

Principal Investigator (Last, First, Middle): **Scotti, Claudia**

BIOGRAPHICAL SKETCH

NAME Claudia Scotti	POSITION TITLE: Researcher, PI
eRA COMMONS USER NAME CLAUDIA_SCOTTI	

EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.*)

INSTITUTION AND LOCATION	DEGREE	MM/YY	FIELD OF STUDY
University of Pavia - Pavia - Italy	MD	07/95	Medicine and Surgery
University of Pavia - Pavia - Italy	Specialist	04/00	Oncology
Institute for Higher Studies - Pavia - Italy	Master	05/01	Molecular Medicine
Birkbeck College - University of London (UK)	PhD	02/08	Crystallography
University of Pavia - Pavia - Italy	Specialist	04/10	Clinical Pathology
Open University – Milton Keynes (UK)	(To be)	12/29	B.Sc. Mathematics

A. Personal Statement

The aim of this research proposal is to define the molecular signature of cancer cells dependent on specific amino acids for survival. My work in the field started with the discovery that L-asparaginase was the molecule responsible for the inhibition of the cell cycle in cells exposed to *Helicobacter pylori* broth culture filtrate. These initial experiments have projected me, from my antibody engineering and structural background, into the world of therapeutic enzymes and of enzyme engineering. Along the way, we started to deeply explore *E. coli* asparaginase, the current form of drug used to treat Acute Lymphoblastic Leukaemia (ALL), with the aim of generating new, improved versions of the enzyme useful for therapy. So far, we have contributed to the field with several publications, and we have filed two patents related to L-asparaginase (PCT/EP2008/006469, PCT/EP2016/076994), becoming a reference laboratory in the world for this field. Our main research interest is towards the deep understanding of asparaginase mechanism of action, in order to improve its efficacy, reduce the number of drug administrations and side effects. In our laboratory, we are also testing exciting ideas in the field of antibody-based drug targeting and asparaginase miniaturization (T-CAN) and we are exploring potential applications of asparaginase to solid tumours.

Access to the national facility of the Human Technopole would provide the information needed to understand the molecular signature of cancer cells dependent on specific amino acids. The consequent deliverables could have a very relevant impact on the survival and the quality of life of leukaemia patients, especially, but not only, of those affected by ALL.

Below and in the "Research support" paragraph I summarise activities and institutional responsibilities which demonstrate my group coordination and networking capabilities, especially between industrial partners and the academic laboratory. The research awards and grants I obtained so far, most of them as principal investigator, have been very useful to select exceptionally brilliant, highly motivated PhD students and post-docs.

Intellectual Property management is normally dealt with in close coordination with the TTO of the University following existing internal regulations and our work has so far led to the filing of five patents. Finally, I have been among the promoters and co-founders of two award-winning academic spin-offs (Ardis S.r.l. and UB-CARE S.r.l.).

The birth of my beautiful daughter Letizia, sixteen years ago, has been a new challenge, which has further increased my daily capacity of organization and management.

All this evidence suggests that the role of PI for this project should be in safe hands.

INSTITUTIONAL RESPONSIBILITIES

- 2024- Member of the PhD teaching board in Translational and Precision Medicine
- 2016- Appointed coordinator of the second year of the Harvey Medical course - University of Pavia, Italy.
- 2016-2023 Member of the board promoting a new national PhD programme in Translational Medicine.
- 2014 Invited member of the PhD Final Examination Evaluation Board - IUSS - Pavia, Italy.
- 2013-2014 Invited member of the PhD Students Admission Board - IUSS - Pavia, Italy.
- 2012-today Member of Graduation Boards, Biology and Biotechnology faculties, University of Pavia, Pavia, Italy.
- 2012-2013 Appointed coordinator of the second year, second semester of the course in Biotechnology - University of Pavia, Italy.
- 2010-2014 Appointed member of the Faculty of Medicine - University of Pavia - Italy.
- 2010-2014 Invited member of the Teaching Board of the PhD course in Life Science - University of Pavia, Italy.
- 2008-2010 Appointed member of the Interfaculty of Motor Sciences - University of Pavia, Italy.
- 2004-2013 Appointed member of the Faculty of Biotechnology - University of Pavia - Italy.

