Immunoprofiling for Prediction of Response to Abatacept in Rheumatoid Arthritis Patients

Prodromos Sidiropoulos, Panagiotis Verginis, Garyfalia Papadaki, George Bertsias, Argyro Repa, Nestor Avgoustidis

MEDITERRANEAN JOURNAL OF RHEUMATOLOGY
ΕΛΛΗΝΙΚΗ ΡΕΥΜΑΤΟΛΟΓΙΑ

RESEARCH PROTOCOLS-PROPOSALS

Immunoprofiling for Prediction of Response to Abatacept in Rheumatoid Arthritis Patients

Prodromos Sidiropoulos[1,3], Panagiotis Verginis[2], Garyfalia Papadaki[3], George Bertsias[3], Argyro Repa[1], Nestor Avgoustidis[1]

[1]Rheumatology Clinic, Medical School, University of Crete, [2]Biomedical Research Foundation of the Academy of Athens (BRFAA), Greece, [3]Laboratory of Rheumatology, Inflammation and Autoimmunity, Medical School, University of Crete

ABSTRACT

Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disease which causes musculoskeletal pain, disability and reduced life expectancy. Activation of immune cells – especially - T helper (Th) lymphocytes - resulting in aberrant production and release of cytokines and chemokines has been demonstrated as one of the major events in the pathogenesis and progression of the disease. Abatacept is a recombinant fusion protein which selectively modulates T-cell activation by blocking the co-stimulation of T cells by inhibiting the CD28-CD80/CD86 pathway between T cells and antigen presenting cells. Abatacept has been licensed for treatment of RA in patients with refractory disease, despite administration of conventional disease-modifying drugs. The current project will be an observational, prospective, single-center study of RA patients starting treatment with abatacept, due to residual disease activity. During the study period of 12 months we aim to investigate whether the peripheral blood immunological profile of RA patients may be used as a biomarker to predict clinical responses to abatacept by characterizing the phenotype and function of pathogenic and regulatory cell subsets and identifying the cytokine and/or chemokine signature in serum of RA individuals receiving this regimen.

Corresponding author:
Prodromos Sidiropoulos, MD, PhD
Associate Professor of Rheumatology
Rheumatology Clinic & Laboratory of
Rheumatology, Inflammation
and Autoimmunity
Medical School, University of Crete
P.O. Box 1352, Voutes, 71003
Heraklion, Crete, Greece
Tel./Fax: +302811115899
e-mail: sidiropp@uoc.gr

BACKGROUND

Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disease which causes musculoskeletal pain, disability and reduced life expectancy. Its prevalence in Western Europe is estimated at 0.63% in females and 0.24% in males, while it increases significantly up to 2% in the adult population aged over 60 years.[1] Globally, among 291 medical conditions, RA is ranked as the 42nd highest contributor to global disability, just below malaria and just above iodine deficiency.[1] In Europe, the total annual costs of the disease are estimated at €45.3 billion (year 2007 data), of which direct medical costs and drugs represent about one-third.[2]

T helper (Th) lymphocytes have been demonstrated to play a central role in disease pathogenesis and progression through the release of cytokines and chemokines. The main pathogenic Th cell subsets are Th1 characterized by the release of IFN-γ and Th17 that secrete significant amounts of IL-17. Both cell subsets direct the recruitment of other pathogenic cells, such as macrophages, dendritic cells and neutrophils, to the site of inflammation. On the other hand, the regulatory networks that should operate in order to inhibit the inflammation and re-establish homeostasis are defective during active RA. The best characterized regulatory cells are CD4+ T regulatory cells (Tregs that characterized by the expression of the transcription factor Foxp3 and/or the secretion of IL-10) as well as myeloid-derived suppressor cells (MDSCs) that exert an immunoregulatory role through release of suppressive factors. Restoration of immune regulation has been shown in RA patients upon effective treatment.

