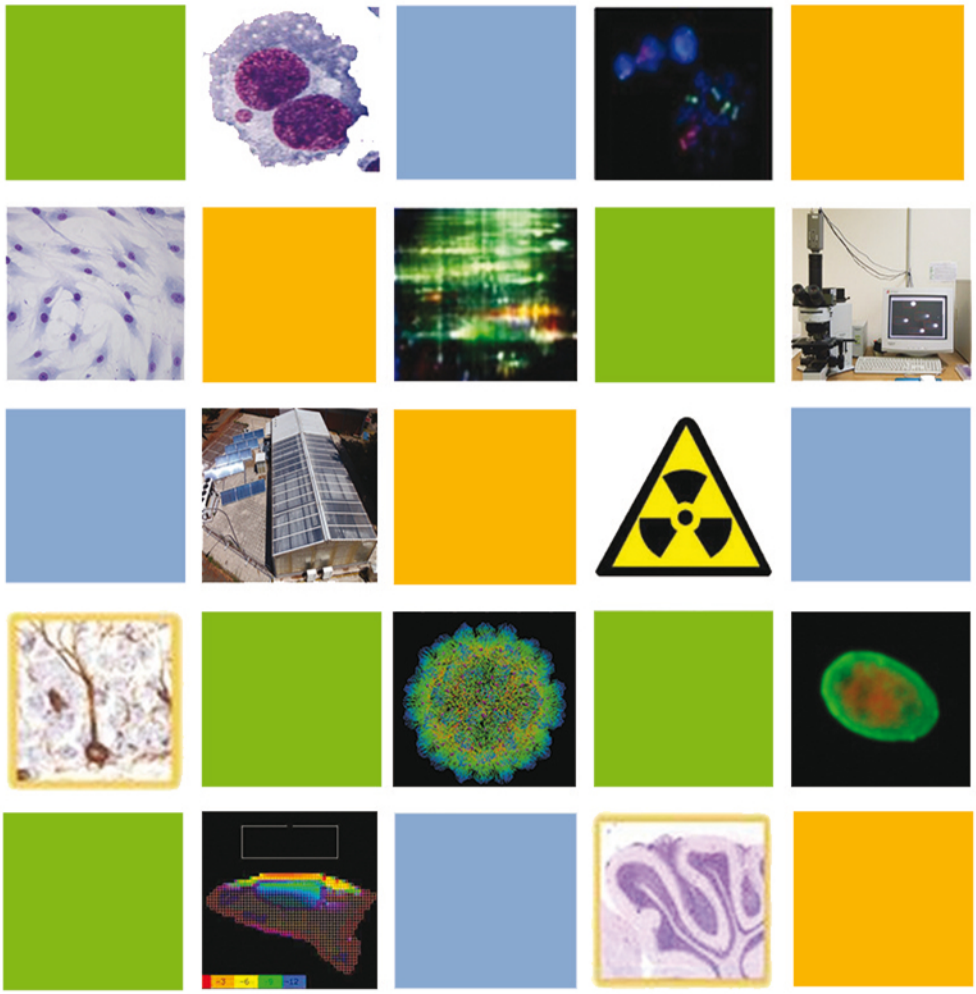
Italian National Agency for New Technologies,
Energy and Sustainable Economic Development

TECHNICAL UNIT OF RADIATION BIOLOGY AND HUMAN HEALTH

Activity Report

2014



TECHNICAL UNIT OF RADIATION BIOLOGY AND HUMAN HEALTH

Activity Report

2014

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Technical Unit Radiation Biology and Human Health (UTBIORAD)

The Technical Unit "Radiation Biology and Human Health" (UTBIORAD) of ENEA Italian Agency, with laboratories located in Casaccia Research Center in Rome, develops processes, products and methodologies aimed to the knowledge of mechanisms and effects of physical and chemical agents and the protection of human health.

UTBIORAD main goals are:

- to setup methodologies, models, technologies in order to study the effects of radiation and of other harmful physical and chemical agents;
- to define the mechanisms of action at cellular and molecular level;
- to transfer products, pharmaceuticals, and systems for diagnosis and treatment of diseases with severe social impact to the national public health system and the corporate environment
- to develop scientific platforms for the production of biopharmaceutical molecules from plants;
- to perform teaching and training activities in the field of radiobiology and biomedical sciences. Long-standing collaborations with Academia, Institutions and Research Laboratories in Italy and in Europe allow us to host students for degree and doctorate, as well as postdocs.

The Unit of Radiation Biology and Human Health includes in its staff about 60 researchers, as well as technicians, research fellows and students.

The scientific production consists of about 50 publications per year in peer-reviewed scientific journals.

Collaborations with other Technical Units within ENEA are active and participation in common projects are encouraged.

Essential for information exchanges and innovation among scientists, the program of seminars by internal staff and invited guest speakers is very active. Successful conference activities by staff members have included keynote and plenary presentations at international and national conferences.

Along with the laboratory research, the UTBIORAD staff is active in chairmanships, congresses organization, scientific committees, tutorships, teaching and dissemination activities.

Long standing collaborations with Academia, Institutions and Research Laboratories in Italy and in Europe allow us to guest students for degree and doctorate, as well as postdocs.

The Unit attracts support from a range of different agencies, at National and local level, national research associations, and from industrial and commercial companies. Currently, UTBIORAD labs are involved in numerous three- or four-years Italian, EU or International ongoing Grants. Research funding comes from international sources, particularly the European Union under the Nuclear Energy (Euratom), Framework Programs, European Metrology RP, NMP, as well as several National Research Programs. Specific programs and projects will be mentioned

and described in the involved laboratories. Interdisciplinary activities has been promoted and in the last years particular attention has been dedicated to focus and to involve different experimental activities (also in different laboratories) in large project to answer to national and international calls.
The Unit takes part in the TOP-IMPLART project, with other Units as Development of Applications of Radiation, the Radioprotection Institute, and Metrology of Ionizing Radiation (and also with Italian Institute of Health and National Cancer Institute Regina Elena - IFO). The project includes biological characterization activities on the biological efficacy in comparison with different proton sources, and *in vivo* and *in vitro* models development, aimed to study cellular and molecular mechanisms involved in the response to protons irradiation.
The Unit owns several patents related to antibodies by biotechnology in plant, vaccines based on genetic chimeras between viral and/or tumoral antigens and vegetal proteins. and a natural compound with cicatrizing, repellent and biocide properties, for external wounds healing

1. UTBIORAD

Modulation of ionizing radiation effects on cells

The activities' lines carried out within UTBIORAD concern the effects of ionizing radiation, in particular oxidative stress, DNA repair and the use of early exposure biomarkers and countermeasures for R/N emergencies' preparedness.

After having investigated the induction by low doses of genomic instability (*in vivo*) and bystander effect (on cultured cells) by analyzing the occurrence of genetic damage, inflammation, toxicity and oxidative stress, the collaboration with Prof. Ghibelli on the use of nanoparticles of cerium oxide (CNPS) as protective/sensitizing of radiation exposure to induce apoptosis of tumour cells while sparing surrounding healthy tissue and. Prof Rufini on the role of Topoisomerase IB in repairing radio-induced DNA breaks are still active.

CNPS are receiving much attention for their antioxidant activity, radio-protective and radio-sensitizing, although the underlying mechanisms of these actions are not known; their surface "defects" (redox couple and oxygen vacancies) can be decoupled from samarium Sm-doping (SDC), thus allowing the study of the mechanisms of their effects. Our aim was to understand the biological basis discriminating the effects of "defects" surface CNPS and locate the biological target. The experimental work was performed on cultured cells HaCaT cells irradiated with increasing doses of X-rays, in the presence / absence of CNPS, SDC and AA861, an inhibitor of 5- lipoxygenase (5 -LOX), by analysing: DNA damage by comet assay; mutagenesis by micronucleus assay; clonogenicity; FACS cell cycle, apoptosis, through the analysis of nuclear vesiculation. In collaboration with the S.

Gallicano hospital: glutathione analysis, the fatty acid composition polyunsaturated membrane and the release of arachidonic acid metabolites. Results demonstrate that the CNPS and SDC protect against oxidative stress and DNA damage induced by X-rays; CNPS and SDC induce cell cycle arrest in G2 phase, promote apoptosis and reduce clonogenicity; mutagenesis is almost completely inhibited. The same results were obtained with inhibitors of 5-LOX. It is interesting to note that the X-rays activate the 5-LOX, and that CNPS and SDC prevent this activation. Our results demonstrate that the CNPS act simultaneously as a radio-protective, as well as radio-sensitizing agents. Noteworthy LOX is activated irradiation, initially inducing DNA damage and oxidative stress, but on the other hand has an anti-apoptotic role by increasing the survival of mutated cells, thus confirming its role in tumour progression. With his work has been shown for the first time that CNPS and SDC act as inhibitors of LOX.

