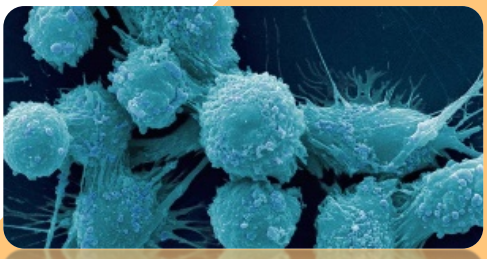
PhD STUDENTS MEETING

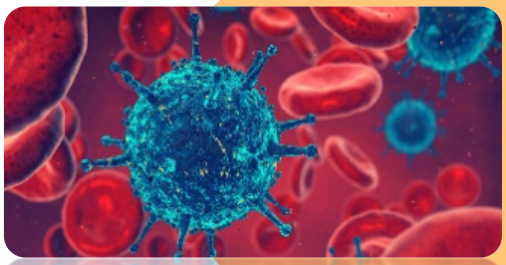
Milan, June 13-14th 2019

ABSTRACT BOOK

Neuroscience

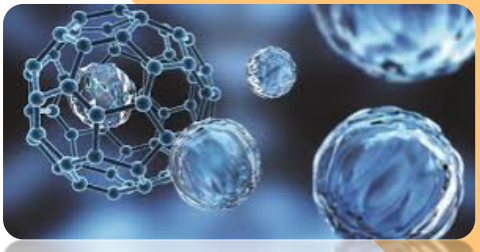


Cancer Biology



Immunology

Molecular Medicine



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ORAL PRESENTATION	June 13th, 10:15 – 10:35
NAME	**Enriquez Claudia**
Course/Year	PhD - 4th year
Institution	Istituto Nazionale Tumori

Adenocarcinoma-neuroendocrine transition of androgen resistant cancer depends on SPARC down-regulation in stromal accessory cells

Authors	Valeria Cancila, Roberta Sulsenti, Renata Ferri, Ivano Arioli, Claudio Tripodo, Mario P. Colombo, Elena Jachetti
Affiliation	Molecular Immunology Unit, Department of Research, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy Tumor Immunology Unit, Department of Health Sciences, University of Palermo, Italy

Background. Treatment-related neuroendocrine prostate cancer often evolves as resistance to androgen deprivation and androgen receptor pathway inhibitors and still lacks effective therapies. Epigenetic and transcriptional regulators control adenocarcinoma cells plasticity toward neuroendocrine differentiation. Moreover, stromal components can provide intrinsic and paracrine signals able to promote this transition. Here we show in a transgenic mouse model that down-regulation of the matricellular protein SPARC in stromal cells is a key event needed for adenocarcinoma-neuroendocrine transition of prostate cancer cells.

Methods. We modeled the pathogenesis of the human disease using the transgenic TRAMP model. Surgical castration was performed at 20 weeks of age. Sparc-/- TRAMP model was generated crossing Sparc-/- mice with TRAMP mice. Immunostaining for SPARC, Cytokeratin-8 and Synaptophysin, RNAScope for Il6 and BaseScope for miR29b were performed on prostates isolated from 30-week old TRAMP, castrated TRAMP and Sparc-/- TRAMP mice. Adenocarcinoma cells derived from TRAMP mice were co-cultured with Sparc-sufficient or deficient fibroblasts. After seven days, tumor cells were tested for Synaptophysin by real-time PCR and fibroblasts for Sparc and Il6 expression.

Results. Crossing TRAMP mice with Sparc-/- mice, we found the appearance of focal areas of neuroendocrine differentiation within adenocarcinoma. Cells undergoing neuroendocrine differentiation displayed double positivity for Cytokeratin-8 and Synaptophysin (normally expressed by adenocarcinoma or neuroendocrine cells, respectively), indicating a trans-differentiation of adenocarcinoma rather than a distinct clonal origin. The same percentage of neuroendocrine differentiation also appeared in castrated TRAMP mice suggesting that SPARC deficiency and castration converge to the same disease outcome. Interestingly, we found that SPARC expression in fibroblasts of TRAMP prostate was dramatically reduced after castration indicating a key role of stromal-SPARC in limiting neuroendocrine differentiation of prostate cancer. Accordingly, adenocarcinoma cells acquired neuroendocrine phenotype after injection in Sparc-/- hosts. Finally, prostate cancer cell lines, regardless from their SPARC expression, acquired neuroendocrine features when co-cultured with Sparc-deficient fibroblasts. This occurs through the effect of IL-6 released by Sparc-deficient, but not sufficient, fibroblasts.

Conclusions. Our results indicate a fundamental role of stromal cells in shaping tumor cell plasticity toward neuroendocrine differentiation unveiling possible prognostic and therapeutic targets. Indeed, our data suggest that after androgen ablation therapy, patients showing SPARC down-regulation or IL-6 up-regulation in fibroblasts could benefit of a therapeutic approach directed to IL-6R.

ORAL PRESENTATION	June 13th, 10:35 – 10:55
NAME	**Vázquez Ríos Abi Judit**
Course/Year	Dott. – 4th year
Institution	Istituto Nazionale Tumori

Identification and study of TAS1R3 in metastatic dissemination of Non-Small Cell Lung Cancer

Authors	Vázquez-Ríos AJ[1,3], Alonso-Nocelo M[1], Tortoreto M[2], Bertolini G[3], Calabuig-Fariñas S[5], Alijas-Pérez S[1], Centonze G[4], Milione M[4], Jantus-Lewintre E[5], López R[1], Sozzi G[3], Roz L[3], de la Fuente M[1]
Affiliation	1) Nano-Oncology Unit, Health Research Institute of Santiago de Compostela. CIBERONC. Spain; 2) Molecular Pharmacology Unit, Fondazione IRCCS-INT, Milan, Italy; 3) Tumor Genomics Unit, Fondazione IRCCS-INT, Milan, Italy; 4) Department of Pathology and Laboratory Medicine, Fondazione IRCCS-INT, Milan, Italy; 5) Molecular Oncology Laboratory, Fundación Hospital General Universitario de Valencia, Spain.

Background. Circulating Tumor Cells (CTCs) are involved in the process of metastatic spread and the establishment of distal foci of the disease; however, it is known that only a small subset of CTCs, endowed with stemness properties, can actually efficiently survive as disseminated tumor cells (DTCs) and eventually progress toward metastasis at distant sites. Understanding the mechanisms by which CTCs can develop new metastasis may provide tools for designing new therapeutic modalities against metastases. We have identified a novel receptor from CTCs of advanced NSCLC patients, TAS1R3 (taste receptor type 1 member 3), and studied its role in metastatic dissemination.

Methods. TAS1R3 expression was analyzed in CTCs obtained by EpCAM-based immunoisolation from NSCLC patients (n=45; stage IIIb-IV). Its expression was further studied by RT-qPCR in primary tumors (n=245; stage I-IIIa) and Patient-Derived Xenograft (PDX) mouse models (n=10). For functional experiments, TAS1R3 was overexpressed in A549 cells and its overexpression confirmed. Proliferation, migration and invasion capability of TAS1R3 positive and control cells was evaluated in vitro as well as their capability to disseminate from the primary tumor and to colonize mouse lungs in vivo. CTCs from mouse models were isolated and characterized phenotypically. Finally, correlation with stem markers was evaluated.

Results. Molecular profiling of CTCs in advanced NSCLC led as to the identification of TAS1R3 as a promising receptor whose role was still to be unraveled. Interestingly, low expression was detected in primary tumors from early-stage patients, suggesting increased expression of TAS1R3 mainly in advanced disease. IHC of TAS1R3 in PDX models, was specifically observed in highly undifferentiated neoplastic cells. Formation of stem-like spheroids from primary cultures and cell lines in vitro led to an increase in the expression of TAS1R3. Upregulation of TAS1R3 in A549 cells led to a more invasive and less migrative phenotype. Further studies in xenograft models revealed that overexpression of TAS1R3 significantly promoted an increase in the number of DTCs in the lungs and CTCs in peripheral blood (all of them TAS1R3). Remarkably, the percentage of TAS1R3 cells was drastically increased in DTCs compared to the primary tumor for both groups and higher levels of stem markers were also found in vivo.

Conclusions. All these results confirm the relevance of TAS1R3 in NSCLC metastatic dissemination and suggest that TAS1R3 plays an important role in the development and progression of human NSCLC metastasis. Correlation with stem markers suggests that TAS1R3 could be a novel diagnostic biomarker for Cancer Stem Cells and may serve as a therapeutic target to impair metastatic dissemination in NSCLC.

ORAL PRESENTATION	June 13th, 10:55 – 11:15
NAME	**Lara Paracchini**
Course/Year	PhD – 4th year
Institution	Istituto di Ricerche Farmacologiche Mario Negri IRCCS

PRKG1, SDF2L1 and PPP1R12A expression profiles are predictive and prognostic factors for therapy response and survival in high-grade serous ovarian cancer

Authors	Paracchini L[1], Benvenuto G[2], Todeschini P[3], Beltrame L[1], Calura E[2], Fruscio R[4], Ravaggi A[3], D'Incalci M[1], Bignotti E[3], Romualdi C[2], Marchini S[1]
Affiliation	1) Laboratory of Cancer Pharmacology, Department of Oncology, IRCCS "Mario Negri" Institute for Pharmacological Research, Milan; 2) Department of Biology, University of Padova, Padova; 3) Division of Gynecologic Oncology, Angelo Novicelli Institute of Molecular Medicine, University of Brescia, Brescia; 4) Division of Obstetrics and Gynecology, San Gerardo Hospital, Monza, Italy

Background. The high mortality rate of high-grade serous epithelial ovarian cancer (HGSEOC) is due to several factors, including resistance to the standard platinum (Pt)-based treatments. The intrinsic resistance to Pt compounds affects 20% of HGSEOC patients leading to their rapid death. Nowadays there are no clinical, pathological or molecular parameters that can be used to distinguish a priori Pt-sensitive (Pt-s) from Pt-resistant (Pt-r) patients. The aim of this work is to identify the molecular pathways responsible for this different sensitivity and their prognostic value.

Methods. HGSEOC tumour samples (n=1080) were obtained from three independent tumour tissue collections, named cohort A, B and C. Coding and non-coding gene expression profiles were analyzed by microarray technologies (cohort A) and data integrated using an in house developed algorithm (Micrographite). Results were validated on an independent cohort of patients (cohort B). Each element of the signature was then associated with progression-free survival (PFS) and overall survival (OS) in multivariate analysis using residual tumour and age as covariates. The prognostic value was further validated in silico using data obtained from the Curated Ovarian Cancer database (Cohort C).

Results. Array data integration (Pt-s=36 and Pt-r=41) identified a network of 131 mRNAs and five miRNAs differentially expressed between Pt-s and Pt-r patients. These elements belong to five major functional biological processes: transcription regulation, transmembrane ion transport, cell cycle regulation and response to DNA damage, fatty acid metabolism and antigen presentation. Orthogonal validation on cohort B (Pt-s=69 and Pt-r=48), confirmed 19 out of 23 selected genes as differentially expressed between Pt-s and Pt-r patients. In order to test whether this signature is not only associated with Pt response, but has also a prognostic value, a survival analysis was performed. 16 out of 19 genes significantly associated with both OS and PFS in multivariate analysis. The prognostic role of three of them (PRKG1, SDF2L1 and PP1R12A) was further confirmed in cohort C (n=838).

Conclusions. The overall data presented in this study can be summarised as follow: i) the expression levels of three genes, PRKG1, SDF2L1 and PP1R12A, shape the biology of intrinsic Pt-resistance in HGSEOC. No data has never been reported before on their role in the biological mechanisms of Pt-r. ii) This three genes based signature is associated as independent prognostic factor to OS and PFS. This finding suggests that despite molecular tumour heterogeneity of HGSEOC it is plausible to identify biomarkers that can predict at diagnosis those cases that will not respond to first line Pt-based chemotherapy and for whom alternative therapeutic strategies should be taken into consideration.

ORAL PRESENTATION	June 13th, 14:05 – 14:25
NAME	**Maria Chiara Trolese**
Course/Year	PhD - 3rd year
Institution	Istituto di Ricerche Farmacologiche Mario Negri IRCCS

Increased macrophage recruitment through mcp1 over-expression prevents skeletal muscle atrophy and motor neuron loss in a mouse model of ALS

Authors	Maria Chiara Trolese, Valentina Melfi, Caterina Bendotti, Giovanni Nardo
Affiliation	Laboratory of Molecular Neurobiology, Department of Neuroscience, Istituto di Ricerche Farmacologiche "Mario Negri" - IRCCS, Milano

Background. The immune system plays a controversial role in the pathoprogression of Amyotrophic Lateral Sclerosis (ALS). We recently found that at the disease onset SOD1G93A mice with a slower disease progression (C57SOD1G93A) expressed higher levels of the MCP1 chemokine within motor neurons and axons compared to fast progressing ALS mice (129SvSOD1G93A). Accordingly, a higher macrophages infiltration was observed within sciatic nerve and hind-limb muscles of C57SOD1G93A mice. Given the pivotal role of MCP1-mediated signaling in driving axonal and muscle regeneration following injury, we investigated whether a further increase of this chemokine in the early disease stages could slow down ALS progression.

Methods. We overexpressed the chemokine in myofibers and MNs through the injection of a single dose (2.18x1010vg/μL) of the self-complementary (sc)AAV9-MCP1 in the skeletal muscles of adult C57SOD1G93A mice. Both hind limb (tibialis anterior, gastrocnemius caput mediale and gluteus maximus) and forelimb (triceps brachii) muscles were injected to induce MCP1 along the related motor unit (motor axons and spinal neuron), as demonstrated by the expression of the green fluorescent protein reporter gene. Mice were treated with the scAAV9-MCP1 at the pre-symptomatic disease stage (8 weeks of age) and sacrificed six and twelve weeks after the intramuscular (i.m.) injection.

Results. Following a single intramuscular injection of scAAV9-MCP1 we recorded a marked chemokine induction along the entire neuromuscular units. This resulted in: i) the protection of spinal motoneurons; ii) the shift of microglia from M1 (pro-inflammatory) to M2 (anti-inflammatory) phenotype in the lumbar spinal cord; iii) a reduced skeletal muscles denervation atrophy, which is associated with an increased phagocytic macrophages recruitment; iv) an increased activation and differentiation of satellite cells within muscles. These led to an amelioration of motor performance and the postponement of symptoms onset in C57SOD1G93A mice treated with scAAV9-MCP1 compared to control group (empty vector).

Conclusions. These results clearly indicate that the recruitment of macrophages induced by the overexpression of MCP1 in the peripheral compartment of the neuromuscular system is essential to maintain the regenerative capacity of skeletal muscles and motor neurons during ALS progression. The characterization of the mechanism underlying this phenomenon could open the way to identify novel targets to promote regeneration. Further studies are underway to examine the effect of the intramuscular injection of scAAV9-MCP1 in fast progressive ALS mice.

ORAL PRESENTATION	June 13th, 14:25 – 14:45
NAME	**Martina Bruna Violatto**
Course/Year	Dott. - 3rd year
Institution	Istituto di Ricerche Farmacologiche Mario Negri IRCCS

Dexamethasone conjugation to biodegradable Avidin-Nucleic-Acid-Nano-Assemblies promotes selective liver targeting and improves therapeutic efficacy in an autoimmune hepatitis murine model

Authors	Violatto MB[1], Talamini L[1], Russo L[1], Passoni A[1], Bagnati R[1], Fumagalli S[1], De Simoni MG[1], Salmona M[1], Christen U[2], Invernizzi P[3], Morpurgo M[4], Bigini P[1]
Affiliation	1) Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milano, Italy; 2) Goethe University Hospital Frankfurt, Frankfurt am Main, Germany; 3) University of Milano-Bicocca, Monza, Italy; 4) University of Padova, Padova, Italy

Background. Autoimmune hepatitis belongs to the group of relative rare, but severe autoimmune liver diseases. Steroid treatment represents the standard therapy. However, mainly due to their off-target effects, steroid long-lasting administration is extremely limited. Alternative methods to improve their liver tropism are therefore required. Among them, the conjugation to nanoparticles could represent a realistic strategy to reduce side effects, allowing a safer chronic care. In this study, we compared the biodistribution, the pharmacokinetics and the efficacy of freely injected dexamethasone or conjugated to a nanoformulation based on Avidin-Nucleic-Acid-Nano-Assemblies in an autoimmune hepatitis murine model.

Methods. An initial in vitro study was performed to verify the stability of the pH sensitive linker, exploited to load dexamethasone to the nanoformulation. Then, healthy and diseased mice were intraperitoneally treated with single or multiple injection of free or nanoparticle-linked dexamethasone and sacrificed at different time point. Optical imaging, confocal and super-resolution microscopy were carried out to localize fluorescently labeled nanosteroids in the whole body and in liver sections, and HPLC MS/MS to measure the level of free drug in plasma and in different organs. Lastly, the clinical signs of the diseased mice were examined with biochemical and histological assays.

Results. The ability of the nanoformulation to release free dexamethasone was demonstrated in vitro. At neutral pH, the drug remains stably tethered to the linker, while at pH below 6 drug release occurs with a kinetics that increases in correlation with the decrease of pH. During in vivo study, if freely injected, the drug circulates in the bloodstream rapidly and then reaches all major tissues, including liver, kidney and brain. On the contrary, when mice are treated with the nanosteroid, the free drug is detected only in the liver. Through super-resolution microscopy of liver sections, we found that nanoparticles segregated inside lysosomes of immunocompetent cells, that are mainly involved in the autoimmune hepatitis progression. Notably, after chronic treatment, nanosteroid administration reduces the overexpression of markers of liver inflammation, the level of circulating autoantibodies and the fibrosis deposition in diseased mice. This is not observed in free dexamethasone treated mice.

Conclusions. Overall, our data show that the dexamethasone conjugated to the nanoparticles is more effective than the free drug in limiting the disease progression. Even though this is only a preliminary outcome, combined with the fact that the nanoformulation does not release the steroid in any body district other than the liver, it suggests that this is a promising carrier for controlling chronic liver inflammatory conditions. The safety and biodegradability of the nanoparticle make this formulation a promising tool for future translation from preclinical to clinical settings.

ORAL PRESENTATION	June 13th, 14:45 – 15:05
NAME	**Barbara Bassani**
Course/Year	PhD. – 2nd year
Institution	Fondazione IRCCS Istituto Nazionale dei Tumori

The role of Zeb-1 in acute myeloid leukaemia development

Authors	Bassani B[1], Portararo P[1], Curti A[2], Simonetti G[2], Tripodo C[3], Lecis D[1], Colombo MP[1], Sangaletti S[1]
Affiliation	1) Molecular Immunology Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy; 2) Department of Experimental, Diagnostic and Specialty Medicine, Institute of Hematology L. and A. Seràgnoli, S. Orsola-Malpighi Hospital, University of Bologna, Bologna, Italy; 3) Tumor Immunology Unit, Department of Health Sciences, University of Palermo, Palermo, Italy

Background. Acute myeloid leukaemia (AML) is a clonal disorder resulting from the neoplastic transformation of hematopoietic stem cells. The most aggressive type of AML expresses the mesenchymal-related gene Zeb1 whose expression correlates with poor overall survival. We speculated that blasts might autonomously create an immunosuppressive environment activating epithelial-mesenchymal transition related genes. We focused on the role of Zeb1 in the orchestration of the tolerogenic microenvironment that allows leukaemia development.