Treatment strategy in RA has radically changed during the last 15 years. Early on, at the time of diagnosis, treatment with non-biologic disease modifying anti-rheumatic drugs (nbDMARDs) is initiated and combinations of nbDMARDs are often used in aggressive forms of the disease. The target of treatment is disease remission or low disease activity.[3] In those patients in whom residual disease activity still persists after optimal doses of nbDMARDs, then biologic DMARDs (bDMARDs) may be used. Biologic DMARDs applied in clinical practice are either cytokine inhibitors (TNFα or IL6 inhibitors), B-cell depleting agents (anti- CD20 antibodies), or T-cell co-stimulation inhibitors (CTLA4Ig). Real-life data from patients' registries have shown that about 50-60% of RA patients treated with those agents may respond to therapy, while the rest will stop treatment either due to inefficacy (primary or secondary) or due to toxicity.[4] Until now, personalized treatment selection based on evidence is extremely limited.[5]

Optimal T cell proliferation and acquisition of effector functions require intracellular signals elicited by both the T cell receptor and by a co-receptor, CD28.[6] CD28 delivers co-stimulatory signals upon engagement of its ligands, B7-1 (CD80) or B7-2 (CD86). Cytotoxic T lymphocyte antigen 4 (CTLA-4), binds to the same ligands as CD28 (with much higher affinity) and restricts T cell activation.[7]

CTLA-4 expression is induced in all T cells transiently after T cell receptor activation. 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.[8] Abatacept, like CTLA4, competes with CD28 for CD80 and CD86 binding, and thereby can be used to selectively modulate T-cell activation. Abatacept has been approved for the treatment of rheumatoid arthritis, based on the results of an extensive clinical development program assessing its effectiveness and safety in different RA populations.[9-11] Abatacept has been also approved for the treatment of juvenile idiopathic arthritis, while belatacept – a modified form of abatacept – has been approved for preventing kidney transplant rejection. Recently, in a randomized controlled trial, abatacept given subcutaneously has been shown to have comparable efficacy to adalimumab (TNFα inhibitor),[12-13] and thus now the sub-cutaneous form is given in clinical practice. Data from registries have shown that in clinical practice, up-to 50% of RA patients starting a bDMARD will stop treatment due to inefficacy or toxicity in the long-term.[4,14] Comparably, although limited, data suggest that in patients with failure to an anti-TNFα agent, abatacept and tocilizumab drug survival in clinical practice is approximately 50% at 1 year in RA patients. Several studies have addressed the issue of predictive markers of response – both clinical or "biomarkers" – to therapy with bDMARDs.[5] Nevertheless, no reliable, clinically applicable tool is yet available. Development of such a tool for everyday clinical practice will be an important step further in optimizing the treatment of autoimmune diseases, since it will save costs and prevent possible toxicities. Available data for predictors of treatment continuation with abatacept are rather limited.[15-17]

PROTOCOL

This will be an observational, prospective, single-center study of RA patients starting treatment with abatacept, due to residual disease activity. All patients will be recruited by the outpatient and inpatient Clinic of Rheumatology, Allergy and Clinical Immunology of the University Hospital of Crete. Treatment decisions will be made by the treating rheumatologist and according to the guidelines for the treatment of RA of the Hellenic Society of Rheumatology and EULAR guidelines.

Patients will be followed for 12 months or until treatment discontinuation (whenever treatment duration is <12 months). Patients will be followed clinically each 3 months, and RA disease activity, function and lab-

oratory parameters will be documented. A series of immunological assays of peripheral blood (cytokines, cell sub-population and functional studies) will be performed at baseline and at 3 months of treatment. These studies will be performed in the Laboratory of Rheumatology, Inflammation and Autoimmunity of the Medical School of the University of Crete.

STUDY POPULATION

Rheumatoid arthritis patients (ACR criteria for RA)[18] will be recruited by the inpatient and outpatient Rheumatology clinic at the University Hospital of Heraklion, Crete. Eligible patients will be all those patients who, according to their treating rheumatologists' clinical judgment, are candidates for starting treatment with abatacept, according to the guidelines of the Hellenic Society of Rheumatology and EULAR guidelines.[3] All participants will consent for participation in the study after being informed.

According to data from clinical trials, approximately 60% of the patients starting abatacept as a first biological DMARD have low disease activity based on DAS28 during the first year of treatment. Of note, these data apply for a selected population recruited for a randomized clinical trial, with a disease duration of <5 years.[13] Assuming that we will have a rather lower rate of response in our population of clinical practice compared to that reported in clinical trials, 40%-50% of our patients will have low DAS28 and will be categorized as responders. Thus, we aim to recruit approximately 30 patients, whom we will follow for 12 months.

