Relazione Scientifica I anno Dottorato di Ricerca

Parte Scientifica

PhD Research Protocol: Study on characterization and evaluation of biological function of different biomarkers, such as Perforin, Osteopontin, anti-Osteopontin antibodies and soluble ICOS, in patients with allergic diseases.

Abstract
This is a cross-sectional study aimed to identify, characterize and evaluate the biological function of different emerging biomarkers of the immune response (such as, for instance, Osteopontin, anti-Osteopontin antibodies, soluble ICOS, Perforin) in common allergic diseases (i.e., bronchial asthma, allergic rhinoconjunctivitis, atopic dermatitis, allergic contact dermatitis, drug allergies, Hymenoptera venom allergies).
All these markers will be evaluated in both serum and DNA in different groups of patients for each allergy type, diagnosed according to criteria provided by the official guidelines, and compared to a healthy control population.
The aim is to identify novel serological and genetic markers that might be useful in the characterization of allergic diseases from a pathophysiological point of view, in the evaluation of the different risk profiles for allergic diseases and in the prediction of both clinical evolution and patients responsiveness to currently available treatments.

Introduction
The immune system is represented by a complex of molecules, cells, tissues and organs targeted to protect against different foreign organisms that are potentially harmful. Schematically, the functional organization of the immune system provides the maintenance of both a state of non-responsiveness to *self* molecules and a protective response against foreign antigens (*not self*). Besides the common peripheral tolerance mechanisms, such as anergy, idiotypic network, cellular apoptosis, anatomical segregation of self-antigens and clonal ignorance, the discovery of regulatory T cells (Treg) in active suppression of immune responses has been crucial. In 1995 Sakaguchi et al. discovered a population of CD4+ T cells characterized by high surface expression of CD25 and ability to prevent the onset of autoimmune phenomena in a murine model. Multiple studies have been conducted afterwards, in order to characterize Treg cells about their immunophenotypic and functional aspects, in physiological and pathological conditions.
At the moment, quantitative and qualitative impairment of immune regulatory functions is supposed to be mainly involved in the genesis of both allergic and autoimmune disorders. The role of Treg cells in the onset of autoimmune diseases is confirmed by immune effects of thymectomy in neonatal period in experimental murine models.
"Allergy" is a pathophysiological condition characterized by an abnormal reactivity to particular substances (haptens) that are generally harmless in healthy subjects. The term *"atopy"* refers to the hereditary tendency to type 1 hypersensitivity manifestations (according to Gell and Coombs classification), with a prevalent involvement of IgE antibodies isotype. Allergic diseases are characterized by the tendency, in atopic subjects, to develop immune responses, in different body districts, with a specific polarization towards type 2 T helper cells (Th2) and production of IgE antibodies against environmental antigens that are commonly harmless. Several studies have demonstrated that atopic patients show a statistically lower percentage of circulating Treg if compared with controls; in addition, Treg isolated from allergic subjects are less effective in suppressing the proliferation of CD4+CD25- T cells induced by allergens. The deviation of naïve T lymphocytes towards Th2 rather than Th1 cytokine pattern (Th1 polarization is pathognomonic for autoimmune diseases) after exposure to allergens, may therefore be related to quantitative and/or qualitative defects of Treg cells.
Allergies and autoimmune diseases are both characterized by multifactorial etiology, with an interaction between a complex immunological network (consisting in cells, cytokines, chemokines, growth factors, receptors and soluble factors) and environmental causes, on the basis of a genetic predisposition linked to multiple genes. HLA molecules are the best known genetic factors at the moment, but also other genes codifying molecules commonly involved in the modulation of immune responses, may be involved. In particular, molecules implicated in the down-regulation of immune response are recently of great interest.
The Research Laboratory of Immunology, Department of Health Sciences, University of Piemonte Orientale "Amedeo Avogadro", has previously identified genetic mutations that may influence the process of the immune down-regulation: among these, genes related to Osteopontin, Perforin and ICOS production are notable.
Osteopontin (OPN) is a proinflammatory cytokine inhibiting the "activation-induced cell death" of lymphocytes (AICD). OPN in humans is encoded by the gene SPP1 (secreted phosphoprotein 1) and was first identified in osteoblasts in 1986. OPN is a negatively charged protein of the extracellular matrix, composed of about 300 amino acids. It is synthesized by a variety of different cell types, including fibroblasts, dendritic cells, macrophages, neutrophils, T and B lymphocytes, pre-osteoblasts, osteoblasts, osteocytes, odontoblasts, chondrocytes, smooth muscle cells, skeletal muscle myoblasts, endothelial cells, inner ear, brain, kidneys, and placenta cells. OPN binds to several integrins (including $\alpha4\beta1$, $\alpha9\beta1$, $\alpha9\beta4$) expressed by leukocytes and involved in adhesion, migration and cellular survival. Furthermore, it provides chemotactic properties, modulates cell activation and cytokines production.
In the Laboratory of Immunology, genetic variants of OPN (-B and-C haplotypes) determining a higher production of basal levels of this cytokine, have been identified. Moreover, the increase of OPN production has been shown to be associated with a significantly higher risk of autoimmune lymphoproliferative syndrome (ALPS), multiple sclerosis (MS) and systemic lupus erythematosus development (Blood, 2004; J Neuroimmunol, 2005; Arthr Rheum, 2005).
The role of OPN has been recently investigated also in allergic inflammation and asthma model. OPN deficiency seems to have a protective role against bronchial hyperresponsiveness and tissue remodeling in an animal model. In addition, it has been shown that OPN expression is statistically higher in patients with asthma than in healthy subjects and is associated with tissue remodeling and severity degree of the disease. Moreover, OPN levels have been observed to be significantly increased in sputum supernatant

