Second year PhD (2014/2015) in Medical Sciences and Biotechnology - Scientific report

Elisa Villa, MD, PhD student

PhD student at the University of Piemonte Orientale "Amedeo Avogadro" - Tutors: Prof. Umberto Dianzani, Prof. Mario Pirisi

ARAP attachment student at the Singapore Immunology Network (SIgN), within the Agency for Science, Technology And Research (A*STAR), Singapore - Tutor: Dr. Olaf Rötzschke

Study on characterization and evaluation of biological function of different biomarkers, such as perforin, osteopontin and anti-osteopontin antibodies, in allergic diseases.

Introduction

This is a cross-sectional project aimed to study various emerging biomarkers of immune response, such as osteopontin, anti-osteopontin antibodies and perforin, in allergic diseases. There is potential to discover novel serological and genetic markers that might be useful in the characterization of allergic disease pathophysiology, in the evaluation of the different risk profiles and in the prediction of both clinical evolution and patients' responsiveness to treatment.

Furthermore, this study would highlight potential differences in genetic and biomolecular risk profiles between Caucasians and Asians. The evaluation of varied genetic patterns and environmental factors exposures could significantly contribute to the understanding of the immune mechanisms leading to the onset of allergic sensitization and the increased prevalence of allergic manifestations.

Results would be also useful in the search for additional tools in clinical monitoring and prediction of responsiveness to treatment of allergic patients, possibly developing a new diagnostic and therapeutic approach.

Project description

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" (Novara, Italy), has previously identified genetic mutations that may impair the process of the immune down-regulation in patients with autoimmune diseases: among these, genes related to osteopontin, perforin and ICOS molecules.

Osteopontin (OPN) is a pleomorphic cytokine known to influence a range of immune cells, including macrophages, neutrophils, dendritic cells, T and B cells. OPN is a proinflammatory cytokine modulating cell activation and cytokines production. Genetic variants of OPN (-B and -C haplotypes) determining a higher production of basal levels of OPN have been identified in the Laboratory of Immunology in Novara, Italy. The increased OPN production has been associated with a sig-

nificantly higher risk of autoimmune lymphoproliferative syndrome, multiple sclerosis and systemic lupus erythematosus, suggesting that OPN is a candidate biomarker of these conditions.

Perforin, contained in cytotoxic lymphocytes granules, plays a key role in cell-mediated cytotoxicity. Mutations of perforin gene have a role in determining familiar hemophagocytic lymphohistiocytosis and autoimmune lymphoproliferative syndrome, as demonstrated by the Laboratory of Immunology in Novara.

In the present cross-sectional study, we aimed to verify if serum levels of OPN and IgG anti-OPN antibodies may qualify as biomarkers of an activated immune response in allergic patients and to investigate the possible presence of rare mutations of perforin gene in allergic patients.

The project will be carried out at the University of Piemonte Orientale and at the Singapore Immunology Network (SIgN), within the Agency for Science, Technology and Research (A*STAR) in Singapore. A Southeast Asian population of allergic subjects has been already studied at the SIgN in order to evaluate both allergic sensitization patterns and respiratory symptoms manifestations in a tropical urban environment. This study, published by Dr. Olaf Rötzschke et al., demonstrated that two independent cohorts of 576 and 7373 ethnic Chinese subjects living in Singapore presented an allergic sensitization pattern that is almost exclusively directed to house dust mites (HDM), since a percentage of 80% of the individuals were HDM-sIgE positive. Of these, less than 30% had sIgE for any other aeroallergens. In addition, HDM-sIgE titers were statistically significant higher in comparison to non-HDM allergen sIgE titers. Interestingly, the population of migrants from non-tropical countries presented low or non-detectable sIgE against HDM, but showed a time-dependent increase of HDM-sIgE levels. Moreover, subjects remaining for a prolonged time in Singapore presented also a significant and remarkable impairment of respiratory symptoms (allergic rhinitis and/or asthma), that are associated with the time-dependent, progressively higher HDM-sensitization. These observations are in line with the ‘hygiene hypothesis’: many scientific evidences nowadays support the hypothesis that in the last decades a decreased microbial exposure due to a life-style “Westernization”, increased cleanliness and reduced family size, could explain the increases in global allergic diseases prevalence.