CLINICAL PARAMETERS

Clinical efficacy will be evaluated every 3 months for the 12 months of follow-up. Patients will be classified as responders (good, moderate or non-responders) based on the DAS28 values.[19] Moreover, patients' disease activity level will be characterized as remission, low, moderate or high when actual DAS28 level is <2.6, ≤3.2, ≤5.1 or >5.1 respectively.[20] We will also apply SDAI/CDAI response criteria.[21] Patients who discontinue treatment will be recorded and reasons of discontinuation will be documented (inefficacy, toxicity, other).

IMMUNOLOGICAL STUDIES

Immunological studies will be performed at baseline and at 3 months. In more detail:

1. Phenotypic characterization of pathogenic and regulatory cell subsets

Initially, we will characterize the T cell responses in abatacept-treated RA patients. To this end, peripheral blood mononuclear CD4+ T cells will be examined for the presence of Th1 and Th17 cells based on the intracellular expression of IFN-γ and IL-17 respective-

ly by flow cytometry. In addition, we will monitor the expression of IL-10 a key cytokine secreted by Treg subsets. Furthermore, using a combination of specific fluorescent-labeled antibodies, we will determine the frequency of myeloid cell populations; macrophages, dendritic cells and neutrophils, as well as myeloid-derived suppressor cells. Finally, intracellular expression of Foxp3 in CD4+ T cells will allow the characterization of natural-occurring Treg cells.

2. Functional characterization of the regulatory cell subsets

To understand whether the regulatory cell networks operate in abatacept-treated RA patients, we will isolate Treg cells (using cell-sorting – 95-99% purity) and will determine their potential to suppress pathogenic autologous Th1 and Th17 cells in vitro. Pathogenic TH1 and TH17 cells will be activated with aCD3/aCD28 beads, and their proliferation will be monitored upon labeling with CFSE dye. Furthermore, detection of IL-2 in culture supernatants by cytokine ELISA will provide evidence for their proliferation ability, and their suppressive activity will be monitored in a similar fashion as described in *(a)*.

3. Identification of the cytokine/chemokine signature in serum of RA patients

Serum from abatacept-treated RA patients will be collected and analyzed for the presence of cytokines and chemokines using the multiplex bead analysis. The multiplex-bead analysis will cover a broad range of cytokines and chemokines such as Interleukin- 1β, 2, 4, 5, 6, 8, 10,12, 17A&F, IFN-γ, IFN-α, INF-β, TNF-α, MCP-1, GM-CSF, TGF-β, MIP1-α, MIP1-β.

SIGNIFICANCE

In this proposal, we will investigate whether the peripheral blood immunological profile of RA patients may be used as a biomarker to predict clinical responses to abatacept.