MEMBERSHIPS OF SCIENTIFIC SOCIETIES

- 2022-today Member of The Antibody Society
- 2014-today Member of the Italian Association of Crystallography.
- 2014-today Member of the European Crystallography Association.

TEACHING ACTIVITIES

- 2016-today Second year coordinator, Course in Medicine and Surgery taught in English, Harvey.
- 2011-today Professor - General Pathophysiology - Faculty of Medicine - Harvey (English) Course, University of Pavia, Italy.
- 2010-today Professor - General Pathophysiology - Faculty of Medicine - Golgi (Italian) course, University of Pavia, Italy.
- 2013-2016 Professor - Molecular Immunology - Faculty of Medicine - Harvey (English) and Golgi (Italian) Courses, University of Pavia, Italy.
- 2004-2013 Professor - Immunological Biotechnologies - Master course in Biotechnology - Faculty of Medicine (Italian course), University of Pavia, Italy.
- 2008-2010 Professor - General Pathophysiology - Faculty of Movement Science (Italian course), University of Pavia, Italy.
- 2000-2004 Teacher - Molecular Immunology practicals for medical students, University of Pavia, Italy
- 2003-today Teacher - Tutorial for medical students: Computer Simulations of Pathophysiological Alterations, University of Pavia, Italy.
- 2003 Teacher - Methods for the production of recombinant proteins - Italian Consortium for Medical research (CIRM), Milan, Italy. Teacher - Monoclonal antibodies - Italian Consortium for Medical research (CIRM), Milan, Italy.

B. Positions, Scientific Appointments and Honors

POSITIONS

- 2023 National Scientific License towards Associate Professor.
- 2009-today Researcher - Department of Molecular Medicine, Unit of Immunology and General Pathology, University of Pavia, Italy. First MD in 30 years to become a researcher in the Unit of Immunology and General Pathology. Now leads a group ("T4 team") including 2 team members plus 6 undergraduate students.
- 1999-2003 Research assistant, University of Pavia, Pavia, Italy.
- 1998-1999 Visiting scientist at the Medical Research Council - Laboratory of Molecular Biology, Cambridge (UK).
- 1998 Clinical License as MD.

FELLOWSHIPS AND AWARDS

- 2010 Winner of the Ricerca.tissimi Award for research and innovation – Regione Lombardia.
- 2008 Winner of the call "Research dowry" – Regione Lombardia.
- 2003-2009 Winner of a EU fellowship for the Post-graduate School in Clinical Pathology.
- 2003 Winner of the Fellowship "Polizzotto Foundation".
- 2002 Winner of the Fellowship "Adriano Buzzati Traverso".
- 2002 Winner of the Young Researchers Funding – University of Pavia.
- 2000-2003 Winner of an "Assegno di ricerca" - University of Pavia.
- 1995-1999 Winner of a EU fellowship for the Post-graduate School in Oncology.
- 1989-1995 University fee reduction for merit.
- 1988 Winner of the "Richard Award" for school merit (Banca Popolare di Lodi).
- 1988 Winner of the "G. Gandini Award" - Appointed by Lodi's Mayor as best student of the year. "Avv. Cesaris" fellowship (Bank "Popolare" di Lodi).
- 1987 Winner of "Torti"s prize for school merit (Bank "Popolare" di Lodi).

COMMISSIONS OF TRUST

- 2016-today Member of the Consultancy Board of the Department.
- 2014-today Invited project reviewer, Italian Ministry of University Research and Education (MIUR).
- 2014 Invited project reviewer, University of Verona, Verona, Italy.
- 2012-today Invited project reviewer, Romanian National Council for Scientific Research, Romania.

C. Contributions to Science

The laboratory of Protein Engineering I coordinate works on proteins with relevance for physiology, pathology and therapy.

