PCCA PROJECT 124/2014

TITLE: **THE TH17 NETWORK - PREDICTOR OF RESPONSE TO ANTI-TNF THERAPY IN RHEUMATOID ARTHRITITS**

ACRONYM: **TH17NET**

PROGRAMMME: **PARTNERSHIPS**

DOMAIN: **HEALTH**

DURATION: **01.07.2014 – 30.09.2017**

CONSORTIUM:

Organization	Type	Role
Centrul Clinic de Boli Reumatismale „Dr. Ion Stoia”	UNI	Coordinator - CO
Spitalul Clinic „Sfanta Maria”	UNI	Partner 1 – P1
Institutul National de Cerectare-Dezvoltare in Domeniul Patologiei si Stiintelor Biomedicale „Victor Babes”	INCD	Partner 2 – P2
Lotus MED SRL	SRL	Partner 3 – P3

BUDGET (LEI):

- PUBLIC BUDGET: **1.250.000 LEI**
- OWN BUDGET: **187.500 LEI**

ABSTRACT

Rheumatoid arthritis (RA) variants and unpredictable responses of patients to therapy (innate or acquired resistance of individual patients) raise important issues in disease management. The detrimental effect of inefficacious therapy in terms of progressive joint destruction and harmful side-effects, along with high healthcare costs, drive the research effort to identify biomarkers for predicting response to therapy. **Aim of the project**: to create and implement innovative medical services based on a proof-of-concept pilot study for clinical research, having as goal to demonstrate that the peripheral Th17 network can predict the response of RA patients to a particular anti-TNF therapy. The project is in line with the «Health» topic 4.1.3 «Investigation and interventional methods based on cellular and molecular medicine, genomics and proteomics». **Consortium**: 2 clinics (the Clinical Center of Rheumatic Diseases "Dr. Ion Stoia" and "Sf. Maria" Clinical Hospital, Bucharest), 1 national R&D institute ("Victor Babes" National Institute of Pathology, Bucharest) and 1 SME (the medical center Lotus Med SRL). The consortium structure responds to the objectives and tasks of the project (development of a pilot clinical study, transfer of results/technology towards the participating SME), and is based on the expertise of the partner institutions. The project is coordinated by the Clinical Center of Rheumatic Diseases "Dr. Ion Stoia" which has the scientific, logistic and managerial capability to implement the project. **Originality**: 1) The network-type approach for biomarker discovery in RA; 2) Integrated cellulomic, proteomic and genomic methodology; 3) Bringing into light granulocytes in RA, as sensitive sensors of inflammatory status changes and active players in the Th17 network. **Main outcomes**: **1)** A panel of Th17 pathway-focused markers and associated laboratory tests for assessing the response of RA patients to anti-TNF therapies, to be initially implemented in the diagnosis centers of the participating SME and national R&D institute; **2)** database and biobank with relevant information and biologic samples, for further study development (biomarkers validation in a larger clinical study, search for additional biomarkers to predict the response of individual RA patients to a particular therapy); **3)** biochemical pathways related to the Th17 network, which may represent targets for new therapeutic intervention in RA; **4)** publications, communications and a project-dedicated workshop. **Benefits** - the study represents a step forward in improving the management of rheumatoid arthritis, in the benefit of a) patients and rheumatologists – a better control of disease and therapy by a personalized approach; b) the Healthcare System – delivery of more cost-effective therapies. Added value for the **SME**: to achieve economic growth and to gain competitive advantage by developing research-driven medical activities, in collaboration with research institutes and rheumatology clinics.

OBJECTIVES

General objective

To create and implement innovative medical services by developing a proof-of-concept pilot study for clinical research, aiming to demonstrate that the peripheral Th17 network can predict responsivity / non-responsivity of RA patients to a particular anti-TNF therapy.

Specific objectives

1. To investigate the Th17 network in patients defined as therapy responders versus patients which are non-responders, using a multi-parametric approach.