of smoking asthmatic patients. Further observations have recently emerged, i.e.: the correlation of OPN with the onset and the chronicization of allergic contact dermatitis; the increase of OPN in long-term bee venom immunotherapy; the suppression of OPN during H1-antihistamine (Levocetirizine) assumption in allergic subjects.

The study of **anti-Osteopontin antibodies** potentially provides several applications because of OPN ubiquity, structural and functional role. In scientific literature, many publications have recently shown a possible usefulness of anti-OPN antibodies evaluation in the treatment of cancer and metastatic conditions. However, at the moment no significant studies have been published in the allergy field yet.

Perforin is contained in cytotoxic lymphocytes (CTLs and NKs) granules and plays a key role in cell-mediated cytotoxicity. It is crucial in the immune responses against viral infections, but it is also involved in the immune system down-regulation through activated lymphocytes or antigen presenting cells killing. Biallelic mutations of Perforin gene have a role in determining familiar hemophagocytic lymphohistiocytosis (HLH), with lymphocytes accumulation and reduced cytotoxic activity. In the Laboratory of Immunology, monoallelic mutations of Perforin gene have been shown to be associated with an increased risk of developing autoimmune lymphoproliferative syndrome (Blood, 2006).

Allergic and non-allergic asthma seems to be associated with a significant increased Perforin production, if compared to controls. In addition, the expression of Perforin in CD4+ T cells in patients with non-allergic asthma has been observed to be significantly higher than in subjects with allergic asthma.

Specifically, the suppression of airways allergic inflammation by CTLs has been demonstrated to be Perforin-dependent: only CTLs with sufficient Perforin expression, in fact, can inhibit the airways eosinophilic infiltration, the mucus production and the accumulation of cytokines in bronchoalveolar fluids. Another study conducted on Perforin and CTLs activity in a cohort of patients with atopic dermatitis and allergic rhinoconjunctivitis has recorded a significantly lower amount of lymphocytes with Perforin positive granules in all the subjects, compared to controls. Additionally, CTLs of atopic patients seem to release Perforin more quickly with respect to healthy subjects. Perforin is likely involved also in the pathogenesis of non immediate allergic reaction (cell-mediated or type IV hypersensitivity reactions) and drug allergies, but currently available studies do not provide conclusive data.

A trial conducted in patients with allergic contact dermatitis, has evidenced an increased lymphocyte expression of Perforin and Granzyme B genes, in comparison to healthy controls; thus a possible pathogenetic role of Perforin in epithelial cells damage, contributing to the onset and the chronicization of contact dermatitis, must be considered.

