Fiorentina Ascenzioni

Curriculum vitae

General Information

Spoken Languages: Italian, native speaker; English, fluent; German, basic
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Education

Graduation: 1983; Master's degree in Biology; University of Rome, La Sapienza (110/100 e lode)
PhD: 1986, PhD in Natural Science; University Eberhard Karls di Tübingen, (DE) (magna cum laude).

Post-doctorate training and fellowships

- 1985-1987 fellowship "Istituto Pasteur-Fondazione Cenci Bolognetti" at Karls Eberhard of Tübingen, Tübingen (DE);
- 1987-1988 Post doc fellowship "Fondazione Giorgio e Loredana Shenker" at Sapienza University, Rome (It);
- 1988-1989, Post doc at University Karls Eberhard of Tübingen, Tübingen (DE);
- 1989-1990 Post doc fellowship "Fondazione Adriano Buzzati Traverso" at Sapienza University, Rome (It);
- 1990-1991 Post doc fellowship "Istituto Pasteur-Fondazione Cenci Bolognetti" at Sapienza University, Rome (It).

Appointments

- 2001 – present: Department of Biology and Biotechnologies Charles Darwin (BBCD), Sapienza University, Rome (It); Associate Professor, SSD BIO19;
- 2017, April 4th, National Scientific Qualification for the functions of Full University Professor in the Sector 05/I2 – Microbiology, SSD BIO/19 (ASN, *Abilitazione Scientifica Nazionale alle funzioni di Professore Universitario di Prima Fascia*);
- 1996-2001: Department of Cellular and Developmental Biology, Sapienza University, Rome (It); Researcher (*Ricercatore a tempo indeterminato*), SSD BIO/19;
- 1992-1996: Department of Cellular and Developmental Biology, Laboratory of Microbiology, Sapienza University, Rome (It); Technicians, VII level (*Tecnico di laboratorio, 7° livello*).

Mobility

1998 - 3 months at Ecole Normale Superieure Lyon (Fr); researcher mobility fellowship UE.

Interruption of work (total 21 months)

- 1992, 5 months, maternity;
- 1995, 5 months, maternity;
- 1996, 5 months, maternity;
- 2015, 6 months, disease.

Teaching experience

Teaching activity carried out at the BBCD Department, formerly Department of Cell and Developmental Biology, Faculty of Mathematical, Natural and Physical Sciences (MNPS), Sapienza University of Rome (It).

- 2018 – present: General Microbiology (6 CFU; SSD BIO/19), Degree Course (*Laurea di primo livello in Biotecnologie Agro-Alimentari e Industriali*);
- 2007 – present: Molecular Genetics of Microorganisms (6 CFU; SSD BIO/19), Master's degree Course (*Laurea Magistrale in Biologia e Tecnologie Cellulari, LM-BTC)*);
- 2008 – present: Microbial vectors and application in gene and cell therapy (6 CFU; SSD BIO/19) Master's degree Course (*LM-BTC*);
- 2004 - 2008, Gene Therapy (3 CFU; SSD BIO/11) Master's degree Course in Genome Biotechnology (*Laurea Specialistica in Biotecnologie Genomiche*);
- 2000 - 2007: Microbial Genetics (6 CFU; SSD BIO/19) Master's degree Course (*Laurea Specialistica in Biologia Cellulare e Applicata*).

Teaching activity carried out at the Department of Environmental Biology, Faculty MNPS, Sapienza University of Rome (It).

- 2017 – 2019, Environmental Microbiology (3 CFU; SSD BIO/19) Master's degree Course Ecobiology (*Laurea Magistrale in Ecobiologia)*;
- 2003 - 2008, Microbiology (6 CFU; SSD BIO/19), Degree Course (*Laurea di primo livello in Scienze Ambientali*).

Activity in higher education (PhD school and master)

- 2001 - present, member of the Scientific Board (*Collegio dei docenti*) of the Doctorate in Cellular and Developmental Biology, BBCD Department, formerly Department of Cell and Developmental Biology, Sapienza University of Rome (It);
  - Mentor of 10 PhD students, including one joint PhD Sapienza University (It) -ENS Lyon (Fr);
  - Examination commission for admission to the Doctorate in the following cycles: 2003, XIX cycle; 2011, XXVII cycle; 2012, XXVIII cycle;
- 2004 - 2010, master M1 (*Master di primo livello*) in "*Applicazioni e controlli biotecnologici*".

Coordination and management

Service activities to the academic community: BBCD Department and MNPS Faculty.