The results of our study thus could have an important impact on the understanding of predisposing factors and pathogenetic mechanisms in common allergies, in terms of genetics and molecular biology. Therefore this project results to be significantly useful in the context of searching for additional tools in clinical monitoring and prediction of responsiveness to treatment of allergic patients.

MATERIALS AND 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, perforin) in common allergic diseases (i.e., allergic asthma, allergic rhinitis, atopic dermatitis, allergic contact dermatitis, drug allergies, Hymenoptera venom allergy).

b. Subjects enrolled in the study

Adults who met the diagnostic criteria established for the allergic diseases mentioned above, were identified both retrospectively and prospectively. For the retrospective recruitment, subjects potentially suffering from allergies were 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 answered a questionnaire regarding demographics, medical history (family and personal) and specific information on their allergic diseases. Patients data and blood samples were immediately made anonymous after their collection and were identified by a code (with deleted sensitive data such as date of birth, address etc.). All the data useful to re-identify patients were 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 (allergic asthma, allergic rhinitis, 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.

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 two genetically different allergic populations: one of Caucasian origins and the other one from Southeast Asia;
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 OPN and perforin genes;
4. to identify new serological markers (i.e., OPN, anti-OPN antibodies) that may be correlated with clinical evolution and responsiveness to currently available treatments for allergies, if possible;
5. to validate and compare genetic and molecular risk profiles for allergic diseases in Caucasian and Southeast Asian cohorts;
6. to evaluate and compare genetic and molecular risk profiles in allergic diseases in different subsets of patients (e.g. gender, migrants from other geographical regions etc.).

Collecting samples for DNA
Genomic DNA has been extracted from an aliquot of whole blood using "Gentra reagents PureGene" (Qiagen). Biological samples (serum/plasma) and DNA have been stored at -80° C for future investigations always related to allergic diseases studies.

Whole exon sequencing of perforin gene
Exons of the perforin gene are sequenced in order to identify rare genetic mutations, missense and nonsense. The study of the genome sequence of DNA (exons) is actually performed using a capillary automatic sequencer.

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

Analysis of molecular markers

Presence and levels of serum biomarkers (such as OPN and IgG anti-OPN antibodies) have been assessed by ELISA (Enzyme-Linked ImmunoSorbent Assay) test (Human Osteopontin Duoset, R&D Systems for OPN assessment; "in house" ELISA kits for anti-OPN antibodies detection).

Bioinformatics analysis

In a primary data analysis, a case/control approach has been used comparing the results obtained in cases with respect to controls. In a secondary analysis, all the data obtained have been compared among the various groups of patients (i.e., subjects with different allergic diseases) to investigate a possible trend of differences in the groups. Serological data have been compared using the nonparametric Mann-Whitney test.

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 OPN, the frequencies of the two above-mentioned polymorphisms will be recorded.

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.

Preliminary results

A series of 121 adult patients, 57 males and 64 females (mean age: 48 years; median age: 49 years), with different allergic diseases was studied: 40 patients (33%) had allergic rhinitis, 25 (21%) allergic asthma, 21 (17%) Hymenoptera venom allergy, 19 (16%) food allergy, 13 (11%) allergic contact dermatitis, and 3 (2%) IgE-mediated hypersensitivity to beta-lactams. 116 healthy subjects with similar demographic characteristics served as controls. Data were analyzed comparing cases to controls, as well as looking for subgroup differences within the group of allergic patients.

OPN prevalence and serum levels were statistically higher in cases with respect to controls (median: 10330.8 pg/ml, interquartile range 25th 5712.49 pg/ml, 75th 16476.21 vs median 6099.12 pg/ml, interquartile range 25th 3122.57 pg/ml, 75th 14519.91 pg/ml; $p = 0.001$ by the Mann-Whitney test). No statistical differences were found between males and females in cases, compared to controls ($p = 0.06$ by the Mann-Whitney test).