ICOS (**I**nducible T-cell **COS**timulator) is a protein encoded by the gene ICOS in humans. ICOS or CD278 is a costimulatory molecule belonging to the superfamily of CD28 cell surface receptors and CTLA-4; it is expressed by activated T lymphocytes and it controls T cells cytokine secretion. The protein produced by ICOS gene forms homodimers and plays an important role in both intercellular signaling and regulation of cellular proliferation, especially regarding Th2 lymphocytes. When T helper cells receive an inappropriate activation stimulus through their receptor TCR (T Cell Receptor), ICOS is up-regulated and determines the production of immunoregulatory cytokines, such as IL-10 and TGF-beta, that provide important anti-inflammatory functions and immunosuppressive properties. At the Laboratory of Immunology a variant of ICOS gene associated with small quantities IL-10 production and with an increased risk of developing multiple sclerosis has been previously identified. The molecular mechanisms controlling ICOS expression are not fully known yet. Different studies have revealed that AP-1 and Fos-related antigen-2 (FRA2) molecules are highly correlated with the expression of ICOS: AP-1 binds the receptor after TCR/CD28 stimulation. Furthermore, many cytokines may increase ICOS expression in naïve and effectors cells, independently from the TCR/CD28 activation, inducing AP-1 to bind its receptor on the promoter (AP1-RE). This study then suggests that AP-1 cytokine activation may be one of the mechanisms that maintain high levels of ICOS expression in chronic inflammations. Regarding the studies on allergic inflammation, ICOS has also been found to be involved in IL-35 production by Treg cells, with consequent reduction of IL-17 production and suppression of airway hyperresponsiveness (IL-17-mediated), in mouse models. This study therefore suggests this immunological pathway as a possible therapeutic target in the treatment of bronchial asthma. In another study, anti-ICOS and anti-CD28 antibodies have found to block IL-4 and IL-13 production, that have a fundamental role in IgE-mediated responses. Furthermore ICOS has been shown to modulate both T cells differentiation and function, particularly among Treg cells, after allergen contact, intervening in the mechanisms of tolerance or development of allergic sensitization.

sICOS is the soluble form of ICOS and represents an emerging biomarker of immune responses; at the moment there are no publications in the allergy field. A previous study, published in the Archives of Dermatological Research, has demonstrated the presence of significantly increased levels of sICOS in sera from patients with diffuse cutaneous systemic sclerosis (SSc), compared to controls. In particular, patients with higher levels of sICOS seem to be more frequently associated with pulmonary impairment and significant deterioration of lung function. In addition, serum sICOS may apparently be correlated with disease severity and activity in localized cutaneous sclerosis. sICOS assessment in sera from patients with early SSc therefore, may represent a useful tool with prognostic meaning.

Another study, conducted in patients with HCV-related chronic liver disease, has correlated sICOS and Programmed Cell Death Protein 1 (SPD-1) levels with chronic liver injury induced by HCV. Recorded sICOS and SPD-1 serum levels are significantly higher in patients with chronic liver disease compared to the control group. In addition, the mRNA expression of these proteins is statistically increased in cases compared to controls and has likely to be correlated with the dysregulation of activated T lymphocytes and the damage from chronic HCV.

In conclusion, our project aims to identify and create innovatively a bio-bank of new biological markers, such as those previously mentioned, in a population of allergic subjects compared with a control group. The results could have a profound impact on the understanding of predisposing factors and pathogenetic mechanisms in common allergies (in terms of genetics and molecular biology). Therefore this study is significantly useful in the context of searching for additional tools in clinical monitoring and prediction of responsiveness to treatment of allergic patients.

METHODS

a. Design

This is a cross-sectional study aimed to identify, characterize and evaluate the biological function of different biomarkers emerging of the immune response (such as Osteopontin, anti-Osteopontin antibodies, soluble ICOS, Perforin) in common allergic diseases (i.e., bronchial asthma, allergic rhino-conjunctivitis, atopic dermatitis, allergic contact dermatitis, drug allergies, Hymenoptera venom allergy).

b. Subjects enrolled in the study

Adults who meet the diagnostic criteria established for the allergic diseases mentioned above, will be identified both retrospectively and prospectively. For the retrospective recruitment, subjects potentially suffering from allergies will be identified on the basis of their clinical history from the Allergology and Clinical Immunology Unit, "Maggiore della Carità" University Hospital, Novara. At the time of informed consent and enrollment, patients will have to answer a questionnaire regarding demographics, medical history (family and personal) and specific information on their allergic diseases. Patients data and blood samples will be immediately made anonymous after their collection and will be identified by a code (with deleted data, date of birth, address). All the data useful to re-identify patients will be stored in a separate archive.