Methods. We selected two AML cell lines, the C1498 and the WEHI-3B that were firstly investigated for Zeb1 expression. Real-time PCR was used to evaluate the expression of immune-modulatory molecules that we hypothesized might be associated with Zeb1. Zeb1 was then silenced using lentiviral vectors in C1498. To strengthen our in-vitro founding, we moved in-vivo injecting the genetically modified cell line C1498shZeb and its negative control (C1498-Scr) into C57Bl/6 mice. To explore the contribution of soluble factors in inducing the immunosuppressive microenvironment, we stimulated leukaemic cells with IFN□. Finally, GEP analysis was performed on 61 AML patients and 7 controls.

Results. C1498 expressed higher levels of Zeb1, pd-l1, il2 and lower levels of cd40, il6, tnfα and il10 compared to WEHI-3B. A different behaviour was observed in response to IFNγ stimulation. Indeed, IFNγ up-regulates cd40, nos-2, pd-l1 and ido-1 in WEHI-3B while in C1498 only the up-modulation of pd-l1 was found. Zeb1 silencing in C1498 was associated with the downregulation of immune-regulatory genes including pd-l1, arg1 and tgfβ and pro-metastatic genes, such mmp9. In-vivo results showed that 34days post-injection, the percentage of GFP cells was higher in C1498-Scr-injected mice and this was associated with the increase of CD11b PD-L1 cells and with the decrease of Tregulatory and Teffector IL17 cells. The relevance of Zeb1 was also confirmed in humans, where AML patients could be divided in two groups according to Zeb1 expression, being Zeb1-high AML characterized by a higher pd-l1 expression compared to Zeb1-low AML and associated with a worse outcome.

Conclusions. Our results highlight the possibility that EMT-related programs and immune suppression could be cross-linked and that Zeb1 AML clones can intrinsically orchestrate the immunosuppressive environment. We showed that Zeb1 down-regulation was associated with an impairment of IL17 axis that is known to display a dual role in tumor progression supporting or inhibiting tumor growth. Zeb1 might be considered as a possible prognostic marker for AML treatment, outcome and survival. Particularly, Zeb1 expression might characterize AML patients that could benefit form the treatment with PD1/PD-L1 blockers.

ORAL PRESENTATION	June 13th, 16:30 – 16:50
NAME	**Silvia Penati**
Course/Year	PhD. – 2nd year
Institution	Humanitas University

Molecular and cellular mechanisms underlying the relationship between metabolic alterations and cognitive decline

Authors	S. Penati[1,2,3], C. Gotti[3], M. Moretti[4], P. Rosa[3], I. Corradini[1,3], M. Matteoli[1,3], M.L. Malosio[1,3]
Affiliation	1) Laboratory of Pharmacology and Brain Pathology, Humanitas Clinical and Research Center, Rozzano (MI), Italy. 2) PhD Programme in Molecular and Experimental Medicine, Humanitas University, Pieve Emanuele (MI), Italy. 3) Institute of Neuroscience (IN), CNR (MI), Italy. 4) Department of Medical Biotechnology and Translational Medicine, Università degli Studi di Milano (MI), Italy

Background. Increasing evidence suggests an association between metabolic disorders, notably insulin-resistance and type 2 diabetes, with cognitive decline and Alzheimer"s Disease. In fact, diet-induced changes in peripheral insulin sensitivity contribute to alterations in brain insulin signaling and cognitive functions. Deranged glucose metabolism in the brain accompanied by elevated fatty acids levels and chronic low-grade inflammation could be the pathogenetic mechanisms associating type 2 diabetes with Alzheimer"s Disease.

Methods. We developed a preclinical animal model of diet induced-glucose impairment. We fed mice with 45% or 60% high-fat diet for several weeks and measured the effects on body weight, glucose-, pyruvate- and insulin-tolerance. Behavioural tests were used to asses the presence of cognitive impairments. The effect of insulin resistance on neurotransmission, myelination, and endoplasmic reticulum stress were studied by western blot of hippocampus and prefrontal cortex. Finally, palmitic acid treatments were used in vitro on primary cell cultures of neurons, astrocytes, and microglia to mimic the metabolic condition determined in the brain by the high-fat diet.

Results. After 2 weeks of diet, a significant increase in body weight was observed in animals fed with fatty diets compared to controls. Metabolic tests showed glucose impairment after 3 weeks of high-fat diet, and insulin tolerance after 5 weeks. Open Field test showed significant alterations in stereotyped activity, rearing activity and anxiety-like behaviour, with no changes in locomotor activity. At the end of the diet, different tissues were taken for biochemical and molecular analyses. Hippocampal tissues showed elevated levels of p-AKT, reduced excitatory subunits receptors and elevated level of BIP, a marker of endoplasmic reticulum stress. Furthermore, after verifying the 24-hour toxicity of increasing doses of palmitate on primary brain cells in culture, we performed a 7-day treatment to mimic the in vivo condition of a prolonged high fat diet.

Conclusions. Hippocampal tissues in mice receiving a high-fat diet are characterized by elevated levels of p-AKT, a hallmark of insulin resistance. Our results suggest that even a short period of exposure to high-fat diet can alter relevant brain functions, including neurotransmitter sensing and myelination. The molecular mechanisms are currently under analysis in our cellular model. Our work will lead to the identification of novel pathways affected by exposure to high-fat diet and may have clinical implications in halting cognitive decline in subjects at risk.

ORAL PRESENTATION	June 13th, 16:50 – 17:10
NAME	**Marco Oggioni**
Course/Year	Dott. – 2nd year
Institution	Istituto di Ricerche Farmacologiche Mario Negri IRCCS

Pentraxin-3 is present in a specific temporal pattern after traumatic brain injury, but its depletion is not sufficient to modify the outcome

Authors	Oggioni M[1]; Minuta D[1]; Mercurio D[1]; Fumagalli S[1]; Sironi M[2], Perego C[2], Garlanda C[2], and De Simoni MG[1]
Affiliation	1) Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milano, Italy; 2) Humanitas Clinical and Research Center, Rozzano, Italy

Background. Pentraxin-3 is a pattern recognition molecule belonging to the family of long pentraxins involved in the humoral immunological response. Pentraxin-3 is known to interact with complement components and regulators including mannose-binding lectin and factor H. Pentraxin-3 is up-regulated following pro-inflammatory stimuli and may be involved in central nervous system diseases, however its role in traumatic brain injury is still unexplored. We investigated plasma and brain presence of pentraxin-3 over time in a mouse model of traumatic brain injury. We then studied brain damage progression in wild-type and pentraxin-3 depleted mice to investigate its role in brain injury.

Methods. Wild-type or pentraxin-3 knock-out male C57BL/6J mice underwent sham surgery or controlled cortical impact brain injury (velocity: 5 m/s; depth: 1mm). Plasma and brain pentraxin-3 presence was assessed in wild type mice, respectively by ELISA assay and immunofluorescence, at different time points after injury. Immunostaining for pentraxin-3 was quantified by segmentation of stained area using Fiji software. Sensorimotor deficits were assessed by neuroscore and beam walk test on a weekly basis for four weeks after traumatic brain injury. At week five brains from both strains were harvested for histopathological analysis.

Results. Plasmatic pentraxin-3 makedly increases at 24 hours after traumatic brain injury (553 %), decreases at 48 hours to pre-injury levels and then slowly increases again from week one to three (max 262 %). Brain pentraxin-3 increases from 48h up to five weeks post-injury. Genetic depletion of pentraxin-3 does not induce any difference in sensorimotor deficits compared to wild-type mice. Lesion volume and neuronal count (on cresyl violet stained sections), axonal damage (luxol fast blue), collagen presence (sirius red), astrocytosis (glial fibrillary acidic protein), microglia activation (CD11b) and fagocytosis (CD68) were not different in knock out compared to wild type mice at 5 week post-injury.

Conclusions. Pentraxin-3 increases in brain and plasma after traumatic brain injury and its activation pattern suggests distinct functions in acute phases versus sub-acute or chronic phases. Its genetic depletion does not modify the outcome following traumatic brain injury. The lack of a clear-cut phenotype in pentraxin-3 knock-out mice may depend on the different roles of this protein, possibly involved in inflammation early after injury and in repair processes later on. Neuronal pentraxins could also be involved in the injury evolution and contribute to the overall outcome.

ORAL PRESENTATION	June 13th, 17:10 – 17:30
NAME	**Daniele Facchi**
Course/Year	PhD. – 3rd year
Institution	Humanitas University

iPSC-derived Midbrain organoids: a novel tool to study GBA-related Parkinson Disease

Authors	Facchi D[1], Frattini E[2], Straniero L[1], Rimoldi V[1], Monzio Compagnoni G[2], Asselta R[1,3], Soldà G[1,3], Aureli M[4], Di Fonzo A[2], Duga S[1,3]
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Background. Biallelic mutations of GBA, encoding the lysosome-resident Glucocerebrosidase (GCase) enzyme, lead to a lysosomal storage disorder, the Gaucher Disease (GD), while heterozygous GBA mutations represent the main risk factor for Parkinson Disease (PD). The penetrance and the severity of GBA-PD are variable and their determinants are still unknown. The available model organisms do not entirely recapitulate the patient phenotype. The recent organoid technology offers a valuable tool to study complex systems overcoming the differences between animal models and human. Here, we generated organoids from PD and GD patients in order to develop a patient-derived model to study GBA-PD.

Methods. Fibroblasts of one healthy control, one PD patient heterozygous for the L444P GBA mutation, and one GD patient homozygous for the same mutation were reprogrammed to iPSC lines and differentiated into organoids. During the 150 days of differentiation, the organoids were collected at different time points and characterized by: Tyrosine Hydroxylase (TH) expression and neuromelanin deposition, by immunocytochemistry and electron microscopy; lipid profile, by [3-3H(sphingosine)]GM3 treatment; GBA and alpha-synuclein expression, by quantitative RT-PCR; GCase activity, by a chemiluminescent enzymatic assay; phospho-alpha-synuclein production, by immunofluorescence.

Results. The organoids showed a nigral dopaminergic identity as they were shown to contain neurons with positive staining for TH and neuromelanin deposits, without differences between control or patients-derived ones. The radioactive lipid species analysis showed a mature neuronal lipid profile after 65 days of differentiation. Interestingly, GD derived organoids showed Glucosylceramide accumulation if compared to the control or PD. The expression of GBA and alpha-synuclein transcripts increased during the differentiation similarly across the groups, but PD and GD-derived organoids showed reduced GCase activity and increased phospho-alpha-synuclein accumulation in TH positive neurons proportionally to the time in culture.

Conclusions. We produced a suitable model to dissect multiple aspects of PD pathogenesis. The obtained midbrain organoids showed a nigral dopaminergic identity and a mature lipid profile. Moreover, they recapitulated the patients' phenotype in terms of GCase activity, Glucosylceramide deposition, and phospho-alpha-synuclein accumulation. Transcriptomic analysis will be performed to better characterize this model and to clarify the molecular events that lead to alpha-synuclein accumulation and the resultant neurodegeneration. Subsequently, we will produce organoids of patients bearing different GBA mutations and we will test specific therapies for GBA-PD.

ORAL PRESENTATION	June 14th, 10:15 – 10:35
NAME	**Marco Campisi**
Course/Year	PhD. – 2nd year
Institution	Politecnico di Torino

Tumor-vascular interactions promote STING-driven inflammation in the lung tumor microenvironment

Authors	Campisi M[1,2], Sundararaman SK[2], Kitajima S[2], Chiono V[1], Kamm RD[3], Barbie DA[2]
Affiliation	1) Dept. of Mechanical & Aerospace Engineering Politecnico di Torino, Turin, Italy; 2) Dept. of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA; 3) Dept. of Biological & Mechanical Engineering, Massachusetts Institute of Technology, Cambridge, MA.

Background. The tumor stroma is an essential component of the tumor microenvironment (TME) and has critical roles in promoting resistance to immunotherapies. Most anticancer therapies target cancer cells specifically, however, it is important to also study signaling contributions from the TME. The recruitment of immune cells following intratumoral administration of Stimulation of Interferon Genes (STING) agonists in the TME is a critical event in the cGAS-STING-driven antitumor immune response, a pathway with great relevance in the context of cancer immunotherapy. Towards this, the infiltration of immune cells rely on functional vasculature to infiltrate into the tumor tissue. We have previously demonstrated that LKB1 mutation is associated with suppression of tumor cell STING levels due to mitochondrial dysfunction and reduced production of T-cell chemoattractants such as CXCL10 in KRAS-driven non-small cell lung cancer (NSCLC). Consistently, immunohistochemical staining of patient samples showed poor infiltration of CD3, CD4, and CD8 T cells into LKB1 negative versus LKB1 intact cancer epithelium, and instead, retention of T-cells in stroma.

Methods. 3-D microfluidic device was fabricated using cyclic olefin polymer (COP) at AIM biotech. NCI-H1355 cells were cultured for 24h in ultra low-attachment culture plates for spheroid formation. To form the tumor microvascular model, cancer spheroids, human lung fibroblasts and human umbilical vein endothelial cells (HUVECs) were resuspended in an extracellular matrix-like fibrin/collagen gel and loaded into the device, and cultured for 7 days. Cytokine profiling (Human Cytokine 40-plex panel, Bio-plex) was performed with media collected from 3D culture.

Results. To examine how LKB1 alters immune cell recruitment in a STING-dependent manner, we used a 3-D microfluidic co-culture system to study interactions between vasculature and tumor spheroids derived from a KRAS/LKB1 mutated (KL) cell line with LKB1 reconstitution /- STING deletion. Co-culturing tumor spheroids with fibroblasts and endothelial cells we identified changes in morphology, cytokine production, and gene expression that occur in coculture. We first observed that co-culture induced synergistic production of multiple immune cell chemo-attractants such as CXCL10, CCL2, IL-6, and G-CSF. Interestingly, this more physiologic ex vivo tumor model of LKB1 reconstitution revealed particularly strong cooperative production of STING-dependent cytokines such as CXCL10 in the vasculature. Moreover, STING depletion in LKB1 reconstituted tumor cells did not significantly attenuate production of CXCL10 and other cytokines in co-culture, suggesting that tumor/vessel interaction may promote STING activation in the vasculature regardless of cancer cell-intrinsic STING function. Furthermore, although there was no appreciable response after treatment of KL cancer cells with cGAMP based STING agonists, treatment of isolated 3-D vascular networks with cGAMP enhanced vascular permeability and increased production of CXCL10 and CCL5, possibly contributing to defective chemokine gradients that retain T cells near the vasculature.

Conclusions. Developing these more complex models that incorporate the vasculature may elucidate important aspects of STING biology and may ultimately aid further development of effective immunotherapies targeting this signaling pathway.

ORAL PRESENTATION	June 14th, 10:35 – 10:55
NAME	**Eleonora Allocati**
Course/Year	PhD. – 1st year
Institution	Istituto di Ricerche Farmacologiche Mario Negri IRCCS

Analysis of the clinical evidence supporting the marketing authorization of biosimilars in Europe

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Background. The development and manufacturing processes of biologic drugs are complex and costly, making such medicines one of the main drivers of costs in healthcare systems. The expiry of patents and/or other data protection certificates of biologics has fueled interest in developing biosimilars, medicines highly similar to biologics already marketed. We reviewed the marketing authorization of biosimilars to provide critical analysis of the pivotal studies supporting their approval by the European Medicines Agency (EMA). This analysis also served to highlight possible gaps and help to propose innovative approaches to optimize the clinical efficacy and safety of biological medicines and their biosimilars.

Methods. We identified biosimilars approved up to December 2018 in the European Union through the EMA database and gathered information on pivotal study design, duration, intervention and control, primary outcome, data on immunogenicity, and comparability margins from the European Public Assessment Report (EPARs). We focused on clinical studies assessing efficacy and safety, when available. Data were extracted by one author and checked independently by a second. Disagreements were solved by discussion.

Results. Up to December 2018, the EMA had approved 52 biosimilars corresponding to 16 biologic products, widely used in several clinical indications such as chronic inflammatory disorders, neoplasms, diabetes, venous thromboembolism, anemia, neutropenia, and osteoporosis. More than half (31/52, 60%) were approved in 2017-2018. For marketing reasons, some biosimilars were licensed as multiple medicinal products, with different commercial names, by the same or different companies. Comparability exercise and subsequent approval of 46/52 biosimilars (88%) were based on one or more pivotal phase III trials testing their clinical efficacy. In all, biosimilars were approved based on 52 clinical trials. Three quarters were phase III trials assessing clinical efficacy (39/52, 75%) and half applied an equivalence design (28/52, 54%). The phase III pivotal trials assessed surrogate measures of clinical effects and the majority reported data about immunogenicity (30/39, 77%).

Conclusions. Analysis of the approval of biosimilars in the European Union depicts a complex and to some extent heterogeneous scenario. The request for showing similarity in terms of clinical efficacy and safety provides a robust demonstration of comparable clinical outcomes but lays a burden for biosimilar manufacturers and may delay their introduction. Moreover, it provides no information about relevant issues, such as interchangeability and switching. The development, licensing and monitoring of biosimilars would benefit from new ways to improve efficiency, speed up access to biosimilars, and reduce uncertainties about their use optimizing their monitoring in clinical practice.

ORAL PRESENTATION	June 14th, 10:55 – 11:15
NAME	**Camilla Galli**
Course/Year	PhD. – 4th year
Institution	IFOM

Dynamic hand-in-hand interaction between spectrin and actin during mammalian cell mechanoadaptation

Authors	Camilla Galli, Andrea Ghisleni, Qingsen Li, Paolo Maiuri, Nils Gauthier
Affiliation	The FIRC Institute of Molecular Oncology, Milan (IFOM)

Background. Multicellular organisms have evolved complex mechanisms to sense and adapt to the surrounding environment by dynamically controlling cell shape and exert forces. In this context, spectrin is a membrane-binding protein involved in cell mechano-sensing which has been mainly described in erythrocytes and neuronal axons, but very few is known about its molecular organization, dynamics and function in other eukaryotic cell types.

Methods. Here, we investigated spectrin dynamics, its regulation and role in membrane adaptation upon chemical and mechanical perturbation of the cell, by different fluorescent microscopy techniques at high spatio-temporal resolution. We used mouse embryonic fibroblasts as model system and applied different mechanical and chemical cues such as cell stretching, compression, osmotic shocks or drug mediated cytoskeleton disruption.

Results. Thanks to all these techniques, we described how spectrin dynamically interplay with both the acto-myosin cytoskeleton and the plasma membrane with complementary functions. Moreover, we observed different responses between actin and spectrin, supporting our hypothesis of a reinforcing mechanism of the plasma membrane mediated by spectrin in regions where the actin cytoskeleton is not established, and a fencing mechanism for actin remodeling and membrane trafficking.

Conclusions. These results potentially unveil why the spectrin family of protein is evolutionary highly conserved and ubiquitously expressed in eukaryotic cells and bring the attention to a possible involvement of spectrin in diseases linked to cell shape rearrangements, such as cancer and the metastasis formation.

ORAL PRESENTATION	June 14th, 15:30 – 15:50
NAME	**Sara Tavella**
Course/Year	PhD. – 2nd year
Institution	IFOM

Targeting genome alterations in cancer cells by CRISPR/Cas9 and dilncRNAs inhibition

Authors	Sara Tavella[1], Fabrizio d'Adda di Fagagna[1,2]
Affiliation	1) IFOM - The FIRC Institute of Molecular Oncology, Milan, 20139, Italy. 2) Istituto di Genetica Molecolare, CNR - Consiglio Nazionale delle Ricerche, Pavia, 27100, Italy.