OPN levels were statistically higher in patients with asthma (median: 13083.83 pg/ml; $p = 0.0269$), followed by those, less significant, observed in the food allergy group (median: 10204.33 pg/ml; $p = 0.046$), in comparison to controls. Not significantly different levels OPN were detected in patients with Hymenoptera venom allergy (median: 13289.95 pg/ml; $p = 0.0624$), rhinitis (median: 9431.2504 pg/ml; $p = 0.1277$) and allergic contact dermatitis (median: 6380.44 pg/ml; $p = 0.39$). Patients with IgE-mediated sensitization to beta-lactams had heterogeneous values, not statistically different in comparison to controls ($p = 0.47$).

Both prevalence and titers of serum IgG anti-OPN autoantibodies were statistically lower in allergic patients with respect to controls (median: 0.179 pg/ml, interquantile range, 25th 0.060 pg/ml, 75th 0.227 pg/ml vs median 0.250 pg/ml, interquantile range, 25th 0.152 pg/ml, 75th 0.367 pg/ml; $p < 0.0001$). Significantly lower levels of anti-OPN antibodies versus controls were found in patients with Hymenoptera venom allergy (median: 0.067 pg/ml; $p < 0.0001$), allergic rhinitis (median:

0.107 pg/ml; p = 0.0009), allergic contact dermatitis (median: 0.078 pg/ml; p = 0.0011) and asthma (median: 0.123 pg/ml; p = 0.0013); on the contrary, the statistical correlation for the food allergy group seems to be less significant (median: 0.185 pg/ml; p = 0.0575). Patients with IgE-mediated sensitization to beta-lactams presented heterogeneous results, not statistically different with respect to controls (p = 0.175). The analysis of OPN gene polymorphisms and the exon sequencing of perforin gene is ongoing, in progress.

Concerning the Singaporean cohort: preliminary data about 476 local individuals affected with allergic rhinitis and asthma have been collected and analyzed. OPN levels have been detected by Luminex, together with other cytokines: no statistical differences between cases and healthy controls have been found. On the contrary, a significant difference between males and females has been observed, as males seem to have remarkably higher OPN levels if compared to females ($p<0.0001$ by the Kruskal - Wallis test). Consequently, we have studied the OPN gene (SPP1) expression in the whole blood: in terms of mRNA production, there is no detectable difference between males and females. Therefore it can be hypothesized that there are some post-transcriptional modifications leading to the gender difference previously mentioned. Among the immune cells, SPP1 is mostly expressed in neutrophils and monocytes, whilst it is weakly expressed in lymphocytes.

Conclusions and further developments

Serum OPN levels seem to be a new potentially useful biomarker of both allergic asthma, reflecting the inflammatory condition and the lung tissue remodeling of the patients that are affected by this disease, and food allergy.

IgG anti-OPN antibodies serum levels were mostly lower in patients affected with Hymenoptera venom allergy, that is the most pure IgE-mediated allergy type with no underlining inflammation. Anti-OPN antibodies were statistically decreased also in patients with allergic rhinitis, asthma and allergic contact dermatitis, that typically is a type 4, cell-mediated, hypersensitivity. On the contrary, anti-OPN antibodies were not significant in the food allergy group. A further analysis of IgA anti-OPN antibodies could be of great interest to clarify this scientific observation. In general, consideration should be given to explore clinical correlates of high OPN and anti-OPN antibodies levels in these conditions.

As research attachment student in SIgN, Singapore, I am evaluating the possible existence of different isoforms of OPN in plasma samples of males and females through Western Blot technique, since in the Singaporean cohort we actually have observed strong eQTLs in SPP1 gene, but we have no pQTLs for OPN.

In parallel, I am evaluating the functional status of basophils (that constitutively express CD44, an OPN receptor) activated by house dust mites (HDM) in the presence of different levels of OPN, in atopic subjects sensitized to HDM. The basophil activation test is currently performed with titrated human recombinant OPN protein (R&D Systems) and with an “in-house” HDM extract, at different concentrations. In a short time, I will perform the same experiment by adding thrombin, a coagulation protease that seems to be important for OPN activation. These experiments are crucial since the role of OPN in basophils activation is not known yet (no data available in literature).

