Foreword

The Doctorate in Molecular Biomedicine is offering a number of positions linked to specific research projects, namely fellowships **1-8** and **11-13**. In contrast, fellowships **9** and **10** are not linked to specific projects; the successful candidates will have to choose from one of several available projects spanning the various topics of the PhD program.
Brief descriptions of all the available projects are provided below.

In their application, candidates may indicate their preference for any of the available projects/fellowships in the motivation letter, and will be asked to express their preference during the interview.
These preferences will be considered by the board, but please note that preferences **are NOT binding.** The positions/fellowships will be assigned by the Selection Committee and the Board of Teachers according to their judgement.

Positions with fellowship

1 - Dr. Baldassare and Dr. Belletti (CRO, Aviano)
Investigating tumor heterogeneity and therapy resistance in Breast and Gynecological cancers

Our project aims to elucidate the mechanisms underlying tumor heterogeneity and therapy resistance in breast and gynecological cancers by integrating molecular, cellular, and preclinical model systems. Tumor heterogeneity—both inter- and intra-tumoral—poses a major challenge to effective treatment, contributing to drug resistance, recurrence, and poor prognosis. By employing multi-omics approaches (e.g., genomics, transcriptomics, and proteomics), we will characterize molecular subtypes and identify key alterations associated with therapy resistance. In parallel, we will use 2D and 3D cell culture systems, including patient-derived organoids (PDO), to model tumor behavior and drug response at the cellular level. These models will be complemented by preclinical in vivo studies using patient-derived xenograft (PDX) and immune-proficient models, such as syngeneic ones, to evaluate therapeutic efficacy and track tumor evolution under treatment pressure.
The integration of these approaches will allow us to map dynamic changes within tumor clones, identify resistant ones, and uncover critical signaling pathways driving resistance. Ultimately, this research aims at the development of more effective, personalized treatment strategies that can overcome or prevent resistance in breast and gynecological cancers.

2 - Dr. Benvenuti (ICGEB)
Harnessing the power of type 1 dendritic cells to improve anti-tumoral response in lung cancer (DCgun)
Checkpoint inhibitors to restore anti-cancer T cells provide benefits to approximately one-third of cancer patients, urging novel combinations to expand the rate of response. The latest evidence highlights the central role of a specific subset of innate immune cells, type 1 dendritic cells (cDC1), in the orchestration of anti-tumoral immunity. However, the potential of harnessing the immunostimulatory properties of type 1 DCs in lung cancer is not yet fully developed. This project will implement relevant lung cancer model, transcriptional analysis, tissue imaging and functional assays to explore strategies to empower the immunostimulatory properties type 1 DCs to complement existing immunotherapies.

3 – Prof. Tiribelli (FIF)
Neurogenomic mechanisms in bilirubin-induced neurological damage

Severe neonatal hyperbilirubinemia may results in severe and permanent disability (auditory, cognitive, and motor), recapped by the term kernicterus spectrum disorder (KSD). The symptoms and MRI suggest that bilirubin induced damage is highly specific for certain areas of the brain, but clinical tools are not sensitive enough to identify the regions selectively involved in the symptoms, their threshold of sensitivity to different bilirubin challenges, and to address the ongoing pathologic molecular processes. The project aims in using a bottom up approach (in silico, in vitro, clinical samples) to answer these question as a basis for a better diagnosis and management of neonates with variable early signs of bilirubin neurotoxicity.

4 - Dr Passafaro (CNR, Milano)
Investigating the role of parvalbumin positive interneurons in PCDH19-related Developmental and epileptic encephalopathy-9 (DEE9)

