NAME: ***BRUNO, ANNALISA***

POSITION TITLE: ***Assistant Professor of Pharmacology***

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
University of Naples Federico II, Napoli, Italy	Master's degree in Pharmaceutical Chemistry and Technology	10/1994	07/2001	Pharmacology
"G. d'Annunzio" University of Chieti-Pescara, Italy	PhD in Medical-Surgical Sciences, Clinical and Experimental Science	01/2010	03/2014	Pharmacological Sciences
"G. d'Annunzio" University of Chieti-Pescara, Italy	Post-doctoral Research Fellow	03/2014	02/2020	Pharmacology

A. Personal Statement

I am a Associate Professor in Pharmacology at the Department of Innovative Technologies in Medicine & Dentistry, University "G. d'Annunzio" of Chieti-Pescara. I hold several teaching positions (BIOS/11 - PHARMACOLOGY) in the following study courses: Psychological Sciences, Midwifery, Nursing, and Psychology of Well-Being and Performance. During my post-doctoral research fellowship, I collaborated on several translational research projects utilizing in vitro cells, mouse models, and clinical trials. I have extensive experience with animal exoerimental models, including conditional transgenic mice, aimed at identifying new disease biomarkers and therapeutic approaches to control inflammation, tumorigenesis, and metastasis formation. I recently contributed to developing a conditional knockout mouse with a specific deletion of cyclooxygenase(COX)-1 in platelets, resembling the pharmacodynamic of the antiplatelet agent low-dose aspirin. The use of these mice led to the identification of platelet COX-1-dependent thromboxane (TX)A2 as an immunosuppressive pathway limiting T-cell immunity to cancer metastasis. These findings, reported in a manuscript recently accepted for publication in Nature, pave the way for more effective anti-metastatic immunotherapies.
More recently, as a senior researcher in the Laboratory of Molecular Pharmacology and Pharmacogenetics, at the Center for Advanced Studies and Technology (CAST) of the University "G. d'Annunzio" of Chieti-Pescara, I have been involved in research projects assessing the impact of micro(nano)plastic exposure on the development of chronic inflammatory and neoplastic diseases in cellular and animal models.

I am a Scientific Member of the Animal Welfare Board at the University "G. d'Annunzio" of Chieti-Pescara. I am well-versed in EU and national laws regulating the management of laboratory animals. I possess strong skills in organizing and managing animal studies and have significant experience in editing and revising research protocols for approval by the Institutional Animal Care and Use Committee. As a staff researcher, I have successfully contributed to several research projects, funded by national and international organizations, including the Italian Association for Cancer Research (AIRC), the Italian Ministry of Education, University and Research (MIUR), and Cancer Research UK. These projects resulted in numerous peer-reviewed publications in high-impact journals. My research activities also included a commitment to education: I have had the opportunity to mentor and guide young researchers

and PhD students. Additionally, I serve as a reviewer for several international scientific journals, contributing to the quality and dissemination of academic research.
My past experiences have underscored the critical role of communication among project members and the need for realistic research plans, timelines, and budgets. This application builds logically on my previous work and ongoing research activities. At this stage of my career, I possess the expertise, comprehensive training, and a cohesive research team to successfully execute the proposed research project.