- To establish for RA patients the activity of disease and its progression, along with their responsivity to anti-TNFα treatment
- To characterize in RA patients the Th1/Th2 and Th17/Treg functional polarization
- To investigate in-depth the functional phenotype and the activation potential of peripheral cells within the Th17 network (Th17 lymphocytes, monocytes and granulocytes)
- To correlate the status of the Th17 network with: 1) the profile of Th17-related cytokines / chemokines/ growth factors in serum; 2) the intracellular oxidative response
- To correlate clinical, biological and immunological data with the response to therapy for the recruited RA patiens

2. To set-up a database with relevant information gained in the study (individual and statistically processed data)

3. To build a biobank with biological samples of the investigated RA patients

4. To design a pathway-focused panel of tests for assessing the response of RA patients to anti-TNF therapies - to be used in research clinical trials, diagnosis centers and hospitals.

5. To disseminate results obtained in the project towards academia, health providers and decision factors, pharmaceutical industry, patients and the general public.

6. To involve young researchers in high level applied research.

Outcomes

1. A **panel of markers** associated to the Th17 network, that correlate with the patients' responsivity / non-responsivity to a particular anti-TNF therapy;

2. A **database** with demographic, clinical and biological information on RA patients on anti-TNF therapy, at which the consortium members will have access after project completion in the frame of the Partnership agreement.

3. Biobank with serum and whole blood (preserved for RNA extraction) from well-characterized RA patients. Biological samples will be available for a larger research clinical trial aiming to validate the candidate maker(s) demonstrated in this pilot study. The biobank is open for the consortium members and for the EU-ROS network (COST action BM1203, 2012-2016).

4. Documentation for a **pathway-focused panel of tests** for assessing the response of RA patients to anti-TNF therapies - to be used in research clinical trials, to be implemented in diagnosis centers and hospitals.

5. at least 1 **publication** in ISI-ranked journals, at least 4 **communications** at national and international congresses of rheumatology, immunology and ROS biology, 1 **workshop** in the topic of the project, the project **website**.

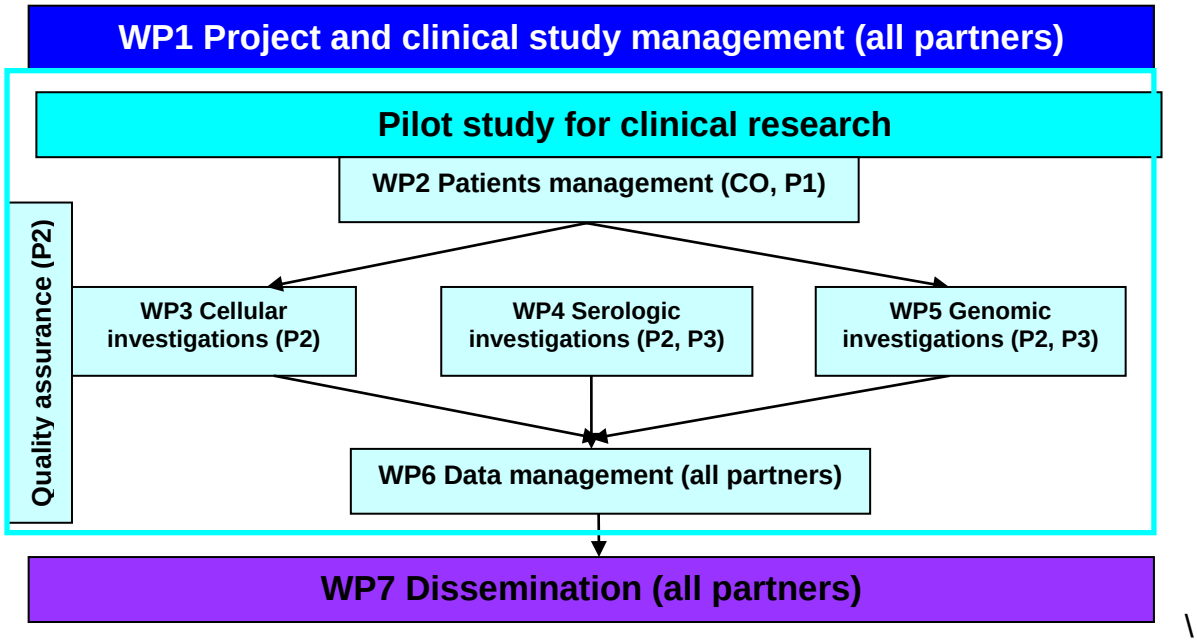
6. A **multi-disciplinary consortium** for developing clinical studies in rheumatic diseases.

7. Innovative, research-driven medical services to be implemented in the diagnosis centers of the participating SME and R&D institute.

