Comparative Medicine
Copyright 2012
by the American Association for Laboratory Animal Science

Vol 62, No 6
December 2012
Pages 548–553

Abstracts of Scientific Papers

2012 AISAL SymposiumTranslational Medicine: Integration between Preclinical and Clinical Studies in Oncology

Rome, Italy • October 4–5, 2012

Main Lectures

Man's Best Friend: The Dog as Translational Model for Cancer Vaccine Evaluation

L Aurisicchio*

Takis SRL, Rome, Italy

Corresponding author. Email: luigi.aurisicchio@gmail.com

The application of vaccines to cancer is currently an attractive possibility thanks to advances in molecular engineering and a better understanding of tumor immunology. Mouse tumor models have proven to be instrumental tools for furthering our understanding of human cancer. Most mouse models are able to accurately recapitulate many aspects of human tumor physiology. Transgenic animal models have played a critical role in establishing basic paradigms of tumor immunology because they provide an in vivo milieu that cannot be reproduced in vitro. As novel immunotherapies and cancer vaccines have been developed, animal models have also played an important role in preclinical testing for therapeutic efficacy. However, scaling up experimental protocols from rodents to humans is often not a straightforward procedure. This particularly is true to cancer vaccines, where vaccination technology must be especially effective to overcome a variety of immune suppressive mechanisms. In light of this, dogs represent a good research model due to their large size, spontaneous tumor occurrence, a gene expression pattern similar to human tumors, and comparable influence of environmental factors. Cancers in pet dogs are characterized by tumor growth over long periods of time in the setting of an intact immune system, interindividual and intratumoral heterogeneity, the development of recurrent or resistant disease, and metastasis to relevant distant sites. Thanks to their large population size, cancer rate in pets is sufficient to power clinical trials, including assessment of new drugs. We will cover advantages and drawbacks of each model, particularly focusing on dogs as translational model in oncology and in immunology.

In Vitro and In Vivo Development of a Novel Pharmacologic Approach to Efficiently Target the Tumorigenicity Mediated by Met Receptor Tyrosine Kinase Signaling

A Furlan[1,†], V Stagni[2,†], A Hussain[1,†], S Richelme[1], F Conti[1], A Prodosmo[3], A Destro[4], M Roncalli[4], D Barilà[2,*], F Maina[1,*]

[1]Developmental Biology Institute of Marseille-Luminy (IBDML), CNRS – Inserm – Université de la Méditerranée, Campus de Luminy, Marseille, France; [2]Laboratory of Cell Signaling, Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Fondazione Santa Lucia, Rome, Italy; Department of Biology, University of Rome "Tor Vergata", Rome, Italy; [3]Laboratory of Molecular Oncogenesis, Regina Elena Cancer Institute, and Institute of Medical Pathology, Catholic University, Rome, Italy; [4]University of Milan, IRCCS Istituto Clinico Humanitas, Rozzano, Italy

†These authors contributed equally to this work
**Corresponding authors. Email: daniela.barila@uniroma2.it and flavio.maina@ibdml.univmed.fr*

Receptor tyrosine kinases are often aberrantly activated in solid tumors and their activity heavily contributes to their tumorigenicity. Their genetic or pharmacologic targeting has been consistently identified as a valuable therapeutic approach in in vitro an in in vivo model systems. However, the success of these strategies has been limited since inhibition of the "primary RTK-addiction" triggers a selective pressure to acquire resistance through RTK switching. As RTK share several effectors that participate in the oncogenic process and in drug response, an alternative strategy would rely on the identification of druggability signals downstream the RTK required for RTK-triggered tumorigenesis. Here, we identified c-Abl nonreceptor tyrosine kinase as an essential modulator of Met tumorigenicity in vitro as well as in vivo. Pharmacologic inhibition of Abl kinase activity through specific inhibitors—such as imatinib, which is currently employed as therapeutic tools in the treatment of Bcr-Abl-dependent leukemia—resulted in a strong reduction of tumor growth in mouse xenograft experiments. Indeed c-Abl allows Met-RTK to modulate p53 activity. Met ensures cell survival through a new path in which c-Abl is crucial to elicit p38-MAPK activation and p53 phosphorylation on Ser_{392}, which in turn triggers the selective upregulation of Mdm2 protein. We found a clinical correlation between activated-Met, phospho-p53, and Mdm2 levels in human hepatocellular carcinoma, supporting the role of this path in tumorigenesis. Our findings highlight the importance of evaluating the relevance of c-Abl antagonists for combined therapies, based on the tumor signaling signature.