Topoisomerases (Tops) are ubiquitous and essential enzymes that solve the topological problems related to the physical structure of the double helix of the DNA by coordinately cleaving, manipulating and religating DNA strands together in the same catalytic event. Although the purpose of cleavage complex formation is still unclear, current data suggest that there might be an involvement in DNA repair correlated with apoptosis. To gain further insight into this hypothesis, as a continuation of our previous work, we investigated, on established human keratinocytes HaCaT irradiated with increasing doses of X rays: DNA breaks, micronuclei count, Top I amount and enzymatic activity. Based on these outcomes, we also figure out the radiation exposure induces a post-translational modification of the enzyme modulating its activity. There is considerable evidence that phosphorylation is critical to the regulation of Topo I activity, hence its activity and ability to associate with DNA is inhibited by treatment with alkaline phosphatase. Our future perspective is to identify these post-translational modifications. Camptothecin is a Topo IB inhibitor used in cancer therapy, showing severe side-effects, the possibility of targeting the post translational modification of Top IB enzyme, instead of targeting the enzyme itself, could explore a new pharmacological insight in the field of treatment of cancer.

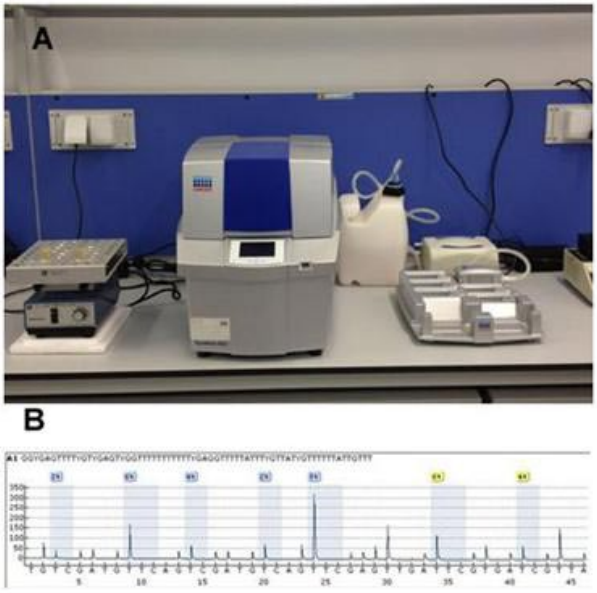
Molecular biology and epigenetics of solid tumors

The modern definition of epigenetics is information heritable during cell division other than the DNA sequence itself. It is now well established that epigenetic changes are implicated in human disease as well as during normal development. Although cancer is regarded as a genetic disease which originates from the accumulation of deleterious mutations, cancer epigenetics, one of the most fast-evolving fields in oncology, involves changes in gene expression that increase the fitness of tumor cells and endow them with the ability to move outside the

tissue/organ of origin. Furthermore, variations in the epigenetic state affect the execution of the cancer stem-cell program providing the tumor with a source of cells that have the ability to reinitiate growth indefinitely.

The group of Dr. Giuseppe Raschellà has worked for many years on the molecular mechanisms underlying proliferation, differentiation, apoptosis and metastasis of solid tumors. Special emphasis has been given to the study of transcriptional control by oncogenes such as MYCN and c-Myb which are important for proliferation and progression of neuroblastoma and of several types of carcinomas. The role of some families of microRNAs in controlling the expression of oncogenes has also been studied as well as how these microRNAs are in turn controlled. These studies have been done in collaboration with several internationally recognized Institutions among which the Thomas Jefferson University in Philadelphia and the University College of London. The results have been published in distinguished peer-reviewed journals.

Recent advances More recently, the research focus has moved on the epigenetic phenomenon of DNA methylation in tumors. The methylation of cytosines (C) by specific DNA methyl transferases (DNMT) is an event that occurs mainly when Cs are followed by guanines (G). Methylation of CpG-rich regions (CpG islands) located within the promoters of genes, affects their transcription. To approach the study of the role of DNA methylation in tumors, we utilize pyrosequencing, a technique that identifies the position and the methylation status of each informative C (those C followed by a G) within the analyzed sequence. The derived pyrogram gives the sequence of the analyzed region and the percent of methylation of each informative C in the sequence.



(A), the Pyrosequencer Qiagen PyroQ24; (B), an example of program which gives the percent of mehtylation of 7 informative C

To carry out this research program we have recently acquired (with funds of Attività di Ricerca per le Finalità dell'articolo 2, comma 44, L. 23 icembre 2009, N.191, research program, "Development of innovative bio-pharmaceuticals for anti-metastatic therapy") the Pyrosequencer Qiagen PyroMark Q24, a machine that allows the processing of 24 different

samples in each run. We published the first results of this research that describe how promoter methylation regulates the expression of microRNA-200 family during the metastasis process of mammary tumor cells (Pieraccioli et al. Cell Cycle 2013, 12: 2309-20).

An additional value of the acquisition of the Pyrosequencer Qiagen PyroMark Q24 derives from the interest for DNA methylation studies of other groups operating in the Unit. At present, Dr. Raschellà is collaborating with the group led by Dr. Marcello Spanò on a project aimed at identifying epigenetic factors involved in transgenerational effects of lifestyle and environment on human reproductive function.

Another important acquisition allowed by the funds of Attività di Ricerca per le Finalità dell'articolo 2, Comma 44, L. 23 Dicembre 2009, N.191, research program, "Development of innovative bio-pharmaceuticals for anti-metastatic therapy", is the multi-reader Promega GloMax Discover. This instrument allows the measure of fluorescence, chemo-luminescence and absorbance of 96 samples in a single run. The GloMax Discover will be used to detect apoptosis, necrosis, oxidative stress, viability and proliferation of cells silenced for the expression of specific genes or treated with drugs whose activity has to be determined.

The Multy-reader Promega GloMax Discover

3. UTBIORAD FARM

The Laboratory of Biotechnology consists of thirteen permanent staff researchers and technicians with an extensive set of expertise ranging from molecular biology to "omic" sciences and from virology to immunology in a synergistic approach to exploit the whole potential of plants mainly in the red biotechnology.

The knowledge of the connections between plants and health is a high-priority task for the generation of novel therapeutics that includes plant-derived recombinant proteins, besides the well-known natural drugs, dietary supplements and functional foods.

Multiple cutting-edge platforms of molecular biology, biochemistry, plant and animal cell biology support ongoing projects. Notably, a fully equipped proteomic platform based on DIGE (Differential In-Gel Electrophoresis) and a mass spectrometer Waters Xevo Tof G2-S and a contained greenhouse (Biosafety Level 2 Containment) designed to be a complete and compact solution for growing plants expressing high value added biopharmaceuticals.

The "Molecular Farming" Concept

Plants seem ideal bioreactors alternative to classical heterologous protein expression systems for overexpression of biologicals in a large scale. This area of biotechnology is also known as "molecular farming", in this way inspiring to applications of crops as bio-factories of high added value molecules.

"Hairy Roots" biofermenters for high-value added molecule production

Plant-derived antibodies.

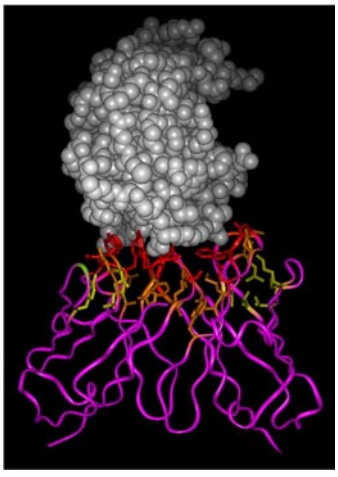
Antibody therapeutics are considered to be among the most promising biopharmaceutical products on a global scale. Antibodies expressed in plants represent a unique example of efficient and low-cost biopharmaceutical and are the historic workhorse of the laboratory. Indeed, different recombinant formats from the simplest to the most complex immunoglobulin, functionally tailored in size and scope, have been successfully expressed in plants with purification yields around 50-100 mg/Kg leaf tissue, in the case of a full-size monoclonal antibody. In this framework, efforts have been made to endow plants with glycosylation patterns as close as possible to those in humans and minimize proteolysis to address the issue

of the substantial equivalence between plant-derived and cognate antibodies.

New molecules have been also designed to enhance antitumor activity of some antibodies. In particular, an immunocytokine based on the C2B8 mAb (Rituximab) fused to the human interleukin 2 (hIL-2). This is the first example of a recombinant immunocytokine based on the therapeutic Rituximab antibody scaffold, whose over-expression in plants may be a formidable tool for the production a low-cost molecule treatment of non-Hodgkin lymphomas.

New attempts to obtain antifungal antibodies directed against Candida albicans in the IgA1 IgA2 subtypes have bee performed with complete plant-derived IgA being tested for fungal protection (Candida adhesion on epithelial cells).

In the field of immunodiagnostics, new high-affinity antibodies have been engineered as screening tools for ultra-sensitive and instantaneous detection of aflatoxins M1 in milk and dairy products with detection capability at concentrations as low as 5 pg/mL (5 ppt).



Modeling of Antibody-Antigen interaction

Plant-derived vaccines.

The expertise in plant molecular virology has led to pioneering multiple approaches to formulate “green vaccines”. These were derived mostly from chimeric plant viruses or large molecular carriers (Heat Shock Proteins, Oleosins) conveying immuno-relevant antigens. By this approach, both humoral and cell-mediated immune responses have been elicited without the need of adjuvant delivery. Main advantage of this approach is that plant viruses are not infectious for animal cells, holding promise to supply large amounts of doses in short times.

In this area of research, in particular on Human Papillomavirus 16 (HPV16)-related cancers, after the fundamental work demonstrating that plant-derived vaccines based on the recombinant E7 antigen strongly affected tumor growth in pre-clinical models, a recent progress highlights that the combination of vaccine formulations with the E5 and E6 oncogenes (besides E7) generates notable anti-tumor effects.