Background. RNA Polymerase II promotes the transcription of damage-induced long non-coding RNAs (dilncRNAs) at the site of DNA double-strand breaks. DilncRNAs are the precursors of small non-coding RNAs called DNA damage response RNAs (DDRNAs) and the interaction between dilncRNAs and DDRNAs is necessary for the recruitment of the proteins involved in the DNA damage response (DDR). The use of antisense oligonucleotides against the pairing of dilncRNAs and DDRNAs is useful to inhibit DDR, including signaling and DNA repair, in a sequence-specific manner, without impacting ongoing DDR activation at other damaged sites in the same cell, both in vitro and in vivo.

Methods. Generation of a GFP-based system to study if introduction of sequence-specific DSBs by CRISPR/Cas9 and inhibition of the repair by ASOs against dilncRNAs can kill the cells. Stable cell line establishment by infecting MCF10A cells with a GFP lentiviral vector in order to mimic a set of genomic aberrations absent in the parental cell line. I have designed a sgRNA targeting the GFP. I designed ASOs targeting the dilncRNAs. I have plated cells, transfected ASOs, infected them with CRISPR/Cas9 and monitored proliferation with a resazurin-based viability assay. Cells infected with a Cas9 carrying a scramble sequence as negative control.

Results. Only the simultaneous generation of double strand breaks inside the GFP sequence by the CRISPR/Cas9 technology and the inhibition of the repair by antisense oligonucleotides matching the transcribed dilncRNAs is causing apoptosis. More specifically, 6 days post infection of MCF10A-GFP promoter with the lentiCRISPR v2 vector, there is about 30% reduction in proliferation of cells in which the repair of the double strand breaks is inhibited by sequence-specific antisense oligonucleotides, compared to the negative controls. Indeed, transfection of MCF10A-GFP promoter with antisense oligonucleotides only or infection with Cas9 alone in not significantly affecting cell proliferation. This preliminary result needs to be confirmed and improved, but it encourages us to keep investigating our initial hypothesis.

Conclusions. I plan to validate the therapeutic potential of this approach in cancer cells carrying aberrant genome alterations. Possible targets are tumour cells carrying gene translocations, gene amplifications or that have undergone chromothripsis; cells infected by an integrated (and silent) proviral genome can be selected too. These results may be of impact in clinical applications, since they could open the possibility to kill different types of aberrant cancer cells, while sparing normal cells.

ORAL PRESENTATION	June 14th, 15:50 – 16:10
NAME	**Jevtic Zivojin**
Course/Year	PhD. – 3rd year
Institution	IFOM

Identification of NUP98-NSD1 and NSD1 interactors and their implication in leukemogenesis

Authors	Jevtic Z, Matafora V, Bachi A
Affiliation	IFOM, the FIRC Institute of Molecular Oncology

Background. Nuclear receptor binding SET domain protein 1 (NSD1) is a chromatin-associated histone methyltransferase which is fused to nucleoporin NUP98 in up to 10% pediatric cases of acute myeloid leukemia as a result of t(5;11)(q35;p15.5) translocation. The fusion protein NUP98-NSD1 activates and maintains the expression of proto-oncogenes HoxA7, HoxA9, HoxA10 and Meis1 in murine myeloid progenitors. The exact mechanism of NUP98-NSD1 proto-oncogene binding and activation still remains elusive. Detailed interactomic studies of NSD1 and NUP98-NSD1 are necessary to pinpoint protein domains and corresponding interactions that are implicated in the activation and maintenance of leukemogenic transcription program.

Methods. We performed proteomic profiling of Flag-tagged NUP98-NSD1 and NSD1 interactors in human embryonic kidney (HEK293) cells. To validate observed interactions, we performed co-immunoprecipitation and immunofluorescence experiments using HEK-293 overexpression system. We also queried published ChIP-Seq datasets for CHD4, SMARCA4, and NUP98-NSD1 to further corroborate interactomic data. Finally, we transduced myeloid progenitor cell line 32D cl3 and established stable expression of Flag-tagged NUP98-NSD1.

Results. We performed biochemical characterization of NSD1 and NUP98-NSD1 in transiently transfected HEK-293 cells using WB, RT-qPCR and immunofluorescence staining. Furthermore, we acquired the interactomes of NSD1 and NUP98-NSD1 in HEK-293 cells using label-free mass spectrometry. Among the direct and specific interactors of the fusion protein, we identified known players in AML such as CHD4, SMARCA4, and RUVBL1, and validated them by co-immunoprecipitation. Moreover, we queried published ChIP-Seq datasets for CHD4, SMARCA4, and NUP98-NSD1, which all showed peak enrichment at the MEIS1 promoter. We plan to investigate the topology of these interactions by cross-linking/mass spectrometry. Finally, we generated a myeloid progenitor cell line (32D cl3) stably expressing NUP98-NSD1, which will be used for the functional validation of targeted proteins.

Conclusions. Taken together, we identified novel, disease-related interactors of the NUP98-NSD1 using proteomic and genomic approaches, and established a physiological model system for the functional studies of the observed interactions.

ORAL PRESENTATION	June 14th, 16:10 – 16:30
NAME	**Valentina Doldi**
Course/Year	PhD. – 3rd year
Institution	IRCCS Istituto Nazionale dei Tumori, Milano

Antiarrhythmic drugs to counteract the activated phenotype of prostate cancer-associated fibroblasts

Authors	Valentina Doldi[1], Paolo Gandellini[2] and Nadia Zaffaroni[1]
Affiliation	1) Molecular Pharmacology Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy. 2) Department of Biosciences, University of Milan, Milan, Italy.

Background. The interplay between cancer cells and adjacent stroma is fundamental for the development and progression of tumours. In the prostate cancer (PCa) context, cancer-associated fibroblasts (CAFs) have been shown to fuel tumour development and metastasis by mutually interacting with tumour cells. By comparatively analysing gene expression profiles of CAFs and normal fibroblasts, we found that CAFs are characterized by positive enrichment of genes that code for calcium, sodium and potassium cation channels. Here, we investigated the effects of cation channel inhibitors, currently used as antiarrhythmics, on CAF activated state and CAF-PCa cell interplay.

Methods. CAFs established from malignant regions of radical prostatectomy specimens from PCa patients were treated with sub-toxical doses of cation channel inhibitors. Upon treatment, the effects on CAF phenotype were assessed by evaluating cell morphology and the expression of specific fibroblast activation markers using immunoblotting. Additionally, CAF contractility was measured in a 3D collagen contraction test and migration was evaluated by scratch assay. The ability of cation channel inhibitors to impair CAF-PCa cell interplay was evaluated in terms of cell growth and expression of epithelial-to-mesenchymal transition (EMT) markers by performing conditioned medium and direct co-culture experiments.

Results. The treatment of CAFs with cation channel inhibitors induced morphological changes, reduced CAF ability to remodel extracellular matrix and impaired CAF migration, although at a different extent depending on the drug. Interestingly, while the conditioned medium of CAF promoted cell growth and induced EMT in PCa cells, as assessed through western blotting and qRT-PCR of epithelial (E-cadherin) and mesenchymal markers (ZEB 1/2 and SNAI1), treatment of CAFs with antiarrhythmics partially reverted CAF–medium protumoral effects. These results suggest that the treatment of CAFs with cation channel inhibitors is able to abrogate CAF ability to promote PCa cell proliferation and plasticity. Noteworthy, these findings were also confirmed in the co-culture setting.

Conclusions. These results indicate that the inhibition of cation channels interferes with crucial features of prostate CAFs, as indicated by changes in cell morphology, modulation of specific activation markers and a substantial reduction of contraction and migration capability. Moreover, indirect and direct co-culture experiments revealed that antiarrthytmics are able to impair CAF-induced proliferative spur and counteract CAF-induced EMT in PCa cell lines. Collectively, such preliminary data suggest new opportunities for the translation of drugs already used in a different medical field, such as the cardiovascular, into the targeting of CAFs with the aim to interrupt their tumour-supportive boost.

POSTER	**C1**
NAME	**Hariprakash Judith Mary**
Course/Year	PhD. – 2nd year
Institution	IFOM

Computational Reconstruction and Analysis of Enhancer-Gene Regulatory Networks Altered in Cancer

Authors	Judith Mary Hariprakash[1], Elisa Salviato[1], Endre Sebestyen[1], Francesco Ferrari[1,2]
Affiliation	1) IFOM, the FIRC Institute of Molecular Oncology, Milan, 2) Institute of Molecular Genetics, National Research Council, Pavia.

Background. Enhancers are cis-acting regulatory elements, that positively regulate the transcriptional output of genes. Enhancer dysregulation has been associated with various diseases including cancer. Thus far, mapping mutations in enhancer regions has proved difficult owing to their distal locations from coding regions and also due to a lack of comprehensive marks that define them. We hypothesize that non-coding mutations in regulatory regions (enhancers) could significantly contribute to cancer prognosis or progression and hence can be exploited for better patient stratification and novel prognostic biomarkers. We study the role of non-coding mutations in lung cancer, as it has higher mutational burden.

Methods. To identify regulatory mutations in lung cancer, using a custom pipeline of 5 variant callers, we call for somatic mutations in 159 matched normal-tumor lung cancer patient samples with whole genome sequencing. To define enhancers across the genome we leverage the epigenomic signatures of enhancers such as the presence of H3K27ac, DNase I sensitivity. We map lung cancer mutations on the enhancers and identify recurrently mutated enhancers in lung cancer patients. To identify target genes for the recurrently mutated enhancers, we developed an enhancer-target gene pairing approach using canonical correlation based on epigenomic data from 44 cell types.

Results. We defined 210,563 enhancer cores in IMR-90 cell line with an average length of 13 bps. 50 percent of lung cancer samples have mutations in at least 25 enhancer cores. The enhancer core mutation frequency and exon mutation frequency are similar in all the patients. Nine enhancer cores are mutated in more than 5 percent of the patients. Of which mutation in the intragenic enhancer of CDH13 gene was further analyzed for its effect in CDH13 gene. Enhancer mutation is mutually exclusive to genic mutations and copy number alterations in the gene. CDH13 promoter in enhancer mutated sample did not show hypermethylation. Though changes in expression is observed in the mutated and non-mutated samples, lack of statistical significance demands further investigation. Other enhancers of CDH13 gene are analyzed for joint effect on the enhancer. The performance of the pairing approach is by far at par with other tools available in literature.

Conclusions. Non-coding mutations in the genome have been overlooked in the context of cancer. Our approach for the first time uses a stringent definition of enhancers that enables identification of core regions within an enhancer for mutations. The enhancer target gene pairing approach that we have developed for the first time uses Hi-C interactions for effective target genes prediction. With this improved and stringent approach we expect to identify non-coding mutations that result in pathway level alterations. Our goal is to better stratify patients to improve prognosis in cases where the prognosis is quite variable after classifying patients just based on coding mutations.

POSTER	**C2**
NAME	**Elisabeth Digifico**
Course/Year	PhD. – 3rd year
Institution	Humanitas University

Tumor Associated Macrophages: toward a better understanding of their cancer driver capability in Malignant Pleural Mesothelioma

Authors	Digifico E[1,2], Erreni M[1], Ceresoli GL[3], Roncalli M[1], Frapolli R[4], D'Incalci M[4], Allavena P[1]*, Belgiovine C[2]*
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Background. Malignant Pleural Mesothelioma (MPM) is an aggressive cancer characterized by chronic inflammation driven by the presence of non-degradable asbestos fibers. MPMs are known to be rich in Tumor Associated Macrophages (TAMs), which are a major source of inflammatory mediators; some studies reported their association with a poor prognosis. We performed a gene expression analysis on human mesothelioma tumor surgical samples and identified two highly upregulated genes: GPNMB, coding for glycoprotein non metastatic B (GPNMB), and SPP1, coding for osteopontin (OPN).

Methods. In order to investigate the mechanisms by which OPN and GPNMB may influence tumor growth, we used three murine mesothelioma cell lines with different histotypes (sarcomatoid, biphasic, epithelioid) as observed with human MPMs: AB1, AB12, and AB22.

Results. The circulating levels of OPN and GPNMB (measured by ELISA) were significantly higher in MPM patients than in healthy donors. These two proteins are known to be produced either by cancer cells or TAMs but their role in cancer is still not completely identified. In vivo experiments confirmed that murine mesothelioma are highly infiltrated by immune cells, especially macrophages. OPN was strongly expressed by the three cell lines while GPNMB levels were very low unlike human MPMs; Accordingly, we silenced OPN and overexpressed GPNMB in AB1, AB12, and AB22 cells to study the role of these proteins in vivo. Ongoing experiments showed that the silencing of OPN strongly decreases tumor growth in vivo, while the overexpression of GPNMB increases tumor progression in some mice.

Conclusions. Our results demonstrate that OPN produced by MPM cells has a strong tumor-promoting activity. Similar results are noted with GPNMB-expressing MPM cells but need to be further confirmed. These two proteins may be useful biomarkers to reach an early detection of mesothelioma, bringing to more effective treatments.

POSTER	**C3**
NAME	**Martina Di Modica**
Course/Year	PhD. – 4th year
Institution	Fondazione IRCCS Istituto Nazionale dei Tumori di Milano

The gut microbiota contributes to effectiveness of HER2-targeted therapy

Authors	Martina Di Modica[1], Viola Regondi[1], Giorgio Gargari[1], Arianna Bonizzi[2], Stefania Arioli[3], Beatrice Belmonte[4], Claudio Tripodo[4], Fabio Corsi[2,5], Simone Guglielmetti[3], Tiziana Triulzi [1]* and Elda Tagliabue [1]*
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Background. Recently, the composition of the gut microbiota, due to its influence on host immune system, has been linked to the effectiveness of chemotherapy and immunotherapy. Since trastuzumab, besides inhibiting the HER2 signaling, recruits innate and adaptive immune cells that mediate its cytotoxic activity in the tumor, we hypothesized that commensal bacteria can be a source of heterogeneity for the response to therapy in patients with HER2-positive breast cancer (HER2 BC).

Methods. The impact of the gut microbiota on anti-HER2 therapy was studied in mice with the intestinal flora alterated by the treatment with vancomycin or streptomycin-two broad spectrum antibiotics poorly absorbed in the intestine. The association between commensal bacteria composition and clinical efficacy of trastuzumab was investigated in a cohort of HER2 BC patients treated with neoadjuvant trastuzumab.

Results. Administration of antibiotics impaired the efficacy of anti-HER2 monoclonal antibodies in FVB mice bearing syngeneic mammary carcinomas expressing HER2. 16S rRNA gene profiling of FVB mouse feces showed that both antibiotics decreased bacterial α-diversity in the gut as evaluated by Chao1, lowering the abundance of Clostridiales bacteria. Mice transplanted with feces from antibiotic treated mice did not benefit from the anti-HER2 treatment supporting a direct relation between intestinal bacteria and therapeutic efficacy. Analysis Immunohistochemistry of tumors showed that alteration of gut microbiota compromised the recruitment of CD4 T cells and granzyme B cells upon anti-HER2 administration. Fecal 16S rRNA gene sequencing demonstrated a significantly higher microbial α-diversity in patients who achieved a pathological complete response compared to non-responders using several indices. Moreover, a clustering effect by patient's response was observed visualizing the β-diversity. OTUs belonging to the Clostridiales and Bacteroides orders were reduced and enriched, respectively, in non-responders.

Conclusions. Our data support that the composition of the gut microbiota, especially as regards the abundance of Clostridiales bacteria, has a role in the therapeutic efficacy of trastuzumab both in mice and patients. Therefore, the manipulation of intestinal bacteria may represent a new strategy to improve the cure of HER2 BC patients. (Supported by Associazione Italiana per la Ricerca sul Cancro).

POSTER	**C4**
NAME	**Armela Huda**
Course/Year	PhD. – 3rd year
Institution	IFOM

Molecular analysis of the replication stress response at human telomeric repeats

Authors	Armela Huda, Hiroshi Arakawa, Giulia Mazzucco, Ylli Doksani
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Background. Telomeres are associated with a protein complex called shelterin, which protects them from the DNA Damage Response and regulates their maintenance. Apart from its role in end protection, the shelterin complex also plays an essential role in promoting efficient telomere replication, which is known to be problematic. Studies in yeast using two-dimensional agarose gel electrophoresis (2D-gels) revealed frequent replication fork pausing in these sequences.

Methods. We are combining 2D-gels and Electron Microscopy analysis to study replication intermediates at telomeric repeats in human cells and the role of different replication stress response pathways involved in telomere replication. In mammalian cells, telomeres are long and highly heterogeneous, which makes it impossible to follow their replication by 2D-gels. We introduced mammalian telomeric repeats in an episomal vector, previously used to study the replication of specific DNA sequences in mammalian cells.

Results. Our preliminary results suggest that replication forks pause at telomeric repeats although the pausing is not prolonged and does not prevent the replication of the plasmid in human cells. We are now following the behavior of these plasmids after the deletion of the shelterin component TRF1, which plays an essential role in telomere replication. To this end we introduced an AID tag at the endogenous TRF1 locus, which allows degradation of the protein upon auxin addition.

Conclusions. We developed an easy and fast system to study the molecular nature of telomere replication abnormalities.

POSTER	**C5**
NAME	**Chiara Pesenti**
Course/Year	PhD. – 1st year
Institution	Istituto di Ricerche Farmacologiche Mario Negri IRCCS

Whole genome CNV analysis depicts the heterogeneous molecular portrait lagging behind the single definition of Stage I Epithelial Ovarian Carcinoma

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Background. Stage I Epithelial Ovarian Carcinoma (EOC) is a rare tumour with a good prognosis, as almost 20% of cases relapse with resistant disease within five years from the diagnosis. To date, little is known about the molecular hallmarks that characterize the biology of stage I EOC, and in particular whether this information can be used as potential biomarkers with diagnostic and prognostic purposes Therefore, we decided to investigate the whole genome Copy Number Variants (CNVs) landscape in Stage I EOC to explore the complex relationship between genomic aberrations, tumour progression and therapy response.

Methods. Shallow Whole Genome Sequencing (sWGS) was exploited to uncover the CNVs status in 165 Stage 1 EOC cases, with a median depth of 0.5x, sufficient to investigate clonal and subclonal events of at least 1Mbp. Recently developed algorithms were applied to identify CNV patterns potentially useful not only to classify the tumour samples, but also to predict patients' prognosis. GISTIC was used to define recurrently altered regions.

Results. The preliminary analysis of sWGS experiments allowed the identification of three different CNVs patterns: a) the Stable (S) profile without any detectable genomic aberration, enriched with low grade tumours; b) the Unstable (U) profile, characterized by the presence of large rearrangements affecting few chromosomes with a homogeneous pattern (either deleted or amplified); c) the Highly Unstable (HU) profile, with a very high level of genomic instability potentially assimilable to chromotriptic events in some cases: substantially all chromosomes are affected by different types of CNV patterns, i.e. large rearrangements or focal amplifications. This last type is enriched with high grade serous histotype and mirrors what has been already described in advanced stages EOC.

Conclusions. This is the first study assessing whole genome CNV profile in Stage I EOC, encompassing all the different histopathological subtypes. Preliminary data suggest a heterogenous genomic landscape lagging behind the conventional histopathological classification of Stage I EOC. Therefore, further ongoing analyses on Stage 1 EOC different CNV patterns could help to better define how they are related to tumorigenesis and if the presence of clonal and subclonal events could be closely associated to tumour type and to patients' prognosis.