Perspectives

- Knowledge and data obtained in the project will inform the design and conduct of a larger research clinical trial aiming to validate candidate marker(s) associated to the Th17 network as surrogate endpoint for distinguishing patients refractory to a particular anti-TNF therapy;
- The study will bring new insight into the cellular pathways underlying the pathological mechanisms in RA; we will draw maps of biochemical pathways related to the Th17 network, which may represent targets for new therapeutic intervention in RA.
- The project may contribute with valuable data to the National Registry for Biological Therapies and to international networks/platforms in the field of the project.

WORKPACKAGES



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CONSORTIUM

CO The **Clinical Center of Rheumatic Diseases „Dr. Ion Stoia”** (CCBR) is a mono-specialty hospital, which *is the sole national medical unit specialized in the diagnosis and treatment of rheumatic diseases*. Founded in 1961, CBR has the longest and the more vast tradition in rheumatology in Romania, having as objective to apply *advanced diagnosis and treatment methods.* Its activity addresses mainly socially active patients, aiming to improve their working capacity and thus to reduce the societal burden of premature retirement due to medical disability. CBR provides in- and out-patients assistance in rheumatology for Bucharest, but also for other regions where rheumatology services are missing. CBR initiated this project addressing by research a major issue in medical practice regarding the early assessment of the patient's response to a particular treatment in rheumatoid arthritis. This is of utmost importance not only for patients, but also for the national health system. Moreover, the project answers to the increasing need for extending the providers of specialized medical services for rheumatic diseases, by including private and R&D medical centers which are committed and have the economic force to implement innovative, research-driven laboratory tests. Having a medical team with exquisite expertise in rheumatic diseases, being endowed with 126 beds and an outpatient unit, along with comprehensive laboratory services, CBR has the capacity to initiate in collaboration with „Sf. Maria” Clinical Hospital a pilot clinical study in rheumatoid arthritis. The participation of the team members in international clinical trials is an important advantage for the project, complemented by the experimental expertise and the state-of-the-art research infrastructure of the participating R&D institute.

Partner 1 - ”Sf. Maria” Clinical Hospital (SCSFMB) through UMF "Carol Davila" Bucharest, the Clinic of Internal Medicine and Rheumatology, is considered a national reference center for the diagnosis and care of patients with rheumatic diseases.

It is considered that from the approximately 10,000 yearly hospitalized patients in the hospital, 50% were hospitalized in the Internal Medicine and Rheumatology Departments, presenting various forms of rheumatic pain. Most of rheumatic patients hospitalized annually represent the patients with rheumatoid arthritis (RA), hospitalized for diagnosis or for disease monitoring.

In the "Sf. Maria " Hospital patients with RA are clinically evaluated by clinical examination, disease and quality of life scores, etc., and by laboratory tests (biochemical investigations, immunological tests, radiology, ultrasound investigation, computer tomography or magnetic resonance imaging) in order to administrate an appropriate treatment, as early as possible, in order to reduce physical disability that characterizes the disease. We have to point out that approximately 15% of the patients with RA from the "Sf. Maria" Hospital, the Department of Internal Medicine and Rheumatology, are treated with biological therapy approved by the National Health Insurance (CNAS), according to the national guidelines developed in agreement with the European ones. For this reason, the patients' evaluation for continuing biological therapy, for switching or for withdrawal, represents a challenge for rheumatologists for whom the primary therapeutic target is disease remission.

The scientific activity carried out in the "Sf. Maria" Clinical Hospital, the Department of Internal Medicine and Rheumatology is commendable. Since 2005, in the clinic has been developed the Research Centre of Systemic Pathology and Treatment of Rheumatic Disease, C Type, approved by CNCSIS. In this context, in the clinic were performed multiple international courses (clinical densitometry, muscular-skeletal ultrasound imaging), held under the auspices of renowned specialist organizations (EULAR, ISCD). The team has actively participated in the multiple national studies of fundamental research applied in clinical practice, in partnership with acknowledged research institutions (National Institute of Pathology "Victor Babes", Institute "Dr. I. Cantacuzino"), or multicentric clinical trials for evaluating the effectiveness and safety of various therapeutic approaches that have entered or will enter later in the clinical practice.

Partner 2 – INCD Victor Babes Founded in 1887, Victor Babes National Institute of Pathology (INCDVB) is the oldest institute of biomedical research in Romania, whose research activity is nowadays focused on human pathology (cancer, neurodegenerative diseases, immune disorders etc).