In addition, after the first demonstration of the higher efficacy of recombinant vaccine formulations with attached sequences of plant origin (thus acting as ‘adjuvants’) (European Patent 2456785 -European Patent Bulletin 14/47 19.11.14), the invention is now being validated in France, Germany as well as in Italy. In this way, new avenues are opening up towards the intrinsic capability of plants components to boost vaccine effectiveness, reinforcing the concept that plants are at the same time, both safe and low-cost production platforms for high-quality bio-therapeutics and sources of undiscovered immune-modulating molecules.

The Proteomic approach

Understanding protein interactions within the complexity of a living cell is challenging, but advanced techniques together with the deciphering of many genomes have enabled important progress to be made in the past 10 years. Proteomics has significantly contributed to the development of systems biology, a new paradigm for the life sciences in which biological processes are addressed in terms of dynamic networks of interacting molecules with key points that become possible targets for intervention.

We have quantified the effects of network perturbation in time and space during expression of foreign proteins in the complex genome of plants or during physiological and pathological conditions both plant or animal cells. In particular, we have addressed the issue of how proteome changes according to c-myb silencing in human chronic myeloid leukemia cells, suggesting molecular mechanisms and putative biomarkers of hematopoietic malignancies. The aberrant expression of c-Myb transcription factor is associated with the suppression of normal differentiation processes promoting the development of the hematopoietic malignancies. Our study reveals a complex network of proteins regulated by c-Myb and the pleiotropic role of this transcription factor as a regulator of genes that are crucial for energy production and stress response in leukemia. This work highlights potential protein biomarkers to look into disease progression in view of translational medicine approaches.

The future in a glance: Virus-derived Nanobiotechnology

Research for the discovery of nature-made nanoparticles is one of the most active areas of nanobiotechnology. Plant viruses have emerged as suitable building blocks in that coat proteins assemble with precise three-dimensional structures to form robust nanoparticles that can be easily manipulated and produced in large amounts. In this area of research, a dual approach that combines both in silico tools and experimental virology was applied for the rational design of immunologically active chimeric virus-like particles (VLPs) carrying immunogenic peptides. Our model is based on the Artichoke Mottled Crinkle virus (AMCV), belonging to the family Tombusviridae. AMCV capsids (30 nm in diameter) have icosahedral symmetry and consist of 180 identical molecules of coat protein (CP). Overall results demonstrated that AMCV CPs expressed in plant tissues were able to self-assemble into VLPs. Due to the efficient and rapid transient expression systems available, AMCV could represent a cheap and safe platform for the production of nanoparticles that could be exploited as a new drug delivery vehicle able to efficiently load the chemotherapeutic drugs.

In addition, we tried to define the safety profile of two structurally different plant viruses produced in plants, the filamentous Potato Virus X (PVX) and the icosahedral Tomato Bushy Stunt Virus (TBSV). To this aim, we performed experiments to test their possible effects on human erythrocytes (to evaluate general cytotoxic effects in vitro) and on chicken embryo development (to assess in vivo toxicity and teratogenicity). The data obtained indicate that these structurally robust particles

possess neither toxic nor teratogenic effects on in vitro animal cells and in vivo animal models.

The future in a glance: "BIOxTREME" Advanced Horticulture and Bioresources for Space

Advances in horticulture research, such as hydroponics, allow farming in places once thought impossible. The most extreme place is a spacecraft where research is active to individuate plants able to provide directly safe food and medicines. In the framework of the BIOxTREME project funded by ASI (Agenzia Spaziale Italiana) we are now exploring and exploiting the potential of plants as "dual" source of anti-oxidant nutriceuticals and microbicide /immunostimulant biopharmaceuticals. To operate in this field, a task force of ENEA scientists with different expertise and backgrounds is involved in the attempt to mimic the extreme environmental conditions of the space (namely, ionizing and non-ionizing radiations, microgravity, altered light and photoperiod conditions, ect.) finding countermeasures to adverse effects of the non-natural conditions of life on Space.

Main objectives of this (just started) project are:

1) developing two plant-based platforms for the production of bioactive molecules in plants and roots of a tomato cultivar (MICRO-TOM) that represents an "ideotype" with extremely favorable characteristics as living material for use in Space (i.e. in the field of 'Bioregerative Life Support');
2) examining the biological effects of the application of physical conditions of 'challenge' while mimicking the actual situation of the cosmos. Changes in the normal physiology of living plant material in extreme conditions will be analyzed according to Systems Biology methods profiling changes induced in the proteome;
3) optimizing the production of bioactive molecules (low molecular weight antioxidant molecules) produced in the berries;
4) optimizing the production of recombinant bioactive molecules of proven microbicide ability (antibacterial and antifungal polypeptides and antibodies);
5) studying the effect of treatment with recombinant immunostimulatory (flagellin) in animal models with radio-induced immunosuppression;
6) developing 'ready-made' pharmaceuticals for early intervention in Space conditions

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Tomato cultivar "MicroTom" raised in an advanced aeroponic system

2. UTBIORAD RAB

As part of the Unit of Radiation Biology and Human Health, the Laboratory of Radiation Biology and Biomedicine carries out multidisciplinary research in the fields of classical and molecular radiation biology, biological dosimetry, carcinogenesis, experimental oncology, molecular immunology, molecular bases of chronic inflammatory disorders, and a line of research investigating the efficacy of natural compounds for human and animal health.

We have a strong interest in the investigation of harmful effects of low-dose ionizing radiation and the definition of risk models, with special emphasis on the analysis of cellular and molecular mechanisms involved in cancer and non-cancer radiation effects. To this aim, the laboratory develops animal models specifically engineered for high radiosensitivity, as well as *in vitro* models and molecular radiobiology approaches, with a view to improve quantification of risk from ionizing radiation exposures. We also focus on the genotoxic effects induced by different qualities of ionizing radiation and their synergy with other environmental genotoxins in human lymphocytes and mammalian cell lines.

We also participate in a European network for biological dosimetry, which has been created to strengthen the emergency preparedness and response capabilities in case of a large-scale nuclear accident or radiological emergency.

The laboratory actively pursues the study of thermal and non-thermal effects of electromagnetic fields, the design and development of experimental models and exposure systems, and the development of applications of electromagnetic fields for therapy and diagnostics. Analysis and evaluation of standards for the protection of workers and general public is carried out.

We also focus on the regulatory mechanisms involved in immune-mediated diseases, including inflammatory diseases, autoimmunity, allergy and other hypersensitivities. Moreover, *in vitro* and *in vivo* work in the laboratory investigates the effects of exposure to RF/microwave radiation on the functions of the immune system and the effects of low-dose ionizing radiation on inflammatory/immune responses.

To understand the relationship between innate immune response and chronic inflammatory processes, we use intestinal inflammation as a reference model, in particular the Inflammatory Bowel Disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), as well as other immune-mediated enteropathies.

An area of research in the laboratory further explores the role of the ENEA-patented remedy of natural extracts in oil from plants (MIX577), which has the capacity to properly regulate most of the complex events of wound healing processes.

We provide support to the realization of an Adrotherapy Center, unique in central Italy, for potentially 1,000 patients per year (AIRO estimate).

Experimental oncology

Estrogens constitute a class of steroid compounds traditionally associated with female reproduction. The last decade has seen a revolution in our understanding of the actions of estrogen in the body. Estrogens play important roles in both reproductive and non-reproductive

systems. They can be synthesized in non-reproductive tissues such as liver, heart, muscle, bone and brain. Very recent data demonstrate a crucial involvement of estrogen signaling in carcinogenesis of non-classical estrogen target tissues (e.g., brain, skin, colon, prostate and lung).

The biological effects of estrogens are mediated by two distinct estrogen receptors (ERs), ERα and ERβ. Although ERα and ERβ have similar structures, they mediate different effects, and there is currently increasing evidence that an imbalanced ERβ expression might play a pivotal role in development and progression of some tumors.

The brain is now recognized to be affected by estrogen, in particular both ERα and ERβ are expressed in the cerebellum. Current literature data suggest that endogenous/exogenous estrogen exert a protective role against the development and progression of some brain tumors (e.g., glioma, medulloblastoma [MB]), while may be detrimental in other tumor types (e.g. meningioma). MB, a highly malignant primitive neuroectodermal tumor of the cerebellum, represents about 20% of all childhood primary central nervous system (CNS) tumors. The presentation peak age is 3-6 years, with only 25% of patients between 15 and 44 years of age. Epidemiological studies have shown that male sex is a risk factor for MB, irrespective of age, race or region of the world with approximately 65% of patients being males. Besides this different susceptibility, female gender is also a significant favorable prognostic factor in MB, with girls having a much better outcome.
In line with these epidemiological findings, using the well-characterized Ptch1+/- mouse model of radiation-induced MB, we previously showed a protective action of estrogen during early stages of MB development; indeed, susceptibility to MB development was significantly increased in ovariectomized Ptch1+/- females, and restored to levels observed in control mice after estrogen replacement. We next investigated the molecular mechanisms by which estrogen might influence tumor progression, and showed that ERβ, but not ERα, is involved in modulation of MB development by estrogens. These effects were achieved via activation of anti-proliferative and pro-apop totic pathways.