- 2022 - 25, President of the LM-BTC (Department BBCD, Faculty MNPS), elected by the Course Council (*Presidente di Corso di Studio, eletto dal Consiglio del Corso di Studi*) and appointed by the Rector, Prof. A. Polinemi (rectoral decree 95/2023);
- 2019 - 22, President of the LM-BTC (Department BBCD, Faculty MNPS), elected by the Course Council (*Presidente di Corso di Studio, eletto dal Consiglio del Corso di Studi*) and appointed by the Rector, Prof. A. Gaudio (rectoral decree 3887/2019);
- 2018-21 Referent of the Double degree programme LM-BTC (Sapienza) – Master in Microbiologie (Aix-Marseille); extended to 2023; designated by the LM-BTC Council (*Consiglio del Corso di Studi*);

- 2013 - 2019, vice President of the LM-BTC, nominated by the President, Prof. D. Bellincampi;
- 2006 - 2009, Coordinator of the Master's degree Course "Laurea Specialistica in Biologia Cellulare", nominated by the Council of area (CAD, *Consiglio di Area Didattica*);
- 2014-16, Member of the BBCD Department Board (*Giunta di Dipartimento*), elected among the Associate Professors of the Department Council;
- 2016-18, Member of the BBCD Department Board (*Giunta di Dipartimento*), elected among the Associate Professors of the Department Council;
- 2018 – present, Department BBCD, Member of the Commission for the elaboration of the Three-year Strategic plan; (strategic plan 2018/2020 and 2023-25), nominated by the Director of the department, Prof. M. Oliverio;
- 2016 - 18 Member of MNPS Faculty Board (*Giunta di Facoltà*), elected among the Associate Professors in the Department Council;
- 2014 - 16, Member of MNPS Faculty Board (*Giunta di Facoltà*), elected among the Associate Professors in the Department Council;
- Exams for the qualification to the profession, Biologist and Biologist Junior (*Esami di stato, Professione Biologo e Biologo iunior*)
  2012, I and II sections; role, President of the commission;
  2009, I and II sections; role, Secretary of the commission;
  2007, I and II sections; role, Secretary of the commission;
  2002, I and II sections; role, Member of the commission.

Ministry of Health - Recognition of the qualification Biologist obtained in non-EU countries (*Riconoscimento qualifica professionale, Biologo*). Activity: organization and execution of compensatory tests for non-EU citizens who have requested recognition of the title of biologist.

- 2022, November 18th; role, President of the commission, appointed by the Ministry of Health, General Directorate of Health Professions; general director Dr. Rossana Ugenti (DGPROF/2/1.5.H.A.7);
- 2023, June 28th; role, President of the commission, appointed by the Ministry of Health, General Directorate of Health Professions; general director Dr. Rossana Ugenti (DGPROF/2/1.5.H.A.5/2023/1).

Service activities to the scientific community: Evaluation activity

- 2021-22, GEV member (*Gruppo Esperti Valutatori, area 5 Biologia*), appointed by ANVUR (*Agenzia Nazionale di Valutazione del Sistema Universitario e della Ricerca*) for the VQR 2015-19;
- Grant evaluator for the following International and National Funding Agencies:
  Welcome Trust (UK);
  Agence Nationale de la Recherche (Francia);
  Research Foundation Flanders (Fonds Wetenschappelijk Onderzoek - Vlaanderen, FWO, Belgio);
  Estonian Research Council;
  Ministry of University and Research, Italy (*Progetti di ricerca di rilevante interesse nazionale*, *PRIN*);
  Università degli Studi di Firenze.
- Reviewer for the many scientific journals including: ACS Infectious Disease; Antibiotics, Spectrum, Virulence, Microorganisms, Frontier in Microbiology, Infection and Immunity, Applied and Environmental Microbiology, J Cystic Fibrosis, Nucleic Acids Research, PlosOne, European Journal of Pharmaceutical Sciences, J Control Release, FEBS Open Bio, Gene, Human

gene therapy, Gene Therapy, International Journal of Pharmaceutics, International Journal Of Environmental Health Research, J Gene Medicine, J of Gene Medicine.

Society memberships, Awards and Honors

National Society

Società Italiana di Microbiologia Generale e Biotecnologie Microbiche;
Società Italiana di Biofisica e Biologia Molecolare (discontinued);
Società Italiana Fibrosi Cistica, since 2003; 2011-12, delegate of the basic research commission; 2005-07 delegate for relations with institutions and companies.

International Society

European Society of Cystic Fibrosis (2010-14; 2021-23).

Awards and Honors

- Best poster "European cystic fibrosis conference" Brest (Fr) 2009;
- Award Annalisa Marzotto, Società Italiana Fibrosi Cistica, Soverato 2009;
- Thesis award Fondazione Istituto Pasteur – Fondazione Cenci Bolognetti, 1983.