We are committed to develop innovative diagnostic tools and personalized treatment strategies, by an integrative approach joining pathology, medical genetics, cellular and molecular medicine, immunology. Still at the forefront of fundamental research, the Institute is today a center of reference for human disease diagnosis and monitoring (advanced medical services provided by the institute's Diagnosis Center). INCDVB has participated in several international projects: NATO Science for Peace, MNT-ERA, FP7-PEOPLE, various bilateral cooperation, and international clinical trials. 2 POSCCE projects coordinated by foreign scientists and 3 POSDRU projects are financed by the European Social Fund. We participate in international research networks within various COST Actions. In the last 6 years, the scientific activity in INCDVB is reflected in more than 180 articles published in ISI-indexed journals (total relative influence score = 195,78), having more than 1220 ISI-citations. 7 national patents were generated by applied research on innovative therapeutic approaches. The institute is certified SR EN ISO 9001:2008 for research and biomedical services and several laboratories are accredited according to SR EN ISO 15189.
Contribution in the project: due to its expertise and state-of-the-art experimental facilities, INCDVB is responsible in this project for the advanced investigations on the Th17 network in RA: a) cellular tests using multi-parametric flow cytometry; b) proteomic investigations by multiplexing using xMAP arrays; c) pathway focused genomic screening using PCR arrays dedicated to the Th17 network and the oxidative stress response. INCDVB will also perform collection and statistical processing of data, and will participate in the validation of selected markers and in transferring technology/knowledge towards the SME partner. This project is a step-forward of the studies we developed since 2001, regarding the immune disturbances in RA and the impact of therapy. INCDVB is committed 1) to develop research-driven innovative medical services for assisting rheumatologists in disease and therapy monitoring; 2) to foster knowledge in the field of oxidative stress (as member of the EU-ROS network, COST Action BM1203).
Partner 3 SC Lotus-Med SRL (LOTUS) is present for 15 years in the market of private laboratory health services. Its haematology, biochemistry, immunology and microbiology departments are monitoring more than 2600 patients for 20 private dialysis centres, and more than 1000 patients for diagnostic centres and health institutions.
In the last five years, Lotus-Med's interest has been extending in the field of *molecular biology techniques* for diagnosing and monitoring infections with human immunodeficiency virus, hepatitis viruses (B, C and D), cytomegalovirus and human papilloma virus.
In the last two years, Lotus-Med has expanded its testing panel with investigations in the field of tumor pathology, with emphasis on predictive and prognostic markers, being able to perform for more than 200 patients / month the assessment of breast cancer markers, using immunohistochemistry and chromogenic *in situ* hybridisation.
Growing and developing its expertise, the company was able and willing to bring its contribution in clinical research; since 2011 Lotus-Med is member of a consortium in a winning PNCDI II project focused on cancer diagnosis (see above).
The main objective of Lotus-Med is to achieve excellence by promoting innovation, quality and accuracy in medical services and by expanding the panel of investigations with state-of-the-art approaches and technologies, in order to provide improved medical care and increased satisfaction for both patients and health-care professionals.
In this project, Lotus-Med aims to address new pathologies (rheumatic diseases) for enlarging the area of medical services it provides, both with specific conventional tests (CRP, ACPA) and innovative molecular investigations at the level of Th17 network. Implementation of a research-driven panel of tests focused on patient's response to a particular therapy will definitely support rheumatologists for establishing an efficient therapeutic strategy. Lotus-Med responds to the need for advanced investigation methods in rheumatic diseases, like molecular ones, which are for the moment not accessible for hospital laboratories.

CONTACT

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USEFUL LINKS

PARTNERSHIPS Programme
http://uefiscdi.gov.ro/Public/cat/593/PARTENERIATE.html

Horizon 2020 - The EU Framework Programme for Research and Innovation
http://ec.europa.eu/programmes/horizon2020/

Romanian Society of Rheumatology
http://www.srreumatologie.ro/

Romanian Society of Immunology
http://societateadeimunologie.ro/ro/

PROJECT'S PROGRESS

Phase 1 / 2014

In the 2015 phase of the TH17NET project activities were mainly focused on preparing the clinical study: procedures regarding RA patients selection and enrolling, biological samples and their flow for cellular and molecular investigations, along with advanced methods to be used in the clinical study (pathway-focused PCR array for genes involved in controlling the redox status of peripheral leukocytes).