References

1. Rutkowski K, et al. Allergic diseases: the price of civilisational progress. *Postepy Dermatol Alergol.* 2014 May;31(2):77-83.
2. Cavkaytar O, Akdis CA, Akdis M. Modulation of immune responses by immunotherapy in allergic diseases. *Curr Opin Pharmacol.* 2014 Jul 22;17C:30-37.
3. Andiappan AK, Puan KJ, Lee B, Nardin A, Poidinger M, Connolly J, Chew FT, Wang DY, Rötzschke O. Allergic airway diseases in a tropical urban environment are driven by dominant mono-specific sensitization against house dust mites. *Allergy*. 2014 Apr;69(4):501-9.
4. Vaschetto R, Navalesi P, Clemente N, Boggio E, Valsecchi S, Olivieri C, Soluri MF, Kroumova V, Fonio P, Dinatale C, Borrè S, Fortina G, Dianzani U, Della Corte F, Chiocchetti A. Osteopontin induces soluble urokinase-type plasminogen activator receptor production and release. *Minerva Anestesiol.* 2014 Jul 3.
5. Quaglia M, Chiocchetti A, Cena T, Musetti C, Monti S, Clemente N, Dianzani U, Magnani C, Stratta P. Osteopontin circulating levels correlate with renal involvement in systemic lupus erythematosus and are lower in ACE inhibitor-treated patients. Quaglia M, Chiocchetti A, Cena T, Musetti C, Monti S, Clemente N, Dianzani U, Magnani C, Stratta P. *Clin Rheumatol.* 2014 Sep;33(9):1263-71.
6. Comi C, Cappellano G, Chiocchetti A, Orilieri E, Buttini S, Ghezzi L, Galimberti D, Guerini F, Barizzone N, Perla F, Leone M, D'Alfonso S, Caputo D, Scarpini E, Cantello R, Dianzani U. The impact of osteopontin gene variations on multiple sclerosis development and progression. *Clin Dev Immunol.* 2012;2012:212893.
7. Cappellano G, Orilieri E, Woldetsadik AD, Boggio E, Soluri MF, Comi C, Sblattero D, Chiocchetti A, Dianzani U. Anti-cytokine autoantibodies in autoimmune diseases. *Am J Clin Exp Immunol.* 2012 Nov 15;1(2):136-46.
8. Barizzone N, Marchini M, Cappiello F, Chiocchetti A, Orilieri E, Ferrante D, Corrado L, Mellone S, Scorza R, Dianzani U, D'Alfonso S. Association of osteopontin regulatory polymorphisms with systemic sclerosis. *Hum Immunol.* 2011 Oct;72(10):930-4.
9. Chiocchetti A, Orilieri E, Cappellano G, Barizzone N, D' Alfonso S, D' Annunzio G, Lorini R, Ravazzolo R, Cadario F, Martinetti M, Calcaterra V, Cerutti F, Bruno G, Larizza D, Dianzani U. The osteopontin gene +1239A/C single nucleotide polymorphism is associated with type 1 diabetes mellitus in the Italian population. *Int J Immunopathol Pharmacol*. 2010 Jan-Mar;23(1):263-9.
10. Cappellano G, Orilieri E, Comi C, Chiocchetti A, Bocca S, Boggio E, Bernardone IS, Cometa A, Clementi R, Barizzone N, D'Alfonso S, Corrado L, Galimberti D, Scarpini E, Guerini FR, Caputo D, Paolicelli D, Trojano M, Figà-Talamanca L, Salvetti M, Perla F, Leone M, Monaco F, Dianzani U. Variations of the perforin gene in patients with multiple sclerosis. *Genes Immun*. 2008 Jul;9(5):438-44.
11. Orilieri E, Cappellano G, Clementi R, Cometa A, Ferretti M, Cerutti E, Cadario F, Martinetti M, Larizza D, Calcaterra V, D'Annunzio G, Lorini R, Cerutti F, Bruno G, Chiocchetti A, Dianzani U. Variations of the perforin gene in patients with type 1 diabetes. *Diabetes.* 2008 Apr;57(4):1078-83.
12. Clementi R, Chiocchetti A, Cappellano G, Cerutti E, Ferretti M, Orilieri E, Dianzani I, Ferrarini M, Bregni M, Danesino C, Bozzi V, Putti MC, Cerutti F, Cometa A, Locatelli F, Mac-

cario R, Ramenghi U, Dianzani U. Variations of the perforin gene in patients with autoimmunity/lymphoproliferation and defective Fas function. *Blood.* 2006 Nov 1;108(9):3079-84.