Third mission

Patent

- Inibitori della antibiotico-resistenza mediata da ArnT; autori, F. Imperi, **F. Ascenzioni**, M. Mori, F. Ghirga, D. Quaglio, S. Corradi, A. Lo Sciuto, B. Botta, A. Calcaterra, N 102019000012888, released 13/07/2021. International extension, WO2021/014422, concluded with regionalization / nationalization in EU (20753432.2, date 04/02/2022) and USA (17/629,654 date 24/01/2022);
- **Ascenzioni, F**., Donini, D., e Gilson, E. Processo di sintesi di DNA telomerico di vertebrato, prodotti così ottenuti e relativi impieghi. N RM2000A0004 Italian patent.

Scientific dissemination to a lay public

2022-2023 "*L'alloro, Conoscenza, controllo e strategie per la vita*", I Edition, July 9th, 10th, 17th 2022, Vignanello (VT); II Edition July 20th, 21st, 2023, Vignanello (VT). Young graduates present their dissertations to the public, with the participation of young start-up. Sponsors: ARCI, Comune di Vignanello; Role, event organizer and host;

2020, October 5th, *GEO Scienza*; RAI-TV (via Asiago, Rm, It), invited to speak about "Plants may help us to combat antibiotic resistance: case study, *Fabiana densa, var. ramulosa*";

2017 - 2014 - Liceo Scientifico E. Majorana, Latina (LT), Giornate sulla ricerca: la Fibrosi Cistica, "*Fare ricerca per comprendere e curare: fibrosi cistica la sfida continua*";

2013 - Bioforum (sponsored by FARMM, Fondazione EBRI, CNR), Roma "*Biofilm microbici: complesse comunità che coordinano le attività dei singoli*" 18/01/2013, Montelibretti (RM).

Scientific journals for a lay public

- Orizzonti FC, vol 2, 2021, For the column: The time capsule, tell the public. Targeted replacement of full-length CFTR in human airway stem cells by CRISPR/CAS9 for panmutation correction in the endogenous locus. F. Ascenzioni and L. Cavinato. Role, author;

- 2022 - present, Editorial board member of "Orizzonti FC", editor of the column "The time capsule";
- Scientific consultant for journalists.
- Among the most recent consultancies: ANSA Scienze e Tech, 25/06/2022, "*Un batterio lungo 1 centimetro"* by Benetta Bianco; Viveresani e Belli, (2021, vol 3) "*I Super Batteri*".

Funding Information

Competitive Grants - role, Principal Investigator (PI)[a]

2021-2023 Pharmacological inhibition of colistin resistance in gram-negative cystic fibrosis pathogens, Grants N FFC#12/2021, Fondazione* Ricerca Fibrosi Cistica, € 65K;
2019-2021 Pharmacological inhibition of colistin resistance in gram-negative cystic fibrosis pathogens, Grants N FFC#15/2019, Fondazione Ricerca Fibrosi Cistica, € 65K;
2017 - 2019 Analisi comparata dei profili di espressione genica nei macrofagi FC in risposta a stimoli batterici, Regione Lazio, Fondi per la ricerca in Fibrosi Cistica, € 40K;
2011 - 2013, Assembly and functional analysis of genomic context vectors containing the human CFTR locus, (36 months), Istituto Pasteur-Fondazione Cenci Bolognetti, € 60K;
2009 - 2011 CFTR gene engineering by means of prokaryotic and eukaryotic artificial chromosome, Istituto Pasteur-Fondazione Cenci Bolognetti, € 60K;
2009 - 2010 Analisi del potenziale patogeno e dei suoi meccanismi in ceppi batterici appartenenti al Burkholderia cepacia complex C26F09THKF, Ateneo federato della Scienza e della Terra, € 4K;
2008 - 2009 Analisi del potenziale patogeno e dei suoi meccanismi in ceppi batterici appartenenti al Burkholderia cepacia complex C26F082EBW, Ateneo federato della Scienza e della Terra, € 4K;
2007 - 2009 Development and analysis of chromosome vectors, Part II, Istituto Pasteur-Fondazione Cenci Bolognetti, € 60K;
2005 - 2006 Evaluation of PEI-albumin for in vitro and in vivo delivery of CFTR chromosomal vectors in airway model systems/Grant N FFC#3/2005, Fondazione Ricerca Fibrosi Cistica, 30K;
2004 - 2006 Development and analysis of chromosome vectors, Part I, Istituto Pasteur-Fondazione Cenci Bolognetti, € 60K;
2002 - 2004 Minichromosomes: a new approach for Cystic Fibrosis gene therapy. Grant N FFC#1/2002, Fondazione Ricerca Fibrosi Cistica, 131K;
2001- 2003 Assembly of mammalian artificial chromosomes and utilization as vector for the CFTR gene, Istituto Pasteur-Fondazione Cenci Bolognetti, € 60K;
2000 - 2002 Functional analysis of the CFTR gene cloned in a human artificial chromosome/ Grant N. A.164 Telethon, 100K;