The project-dedicated database was created (a preliminary form), that will be improved next year when a larger volume of data will be obtained.

For promoting the project towards the academic community and the health system, the project-dedicated web page was created.

We have started selction and enrollment of RA patients, according to criteria defined specifically for this study. Biological samples from 15 RA patients (serum, plasma, whole blood RNA, and RNA from isolated mononuclear cells and granulocytes were collected, processed and stored. Samples stored will be used in future stages of the project for proteomic and genomic investigations.

Preliminary tests were conducted for methodology development regarding the identification of particular characteristics of RA patients unresponsive to synthetic DMARDs therapy (methotrexate and leflunomide)

- 1 RA patient was investigated before the anti-TNF therapy onset, at 4 weeks and 13 weeks thereafter. For this patient a genomic scereening was done on the impact of the anti-TNF therapy on the expression of 84 genes in mononuclear cells, involved in oxidative stress and ntioxidant response. Results revealed that anti-TNF therapy alters the expression of genes involved in: a) the generation of ROS (NOX4, NOX5, DUOX1, MPO, NOS2); b) endogenous antioxidant system (catalase, peroxireoxine, thioredoxin), c) the protection of cells against oxidative stress; c) recruitment of leukocytes.

- 7 sera of RA patients were tested for the concentration of the in "danger" molecule HMGB1, and of the soluble form of the receptor it binds to (sRAGE). 9 healthy subjects constituted the control group in this stage of the method development.

Phase 2 / 2015

In the 2015 phase of the project 52 RA patients were investigated. For 43 patients anti-TNF therapy was initiated, and 11 patients would represent therapy controls (MTX, anti-TNF). Patients were characterized clinically, radiologically and biologically.

Blood was collected, and serum, plasma, and RNA from mononuclear cells, granulocytes and total leukocytes were processed. Biological samples were stored in the study-associated biobank.

A preliminary serological study was conducted to determine the serum levels of soluble factors specific for the Th17network (cytokines, chemokines and growth factors): IL-1β, IL-6, IL-10, IL-12p70, IL-13, IFNγ, GM -CSF, TNF, IL-21, IL-23, IL-28α, MIP3α / CCL20, IL-15, IL-17, IL-17 F, IL-22, IL-33, IL-17E, IL-27, IL-31, TNFβ. The investigations were conducted by protein multiplexing (Luminex technology) for 14 patients during anti-rheumatic therapy (3 time points during 6-8 months). 3 healthy subjects, volunteer blood donors were the normal control group.

To validate the results obtained by protein multiplexing, some soluble factors (TNF, IL-6, IL-6R, IL-8, IL-17A / F) were evaluated by ELISA.

Gene expression changes involved in Th17 cell differentiation and function were investigated by PCR array using the "Human Response Th17 PCR Array" kit (Qiagen) that contains 84 genes relevant to the Th17 network. The study was conducted in 3 patients on adalimumab therapy, and. 3 healthy subjects were used as controls.

The database was initiated in Phase 1 completed demographic parameters, clinical, biological and molecular study of patients would take.

Some of the data contained so far in the study were interpreted statistically to identify statistical trends of the investigated biological parameters, and to screen for a panel of candidate biomarkers associated to the Th17 network for describing the individualized therapy response of RA patients.

The knowledge gained in the project were disseminated through 4 communications at national and international scientific meetings.

Phase 3 / 2016

29 RA patients were investigated in 2016, and hence the group of recruited RA patients reached 81 patients with a total of 151 visits. RA patients were clinically, radiologically and biologically characterized by the participating clinics. Blood was harvested and processed for obtaining serum, plasma and RNA isolated from peripheral mononuclear cells (PBMC), granulocytes and whole leukocytes population. Biologic samples were stored in the study-dedicated biobank.

The cellular study was initiated this year, going beyond the assessment of PBMC, lymphocytes, monocytes and granulocytes counts in peripheral blood. The study had as main focus to clarify particular issues revealed by the molecular study, such as the effect of anti-TNFα therapies on the traffic of leukocytes between the inflamed synovium and blood of RA patients. For 29 patients we performed immunophenotyping by flow cytometry of blood lymphocytes and monocytes. For validating molecular data using an alternative method, PBMCs from 27 RA patients were prepared for flow cytometry investigations on Th17-Treg polarization in the blood of RA patients.