Translational Cancer Research: Critical Aspects and National and European Initiatives

F Belardelli*, F Moretti

Department of Hematology, Oncology and Molecular Medicine, Istituto Superiore di Sanità, Rome, Italy

*Corresponding author. Email: filippo.belardelli@iss.it

Since cancer represents a worldwide health priority in terms of human deaths and social-economic impact, fostering translation cancer research is a major priority of both scientific community and national governments. It is generally believed that the discoveries stemming from basic research laboratories take too much time for their development into clinically valuable new drugs and/or interventions. This is due to important bottlenecks for the clinical development of new drugs, including the complexity of the regulatory framework, high costs, needs for GMP facilities and adaptation of clinical trial endpoints to the type and mechanism of action of the new substances. While some aspects of the developmental chain depend on the type of product (small molecules, biologics, advanced therapy medicinal products), the development and correct use of animal models remain issues of fundamental importance for translational cancer research. Recently, some national (the ISS per ACC program) and European (the TRANSCAN ERANET project) initiatives have been launched to support translational cancer research. Moreover, a great importance has been given to the need for establishing European research infrastructures dedicated to translational medicine, such as EATRIS (European Advanced Translational Research Infrastructure in Medicine). EATRIS will be interacting frequently with other European research infrastructures such as ECRIN (clinical trials and biotherapies) and BBMRI (biobanks). These initiatives are expected to exert a positive impact on the promotion of translation cancer research at both the national and international level, and have potential to greatly impact public health and cancer patients.

Teleosts as Models in Molecular Oncology

A Cellerino*

Scuola Normale Superiore Pisa, Italy and Leibniz Institute for Age Research, Jena, Germany

*Corresponding author. Email: a.cellerino@sns.it

Rodents are widely used as models in molecular oncology but the cost of rodent colonies limits the use of these model species for pharmacologic screening and lead development. Small teleost species are emerging as alternative models due to their reduced housing cost and high fecundity. In zebrafish, hepatic overexpression of an oncogenic form of mutated RAS (V12) initiated liver tumorigenesis, which progressed from hyperplasia to benign and malignant tumors with activation of the Ras-Raf-MEK-ERK and Wnt-β-catenin pathways. Histologic diagnosis of zebrafish tumors identified HCC as the main lesion. In our laboratory, we have studied the short-lived killifish *Nothobranchius furzeri*, which is the shortest-lived vertebrate that can be cultured in captivity. A high incidence of neoplasias was observed *postmortem* in liver (approximately 35%) and kidney (approximately 25%). Different laboratory strains of *N. furzeri* show large genetic differences in longevity. Cross-sectional analysis revealed a clear age-dependent increase in the incidence of liver neoplasias, which was accelerated in a short-lived strain. Liver neoplasias, like in human and zebrafish HCC, were characterized by nuclear translocation of β-catenin. Therefore, the short life span in *N. furzeri* is a consequence of a typical teleost aging process but unlike other teleosts, this scenario is reinforced by high incidence of age-dependent neoplasias. Therefore, HCC in teleosts reflects many molecular hallmarks of human HCC and may represent a convenient model system for a first screening of molecules targeting HCC.

The Benefits and Limitations of Animal Models for Translational Research in Neoplastic Diseases

M Cilli*

IRCCS Azienda Ospedaliera Universitaria San Martino, IST, Istituto Nazionale per la Ricerca sul Cancro, Genoa, Italy

*Corresponding author. Email: Michele.cilli@istge.it

Basic research is proving that cancer is not a homogeneous disease, but is complex and heterogeneous. The mouse has proved to be an excellent tool for modeling cancer in a mammalian system. This has traditionally been accomplished with the use of xenograft models incorporating primary or genetically altered cell lines derived from primary and metastatic tumors. Implantation of tumor cells into the organ of origin (orthotopically) allows organotypical interaction between tumor cells and surrounding stroma. It has been shown that this interaction affects growth, differentiation, and drug sensitivity of tumor cells. Moreover, tumor cells can spread to metastatic sites in other organs, with features comparable to the human situation. In the same way, the development and exploitation of animal imaging procedures is providing new means for preclinical studies. Genetically engineered mouse models make it possible to evaluate specific molecular pathways involved in carcinogenesis through the expression of oncogenes, specific genetic mutations, or the inactivation of tumor suppressor genes. These experiments have begun to provide us with an understanding of the molecular pathways involved in tumor initiation and progression. Additionally, these mouse models serve as an excellent system to evaluate the efficacy of currently developed molecular targeted therapies and identify new potential targets for future therapies. A new strategy called "coclinical mouse/human trial" has been conceived for improving mouse models of human cancer: mice with similar disease characteristics are treated in a similar way to the humans, improving human clinical trials of drugs for these cancers.

