

BIOGRAPHICAL SKETCH

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NAME: CRISTINA SOBACCHI

eRA COMMONS USER NAME (credential, e.g., agency login):

POSITION TITLE: Primo Ricercatore CNR

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	Completion Date MM/YYYY	FIELD OF STUDY
Università degli Studi di Milano, Milan, Italy	Single-cycle master's degree	10/1998	Pharmaceutical Chemistry and Technologies, Training in Molecular Biology

A. Personal Statement

The general objective of my research is to unravel the molecular and cellular mechanisms involved in bone pathophysiology, which I investigate by means of *in vitro* (2D and 3D) and *in vivo* preclinical models and studies in patient cohorts. I have a longstanding interest on the genetics and pathophysiology of RARE BONE DISEASES, particularly Osteopetrosis. In this framework, I was involved in studies exploiting the iPSC technology (Neri et al, Stem Cell Reports 2015; Lanzi et al, Stem Cell Res 2020).

In the framework of the molecular analysis of patients affected by rare bone diseases, I became interested by ACROFRONTOFACIONASAL DYSOSTOSIS TYPE 1 (AFFND1), for which I received an Exploratory Telethon Project in 2014/2015; this led me to the identification of variants in the *NBAS* (Palagano et al, Bone 2018) and in the *PIGB* gene (Palagano et al, Bone 2021) as associated to AFFND1. I also conducted and contributed to additional studies related to NBAS and inherited diseases (Ritelli et al, Bone 2020; Hammann et al, Mol Genet Metab 2024).

The Host Institution where I have been conducting my research since 2007 (Humanitas Research Hospital) is a vibrant and very stimulating environment devoted to basic, translational, and clinical research in different fields of life sciences. The presence of a variety of facilities equipped with cutting-edge technologies warrants me wide technological support. Moreover, during my research I have taken advantage from the collaboration with physicians, expert in rare inherited skeletal disorders and in other musculoskeletal and metabolic bone disorders, and with basic/translational researchers with different/complementary technical expertise, as witnessed by my publications. I have strong collaborations with CNR Researchers of other CNR Institutes, such as Dr Monica Sandri, Istituto di Scienza, Tecnologia e Sostenibilità per lo Sviluppo dei Materiali Ceramici (ISSMC, previously ISTE), Faenza; Dr Renzo Vanna, Istituto di Fotonica e Nanotecnologie (IFN), Milan; Dr Daniele Panetta, Istituto di Fisiologia Clinica (IFC), Pisa; this network of collaborations contributes to giving my research a multidisciplinary character. In particular, thanks to the collaboration with Dr Sandri, I approached 3D cultures on biomimetic scaffolds and exploited them for proof-of-concept therapeutic purposes and for the development of an *in vitro* model of bone infection. Moreover, in the framework of the collaboration with Dr Vanna (IFN) and Dr Dario Polli (Politecnico di Milano), I approached the application of vibrational microscopy approaches (Raman, Brillouin) to murine models of skeletal pathologies.

Since 2007, I have been awarded 11 GRANTS as PRINCIPAL INVESTIGATOR, which I have always successfully completed.

B. Positions, Scientific Appointments, and Honors**Positions:**

2010 to present: Tenured Researcher at CNR

2007-2010 Researcher of CNR. Topic: Genetic analysis of human osteopetroses; development of new cellular and pharmacological approaches to autosomal recessive osteopetrosis in murine models.

2005-2007 Research Assistant in the lab directed by Dr Anna Villa. Topic: Genetic analysis of human osteopetroses; cellular approaches to autosomal recessive osteopetrosis in a mouse model.

2001-2003 FIRC Fellowship in the lab directed by Dr Anna Villa. Topics: Molecular and cellular mechanisms controlling antigen receptor rearrangement and potentially involved in the pathogenesis of lymphoid tumors.

Genetic analysis of human osteopetroses.