Summary of inclusion and exclusion criteria

Inclusion criteria

• Male or female patients, aged 18 years and over, suffering from allergic diseases (bronchial asthma, rhino-conjunctivitis, atopic dermatitis, allergic contact dermatitis, drug allergies, Hymenoptera venom allergy) diagnosed according to criteria and methods established by official guidelines; • signed and dated informed consent.

Exclusion criteria

• Patients under 18 years of age; • inflammatory and/or infectious episodes during the last month • immunodeficiency / immunosuppression; • systemic autoimmune diseases; • malignancies.

Sample Size

The project will be carried out at the Allergology and Clinical Immunology Unit, "Maggiore della Carità" University Hospital, Novara, in collaboration with the Research Laboratory of Immunology of the Eastern Piedmont "Amedeo Avogadro" University and aims to recruit 20 subjects at least for each type of allergic diseases, for a total amount of about 120 subjects. The control population will be represented by an equal number of subjects with similar demographic characteristics, already available in the biological bank of Health Sciences Department.

Main problem

•To evaluate the involvement in susceptibility to allergic diseases of certain gene variations that have been associated with various autoimmune diseases, i.e. sequencing of genomic DNA (exons) for the detection of Perforin gene rare mutations and typing for Real Time PCR some polymorphisms of Osteopontin gene;

• To assess the role of new serological markers (previously identified in various autoimmune diseases) in different allergic diseases, such as Osteopontin, anti-Osteopontin antibodies and soluble ICOS.

The aim is to identify novel serological and genetic markers that can be useful in the characterization of allergic diseases by a pathophysiological point of view and allow to predict the clinical evolution and the responsiveness to currently available treatments (such as immunotherapy to common inhalant allergens and Hymenoptera venoms).

Main purpose:

1. to create a case record of subjects phenotypically compatible with allergic diseases, diagnosed through officially recognized methods;
2. to create a bio-bank of DNA and sera representing a common allergic population;
3. to generate a catalogue of genetic variations in patients with allergic diseases, by sequencing of genomic DNA (exons) for the detection of rare mutations such as, for instance, those of the Osteopontin and Perforin genes;
4. to identify new serological markers (i.e., Osteopontin, anti-Osteopontin antibodies, soluble ICOS) that may be correlated with clinical evolution and responsiveness to currently available treatments for allergies, if possible.

Secondary purpose:

1. to validate genetic risk profiles in allergic diseases in different populations (e.g. gender, ethnicity);
2. to characterize, from a functional point of view, identified genetic variations and biomarkers in order to increase the knowledge on allergic diseases pathophysiology and in particular the immunological mechanisms involved in the development of allergic sensitization;
3. to develop a new diagnostic/therapeutic approach.

Collecting DNA samples

Blood samples collected from the Allergology and Clinical Immunology Unit, "Maggiore della Carità" University Hospital, Novara, will be sent and analyzed at the Research Laboratory of Immunology, Health Sciences Department, University of Piemonte Orientale "Amedeo Avogadro". Genomic DNA will be extracted from an aliquot of whole blood using "Gentra reagents PureGene" (Qiagen). Both biological samples (serum/plasma) and DNA will be stored at -80° C for future investigations always related to allergic diseases studies.

Whole exon sequencing of Perforin gene
Exons of the Perforin gene will be sequenced in order to identify rare genetic mutations, missense and nonsense. The study of the genome sequence of DNA (exons) will be performed at the Research Laboratory of Immunology, using a capillary automatic sequencer.

Analysis of Osteopontin gene polymorphisms
Genomic DNA polymorphisms + 1239A> C and -156G> GG of Osteopontin gene, that have been previously associated with various autoimmune diseases, will be analyzed through Real-time PCR.

Analysis of molecular markers
Presence and levels of plasmatic biomarkers (such as Osteopontin, anti-Osteopontin antibodies, soluble ICOS) will be assessed through ELISA test (Enzyme-Linked ImmunoSorbent Assay) in the Laboratory of Immunology.