In this context, work carried out in 2014 evaluated the impact of gender and the role of ERβ in preclinical models of human MB. We found that the growth of D283Med human cell line, xenotransplanted in male and female nude mice, was strongly dependent on gender. Indeed, tumors growing in females were significantly smaller compared to those growing in males. Moreover, at microscopic examination, tumors from females showed a shift towards differentiation, as evaluated by a decreased stem cell population component (evidenced by expression of the stem-like cell marker for MB, nestin), and a substantial increase of neuronal and glial differentiation markers.

Hormone receptor expression profile in tumors from both female and male mice showed that the expression of ERβ isoforms was differentially regulated in tumors from males and females. Indeed, while ERβ2 and ERβ5 were consistently expressed in all tumors independently of gender, ERβ1 expression was significantly higher in tumors from females compared to males.

In summary, we have shown a significant sex effect on MB growth in a preclinical mouse model of the human disease,

reflecting the greater prevalence of MB in males compared to females in the human population. We have also provided mechanistic evidence supporting the idea that ERβ1 signaling may have pro-differentiation and tumor suppressive functions in MB, thus suggesting that functional activation of the ERβ pathways may be a potential therapeutic option for MB.

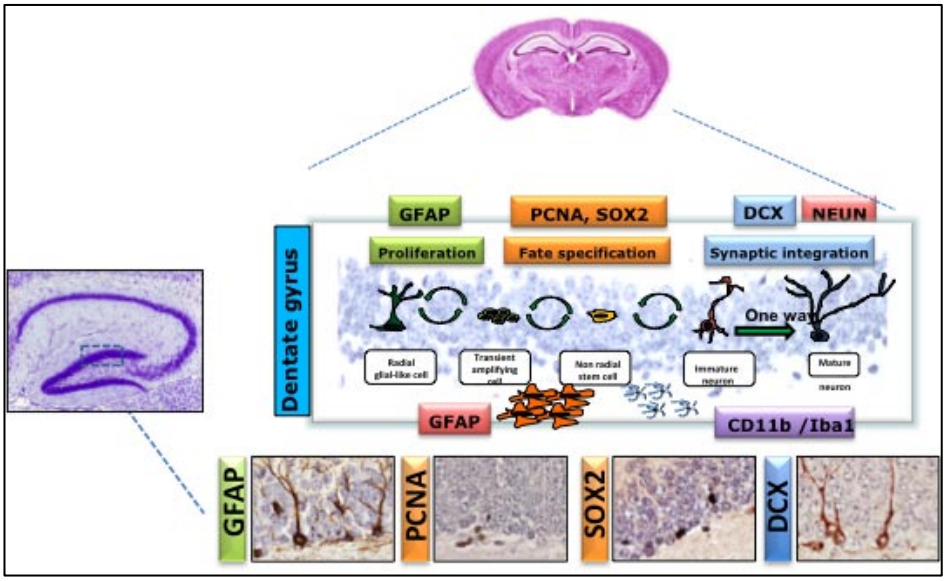
Mechanisms of non-cancer effects of ionizing radiations

In addition to cancer, ionizing radiation exposure can induce non-cancer effects. These effects occur when cell death is so extensive to cause functional impairment of the tissue or organ.

The main non-cancer effects are cardiovascular disease, neurocognitive effects and lens opacities. It has been traditionally assumed that health effects other than cancer show a threshold at doses that are well above the levels of exposures typically encountered in the public environment, at work, or from medical uses of ionizing radiation. Results from recent studies indicate increased risks from cardiovascular diseases, cataracts, and cognitive effects not only at doses above 5 Gy but also in a range of doses from 5 to 0.5 Gy and, possibly even at lower doses (<0.5 Gy). In 2011, the International Commission on Radiation Protection (ICRP) recognized a new dose limit of 0.5 Gy for the lens of the eye, and a recommendation was made for a reduction in the annual absorbed dose limit to 20mSv. The mechanisms responsible for the non-carcinogenic effects of radiation are for the most part unknown. Research to understand biological mechanisms underlying radiation-related non-cancer effects is necessary and animal models may help identifying these mechanisms. Examples of experimental mouse studies on cardiovascular diseases, cognitive effects and cataract are provided below.

Neurocognitive effects

Neurological deficits including impairment in memory, attention and executive function are frequent after high-dose cranial irradiation for radiotherapy with effects more pronounced in children than in adults. The increasing use of radiation procedures such as computed tomography (CT) scans raised concerns on potential harmful effects of low radiation doses to the brain in the general population, and especially in children. To assess long-term cellular and molecular alterations induced by low-dose irradiation, newborn mice (NMRI) of 10 days of age were exposed to 20-1000 mGy of γ-Rays. Six months later, they were subjected to cognitive evaluation and immunohistochemical analysis of the dentate gyrus (DG) of the hippocampus to stage neuronal differentiation and to identify modification of the microenvironment. We focused on the hippocampus because the learning memory and spatial information processing abilities are dependent on proper hippocampus functionality, and because the DG is one of the two structures of the CNS where continuous neurogenesis is observed throughout life. Our analyses revealed a strong association between cognitive dysfunction, impaired neurogenesis and neuroinflammation in the hippocampus after irradiation at low/moderate doses. No single cell type alteration can fully explain the complexity of the long-term consequences of irradiation. A dynamic interaction between multiple cell types (i.e., neurons, microglia and astrocytes) is probably involved in the pathogenesis of radiation-induced cognitive injury.



Immunohistochemical and molecular analysis of the hippocampus

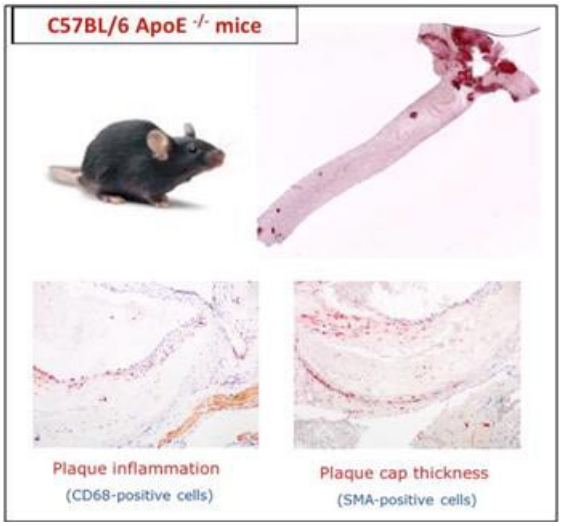
Cardiovascular diseases

Radiation is a risk factor in human vascular disease and an increased incidence of atherogenesis is observed in patients with Hodgkin's disease, breast cancer and head and neck cancer after radiotherapy. The apolipoprotein E-deficient (ApoE-/-) mice, exhibiting a marked increase in the levels of plasma cholesterol when fed a normal chow diet (4.2% fat), are predisposed to spontaneous atherogenesis and develop lesions resembling those seen in humans. As a consequence, they represent an ideal mouse model to establish the role of ionizing radiation in the atherogenic process *in vivo*. To evaluate cardiovascular alterations, aortas from irradiated ApoE-/- mice were subjected to morphometric and histological analyses.

We found that acute exposure of ApoE-/- mice to high (6 Gy) or low (0.3 Gy) radiation dose enhanced atherogenesis by increasing the percentage of aortic area covered by plaques. Interestingly, irradiation failed to cause an increase in plaque size, yet causing increase in plaques density. This suggests that radiations act mainly as an initiating stimulus for atherosclerotic plaque development.

Histopathologic and vascular studies show that plaque composition and vulnerability (type of lesion) are crucial factors that may lead to sudden rupture of the plaque surface, underlying the great majority of infarctions. As markers of vulnerability of the plaque we examined, through immunostaining, the thickness of the fibrous cap (SMA) and the inflammation index (CD68). We observed an increase in

percentage of total plaque area occupied by macrophages (CD68+ cells) and decrease of smooth muscle cells (SMA+), indicating a thinning of fibrous cap after irradiation with 6 Gy. A thinning of the fibrous cap decreases lesion stability and is a typical feature of advanced lesions



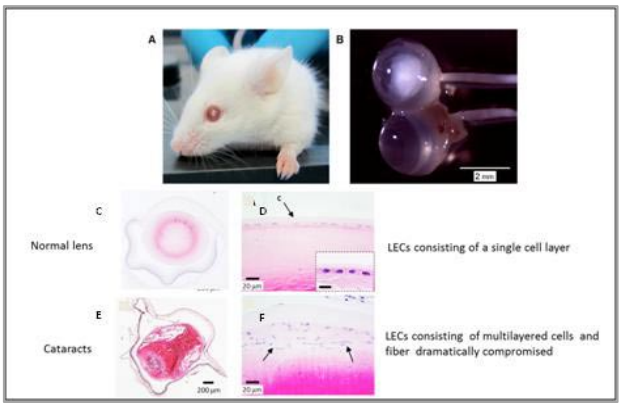
Markers of plaque vulnerability examined through immunostaining for SMA and CD68

Lens opacity

Cataract (the opacification of the ocular lens) is the most frequent cause of blindness worldwide.

The eye is well known as one of the most radiosensitive body-tissues and it is clearly recognized that cataracts can be induced by ionizing radiation exposure. We investigated the mechanisms of radiation-induced cataract development in Ptch1$^{+/-}$ mice, a well-known mouse model of radiation-induced oncogenesis. Irradiation of Ptch1$^{+/-}$ and wild-type mice with 3Gy of X-ray at postnatal day 2 (P2), a very early stage of lens development, significantly increased cataract development compared to unirradiated Ptch1$^{+/-}$ mice. Instead, irrespective of Ptch1 status, very low or no induction of cataracts was observed when radiation was delivered at P10, when lens development is near to completion, or at P56, when development is completed.

