domain of human CTLA4 and a fragment of the Fc domain of human IgG1, which has been modified to prevent complement fixation. The drug competes with CD28 for CD80 and CD86 binding and thus can be used to selectively modulate T cell activation[xxii].

The most common adverse effects experienced by more than 5% of patients include headache, nasopharyngitis, upper respiratory tract infection, diarrhea, bronchitis, sinusitis and nausea. Serious adverse events occurred in 10.5% of patients on abatacept in one study[xxiii] and 4.2% of patients in another[xxiv]. The most

serious adverse events are infection and malignancy. Acute infusion reactions occurred in about 5% of patients but were not significantly more frequent than following placebo. The most commonly reported infusion-related symptoms were dizziness and headache [xxv]. Patients with chronic obstructive pulmonary disease (COPD) treated with abatacept had a higher incidence of adverse events and serious adverse events, especially respiratory disorders[xxvi].

Treatment with abatacept did not increase the risk of inducing autoantibodies including ANA and anti-dsDNA antibodies. In addition, only 1.3% of patients developed neutralizing antibodies to abatacept, and in all cases they showed low level reactivity[1].

There are no black box warnings.

5) Study Questionnaires

Patient pain score, patient global assessment, and physician global assessment are all conducted using a visual analog scale (VAS), which is measured with a ruler. Visual analog scales have been validated for RA, and in fact are one of the recommended measures of disease activity[xxvii].

Health Assessment Questionnaire (HAQ) – The HAQ was originally developed in 1978 by James F. Fries, MD, and colleagues at Stanford University. It serves as a comprehensive measure of outcome in patients with a wide variety of rheumatic diseases, and has become the dominant instrument in arthritis. Its focus is on self-reported patient-oriented outcome measures.

6) Study Subjects

Patients being followed at the outpatient Rheumatology clinic at Columbia/NY Presbyterian Hospital will be screened for eligibility.

Inclusion criteria:
1) Patients ≥ 18 yrs with diagnosis of RA.
2) Active RA (despite MTX) defined by ≥ 6 swollen joints and ≥ 8 tender joints and ESR ≥ 28 or CRP ≥ 15mg/L. These parameters will yield a minimum DAS 28 score of approximately 5.1 (high disease activity).
3) Patients must have tried and failed methotrexate therapy, and been on a stable dose of methotrexate for at least 2 months.

Exclusion criteria:
1) Current or past treatment with any biologic agent.
2) Oral corticosteroid use within the past 30 days.
3) A change in methotrexate dose over the past 2 months.
4) A prior diagnosis of COPD.

7) Recruitment of Subjects

Subjects will be recruited from the outpatient Rheumatology clinic at Columbia/NY Presbyterian Hospital according to the criteria outlined above. Faculty in the Rheumatology department will be notified of the study and asked their

opinion on patients' suitability for the study. Possible participants will be given information regarding the study as well as consent forms prior to their inclusion in the study.

8) Confidentiality of Study Data
All patient data will be de-identified and stored appropriately.

9) Conflict of Interest
No potential conflicts of interest to disclose

10) Location of Study
This study will take place at Columbia/NY Presbyterian.

11) Potential Risks

The risks of this study are attributed to side effects of abatacept which are listed under the section "study drugs". In addition, participants taking abatacept are precluded from taking other biologic therapies during the study period. While there is the chance that abatacept may not be as effective as other biologics such as etanercept or infliximab, prior studies show that abatacept is equally if not more effective than infliximab with fewer adverse effects[xxviii].

12) Potential Benefits

You may or may not benefit from your participation in this study. It is possible that your condition may improve with abatacept. However, even if you do not directly benefit from abatacept, your participation in this study will help other patients by providing new information about which patients might benefit most from abatacept.

13) Alternatives Therapies

Other biologic agents including etanercept, infliximab, rituximab, adalimumab, and certolizumab. All participants have the option not to participate in this research.

14) Compensation to subjects:
None

15) Cost to subjects:
None

16) Minors as research subjects:
Not applicable

17) Radiation or radioactive substances:
Not applicable

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