Use of Xenografts Models for Preclinical Testing in New Antitumor Strategies: The Example of Proton Pump Inhibitors

S Fais*

Istituto Superiore di Sanità, Rome, Italy

*Corresponding author. Email: stefano.fais@iss.it

Antitumor strategies have unfortunately shown an unpredictable failure, although there has been tremendous investment in terms of both human and economic resources. To understand this failure we should begin by considering cancer a curable disease, with the purpose to understand as much as possible of the pathogenesis of the disease. This should become the starting point, rather than the ocean of potential molecules that should be targeted by "new drugs." One of the most important features of tumors is that they create and maintain an acidic microenvironment that makes tumors entirely isolated from the normal tissues, which do not bear acidic milieu. Tumors are able to resist the low pH thanks to proton pumps that avoid internal acidification. We tested the effect of a class of antiacidic drugs that inhibit some proton pumps (PPI), evaluating their ability to both increase sensitivity of human tumors to drugs and to inhibit their growth, through the use of xenograft models as represented by human tumor/SCID mice. The results have shown that: 1) pretreatment with PPI renders tumors sensible to a wide panel of antitumor drugs, 2) high dosage PPI markedly inhibit tumor growth through an unconventional cell death, and 3) markedly increase survival of the xenografts. These results represented the background of presently ongoing clinical studies.

CBX7 is a Tumor Suppressor Gene in Lung Carcinogenesis

F Forzati[1], A Federico[1], P Pallante[1], C Arra[2], M Scarfò[3], M Fedele[1], A Fusco[1,*]

[1]Istituto di Endocrinologia ed Oncologia Sperimentale del CNR, Naples, Italy; [2]Istituto Nazionale dei Tumori, Fondazione Pascale, Naples, Italy; [3]IRGS-Biogem, Ariano Irpino (AV), Italy

[*]*Corresponding author. Email: alfusco@unina.it*

The *CBX7* gene encodes a polycomb group protein whose role in carcinogenesis is still controversial. To shed light on this issue, we generated mice null for the *cbx7* gene. These mice develop liver and lung adenomas and carcinomas, which suggests that CBX7 plays a tumor-suppressor role. Accordingly, mouse embryonic fibroblasts derived from the *cbx7*-knockouts have a higher growth rate and a reduced susceptibility to senescence than their wildtype counterparts. We demonstrate that CBX7 negatively regulates *Cyclin E* expression, and this likely accounts for the phenotype of the *cbx7*-null mice. Finally, we show the lack of CBX7 protein expression in human lung carcinomas that correlates with the overexpression of *Cyclin E*, already known to play a key role in lung carcinogenesis.

Premio AISAL 2011

Laboratory Culture of *Ciona intestinalis* in Closed Systems

O Migliaccio[1,*], C Agnisola[2], A Palumbo[1]

[1]Laboratory of Cellular and Developmental Biology, Zoological Station Anton Dohrn and [2]Department of Biologic Sciences, University of Naples Federico II, Naples, Italy

[*]*Corresponding author. Email: oriana.migliaccio@szn.it*

Marine invertebrates are essential for different fields of research. One major problem in their use as experimental models is the difficulty in their maintenance and breeding, which influence their availability and manageability directly. One of the most used animals in science is the tunicate *Ciona intestinalis*. *Ciona*'s maintenance started from the middle of the last century. In the last decade protocols for the culture of *Ciona* in open system have been set, which requires an unlimited supply of sea water. This culture's system, however, cannot be realized in research facilities located far from the sea; hence, the need to create closed systems for the maintenance and breeding of *Ciona*. Closed systems, in addition to requiring a limited quantity of water, would also prevent the spread of specimens OGM in the natural environment. The main goal of this work was to culture *Ciona* in a closed system, using artificial seawater and modulating some major chemical and physical parameters, that is, temperature, photoperiod, dimensions of the tanks, and density. Larvae were obtained by in vitro fertilization, using gametes freshly collected from adults. With this protocol it was possible to follow the development of *Ciona* up to 2 mo after hatching.