Competitive Grants - role, Responsible of Sapienza University Unit/Partner [a]

2009 - 2010 Mechanisms of bactericidal activity of human macrophages and influence of CFTR mutations. Grant FFC #21/2009 Fondazione Ricerca Fibrosi Cistica (PI P. Del Porto, € 25K), Partner, € 10K;
2009 - 2007 Mechanisms of bactericidal activity of human macrophages and influence of CFTR mutations. Grant N FFC #14/2007, Fondazione Ricerca Fibrosi Cistica, (PI P. Del Porto, € 58K), Partner, € 20K;

[a]the amounts are reported in thousands (K) €

2008 - 2010 *Burkholderia cenocepacia* pathogenicity: synergistic interactions with *Pseudomonas aeruginosa* and adaptation to CF host. Grant N FFC #17/2008, Fondazione Ricerca Fibrosi Cistica (PI AM Bevivino, € 65K), Partner, € 20K;
2006 - 2008 Influence of *Pseudomonas aeruginosa* and CF host on *Burkholderia cenocepacia* pathogenicity. Grant N FFC#7/2006, Fondazione Ricerca Fibrosi Cistica (PI AM Bevivino, € 35K), Partner, € 10K;
2004 - 2005 Evaluation of the pathogenicity of environmental and clinical isolates of *Burkholderia cepa complex* alone and in the presence of *Pseudomonas aeruginosa*. Grant N FFC #11/2004 Fondazione Ricerca Fibrosi Cistica (PI AM Bevivino, € 30K), Partner, € 10K;
2004 - 2007 Improved precision of nucleic acids-based therapy of cystic fibrosis, FP6-2003-LIFESCIHEALTH-I, Contract no: 005213; PI Joseph Rosenecker (Ludwig Maximilians University, München, Germania), responsible of Sapienza University Unit, € 300K;
2002 - 2006 Development and application of chromosome-based gene transfer vectors for cell therapy, UE, FP5 LIFE-SCIENCE, contract no: QLK3-CT-2002-02119, PI Dr. Massimo Conese (San Raffaele Hospital), responsible of Sapienza University Unit, € 350K;

Competitive Grants - role, Participant[b]

2022 - PNNR, Centro Nazionale 3, Spoke 2. RNA based therapeutics in cancer: from discovery to preclinical studies. PI Alberto Boffi, Sapienza University, Rome (It), 4400K;
2018 – 2020 Functional characterization and pharmacological inhibition of colistin resistance in *Pseudomonas aeruginosa*, Istituto Pasteur-Fondazione Cenci Bolognetti, PI, F. Imperi, Sapienza University, Rome (It), University of Verona, Verona (It), 40K;
2017 - 2019 Epigenetic modulation of inflammation: evaluation of the role of microRNAs in cystic fibrosis macrophages, Vaincre le Mucoviscidose (Fr). PI, P. Del Porto, Sapienza University, Rome (It), € 50K;
2012 - 2014 Study of the pathogenetic and therapeutic role of the epithelial Na+ channel (ENaC) in CF and CF-like disease. Gran N FFC#3/2012, Fondazione Ricerca Fibrosi Cistica. PI M. Lucarelli, Sapienza University, Rome (It), € 85K;
2010 - 2011 Molecular and functional study of the epithelial Na+ channel (ENaC) in CF and CF-like disease. Gran N FFC #1/2010, Fondazione Ricerca Fibrosi Cistica. PI, C. Bombieri, € 60K.

Grants financed by Sapienza University - role, PI[a]

2023 - Fighting antibiotic resistance by exploiting innovative strategies and targets, Sapienza University; Project class, *Progetti grandi*; pending;
2022 - 2023 Inhibition of colistin resistance by optimized inhibitor of ArnT in planktonic and biofilms, 10K;
2019 - 2020 Inhibition of colistin resistance in gram-negative pathogens by natural compounds, Sapienza University, € 10K + 23.78 K (post-doc fellowship);
2014 - 2015 Telomere maintenance in the absence of essential telomere-capping proteins: analysis of the molecular mechanisms involved in telomere resection, C64A14HF8A, Sapienza University, 12 months, 3K;
2013 - 2014 Nitric oxide in macrophage response to *P. aeruginosa*: modulation of microbicidal activity and autophagy. C26A137YST, Sapienza University, 5K;
2012 – 2011 Lung Inflammation and defence against bacteria in CF: contribution of autophagy to macrophage dysfunction, Grant N C26A124W28, Sapienza University, 3K;

[a]the amounts are reported in thousands (K) €
[b]the total amount of each project is reported

2011- Rimozione di azoto da digestati di origine zootecnica in reattori MFC (Microbial Fuel Cell): analisi dei batteri elettrogenici e denitrificatori, 12 mesi, Grant N C26A11HBR2 Sapienza University, 12 months, € 12K+ 22.94 K (post-doc fellowship);
2010 – 2011 Analisi molecolare di batteri elettrogeni isolati da un prototipo in scala-laboratorio di una cella a combustibile microbiologica, C26A107ENC, Sapienza University, 4K.