13. Chiocchetti A, Comi C, Indelicato M, Castelli L, Mesturini R, Bensi T, Mazzarino MC, Giordano M, D'Alfonso S, Momigliano-Richiardi P, Liguori M, Zorzon M, Amoroso A, Trojano M, Monaco F, Leone M, Magnani C, Dianzani U. Osteopontin gene haplotypes correlate with multiple sclerosis development and progression. *J Neuroimmunol.* 2005 Jun; 163(1-2):172-8.
14. D'Alfonso S, Barizzone N, Giordano M, Chiocchetti A, Magnani C, Castelli L, Indelicato M, Giacopelli F, Marchini M, Scorza R, Danieli MG, Cappelli M, Migliaresi S, Bigliardo B, Sabbadini MG, Baldissera E, Galeazzi M, Sebastiani GD, Minisola G, Ravazzolo R, Dianzani U, Momigliano-Richiardi P. Two single-nucleotide polymorphisms in the 5' and 3' ends of the osteopontin gene contribute to susceptibility to systemic lupus erythematosus. *Arthritis Rheum.* 2005 Feb;52(2):539-47.
15. Chiocchetti A, Indelicato M, Bensi T, Mesturini R, Giordano M, Sametti S, Castelli L, Bottarel F, Mazzarino MC, Garbarini L, Giacopelli F, Valesini G, Santoro C, Dianzani I, Ramenghi U, Dianzani U. High levels of osteopontin associated with polymorphisms in its gene are a risk factor for development of autoimmunity/lymphoproliferation. *Blood.* 2004 Feb 15;103(4):1376-82.
16. Perkins JR et al. The study of severe cutaneous drug hypersensitivity reactions from a systems biology perspective. *Curr Opin Allergy Clin Immunol.* 2014 Aug;14(4):301-6.
17. Enomoto N, Hyde E, Ma JZ, Yang J, Forbes-Blom E, Delahunt B, Le Gros G, Ronchese F. Allergen-specific CTL require perforin expression to suppress allergic airway inflammation. *J Immunol*. 2012 Feb 15;188(4):1734-41.
18. Arnold V, Balkow S, Staats R, Matthys H, Luttmann W, Virchow JC Jr. Increase in perforin-positive peripheral blood lymphocytes in extrinsic and intrinsic asthma. *Pneumologie*. 2000 Oct;54(10):468-73.
19. Ambach A, Bonnekoh B, Gollnick H. Perforin hyperreleasability and depletion in cytotoxic T cells from patients with exacerbated atopic dermatitis and asymptomatic rhinoconjunctivitis allergica. *J Allergy Clin Immunol.* 2001 May;107(5):878-86.
20. Yawalkar N, Hunger RE, Buri C, Schmid S, Egli F, Brand CU, Mueller C, Pichler WJ, Braathen LR. A comparative study of the expression of cytotoxic proteins in allergic contact dermatitis and psoriasis: spongiotic skin lesions in allergic contact dermatitis are highly infiltrated by T cells expressing perforin and granzyme B. *Am J Pathol.* 2001 Mar;158(3):803-8.
21. Seier AM et al. Antigen-specific induction of osteopontin contributes to the chronification of allergic contact dermatitis. *Am J Pathol.* 2010 Jan;176(1):246-58.
22. Weiss JM et al. Osteopontin is involved in the initiation of cutaneous contact hypersensitivity by inducing Langerhans and dendritic cell migration to lymph nodes. *J Exp Med.* 2001 Nov 5;194(9):1219-29.
23. Kanemitsu Y et al. Osteopontin and periostin are associated with a 20-year decline of pulmonary function in patients with asthma. *Am J Respir Crit Care Med.* 2014 Aug 15;190(4): 472-4.