Bioinformatics analysis
In a primary data analysis, a case/control approach will be used comparing the results obtained in all the cases with respect to controls. In a secondary analysis, all the data obtained will be compared among the various groups of patients (i.e., subjects with different allergic diseases) to investigate a possible trend of differences in the various groups; a trend identification will lead to a casuistry expansion in the diseases of interest and to a satisfying statistical power. The frequencies of genetic variations will be analyzed through Chi-Square test in the primary analysis and Fisher's test in the secondary one. Regarding Perforin, the total frequency of rare missense and nonsense mutations will be analyzed in the different study groups. The possible functional effect of each change will be assessed *in silico* using the software SIFT, PolyPhen and VAAST. Considering Osteopontin, the frequencies of the two above-mentioned polymorphisms will be recorded. Serological data will be compared using the nonparametric Mann-Whitney test.
Further evidence for a possible pathogenetic role of possible genetic mutations observed, will be evaluated through a comprehensive literature search for studies concerning function and structure of relevant genes/proteins, comparison with similar proteins in different species, protein modeling programs and functional studies in vitro.

PROTECTION OF PATIENTS
This is a basic research study that aims to find a genetic and biomolecular pattern that identifies allergic diseases innovatively. If during the genetic study strong data significantly emerge and may have a beneficial aspect for the patients, they will be informed.

Protection of sensitive data
All the subjects enrolled in the present study will have to sign an informed consent approved by the Ethics Committee. All the clinical information will be stored on PC whose access will be possible only with a custom password.

Potential short-term benefits for patients enrolled in the study
The possible risks for participating patients are minimal (related to venipuncture). There are no immediate benefits for the subjects of the study, since it consists in a basic research.

Importance of possible knowledge arising from the study
The potential long-term benefits of the present study are:
• a greater comprehension of the role of genetic factors and emerging biomarkers in allergic diseases;
• new knowledge on the possible mechanisms underlying the alteration of immune tolerance and leading to the onset of allergic sensitization and clinical manifestations;
• a possible new approach in terms of prevention and treatment.

Study expected end date: 31/12/2017

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43. Wang D, Zhou D, Du Q, Liang Q, Wang Q, Fang L, Wang G, Fan Q, Liu B, Zhou J, Tang Z, Wu H, Guo X, Jiao Y, Zhang G. Aberrant production of soluble inducible T-cell co-stimulator (sICOS) and soluble programmed cell death protein 1 (sPD-1) in patients with chronic hepatitis C. Mol Med Rep. 2013 Apr;7(4):1197-202.

Elenco congressi

Corsi di formazione professionale e di aggiornamento

1) Dal 26/06/2014 al 26/06/2014: **uditore** al corso "Progetto Genesi - Come tradurre un'ipotesi clinica in uno studio?" (7 ore), organizzato da Novartis Pharmaceuticals a Origgio (VA) – senza esame finale;
2) dal 09/05/2014 al 09/05/2014: **relatore** al corso "Advisory Board - Il Paziente Asmatico: La Nuova Gestione" (2 ore), organizzato da Mundipharma Pharmaceuticals S.r.l., presso Hotel San Carlo - Arona (NO) – senza esame finale. Topic: "Asma e BPCO: due volti di una stessa malattia?";
3) dal 28/02/2014 al 01/03/2014: **uditore** al corso "Med On Stage - Theatrical Based Medicine" (11 ore), organizzato da Alfa FCM presso Complesso Monumentale Santo Spirito in Sassia - Roma - esame finale superato, 11 crediti ECM. Corso di comunicazione;
4) dal 25/02/2014 al 25/02/2014: **uditore** al corso "Allergia Respiratoria: i nuovi Algoritmi Molecolari Diagnostico-Terapeutici" (8 ore), organizzato da Phadia S.r.l. e Stallergenes S.r.l. presso Starhotels Majestic - Corso Vittorio Emanuele II, 54 – 10123 Torino – senza esame finale. Aggiornamenti sull'allergologia molecolare e l'uso dei ricombinanti per diagnosi e trattamento delle allergopatie;
5) dal 21/10/2013 al 28/10/2013: **relatore** al "Corso Teorico Pratico di Allergologia" (7 ore), organizzato da A.O.U. Maggiore della Carita', Via Solaroli 17 - 28100 Novara - esame finale superato, 1 crediti ECM. Topic: "Allergologia Molecolare e test diagnostici innovativi";
6) dal 17/10/2013 al 17/10/2013: **relatore** al corso "Advisory Board - Soluzioni per un miglior controllo dell'asma" (2 ore), organizzato da Mundipharma Pharmaceuticals S.r.l. presso UNA Golf Hotel, Cavaglia' (BI) – senza esame finale. Topic: "Asma e BPCO: 2 malattie, 1 solo paziente?"