Cooperation funds

2021-2022 CIVIS, Short mobility courses; Hub 1, Climate, Environment and Energy; The project comprised 4 blended-learning courses and 2 blended-learning and practical courses; Topics: microorganisms for biofuel production; Current Methods in Molecular Microbiology: from theory to applications; plant-microorganism interactions; Radionuclides, radiation and microorganisms. Aix-Marseille University (Marseille, FR); Sapienza University (Rm, IT); University of Tübingen (Tübingen, DE). Role: proponent: The project was supported by CIVIS; mobility grant for Students and Professors;
2023 - Proponent of the Visiting Professor Grant for Dr Stefano Mattarocci, INSERM Paris (FR), pending;
2020 - Proponent of the Visiting Professor Grant for Dr Stefano Mattarocci, INSERM Paris (FR);
2009, Proponent of the Visiting Professor Grant for Dr J. Rosenecker, Dep. of Pediatrics, Ludwig-Maximilians, University of Munich (DE);
2009 - "Messa a punto di tecniche di trasferimento genico in cellule staminali epiteliali". Vigoni, Scientific exchange programe; exchange researcher: Dr J. Rosenecker, Dep. of Pediatrics, Ludwig-Maximilians, University of Munich (DE); Fiorentina Ascenzioni, Sapienza University, mobility grant;
2005- Eurocare CF, financed by EU in the frame of the concerted action 2005-2009, Work Package, Gene Therapy. PI, Massimo Conese (HS Raffaele, Mi, IT) and David Sheppard (University of Bristol, Bristol, UK); role, participant, mobility grant.

International Workshop Organization

2005, 29 September to 3 October - EMBO workshop: "Chromosome structural elements: from DNA sequence to function", Villa Mondragone, Rome (It). Scientific committee Fiorentina Ascenzioni, Silvia Bacchetti, Giuseppe Novelli, Maria Savino. The workshop was granted by: EMBO, MIUR, Istituto-Pasteur Fondazione Cenci Bolognetti; Sapienza University; Applied Biosystem. Event report: https://doi.org/10.1038/sj.embor.7400661. Role, Organizer and Responsible for funds.

Third party activities[a]

- 2010, Todini SpA; " Execution of biological tests on soil samples taken from the Sardara ss131, Sardinia (It): identification of Thiobacillus ferrooxidans " Todini Protocol: PST/WS/pm/2069; € 3K;
- 2010, ENAMA, Research contract ENAMA-DIE (*Dip. di Ingegneria Elettrica*, Sapienza University). "Implementation and characterization of a laboratory-scale prototype of a microbial fuel cell (MFC) fed by manure, scraps of food industries and/or products of crops; subsequent preliminary design, assistance for the implementation and performance analysis of a prototype 'scale-up' for industrial application". PI, A. Geri and F. Gatta; Participants L. di Palma (*Dip. Ingegneria Chimica Materiali Ambiente*, Sapienza University) and F. Ascenzioni (Dip. BBCD), € 45K.

[a] the amounts are reported in thousands (K) €

Invited Speaker and Seminars (speaker: Fiorentina Ascenzioni)
A selection of the most relevant events, in relation to the research topics is reported.

- VI Congress Italian Cystic Fibrosis Society (SIFC), 18th-21st Novenber, 2010, Rimini (IT) "Macrophage activity in CF: bacterial killing and cytokine production";
- Human artificial chromosomes: Development and Prospects for Gene Therapy, 18th-19th February 2007, NIH Bethesda, USA - "Artificial chromosomes assembly for CF gene therapy";
- Cystic Fibrosis Basic Reserach Conference,18th-20th April 2006, Algarve (PT). "Downregulation of human and murine ENaC channel by RNA interference";
- ENEA La Casaccia (Rm), 25/02/05. Chromosomal vectors for cystic fibrosis gene therapy;
- EMBO workshop on Nuclear Organization, Elmau (DE) 7-10/10/2004 "Minichromosomes: structural analysis and engineering";
- INSERM, Ateliers de formation, Telomeres and Telomerases: from basic research to clinical application. La Londe (FR) 19- 20/06/2003 "Artificial Chromosomes, new tool for basic and applied research;
- ECFC (European Cystic Fbrosis Conference) 6th-9th June 2001 Wien (AT) "CFTR cloning into a human mini chromosome";
- Functional analysis of the entire human CFTR gene cloned in a human artificial chromosome Telethon, Convention 2001, Riva del Garda (IT);
- ENI Ricerche, Monterotondo, 1998 "Modificazioni del metabolismo telomerico del lievito *Saccharomyces cerevisiae*";
- DIBIT, HS Raffaele, Mi (IT), 1996 "Cromosomi Artificiali, un nuovo approccio per la terapia genica";
- University of Witten (DE), 1996 "Assembly of therapeutic mammalian artificial chromsomes".