Oral Presentations

Integration of Bioluminescence Imaging and Histology as a Targeted Approach in Evaluation of Anticancer Drugs Efficacy in Human Malignant Mesothelioma Xenograft Mice Model

M Ardizzone[1,†,*], L Emionite[2,†,*], M Cilli[2]

[1]RBM SPA, Merck Serono, Istituto di Ricerche Biomediche "A Marxer," Colleretto Giacosa, Italy; [2]IRCCS Azienda Ospedaliera Universitaria San Martino, IST, Istituto Nazionale per la Ricerca sul Cancro, Genoa, Italy

[†]*These authors contributed equally to this work*
[*]*Corresponding authors. Email: michele.ardizzone@merckgroup.com and laura.emionite@istge.it*

Malignant mesothelioma (MMe) is an asbestos-related neoplasm with poor prognosis, refractory to current therapies, the incidence of which is expected to increase over the next few decades. There are 3 traditional kinds of treatment for patients with malignant mesothelioma of the pleura: surgery, chemotherapy, and radiation therapy; the folate antimetabolites/cisplatin chemotherapeutic regimen is currently considered the most effective first-line treatment; however, additional combinations are potentially welcome to increase efficacy in combined chemotherapy. To better evaluate the efficacy of combined treatments on human MMe cell lines in intraperitoneal xenograft orthotopic mice, we set up a study protocol in which quantitative and qualitative data were integrated, to compare the effectiveness of an EGFR monoclonal antibody, both as single agent and in combination with folate antimetabolite/cisplatin regimen. In vivo bioluminescence imaging (BLI) of the tumor growth spreading in the abdominal cavity and consequent histology on tumor masses generally showed the photon emission measurement reflected the quantification of neoplastic tissue observed at histology. Indeed, histology of the main peritoneal cavity organs was performed on whole mount liver, GI tracts, spleen, and pancreas. This innovative particular procedure enabled evaluation of the smallest tumoral masses formed, scattered around the peritoneum and unrelated to parenchymal struc-

tures, indicated by the peculiar and insidious nature of mesothelial tumor. Our results show that integration of quantitative data (BLI) and qualitative data (histology) offer an interesting platform to compare new treatment regimens in MMe animal models, which can be translated into clinic use.

Chronic Stress Induces an Increase of Metastasis in B16F10 and DU145 Cell Line In Vivo

A Barbieri[1,*], G Palma[1], A Luciano[1], D Rea[1], S Bimonte[1], G Scognamillo[2], E La Mantia[2], A Giudice[1], C Arra[1]

[1]Animal Facility Core and [2]Pathology Unit, National Cancer Institute "G Pascale" Foundation, Naples, Italy

[*]*Corresponding author. Email: barbieriant@gmail.com*

Metastasis causes most of the deaths for cancer and this process still represents an enigmatic aspect of the disease. It is noted that catecholamines, such as norepinephrine, are released by the sympathetic nervous system in chronic stress conditions. Since it has also been demonstrated that norepinephrine stimulates the motility of breast and colon cells through β-adrenergic receptor, we decided to examine their possible role in the development and metastasis formation through in vivo and in vitro studies. For this purpose, we used 2 different cell lines injected in mice: murine melanoma cells (B16F10), and human prostate cancer cells (DU145). We demonstrate that the modifications of microenvironment induced by chronic stress (restraint) and norepinephrine treatment of mice injected with cancer cell lines increase the metastatic potential of both B16F10 and DU145 cells. We also show that the treatment of mice with norepinephrine, leads to a significant increase of the migratory activity of cancer cells and that this process can be blocked by the β2-adrenoceptor (β2AR) propranolol. Then we showed that mice treated with norepinephrine, displayed an increased number of metastatic foci of DU145 in inguinal limphonodes, if compared to mice controls. In conclusion, these data show that β2AR plays an important role in the formation of metastasis in prostate cancer and melanoma cells. They also suggest that the treatment with antagonist propranolol could represent an interesting tool to control metastasis formation in cells overexpressing β2AR. Further investigations will be performed to shed light on this important point.