1999-2000 Fellowship in the lab directed by Dr Anna Villa. Topic: Development and application of technologies of industrial interest in the field of genomics (Biosearch Italia S.p.A.)

1998-1999 Fellowship in Molecular Biology, National Research Council (CNR), in the lab directed by Dr Anna Villa. Topic: Genetic analysis of primary immunodeficiencies.

Honors:

Awards: ECTS/ABBH Iain Boyle Award (2009)

Young Investigator Award, from the European Calcified Tissues Society (2007)

Grants:

(CURRENT) PNRR-Ministero della Salute. Project PNRR-MAD-2022-12376568: "Dipeptidyl peptidase 3 and bone health: mechanistic insights and clinical assessment for fracture risk prediction in Type2 Diabetes". Role: Principal Investigator. 2 Years Grant. Founded €740.000

Ministero della Salute, Ricerca Finalizzata ordinaria - "Theory Enhancing". Project RF-2018-12367680: "Dissecting the neglected link between RANKL cytokine and stemness features with high relevance in osteoporosis" Role: Principal Investigator. 3 Years Grant. Founded €450.000

Telethon Rare Diseases and COVID-19, Grant n. GSP20006Covid025: "Assessing the role of the gene associated with Acrofrontofacionasal Dysostosis type 1, NBAS, in the nonsense mediated decay and Golgi-to-ER retrograde transport functions in Sars-CoV-2 infected cells". Role: Principal Investigator. 1 Year Grant. Founded €50.000

"Giovani Ricercatori" Grant: Exome sequencing of osteoimmunological diseases: from diagnosis to cure of osteopetrosis and other genetic skeletal diseases. Role: Principal Investigator. 3 Years Grant. Founded €225.987,76

Telethon Exploratory Project n.GGP13060: Understanding the genetic basis of Acrofrontofacionasal Dysostosis 1. Role: Principal Investigator. 1 Year Grant, founded €21.350

Telethon Grant n.GGP12178: Mesenchymal Stem Cell Transplantation as a therapeutic approach to RANKL-dependent Osteopetrosis. Role: Principal Investigator. 2 Years Grant, founded €123.000

Telethon Grant n.GGP10116: A pharmacological approach to RANKL-dependent osteoclast-poor Autosomal Recessive Osteopetrosis. Role: Principal Investigator. 2 Years Grant, founded €138.000

"Giovani Ricercatori" Grant GR-2008-1134625: New therapeutical approaches to RANKL-dependent Autosomal Recessive Osteopetrosis. Role: Principal Investigator. 3 Years Grant, founded €460.000

"Giovani Ricercatori" Grant GR-2007-686104: HLA-G: a new tolerogenic marker for tolerance induction mediated by regulatory T cells. Role: Head of Unit. 3 Years Grant, founded €150.000

Fondazione Cariplo Grant n.2008-2218: Understanding and treating osteoclast-poor autosomal recessive osteopetrosis: RANKL-RANK axis. Role: Principal Investigator. 3 Years Grant, co-founded €232.999,94

Telethon Grant n.GGP08176: Understanding and treating osteoclast-poor autosomal recessive osteopetrosis: RANKL-RANK axis. Role: Principal Investigator. 2 Years Grant, founded €120.000

Telethon Grant n.GGP07059: Characterization of the molecular basis of Osteopetrosis: the osteoclast-poor form. Role: Principal Investigator. 1 Year Grant. Founded €40.500

Scientific appointments:

Affiliation to Scientific Societies: Member of the European Calcified Tissue Society (ECTS), where she serves as CoChair of the ECTS PhD Training Action Group. Local Organizer of the ECTS PhD Training

Course 2015. Member of the Scientific Program Committee for the ECTS 2021 Annual Congress and of the Scientific Program Committee for the ECTS 2025 as co-chair of the Basic Program.

Member of the International Federation of Musculoskeletal Research Societies (IFMRS) International Education Committee (since July 2024).