Research activity

A brief description of the most recent and relevant research topics with related reference publications by FA.
Host-pathogen interaction. Study of the molecular basis of macrophage microbicidal activity and inflammatory response, against *Pseudomonas aeruginosa.* Our interest is focused on Cystic Fibrosis (CF), a genetic disease characterized by chronic lung *P. aeruginosa* infection and hyperinflammation, both of which contribute to morbidity and mortality. In the last years, and for the first time, we have demonstrated that the *CFTR* gene, mutated in persons with CF (pwCF), is expressed in human macrophages and, similarly to epithelial cells, it acts as a chloride channel (Del Porto et al 2010). Further, we have demonstrated that CF macrophages were defective in killing *P. aeruginosa* which suggest a role of CFTR in macrophage function (Del Porto et al 2010). The microbicidal defect against *P. aeruginosa* was also observed in lung macrophages, isolated from the lung of pwCF undergoing lung transplantation (Cifani et al 2013). Very quickly our data was confirmed by others. More recently, we have investigated whether the new triple therapy, Kaftrio/Trikafta, which consists of a combination of three CFTR modulators, is also effective in restoring the activity of phagocytic cells. As expetct, Trikafta improved markedly the clinical conditions of pwCF as well as the activity of their phagocytic cells (Cimino et al 2023). This result may have multiple indication: a) Trikafta therapy, directly or indirectly, improves the activity of phagocytes against *P. aeruginosa*; b) functional phagocytes are important for the rescue of CF lung disease which in turn further confirm the notion that phagocytes deficiency contributes to CF disease (Cavinato et al 2023; Del Porto et al 2010; Cifani et al, 2013).
The microbicidal activity of macrophages depends on different mechanisms including ROS production, which contributes to the elimination of intracellular *P. aeruginosa* by CF macrophages

(Cifani et al 2013) although it doesn't appear to be optimal (Cavinato et al 2023).
Bacteria respond to the phagocytic oxidative burst by expressing different ROS scavenger enzymes. In this context we have investigated the role of the *P. aeruginosa* SODs (Super Oxide Dismutase) highlighting a direct effect of SODB on the intracellular bacteria survival and on the activation of other microbicidal mechanisms (Cavinato et al 2020). More recently, in collaboration with Prof. L. Leoni, we have also investigated the role of the transcriptional regulators DksA1-A2 on H_2O_2 tolerance and intra-macrophage survival (Fortuna et al 2022).
Currently, aiming at the identification of dysfunctional mechanisms in CF macrophages, we are investigating other ROS-dependent and -independent microbicidal mechanisms, in *ex-vivo* and *in-vitro* cellular models, with the final goal to find new therapeutic targets.

Molecular mechanisms leading to hyperinflammation. CF lungs are dominated by high levels of proinflammatory cytokines, which significantly drop after lung transplantation, suggesting that CFTR expression and function are required to control inflammation in the lung environment by (Patella et al 2015). Accordingly, we have observed a reduction of the cytokines production by monocytes after therapy with Kaftrio/Trikafta, supporting a broader anti-inflammatory property of this therapy (Cavinato et al, 2023).
Deregulated microRNA (miRNA) expression has been implicated in the pathogenesis of inflammatory lung disease. By miRNA profiling we have identified 22 miRNAs as differentially expressed between CF and non-CF macrophages and among these, miR-146a was associated with a significant enrichment of validated target genes involved in responses to microorganisms and inflammation (Luly et al, 2019). This research topic is carried out in collaboration with an immunologist, Prof. Paola Del Porto (BBCD Dep. Sapienza University). At present we are investigating the molecular effects of miR-146a upregulation on macrophage and monocyte activity in response to bacteria.
The above topics are also based on the collaboration with the clinicians of the FC Center of the Lazio Region (Policlinico Umberto I), first Dr. S. Quattrucci and more recently Dr. G. Cimino, as Center Manager.