Mutations in *PCDH19* cause DEE9, a severe neurodevelopmental disorder (NDD) that mainly affects females and is characterized by early-onset seizures, intellectual disability (ID), autism spectrum disorder (ASD), and other neuropsychiatric dysfunctions.
PCDH19 encodes protocadherin-19 (PCDH19), an adhesion molecule of the cadherin superfamily. The adhesive properties and synaptic expression of PCDH19 make this protein well equipped for neuronal circuit organization, as suggested by its involvement in neuronal migration, sorting and clusterin. Furthermore, PCDH19 mismatch at synapses has recently been shown to affect NCAD-dependent signaling at presynaptic terminals of mossy fibers, impairing their function. PCDH19 is present at both inhibitory and excitatory synapses, where it mediates cell adhesion. Functionally, we have observed that altered expression of PCDH19 in the mouse hippocampus affects synaptic transmission, as well as network activity and connectivity.
In this project, we will investigate the functional effects of PCDH19 deficiency in hippocampal parvalbumin interneurons (PVIs) of conditional *Pcdh19/SynCre* mice. This will be studied using electrophysiological tools in combination with biochemistry, multi-electrode arrays (MEA), and in vivo calcium imaging techniques.

5 – Prof. Del Sal (ICGEB)
The role of the tissue microenvironment in the early phases of tumor development and in metastatic disease
Abstract not available yet

6 - Prof. Cingolani (DSV)

Advancing brain repair: Synaptic magneto-genetic tools for restoring neural connectivity

This project, part of the European research initiative SynMech (www.synmech.eu), aims to assess the efficacy of a novel mechanogenetic toolkit to regulate brain connectivity, with a focus on therapeutic applications for fragile X syndrome, a severe neurodevelopmental disorder. Mechanogenetics merges the precision of optogenetics with the versatility of magneto-mechanical stimulation.
The candidate will employ calcium imaging and electrophysiological techniques, including patch-clamp and MEA recordings, in primary neuronal cultures and brain slices.
The ideal candidate holds an MSc in biology, physics, neuroscience or a related field. Experience in electrophysiology and/or live-cell imaging, along with a background in neuroscience, is desirable but not required.
Our laboratory employs an integrative experimental approach, combining molecular, imaging and electrophysiological methods in mouse models of neurological disorders (Thalhammer et al., 2017 Cell Rep 20, 333-343; Ferrante et al., 2021 Cell Rep 35 109248, Jaudon et al., 2022 Mol Ther Nucleic Acids 29 462-480, Moretto et al. 2023 Elife 12, Jaudon and Cingolani, 2024 Trends Cell Biol 34(12): 1029-1043, Vitale et al. 2025 Brain). The position includes the possibility for training periods in Germany and the Netherlands.

7 – Prof. Fornasiero (DSV)

Identification of the molecular functions of CCDC32: a tDARK gene associated with nonspecific syndromic intellectual disability and other congenital anomalies

The coiled-coil domain containing protein CCDC32 is encoded by an enigmatic gene (tDark). Mutations in its sequence are linked to a rare intellectual disability syndrome. Despite its putative neuronal expression and possible roles in ciliogenesis, endocytosis, and synaptic vesicle dynamics, its molecular function, subcellular localization, and brain-interacting partners remain largely unknown. This Ph.D. project aims to address these gaps using a multidisciplinary experimental strategy.
The selected candidate will develop and apply new molecular tools, including CRISPR-Cas9-mediated CCDC32 knockout and knock-in models, to precisely track this protein's subcellular localization. Super-resolution imaging will visualize CCDC32 localization at the nanoscale in neurons and glial cells. Multiplexed marker analysis will establish its spatial and functional context. Concurrently, the project will uncover CCDC32's interactome via immunoprecipitation and mass spectrometry using both endogenous and tagged protein approaches.
This wide-ranging project combines genome editing, molecular biology, proteomics, and super-resolution imaging to explore CCDC32's role in brain disease and development. The project offers extensive hands-on experience with the latest methods and aims to contribute to basic neurobiology and rare disease research. It requires strong motivation and commitment.

8 – Prof. Sblattero (DSV)
Diagnostic antibody selection by in vitro display technologies

Modern immunodiagnostics relies on the availability of “specific detection reagents”, which provide information about the presence of a particular component in a biological sample, its quantity and its location. Monoclonal antibodies are by far the most commonly used class of diagnostic reagents. Even today, obtaining a “functional” monoclonal antibody is a process that poses several problems. The proposed PhD project will take an innovative approach to the problem described above by following two paths:
- firstly, the use of the so-called “antibody display technologies”, phage display and yeast display. These technologies today provide powerful tools to isolate “recombinant monoclonal antibodies (rAbs) with high affinity for any target molecule for both diagnostic and therapeutic use, starting from "antibody libraries".
- On the other hand, the methods for the production and purification of protein antigens are to be optimized.
- The aims of the project are:
A) To develop an innovative integrated selection pipeline (high throughput) of recombinant monoclonal antibodies that are of high quality and can be used immediately by industrial partners for diagnostics.
B) Development of technologies for the cloning, production and purification of antigen molecules.