(A, B) Macroscopically visible cataract is recognizable as a white pinpoint focus in the pink eyes. (C) Representative image of a normal unirradiated mouse lens. (D) Representative image of lens epithelial cells (LECs) of a normal unirradiated mouse lens. (E) Extensive disorganization of fiber cells in severe anterior subcapsular cataract (ASC). (F) Severe ASC, characterized by a typical multilayered plaque

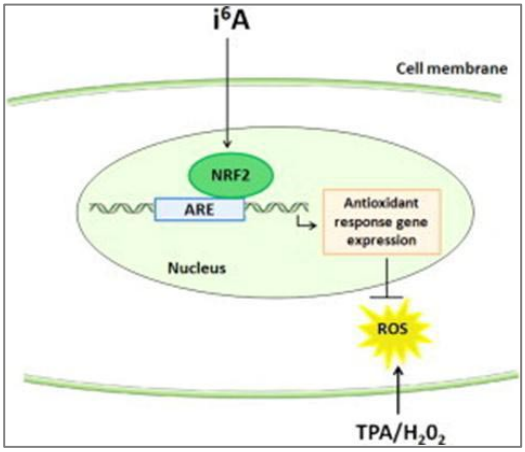


These results indicate a clear age-related window of susceptibility in cataract induction by ionizing radiations. In addition, these data highlight a novel function of Sonic Hedgehog (Shh) signaling unrelated to cancer, and provide a new relevant animal model to investigate the molecular pathogenesis of cataract formation.

Topical anti-inflammatory drugs against oxidative stress-induced tissue damage

N6-isopentenyladenosine (i6A), a naturally occurring modified nucleoside, inhibits the proliferation of human tumor cell lines *in vitro*, but its mechanism of action remains unclear. Treatment of MCF7 human breast adenocarcinoma cells with i6A or with three synthetic analogs (allyl6A, benzyl6A, and butyl6A) inhibited growth and altered gene expression. About 60% of the genes that were differentially expressed in response to i6A treatment were also modulated by the analogs, and pathway enrichment analysis identified the NRF2-mediated oxidative stress response as being significantly modulated by all four compounds. Luciferase reporter gene assays in transfected MCF7 cells confirmed that i6A activates the transcription factor NRF2.

Assays for cellular production of reactive oxygen species indicated that i6A and analogs had antioxidant effects, reducing basal levels and inhibiting the H_2O_2- or 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced production in MCF7 or dHL-60 (HL-60 cells induced to differentiate along the neutrophilic lineage) cell lines, respectively. *In vivo*, topical application of i6A or benzyl6A to mouse ears prior to TPA stimulation lessened the inflammatory response and significantly reduced the number of infiltrating neutrophils. These results suggest that i6A and analogs trigger a cellular response against oxidative stress and open the possibility of i6A and benzyl6A being used as topical anti-inflammatory drugs. In particular, they might be an attractive approach to alleviate skin inflammation and oxidative stress-induced tissue damage caused, for example, by UV radiation, or to prevent UV-related skin tumors.



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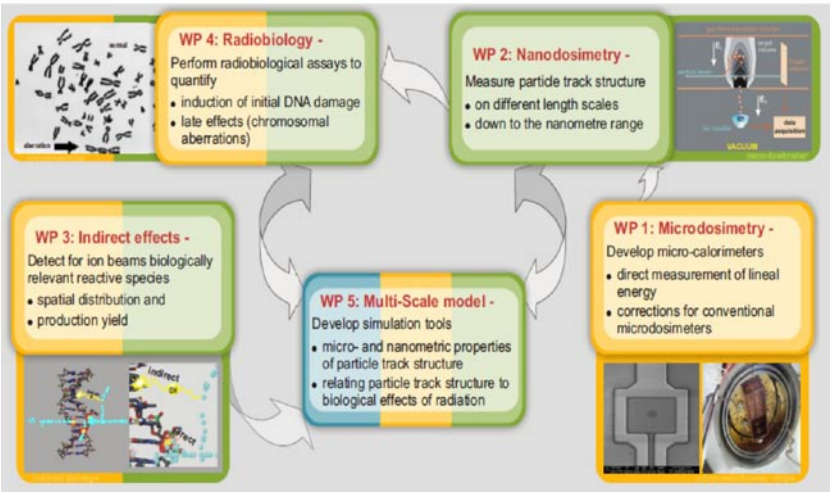


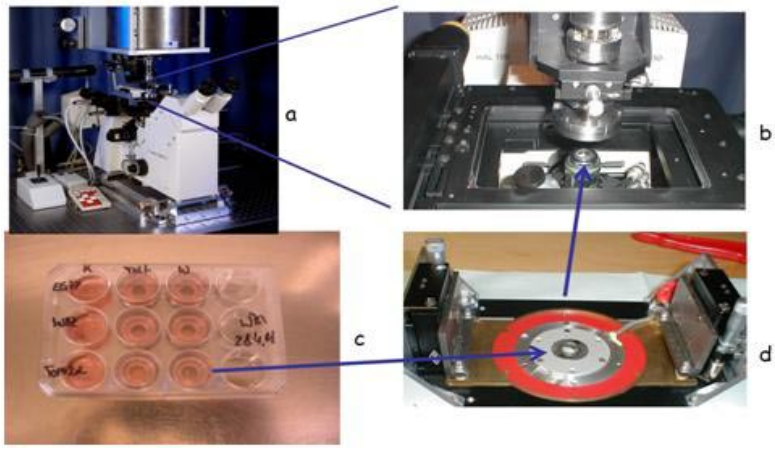
JRP-s04 BioQuart "Biologically weighted Quantities in Radio Therapy (project budget 3.5). By courtesy of H.. Rabus, 2011

Genotoxic effects induced by ionizing radiations of different qualities

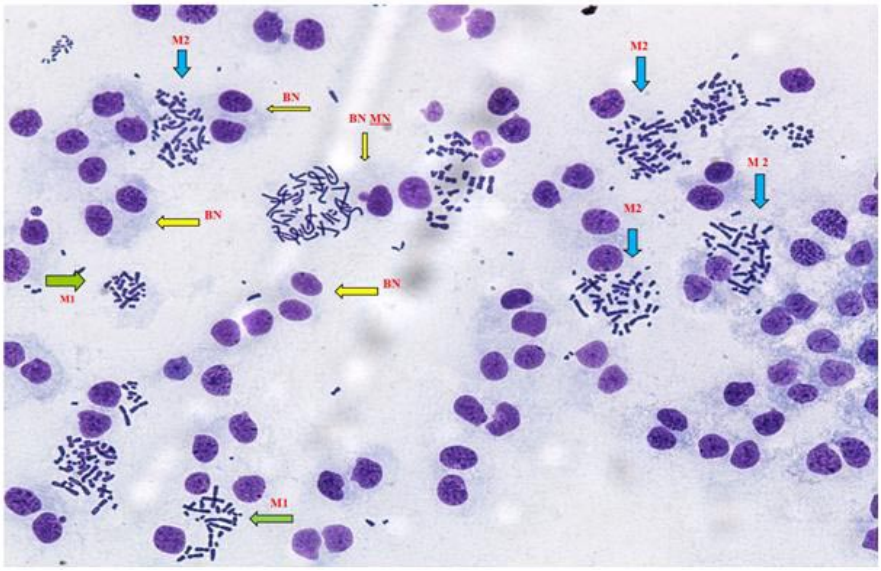
Our laboratory is a partner of the EMRP Joint Research Project "BioQuaRT" (Biologically weighted Quantities in RadioTherapy) with the Metrology ENEA Unit.

The aim of "BioQuaRT" is to develop measurement techniques for a multi-scale characterization of charged particle track structure that correlates, at the cellular level, the track structure properties with the biological effects of radiation qualities used in radiotherapy.

We are involved in "BioQuaRT" Radiobiology Work.Package (WP4) which is concerned in the production of biological data to be used as benchmarks to validate the multi-scale model developed in WP5. Our task is to evaluate the misrepaired and/or unrepaired chromosome damage induced by different qualities of charged-particle radiations, provided by a single-ion microbeam facility. For this purpose we chose to perform two radiobiological assays, the dicentric chromosome assay (CA) and the micronucleus test (MN) and we developed a novel in situ protocol on mammalian cells for the simultaneous scoring of CA and MN, adapted to the irradiation system in use at the Physikalisch-Technische Bundesanstalt (PTB) microbeam facility. This protocol, specifically developed for radiobiology experiments with single-ion microbeam, may be used for other radiobiological applications requiring in situ cytogenetic assays.