Antibiotic resistance. Antimicrobial resistance (AR) is now putting at risk the benefits gained using antibiotics. At present, the development of new antimicrobial drugs is proceeding much more slowly than AR spreading, and new approaches are required to contain AR and to balance the very low number of new antibiotics. Among these, drug repurposing and delivery are two strategies under extensive investigation. In this context we have investigated delivery by nanoparticles to improve antibiotic activity against *P. aeruginosa* biofilms (Sardo et al 2019; Chronopoulou et al 2016).
Drug repurposing is addressed in Costabile et al (2015); in this work, the anthelmintic drug niclosamide (NCL), which has strong quorum sensing (QS) inhibiting activity against *P. aeruginosa*, has been tested as an antivirulence drug. Accordingly, we have reported that NCL repurposed in the form of inhalable nanosuspensions has great potential for the local treatment of *P. aeruginosa* lung infections. (Costabile et al 2015).
A further approach to combat AR, is the use of adjuvants, i.e. molecules that do not have antimicrobial effect *per se*, but potentiate the antibiotic activity. Colistin, a last resource antibiotic against multi drug resistant Gram-negative bacteria, interacts electrostatically with the outer membrane (OM) causing bacterial death. Gram-negative bacteria, such as *P. aeruginosa* and *K. pneumoniae*, may resist to colistin by aminoarabinosylation of lipid A, which in turn prevent colistin binding to OM. Recently, by a structure-guided virtual screening targeting ArnT, the last enzyme of the pathway leading to lipidA aminoarabinosylation, we have identified putative ArnT inhibitors that selectively potentiate colistin activity in *P. aeruginosa* and *K. pneumoniae* resistant strains (Ghirga et al 2020; Quaglio et al 2020, JOC; Quaglio et al 2020). Presently, we are working at optimization of the compounds and development of nanoparticle-systems for the combined delivery of colistin and adjuvants. The targets are colitin-resistant *P. aeruginosa* and *K. pneumoniae* strains, either reference and clinical isolates,

in planktonic cultures and biofilms. This research is made possible thanks to a multidisciplinary team that FA has put together based on specific skills that include microbiology, medicinal chemistry, biochemistry, computational chemistry, bioinformatic (molecular modelling) and drug delivery.

Microbial communities and bacterial biofilm. Bacteria may be organized in biofilms, a complex structure in which the microbial cells are embedded within an extracellular matrix that mediates adhesion to abiotic/biotic surfaces, decreases drugs penetration, and favour the exchange of resistance genes; these features make biofilms critical determinant of persistent infections and pose a significant challenge to healthcare systems (Ascenzioni et al 2021). Despite their importance, the early recognition of biofilm-associated infections still represents an unmet need in clinical microbiology. Therefore, the development of novel diagnostic and therapeutic strategies is urgently needed to manage biofilm-associated infections effectively. More recently, this research topic has been further explored through collaboration with Dr. Enea Gino Di Domenico by studying biofilms in relation to dysbiosis and disease (Cavallo et al 2022; Di Domenico 2019). Importantly, as growing body of evidence highlight the relevant role of biofilms in different infectious diseases, a rapid and robust method for the quantitative analysis of bacterial biofilms was set up. Due to its simplicity and time of execution, this method has been designed also for application in clinical microbiology, in which it may help the selection of the most effective antibiotic therapy for the treatment of difficult-to-eradicate infections (Di Domenico et al 2016).
Bacterial biofilms are being considered also in the ArnT-inhibitor project as reported above.
Biotechnological application of biofilms was also explored in a multidisciplinary study, supported by electrical and chemical engineers, aimed at biodegradation of agriculture waste by electroactive bacteria (Anammox) which, in addition to the metabolization of recalcitrant substrates, release electrons that are captured by an electric circuit (Di Domenico et al., 2015). Such systems have been proposed, and in some cases realized, for the treatment of different kind of waste, such as municipal and industrial wastewater.

Gene Therapy: vector development and targets. CF is a single gene disorder (autosomal, recessive) caused by mutations in the *CFTR* gene, supporting the notion that the introduction of the *wt* copy of the gene would cure CF disease. This approach has the advantage to be mutation-independent and applicable to all CF patients, which are highly diverse due to the many different mutated alleles that have been recognized in CF. Additionally, as mutations in the CFTR gene have been causatively linked to CF, development of genetic medicine is possible even though the disease pathophysiology is not completely understood. FA started her independent research group, working on the development of vector for the delivery of the *CFTR* gene to airway epithelial cells, whose physiological function is heavily compromised by dysfunctional CFTR. By that time the gene was identified and the first attempts to develop gene therapy, based on viral vectors, very soon revealed the limit of this approach due to the host's immune response against the vector. As an alternative, we and others, proposed the development of human artificial chromosomes as expression vector of *CFTR* gene. Human artificial chromosome assembly required expertise in the genetic manipulation of yeast that FA acquired during her studies (PhD and post-doctorate training). With a small, but very motivated, group which, in addition to FA, included geneticist and electrophysiologists from Gaslini Hospital (Ge; It) and San Raffaele (Mi, It) we have been successful in assembling the first human artificial chromosome with the entire CFTR locus (Auriche et al 2002; Auriche et al 2001; Conese et al 2007). This research topic continued with the development of simplified CFTR locus, in BAC vectors, to complement CF mutant cells (Auriche et al 2010; Rocchi et al 2010) and more recently the development of episomal vectors, based on the S/MAR element (De Rocco et al 2018).
We have also exploited other targets involved in CF disease, such as the ENaC (Epithelial sodium channel) which is upregulated in CF and appears to contribute to CF lung disease. Downregulation of ENaC is a valid strategy to improve lung function in CF that we have addressed by developing

silencing viral-vectors (Tilesi et al 2010; Aarbiou J et al 2012) and epigenetic modulators (Pierandrei et al 2021; Balconà et al 2022) of the ENaC genes.