Member of the Forum in Bone and Mineral Research (FBMR), of Società Italiana dell'Osteoporosi, del Metabolismo Minerale e delle Malattie dello Scheletro (SIOMMMS) and of the International Scientific Society of Ectopic Calcification (ISSEC).

Reviewer for different peer-reviewed journals, such as Journal of Bone and Mineral Research, Bone, Calcified Tissue International, eLife, Physiology, American Journal of Medical Genetics, International Journal of Molecular Science, Cancers, Liver International, Frontiers, Journal of Leukocyte Biology, and others.

Guest Associate Editor for the Research Topics "Innate Immunity in the Context of Osteoimmunology" and "Regulation of Osteoclast Differentiation in Autoimmune and Autoinflammatory Disorders", both in Frontiers in Immunology, and for the Special Issue "The Endocrine Role of the Skeleton" in the International Journal of Endocrinology.

Reviewer of Scientific Proposals submitted to different funding agencies, such as the LifeArch Philanthropic Fund; NC3Rs – National Centre for the Replacement, Refinement and Reduction of Animals in Research; the Israel Science Foundation; the International Federation of Musculoskeletal Research Societies; the Austrian Science Fund (FWF); the Biotechnology and Biological Sciences Research Council (BBSRC); the Arthritis Research UK; AFM-Telethon.

Participation in doctoral thesis evaluation commissions: assignments by the Department of Translational Medicine, DIMET, of Università degli Studi di Milano-Bicocca, a.y. 2017-2018; and supervisor of a PhD student in the framework of the Doctoral program on Experimental Medicine and Medical Biotechnologies, Department of Medical Biotechnologies and Translational Medicine, DIMETRA, of Università degli Studi di Milan (2014-2017).

2013-2020: Member of Ethic Committee Associazione "La Nostra Famiglia", IRCCS Eugenio Medea (as the expert in genetics)

Elected Member of the Governing Board (GB) representing Partner 7 (CNR) in the framework of the project "Systems biology for the functional validation of genetic determinants of skeletal diseases" (SYBIL), funded by the European Community (EU, FP7/2007-2013).

Invited Speaker to several National and International Conferences and Master courses:

51st ECTS Congress, Marseille, France, May 25-28, 2024. Invited talk: "Inflammation beyond arthritis – the case of osteomyelitis"

Master in Gestione del Paziente con Osteoporosi e Malattie Osteometaboliche, Humanitas University, Rozzano (MI), Italy, October 4th, 2022. Invited talk: Osteopetrosi.

ECTS PhD Training Course July 4-7, 2022 – Nice, France. Invited talk: "Basics on bone cells: osteoclasts"

9th International Conference on Children's Bone Health, Salzburg, Austria, June 22-25, 2019. Invited talk: "Osteoclast disorders"

Digital summer school, Université Côte d'Azur, 7-9 July 2021 "From Bones to Oceans and Space: the untold stories of Biomineralization", supported by COST Action European Network for Connective Tissue Calcifying Diseases, EuroSoftCalcNet. Invited talk: "Lessons from rare bone diseases to understand the pathophysiology of common bone diseases"

ECTS 2020 Digital Congress, ECTS@Home Programme. Invited talk: "Novel roles of RANKL in bone biology and beyond"

46th ECTS Congress, Budapest, Hungary, May 11-14, 2019; pre-congress Friday 10 May 2019. Invited talk: "Genetics of Osteopetrosis and Osteoclast Biology"

Master in Medicina Rigenerativa, Humanitas University, Rozzano (MI), Italy, January 16th, 2019. Invited talk: Immunologia e medicina rigenerativa - Ricerca di base.