POSTER	**C6**
NAME	**Valentino Ribecco**
Course/Year	PhD. – 1st year
Institution	Humanitas Clinical and Research Center – IRCCS

Exosomes from irradiated glioblastoma stem cells support tumor growth and invasiveness in patient-derived xenograft disease model

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Background. Glioblastoma is the most common primary brain tumor, highly proliferative and invasive, characterized by remarkable biological heterogeneity and poor response to present treatments (overall 5-year survival rate less than 3%). Exosomes are cell-secreted vesicles, involved in both autocrine/paracrine intercellular communication through the transfer of a wide variety of biomolecules. Exosome quantity and composition vary respect to cell of origin and physiologic/pathologic status, modulating tumor-host interaction. This study focused on the molecular features and the functional activities of exosomes released by patient-derived Glioblastoma Stem Cell lines under basal conditions and under radiation treatment on invasiveness, migration and resistance to therapies.

Methods. The conditioned medium are centrifuge at 300g x 5min to isolate cells, after that the supernatant are centrifuge at 10000g x 30 mins to isolate microvescicles, then the supernatant are centrifuge at 110000g x 65mins. Exosomes are characterized through western blot and were analyzed through Nanoparticle Tracking Analysis. Luciferase-transfected GSCs (ICH001-luc cell line) were intracranially implanted in CD1/male nude mice. Exosomes isolated from irradiated (2Gy) or non-irradiated parental ICH001 cells (not-trasfected) were administered to the mice starting 2 weeks after the orthotopic xenografts. Tumor growth was evaluated by bioluminescence detection 24 hours and 5 days after the last exosomes treatment.

Results. Results demonstrated an increased invasive capacity of Glioblastoma Stem Cell upon incubation with exosomes both in vitro and in vivo. In vivo administration of exosomes derived from irradiated (2Gy) Glioma Stem Cells significantly augment tumor growth and expansion compared to administration of exosomes from non-irradiated cells and not treated tumors, indicating a key role for radiotherapy and exosomes in glioblastoma tumor survival and progression. Nanoparticle Tracking Analysis indicated a noteworthy higher amount of exosomes released by irradiated Glioblastoma Stem Cells compared to non-irradiated ones. Furthermore, proteomic analysis revealed an enrichment of the proteins involved in motor activity and DNA repair mechanisms in exosomes derived from irradiated Glioblastoma Stem Cells.

Conclusions. These results are able to highlight a more clear picture about the contribute of exosomes in glioblastoma multiforme. The exosomes are capable of influence both the microenvirorment and the tumor cells to enhance and improve the highly capacity to infiltrate in the brain parenchyma. Furthermore, studying exosomes, we highlighted how the highly heterogeneous content, in terms of proteins, could have a key role in determining all this. Importantly, these study may lead to future insights on the exosomes role in the standard treatments failure.

POSTER	**C7**
NAME	**Luca Varinelli**
Course/Year	PhD. – 2nd year
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Microenvironment and Metastatic Spread: 3D-decellularized Matrix as a Tool to Dissect the Main Features of the Colorectal Cancer Peritoneal Metastasis

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Background. Metastatic dissemination to the peritoneal cavity is common in colorectal cancer and is still a lethal disease. The development of peritoneal metastases is due to the crosstalk between cancer cells and host elements which involves several steps, known as “peritoneal metastatic cascade”, characterized by a fine-tuned interaction between biochemical factors, released by the tumor (such as secretome/exosomes) and biomechanical events (such as ECM remodelling) that generate the so-called “metastatic niche”. The development of an innovative 3D model that integrates all the elements involved in the metastatic dissemination could be the ideal strategy to model the peritoneal metastatic environment.

Methods. Peritoneal-derived 3D-matrices were decellularized following a procedure that uses detergents and enzymatic cycles. The effectiveness of the methodology was tested by determining the DNA loss (absence of nuclei), the presence of structural proteins and the preservation of the tissue architecture (immunohystochemistry, fluorescence and histological approaches). The obtained 3D-matrices were repopulated through direct seeding of organoids on their top. The proliferation rate of organoids and the maintenance of the stem cell pool were estimated by immunostaining of Ki67 and LGR5 respectively. Staining of the ex-vivo 3D-peritoneal metastasis models with cytokeratins and CDX2 confirmed their similarity with the surgical sample of origin.

Results. The decellularized 3D-matrices obtained both from normal and neoplastic peritoneum resulted free of DNA and lipids (WGA staining). Haematoxylin and eosin staining revealed that they maintained their original architectural structure. Immunohistochemistry analyses indicated the absence of epithelial and mesenchymal cells and maintenance of the distribution of stromal components. The repopulation of 3D-matrices with organoids derived from peritoneal metastases at different time points (5-12-21 days) showed that the organoids were widely distributed throughout the 3D-matrix, with an evident diffusion at later time points, characterized by stromal infiltration. Immunofluorescence analysis showed that neoplastic-derived 3D-matrix enhanced the proliferation of the organoids and the staminality was higher at later time points. The presence of specific colorectal cancer markers confirmed that the repopulated 3D-matrix models resemble their tumor of origin. Repopulation of 3D-matrices derived from colon tissue (normal or neoplastic mucosa) showed a preferential growth of organoids derived from peritoneal metastasis on peritoneal-derived matrices.

Conclusions. Our results suggest that 3D-decellularized matrices can be used as a natural biological scaffold for culturing organoids. We observed that the repopulated 3D-matrix models reproduce the lesions found in vivo. In fact they retain the expression of specific colorectal markers and recapitulate the morphological characteristics observed in their tumor samples of origin. The 3D-matrix models we generated recapitulate the main features of the peritoneal niche, and can be used to deeply characterize the molecular events that sustain the development of the peritoneal metastasis disease.

POSTER	**C8**
NAME	**Roberta Vazzana**
Course/Year	PhD. – 2nd year
Institution	IFOM

The Interplay Between Fatty Acid and Glucose Metabolism in Breast Cancer

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Background. Metabolic pathways are frequently dysregulated in cancer. Breast cancer cells show high glycolytic rates, intense anabolic growth and de-novo fatty acid synthesis. A competitive relationship between glucose and fatty acid usage at the level of tissue metabolism is well known; here, we investigate how this balance is maintained and contributes to tumour progression in breast cancer.

Methods. We examined the effects on glycolysis of incubating cells with high levels of fatty acids, forcing an accumulation of cellular fat stores. Incubation of cancer cell lines in media supplemented with fatty acids led to an accumulation of lipid droplets, the lipid storage organelle. To determine the effect of the increased lipid stores on glycolysis, we assayed the activity of Pyruvate Kinase Muscle Isozyme M2 (PKM2), the limiting enzyme that catalyses the last reaction in glycolysis.

Results. The presence of lipid droplets was accompanied by a significant reduction in PKM2 activity in cellular lysates. Moreover, utilizing a fatty acid-pulldown assay, we detected a robust association between PKM2 and long chain fatty acids. Proteomic profile of lipid droplets-associated proteins in breast cancer cells showed the presence of PKM2.

Conclusions. The strong interaction between fatty acids and PKM2 and its sub-localization into lipid-droplets suggests a hypothetical sequestration of the enzyme mediated by fatty acid-rich organelles. This result provides an attractive mechanism to link the uptake of fatty acids to the regulation of the glycolytic pathway in breast cancer cells.

POSTER	**C9**
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Course/Year	PhD. – 2nd year
Institution	Fondazione IRCCS Istituto Nazionale dei Tumori

Activating Transcription Factor 3: a New Player in Cancer-Adapted Myelopoiesis

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Background. Cancer is a systemic disease able to skew the host immune system towards a pro-tumorigenic state. Yet, little is still known about the timing in which bone marrow starts to sense developing distant tumors. A gene expression profile performed on bone marrow collected from MMTV-NeuT mice, which develop mammary carcinomas, identified the activating transcription factor 3 (Atf3) as one of the genes significantly induced in pre-invasive lesions. Atf3 is a hub of inflammatory response signals to stress and danger sensing, and it is markedly expressed in a subset of immature myeloid cells. Therefore, we investigated the activity of Atf3 in the bone marrow, also focusing on its potential target genes.

Methods. Colony-forming unit assay was performed with lineage negative BM cells isolated from naive BALB/c mice either infected or not with a lentiviral vector carrying Atf3 under the control of the myeloid-specific promoter CD68. Bone marrow transplantation experiments were performed with Atf3-transduced or WT hematopoietic stem cells transferred into lethally irradiated NeuT mice. Gene expression profile performed on total bone marrow samples of transgenic NeuT and BALB/c control mice collected at different stages of disease was analyzed in silico, to further investigate Atf3-related pathways. qRT-PCR was performed on CD11b Gr1dimLy6Gneg cells isolated by immunomagnetic beads from bone marrow of the same animals. CRISPR/Cas9 technology and lentiviral vectors were employed to deplete and to over-express, respectively, ATF3 in a monocyte/macrophage cell lines (J774).

Results. Colony-forming unit assay revealed that over-expression of ATF3 in early bone marrow progenitors promotes the expansion towards monocytic and/or granulocytic progenitors. Bone marrow transplantation with Atf3-transduced hematopoietic stem cells induced an exacerbated myelopoiesis in NeuT mice, boosting a subset of immature and anti-inflammatory cells (CD11b Gr1dimLy6Gneg), accelerated tumor onset and increased the migration of neutrophils from the bone marrow to the periphery. Through in silico analysis, we identified Jdp2 and Tiam2 as potential ATF3 target genes involved in a systemic tumor-promoting myeloid response. In the NeuT myeloid subpopulation (CD11b Gr1dimLy6Gneg), in which we had previously described the up-regulation of Atf3, we observed the down-modulation of Jdp2 and the simultaneous increase of Tiam2 expression. Finally, by engineering macrophage cell lines through ATF3 over-expression and knock-out, we confirmed that this transcription factor promotes Tiam2 and inhibits Jdp2 expression.

Conclusions. Our findings support the idea that, under persistent inflammatory conditions, Atf3 drives the expansion of the CD11b Gr1dimLy6Gneg immature myeloid cells through the inhibition of Jdp2, which is involved in neutrophil differentiation. Moreover, Atf3 could regulate a number of proteins necessary for chemotaxis, such as Tiam2 that is involved in cell migration. Overall, Atf3 up-regulation in the myeloid compartment may play a central role in cancer-adapted haematopoiesis able to sustain a systemic tumor-promoting myeloid response.

POSTER	**C10**
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Course/Year	PhD. – 1st year
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Unveiling the role of PLEKHA7 during the progression of epithelial ovarian cancer

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Background. PLEKHA7, localized apically at the site of the Zonula Adherens in the adherens junctions, is involved in the maintenance of cell polarity. In breast and renal cancers, PLEKHA7 protein was found to be de-localized and/or downregulated. In vitro, PLEKHA7 appeared to contribute to the processing of several miRNAs/mRNAs by recruiting the microprocessor and RISC complexes at the site of adherens junctions. In ovarian carcinoma, we have found that higher levels of PLEKHA7 transcript are associated with better prognosis and PLEKHA7 protein expression in a low-grade ovarian carcinoma cell line lead to a less aggressive phenotype by negatively impinging EGFR activation.

Methods. Gene expression and immunohistochemical analysis were performed to assess PLEKHA7 expression and localization in a cohort of early-stage epithelial ovarian cancer patients (n=90). Two stable-infected overexpressing PLEKHA7 cell lines (OAW42 and OVCAR5), representative of low- and high-grade ovarian cancer, were used as in vitro models. Biochemical, functional and molecular characterization of the two in vitro models was assessed by immunofluorescence, western blot analysis, proliferation and drug sensitivity analysis, 3D cell growth in different matrix (Matrigel® and Algimatrix), gene profiling. Ingenuity Pathway and Gene Set Enrichment Analyses were conducted on gene profiling data.

Results. In the early-stage ovarian carcinoma cohort, a quite heterogeneous PLEKHA7 expression in terms of both intensity and staining patterns was observed. When expressed, PLEKHA7 was found apically on the cell membrane or focally accumulated in the perinuclear region. Gene expression profiling of the same cohort highlighted that PLEKHA7 expression intensities did not correlate with histotype or grading of samples. In the low-grade PLEKHA7 overexpressing cell line, we observed that PLEKHA7 is localized apically in cell-cell adhesion structures likely recapitulating the zonula adherens of normal epithelial cells, overall conferring a less aggressive phenotype. Conversely, high grade overexpressing cells retain the protein in the cytoplasm, where it co-localizes with the Golgi network. The latest cell model acquires a more aggressive phenotype in terms of migration and invasion, together with an increased resistance to platinum compounds. The gene expression profiling confirmed the different molecular portraits led by PLEKHA7 overexpression in the two in vitro models.

Conclusions. PLEKHA7 might modulate cell behaviour depending on its localization and post-traslational modulation. Indeed, in the low-grade model, PLEKHA7 overexpression leads to a less aggressive phenotype. Conversely, in the high-grade model, PLEKHA7 is found in the Golgi apparatus, likely representative of those tumors in which the protein is retained in the cytoplasm. Further molecular analysis of the gene expression data of our cohort together with biochemical and functional characterization of the in vitro models will help to refine PLEKHA7 role during the progression of ovarian carcinoma. Supported by Ministry of Health 5 x 1000 -2013 Fund.

POSTER	**C11**
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Course/Year	Dott. – 1st year
Institution	Istituto di Ricerche Farmacologiche Mario Negri IRCCS

Multisite analysis of high-grade serous epithelial ovarian cancers identifies genomic regions of recurrent copy number variation in 3q26.2 and 8q24.3

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Background. High grade serous epithelial ovarian cancer (HGS-EOC) is a systemic disease with a marked intra and inter patients tumor heterogeneity. The issue of spatial and temporal heterogeneity has long been overlooked, hampering the possibility to identify those genomic alterations that persist, before and after therapy, in the genome of all tumor cells across the different anatomical districts. In this study, spatially and temporally different tumor biopsies of the same patient have been investigated to identify in the genome of primary tumor lesions those genomic abnormalities shared by all lesions that characterize the genomic landscape of matched relapsed disease.

Methods. Multisite tumor biopsies from HGS-EOC stage III/ IV patients were gather together from two hospitals. Cohort A consists of 27 biopsies from 7 women taken at primary surgery while cohort B is composed by 52 biopsies from 17 patients underwent to primary and second surgery, after chemotherapy. Whole exome sequencing (WES) and array CGH (aCGH) experiments were done and recurrent regions of copy number alterations (SCNA) were identified. In silico data validation was performed on independent cohorts: cohort C consists of 494 biopsies from the Cancer Genome Atlas consortium while 91 biopsies from a published database compose cohort D.

Results. WES analysis shows that the majority of single nucleotide variant are private to each single tumor sample, with core mutations ranging between 0.2-25%. Differently, SCNA profiles depict a more homogenous and stable portrait than the mutational landscape. GISTIC analysis identifies two focal and minimal common regions of amplification (FMCR) in cytobands 3q26.2 and 8q24.3, respectively called regions α and β, shared by synchronous lesions and which persist at relapse, after chemotherapy. Analysis in external cohorts confirm that regions α and β are features of HGS-EOC. WES data was further analyzed to investigate the proportion of tumor cells harbouring the two selected FMCR of amplification in the overall tumor cell population: in the majority of tumor lesions (85%) α and β regions are detected in all tumor cell populations. This analysis confirm that SCNA and particularly the selected α and β regions are at the root of tumor evolution in HGS-EOC.

Conclusions. In conclusion, by dissecting the molecular architecture of primary and matched synchronous and metachronous lesions of HGS-EOC patients, this study demonstrates: 1. a marked intra patient tumor heterogeneity of SNV loci across different tumor lesions of the same patients; 2. an homogeneous SCNA profile, allowing to identify two recurrent regions of focal amplification (3q26.2 and 8q24.3) that are a common hallmark of HGS-EOC. The clonal nature of these focal amplifications paves the way for their important biological role in the etiopathogenesis of HGS-EOC.

POSTER	**C12**
NAME	**Marinella Corbetta**
Course/Year	PhD – 2nd year
Institution	Humanitas University

RNA-based molecular biomarkers in primary prostate cancer

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Background. The current diagnostic schedule to detect prostate cancer (PCa) consists of blood serum prostate specific antigen (PSA) test. A PSA level >4 ng/mL can be a sign of organ-confined PCa, and prostatic biopsy is recommended, even though 60-75% of PSA-driven biopsies are unnecessary and in 10% of cases, aggressive low-volume tumors may not be detected. In this frame, new non-invasive methods for early detection of primary PCa are needed. We aimed at performing a molecular characterization of PCa to identify novel biomarkers detectable in liquid biopsies, including fusion transcripts and circular RNAs (circRNAs), possibly representing specific signatures of the disease.

Methods. We collected 270 specimens of fusion-guided prostate biopsies. Samples were homogenized using a Tissue Lyser and total RNA extraction was performed. The presence of the TMPRSS2-ERG translocation was tested by RT-PCR assays and confirmed by Sanger sequencing. A targeted RNA-seq experiment was performed on 9 samples using the TruSight RNA PanCancer Panel (Illumina) able to provide a comprehensive analysis of the cancer transcriptome detecting, in a single experiment: single nucleotide variants, gene expression levels and fusion transcripts. Prostate-expressed circRNAs were tested both in biopsy specimens and in seminal plasma by RT-PCR assays using divergent primers.

Results. The TMPRSS2-ERG translocation, the most frequent rearrangement in PCa, was detected in 20% of analyzed samples (86), corresponding to 24% of patients positive for PCa. This frequency is lower than the expected 40%-50%. This is due to the heterogeneity of PCa: the lower the number of cells expressing the translocation in a single sample, the more difficult it is to detect the translocation. All biopsies carrying the translocation were positive for PCa, although in some cases (40% of confirmed positive samples) the companion (closest position) biopsy analyzed at histopathology showed only hyperplasia. The targeted RNA-seq experiment identified other translocations in addition to TMPRSS2-ERG (identified in one sample), such as SLC45A3-ERG and FOXA1-ETV1. SLC45A3 is an already known androgen-responsive partner and FOXA1 is a frequently mutated gene in PCa. Concerning circRNAs, we demonstrated the feasibility to detect specific circRNAs in seminal plasma, suggesting their use as biomarkers of the disease.

Conclusions. We previously showed the possibility to detect the TMPRSS2-ERG fusion transcript in the sperm of men with PCa. Under such light our preliminary results confirm and support the possibility of using RNA-based molecular biomarkers for the detection of PCa, with the final aim to reduce the number of unnecessary invasive diagnostic procedures. The presence of fusion transcripts, together with the mutational and gene expression profile of biopsies, will be in future correlated with clinical outcomes as well as radiomic features extracted from multi-parametric magnetic resonance imaging.

POSTER	**C13**
NAME	**Pierluigi Di Chiaro**
Course/Year	PhD – 2nd year
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Molecular characterization of cellular heterogeneity in pancreatic adenocarcinoma by transcriptomic profiling

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Background. Pancreatic ductal adenocarcinoma (PDAC) is a lethal disease with an extremely aggressive behavior due to both the advanced stage of the disease at diagnosis and the peculiar biology of this tumor. PDAC is characterized by foci of poorly differentiated cell nests (high-grade) frequently coexisting with well- or moderately differentiated glandular structures (low grade). This histological intratumor variability reflects distinct gene-regulatory networks and transcriptional outputs and might largely account for the clinical properties of this tumor, including resistance to the therapeutic treatments. The aim of this project is to obtain a mechanistic understanding of cellular heterogeneity in human PDACs.