Characterization of the Neurotoxicity and Antineoplastic Activity of Bortezomib in a New Myeloma-Bearing Murine Model

VA Carozzi[*], C Meregalli, A Chiorazzi, B Sala, N Oggioni, G Cavaletti

Neuroscience and Biomedical Technologies Department, University of Milan-Bicocca, Monza, Italy

[*]*Corresponding author. Email: valentina.carozzi1@unimib.it*

Bortezomib (BTZ) is an effective antineoplastic drug for the treatment of multiple myeloma. Its clinical use induces the development of a peripheral neuropathy characterized by sensory alterations and neuropathic pain. Rat models of BTZ-induced peripheral neuropathy have been established. However, since only a few cancer cell lines induce the development of cancer in the rat, these models do not represent the most effective way to study, at the same time, the antineoplastic activity and the neurotoxic effects of BTZ. Here, immunodeficient SCID mice were injected subcutaneously with RPMI8266 human myeloma cells. Three weeks after tumor injection, mice were treated intravenously with BTZ 1 mg/kg once a week for 5 wk. The tumor volume was assessed as a measure of BTZ antitumor activity; the mechanical nociceptive threshold and nerve conduction velocities were measured to assess the neuropathic pain and the toxic effect of BTZ on the peripheral nervous system, respectively. Starting from the first administration, BTZ 1 mg/kg was able to block tumor growth, inducing the development of mechanical allodynia and an impairment of nerve functions. Interestingly, myeloma itself seemed to be able to induce mild functional alterations of peripheral nerves. This mouse model and treatment schedule allowed the study of the antineoplastic activity and of the neurotoxic effects of BTZ at the same time. Moreover, a preliminary evaluation of the effect of myeloma itself on the peripheral nervous system was assessed. This animal model should be used in the preclinical discovery of new neuroprotective as well as of analgesic compounds

Role of MDM4 in DNA Damage Response in a Mouse M4-Transgenic Model

L Fici[1,3,*], M Pellegrino[1,2], M Natoli[1], G Di Conza[1,4], F Mancini[1,2], A Apollo[1], M Buttarelli[1,3], R Masetti[3], A Pontecorvi[2], S Xiong[5], G Lozano[5], F Moretti[1]

[1]Cell Biology and Neurobiology Institute, National Research Council of Italy (CNR), Rome, Italy; Departments of [2]Endocrinology and [3]Surgical Science, Catholic University, Rome, Italy; [4]Department of Experimental-Clinical Medicine and Pharmacology, University of Messina, Messina, Italy; [5]The University of Texas, MD Anderson Cancer Center, Houston, Texas

[*]*Corresponding author. Email: laura.cc@alice.it*

MDM4 is an important regulator of the oncosuppressor p53 function. In normal growth conditions, MDM4 binds to p53 and prevents its transcriptional activity. Furthermore, it cooperates with MDM2 to ubiquitinate and degrade p53. Conversely, upon DNA damage, MDM4 acts as a positive regulator of p53; cytoplasmic MDM4 promotes stabilization of p53 and increases its protein levels. Moreover, during apoptosis MDM4 facilitates p53 phosphorylation at Ser46 (a proapoptotic modification of p53) its mitochondrial location, binding between p53Ser46P and BCL2, release of cytochrome C, and apoptosis. To confirm the positive regulation of MDM4 towards p53 in vivo, we have used 2 different strains of a transgenic mouse model overexpressing Mdm4 (Mdm4-Tg mouse). Particularly, we have analyzed thymocite apoptosis 4 to 6 h after a sublethal dose of total body γ-irradiation (6 Gy), a cell response mediated by p53-mediated apoptotic activity. Noteworthy, IR irradiation of Mdm4-Tg mice causes a significant decrease in thymocite viability compared to normal littermates (CTR) with a profound difference between males and females. Male Mdm4-Tg mice showed indeed a significant increase of cell death in comparison to littermates CTR mice. Conversely, this was not observed in female mice. Ovariectomization of female mice partly reversed these effects, suggesting that estrogens

modulate MDM4-mediated p53 dependent apoptosis. Overall, these data confirm in vivo the proapoptotic function of MDM4 and evidence a new control of it by female hormones.