XVIII Congresso Nazionale SIOMMMS, Basic and Translational Research Symposium, October 26th, 2018 - Naples, Italy. Invited talk: "Osteopetrosis: from gene to therapy"

Convegno Auxologico Focus on: Interrelazioni tra syndrome metabolica e fragilità scheletrica: dalla Ricerca di base alle problematiche cliniche. October 11, 2018, Milan. Invited talk: "Stress ossidativo e tessuto osseo".

"Osteoclasts and Bone Resorption in Health and Disease" Meeting, Weizmann Institute of Science, Rehovot, Israel; June 10-12, 2018. Invited talk: "Genetics of Osteopetrosis".

ECTS PhD Training Course July 8-11, 2017 - Paris, France. Invited talk: "Bone phenotype and applications to rare diseases".

16th Forum in Bone and Mineral Research Meeting, June 15-16, 2016 - Turin, Italy. Invited talk: "Iron metabolism and bone"

XV Congresso Nazionale SIOMMMS, Basic and Translational Research Symposium, November 12th, 2015 - Bologna, Italy. Invited talk: "Osteopetrosis: diagnosis, prognosis, treatment"

ECTS PhD Training Course September 13-16, 2015 - Siena, Italy. Local organizer and invited speaker. "Rare bone diseases"

5th International Conference on Osteoimmunology: Interactions of the Immune and Skeletal Systems, June 15-20, 2014 – KOS, Greece. Invited talk: "New insights on bone biology from exome sequencing of osteopetrotic patients with atypical presentations"

ECTS PhD Training Course September 15-18, 2013 - Hamburg, Germany. Invited talk: "Bone cells: osteoclasts".

16° convegno "Patologia immune e malattie orfane" January 17-19, 2013 - Turin, Italy. Invited talk: "Osteopetrosi" Convegno Scientifico Nazionale "FOCUS ON: OSTEOPOROSI ED ENDOCRINOPATIE - Approccio multidisciplinare per la prevenzione ed il trattamento delle fratture secondarie ad osteoporosi", March 8-9, 2012 - Ancona, Italy. Invited talk: "Forme genetiche con aumento della massa ossea: osteopetrosi ed altre forme"

C. Contributions to Science

1) GENETIC DISSECTION of inherited **RARE DISEASES**: I contributed to the molecular characterization of T-B⁺ Severe Combined Immunodeficiency (T-B⁺ SCID), took part in the identification of the first gene responsible for human OSTEOPETROSIS (i.e., TCIRG1) and first described the RANKL-deficient subset of Osteopetrosis in patients. More in general, I contributed to characterize the genetic basis of human osteopetrosis and to define genotype-phenotype associations. For this activity I have been involved in the definition and periodical review of the Consensus Guidelines for diagnosis, therapy, and follow-up of Osteopetrosis, on behalf of the ESID and the European Bone Marrow Transplantation-Inborn Error Working Party (EBMT-IEWP). In the framework of this activity, in recent years I exploited exome sequencing in patients with osteopetrosis of unknown molecular diagnosis. This led to the identification of deep intronic mutations and of synonymous not silent mutations in known osteopetrosis-associated genes in patients with atypical phenotypes; and to the identification of the genes (i.e., *NBAS* and *PIGB*) responsible for a neglected inherited disorder called Acrofrontofacionasal dysostosis type I.

References: Frattini A et al, Nat Genet 2000 [coauthor]; Sobacchi C et al, Nat Genet 2007; Palagano E et al, Bone 2018 [last author]; Palagano E et al, Bone 2021 [last author]