Microbeam and cell dishes at PTB facility (By courtesy of U. Green, 2011)

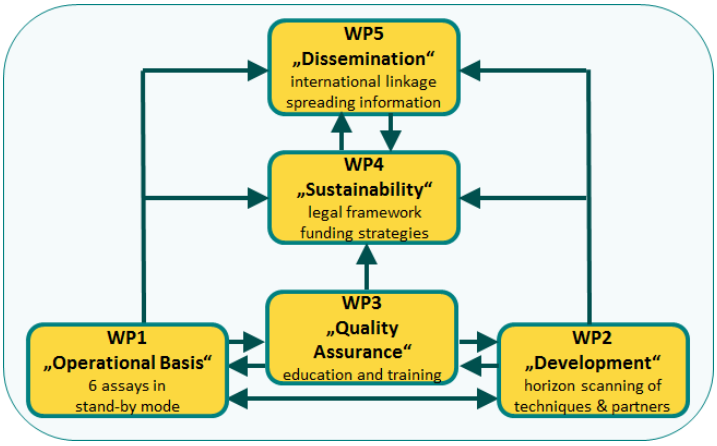


In situ CA and MN assay. M1, metaphase in tm cellhe first cell cycle; M2, metaphase in the second cell cycle; BN, binucleated cell; MN , micronucleus

Biological Dosimetry

Our laboratory is part of the European network "RENEB" (Realizing the European NEtwork of Biodosimetry), a FP7 - EURATOM project for biological dosimetry, created to strengthen the emergency preparedness and response capabilities in case of a large-scale radiological accident or radiological emergency. "RENEB" involves 23 experienced laboratories from 16 European countries that will establish a sustainable network for rapid, comprehensive and standardised biodosimetry provision that would be urgently required in an emergency situation on EU ground.

Geographic distribution of laboratories involved in RENEB



RENEB Structure

The foundation of the network is formed by five main pillars, 1) the ad-hoc operational basis, 2) a basis of future developments, 3) an effective quality management system, 4) arrangements to guarantee the long-term sustainability and 5) awareness of the existence of RENEB. RENEB will thus provide a mechanism for quick, efficient and reliable support within the European radiation emergency management. The scientific basis of RENEB will concurrently result in contributions to increased safety in the field of radiation protection.

Bioelectromagnetics

Medical Applications

Research activities on medical applications of EM fields are addressed to radiofrequency (RF) and microwave (MW) thermal ablation for clinical therapy, to emerging applications of magnetic nanoparticles for drug delivery, and to RFId technologies for healthcare applications.

RF/MW thermal ablation

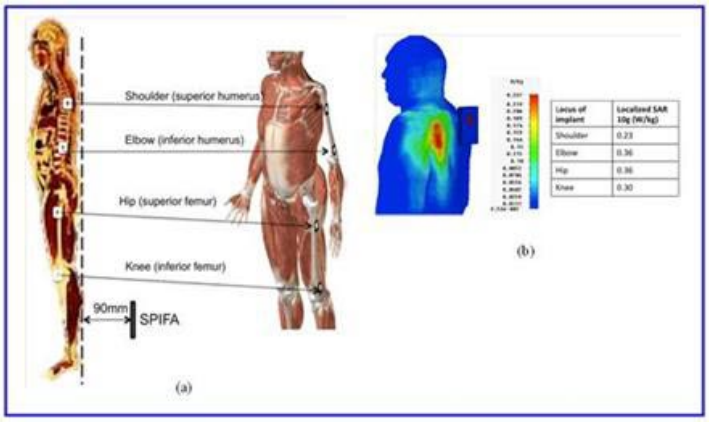
RF/MW thermal ablation is an EMF-based therapeutic technique offering the possibility of destroying relative large tissue areas with minimally invasive applicators, exploiting the irreversible cellular damage due to high temperature heating induced by the absorption of EM energy. The research activity on thermal ablation is focused both on the experimental investigation of the changes in the dielectric and thermal properties of tissues, and on the study of morphologic changes induced by tissue contraction associated with the very high temperatures reached during ablation procedures. This activity is supported by a scientific collaboration with a biomedical company (HS Hospital Service S.p.A.), DIET Dept. of University of Rome "La Sapienza", IRE Italian National Cancer Institute, and the Hadassah Hebrew University Medical Centre, Israel.

Magnetic drug delivery

The BioEM Group cooperates with the DDCT and DIET, University of Rome "La Sapienza, in the characterization of drug delivery systems for disease treatment based on magnetoliposomes (MPLs). A coil system was designed to test the capability of MLs to respond to an alternating magnetic field in the kHz range.

RFId technologies

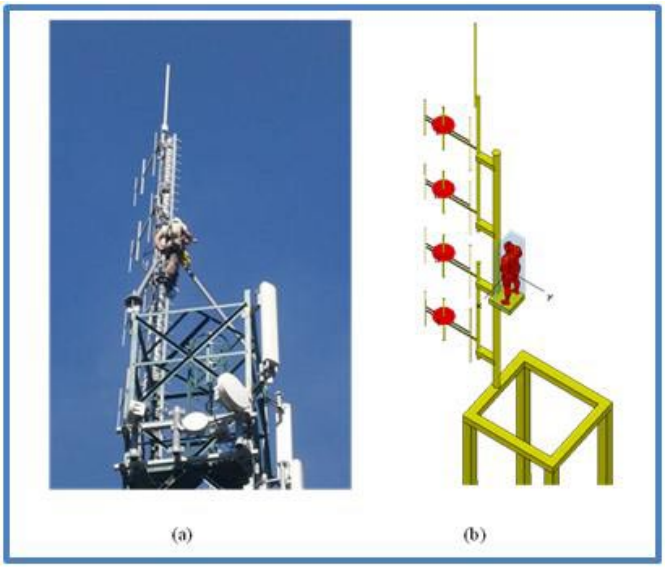
RFId technology is a challenging application for monitoring of tumours, which cannot be surgically treated, for real-time acquisition of physiological parameters on the pathology evolution. The feasibility of direct and forward links for UHF-RFID (860 – 960 MHz) tags, implanted into human limbs, was investigated in collaboration with the DISP, University of Rome "Tor Vergata". Performance gain indicators of through-the-body RFId channel were numerically evaluated in an anthropomorphic phantom, and experimentally validated by developing a real RFId communication link in a simplified *in vitro* setup.



(a) Considered position of the loop tags over the bones; (b) SAR distribution produced in shoulder by the reader's antenna, and table of localized SAR averaged over 10 g of tissue. Lodato et al, 2014

Exposure assessment of EM fields in occupational environments

The European Parliament and the Council of the European Union have recently approved the Directive 2013/35/EU on the minimum health and safety requirements regarding the exposure of workers to the risks arising from EM fields. The Directive establishes exposure restrictions articulated in exposure limit values (ELVs) and action levels (ALs): radiometric measurements (for ALs) and dosimetric analysis (for ELVs) are the tools to assess compliance with the restrictions. Industrial and medical applications represent a critical issue in occupational exposure due to the employed EM powers, the lack of standardized procedure for exposure assessment, and the large number of workers involved.



(a) multi-frequency occupational exposure scenario; (b) simulation model

BioEM Group is involved in funded research projects on the evaluation of health and safety risks related to the exposure of workers to EM fields, with particular reference to hospital environments (MRI tomography and electrosurgical units), in collaboration with INAIL and CNR-IFAC, and industrial applications (wireless power transfer systems (WPT) in collaboration with DIE, University of Padova, and to broadcast and cellular network systems, in collaboration with Tecnorad Italia S.p.A. Measurement procedures and methods of data analysis were set up to assess the compliance of EM occupational exposure. Measurement surveys were conducted at several hospitals in Rome considering different MRI scanners (1.5 and 3.0 T, partial and whole-body) and electrosurgical units.

With reference to industrial applications, a bench prototype of WPT was characterized; emitted stray EM fields were measured to assess the exposure levels of

workers, operating in proximity of the prototype. In the future, stray EM fields will be characterized in an actual exposure scenario, with the WPT system mounted on a city car. Moreover, a case study was investigated to assess the occupational exposure to multiple frequencies EM sources from broadcast and cellular network stations; radiometric measurements were performed to characterize the exposure scenario, which was numerically simulated to calculate the power absorbed per unit of mass in a numeric anthropomorphic model; compliance with relevant ELVs was assessed in terms of an index of compliance.

Exposure systems

In the last years, great efforts have been addressed to the experimental research on thermal and non-thermal effects on the frequencies of social interest, such as the extremely low frequencies (ELF) used for power lines (50/60 Hz), the intermediate frequencies (IF) with particular reference to those of gradient fields from MRI scanners and of industrial equipments, as well as the radiofrequency (RF) and microwave (MW) frequencies typical of wireless communications (GSM, UMTS, WiFi). In this framework, BioEm Group designed and realized specific controlled exposure setups according to the international guidelines for quality in research.

Recently, two different *in vitro* exposure systems were developed. The first setup is constituted by a couple of square coils providing a uniform magnetic field exposure volume of $21 \times 21 \times 11$ cm^3. This system can be used either for ELF exposure to power lines magnetic fields (50 Hz) or for exposure to non-sinusoidal magnetic fields from actual IF sources as, for instance, of MRI scanners; the system can fit into a standard incubator to perform *in vitro* experiments with appropriate environmental conditions. A second set up was designed to expose biological samples to selected levels of static magnetic fields. The system consists in a magnetic circuit, constituted by two neodymium-iron-boron permanent magnets at a fixed distance according to the required magnetic field intensity; a similar structure, but without magnets, was realized to achieve sham exposure in blind way. The static magnetic field exposure system will be used to mimic chronic exposure of vegetable cultures in a challenging environment, as the habitat of a spacecraft in presence of active magnetic shielding.