Dual Energy X-ray Absorptiometry to Evaluate Body Composition in *Cbx7*-KO Mice

S Gargiulo[1,2,*], F Forzati[2,3], A Greco[2,4], M Gramanzini[1,2], E Vergara[4], R Liuzzi[1], M Fedele[2,3], A Fusco[2,3], A Brunetti[2,4]

[1]Consiglio Nazionale delle Ricerche, Istituto di Biostrutture e Bioimmagini, Naples, Italy; [2]CEINGE Biotecnologie Avanzate SCA RL, Naples, Italy; [3] Consiglio Nazionale delle Ricerche, Istituto di Endocrinologia ed Oncologia Sperimentale, Naples, Italy; [4] Dipartimento di Scienze Biomorfologiche e Funzionali, University Federico II, Naples, Italy.

[*]*Corresponding author. Email: sara.gargiulo@ibb.cnr.it*

Activated oncogenes and loss of tumor suppressors alter metabolism. Leptin-induced signals and oxidative stress may be the link among energy balance and adiposity regulation, as well as endocrine systems and oncogenesis. The *CBX7* gene encodes a polycomb group protein downregulated in many types of human cancers, suggesting a tumor suppressor role. Consistently, *Cbx7*-KO mice develop liver and lung carcinomas. A greater body weight, subcutaneous and omental fat were also observed in $Cbx7^{+/-}$ and $Cbx7^{-/-}$ compared with wildtype, suggesting *CBX7* has a role in adipose tissue metabolism. To better evaluate differences in body weight, fat, and lean body mass (LBM), we examined cohorts of age- and sex-matched mice by dual-energy X-ray absorptiometry (DEXA). DEXA was performed with a dedicated scanner on 29 5-mo-old and 83 10-mo-old, C57Bl/6J mice, genetically modified for *Cbx7*. In 5-mo-old mice, body weight were significantly different between wildtype and $Cbx7^{+/-}$ ($P < 0.01$) and wildtype and $Cbx7^{-/-}$ ($P = 0.009$), as well as the LBM between wildtype and $Cbx7^{-/-}$ ($P = 0.02$). In 10-mo-old mice, body weight and fat were significantly different between wildtype and $Cbx7^{+/-}$ ($P < 0.01$), and $Cbx7^{+/-}$ and $Cbx7^{-/-}$, as well as the LBM between wildtype and $Cbx7^{-/-}$ ($P < 0.01$). There were no significant differences in body fat accumulation or in LBM between male and female animals. These results support a key role of the *Cbx7* gene in the control of both fat and lean body mass.

[^{18}F]FLT Preclinical PET to Evaluate the Role of a Novel Cancer-Associated Gene in the Aggressiveness of Human Glioma

M Gramanzini[1,2,*], S Gargiulo[1,2], L Lignitto[3], A Greco[2,4], A Feliciello[3], A Brunetti[2,4]

[1]Consiglio Nazionale delle Ricerche, Istituto di Biostrutture e Bioimmagini, Naples, Italy; [2]CEINGE Biotecnologie Avanzate SCA RL, Naples, Italy; [3]Dipartimento di Biologia e Patologia Cellulare e Molecolare, "L Califano" and [4]Dipartimento di Scienze Biomorfologiche e Funzionali, University Federico II, Naples, Italy

[*]*Corresponding author. Email: matteo.gramanzini@ibb.cnr.it*

The recently identified praja2 gene is related to the potential aggressiveness of glioma. Genetic knockdown of praja2 significantly inhibited proliferation of glioblastoma cells in vitro and impaired xenograft and orthotopic growth in vivo. We investigated the use of ^{18}F-FLT in an ortothopic murine model of glioblastoma, silencing endogenous praja2 in UM87G cells prior to injection. U87MG cells were stereo-taxically implanted into the left caudate nucleus of 8 male CD1 nude mice. Animals were injected in tail vein with 9.5 MBq of ^{18}F-FLT, and after a 60-min biodistribution, a static PET was performed with a dedicated scanner (1.6 mm FWHM). Data were corrected for random coincidences, scatter and physical decay. SUV maximum and mean and the volume of lesions were calculated, by a "region growing" procedure. SUV mean and max were 0.017 ± 0.10 and 0.35 ± 0.29 in the control group and 0.12 ± 0.10 and 0.19 ± 0.29 in the treated subjects 2 wk after the cells were transplanted. SUV mean and max resulted 30% and 45% greater in control compared with siRNA treated group. SUV mean and max were 0.77 and 1.13 in control subject, but they were 0.68 and 1.1 in treated subject 4 wk after the cells were injected. SUV mean and max results were 11% and 3% greater in control than siRNA treated group. Tumor volume increased from 0.012 ± 0.002 to 0.12 cc in control group and decreased from 0.013 to 0.09 cc in the siRNA-treated one. The metabolic volume results were 7% greater in the siRNA-treated group 2 wk postinjection and 25% greater in the control group 4 wk after tumor implantation.