9, 10 – Multiple projects/supervisors (**see below**)

11 – Dr. Buratti (ICGEB)
Co-regulation of splicing processed by TDP-43 and RGNEF
Abstract not available yet

12 – Dr. Benvenuti (ICGEB)
Dissecting early stages of alveolar macrophage-cancer cell interactions in 3D lung organoids
Non-small cell lung cancer (NSCLC), is the main cause of cancer-related deaths worldwide, accounting for the highest mortality rates among both men and women. Macrophages represent the most abundant infiltrate of innate cells in lung tumor tissues. However, the subset- and stage-specific impact of macrophages on tumour development has been difficult to disentangle, due to the plasticity and complexity of the compartment and the lack of experimental models to uncouple the action of macrophages from the overall tissue imprinting in humans and preclinical in vivo models. Here we propose to reproduce the stepwise progression of tumor evolution ex-vivo using 3D normal lung organoids in which oncogenic mutations (KrasG12D and p53 deletion) can be activated to control the initiation and evolution of oncogenesis. This system allows tracking of initial phases of tumorigenesis and complementation with desired cellular or soluble factors to isolate their precise contribution to the process.

13 – Prof. Sblattero (DSV)

Validation of the combined anti-osteolytic and anti-tumor therapeutic potential of the ICOS-Fc molecule

ICOS-Fc is a patented biologic drug that targets the costimulatory ICOS/ICOSL pathway of the immune system and has effectively blocked osteolysis in osteoporosis models and the growth of established primary tumors in various experimental settings.

This project aims to bring these effects together by investigating the effect of ICOS-Fc on bone metastases, as it is expected to both reduce the incidence of bone fractures and inhibit the growth of tumor metastases, thus improving patient survival and quality of life.

The idea will be developed by a series of steps that will first confirm the effect of ICOS-Fc on in vivo models of bone metastases and later improve its efficacy and deliverability by incorporating it into nanoparticles.

Key steps of the project include:

1. Testing the efficacy of ICOS-Fc in a mouse bone metastases model.
2. Incorporation of ICOS-Fc into poly lactic-co-glycolic (PLGA) nanoparticles.
3. Functionalization of nanoparticles loaded with ICOS-Fc with biphosphonates (BPs).
4. Assessment of tumor burden and bone resorption.
5. A toxicology study will be performed for all tested doses.

The combined antiosteolytic and antitumor activity of ICOS-Fc represents a fundamental advance in the treatment of bone metastases, ICOS-FC would be the first drug with these activities.

Positions without fellowship

SB1 – Prof. Sblattero (DSV)

Characterization of tumor microenvironments in the development of antibody-based therapeutic approaches

Antibody-based immunotherapy remains a pivotal therapeutic approach for cancer. In this contest the definition of a correct tumor-associated antigen and the analysis of its expression in cancer microenvironment in comparison with normal tissues represent the starting point for the development of new therapeutic antibodies. The characterization of tumor microenvironment allows to understand the context in which the immunotherapy has to work. Finally, understand the mechanism of action involved in Ab-based immunotherapy allows to tune the therapy and design more efficient approaches that exploit recombinant antibodies, like CAR T-cells, Ab-Drug Conjugated, targeted nanoparticles or bispecific antibodies.

The project is based on the set up of methods to analyze tissue sections form human or mouse origin, to fully characterize cancer microenvironment before and during immunotherapy of solid tumors. General histological analysis will be associated to the presence of specific protein markers and, if required, to RNA analysis.

The final aims of the projects include:

- the analysis of new tumor-associated antigens,
- the contribution in the characterization of new recombinant antibodies,
- the evaluation of the effect of novel therapeutic approaches based on the association between recombinant antibodies and other drugs or drug-delivery systems.