B. Positions, Scientific Appointments and Honors

Positions

- ***2026 – Present*** *Associate Professor* of Pharmacology, "G. d'Annunzio" University of Chieti-Pescara, Italy
- **2024 – Present** Member of Teaching and research staff of PhD program in Molecular Oncology and Tumor Immunology, "G. d'Annunzio" University of Chieti- Pescara, Italy
- **2023 – Present** Senior Research Assistant of Pharmacology, "G. d'Annunzio" University of Chieti- Pescara, Italy
- **2020 – 2023** Young Research Assistant of Pharmacology, "G. d'Annunzio" University of Chieti-Pescara, Italy
- **2019-present:** Scientific Member of the 'Committee for Animal Care' (OpBA), established by Resolution No. 213 of the Academic Senate of the 'G. D'Annunzio' University of Chieti - Pescara of 17/06/2014, in compliance with Art. 25 of Italian Legislative Decree No. 26 of 04/03/2014, following European Directive No. 63 of 22/09/2010 on the protection of animals used for scientific purposes
- **2014 – 2020** Post-doctoral Research Fellow, "G. d'Annunzio" University of Chieti-Pescara, Italy
- **2010 – 2014** PhD Student in Medical-Surgical Sciences, Clinical and Experimental Science, "G. d'Annunzio" University of Chieti-Pescara, Italy
- **2008 – 2009** Pre-doctoral Research Fellow, "G. d'Annunzio" University of Chieti-Pescara, Italy
- **2005 – 2008** Junior Researcher, Dompé pha.r.ma. s.p.a. Research & Manufacturing, L'Aquila, Italy
- **2002 – 2005** Pre-doctoral Research Fellow, Institute of Pharmacological Sciences "Consorzio Mario NegriSud", Santa Maria Imbaro (CH), Italy

Scientific Appointments and Honors

- **2007 – Present** Member of Italian Society of Pharmacology
- **2021** Obtained the National Scientific Qualification (ASN) as Associate Professor in Pharmacology, awarded Italy by MIUR – Italian Ministry of Education, Italy
- **2021** Awarded with "Fondi di Ateneo ex 60%" Research Grant for the project "Effect of low-dose aspirin on the lipidomic profile of colorectal cancer" funded by MIUR – Italian Ministry of Education, Italy

C. Contributions to Science

My scientific production is documented by:

- **57 publications in Peer-Reviewed Journals:**

Complete List of Published Work in: *https://ricerca.unich.it/cris/rp/rp02351#.WOS8J5VPrIU*

Bibliometric indicators:

- Total citations: **2170** ***(from Scopus)***
- H-index: **29** ***(from Scopus)***

Main Scientific Interests:

i) Development of biomarkers predictive of the response to anti-inflammatory drugs. During my PhD program, I contributed to the realization of a clinical study aimed at exploring the pharmacodynamic interactions occurring in the co-administration of nonsteroidal anti-inflammatory drugs (NSAIDs) naproxen and low-dose aspirin (Anzellotti et al., Arthritis Rheum. 2011,63:850-859). Moreover, I contributed to the pharmacological characterization of new anti-inflammatory agents: a)selective inhibitors of the enzyme mPGES1, the synthase involved in prostaglandin E2 generation (Bruno et al., Biochem Pharmacol. 2010, 79: 974-81).

ii) Role of platelets in inflammation and cancer. The biological mechanisms that lead to the development of colorectal cancer also seem to be involved in the development of cardiovascular diseases, such as atherosclerosis and thrombosis. In particular, several experimental studies have shown that low-dose aspirin, used to prevent cardiovascular events, also has anti-tumor effects (mainly against colorectal cancer). The drug primarily acts by inhibiting platelet function. Therefore, it has been hypothesized that these cells may also play a role in cancer development and progression. During my post-doctoral fellowship, I conducted several studies on this topic, which contributed to clarifying the mechanisms by which activated platelets promote cancer development and metastasis formation.

- By generating a mouse with a specific deletion of cyclooxygenase-1 in megakaryocytes/platelets, which reproduces the human pharmacodynamics of low-dose aspirin (COX-1 cKO mice), I contribute to demonstrating that platelets can participate in the initial phase of tumor formation by inducing an intestinal inflammatory microenvironment (Sacco & Bruno, J Pharmacol Exp Ther. 2019; 370:416–426, Bruno et al., Pharmacological Research 2022; 185:106506).

- Additionally, platelets can contribute to metastasis formation due to their ability to interact with tumor cells through the induction of epithelial-mesenchymal transition (EMT) and the expression of cyclooxygenase-2; this enzyme produces prostaglandin E2, involved in many pro-tumorigenic responses. Furthermore, tumor cells activate platelets, thus promoting the release of thromboxane A2, a potent prothrombotic agent (Guillem-Lobatt, Dovizio & Bruno, Oncotarget. 2016; 7:32462-77).