Metformin Elicits Anticancer Effects through the Sequential Modulation of DICER, MicroRNA33, and c-MYC

F Mori[1,*], M Cioce[2], MC Valerio[3], L Casadei[3], C Pulito[1], A Sacconi[2], F Biagioni[2], G Cortese[6], S Galanti[2], C Manetti[3], G Citro[6], P Muti[4,5], G Blandino[2], S Strano[1,4]

[1]Molecular Chemoprevention Unit and [2]Translational Oncogenomics Unit, Italian National Cancer Institute, Rome, Italy; [3]Department of Chemistry, University of Rome "La Sapienza", Rome, Italy; [4]Department of Oncology Juravinski Cancer Center, McMaster University Hamilton, Ontario, Canada; [5]School of Public Health, Harvard University, Boston, MA; [6]SAFU Department, Regina Elena Cancer Institute, Rome, Italy

[*]*Corresponding author. Email: federicamori78@yahoo.it*

Diabetic patients treated with metformin have a reduced incidence of cancer and cancer-related mortality. We show that metformin affects engraftment and growth of breast cancer tumors. This correlates with the induction of metabolic changes compatible with clear anticancer effects. We demonstrate that microRNA modulation underlies the anticancer metabolic actions of metformin. Indeed, metformin induces dicer expression and its effects are severely impaired in dicer knocked down cells. Conversely, ectopic expression of dicer recapitulates the effects of metformin in vivo and in vitro. The microRNAs upregulated by metformin belong mainly to energy metabolism pathways. Among the mRNAs downregulated by metformin, we found c-Myc, IRS-2, and HIF1α. Downregulation of c-Myc requires AMPK-signaling and mir33a upregulation by metformin. Ectopic expression of c-Myc attenuates the anticancer metabolic effects of metformin. We suggest that dicer modulation, mir33a upregulation and c-Myc targeting play an important role in the anticancer metabolic effects of metformin.

Triple-Negative Breast Cancer: Preclinical Model of Core-Shell Biodegradable Nanoparticles for Sustained Delivery of

Docetaxel in Neoadjuvant Chemotherapy

G Palma[1,*], A Barbieri[1], D Rea[1], A Luciano[1], C Conte[2], F Ungaro[2], P Tirino[3], G Maglio[3], R Palumbo[3], F Quaglia[2], C Arra[1]

[1]Animal Facility, National Cancer Institute "G Pascale" Foundation, Naples, Italy; [2]Department of Pharmaceutical and Toxicological Chemistry and [3]Department of Chemistry "Paolo Corradini", University of Naples Federico II, Naples, Italy

[*]*Corresponding author. Email: biopeppe@hotmail.com*

"Triple-negative" tumors lack expression of estrogen and progesterone receptors and HER2. The subtype comprises some 15% of all breast cancers, with tumors of a typically larger size and higher grade. Clinically, triple-negative breast cancer has a relatively high rate of recurrence, distant metastasis, and poor overall survival. The standard of care is chemotherapy, although recent research suggests a sound rationale for the use of targeted agents with antitumor and/or antiangiogenic activity such as receptor tyrosine kinase inhibitors. We designed long-circulating nanoparticles (NPs) for the sustained delivery of docetaxel (DTX). Biodegradable block copolymers of poly(ε-caprolactone) (PCL= 4.2 kDa) and monomethoxy-poly(ethylene oxide) (PEO=2 kDa) with diblock architecture (mPEO-b-PCL) were assembled in core-shell NPs by a melting-sonication technique (MeSo). NPs cytotoxicity was evaluated in breast cancer cells (MDA-MB231) with increasing concentrations of free DTX and DTX-NPs (1 to 500 ng/mL). NPs highlighted an antitumor effect similar to that of free drug inducing cell growth inhibition up to 80% after 72 h of treatment. In vivo antitumor efficacy of DTX-loaded NPs (at a dose of 10 mg/kg) were carried out in female athymic nude *Foxn1*^{nu} mice bearing MDA-MB231 cells by lateral tail vein injection. Tumor growth, body weight, and survival (Kaplan–Meier) of mice were monitored. Empty NPs did not show any obvious toxicity, whereas DTX-loaded NPs showed strong tumor regression after a single administration without a significant difference as compared with the DTX commercial formulation. We have demonstrated that core-shell PCL/PEO NPs can be a promising strategy for the treatment of solid tumors allowing alleviation of toxicity profile, and the DTX-loaded NPs can be used for the neoadjuvant chemotherapy in triple negative breast cancer.