SB2 – Prof. Tiribelli (FIF)

Translational approaches for molecular and cellular study for liver pathologies

Liver pathologies, including prolong chronic liver diseases leading to liver cancer (hepatocellular carcinoma; HCC) represent a major global health burden due to their increasing prevalence and complex molecular mechanisms. Despite advancements in diagnostics and therapy, the molecular mechanisms underlying liver pathologies remain incompletely understood. This translational research project aims to integrate transcriptomic, genetic, and proteomic analyses to characterize the cellular heterogeneity and molecular drivers of liver pathologies, with focus on liver cancer. Multi-modal approaches combining *in vivo* (human clinical samples and transgenic murine models), *in vitro* (cellular manipulations), and *in silico* (bioinformatics pipelines) will be employed to identify disease-specific regulatory networks, validate biomarkers, and assess therapeutic targets. Recent advances in high-throughput sequencing, patient-derived organoids, genome editing, and computational biology can be employed to provide robust platforms for modelling liver disease and predicting treatment outcomes.

Through this multidisciplinary framework, the project aims to advance a systems-level understanding and support the development of personalized medicine strategies for liver cancer. A doctorate candidate will be actively involved in the design, implementation, and analysis of these translational studies, contributing to both experimental and computational components of the research.

Available projects for fellowships 9 and 10

Successful candidates will choose one of these projects/positions

The central role of endothelium in hereditary angioedema
Prof. Roberta Bulla (DSV)

Hereditary angioedema (HAE) due to C1 inhibitor deficiency is a condition characterized by recurrent episodes of increased vascular permeability that can be extremely dangerous when the upper airways are involved. The aim of our project is to investigate the central role of the endothelium in the development of hereditary angioedema type I. In particular, we aim to identify new mediators of endothelial permeability, the factors responsible for the increase in permeability in certain areas and the enzymatic mechanisms associated with these effects.
The project proposal is divided into three main points:
1. Investigation of the expression of C1-inhibitor by endothelial cells and the role the receptor for high molecular weight kininogen, C1q and hyaluronic acid in HAE.
2. Investigation of the regulation of the expression of different SERPING1 isoforms by RT-qPCR with particular regard to the expression of some microRNAs predicted by bioinformatics tools as specific target of the different isoforms.
3. Characterization of endothelium isolated from umbilical cords of newborns with C1-INH deficiency (RNA-Seq, gC1qR expression, sensitivity to BK, production of pro-inflammatory cytokines).

Studying the role of aging-related microenvironmental stress in oncogene-driven cell competition during tumor initiation and progression
Prof. Licio Collavin (DSV) and Prof. Gianni Del Sal (ICGEB)

Tumors develop through genetic and epigenetic alterations that accumulate in cells over time and are selected by tissue microenvironmental conditions that favor the survival and expansion of best-adapted cell clones. In healthy tissues, cells harboring cancer-driving mutations are held in check by wild-type neighbors through cell competition (CC), an evolutionarily conserved process in which fittest cells eliminate less fit cells. As tissues age, mutant cells circumvent tumor-suppressive CC, behaving as *supercompetitors* that clonally expand; some of these clones eventually progress to malignancy. Previous observations suggest that the supercompetitor behavior of mutant preneoplastic cells is exacerbated by aging-associated conditions that increase cancer susceptibility, such as fibrosis, mechanical stress, inflammation, and metabolic dysfunction. This project aims to investigate the impact of microenvironmental cues in shaping the evolutionary trajectory of preneoplastic cells bearing cancer-related alterations such as TP53 missense mutation, YAP activation, and loss of selected tumor-suppressors. Uncovering mechanisms and actionable targets involved in the supercompetitor behavior of preneoplastic cells, and in aggressiveness of fully neoplastic cells, may provide tools to block cancer from its earliest stages - or even prevent it - and to better address advanced stages of the disease and potential therapy resistance.