24. Liu W, Xia W, Fan Y, Wang H, Zuo K, Lai Y, Li H, Liu Z, Shi J, Xu G. Elevated serum osteopontin level is associated with blood eosinophilia and asthma comorbidity in patients with allergic rhinitis. *J Allergy Clin Immunol.* 2012 Dec;130(6):1416-8.e6.
25. Hillas G et al. Increased levels of osteopontin in sputum supernatant of smoking asthmatics. *Cytokine.* 2013 Jan;61(1):251-5.
26. Akelma AZ et al. Elevated level of serum osteopontin in school-age children with asthma. *Allergol Immunopathol (Madr).* 2014 Jul-Aug;42(4):275-81.
27. Barreno RX et al. Endogenous osteopontin promotes ozone-induced neutrophil recruitment to the lungs and airway hyperresponsiveness to methacholine. *Am J Physiol Lung Cell Mol Physiol.* 2013 Jul 15;305(2):L118-29.
28. Konno S et al. Increased expression of osteopontin is associated with long-term bee venom immunotherapy. *J Allergy Clin Immunol.* 2005 May;115(5):1063-7.
29. Komatsuzaki T et al. Suppression of osteopontin functions by levocetirizine, a histamine H1 receptor antagonist, in vitro. *Biomed Res Int*. 2013;2013:735835.
30. Seier AM et al. Antigen-specific induction of osteopontin contributes to the chronification of allergic contact dermatitis. *Am J Pathol.* 2010 Jan;176(1):246-58.
31. Cabrera CM, Urra JM, Alfaya T, Roca Fde L, Feo-Brito F. Expression of Th1, Th2, lymphocyte trafficking and activation markers on CD4+ T-cells of Hymenoptera allergic subjects and after venom immunotherapy. *Mol Immunol.* 2014 Nov;62(1):178-85.

Second year PhD (2014/2015) - Training report

List of attended conferences

1) 20/11/2014: “First European Workshop on Tolerogenic Vaccination in Autoimmune Diseases” (chairman: Prof. U. Dianzani) - Università degli Studi del Piemonte Orientale, Via Solaroli 17, 28100 Novara (NO);

2) 29/11/2014: “Novità diagnostico-terapeutiche in Allergologia ed Immunologia Clinica” - Asmallergie 2014 - Torino;

3) 25-26/03/2015: "Allergo 2015: Right treatment to right patient" - Genova.

Oral communications/posters

Elisa Villa, Giovanni Melioli, Giorgio Walter Canonica. **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);

Elisa Villa, Luisa Bommarito, Giuliana Zisa, Francesca Riccobono, Cristian D’Antonio, Ambra M. Calamari, Mariangela Poppa, Adele Moschella, Carlo Di Pietrantonj, Maurizio Galimberti. **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);

Elisa Villa, Giuliana Zisa, Valentina Zanotti, Umberto Dianzani, Mario Pirisi. **A 9-year-old boy presenting with hypothermia during specific immunotherapy for Gramineae.** (Immunotherapy, Rhinitis, Sinusitis, Ocular Diseases and Cough session, poster n° 2118).

Abstracts accepted as posters at the III World Allergy Organization Scientific International Conference 2014 (WISC 2014), 06-09/12/2014, Rio de Janeiro (Brasil). Scientific program and abstracts available at: http://www.worldallergy.org/wisc2014/scientific_program/abstracts.php.

Villa E, Zisa G, Zanotti V, Dianzani U and Pirisi M. "A 9-year-old boy presenting with hypothermia during specific immunotherapy for Gramineae". Poster presented at the "First European Workshop on Tolerogenic Vaccination in Autoimmune Diseases", 20/11/2014 - Università degli Studi del Piemonte Orientale, Via Solaroli 17, 28100 Novara (NO).

Elisa Villa, Rosalba Minisini, Olaf Rötzschke, Anand Andiappan, Elena Boggio, Luca Gigliotti, Nausicaa Clemente, Annalisa Chiocchetti, Umberto Dianzani, Mario Pirisi. **Evaluation of Serum Levels of Osteopontin As a Potential Biomarker of Immune Activation in Patients with Allergic Diseases.** Abstract accepted as poster at the XXIII World Allergy Congress 2015 (WAC 2015), 14-17/10/2015, Seoul, Republic of South Korea (Immunology session, poster n° 8572).