(2) PRECLINICAL STUDIES aimed at the development of **NEW THERAPIES** for osteopetrosis, by using pharmacological, cell-based and gene therapy approaches, particularly related to RANKL-deficient Osteopetrosis. Specifically, I demonstrated that the pharmacological administration of soluble RANKL in the Rankl knock-out (Rankl ko) animal model of disease corrected the skeletal defects and the secondary hematological alteration, providing proof-of-concept of the effectiveness of this therapeutic approach. I demonstrated that biomimetic scaffolds seeded with mesenchymal stromal cells overexpressing soluble RANKL are a source of RANKL able to activate (though at a limited extent) osteoclast formation in Rankl ko mice. I contributed to demonstrate that targeted gene correction in induced Pluripotent Stem Cells generated from the oc/oc mouse model of osteopetrosis allows the generation of functional osteoclasts, pointing to the possible future exploitation of this approach into the

human clinical setting, to possibly overcome limitations of the current therapy (i.e., allogeneic hematopoietic stem cell transplantation). Finally, I contributed to characterize circulating HSPCs from patients with TCIRG1-deficient osteopetrosis and to show that expansion of these cells coupled to gene therapy can overcome the limit of stem cell harvest in osteopetrotic patients, which paves the way to future gene-based treatment of skeletal diseases caused by bone marrow fibrosis.

References: Lo Iacono N et al, J Bone Miner Res 2012 [last author]; Neri T et al, Stem Cell Reports 2015 [coauthor]; Menale et al, Stem Cell Transl Med 2019 [last author]; Capo V et al, Haematologica 2021 [coauthor]

(3) Characterization of the ROLE of RANKL in SKELETAL STEM and PROGENITOR CELLS: In the Rankl knock-out murine model, I have very recently demonstrated an unexpected role of RANKL in bone homeostasis: building upon previous results showing a partial osteogenic defect in bone marrow-derived mesenchymal stromal cells lacking RANKL which could be improved by restoring RANKL production through lentiviral transduction, I demonstrated that in the absence of RANKL the skeletal stem cell hierarchy is altered and that this translates into impaired stemness features and reduced mineralization capacity. Further deepening of this mechanism, as planned in the present proposal, will expand the understanding of the osteopetrotic bone phenotype and possibly identify new targets for therapy.

References: Schena F et al, Stem Cells 2017 [last author]; Menale C et al, Stem Cell Transl Med 2019 [last author]; Schiavone ML et al, Stem Cell Res Ther 2024 [last author]

(4) Generation of NEW MURINE MODELS: I generated a new mouse model of osteopetrosis, which we named NSG oc/oc, presenting severe autosomal recessive osteopetrosis owing to the Tc1rg1oc mutation, and profound immunodeficiency caused by the NSG background. This study paved the way to generating an improved xenograft model for in vivo evaluation of functional rescue of patient-derived corrected cells. Moreover, I generated the dipeptidyl peptidase 3 knock-out (Dpp3 ko) mouse and by this mean demonstrated for the first time the important role played by DPP3 in bone pathophysiology; indeed, Dpp3 ko showed low bone mass owing to increased osteoclast activity caused by sustained oxidative stress and local inflammation. This evidence was corroborated by the result of a clinical study in postmenopausal women affected by severe osteoporosis. On this line, recent (and ongoing) activities are aimed at addressing DPP3 function with respect to bone health in the framework of common osteoarticular pathologies with the support of various clinical collaborations and markedly technological and innovative approaches, thanks to the collaboration with Politecnico of Milan.

References: Palagano E et al, Bone Rep 2020 [co-last and corresponding author]; Menale C et al, J Bone Miner Res 2019 [last author]; Menale C et al, Molecules 2022 [corresponding author]; Talone B et al, Front Bioeng Biotechnol 2022 [corresponding author]

(5) 3D CULTURE systems: thanks to the collaboration with CNR Researchers of the ISSMC (previously ISTECC), in Faenza, I approached 3D cultures on biomimetic scaffolds, exploited for proof-of-concept therapeutic purposes in the Rankl ko mouse and in recent works aimed at the development of an *in vitro* model of bone infection (osteomyelitis).

References: Schena F et al, Stem Cells 2017 [last author]; Sobacchi C et al, Int J Mol Sci 2018; Menale C et al, Stem Cell Transl Med 2019 [last author]; Parente R et al, Pathogens 2021 [corresponding author]