Interplay between TRIM ubiquitin ligases and deubiquitinating enzymes in the regulation of ciliogenesis in rare genetic diseases
Prof. Germana Meroni (DSV)

Ubiquitination is a post-translation modification process that changes protein fate and that is highly coordinated to proper balance activity and stability of proteins in cells. The tripartite motif (TRIM) proteins represent a large family of RING E3 ubiquitin ligases (E3s) that mediate protein ubiquitination, activity that is counteracted by deubiquitinating enzymes (DUBs) that reverse this modification. We are interested in studying the dynamics of ubiquitination in the pathogenesis of rare genetic diseases.
In this project, we aim at investigating the interplay between TRIM E3s and DUBs in ciliogenesis, a process frequently altered in genetic diseases. Almost all cell types possess a primary cilium that serves as antenna, especially during embryonic stages, to sense morphogens. We will perform an initial screening through down-regulation and/or pharmacological inactivation of DUBs and TRIM E3s examining %age of cells presenting the cilium, its length, and its assembly/disassembly dynamics. Preliminary data indicate that some TRIM E3s and DUBs have indeed a role in ciliogenesis. In the second phase, selected members of the two families whose perturbation affect ciliogenesis, will be further studied in terms of possible functional TRIM E3-DUB pairs within the same ciliogenesis pathway. As several of these proteins are implicated in genetic disorders, these data will be instrumental for the identification of novel therapeutic targets.

The role of CTL in 3D stem cell culture systems for faster chondrogenic differentiation to support cartilage regeneration
Prof. Pasquale Sacco (DSV) and Prof. Ivan Donati (DSV)

Among biopolymers, polysaccharides are particularly interesting for various applications due to their compatibility with the human body and the possibility of chemically functionalizing them to give them additional properties. Over the past 20 years, the Research Group in which the PhD Student will be working has developed and studied an engineered polysaccharide known in the literature as CTL with advanced properties for various high-tech applications, particularly for biomedical applications in the field of cartilage regeneration.
The research project in which the PhD Student will be involved focuses on the development and characterization of 3D networks based on stem cells and the CTL biopolymer, which can be grafted onto damaged cartilage in combination with other polysaccharides or other bioactive compounds to promote its regeneration. The development of these biomaterials is carried out through the parallel characterization of the intrinsic aspects of the material and the resulting biological properties to optimize their overall performance. The materials are evaluated based on their chemical-physical, morphological and mechanical properties. The biological effects of the biomaterial will be tested *in vitro* on eukaryotic cells. Molecular biology techniques will be used to identify the biological mechanisms underlying the observed cellular responses.

Understanding molecular mechanisms that prevent R-loop mediated DNA repeat instability in cancer cells
Prof. Stefan Schoeftner (DSV)

The instability of repetitive DNA elements contributes to tumor formation and progression. Research from our laboratory demonstrates that aberrant transcription of repeat elements leads to the formation of atypical nucleic structures known as R-loops. These R-loop structures consist of an RNA:DNA hybrid duplex and an unpaired single-stranded DNA loop. They predominantly form at CG-rich and repetitive sequences, driving replication-transcription conflicts that result in DNA damage and genomic instability.
We have shown that the SFPQ protein exhibits binding specificity for R-loop structures and facilitates histone H3.3 chaperone activity at telomeres, (peri-)centromeres, and LINE and SINE repeats to preserve chromatin integrity. Loss of SFPQ function exacerbates genomic instability, promotes cytoplasmic DNA accumulation, and triggers innate immune activation in human sarcoma (Petti et al., 2019; Ferrando et al., under revision in Nature Communications).
The current project focuses on a detailed molecular analysis of SFPQ and its mutant variants identified in human sarcoma. We will investigate the regulatory pathways that govern SFPQ accumulation at R-loops, its protein complex formation and the downstream mechanisms responsible for RNA removal from R-loops and the incorporation of histone H3.3-containing nucleosomes at repetitive DNA sequences. Furthermore, we aim to develop strategies to disrupt R-loop binding and histone chaperone recruitment.