Scientific publications

Elisa Villa, Rosalba Minisini, Olaf Rötzschke, Anand Andiappan, Elena Boggio, Luca Gigliotti, Nausicaa Clemente, Annalisa Chiocchetti, Umberto Dianzani, Mario Pirisi. **Evaluation of Serum Levels of Osteopontin As a Potential Biomarker of Immune Activation in Patients with Allergic Diseases.** Accepted for publication as a supplement to the World Allergy Organization Journal.

List of seminars/courses attended at the University of Eastern Piedmont, Novara

06/11/2014 "Dysregulated antigen receptor signaling: molecular lessons from two congenital lymphoproliferative disorders." - Dr. A. Snow;

14/11/2014 "Tissue engineering: the state of the art" - Dr. Boccafoschi;

21/11/2014 "Stem cell in the regeneration and repair of the tissues and organs" - Prof. Prat;

28/11/2014 "Humoral responses to HCV infection and clinical outcomes" - Prof. Arvind Patel;

01/12/2014 "Beta Human Papillomavirus infection and skin cancer in the immunocompromised host" - speaker: Lanfredini Simone (tutor: Prof. Gariglio);

04/12/2014 "Uncovering the role of β-HPV in field cancerization: a collaboration in progress" - Prof. G. Patel;

05/12/2014 "Focus on the liver: from basics of NAFLD to hot topics in HBV & HCV infections" - Prof. Rifaat Safaadi;

16/12/2014 "From the legend of Prometheus to regenerative medicine" - Prof. Antonio Musarò;

17/12/2014 "Microglia microvesicles: messengers from the diseased brain" - Prof. Furlan;

19/01/2015 “Anticancer strategy Targeting cancer cell metabolism in ovarian cancer” - Prof. Yong-Sang Song;

20/01/2015 “Regulation of hepatocytes differentiation during the transitions between epithelial and mesenchymal states” - Dr. Tonino Alonzi;

27/01/2015 “Myeloid cells as therapeutic target in cancer” - Prof. Antonio Sica;

11/03/2015 “Proof of principle for cell therapy: from autologous transplantation of tissue specific progenitors to gene corrected patient specific injured pluripotent stem cells ” - Prof. Bosnakovski;

07/05/2015 “An Integrated Approach to the Diagnosis and Treatment of Ovarian Cancer” - Prof. John McDonald;

14/05/2015 “Conflicting interests and scientific communication. What ethical standards to apply? How effective are these standards in practice?” - Kathleen Ruff;

25/05/2015 “Ribosomopathies" - Prof. Steve Ellis.

List of seminars/courses attended at the Singapore Immunology Network - A*STAR institute, Singapore

15/07/2015 “Thermogelling polymers – What lies ahead?” - Dr. Loh Xian Jun;

04/08/2015 “Targeting BCL-2 Pathway as a Future Therapy for Paediatric Leukaemia” - Dr. Santi Suryani;

05/08/2015 “Conquering TB: The NIH Research Portfolio” - Dr. Jing Bao;

05/08/2015 “Molecular Targeting of Key Pathogenic Effects on Host Defense Mechanisms, Focusing on Tuberculosis” - Dr. Richard Hafner;

05/08/2015 Informal progress, Dr. Olaf Rötzschke’s lab - Dr. Olaf Rötzschke;

06/08/2015 “Architectural regulation of cytotoxic T cell function” - Dr. Morgan Huse;

02/09/2015 “Tetraspanins as therapeutic targets in Leukemia” - Dr. Natarajan Muthusamy;

04/09/2015 “Science Talks” within the Science Jubilee, Singapore 50 - Dr. Yuko Nakamura and Dr. Bernard Cher on behalf of Procter & Gamble; Thibault Gauriau on behalf of Lucasfilm Singapore.

On Tuesdays: lab meeting.

I hereby declare that the above written particulars are true to the best of my knowledge and belief. Supporting documents and references available on request.

Singapore, 9th September 2015

Elisa Villa