- Recently, by performing experiments in mice with COX-1 cKO mice, I contributed to revealing a novel immunosuppressive pathway limiting T cell immunity to cancer metastasis. These findings provide a mechanistic basis for aspirin's anti-metastatic activity and pave the way for more effective anti-metastatic immunotherapies (Jie Yang, Nature, 2022-11-17401C, accepted for publication10th January 2025).

- Platelets can ingest circulating plasma molecules and, upon activation, release them in extracellular vesicles (EV). Notably, I contributed to demonstrating that EVs can transfer their molecular content to tumor cells, which acquire a migratory and, thus, pro-metastatic phenotype (Grande et al., Front Pharmacol. 2019;10:7, Contursi et al., Cancers 2023, 15: 350). The results of these studies support the hypothesis that the characterization of EV content released into the blood by activated platelets may have diagnostic and prognostic relevance for various diseases, including cancer.

- Chronic administration of low-dose aspirin in humans is associated with a reduction in the incidence and mortality from colorectal cancer, suggesting that the drug may influence the early stages of intestinal tumorigenesis by inhibiting platelet function. Therefore, I was involved in a clinical study in patients with familial adenomatous polyposis (FAP), showing that the presence of colorectal adenomas is associated with platelet activation (Dovizio et al., J Pharmacol Exp Ther. 2012; 341: 242-50).

- An open clinical question regarding the use of aspirin as an anti-tumor agent concerns the choice of the appropriate dose to administer to patients. In particular, it is necessary to understand whether the drug acts by inhibiting only platelets or exerts direct effects on the neoplastic lesion. In this context, I contributed to the realization of clinical studies in which direct biomarkers of aspirin action were developed, such as the evaluation of the degree of acetylation of cyclooxygenase-1 and -2, using mass spectrometry (Patrignani et al., Clin Pharmacol Ther. 2017; 102:52-61, Tacconelli et al. Biochem Pharmacol. 2020;178: 114094). These new biomarkers have highlighted that low-dose aspirin exerts a preferential inhibitory action on platelet function but simultaneously causes an inhibitory, albeit incomplete, action on the synthesis of colorectal prostanoids.

iii) The Toxic effects of micro- and nanoplastics (MNPLs) on respiratory and gastrointestinal systems. As a senior researcher, I've recently focused on studying MNPLs' toxic effects on human health.

- Our research team recently showed that polystyrene (PS)-NPs, which account for over 5% of global plastic demand, can penetrate the cytoplasm of human lung adenocarcinoma cells (A549), affecting cell viability and inducing oxidative stress, cellular senescence, and apoptosis (Milillo et al., Front Public Health. 2024; 12:1385387). While this study contributed to clarifying the mechanisms of PS-NP toxicity in the respiratory tract, the use of submerged cultures limited these findings. Thus, more recently, we assessed the toxic effects of PS-NPs on bronchial adenocarcinoma cells (Calu-3) grown at the air-liquid interface (ALI). This validated 3D-cellular model mimics an impaired airway epithelium. Preliminary results from this study showed that PS-NP exposure reduced cell viability and altered epithelium integrity, thus suggesting that MNPL exposure may exacerbate pre-existing pulmonary conditions in impaired bronchial epithelium. These effects were linked to increased biogenesis and release of EVs from Calu-3 cells exposed to PS-NPs, indicating that EV biogenesis and release might complicate the biological impacts of MP exposure.

- Accumulating evidence indicates that ingested MNPLs are toxic to the intestine and may contribute to the rising incidence of colorectal cancer. Disruption of the colonic mucus layer can facilitate MNPL entry into the bloodstream, exacerbating their toxic effects on various organs. We recently reviewed how MNPL oral exposure affects healthy individuals and those with compromised intestinal barriers, such as inflammatory bowel disease (IBD) and CRC patients (Bruno et al., Cancers. 2024; 16:3079). In this context, we have recently investigated the potential role of MNPL-induced platelet activation in these processes (preliminary unpublished data).