Methods. We used immunohistochemistry and immunofluorescence techniques to morphologically identify low- and high-grade tumor areas from individual human FFPE PDAC samples of a cohort of patients. Due to lack of reliable techniques to perform transcriptional profiling in intact tissues, we performed multiplexed single molecule fluorescence in situ hybridization for the detection and quantification of a panel of spatial distributed mRNA transcripts in their native environment with a single cell resolution.

Results. Our experimental approach showed high efficiency and accuracy in the detection of mRNA targets in complex tissues like human FFPE PDAC samples preserving RNA integrity. We chose candidate targets characterized by the selectively expression in primary PDACs and cell lines of different grades as shown in previously published RNA-seq transcriptomic analysis. Therefore, we displayed that low- or high-grade tumor areas are transcriptionally distinct regions in a context of human PDAC specimens.

Conclusions. Our first analysis provides an initial approach to study transcriptional differences between high- and low-grade tumor areas. We will improve this experimental strategy for the analysis of few hundred of mRNA targets and we will apply it to dissect the cell-type- and spatial-domain-associated heterogeneity in an independent cohort of PDAC patients.

POSTER	**C14**
NAME	**Andrea David Re Cecconi**
Course/Year	Dott. – 3rd year
Institution	Istituto di Ricerche Farmacologiche IRCCS

Musclin, a myokine induced by aerobic exercise, retards muscle atrophy during cancer cachexia

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Background. Physical activity ameliorates the prognosis of cancer patients, also by contrasting the associated muscle wasting (i.e. cachexia). Since aerobic exercise seems to be the most effective to preserve muscles during cancer, we asked whether it promotes secretion of proteins by muscles (i.e. myokines) that may contrast cachexia.

Methods. To mimic aerobic exercise, we infected C2C12 myotubes with PGC1α expressing adenoviruses. In vitro we evaluated the effects of supernatants from GFP or PGC1α-overexpressing cells on protein synthesis and degradation of atrophying myotubes and in LucAssay. By microarray, we identified putatively secreted proteins inducible by PGC1α and confirmed by Q-PCR. We measured by Q-PCR their expression in Tibialis Anterior (TA) muscle of C26-bearing mice during cachexia and plasma levels by ELISA. To induce aerobic exercise adaptations, mice were run on treadmill. Anaerobic exercise-like effects were obtained in vivo in overloaded Plantaris muscle and in vitro in myotubes expressing myristoylated AKT.

Results. Our microarray and Q-PCR analyses showed musclin as a PGC1α-induced myokine. Conversely, its expression was unchanged in myotubes hypertrophying because of activated AKT. Dexamethazone-treated myotubes or constitutively active (ca)FoxO3-expressing ones undergo atrophy as measured by increased proteolysis and MuRF1 induction. Unlike to GFP, musclin restrained the dexamethazone-induced MuRF1 expression in Luciferase assays. Consistently, musclin-containing supernatants reduced the caFoxO3-induced rates of long-lived protein degradation. Among the newly identified PGC1α-induced myokines, we found that only musclin (and its receptor Npr3) was strongly downregulated in cachectic muscles and plasma of C26-bearing mice. Thus, we electroporated TA of C26-bearing mice with musclin or Npr3-encoding plasmids and found either musclin or Npr3 to preserve fiber area. Interestingly, treadmill exercise protected C26-bearing mice from muscle loss, with no effect on tumor growth, and rescued the C26-induced downregulation of musclin in muscles and plasma. By contrast, musclin expression did not change in overloaded Plantaris of adult mice.

Conclusions. Musclin is a myokine induced specifically by PGC1α, typically increased upon aerobic exercise and preserves muscles from wasting during C26 growth or myotubes from atrophy. Overall, musclin could be beneficial to cancer patients that cannot exercise and are at risk of developing cachexia.

POSTER	**C15**
NAME	**Sara Ballabio**
Course/Year	SRB – 2nd year
Institution	Istituto di Ricerche Farmacologiche IRCCS

Deciphering the mechanism of resistance to trabectedin in a model of myxoid liposarcoma by NGS approach

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Background. Myxoid/round cell liposarcoma (MRCL) is a rare tumor representing 30-35% of all liposarcomas. The molecular hallmark of MRCL is the presence of the FUS-DDIT3 chimera gene which drives malignant transformation by blocking adipocyte differentiation. Trabectedin, a DNA minor groove-binding agent with a complex mechanism of action, has demonstrated a remarkable antitumor activity against MRCL although most patients, after remission, become progressively resistant to the drug. To decipher the mechanisms leading to trabectedin resistance, we have developed a patient-derived xenograft (PDX) model resistant to trabectedin and studied its genomic landscape in comparison to its parental sensitive counterpart.

Methods. Patient-derived xenograft model ML017/ET resistant to trabectedin has been developed from the ML017 sensitive PDX model by prolonged in vivo treatments. Next generation sequencing (NGS) experiments were performed using the OneSeq Constitutional Panel (Agilent Technologies) to determine structural variants and mutations of almost 6000 disease-related genes. Analysis was performed on samples at baseline and after different trabectedin administrations (24 and 72 hours after the first dose and 15 days after the third dose of trabectedin). Matched healthy tissue was used as control. Orthogonal validation was performed by digital droplet PCR approach (ddPCR).

Results. Copy number variation (CNVs) and single nucleotide variant (SNVs) profiles are in line with databases reported for the MRCL's literature. No pathogenic germline variants are detected. Detailed analysis reveals gain in 2q31.1 region in treated ML017 samples compared to control. Differently from the parental sensitive counterpart, ML017/ET is characterized by loss in 4p16 region. Further investigations of genes mapped in this region reveals that the UVSSA gene, a component of the Transcription-Coupled Nucleotide Excision Repair (TC-NER), harbors both a CNV and a SNV in the ML017/ET tumors only. The CNV is a heterozygous deletion and the SNV (c. 256_261del) is predicted to impact on protein function with an allelic fraction of 100%. qRT-PCR and Western Blot experiments confirm the lack of expression of UVSSA gene. To note, loss of function of this gene is observed also during induction of resistance.

Conclusions. In our MRCL PDX model, the induction of resistance to trabectedin didn't modify the genome architecture. The loss of UVSSA gene, which interacts with the TC-NER factors (as RNA pol II, CSA, CSB and TFIIH), might result in destabilization and in impaired recruitment of the TC-NER complex. NER-deficient tumor cells could be resistant to trabectedin because they are unable to recognize and process the DNA damage induced by the drug and, therefore, able to survive. We suggest that, probably, evaluation of the status of NER factors in clinical samples of myxoid liposarcoma might help guiding the choice of chemotherapeutic agents.

POSTER	**C16**
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Course/Year	PhD – 2nd year
Institution	Fondazione IRCCS Istituto Nazionale dei Tumori - Milano

Detection and clinical relevance of circulating tumor DNA in patients with non invasive and invasive breast cancer

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Background. Breast cancer (BC) treatment should optimally be reserved to patients (pts) who eventually relapse and within the earliest possible timeframe. Here, we investigated the value of circulating tumor DNA (ctDNA) as a timely and repeatable biomarker for the clinical management of pts with non invasive, or invasive non metastatic BC.

Methods. The non invasive BC pt cohort was composed of 20 cases with ductal carcinoma in situ (DCIS); the invasive BC pt cohort was composed of 12 cases receiving neoadjuvant chemotherapy, surgery and post-surgical follow up (FU). Mutation analysis of BC tissues was performed by targeted next generation sequencing, and the identified Single Nucleotide Variations (SNVs) were first validated and then tracked in serial plasma samples using ad hoc digital polymerase chain reaction assays.

Results. One or more SNVs were identified in primary tumor tissue specimens and validated in 9/18 (50%) cases of DCIS at the time of initial diagnosis. Recurrent mutated genes were PI3KCA and TP53 (two cases each). At the reporting census date (May 15th, 2019), ctDNA was analyzed in 6/9 cases. Among 4 pts with detectable levels, 2 pts relapsed locally with invasive (1 pt) and in situ followed by invasive (1 pt) BC after 1.6 and 1 year, respectively. Notably, ctDNA levels at the time of relapse resembled those of initial diagnosis. In parallel with these analyses, additional 12 pts with invasive triple negative breast cancer were analyzed with respect to ctDNA prior, during, and after neoadiuvant chemotherapy (NAC). ctDNA was detectable in 81% of cases at baseline, and disappeared after NAC and surgery in 83% of cases. Among pts who eventually relapsed , ctDNA anticipated overt metastases with a lead time up to 13 months.

Conclusions. Although additional analyses and longer follow-up are necessary, it is a matter of fact that ctDNA may be detected in pts diagnosed with DCIS both at the initial diagnosis and at the time of non- and/or invasive recurrences. The clinical value of ctDNA is more clear in TNBC cases where earlier detection of relapse provides a possible window for therapeutic intervention.

POSTER	**N1**
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Course/Year	PhD – 1st year
Institution	Istituto di Ricerche Farmacologiche Mario Negri IRCCS

Specific contribution of mannose-binding lectin murine isoforms to brain ischemia/reperfusion injury

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Background. Mannose-binding lectin (MBL), an initiator of the lectin pathway of complement activation, is detrimental in ischemic stroke, as shown by clinical studies and rodent models. While humans have one functional MBL, rodents have two isoforms, MBL-A and MBL-C, whose function compared to human MBL is unknown. To foster the clinical transferability of pre-clinical data, we aimed at defining the specific contributions of MBL-A and MBL-C to brain ischemia.

Methods. We used double (MBL-/-) and single MBL isoform (MBL-A-/- or MBL-C-/-) knock-out mice subjected to transient (60 min) occlusion of the middle cerebral artery (tMCAo) to induce focal cerebral ischemia. At 48h after the induction of ischemia, we evaluated behavioral deficits by composite neuroscore, ischemic volume and neuronal viability on cresyl violet stained sections, hepatic Mbl1 and Mbl2 gene expression (coding for MBL-A and MBL-C respectively) by real-time PCR, MBL isoform plasma and brain levels by ELISA, complement system activation by C3 deposition, lectin pathway activity by functional assay.

Results. MBL-/- mice had less neurological deficits and smaller ischemic lesions than wild-type (WT). MBL-A-/- had a smaller lesion, with no significant behavioral effects, while MBL-C-/- did not differ from WT. The induction of Mbl1 and Mbl2 (MBL-A and -C genes) 48h after tMCAo was similar across genotypes. The time course of Mbl1 and Mbl2 expression in WT ischemic mice showed earlier Mbl1 (24h) than Mbl2 (48h). The plasmatic presence of MBL-A and -C in MBL-C-/- and MBL-A-/- was similar to WT either at baseline or at 48h after tMCAo. At 48h MBL-A-/- ischemic mice showed increased MBL-C in the brain than WT. WT and MBL-C-/- ischemic mice had higher lectin pathway activity in plasma and, accordingly, higher C3 brain deposition than MBL-A-/- and MBL-/-.

Conclusions. Depletion of a single MBL isoform induced low-level (MBL-A-/-) or negligible (MBL-C-/-) neuroprotection from ischemic injury compared with that achieved when both isoforms were depleted. Among the two isoforms, MBL-A appears as the main participating in the development and evolution of ischemic brain injury, but pathophysiological roles for both MBL isoforms should be considered.

POSTER	**N2**
NAME	**Domenico Mercurio**
Course/Year	SRB – 2nd year
Institution	Istituto di Ricerche Farmacologiche Mario Negri IRCCS

Mannan-binding lectin-associated serine protease-2 (MASP-2) deletion is associated with better outcome in a mouse model of traumatic brain injury

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Background. Lectin pathway (LP) of complement activation is a key mechanism behind tissue inflammation after traumatic brain injury (TBI). Studies in TBI patients have reported that an increase in circulating mannan-binding lectin-associated serine protease-2 (MASP-2), the essential enzyme driving LP activation, and its presence in the brain, is associated with increased TBI severity. This work defines the neuropathological and functional responses of traumatized mice with a gene-targeted disruption of the MASP-2 gene (MASP-2 KO) to get a further insight in its role in TBI.

Methods. WT or MASP-2 KO male C57BL/6J mice underwent sham surgery or TBI by controlled cortical impact (velocity= 5 meters/sec, depth= 1 mm). The sensorimotor response was evaluated by neuroscore and beam-walk test on a weekly basis for 4 weeks. Brains were harvested 6 weeks after injury for histopathological analysis. A group of mice was sacrificed 30 minutes post-TBI to evaluate mannose-binding lectin (MBL) presence in the brain.

Results. MASP-2 deficient TBI mice showed reduced sensorimotor deficits (by 33% at 3 weeks and by 36% at 4 weeks, beam-walk test) compared to WT mice. At 6 weeks after TBI, MASP-2 KO mice retained higher neuronal density in the ipsilateral cortex with a 13% increase compared to WT mice. Lesion volume and inflammation markers such as glial fibrillary acidic protein (GFAP) and CD11b were not different. MBL deposition in brains of MASP-2 KO mice was similar to that in WT when assessed at 30 minutes post-TBI, a time point where significant MBL deposition is seen in the lesioned brain.

Conclusions. This study demonstrates that the absence of MASP-2 or MASP-2 functional activity is neuroprotective after TBI, ameliorating the sensorimotor performance and limiting neuronal loss.

POSTER	**N3**
NAME	**Giada Lavigna**
Course/Year	SRB – 2nd year
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Doxycycline treatment in transgenic fatal familial insomnia mice

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Background. Fatal familial insomnia (FFI) is a genetic prion disease linked to the D178N/M129 mutation in the PRNP gene encoding the cellular prion protein (PrP^C). Symptoms, including insomnia, autonomic dysfunction, motor abnormalities and memory loss, appear in the 5th decade of life, leading to death within two years from onset. No treatment is available. PRNP mutations promote PrP^C conversion into an infectious isoform (PrP^{Sc} or prion) which propagates by imprinting its abnormal conformation onto PrP^C molecules. Doxycycline favors PrP^{Sc} degradation and extends survival of prion-infected hamsters. We tested the therapeutic potential of doxycycline in a transgenic (Tg) mouse model of FFI.

Methods. We used Tg(FFI-26) mice, expressing the mouse PrP homologue of the D178N/M129 mutation (moPrP D177N/M128) at two-fold the physiological level. These mice develop a fatal neurological illness with clinical and neuropathological abnormalities reminiscent of FFI. Groups of Tg(FFI-26) mice and non-Tg controls were treated daily with doxycycline (10 mg/kg) or the vehicle (saline) via intraperitoneal (i.p.) injection. Onset and progression of motor dysfunction was assessed by the beam walking and rotarod tests. The novel object recognition task was used to assess the effect of the treatment on memory deficits.

Results. In a first experiment mice were treated starting from a symptomatic stage (120-160 days of age) for three months. Chronic treatment with 10 mg/ml doxycycline was well tolerated, with no detectable adverse effects. However, no differences in progression of motor dysfunction were found between doxycycline- and vehicle-treated Tg(FFI-26) mice. In a second experiment Tg(FFI-26) mice were treated starting from 30-60 days of age, when there was no significant difference in motor performance compared with the Non-Tg controls. This experiment is still ongoing. Mice are assessed every 15 days by the beam walking and rotarod tests, and will be tested by the novel object recognition task after one and three months of treatment.

Conclusions. Doxycycline had no significant effect on the progression of disease in Tg(FFI) mice when administered in an advanced stage of disease, in line with two double blind clinical trials which did not show beneficial effects in symptomatic prion disease patients. A preventive treatment with doxycycline in currently ongoing in asymptomatic carriers of the FFI mutation. It will be interesting to see the results of the presymtomatic doxycycline treatment in Tg(FFI-26) mice, and if it will be predictive of the outcome in humans.

POSTER	**N4**
NAME	**Gloria Vegliante**
Course/Year	PhD – 1st year
Institution	Istituto di Ricerche Farmacologiche Mario Negri IRCCS

Old age worsens neurological outcomes following traumatic brain injury and is associated with an exaggerated astroglial response in mice.

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Background. Traumatic brain injury (TBI) shows a second peak of incidence in the elderly and is associated with worse outcome. The mechanisms underpinning the higher susceptibility to progressive neurological deterioration and neurodegeneration are uncertain. We assessed the susceptibility of adult and aged mice to different degrees of TBI, by analysing functional outcome, structural damage and inflammatory response to understand whether an exacerbated aged dependent inflammatory activation may contribute to brain dysfunction and white matter damage in the elderly.

Methods. Adult (8 weeks old) and aged (18 months old) mice were subjected to sham, moderate (m) or severe (s) TBI by controlled cortical impact. Sensorimotor deficits were longitudinally assessed weekly by neuroscore and Simple Neuroassessment of Asymmetric imPairment (SNAP) up to 6 weeks post-TBI. Magnetic resonance imaging (MRI) images were acquired on a 7T small-bore animal scanner (Bruker Biospec). Contusion volume was measured using T2-weighted MRI, while white-matter integrity was evaluated by diffusion tensor imaging. Histopathology was assessed at 6 weeks by cresyl violet (neuronal viability staining), GFAP (astrocytes marker), Iba1 (microglial marker).

Results. TBI aged mice showed greater sensorimotor deficits than adult mice, with mTBI in the elderly producing a degree of functional impairment similar to that observed in adult sTBI mice. We found a high correlation between SNAP and contusion volume in adult and aged mice at 1 week. Functional deficits at 6 weeks only correlated with contusion volume in aged mice, suggesting a higher recovery potential in the adult. TBI induced loss of white matter integrity highlighted by a reduction of fractional anisotropy with no age-related differences. In contrast, an increased axial and radial diffusivity was observed in aged compared to adult sTBI mice. Histopathology showed similar neuronal damage in the pericontusional cortex across ages and an increased neuroinflammatory response. In the ealderly, increased microgliosis was observed exclusively in the lesioned cortex while astrogliopathy was widespread involving white matter and contralateral brain structures.

Conclusions. Worse post-traumatic behavioral outcomes in aged animals were associated with exaggerated microglial and astroglial responses, with the latter showing a more persistent and widespread activation in the aged.

POSTER	**N5**
NAME	**Carmina Natale**
Course/Year	SRB – 2nd year
Institution	Istituto di Ricerche Farmacologiche Mario Negri IRCCS

A new cellular system to uncover the prion-like properties of TauP301L.

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Background. Tauopathies are a family of neurodegenerative diseases defined by the accumulation of fibrillar deposits of the microtubule-associated protein Tau. One hypothesis for the progression of Tauopathies is a prion like mechanism, where Tau aggregates in one cell spread to another to trigger aggregation of previously soluble Tau. The mechanisms underlying this process are still largely unknown. We characterized a novel cell-based system to study Tau spreading, using the controlled expression of a mutated form of Tau associated with Frontotemporal dementia, TauP301L. Specifically, we studied the different propensity of aggregated and not aggregated forms of Tau P301L to spread and induce toxicity.

Methods. In our studies we used HEK T-REx cells in which TauP301L expression is regulated by a Tet ON system where the gene can be induced by doxycicline (1µg/mL). To study the spreading of Tau, recombinant human TauP301L (rhTauP301L) was purified from E. coli and its aggregation in vitro was induced with heparin. Cells were treated with aggregated or monomeric rhTauP301L and six days after treatment cells were either harvested, lysed and subjected to a detergent insolubility paradigm, or analyzed by confocal imaging. To study toxicity induced by rhTau P301L, cells were treated with recombinant proteins and survival assay were performed.