Development of an In Vivo Platform to Elucidate the Mode of Action of Antimicrobial Peptides Targeting Bacterial Vital Functions
Prof. Marco Scocchi (DSV)

The rise of multidrug-resistant (MDR) bacterial pathogens necessitates innovative strategies for antibiotic development. Antimicrobial peptides (AMPs) offer a promising avenue, but understanding their in vivo mode of action (MoA) is crucial for therapeutic advancement.
Objectives: Establish a versatile in vivo platform to monitor the effects of AMPs on bacterial vital functions. Elucidate the MoA of selected AMPs by assessing their impact on translation, transcription, replication, and efflux mechanisms within live bacterial cells.
The methodology include : i) use of BONCAT to incorporate non-canonical amino acids into newly synthesized proteins, enabling visualization of protein synthesis and assessment of transcriptional activity; ii) Employ fluorescent probes, such as 7- aminoactinomycin D, to monitor DNA replication dynamics in real-time; iii) Assess efflux pump activity using fluorescent antibiotic derivatives, like fluorophore-coupled ciprofloxacin, to evaluate accumulation and efflux inhibition. Integrate these techniques with advanced imaging methods, for high-resolution spatial and temporal analysis.
The Expected Outcomes: a validated in vivo platform capable of dissecting the MoA of AMPs on bacterial functions and efflux mechanisms. Identification of AMPs with specific or multi-target effects, enhancing their potential as broad-spectrum antibiotics. This integrated approach will advance our understanding of AMP interactions with bacterial targets, informing the development of novel therapeutics against MDR pathogens.

Reactivating neuronal development in induced pluripotent stem cells (iPSC)-derived neurons from Rett syndrome patients

Prof. Enrico Tongiorgi (DSV)

Rett syndrome (RTT) is the second most frequent cause of mental disability in females worldwide. RTT is a genetic, progressive neurodevelopmental disorder caused by mutations in the X-linked MECP2 gene, having an incidence of 1/10.000 newborn females. MECP2 mutations cause developmental arrest, loss of speech and motor abilities, seizures, breathing abnormalities and brain atrophy characterized by more closely packed neurons and decreased dendritic complexity. Previous studies, have shown that RTT patients' iPSCs are able to undergo X-inactivation and generate functional neurons which recapitulate the main disease features, including fewer synapses, reduced spine density, smaller soma size, altered calcium signaling and electrophysiological defects when compared to controls. We recently identified (and patented) three FDA-approved drugs able to rescue RTT-associated neuronal atrophy in an in vitro model of the pathology. Bioinformatic investigation of the protein pockets bound by these drugs led to the identification of novel, unexpected signaling pathways involved in inflammation, cholesterol homeostasis, apoptosis, neuronal maturation and synaptic plasticity. The project will investigate these new molecular mechanisms in fibroblasts and iPSC-derived neurons from RTT patients to explain how the drugs can reactivate neuronal development in RTT. Main techniques include: biochemistry (WB, ELISA), cell biology (cell cultures, IF, advanced microscopy), neurophysiology (Ca^{2+}-imaging).

Structure/function studies on the antimicrobial and immunomodulatory activities of Host Defence Peptides (HDP)

Prof. Alessandro Tossi (DSV) and Prof. S. Pacor (DSV)

The human cathelicidin HDP, LL-37, plays an important role in preventing infection as a direct antimicrobial agent and immune signalling molecule. Its activity is based on a complex interplay of intra- and intermolecular interactions that determine its oligomerization and subsequent interaction with microbial or host cell membranes. It depends on the physiological environment and varies markedly in other primate orthologs, significantly altering their activity. The project continues an ongoing systematic investigation of the effects of rational, minimal variations in ortholog sequences on oligomerization and activity, to establish a comprehensive model for structure-activity relationships. The successful candidate will apply a broad range of biochemical and biophysical techniques, including design, automated preparation and purification of peptides, structure prediction and spectroscopic determination, microbiological assays, flow cytometry studies on bacterial and host cells, and various types of interaction and oligomerization assays, working in DSV and CNR-IC and availing of an international network of collaborations. A separate aspect of the project concerns the study of HDPs from helminths, which have interesting properties due to the complex life-cycles of these parasites, with stages in different animal hosts, including humans, with which they must coexist. The developed AMPs may have useful applications as novel antibiotics, immune modulation and wound healing.