The genomic study performed by pathway-focused PCR array was continued in 2016 aiming to characterize the dynamics of the expression profile for genes related to the differentiation and activation of the Th17 network, or related to the oxidative stress and antioxidant response in PBMCs from RA patients on adalimumab therapy.

Molecular data were correlated with the concentration of cytokines, chemokines and growth factors in serum, determined by protein multiplexing using the Luminex technology. ELISA was used for validating the protein multiplexing data.

Experimental conclusions Molecular data show that responsivity of RA patients to adalimumab is associated with a pro-inflammatory phenotype in blood, characterized by: 1) increase of pro-inflammatory Th17 cells in blood; 2) increase of activated T cells; 3) increase of cells with enhanced intracellular oxidative activity in blood. These results sustain the hypothesis that adalimumab might impact leukocyte traffic between synovium and blood. It is possible that adalimumab triggers the egress of pro-inflammatory cells from the synovium, hence reinforcing pro-inflammatory processes in blood despite the decrease of disease activity.

Our study reveals that correct evaluation of the immune response related to the Th17 network and the intracellular oxidative stress in RA patients on anti-rheumatic therapy should be performed in dynamics, each patient being its own control. Such an approach is necessary considering the huge individual variability in RA, including several disease variants, and due to the fact that during therapy important changes of immune parameters occur, reflecting reaction to therapy and development of cellular and humoral responses for counteracting inflammatory processes at local and systemic levels.

DISSEMINATION

1. **Annual Immunology Conference with international participation**, 22-24 Octomber 2015, INCD "Victor Babeş", Bucharest

HETEROGENEITY AND PLASTICITY OF THE INNATE AND ADAPTIVE IMMUNE RESPONSE IN RHEUMATOID ARTHRITIS

Gina Manda, Gheorghita Isvoranu, Dobre Maria

2. **Annual Meeting of „ Victor Babes" National Institute of Pathology, and 8th National Symposium of Pathology**, 19-21 November 2015, INCD "Victor Babeş", Bucharest.

PARADIGM SHIFT IN RHEUMATOID ARTHRITIS

Gina Manda, Gheorghita Isvoranu, Maria Dobre

3. **ACR Annual Meeting, San Francisco, USA, 7-11 noiembrie 2015**

WHEN COMPARED WITH A COMPUTERIZED SYSTEM BOTH EXPERIENCED AND IN-TRAINING SONOGRAPHERS HAVE DIFFICULTIES TO SELECT THE BEST DOPPLER IMAGE FROM A CINE-LOOP

Florian Berghea, Violeta Vlad, Lavinia Palanciuc, Violeta Bojinca, Florentin Vreju, Luminita Enache, Monica Copotoiu, Alexandra Kosevoi, Teodora Serban, Denisa Stanciu, Mihai Abobului, Andreea Borangiu, Andra Rodica Balanescu and Ruxandra Ionescu

POWER DOPPLER CONTINUOUS QUANTITATIVE ASSESSMENT TECHNIQUES IS FASTER THAN SEMI QUANTITATIVE ASSESSMENT IN IDENTIFICATION OF THERAPEUTIC RESPONSE

Florian Berghea, Violeta Vlad, Mihai Bojinca, Luminita Enache and Ruxandra Ionescu.

4. **Congresul National de Reumatologie 2016, organizat în perioada 13-15 Octombrie 2016 la Biblioteca Națională, Bucureşti**

THE MOLECULAR FINGERPRINT OF THE TH17 NETWORK IN PERIPHERAL MONONUCLEAR CELLS FROM RHEUMATOID ARTHRITIS PATIENTS

Manda Gina, Dobre Maria, Mocanu Mihaela, Neagoe Ionela Victoria, Groseanu Laura Maria, Berghea Florian, Mogosan Corina Delia, Predeteanu Denisa, Codreanu Catalin

THE MOLECULAR PROFILE OF OXIDATIVE STRESS AND ANTIOXIDANT RESPONSE IN PERIPHERAL MONONUCLEAR CELLS FROM RHEUMATOID ARTHRITIS PATIENTS

Manda Gina, Dobre Maria, Denisa Predeteanu, Codreanu Catalin

5. **7th EULAR Course on Capillaroscopy**, 8-10 September 2016, Genoa, Italy.