1. L-asparaginase

This research originated from observations performed with my colleagues Sommi et al. (2002) regarding the cell cycle inhibition induced by the culture supernatant of various *Helicobacter pylori* strains. These observations led us to identify the enzyme L-asparaginase as the factor responsible. Subsequent biochemical characterization revealed that its properties were potentially interesting from a biotechnological and applicative point of view in various fields. Our research then focussed on the corresponding *E. coli* enzyme, which is used in the clinics for the treatment of Acute Lymphoblastic Leukaemia A (ALL). By site-directed mutagenesis, we isolated an enzyme variant which showed a much higher resistance to proteases, temperature and time-driven degradation, and which is endowed with a much lower toxicity compared to the molecule currently in use. Using this molecule as a tool, we are learning more about the biology of ALL and solid tumors.

Selected references

1. Pessino G, Lonati L, Scotti C et al. Differential effect of asparagine and glutamine removal on three adenocarcinoma cell lines. *Heliyon*. 2024 Aug 3;10(15):e35789.
2. Maggi M, ..., Scotti C. A protease-resistant *Escherichia coli* asparaginase with outstanding stability and enhanced anti-leukaemic activity in vitro. *Sci Rep*. 2017 Nov 3;7(1):14479.
3. Scotti, C et al. Cell-Cycle Inhibition by *Helicobacter pylori* L-Asparaginase, *PLoS ONE*, 5(11): e13892, 2010.
4. Sommi P., Savio M., Stivala LA., **Scotti C.**, Mignosi P., Prosperi E., Vannini V., Solcia E., *Helicobacter pylori* releases a factor(s) inhibiting cell cycle progression of human gastric cell lines by affecting cyclin E/cdk2 kinase activity and Rb protein phosphorylation through enhanced p27(KIP1) protein expression, *Exp. Cell. Res.*, 281, 128-39, 2002.

2. Lipoprotein (a)

The research project focuses on the development and engineering of monoclonal antibodies targeting lipoprotein(a) [Lp(a)], a newly recognized risk factor for cardiovascular diseases, particularly ischemic heart disease. Cardiovascular diseases are the leading cause of death in Italy, with hyperlipidemia being a major risk factor due to its role in promoting atherosclerosis. Lp(a) has emerged as a significant marker in identifying cardiovascular risk, especially in familial cases where specific Lp(a) phenotypes are present. The project aims to create high-affinity monoclonal antibodies against Lp(a) that could be used for both diagnostic and therapeutic purposes. The research utilizes advanced techniques such as phage display, bioluminescence, and structural biology. So far, methods for antigen and antibody purification have been established, along with in vitro selection techniques for antibodies that can increase Lp(a) internalization by macrophage cells. Ongoing tests are evaluating these antibodies ability to interfere with Lp(a)'s anti-thrombolytic activity.

Selected references:

1. Santonastaso A, ..., Scotti C. Interfacial Activity of Lipoprotein (a) Isoforms with a Variable Number of Kringle IV Type 2 Repeats: A New Indicator of Cardiovascular Risk? *Ann Clin Lab Sci*. 2021 Nov;51(6):795-804.
2. Santonastaso A, ..., Scotti C. High resolution structure of human apolipoprotein (a) kringle IV type 2: beyond the lysine binding site. *J Lipid Res*. 2020 Dec;61(12):1687-1696. doi: 10.1194/jlr.RA120001023. Epub 2020 Sep 9.
3. Santonastaso A., Scotti C., Real Time Cell Analysis of Model Target Cell Lines Exposed to Purified Lipoprotein (a), *British Journal of Medicine and Medical Research*, 2016, 16(4), 1-12.

3. Structural Bases of Antibody Affinity Maturation

This research focuses on antibody affinity maturation, building upon my previous work in macromolecular crystallography. Antibody affinity maturation, a crucial aspect of the immune response, enhances antigen-binding affinity and is of significant therapeutic interest. The process involves genetic recombination and somatic hypermutation in B cells, leading to structural changes in the antibody's complementarity-determining regions (CDRs), which improve binding kinetics. My project studies the structural variations during maturation using the 2-phenyloxazolone system. I successfully cloned, sequenced, and expressed scFv constructs from seven out of ten antibodies under study. Structural determination through X-ray crystallography of two class III antibodies (NQ10/1.12 and NQ16/113.8) provided insights into their maturation, with findings published in the *Journal of Molecular Biology* (PDB IDs: 2cju, 2uud).