Immunology

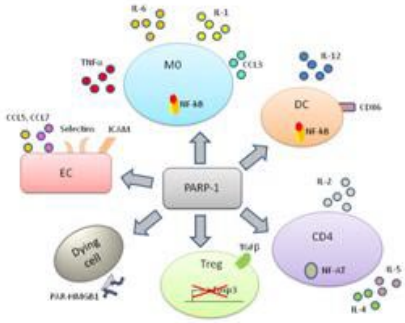
ImmuReg - Molecular and cellular mechanisms of IMMune-REGulation: Seeking new therapeutic targets

Immune-mediated diseases, including inflammatory diseases, autoimmunity, allergy and other hypersensitivities, have a dramatic effect on the quality of life of the affected individuals and a huge socio-economic impact, representing one of the major costs for therapies in western countries. A better understanding of the regulatory mechanisms involved in these diseases, with particular reference to mechanisms common to several disorders, would allow to identify new therapeutic targets with great benefits for patients and for the socio-economic burden.

T cells, which represent a key component of immune response, need to recognize and fight pathogens while remaining tolerant to host molecules, harmless commensal microorganisms and food antigens. Differentiation and functions of effector helper T (Th) cells are kept under control by a specific T cell subset known as regulatory T cells (Treg), which prevent chronic inflammation and maintain tolerance. Alterations in regulatory T cell differentiation and functions lead to over-exuberant responses and are associated with immune-mediated diseases.

In recent years, an increasing series of results has shown that the ADP-ribosylation plays an important role in inflammatory processes and in the immune response. The enzyme PARP-1 (poly(ADP-ribose)polymerase-1), also known for its role in DNA damage recognition and repair, synthesizes and binds branched polymers of ADP-ribose to target proteins including the same PARP, histones, proteins for DNA repair, transcription factors and epigenetic regulators of chromatin. PARP-1 regulates the transcription of several genes in cells of innate and acquired immunity. PARP-1 modulates the expression of several inflammatory cytokines, is activated during stimulation of T cells, and plays an important role in the differentiation of T and B lymphocytes as demonstrated also by our group.

Although other factors are also involved, the expression of the transcription factor Foxp3 is required for the differentiation of Treg cells and the maintenance of their inhibitory function. The absence of a functional Foxp3 protein determines the scurfy phenotype in mice and the IPEX in humans. Recently we have found that PARP-1 inactivation resulted in increased regulatory CD4+CD25+/Foxp3+ T cells in thymus, spleen and lymph nodes. More striking, purified naïve CD4 cells from mice carrying PARP-1 null gene (knock out, KO) when stimulated *in vitro* expressed Foxp3 mRNA at higher levels and generated higher numbers of Foxp3+ cells (inducible Treg cells) than the WT counterpart. *In vitro*, PARP-1 KO Treg cells were able to suppress cell proliferation and cytokine production in freshly isolated CD4+CD25- cells as well as in differentiated effector Th1 and Th2 cells. *In vivo*, transplanted PARP-1 KO Treg cells protected from the graft versus host disease MHC-mismatched bone marrow chimeras that received allogenic T cells. Preliminary results performed with PARP-1 enzymatic inhibitors confirmed the findings found in PARP-1KO mice. Currently, we are investigating how PARP-1 regulates Foxp3 expression and how its activity can be targeted for therapeutic purposes.



In our Laboratory several techniques are used to assess therapeutic and toxic effects of pharmaceutical products. In the ADCC assay, antibodies specifically recognizing antigens expressed on tumor cells activate NK cells for tumor killing, confirming their functional integrity.

ImmuNIR - Effects of Non-Ionizing Radiation on development and functions of the immune system

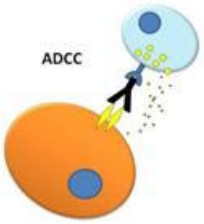
The immune system protects the organisms from invading pathogens by carrying out effective not harmful responses. It develops during embryogenesis and completes its maturation after birth, becoming fully competent during infancy. Perturbing factors such as toxic compounds and ionizing radiation can compromise the integrity of the immune system, leading to ineffective responses or to immune-mediated diseases.

Wireless local area networks are an increasing alternative to wired data networks in workplaces, homes, and public areas. Concerns about possible health effects of this type of signals, especially when exposure occurs early in life, have been raised.

We study the potential effects of radiofrequency (RF) electromagnetic-field (EMF) exposure on the immune system in animal models. Our studies investigated the effects of the exposure to GSM-modulated 900 MHz EMF on peripheral lymphocytes, B cell differentiation and antibody production), and on generation of lymphocytes from bone marrow cells. More recently, we extended our researches to the effects of 2.45 GHz WiFi signals. In particular, we studied the early and late effects of pre-natal exposure on the development of the immune system during embryo life. The exposure period included all the phases of de novo hematopoietic cell generation in the embryo. Moreover, we also analyzed the effects of post-natal early life (childhood) exposure on maturation of the immune system. Our results on the exposure during perinatal life represent the first studies on the effects of WiFi signals in such a critical developmental phase. In all of our studies several parameters on lymphocytes from central and peripheral lymphoid organs were analyzed. Animals were exposed to several SAR levels, including levels much higher than those established for human exposure. Data on cell proliferation, phenotype, antibody production, cytokine production, activation markers and others were analyzed.

ImmuTransfer - Development of immunological assays to assess functional integrity, activity and immunotoxic effects of biotech products.

Generating new knowledge and turning it into new products and services is crucial to maintain and enhance competitiveness. In the context of collaborative partnerships, our group has provided and provides support to companies in the assessment of therapeutic and/or toxic effects of candidate pharmaceutical products targeting immune functions. We complement the assessments of immuno-therapeutics with already optimized *in vitro* and *in vivo* assays and models or sustaining the development of new assays with our knowledge and technical expertise.



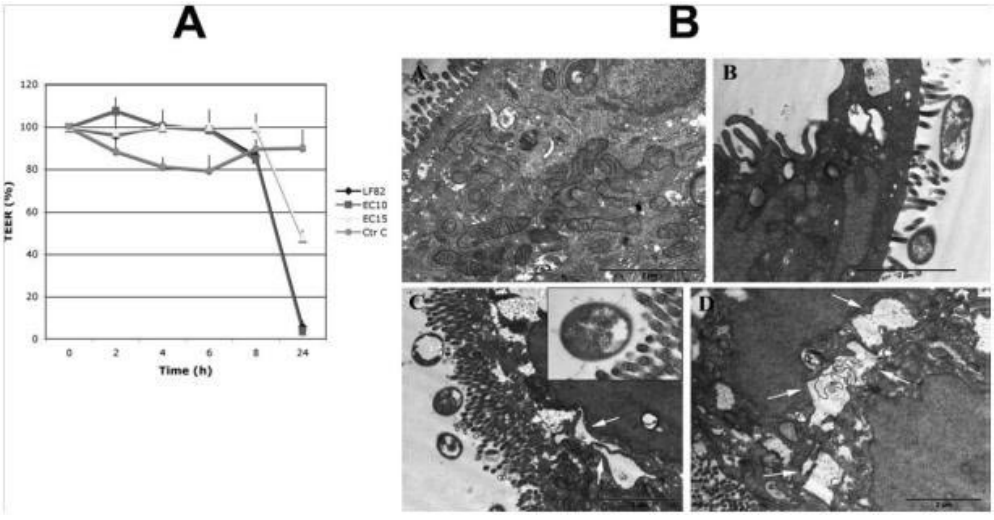
PARP-1 plays a relevant role in inflammatory/immune responses and may represent a new potential therapeutic target. We found that PARP-1 dampens regulatory T (Treg) cell differentiation. Alterations in Treg cell functions lead to several immune-mediated diseases, including autoimmunity, allergy, type 1 diabetes and rheumatoid arthritis.

Immunity and inflammation

Our studies are focused on investigating the relationship between innate immune response and chronic inflammatory processes. For this purpose, we use as a reference model the intestinal inflammation, in particular the Inflammatory Bowel Disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), as well as other immune-mediated enteropathies.

Host-microbiota interactions:

The main goal of this topic is the study of the interaction between the intestinal mucosa-associated bacteria and the innate immune response in IBD patients with different disease activity, grade and phase. In particular, we focused on the intestinal population of Escherichia coli (E. coli), which is strongly increased in IBD patients, and more specifically on a subgroup of E. coli, called AIEC (adherent invasive E.coli), able to adhere and invade the intestinal epithelium, triggering the inflammatory response. We isolated and characterized from the intestinal mucosa of CD and UC pediatric patients several strains of E. coli with peculiar features of adhesiveness and/or invasiveness; in particular, we isolated 2 AIEC strains and 12 adhesive strains, that are currently used to induce an inflammatory immune response in our experimental models.



A: *AIEC strains are able to strongly reduce the epithelial barrier functions and cause morphological alterations.* ***B****: TEM analysis in control CACO2 cells (A) and in AIEC infected CACO2 cells (B-D).*

Innate immune response and inflammation

It is now well accepted that an altered innate immunity, unable to effectively eliminate bacteria penetrating the mucosa, forms the basis of pathogenetic mechanisms leading to IBD. Moreover, excessive or misplaced epithelial cell death can result in barrier dysfunction and uncontrolled translocation of components of the microbial flora from the lumen into the bowel wall.

Our goals are to study *in vitro*, *ex vivo* and *in vivo* the role of several components of the innate immune response and programmed cell death during inflammation.