Results. We induced formation of high molecular assemblies by incubating rhTauP301L with Heparin, and confirmed the insoluble nature of the oligomeric assemblies of rhTauP301L by performing detergent insolubility assay. Next, we treated HEK T-REx cells expressing TauP301L with monomeric or oligomeric rhTauP301L and found that both monomeric and oligomeric rhTauP301L can enter the cells. We also monitored aggregation of endogenous TauP301L by detergent insolubility assays. Differently from control cells and cells treated with monomeric rhTauP301L, treatment with oligomeric rhTauP301L induced a transition from a soluble state to an aggregated and insoluble one of endogenous Tau, thus mimicking the seeding effect occurring during the spreading of aggregates in animal models of Tauopathies. Additionally we performed viability assay (MTT reduction) on cells exposed to rhTauP301L and we found that Tau oligomers but not monomers reduced the viability of cells, therefore showing a selective cell toxicity of oligomeric rhTauP301L.

Conclusions. We created a reproducible and easy to use cell system to study the prion-like properties of TauP301L. We showed that exogenous rhTauP301L, either in monomeric or oligomeric form, can be uptaken by recipient cells and, once inside the cells, oligomeric TauP301L, but not monomeric, can trigger aggregation of endogenous TauP301L and induce toxicity. In future studies we plan to use this system to uncover how the process of uptake and seeding is regulated.

POSTER	**N6**
NAME	**Silvia Luotti**
Course/Year	SRB – 2nd year
Institution	Istituto di Ricerche Farmacologiche Mario Negri IRCCS

Validation study of PBMC protein biomarkers for amyotrophic lateral sclerosis

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Background. Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disorder that selectively affects motor neurons, causing gradually weakness of all voluntary muscles. There are currently no validated biomarkers for ALS, due to its clinically, genetically and neuropathologically heterogeneous characteristics, but they would be useful for early diagnosis, monitor disease progression and evaluate the efficacy of new treatments. Peripheral blood mononuclear cells (PBMCs) display pathological features mirroring those occurring in the central nervous system. In this study, we verified changes in a panel of protein biomarkers, identified in previous studies, in PBMCs samples of a large cohort of ALS patients and controls.

Methods. The study was done on totally 305 PBMC samples from ALS patients (n=90), healthy subjects (n=104), and patients with other neurological/neuromuscular diseases (n=111), stratified in diseases affecting the central nervous system (CNS) (n=63) or muscle/nerve (Periphery) (n=48). PBMC samples were subjected to a two-step sequential protein extraction that implied low and high % of detergents, generating a soluble and an insoluble fraction. We next analysed by a slot blot immunoassay the levels of cyclophillinA (CypA), heat shock cognate protein 71kDa (HSC70), TAR DNA-binding protein 43 (TDP-43), heterogeneous nuclear ribonucleoprotein A2/B1(hnRNPA2/B1) and superoxide dismutase 1 (SOD1) in both soluble and insoluble fractions.

Results. Soluble CypA and HSC70 levels in PBMCs of ALS patients were respectively significantly lower and higher than Healthy controls. Protein levels of CypA and hnRNPA2/B1 were significantly lower respect to CNS and Periphery controls, while TDP-43 and SOD1 were lower respectively versus CNS and periphery controls. Insoluble CypA, TDP-43 and hnRNPA2/B1 protein levels were higher in ALS patients than in Healthy controls. Insoluble TDP-43 was also significantly lower in ALS than in CNS and Periphery controls. We also defined an index of protein insolubility as the ratio of the amount of protein in PBMC-insoluble to PBMC-soluble fraction. We observed that it was significantly higher in ALS patients than in Healthy and Periphery controls. Interesting, the combination of some of these protein biomarkers distinguishes, with high discriminatory power, ALS patients from controls (AUC = 0.92).

POSTER	**N7**
NAME	**Rossella Di Sapia**
Course/Year	PhD – 1st year
Institution	Istituto di Ricerche Farmacologiche Mario Negri IRCCS

Glia activation, neuroinflammation and oxidative stress are concomitant pathologic events in an infantile rat model of epileptic encephalopathy

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Background. Paediatric status epilepticus (SE) results from acquired, metabolic, immune, genetic or unknown causes. The mechanisms of pathology are unclear and effective treatments are lacking for children with severe seizures triggered without an identifiable cause. There is urgent need of animal models to study the pathologic mechanisms induced by unremitting seizures in the immature brain that lead to cognitive deficits and epilepsy. Since neuroinflammation and oxidative stress are ignited by activated glial cells and may contribute to disease development, we investigated this brain response in an infant rat model of de novo SE and epileptogenesis.

Methods. Postnatal day (P)13 rat male pups were electrode implanted for EEG analysis and injected intra-amygdala with 2μg kainic acid to evoke SE lasting for about 4 h. Immunohistochemical analysis of forebrain was used to determine the cellular origin of neuroinflammation in rats killed 6 h-1 week post-SE (n=6/group). Markers of neuroinflammation and oxidative stress were measured in hippocampus of SE-exposed rats by RT-qPCR. A cohort of SE-exposed rats (n= 10) was longitudinally video-EEG monitored and exposed to MRI. Control animals were similarly injected with saline (n=5-8).

Results. The activation of astrocytes and microglia, together with the induction of the ictogenic molecules IL-1β and HMGB1 and the oxidative stress marker Nrf2, occurred during one week post-SE. Degenerating neurons were detected 72 h post-SE. Spontaneous seizures (i.e., epilepsy) developed in 60% of P65-old rats. MRI analysis showed a significant reduction in cortical thickness which was often associated with reactive gliosis and occurred before epilepsy onset. Rats displayed impairment in the Morris Water Maze.

Conclusions. This rat model reproduces salient features of the encephalopathic effects of seizures in early life. It highlights the potential role of reactive gliosis in disease development and can be used for further mechanistic studies, to test novel drugs and for developing biomarkers of disease progression.

POSTER	**N8**
NAME	**Susanna Pucci**
Course/Year	PhD – 2nd year
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Neuronal nicotinic acetylcholine receptors as new targets for glioblastoma therapy

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Background. Non-neural tumours express neuronal nicotinic acetylcholine receptors, whose α7 and/or α9-containing receptors play a role in tumour angiogenesis, growth and metastasis formation. Human glioblastoma U87MG cells and primary cell lines derived from patients express mRNAs for α7 and α9 subunits and nicotine activation of receptors containing these subunits increases cell proliferation, which is selectively blocked by α7/α9 antagonists.

Methods. We counted manually U87MG cells to determine their proliferation rate. Cell viability assay was based on the reduction of water-soluble MTS tetrazolium salt by viable cells. The affinity of the compounds was determined by means of binding studies using the α7/α9-specific ligand αBungarotoxin on SH-SY5Y cells transfected with the human α7 subunit, while their potency was assessed through an electrophysiological assay on Xenopus laevis oocytes heterologously transfected with human neuronal nicotinic acetylcholine receptor subtypes. ATP concentrations were determined using a luciferin-luciferase colorimetric assay.

Results. The triethylammoniumethyl ether of 4-stilbenol MG624, an α7,α9-neuronal nicotinic acetylcholine receptor antagonist, has antiproliferative activity on U87MG cells. The structural analogy of MG624 with the mitocan RDM-4'BTPI, a triphenylphosphoniumbutyl ether of pterostilbene that is more potent than MG624 in reducing U87MG cell viability, although it does not possess nicotinic activity, suggested that molecular hybridisation might result in antitumor agents with higher potency and neuronal nicotinic acetylcholine receptor selectivity. We found that the replacement of MG624 ethylene with butylene resulted in more potent and selective glioblastoma toxicity, which was paralleled by increased α7,α9-neuronal nicotinic acetylcholine receptor antagonism and decreased mitochondrial ATP production. Further elongation of the alkylene linker enhanced neuronal nicotinic acetylcholine receptor selectivity and glioblastoma cell viability reduction. Moreover, MG624 and its elongated derivatives decrease U87MG cell proliferation, which was only partially restored by co-incubation with selective α7/α9 antagonists, revealing that they may also have non-nicotinic-mediated effects.

Conclusions. Overall, we enhanced the effectiveness of MG624 towards glioblastoma cells by lengthening its alkylene linker, resulting in more α7,α9-selective derivatives. We are now in vivo testing α7,α9 selective antagonists in mice xenografted with U87MG cells and in mice xenografted with α7/α9-knockout U87MG cells to determine whether neuronal nicotinic acetylcholine receptors represent targets for glioblastoma therapy.

POSTER	**N9**
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Course/Year	PhD – 3rd year
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Inflammation and synaptogenesis: The role of Interleukin-6 in developing neurons

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Background. The brain developmental follows a precise genetic program that ensures the establishment of a functional network system in adulthood. However, this process is influenced by several environmental insults, including inflammation. An increase in the amount of cytokines upon an inflammatory challenge during pregnancy, the so called Maternal Immune Activation, can lead to deleterious effects on this process. The pro-inflammatory cytokine interleukin 6 was found to be central in Maternal Immune Activation, although its precise role in neuronal development is still unknown. The aim of this work is to understand the molecular mechanisms by which Interleukin 6 impacts on the formation of synapses.

Methods. We used both in vivo and in vitro models. Our in vivo model of Maternal Immune Activation was based on a single intraperitoneal injection of Interleukin 6 in pregnant mice at gestational day 15. The in vitro model was primary hippocampal culture stimulated with different concentration of interleukin 6 in specific time windows. We used a multidisciplinary approach based on functional and morphological techniques like confocal imaging, patch clamp, western blot, real time PCR, single cell RNA sequencing and connectomic analysis in order to verify the long-lasting effects of Interleukin 6 on synaptic formation and maturation.

Results. A single injection of Interleukin-6 in pregnant mice at gestational day 15 induced an increase of excitatory synapse density in pyramidal hippocampal neurons in the offspring at post-natal days 15 and 30. This effect was accompanied by an increased functional connectivity at hippocampal level at adult stages and was due to a direct action of Interleukin-6 on brain embryos. The increase of excitatory synapses was recapitulated in primary hippocampal cultures through a single application of Interleukin-6 at early presynaptic stages, 1-5 days in vitro. We also identified the critical role of the transcription factor STAT-3 in the IL-6-dependent increase of glutamatergic synapses. Eventually, using single-cell sequencing we found the neuronal-specific gene RGS4 as a possible downstream gene of STAT-3 whose activity was required for the pro-synaptogenic effect of Interleukin-6. We validate the involvement of RGS4 taking advantage of its inhibitor together with interleukin 6 to prevent the increase of glutamatergic transmission.

Conclusions. This work highlights the effects of Interleukin 6 in brain development and unravels a functional role of the cytokine in the glutamatergic synaptogenesis both in vivo and in vitro through the engagement of STAT-3 activity via RGS4-mediated signaling. Our work sheds light on the critical role played by Interleukin 6 in shaping the early phases of brain development with long-lasting consequences on hippocampal transmission.

POSTER	**I1**
NAME	**Giulia Vandoni**
Course/Year	PhD – 1st year
Institution	Fondazione IRCCS Istituto Nazionale dei Tumori

Role of diet and gut microbiome on clinical outcomes in patients with advanced melanoma undergoing immunotherapy

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Background. Many studies have highlighted the correlations between diet and gut microbiome: diet regulates the composition of microbiome, which transduces nutrient signals to the host favouring homeostasis. Interestingly, a role for the gut microbiota has been recently described in the efficacy of immunotherapy due to its immunomodulatory properties. One of the major links between diet and gut microbiome is the shaping of the innate and adaptive immunity. Therefore, we hypothesize that a correct diet may improve the efficacy of immunotherapy by maintaining a balanced and biodiverse gut microbiome profile and a favourable immune status.

Methods. All patients with advanced melanoma treated with ANTI-PD-1 will be prospectively enrolled. The following elements will be analysed and compared in responder and non responder patients: usual diet collected with EPIC FFQ and a 3-day food diary; gut microbiota composition evaluated by 16S rRNA gene sequencing and microbiome profile using shotgun metagenomics sequencing; immune profile by multiparametic flow cytometry; clinical benefit in terms of response rate according to RECIST 1.1 and 3-4° immune-related adverse events as determined by CTCAE 4.3. Possible confounding factors will be also considered. Significant differences and correlations among variables will be assessed by appropriate statistical tests.

Results. We expect to find out useful results that will help to better understand the complex relation among diet, gut microbiome, immunity, and clinical outcome in advanced melanoma undergoing immunotherapy. Preliminary results are needed to plan future diet-specific intervention studies that could be undertaken in different and numerous types of cancer patients. More specifically, in patients with advanced melanoma with an objective response rate to immunotherapy, we expect to find a positive relationship between fibre intake and gut microbiota alpha-diversity. In these patients, a high alpha-diversity is more likely to be present from the diagnosis onwards and related to a various diet with adequate intake of dietary fibre. The maintenance of a various diet and an adequate dietary fibre intake during treatment may help in preserving an eubiotic state of gut microbiome and improving clinical outcome.

Conclusions. These preliminary findings will enable us to better understand the role of diet and gut microbiome in modulating response to immunotherapy. The main challenge today is to fully utilize the potential of immunotherapy treatment in order to limit the immune-related events and toxicity,to obtain the objective response rate and to have long-standing effectiveness of treatment. The development of diet-specific strategies able to modulate gut microbiome and to improve clinical outcome in patients undergoing immunotherapy is extremely challenging. Dietary intervention could be a simple and safe opportunity in order to obtain the best clinical benefits from drugs, while minimizing side effects.

POSTER	**I2**
NAME	**Celeste Rizzello**
Course/Year	PhD – 2nd year
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Osteopontin protects from autoimmunity-driven DLBCL lymphomagenesis

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Background. Autoimmune diseases have been associated with increased risk of lymphoid malignancies, particularly Non-Hodgkin's Lymphomas, yet the mechanisms driving the evolution from autoimmunity towards lymphomagenesis remain elusive. We have previously demonstrated that a defective stromal remodeling in the secondary lymphoid organs (SLO) due to the absence of the matricellular protein SPARC, favors the transition from systemic lupus erythematosus (SLE) to a CD5 B cell malignancy in the autoimmunity-prone Faslpr/lpr mouse model. Another matricellular protein, osteopontin (OPN) has been associated with SLE pathogenesis, as SLE patients are characterized by increased serum levels of OPN and, in some cases, polymorphisms in the OPN gene, Spp1.

Methods. To test the role of OPN in autoimmunity-driven lymphomagenesis, the Faslpr/lpr mutation has been transferred onto a OPN-deficient background. The development of signs of autoimmunity was analysed by measuring lymph node swelling. Cellular composition within SLO and lymphomatous B cells were characterized by flow cytometry analysis. Histopathological analysis was performed to characterize SLO architecture and confirm lymphoma development. Gene expression profile (GEP) of CD19 B cells sorted from mouse spleens was performed to assess potential difference in molecular patterns between Faslpr/lpr and OPN-/-Faslpr/lpr transforming B cells.

Results. OPN-/-Faslpr/lpr mice showed an accelerated autoimmunity and a higher percentage of development of lymphomas in comparison of Faslpr/lpr mice upon ageing (6-8 months of age). Flow cytometry analysis indicated an accumulation of a CD19 B-cell population with no IgM and variable B220 expression, with typical markers of the activated type of DLBCL (ABC-DLBCL): Bcl6-, Bcl2 , IRF4/MUM1 . Histopathological evaluation confirmed the phenotype of large-cell lymphoma with diffuse architecture and immunoblastic morphology. In addition, from a very preliminary analysis of GEP performed on CD19 B cells, it seems that lymphomas occurring in OPN-/-Faslpr/lpr mice derived from B cells in a post-activated state.

Conclusions. These results are suggestive of an unexpected, protective role of the matricellular protein OPN in DLBCL lymphomagenesis associated with deregulated immune responses.

POSTER	**I3**
NAME	**Adriana Salvaggio**
Course/Year	PhD – 1st year
Institution	Fondazione IRCCS Istituto Nazionale dei Tumori

Targeting tumor-associated macrophages in osteosarcoma: depletion versus re-direction

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Background. The pro-tumorigenic role of tumor-associate macrophages has been widely demonstrated in a variety of tumor types. Few data exist regarding the activity of tumor-associate macrophages and other immune cells in osteosarcoma. In immune competent osteosarcoma mouse models, we have recently demonstrated that trabectedin, a marine-derived chemotherapy, exerts a potent anti-tumor activity that is further enhanced in combination with anti-PD-1 antibody. Besides targeting neoplastic cells, trabectedin modifies the tumor immune environment recruiting T lymphocytes and, contrary to what expected from the literature, without depleting tumor-associate macrophages.

Methods. To investigate the role of tumor-associate macrophages in osteosarcoma we tested the growth capacity of osteosarcoma cells co-injected with macrophages previously differentiated in vitro toward classical M1 or M2 phenotype, or left undifferentiated (M0). Then, to further clarify the involvement of tumor-associate macrophages, mice bearing osteosarcoma tumors implanted on both flanks were treated, locally, in only one lesion, with either liposome-encapsulated clodronate to deplete tumor-associate macrophages, or with SD101, a synthetic oligonucleotide with immunostimulatory CpG motifs, to favor the M2 > M1 shift. The size of treated and untreated contralateral tumors was monitored.

Results. The co-injection experiment showed that in presence of M1 macrophages osteosarcoma growth was largely inhibited whereas unchanged in presence of M0 and M2 macrophages. These data suggest that tumor-associate macrophages, per se, do not influence osteosarcoma outgrowth and that tumor-associate macrophages re-direction toward M1-like phenotype could exert therapeutic activity. Accordingly, the clodronate treatment while reducing tumor-associate macrophages infiltration was almost ineffective in reducing the growth of treated and untreated tumors. Differently, SD101 administration halted osteosarcoma growth of both treated and contralateral untreated tumors. FACS analysis showed unaltered tumor-associate macrophages number after SD101 treatment, although the M2 marker CD206 was considerably reduced. Moreover, tumor infiltration by CD8 T cells was increased in both SD101 treated and untreated tumors.

Conclusions. Our results, although preliminary, support the hypothesis that tumor-associate macrophages re-direction toward the M1 phenotype could be therapeutically more effective than their direct depletion.

POSTER	**I4**
NAME	**Giovanna Talarico**
Course/Year	PhD – 2nd year
Institution	Fondazione IRCCS Istituto Nazionale dei Tumori

Extracellular matrix regulation of breast cancer immune microenvironment

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Background. Breast cancer (BC) is one of the worldwide leading causes in women. Besides cellular components of the tumor microenvironment (TME), also the extracellular matrix (ECM) has been shown to exert regulatory functions on both tumor and stromal cells. The crucial role of ECM in cancer progression is strengthened by its prognostic relevance such as in the case of high-grade (HG)BC, where the ECM-gene profile classification correlates with clinical outcome and therapy response. Since the recruitment of different immune suppressive cells is responsible for worse prognosis, we investigated whether zoledronic acid (ZA) can revert this phenotype in HGBC patients.

Methods. BALB/c mice were injected with different BC cell lines. Flow cytometry analysis (FACS) and immunohistochemistry (IHC) analysis were performed onto primary tumors. Peripheral blood samples were collected from 6 metastatic patients before and after (28 and 56-days) ZA administration. Samples were processed using conventional Ficoll separation. Peripheral Blood Mononuclear Cell (PBMC) was analyzed by FACS analysis for different immune cell populations. Through FACS sorting MDSC fraction was isolated and RT-PCR analysis performed for PD-L1 and STAT3.