Selected references:

1. Scotti, C., Gherardi, E. Structural Basis of Affinity Maturation of the TEPC15/VK45.1 Anti-2-phenyl-5-oxazolone Antibodies, *J. Mol. Biol.*, 359, 1161–1169, 2006.
2. Maggi M., Scotti C., Enhanced expression and purification of camelid single domain VHH antibodies from classical inclusion bodies, 2017, *Protein Expression and Purification*, 2017, pii: S1046-5928(17)30017-7. doi: 10.1016/j.pep.2017.02.007. [Epub ahead of print].

4. Bioinformatic techniques for the analysis of point mutations

In collaboration with the Unit of Human Genetics, we have started to systematically apply bioinformatic techniques for the analysis of the effect of point mutations on the extracellular domain of ALK1, a protein in which they can lead to the development of a pathology called Rendu-Osler-Weber disease or Hereditary Haemorrhagic Telangiectasia (Type 2 Hereditary Haemorrhagic Telangiectasia, HHT-2). Bioinformatic analysis applied to structures has allowed to gain information also on other genetic diseases, like Pompe Disease.

Selected references:

1. De Filippi P., ..., Scotti C. et al. Genotype-Phenotype correlation in Pompe disease, a step forward, *Orphanet Journal of Rare Diseases*, 9:102, 2014.
2. Vecchia L., Olivieri C., Scotti C., Activin Receptor-Like Kinase 1: a Novel Anti-angiogenesis Target from TGF-beta Family, *Mini-Reviews in Medicinal Chemistry*, 13, 1398-1406, 2013. Invited review.
3. Scotti, C. et al. Bioinformatic Analysis of Pathogenic Missense Mutations of Activin Receptor Like Kinase 1 Ectodomain. *PLoS ONE* 6(10): e26431. doi:10.1371/journal.pone.0026431, 2011.

5. Patented inventions

1. PCT/EP N. 2008/006469, WO 2010015264, Scotti, C., Valentini G., Cappelletti D., Pasquetto M.V., Stivala S., Chiarelli L.R., *Helicobacter pylori* L-asparaginase, 2008
2. MI2013A001094, Method to predict allergies, 2013.
3. PCT/EP N. 2016/076994, Maggi Maristella, Claudia Scotti, A highly stable, protease resistant *E. coli* asparaginase, 2016.
4. PCT/IB N. 2018/057259, Maristella Maggi, Greta Pessino, Claudia Scotti, An antibody drug conjugate based on asparaginase, 2018.
5. PCT/IB N. 2018/057037, Claudia Scotti, Maristella Maggi, Targeted single domain antibodies with catalytic activity (T-CAN), 2018.

D. Research Support

See full list in "Applicant's publications and relevant funding" section of the application.

Selected grants:

From 03-04-2024 to today

Role: Unit member. Project title: "IMAGEOMICS: Optimizing Benefit/Risk Ratio in Breast Cancer Diagnosis and Radiotherapy: Identifying Molecular, Cellular and Imaging Signatures of Breast Cancer Heterogeneity to Improve Personalized Therapeutic Strategies for Synergistic Treatment Combinations. Funding body: PIANOFORTE, European Partnership for Radiation Protection Research Amount: 50.000 euros.

From 28-09-2023 to today

Role: Unit member. Project title: "The PIANO project, Pathways Involved in the action of ANTI-MDA5 antibodies: impact On innate immunity". Funding body: MIUR. Amount: 60.000 euros.

From 19-07-2022 to today.

Role: Co-PI with Dr. Maristella Maggi. Project title: "Analysis of the expression of the MET oncogene in selected subtypes of leukaemia: evaluation of a new potential target for CAR-T therapy". Funding body: Gilead Fellowship program 2022. Amount: 20.000 euros.

From 30-03-2016 to today.

Role: PI. Project title: "Optimization of physico-chemical properties of L-asparaginase for the therapy of Acute Lymphoblastic Leukaemia". Funding body: Associazione Gian Franco Lupo - Un sorriso alla vita – ONLUS. Amount: 10.000 euros.

From 14-11-2012 to 07-07-2014

Role: PI. Project title: "Immunological effects of the JAK inhibitor INCB018424". Funding body: Novartis. Amount: 71.412 euros.