Participation in international and national conferences

I contributed to 23 scientific works presented at 19 national conferences and 4 international conferences. As a speaker, I presented 11 of them as oral communications and 5 as posters

Participation in national and international research projects:

- **2008-2012**: *Staff Scientist* for a project funded by AIRC (Associazione Italiana per la Ricerca Sul Cancro) [Grant IG-1262, “G.d’Annunzio” University of Chieti-Pescara, ***Project title***: “Determinants of colorectal adenoma recurrence in patients with FAP in response to celecoxib”].
- **2012-2014**: *Staff Scientist* for a project funded by AIRC (Associazione Italiana per la Ricerca Sul Cancro) [Grant IG-12111, “G.d’Annunzio” University of Chieti-Pescara. ***Project title***: “Platelets in colorectal cancer development].”

- **2013-2016**: *Staff Scientist* for a PRIN project, funded by Ministero dell'Istruzione, dell'Università e Della Ricerca (MIUR) [protocol number 2010FHH32M, 2010-2011, "G.d'Annunzio" University of Chieti-Pescara). ***Project title***: "A translational medicine research programme exploring early events in cancer development: the role of platelets in intestinal tumorigenesis."
- **2013**: *Staff Scientist* for a project funded by the pharmaceutical company Bayer Pharma AG ["G.d'Annunzio" University of Chieti-Pescara]. ***Project title***: "Development of biomarkers of prostanoid inhibition using metabolomics and proteomics strategies"
- **2014**: *Staff Scientist* for a project funded by the pharmaceutical company Bayer Pharma AG ["G.d'Annunzio" University of Chieti-Pescara]. ***Project title***: Aspirin and colorectal cancer prevention: exploring the platelet hypothesis of its mechanism of action"
- **2017-2020**: *Staff Scientist* for a project funded by AIRC, Associazione Italiana per la Ricerca Sul Cancro [Grant IG-20365, "G.d'Annunzio" University of Chieti-Pescara. ***Project title:*** "Extracellular vesicles in colorectal cancer: diagnostic and therapeutic implications].
- **2018-2020:** *Staff Scientist* for a project funded by Cancer Research UK Grant, AsCaP Collaboration. ***Project title***: "Understanding the mechanisms of aspirin chemoprevention of cancer through population research".
- **2024-2025:** *Staff Scientist* for a project funded by "THE - TUSCANY HEALTH ECOSYSTEM" - SPOKE 7 "INNOVATING TRANSLATIONAL MEDICINE", PNRR-MUR, BANDO A CASCATA SU "PRECISION MEDICINE & PERSONALIZED HEALTHCARE"-NEXTGENERATIONEU (CODICE PROGETTO ECS00000017 - CUP B63C22000680007), PI: Giovanni Di Bonaventura, "G.d'Annunzio" University of Chieti-Pescara. *Project title*: Optimized in vivo and ex-vivo Models as Platforms for the Preclinical Evaluation of Antimicrobial lead Compounds) (OMPEAC)
- **2024-2025:** *Staff Scientist* for a project funded by "THE - TUSCANY HEALTH ECOSYSTEM", Piano Nazionale per la Ripresa e Resilienza (PNRR), emanato con D.D. n. 2004/2023 – Prot. 315887 del 22 dicembre 2023. SPOKE 3. Codice progetto ECS00000017. CUP B83C22003920001. PI: Rossella Grande, Co-PI Patrizia Ballerini, "G.d'Annunzio" University of Chieti-Pescara. *Project title*: "Microvescicole Batteriche: Implicazioni nella Fibrillazione Atriale" (MICROFIBRA)

Editorial Activity

Associate Editor for Inflammation Pharmacology (Frontiers in Pharmacology).
Reviewer for the following peer-reviewed journals: Advances in Pharmacological Sciences, Biochemical Pharmacology, European Journal of Medicinal Chemistry, Journal of Physiology and Pharmacology
2010-present: Associate Editor for *Frontiers in Inflammation Pharmacology* (IF: 3.8)
2019-present: Special Issue Editor for *Cancers* (IF: 5.3)(***Special Issue Title: Platelets and cancer***)
2016: Co-author of the book chapter entitled "Acetylsalicylic acid as platelet aggregation inhibitor" published in PHARMAKON. 2017; 5:32-40 Edited by Avoxa-Mediengruppe Deutscher Apotheker GmbH(***First author)***

Chieti, July 2nd, 2026