Results. IHC analysis of tumors highlights the reduction of collagen (type I and IV) deposition in mice treated with ZA compared to untreated ones. In addition, we found that the expression of PD-1 is higher in mice treated with ZA compared to control. In situ immunostaining of Gr-1 markers showed that tumors treated with ZA exhibited more Gr-1 myeloid cell infiltration than untreated mice. We performed a semiquantitative PCR analysis of PD-L1, a suppressive marker, on myeloid cells isolated from tumors cells of ZA-treated mice. Results showed a significant down-modulation of PD-L1 in G-MDSC subsets. Instead, we found a trend of reduction in MDSC frequency in patients treated with ZA. FACS-sorted MDSC from ZA treated patients showed a decreased expression of key immune suppressive genes, including STAT3 and PD-L1.

Conclusions. Our results suggest that ECM profile can contribute to tumor progression and promote an immunosuppressive environment. Notably, ECM-related features can potentially impact on immunotherapy. Since we found that ZA is able to revert the immunosuppressive phenotype of MDSCs in mouse model of BC, the employment of ZA on human immune cells alone or in combination with immune checkpoint blockade (ICB) therapy can improve the efficacy of immunotherapy.

POSTER	**M1**
NAME	**Ignacio Fernando Hall**
Course/Year	PhD – 3rd year
Institution	Humanitas University

Circ_Lrp6, a circular RNA enriched in vascular smooth muscle cells, acts as a sponge regulating miRNA-145 function

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Background. Vascular smooth muscle cells (VSMCs) represent a fundamental component of vessels and possess the capacity to switch between a contractile (differentiated) and a synthetic (proliferative/migrating) phenotype. Non-coding RNAs (ncRNAs) are key players in the orchestration of this phenotypic transition. However, while the role of short ncRNAs such as miRNAs, has been extensively studied, the knowledge about the involvement of long ncRNA is still limited. Among these molecules, circular RNAs (circRNAs) represent a very interesting class. Therefore, the aim of this work is to investigate the VSMC epigenetic panorama regulated by circRNAs and their putative interactions with miRNAs.

Methods. RNA sequencing and bioinformatics identified VSMC-enriched circRNAs conserved between human and mouse. Screening for those bearing putative binding sites for VSMC-enriched miRNAs, we identified one conserved circRNA, circ_Lrp6. It was validated by RT-qPCR analysis, sequencing and biochemical assays. Then, we assessed circ_Lrp6 enrichment in vessels by RT-qPCR and RNA-FISH analysis. The cellular localization of circ_Lrp6 and miR-145 was determined by RNA-FISH and fractionation assays; the sponge-like activity was evaluated through luciferase reporter assays. To study circ_LRP6 impact on VSMC biology, we adopted a loss- or gain-of-function approach. Finally, we evaluated the circ_Lrp6/miRNA-145 axis in mouse and human injured vessels.

Results. Exploiting a bioinformatic pipeline, we identified only one conserved circRNA candidate bearing binding sites for miR-145. This circRNA candidate, named circ_Lrp6, was validated by qPCR analysis, Sanger sequencing and biochemical assays. To assess the role of circ_Lrp6, we performed reciprocal interaction studies with miR-145, unveiling their direct interaction. To further corroborate these results, we performed RNA-FISH experiments and super resolution microscopy acquisitions. Moreover, we studied circ_Lrp6 sponge-like activity on miR-145 through competitive luciferase assays. The functional inhibition of miR-145 was evaluated by measuring the expression of several target genes. Then, using loss- and gain-of-function approaches, we found that circ_Lrp6 buffers miR-145-mediated regulation of VSMCs. Differential expression of miR-145 and circ_Lrp6 in diseased specimens, from murine and human models, suggests that the ratio of circ_Lrp6 bound to miR-145 could play a role in vascular pathogenesis. Finally, viral delivery of circ_Lrp6 shRNA prevented intimal hyperplasia in mouse carotids undergoing perivascular collar placement

Conclusions. Thus, circ_Lrp6 is a novel intracellular modulator of miR-145, counterbalancing its biological function in VSMCs by acting as its natural sponge. These findings add another layer of complexity to the mechanism through which miRNAs are regulated in VSMCs, showing that ncRNA co-regulatory mechanisms play a fundamental role. Nonetheless, further studies are needed to define circRNA molecules role in vascular diseases. Since we found that ZA is able to revert the immunosuppressive phenotype of MDSCs in mouse model of BC, the employment of ZA on human immune cells alone or in combination with immune checkpoint blockade (ICB) therapy can improve the efficacy of immunotherapy.

POSTER	**M2**
NAME	**Daria De Giorgio**
Course/Year	SRB – 2nd year
Institution	Istituto di Ricerche Farmacologiche Mario Negri IRCCS

Effects of trabectedin and lurbinectedin in a doxorubicin-induced cardiotoxicity model on mice

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Background. Anthracycline-based chemotherapy is the standard first-line chemotherapy for advanced soft-tissue sarcoma. In 2007, the marine alkaloid trabectedin was approved for treatment of adult anthracyclines-resistant patients. Trabectedin is not considered per se cardiotoxic, nevertheless cardiac events were described in few cases from medical records. Since high cumulative doses of doxorubicin are known to be associated with cardiotoxicity leading to left ventricular dysfunction, we hypothesize that doxorubicin pretreatment may predispose patients to trabectedin-related cardiotoxicity. The aim of this study was to evaluate cardiotoxicity of doxorubicin pretreatment associated to trabectedin and its less-toxic analogue lurbinectedin, in a murine model.

Methods. Twenty female C57BL6 mice were randomized in equally distributed groups. Five mice received 10 ml/kg of saline solution weekly (VEHICLE). The remaining animals were treated with doxorubicin (10 mg/kg). After 5 days they were randomized to: trabectedin 0.15 mg/kg q7dx2 (DOX ET), lurbinectedin 0.2 mg/kg q7dx2 (DOX PM), and saline solution (DOX VEHICLE),. All medications were administered intravenously. Conscious mice underwent echocardiographic exams on day 5 and 19. After the second echocardiographic exam and blood sampling, animals were euthanized. Hearts were excised for hematoxilyn-eosin staining. High-sensitivity-cardiac-Troponin-T was assayed in plasma. T-test and one-way-ANOVA statistical analyses with Bonferroni post-test were performed.

Results. Shortening fraction and cardiac output decreased in doxorubicin-treated mice versus VEHICLE (respectively -8% and -21%). E/e' increased of 44% and ventricular arrhythmias were observed. During the second echocardiographic evaluation, a reduction in shortening fraction and cardiac output was found in DOX ET group compared to DOX VEHICLE one (respectively -19% and -21%), although these differences did not reach a statistical significance. DOX PM treated mice showed higher and not significant reductions in shortening fraction and cardiac output compared to DOX VEHICLE ones (-8% and -4% respectively). Plasma concentration of high-sensitivity-cardiac-Troponin-T was two-fold higher in DOX PM and DOX ET groups compared to DOX VEHICLE. No relevant histological alterations were found in LV myocardium.

Conclusions. A trend toward a mild and transient impairment of cardiac function was observed after doxorubicin administration. Treatment with marine alkaloids after doxorubicin appeared to worsen cardiac function, mainly after trabectedin. Given the small number of animals studied, new experiments are needed to confirm these findings. In future experiments we will also consider the need for including a treatment group where the cumulative dose of doxorubicin will be higher, but divided over time similarly to clinical practice.

POSTER	**M3**
NAME	**Benjamin Hershey**
Course/Year	PhD – 1st year
Institution	FIRC Institute for Molecular Oncology - IFOM

Tumor Ecology, an Investigation into the Functional Role of Intra-Tumoral Metabolic Heterogeneity

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Background. From tide pools to grasslands, ecological diversity drives the fitness of entire communities through webs of interactions. Elimination of certain members of these communities can catalyze the collapse of the entire community. Tumors, like larger ecological communities, are highly heterogeneous in their composition. The significance of immune cells, the stroma and resident microbial populations on tumorigenesis and metastases remains an active area of study. However, these lines of questioning fail to address the potential for the existence of meaningful cooperative interactions between clonal populations of metastatic cells and the impact of such cooperative networks on the pathogenesis of the tumor. This led us to ask whether heterogeneity could be stabilized through functional intra-tumoral interactions.

Methods. We choose to address this question using three cell lines as model systems: MDA-MB-231, MCF7 and BT474, representative of the Triple Negative, Luminal A and Luminal B subtypes of breast cancer respectively. First we asked whether the exposure to media conditioned by a population of cells could enhance the fitness of a single cell seeded in isolation. Next we ask whether combinations of clonal populations derived from our model cell lines can result in altered growth rates. Finally we will ask whether inducing aneuploidy, a hallmark of cancer, in a euploid clone derived from the MCF10a cell line will result in a population which exhibits dependence on the population.

Results. We have found that across all three of our model cell lines, culturing single cells in media conditioned by the parental population results in a greater number of cell being able to outgrow into a colonies. Furthermore colonies established in conditioned media are larger than those established in non-conditioned media.

Conclusions. The results of our initial experiments, using the three aforementioned model cell lines, suggest that a single tumor cell does benefit from being exposed to soluble factors from the parental population from which it was derived. We see that individual cells are more likely to develop into a colony when cultured in conditioned media across all three cell lines. Furthermore the colonies that are established are larger than those which grow in regular growth media.

POSTER	**M4**
NAME	**Mariaelena Valentino**
Course/Year	PhD – 1st year
Institution	FIRC Institute for Molecular Oncology - IFOM

Epigenetic rewiring in Cerebral Cavernous Malformation disease: the Endothelial-to-Mesenchymal transition

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Background. Cerebral Cavernous Malformation is a rare neuro-vascular disease, characterized by enlarged and irregular blood vessels, often resulting in cerebral hemorrhages. One of the major hallmark of the disease is the Endothelial-to-Mesenchymal transition (EndMT), a de-differentiation process to which undergo the endothelial cells (ECs) lining the vascular malformations. During EndMT, ECs progressively lose their endothelial features and acquire a mesenchymal phenotype, becoming more proliferative and invasive and expressing mesenchymal/stem cell markers. Given the importance of the Polycomb machinery in the mesenchymal transition, we have hypothesized its implication in Endothelial-to-Mesenchymal transition as well as cavernoma formation and development.

Methods. Biochemistry: protein extraction, western blotting, chromatin immunoprecipitation. Molecular Biology: nucleic acids extraction and purification, retro-transcription and RT-qPCR. Cell Biology: establishment/maintenance and transfection of immortalized lung-derived endothelial cells. Imaging Techniques: fluorescence and confocal microscopy.

Results. Preliminary data showed that two pivotal players of the Polycomb Repressive Complexes (PRCs), Bmi1 and Ezh2, are up-regulated in a Ccm3-/- environment, together with their histone targets, H2AK119Ub and H3K27me3. Furthermore, the administration of chemical inhibitors of BMI1 (PTC-209) and EZH2 (UNC-1999), was able to rescue the EndMT of Ccm3-/- cells in vitro and to reduce the number and size of cavernomas in vivo. To fine-tune the role of Bmi1 and Ezh2 during EndMT, we have tried a si-RNA mediated knock-down approach; we have been able to down-regulate some mesenchymal markers such as SCA-1 and FIBRONECTIN-1. To figure out the molecular mechanism through which the PRCs regulate the EndMT in Ccm3-/- conditions we have performed a Chromatin ImmunoPrecipitation assay: preliminary experiments showed that the promoters of Claudin-5, vWf and VE-PTP are enriched in the histone modification H3K27me3, making us argue that these could be targeted by the PcG proteins.

Conclusions. We report here that two PcG proteins, BMI1 and EZH2, play an important role in driving EndMT and CCM lesions development. The transient knockdown of Bmi1 and Ezh2 partially rescued the EndMT process in Ccm3 -/- endothelial cells. The ChIP experiments showed that the promoters of the specific endothelial genes claudin-5, vWf and VE-PTP are enriched in the histone modification H3K27me3 in Ccm3 -/- cells. This means that EZH2, the PcG protein catalyzing this modification, mediates the repression of endothelial genes. Taken together these data support a pivotal role of the PcG proteins in the EndMT induced by Ccm3 deletion.

POSTER	**M5**
NAME	**Roberta Sulsenti**
Course/Year	PhD – 1st year
Institution	Fondazione IRCCS Istituto Nazionale dei Tumori

Mast cells- derived osteopontin protects from neuroendocrine prostate cancer

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Background. Cancer development is influenced by the interactions between tumor cells and the microenvironment. We found in the TRAMP mouse model of prostate cancer that mast cells support the growth of adenocarcinoma whereas their inhibition encourage onset of neuroendocrine tumors. In prostate cancer patients, these tumors emerge after androgen deprivation therapies but can arise also as de novo entities, albeit very rarely. We observed an abnormal frequency of neuroendocrine tumors also in TRAMP mice genetically deficient for the non-structural matricellular protein osteopontin. We therefore hypothesized that mast cells can limit the growth of neuroendocrine prostate cancer through osteopontin production.

Methods. We used TRAMP mice on C57BL/6 background that spontaneously develop prostate adenocarcinoma (90% of mice) or prostate neuroendocrine tumors (10% of mice). TRAMP mice rendered genetically deficient for mast cells (KitWsh-TRAMP) or for osteopontin were reconstituted at 8 weeks of age with wild type or osteopontin-deficient mast cells. Mice were killed at 25 weeks of age and prostates collected for histopathology and immunohistochemistry for osteopontin. Tumor cells with adenocarcinoma or neuroendocrine phenotype were co-cultured in the presence of wild type or osteopontin-deficient bone marrow-derived mast cells. Tumor cells growth was evaluated after 4 days by trypan blue cell count.

Results. We detected osteopontin expression by immunohistochemistry in mast cells infiltrating TRAMP prostates. In vitro experiments showed that osteopontin sufficient, but not osteopontin-deficient, bone marrow-derived mast cells inhibited the proliferation of neuroendocrine prostate cancer cells, while they boost adenocarcinoma cell growth. Adoptive transfer of wild type mast cells in KitWsh-TRAMP and osteopontin-deficient TRAMP mice reduced neuroendocrine tumors to a similar frequency to that observed in TRAMP mice. On the contrary, transfer of osteopontin-deficient mast cells did not modify neuroendocrine tumor incidence.

Conclusions. Neuroendocrine prostate cancer is still incurable. Our data indicate a dual role of MCs in promoting or preventing prostate adenocarcinoma or neuroendocrine tumors, also suggesting a role of osteopontin in this mechanism. These results imply that mast cells could be a possible target to block prostate adenocarcinoma, but only in combination with therapeutic approaches direct against neuroendocrine variants. We are now investigating which molecular pathways are involved in the control of neuroendocrine differentiation mediated by mast cells-derived osteopontin.

POSTER	**M6**
NAME	**Giulia Mazzucco**
Course/Year	PhD – 3rd year
Institution	IFOM

Structural analysis of mammalian telomere

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Background. Mammalian telomeres are made of short tandem TTAGGG repeats, bound by a six-protein complex called Shelterin. Telomeres prevent the recognition of linear chromosome ends by the DSB response, thereby permitting the maintenance of linear chromosomes. Telomeric repeats are notoriously hard to replicate and behave like fragile replication sites. Their repetitive nature, the tendency to form secondary structures (e.g G4 DNA) and their ongoing transcription (TERRA), are thought to interfere with their replication, but the molecular nature of telomere replication problems has not been elucidated.

Methods. We have developed a new protocol for the purification of mammalian telomeric repeats and are using it to study telomere structure and telomere replication intermediates in electron microscopy.

Results. Using this approach, we have found that damaged telomeres tend to form intramolecular loops. These structures can be induced by nicking with DNaseI telomeres from normal cells and accumulate spontaneously in ALT telomeres, deriving from cancer cells that maintain telomeres by recombination and are known to accumulate telomere damage.

Conclusions. We propose that these intramolecular loops resemble homologous recombination intermediates that form spontaneously at damaged telomeres due to their high abundance in homology. Formation of these structures as a consequence of telomere damage might have important consequences in telomere replication and telomere maintenance. Our preliminary results with the imaging of telomere replication intermediates will also be discussed.

POSTER	**M7**
NAME	**Federica Guffanti**
Course/Year	PhD – 3rd year
Institution	Istituto di Ricerche Farmacologiche Mario Negri IRCCS

RAD51 foci quantification in ovarian cancer patient-derived xenograft FFPE samples to predict the response to therapy

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Background. Epithelial ovarian carcinoma (EOC) is characterized by impaired DNA repair systems. Platinum and poly ADP-ribose polymerase inhibitors (PARPi) based therapy exploits these defects inducing DNA double-strand breaks (DSBs) that, if not repaired by homologous recombination (HR), are lesions able to kill cancer cells. RAD51 is a protein involved in HR, which forms nuclear foci at the site of DSBs and their number has been shown to predict olaparib (PARPi) sensitivity in breast cancer. The aim of this study was to investigate if the number of RAD51 foci was able to predict both olaparib and cisplatin response in ovarian cancer xenografts.

Methods. We have established a xenobank of patient-derived xenografts (PDXs) from fresh ovarian carcinomas, whose sensitivity to cisplatin and olaparib has been characterized. RAD51 and γH2AX foci were quantified in 47 not-treated formalin-fixed-paraffin-embedded (FFPE) tumours by immunofluorescence. The co-staining with geminin, a human S-phase marker, allows the quantification of the foci only in proliferative cells. Foci score (percentage of geminin-positive cells that were also positive for the protein of interest) was calculated considering at least one hundred geminin-positive nuclei per sample. Samples with a percentage of ≤10% and ≤25% were considered as RAD51 and γH2AX negative respectively.

Results. Eleven samples were excluded from the analyses because murine cells were predominant in the tissue or for the lack of at least one hundred geminin-positive cells. Thirty-two and 16 out of 36 PDXs were known for cisplatin and olaparib in vivo response respectively. On the basis of their drug response, they were classified in very sensitive, sensitive and resistant PDXs. 61.2% of the samples were considered RAD51-positive (range from 10 to 78% of cells RAD51- positive per sample). The RAD51-negative PDX's group was enriched in olaparib-responsive ones, while all the 10 olaparib-resistant were RAD51-positive. Statistical analysis confirms a significant association between RAD51-positivity/negativity and olaparib response (p-value=0.0005, Fisher exact test). The quantification of RAD51-foci did not predict cisplatin response. 95% of the samples were found γH2AX positive (range from 30 to 100%) suggesting a high level of endogenous DNA damage; however, no association with drugs' response was observed.

Conclusions. Our data confirm those reported in the literature in breast cancer, which showed an association between RAD51 positivity and olaparib resistance. These data, if corroborated in a wider panel of PDXs and/or in FFPE tumour patients' samples, would suggest RAD51 foci quantification as a predictive biomarker of response to olaparib treatment.

POSTER	**M8**
NAME	**Giulia Terribile**
Course/Year	Undergraduate Student
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Dissecting the possible role of p97 in muscle wasting during cancer cachexia

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Background. Half of patients with malignancies develops muscle wasting (i.e. cachexia) and aerobic exercise ameliorates their prognosis. The p97/VCP ATPase complex facilitates the rapid degradation of myofibrillar proteins during muscle atrophy caused by denervation or fasting. The aim of this study was to investigate if p97 plays a role also during cancer cachexia and if it is modulated by physical exercise.