New Orthotopic Mouse Models for Testing Therapeutic Vaccines in HPV-Associated Head and Neck Cancer

F Paolini[1*], S Massa[3], I Manni[2], R Franconi[3], A Venuti[1]

[1]Laboratory of Virology and [2]Laboratory of Molecular Oncogenesis, Regina Elena Cancer Institute, Rome, Italy; [3]ENEA, Italian National Agency for New Technologies, Energy and Sustainable Economic Development, CR Casaccia, UT BIORAD-FARM Unit, Rome, Italy

[*]*Corresponding author. Email: paolini@ifo.it*

Head and neck squamous cell carcinoma (HNSCC) is a significant cause of cancer worldwide. In HNSCC there is a subgroup of tumors associated with human papillomavirus (HPV) infection, and data recently reported shows that the HNSCC immune system can recognize HPV-induced cellular changes. HPV presence could be useful for the prognostic assessment of HNSCC with indications for a better prognosis of HPV-associated tumors. Presence of viral proteins suggests an active role of HPV and validates a therapeutic intervention with vaccines against these oncoproteins. Specifically, the E7 of HPV 16 is an attractive candidate for anticancer vaccine development. To evaluate the role of therapeutic vaccines against HPV proteins we used an orthotopic mouse models. The AT-84 E7 cells offer an attractive possibility for the development of a realistic murine model of oral cancer. This cell line was obtained by transfection of a vector carrying the E7 oncogene into the AT-84 cells derived from a squamous cell carcinoma of C3H mouse. For this study, we used a fluorescent reporter gene and a bioluminescent reporter gene luciferase to render the preclinical model more suitable for a fast and easy monitoring of the immuno treatments. The fluorescent or bioluminescent AT-84 E7 were injected into the floor of the mouth via an extra-oral route in C3H mice to obtain orthotopic tumors. Thereafter, the mice were challenged with E7 therapy and monitored with an imaging machine that offers unique opportunities to measure tumor growth and metastasis in vivo as well as by ELISPOT to evaluate the specific immune response.

Generation of Small Cell Lung Cancer Bioluminescent Xenograft Orthotopic Mouse Models and Evaluation of Antitumoral Activity of Different Chemotherapy Agents

M Ruocco[1,*], S Angelucci[1], E DeMarco[3], A Roda[2], P Hrelia[1], R Tonelli[1]

Departments of [1]Pharmacology and [2]Pharmaceutical Sciences, and [3]Department of Pediatric Oncology and Hematology, Sant'Orsola Hospital, University of Bologna, Bologna, Italy

[*]*Corresponding author. Email: margheruocco@gmail.com*

Each year approximately 1.4 million people worldwide are diagnosed with lung cancer. Small cell lung cancer (SCLC) is initially chemosensitive, but rapidly relapses into a chemoresistant form with an overall survival of less than 5%. The purpose of this work is to develop a preclinical mouse model of SCLC to further investigate the mechanisms involved in the neoplasia and to evaluate new therapeutic agents. The mouse model was created by inoculation of SCLC H69 human cell line. The tumor onset and progression were evaluated with a bioluminescence imaging (BLI) technique based on luciferase reporter gene (*Photinus pyralis).* In this regard, before being grafted in mouse lung, the tumor cells were transfected to permanently express luciferase gene. The mouse model has shown an incidence of a 100% and a 2-wk latency. Mice were monitored for 3 mo with an endpoint at 70 d from cells implantation. The BLI showed the presence of bone metastasis in mice, reflecting the behavior of human SCLC evolution. To validate the mouse model as an instrument to evaluate the therapy response using BLI, we used cisplatin and etoposide in combination. After 4 wk of drug administration, the mice showed a decrease of BLI signal and a prolonged survival. In vivo molecular BLI allowed the noninvasive, fast, and accurate monitoring of tumor evolution, from the first phases of tumor development. Finally, the BLI proved to be a suitable means to evaluate new drugs in preclinical studies for the treatment of this highly aggressive cancer.