Telomere biology. Telomere biology was a fundamental topic in FA's scientific career, which marked her formation in degree and doctoral studies. The study of telomeres originates and develops in unicellular model organisms, in particular ciliates and yeasts. Sa*ccharomyce cerevisiae* is still a valid eucaryotic model for the study of telomeres which have many implications in cell biology, including cell cycle regulation and genome stability. Due to differences in telomeric sequences among eukaryotes, it may help the use of yeast engineered with humanized telomeres, which we have constructed by manipulating the *S. cerevisiae* telomerase, and used to study fundamental aspect of telomere biology such as the activation of DNA damage checkpoints (Saint-Léger A et al 2014; Di Domenico et al 2013; Di Domenico et al., 2009) and the molecular basis of telomere-length maintenance (Brevet et al 2003).
Currently, the interest in telomere biology has been renewed thanks to the collaboration with Dr. Stefano Mattorocci (INSERM, Fr) visiting professor in our department in 2020, with whom we are developing a new line of research aimed at studying telomere damage and oxidative stress in immune cells infected by bacteria.

Summary of Scientific Achievements

Database: Scopus; consultation date, June 21st, 2023

- Publications (International): 64, from 1985 to 2023;
- Total citations: 1202;
- Average Citations/publication: 18.781;
- *h-i*ndex: 20.

Database: JCR-Clarivate; consultation date, June 21st, 2023

- Total IF: 286.939[c];
- Average IF/publication[c]: 4.554,

Patents: 2

Publications (complete list)

*last and/or corresponding author; IF, Impact Factor of the year of publication; source, Journal Citation Reports (JCR) - Clarivate

1. Cavinato L, Luly FR, Pastore V, Chiappetta D, Sangiorgi G, Ferrara E, Baiocchi P, Mandarello G, Cimino G, Del Porto P and **Ascenzioni F***. Elexacaftor/tezacaftor/ivacaftor corrects monocyte microbicidal deficiency in cystic fibrosis. Eur Respir J. 2023; 61(4): 2200725. doi: 10.1183/13993003.00725-2022. IF 24.3;
2. Cimino G, Sorrenti S, Murciano M, Galoppi P, **Ascenzioni F**, Botta B and Brunelli R; Sapienza University Working Group on Cystic Fibrosis in Pregnancy. Use of elexacaftor/tezacaftor/ivacaftor combination in pregnancy. Arch Gynecol Obstet. 2023. doi: 10.1007/s00404-023-06962-5. IF 2.6;
3. Cavallo I, Sivori F, Truglio M, De Maio F, Lucantoni F, Cardinali G, Pontone M, Bernardi T, Sanguinetti M, Capitanio B, Cristaudo A, **Ascenzioni F,** Morrone A, Pimpinelli F and Di Domenico EG. Skin dysbiosis and Cutibacterium acnes biofilm in inflammatory acne lesions of adolescents. Sci Rep. 2022; 12(1): 21104. doi: 10.1038/s41598-022-25436-3. IF 4.997;

[c] calculated on the number of publications with IF (63 out of 64)

4. Fortuna A, Collalto D, Schiaffi V, Pastore V, Visca P, **Ascenzioni F**, Rampioni G and Leoni, L. The *Pseudomonas aeruginosa* DksA1 protein is involved in H2O2 tolerance and within-macrophages survival and can be replaced by DksA2. Scientific Reports 2022; 12(1):10404. doi: 10.1038/s41598-022-14635-7. IF 4.6;
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7. Pierandrei S, Truglio G, Ceci F, Del Porto P, Bruno SM, Castellani S, Conese M, **Ascenzioni F*** and Lucarelli M*. DNA Methylation Patterns Correlate with the Expression of *SCNN1A*, *SCNN1B*, and *SCNN1G* (Epithelial Sodium Channel, ENaC) Genes. Int J Mol Sci. 2021; 22(7): 3754. doi: 10.3390/ijms22073754. IF 6,208;
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Book chapter

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(Fiorentina Ascenzioni)

[d] 1997 IF, the first available in JCR-Clarivate