Methods. To induce cachexia in mice, we injected subcutaneously colon adenocarcinoma (C26) or Lewis Lung Carcinoma (LLC) cells. To understand if aerobic exercise improves cancer cachexia through p97 modulation in muscle, C26-bearing mice were run on treadmill for 5 days at 12 m/min and 15° inclination for 45 min/day. By Q-PCR or Western Blotting we measured the expression of p97 and its main adaptor proteins (Ufd1, Ufd2, p47) in cachectic Tibialis Anterior (TA) muscle. In vitro we performed luciferase assay to test the possible effect of p97 or its dominant negative mutant (DNp97) on dexamethasone-induced MuRF1 signalling.

Results. In vivo, we found that the mRNA levels of p97 and its interactors Ufd1, Ufd2 and p47 were induced in cachectic TA muscle from C26-carrying mice, undergoing body weight loss (i.e. cachexia). The protein levels of p97 and Ufd1 were also enhanced. Interestingly, treadmill exercise protected C26-bearing mice from muscle loss, with no effect on tumor growth, and rescued the C26-induced upregulation of p97 and its adaptors in muscles. As expected, MuRF1 was induced in dexamethasone-stimulated myoblasts. Surprisingly, we found that, unlike WTp97, exogenous DNp97 further upregulated dexamethasone-induced MuRF1 signalling.

Conclusions. Our preliminary data suggest that p97/VCP ATPase may play a role in muscle wasting also during cancer in mice but it remains to be established whether -and how- DNp97 is able to recapitulate the beneficial effects of aerobic exercise in vivo.

POSTER	**B1**
NAME	**Davide Olivari**
Course/Year	Dott – 2nd year
Institution	Istituto di Ricerche Farmacologiche Mario Negri IRCCS

A new preclinical porcine model for the study of acute congestive heart failure

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Background. Acute heart failure is a common cause of pulmonary edema and respiratory distress. Over the years, several animal models have been employed, but none of them were able to replicate the clinical conditions of this disease. The present study aimed at setting up a novel, highly reproducible and preclinical swine model of acute heart failure. Specifically, we (1) characterized the progression of the disease, using hemodynamic measurements, together with echocardiography and circulating biomarkers evaluation; (2) evaluated the effects of Serelaxin on the severity of acute heart failure, with the intent to validate the utility of the proposed animal model.

Methods. Myocardial infarction was induced by left anterior descending coronary artery occlusion (n=17). Two weeks later, a second myocardial infarction was achieved occluding the circumflex coronary artery (n=15). Intravenous infusion of crystalloids was performed in order to obtain volume overload, while phenylephrine infusion was used to cause an acute arterial crisis. Acute heart failure arose when the following conditions occurred concurrently: left ventricular ejection fraction < 30%; increases in thoracic fluid content > 25% and a pulmonary capillary wedge pressure > 30 mmHg. Finally, animals were divided to receive continuous intravenous infusion of serelaxin (n=9) or saline (n=6) over 48 hours.

Results. Thoracic fluid content significantly increased, with presence of pulmonary edema, at the onset of acute heart failure and such an increase persisted until the sacrifice. Intriguingly, the rate of thoracic fluid content in animals treated with Serelaxin was different compared to the ones treated with placebo. In both groups, arterial blood gases showed the conventional modifications occurring in patients with acute heart failure clinically with significantly decreased of the oxygen partial pressure and increased of the carbon dioxide partial pressure. Moreover, pulmonary arterial pressure, pulmonary capillary wedge pressure and right arterial pressures significantly increased, while left ventricular ejection fraction decreased during the induction of acute heart failure, and left ventricle end-diastolic and end-systolic volumes increased. Importantly, after 48 ours the recovery of cardiac function was incomplete. Serelaxin ameliorated the severity of the disease, reducing the pulmonary edema calculated by the ratio of the wet and dry pulmonary weights.

Conclusions. In conclusion, our model was able to replicate the condition of acute heart failure. In particular, it reproduced the physiopathology of the human disease, including alterations in hemodynamic paramenters and myocardial function. Moreover, the porcine model showed an increase of biomarkers typically involved in this disease. Finally, the swine treated with Serelaxin exhibited a drug response similar to the one observed on the patients in clinical studies. Thus, our models will be useful for the future molecular and functional studies of acute heart failure and for the investigation on new pharmacological approach to ameliorate patients survival and quality of life.

POSTER	**B2**
NAME	**Alice Passoni**
Course/Year	PhD – 1st year
Institution	Istituto di Ricerche Farmacologiche Mario Negri IRCCS

Mass spectrometric quantitation of intranasal cholesterol delivery in a mouse model of Huntington disease

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Background. Since the current pharmacological treatment for Huntington disease is only palliative, there is a need for therapies to restore function in patients. Recent results indicate that exogenous cholesterol administration into the brains of mice model of Huntington disease rescues synaptic communication and protects the animal from cognitive decline, suggesting that this can be translated into clinical therapy. Intranasal administration of cholesterol may be an effective means to reach the brain, so we developed a fully validated method based on liquid chromatography and mass spectrometry for the quantitation of cholesterol-D6 levels in brain, bulbs and plasma.

Methods. Two experimental groups, well-timed and R6/2 mice, were treated with a single or two injections of liposomes loaded with cholesterol-D6 (200 ug cholesterol-D6/mouse). The delivery of cholesterol-D6 to the brain after intranasal treatment was verified following the determination of cholesterol-D6 concentrations in mouse brain, plasma and bulbs. The analytical method was developed using a liquid chromatographic system coupled to a triple quadrupole mass spectrometer and using a Selected Reaction Monitoring (SRM) method. The method was fully validated according to EMA guidelines in both plasma and brain.

Results. The separation of cholesterol was performed using a fast chromatographic method, which allowed the analysis of cholesterol-D6 in only 6 minutes. The method was sensitive and reproducible allowing the quantitation of the analyte starting from concentrations of 0.1 ng/mg in tissues and 7.5 ng/ml in plasma. The evaluation of time-course profiles after acute treatment confirmed delivery of cholesterol to the brain of animals, reaching the highest concentration 48 hours after treatment. A significant amount of cholesterol-D6 was still detected 72 hours after treatment, suggesting a possible accumulation in the target tissue. The brain concentrations of cholesterol-D6 are consistent with those found in plasma: time-course profile in plasma reached the maximum concentration 24 hours after treatment with a decrease of cholesterol-D6 levels 72 hours after treatment. Finally, the levels of cholesterol-D6 in bulbs were stable at different time-points, confirming delivery of cholesterol with a minimum accumulation at the site of administration.

Conclusions. A reproducible and reliable liquid chromatography-mass spectrometry method was successfully validated for the quantitation of cholesterol-D6 in biological samples of treated mice. This method meets the acceptance criteria of EMA guidelines, showing good linearity in a wide concentration range with acceptable precision and accuracy. This validated analytical method enabled an accurate quantitation of cholesterol-D6 in biological samples and the evaluation of its biological distribution after intranasal treatment, confirming the delivery of cholesterol-D6 into the brain. The plasma, brain and bulbs time-course profile will offer useful information for further investigation of its behavioural effects.

POSTER	**B3**
NAME	**Laura Mannarino**
Course/Year	PhD – 2nd year
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Deciphering the transcriptional modulation program of trabectedin in sensitive and resistant patient derived xenograft models of myxoid liposarcoma

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Background. Myxoid liposarcoma is a soft tissue sarcoma whose pathogenesis is related to the expression of FUS-DDIT3 fusion gene which prevents adipocytic differentiation. Evidences suggested that in myxoid liposarcomas trabectedin, a natural compound with a complex and heterogenous mechanism of action, is able to restore differentiation program. However the mechanisms which underlie its antitumor activity are largely unknown. Since trabectedin acts as transcriptional regulator, we used RNA-Seq approach to analyze the transcriptomic modulation of the drug on two patient-derived xenograft models either sensitive (ML017), or resistant (ML017/ET) to trabectedin.

Methods. Samples from both sensitive and resistant models were collected at 24 and 72 hours after the first dose and 15 days after the third dose of trabectedin, giving 4 replicates per condition. RNA-Seq data were processed on a high performance computing platform provided by the Cloud4Care project counting at least 60 million of reads per sample. Alignment was performed with HISAT2 (Kim, 2015) and gene expression was quantified with Salmon (Patro, 2017). Low counts genes were filtered out and differential expression analysis and data processing were led through DeSeq2 (Love, 2014) applying both Wald test and likelihood ratio test for time course analysis.

Results. In the ML017 patient derived xenograft model, the highest gene modulation was registered at 15 days after the third dose, with more than 3500 differentially expressed genes. At 24 and 72 hours after the first dose most of the genes with altered expression were related to the TP53 pathway, while the biosynthesis of cholesterol and the metabolism of steroids were inhibited. The major effect at 15 days, the late time point, was the activation of extracellular matrix organization, collagene and elastic fibres formation, and the metabolism of glycosaminoglycan. On the other hand, the resistant model ML017/ET responded only at 24 hours after the first dose, involving the activation of DNA repair, homology direct repair and homologous recombination, while no modulation was found at later time points.

Conclusions. In the sensitive model trabectedin induces a great transcriptional modulation after different cycle of treatment. The remodeling of the extracellular matrix and the activation of collagene and elastic fibres formation suggest a change in the phenotype of the tumoral cells, in line with the known ability of trabectedin to restore adipose tissue differentiation. Instead, a part from a very early activation of DNA repair systems, the resistant model is no longer able to respond to the treatment, as expected. The mechanisms underlying this aspect should be further investigated by the integration of different genomics data.

POSTER	**B4**
NAME	**Laura Talamini**
Course/Year	PhD – 4th year
Institution	Istituto di Ricerche Farmacologiche Mario Negri IRCCS

Repeated administration of the food additive E171 to mice results in accumulation in intestine and liver and promotes an inflammatory status

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Background. Titanium dioxide is widely used in food as additive (E171). It contains micro- and nano-particles which can be absorbed systemically by humans after ingestion. In spite of several toxicological studies, a solid risk assessment of oral exposure to E171 has not been satisfactorily achieved. We investigated whether repeated oral administration of E171 to mice at a dose level comparable to estimated human dietary exposure, results in titanium deposition in the digestive system and internal organs and in molecular and cellular alterations associated with an inflammatory response.

Methods. E171 powder was characterized in terms of particle size and morphology. To reproduce closely the physiological digestion process, E171 was directly dripped into the mouth of mice. Healthy mice were orally treated (5 mg/kg) 3 days/week for 3 weeks. On day 21, mice were anesthetized and blood was collected. Then, mice were killed and lung, liver, stomach, spleen, kidney, brain, testes and gut were excised. Before subsampling, tissues were cleaned to remove any adhering blood or gastrointestinal residue. Tissues were appropriately processed for histological examination, inductively coupled plasma-mass spectrometry determination, superoxide and gene expression analyses.

Results. E171 is formed of particles with irregular sphericity, 35% with a diameter < 100 nm and 63% ranging from 100 to 200 nm. Repeated oral administration of E171 resulted in a significant accumulation in the large intestine and in the liver. The intestine showed a 3-fold increase in the number of titanium particles. In the liver, titanium accumulation was associated with necroinflammatory foci infiltrated with F4/80 positive cells. Increased superoxide production was observed in stomach and intestine. Gene expression analysis revealed an upregulation of IL-1β in the stomach and gut, on the contrary TNF-α level was not modified in the stomach and liver but was reduced in the gut. The tissue expression levels of IL-10 were significantly modified only in the liver. Circulating cytokines were also determined. Repeated E171 doses did not affect the concentrations of IL-1β and TNF-α whereas IL-6 and SDF-1 increased 2.2- and 3.1-fold respectively.

Conclusions. In summary, the present data indicate that repeated administration of E171 results in titanium dioxide deposition in the gastrointestinal tract and the liver, and is associated with molecular and cellular alterations in the inflammatory response. These new findings indicate that the risk for human health associated with dietary exposure to E171 needs to be carefully considered.

POSTER	**B5**
NAME	**Patricia Mondelo-Marcia**
Course/Year	Dott. – 2nd year
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Droplet digital PCR to detect MET copy number status in liquid biopsy

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Background. MET oncogene is a transmembrane tyrosine kinase receptor, implicated in embryonic development and organ regeneration. However, the deregulation of MET pathway, like amplification, is associated with metastasis and cancer growth. In addition, MET amplification is a common mechanism of resistance to other therapies. Several inhibitors of MET are undergoing testing in early-phase clinical trials. Thus, MET amplification can be a good biomarker for these therapies. Sometimes, the re-biopsy or tumor biopsy is not possible. Therefore, it's important to develop a good platform to determine MET gene copy number based on liquid biopsy to select patients to can received MET-targeted therapy.

Methods. Digested genomic DNA of 8 cancer cell lines were tested by droplet digital PCR (ddPCR) and compared with the MET gene copy number data, determined using the Affymetrix Genome-Wide Human SNP Array 6.0 platform (http://www.broadinstitute.org/ccle/home). We used ddPCR to detect MET amplification in the plasma circulating cell-free DNA (cfDNA) of 68 patients with different metastatic cancer (head and neck, lung, melanoma, pancreatic and colorectal cancer) from the Hospital Clínico Universitario of Santiago de Compostela. All patients had progressed to at least one line of therapy. Control plasma samples were also obtained from 39 healthy volunteers.

Results. The linear association for MET copy number measurements between ddPCR and SNP 6.0 is strong based on Pearson's correlation ($r = 0.98$; $P < 10^{-6}$). Controls values were between 1,9 and 2,66 copy numbers We set a cutoff value for MET amplification in plasma of 2,7 based on the healthy controls values. Patients' values were between 1,75 and 4,09 copy numbers. Seventeen patients with values higher than the cutoff were considered as positive for MET amplification. We observed a positive correlation between cfDNA concentration and MET copy number in cancer patients ($r = 0.60$; $P < 10^{-5}$). A similar tendency between the MET copy number and the number of treatments prior to MET analysis was also observed.

Conclusions. We establish a strategy to assess MET copy number status in patients with advanced cancer using circulating tumor DNA in order to identify patients eligible for MET inhibitors therapy. Our data with cancer cell lines demonstrate that ddPCR is validated to detect MET copy number. Moreover, controls values set a cutoff for the MET amplification in cell free DNA. Overall, our preliminary data demonstrate that ddPCR is a good option to detect MET amplification in cell free DNA metastatic cancer patients.

POSTER	**B6**
NAME	**Francesco Riva**
Course/Year	PhD – 1st year
Institution	Istituto di Ricerche Farmacologiche Mario Negri IRCCS

Assess the adherence to the pharmacological therapy: a wastewater-based epidemiology approach

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Background. The scarce adherence to the pharmacological therapies can lead to the onset of co-morbidity and can increase the risk of treatment failure. Here we propose a method to assess the overall medical adherence in a population through a new approach called Wastewater-Based Epidemiology. This approach assumes that any substance an individual ingest directly or indirectly is excreted unchanged and/or as a mixture of metabolites, and end up in wastewaters. Measuring specific pharmaceuticals' metabolic residues in raw wastewater will be possible estimate their real use and, comparing these data with the prescriptions figures, to assess the adherence to medical treatments.

Methods. Firstly, we defined the eligibility criteria for the biomarkers, and collected the pharmaceuticals' prescription figures from the report of the Italian Medicine Agency. Then we collected wastewater influent samples from 6 Italian cities and set up an analytical method to quantify the selected biomarkers in wastewater. Biomarkers stability in this matrix was also assessed. In order to back-calculate pharmaceuticals consumption a correction factor has been developed using excretion data available in the literature. A ratio between the consumption expected from prescription data and the one estimated through wastewater analysis has been calculated for each biomarker to compare the different figures.

Results. Among the pharmaceuticals listed in the report of the Italian Medicine Agency, only few were suitable as biomarkers to assess the medical adherence. The pharmaceutical selected were 3 cardiovascular drugs, ramipril, enalapril and losartan, and the antidepressant citalopram. Their respective main urinary metabolites selected were ramiprilat, enalaprilat, EXP-3174 and N-desmethylcitalopram. The analytical method allowed a good separation and quantification of the biomarkers investigated, the recovery rates ranged 73 - 119%, and the limits of quantifications were in the 0.67 - 5.2 ng/L range. The biomarkers selected were rather stable in wastewater at room temperature for 24 hours and at -20 °C up to three months. The ratios between the consumption expected from prescription data and the one estimated through wastewater analysis were in the 0,5 – 2 range with the sole exception of ramiprilat (0.34) and losartan (0.39).

Conclusions. Overall, the pharmaceuticals' back-calculated consumptions are quite in accordance with medical prescriptions. This preliminary study showed how the Wastewater-based Epidemiology approach can be considered reliable to estimate pharmaceuticals use in a population. This method can be further improved expanding the investigation to other pharmaceuticals and widening the number of samples analysed. The Wastewater-based Epidemiology approach developed was useful to investigate the use of pharmaceuticals in a defined area and can be combined with classical epidemiological studies to investigate the habits of the patient.

POSTER	**B7**
NAME	**Antonio Masone**
Course/Year	PhD – 1st year
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Generation of a PrP-HaloTag chimera to study the cellular trafficking and metabolism of PrP

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Background. The conformational conversion of the cellular prion protein (PrP^C) into a β-sheet-rich, infectious isoform (PrP^{Sc} or prion), which replicates by inducing misfolding of native PrP^C, is the key pathogenic event in prion diseases, a group of fatal neurodegenerative disorders for which there is no cure. To investigate PrP^C metabolism we have generated a PrP chimera containing a HaloTag (PrP-Halo) derived from a Rhodococcus rhodochrous haloalkane dehalogenase that can covalently bind specific fluorescent ligands.

Methods. The PrP-Halo construct was cloned in pCDNA3.1. HEK293 cells were transfected using Fugene HD and selected with hygromycin (200 μg/ml). PrP-Halo expression was analyzed by Western blot or SDS-PAGE and typhoon imaging after HaloTag labeling. The cellular localization of PrP-Halo was studied by confocal and super-resolution (SIM) microscopy using fluorescent ligands that were permeant or impermeant to the cell membrane.

Results. We designed two different PrP-Halo constructs by inserting the HaloTag sequence at either the N or C terminus of PrP^C. We transiently transfected HEK293 cells with these constructs. The C-terminally tagged PrP-Halo showed a better band profile on Western blot and was more efficiently expressed on the cell surface than the N-terminally tagged PrP-Halo. Therefore, we used this construct (hereafter PrP-Halo) to generate stable HEK293 cell lines. PrP-Halo was efficiently expressed and glycosylated. It could be easily detected by Western blot with anti-PrP antibodies, and SDS-PAGE and typhoon imaging. We labeled PrP-Halo cells with a cell permeant fluorescent ligand and observed that the protein was correctly exposed on the plasma membrane like untagged PrP^C. To confirm that PrP-Halo was normally degraded through the lysosomal pathway we treated cells with the lysosomal inhibitor bafilomycin A1. We observed accumulation of PrP-Halo in lysosomes.

Conclusions. Our analysis indicates that PrP-Halo is correctly expressed on the plasma membrane of HEK293 cells, and can be detected by fluorescence microscopy, Western blot and SDS-PAGE typhoon imaging. The possibility of specifically labeling different subsets of PrP^C molecules with cell permeant and impermeant ligands, and to analyze the protein by imaging and biochemical approaches, will allow to precisely define the cellular trafficking and metabolism of PrP^C.

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