Approximately 40-50% of RA patients who start treatment with abatacept in clinical practice will discontinue this agent due mainly to inefficacy during the first year of treatment. Most of them will be "primary failure" to abatacept. On the other hand, there is a percentage of patients who respond later to abatacept, between the 3rd and 6th month of therapy. According to EULAR guidelines for the treatment of RA, rheumatologists should decide about treatment efficacy during the first 3-6 months of therapy.[3] Decisions for treatment discontinuation are now based only on clinical disease activity. No biomarkers that predict clinical responses are available, and this represents a significant unmet need for optimizing therapy of RA. Developing such a predictor of response is of clinical and immunological significance.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Cross M, Smith E, Hoy D, Carmona L, Wolfe, F, et al. The global burden of rheumatoid arthritis: estimates from the global burden of disease 2010 study. Ann Rheum Dis 2014;73:1316-22.
2. Lundqvist J, Kastang F, Kobelt G, and Jonsson B. The burden of rheumatoid arthritis and access to treatment: determinants of access. Eur J Health Econ 2008;2:S87-93.
3. Smolen J S, Landewe R, Breedveld F, Buch M, Burmester G, Dougados M, et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2013 update. Ann Rheum Dis 2014;73;492-509.
4. Flouri I, Markatseli T E, Voulgari P V, Boki K A, Papadopoulos I, Settas L, et al. Comparative effectiveness and survival of infliximab, adalimumab, and etanercept for rheumatoid arthritis patients in the Hellenic Registry of Biologics: Low rates of remission and 5-year drug survival. Semin Arthritis Rheum 2014;43:447-57.
5. van den Broek M, Visser K, Allaart C F, Huizinga T W. Personalized medicine: predicting responses to therapy in patients with RA. Curr Opin Pharmacol 2013;13:463-9.
6. Carreno B M, Collins M. The B7 family of ligands and its receptors: new pathways for costimulation and inhibition of immune responses. Annu Rev Immunol 2002;20:29-53.
7. Tivol E A, Borriello F, Schweitzer A N, Lynch W P, Bluestone J A, Sharpe A H. Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4 Immunity 1995;3:541-7.
8. Linsley P S, Brady W, Urnes M, Grosmaire L S, Damle N K, Ledbetter J A. CTLA-4 is a second receptor for the B cell activation antigen B7. J Exp Med 1991;174:561-9.
9. Bathon J, Robles M, Ximenes A C, Nayiager S, Wollenhaupt J, Durez P, et al. Sustained disease remission and inhibition of radiographic progression in methotrexate-naive patients with rheumatoid arthritis and poor prognostic factors treated with abatacept: 2-year outcomes. Ann Rheum Dis 2011;70;1949-56.
10. Genovese M C, Becker J C, Schiff M, Luggen M, Sherrer Y, Kremer J, et al. Abatacept for rheumatoid arthritis refractory to tumor necrosis factor alpha inhibition. N Engl J Med 2005;353:1114-23.
11. Kremer J M, Genant H K, Moreland L W, Russell A S, Emery P, Abud-Mendoza C, et al. Results of a two-year follow-up study of patients with rheumatoid arthritis who received a combination of abatacept and methotrexate. Arthritis Rheum 2008;58:953-63.
12. Schiff M, Weinblatt M E, Valente R, van der Heijde D, Citera G, Elegbe A, et al. Head-to-head comparison of subcutaneous abatacept versus adalimumab for rheumatoid arthritis: two-year efficacy and safety findings from AMPLE trial. Ann Rheum Dis 2014;73;86-94.
13. Weinblatt M E, Schiff M, Valente R, van der Heijde D, Citera G, Zhao C, et al. (2013) Head-to-head comparison of subcutaneous abatacept versus adalimumab for rheumatoid arthritis: findings of a phase IIIb, multinational, prospective, randomized study. Arthritis Rheum 2013;65:28-38.
14. Hetland M L, Christensen IJ, Tarp U, Dreyer L, Hansen A, Hansen I T. et al. Direct comparison of treatment responses, remission rates, and drug adherence in patients with rheumatoid arthritis treated with adalimumab, etanercept, or infliximab: results from eight years of surveillance of clinical practice in the nationwide Danish DANBIO registry. Arthritis Rheum 2010;62:22-32.
15. Maneiro R J, Salgado E, Carmona L, Gomez-Reino J J. Rheumatoid factor as predictor of response to abatacept, rituximab and tocilizumab in rheumatoid arthritis: Systematic review and meta-analysis. Semin Arthritis Rheum 2013;43:9-17.
16. Scarsi M, Ziglioli T, Airo P. Baseline numbers of circulating CD28-negative T cells may predict clinical response to abatacept in patients with rheumatoid arthritis. J Rheumatol 2011;38;2105-11.
17. Takahashi N, Kojima T, Kaneko A, Kida D, Hirano Y, Fujibayashi T, et al. Use of a 12-week observational period for predicting low disease activity at 52 weeks in RA patients treated with abatacept: a retrospective observational study based on data from a Japanese multicentre registry study. Rheumatology (Oxford) 2015;54:854-9.
18. Arnett F C, Edworthy S M, Bloch D A, McShane D J, Fries J F, Cooper N S, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis, Arthritis Rheum 1998;31;315-24.
19. Kavousanaki M, Makrigiannakis A, Boumpas D, Verginis P. Novel role of plasmacytoid dendritic cells in humans: induction of interleukin-10-producing Treg cells by plasmacytoid dendritic cells in patients with rheumatoid arthritis responding to therapy. Arthritis Rheum 2012;62:53-63.
20. van Gestel A M, Haagsma CJ, van Riel P L. Validation of rheumatoid arthritis improvement criteria that include simplified joint counts. Arthritis Rheum 1998;41;1845-50.
21. Prevoo M L, van't Hof M A, Kuper H H, van Leeuwen M A, van de Putte L B, van Riel P L. Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis Rheum 1995;38:44-8.
22. Smolen J S, Breedveld F C, Schiff M H, Kalden J R, Emery P, Eberl G, et al. A simplified disease activity index for rheumatoid arthritis for use in clinical practice, Rheumatology (Oxford) 2003;42;244-57.