Convegni

1) Dal 16/05/2014 al 21/05/2014: **uditore** al congresso "American Thoracic Society (ATS) 2014 International Conference" (35 ore), organizzato da American Thoracic Society a San Diego, California (USA) – senza esame finale. Update sulle malattie toraco-mediastiniche, in particolare quelle cardio-polmonari (aspetti clinici, diagnostica, terapie, linee guida americane);
2) dal 28/03/2014 al 02/04/2014: **uditore** al congresso "World Congress of Asthma" (WCA) (24 ore), organizzato da INTERASMA (Global Asthma Association) presso Hilton Hotel, Mexico City, Mexico – senza esame finale. Aggiornamento e revisione delle conoscenze e degli studi sulla patologia asmatica;
3) dal 16/11/2013 al 16/11/2013: **uditore** al convegno "Novità diagnostico-terapeutiche in allergologia e immunologia clinica" (5 ore), organizzato da Associazione AsmAllergie presso Starhotels Majestic - Corso Vittorio Emanuele II, 54 - 10123 Torino – esame finale superato, 5 crediti ECM. Update su: reazioni avverse ad alimenti, probiotici, eczema, gestione delle alte vie aero-digestive, asma bronchiale.

Comunicazioni/poster presentati

Accettati 3 abstract in qualità di poster al congresso WISC 2014 (WAO Scientific International Conference), che si terrà dal 6 al 9 Dicembre 2014 a Rio de Janeiro (Brasile). Titoli:
1) The results of the clinical and laboratory trial of the Italian board for ISAC: A cluster analysis. (Immunotherapy, Rhinitis, Sinusitis, Ocular Diseases and Cough session, poster n° 2026);
2) Avoidance of nonsteroidal anti-inflammatory drugs after negative provocation tests in urticaria/angioedema reactions: Real-world experience. (Dermatology and Drug allergy session, poster n° 3047);
3) A 9-year-old boy presenting with hypothermia during specific immunotherapy for Gramineae. (Immunotherapy, Rhinitis, Sinusitis, Ocular Diseases and Cough session, poster n° 2118).
Programma scientifico congressuale consultabile su internet: www.wao.confex.com.

Pubblicazioni scientifiche

Bommarito L, Zisa G, Riccobono F, Villa E, D'Antonio C, Calamari Ambra M, Poppa M, Moschella A, Di Pietrantonj C, Galimberti M. Avoidance of nonsteroidal anti-inflammatory drugs after negative provocation tests in urticaria/angioedema reactions: Real-world experience. Allergy Asthma Proceedings. 2014 Jul-Aug;35(4):303-6.

Parte didattica

Elenco seminari/corsi o altre attività didattiche seguite nel corso del dottorato

9 giugno 2014: "Assessment of cervical cancer control in Rwanda and Bhutan" – Dott. Iacopo Baussano
11 giugno 2014: "Ribosome alteration in cancer: effect or cause?" – Prof. Fabrizio Loreni
12 giugno 2014: "Metformin rewires the signaling network of breast cancer cells and changes their sensitivity to growth and apoptotic stimuli" – Prof. Gianni Cesareni
15 luglio 2014: "Gene therapy application" – Prof. Follenzi
Dall'8 al 22 settembre 2014: The Borghese Sessions. Relatore: Prof. Steven E Ellis

20 ottobre 2014: “The Kruppel-like factor 2 transcription factor is a novel tumor suppressor gene recurrently mutated in Splenic Marginal Zone Lymphoma” – Dott.ssa Famà Rosella (Tutor: Prof. Gariglio).

Dal 2 al 4 ottobre 2014: 5th International Master in Nasal Cytology, organizzato da Ellecenter a Parigi, Hotel Hyatt Regency Paris Etoile. Direttore del corso: Dr. Matteo Gelardi, Presidente AICNA (Accademia Italiana Citologia Nasale) e Vice-Presidente Accademia Italiana di Rinologia. Conseguiti 12 CME credits.

In fede

Novara, lì 22 Ottobre 2014

Elisa Villa, MD, PhD student