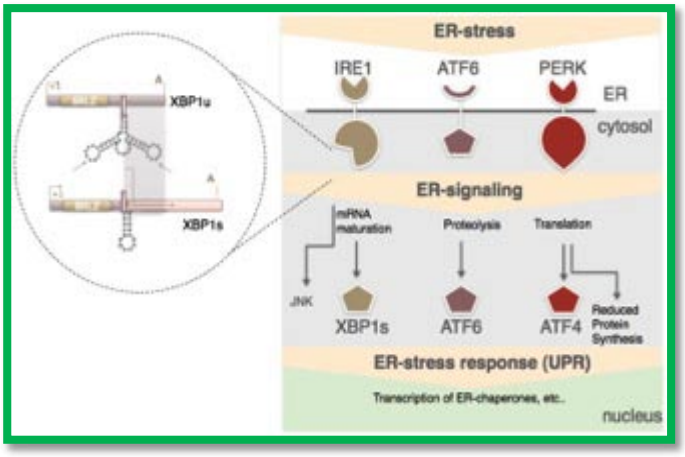
In particular, proteins regulating the following signaling are focused:

bacterial sensing (NOD-like receptor (NLRs) family–Toll like receptors (TLRs) family;

Autophagy (ATG16L1, LC3);

Programmed cell death (RIP3, mixed lineage kinase domain–like-MLKL);

Endoplasmic Reticulum (ER) stress/Unfolded Protein Response (UPR) (IRE1-XBP1, PERK-ATF4, ATF6p90-ATF6p50).

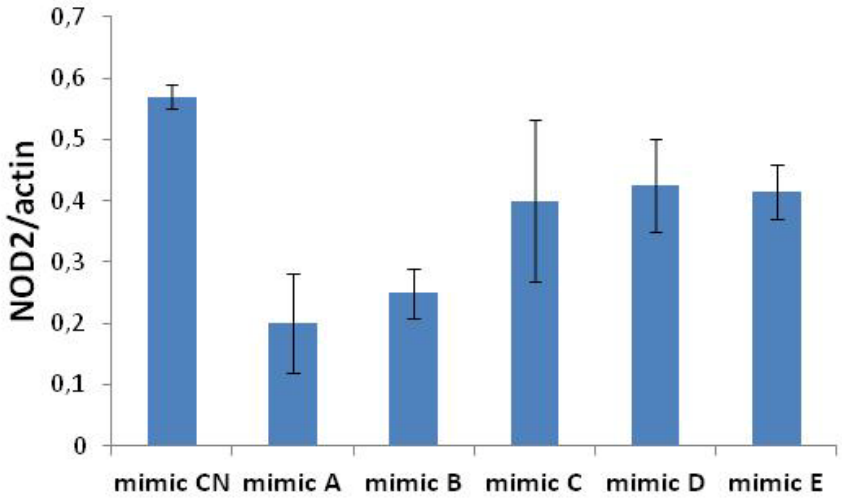


A: UPR signaling pathways. B: UPR signaling pathways (IRE-XBP1 and ATF6-PDIA4) are significantly over-expressed in the inflamed colonic mucosa of IBD patients. UC:ulcerative colitis; CD:Crohn's disease; Uninfl: uninflamed; Infl: inflamed.

UPR Pathways:	Uninfl Colon UC	Infl Colon UC	Uninfl Colon CD	Infl Colon CD
IRE-XBP1	-	+	-	+
ATF6-PDIA4	-	+	-	+
PERK-ATF4	-	-	-	-

We recently demonstrated that NOD2 overexpression activates autophagy, a cellular self-digestion and bacteria killing process that may lead to either cell death or survival. We also showed that necroptosis, a caspase-independent programmed cell death, is strongly represented in the inflamed intestinal mucosa of pediatric patients with IBD, confirming that it is a main form of inflammatory cell death.

Finally, we showed that UPR (unfolded protein response), an adaptive response focused on resolving the ER stress, is significantly increased in the inflamed colonic mucosa of pediatric patients with IBD and may be involved in the disease pathogenesis.



NOD2 expression is down-regulated by miR320-family members

miRNAs and inflammation

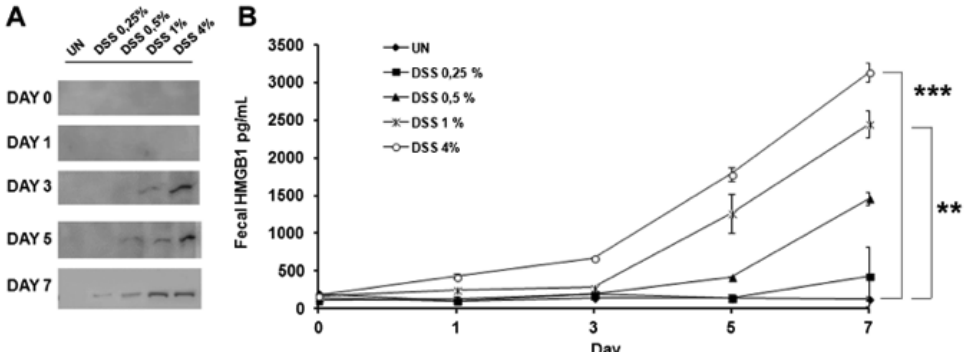
There are several evidences that miRNA-mediated gene control plays a crucial role in the breaking-down of intestinal immunological homeostasis and in the development of chronic inflammatory disorders.

We have recently showed that miR-320 family targets NOD2 expression and that, during inflammation, miR-320 down-regulation leads to the increase of NOD2 expression. Currently, we are focusing on other targets of miR-320 involved in inflammatory pathways

Non-invasive biomarkers of intestinal inflammation

The identification of non-invasive biomarkers (serological or fecal) of inflammation is essential for clinical assessment and management of human inflammatory diseases involving the gut. Hence, we previously identified the alarmin high mobility group box 1, (HMGB1), as a novel fecal biomarker of intestinal inflammation.

More recently, we showed in vivo that HMGB1 may be also used as a marker of sub-clinical intestinal inflammation. Currently, other inflammatory intestinal markers are under investigation



HMGB1 is a reliable marker of high- and low-grade intestinal inflammation in mice with a DSS-induced colitis. A: Immunoblotting B: ELISA

Natural compounds for solutions to human health problems

The activity is dedicated to the solution-oriented approach of applying Neem (Azadirachta indica (A. Juss.) derived compounds (NDC) to various problems that directly or indirectly impact on either human/animal health or the environment.

In the past the R&D activity led to the development of an innovative wound healing dressing, based on Neem and St John's Wort (Hypericum perforatum (L.)) oil extracts. A patent was obtained (Patent family: EP1773365) which has been further developed by Phytoceuticals Ltd. (CH), together with this research unit, and is now commercialized under the name of "1 Primary Wound Dressing®" (1PWD®) as a medical device of class 2b.

The researchers also contributed significantly to the development of a Neem-based preparation for the control of ecto-parasites (lice, fleas and ticks) in both production and affection animals and humans. Trifolio-M GmbH (DE) commercializes these products (NeemPro®Tierpflege and Neem Extrakt Shampoo). Other interesting problems addressed by this unit were (i) fertility control in pigeons (ii) vector control of various disease spreading insects (Malaria etc…) (iii) control of endo-parasites in production animals.

Ongoing R&D activities focus on: (a) further development of application protocols for 1PWD® both in humans (acute and chronic wounds) and animals; (b) application of NDCs to the control of Varroatosis and Colony Collapse Disorder (CCD) in bees (Apis mellifera); (c) solving extraction, purification and analytical problems of natural compounds for other research units of our organization (ENEA).

Development of application protocols for the medical device "1 Primary Wound Dressing®"

In collaboration both with Medical and Veterinary Institutions protocols have been developed, or are under development, for the application of 1PWD® on pediatric burns, chronic wounds (venous ulcers, diabetic foot, etc) and acute wounds (bed sores, burn wounds, dehiscence, traumatic wounds, etc.) in humans.

An observational study at the CTO Hospital of ROME (IT) on the efficacy of 1PWD® is running since 2 years. During this study, two dissertations detailing the use of the medical device 1PWD® were produced.

In the veterinary field, together with the Veterinary Faculty of Perugia, a protocol is being developed for the use of 1PWD® for treating pyoderma in dogs thus avoiding the use of antibiotics and cortisone. In horse, protocols have been developed for the treatment of simple wounds and those presenting with keloid formation. As for the repellent and biocide effects associated with 1PWD®, a study for the treatment of cutaneous myiasis of pets and production animals, especially on sheep in organic production that cannot be treated with synthetic chemical compounds such as pesticides, is ongoing. The results will be used for a specific thesis and publication shortly.

Application of NDCs to the control of Varroatosis and Colony Collapse Disorder (CCD) in bees (Apis mellifera)

Modern beekeeping has been facing, in the last 30 years, two major challenges that are seriously threatening bee colony survival. The first is the spreading from Asia of the Varroa destructor honeybee mite, which started in the late 1970's and beginning 1980's and is known as Varroatosis. The second threat to colony survival is called Colony Collapse Disorder (CCD), which has also been observed on a worldwide scale. Since three years, in very close collaboration with the "Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana" (IZSlt) of Rome, both *in vitro* and *in vivo* experiments are being carried out in order to evaluate the possibility to control Varroatosis through

the application of NDCs. *In vitro* results confirmed that NDCs could be a valid biocide towards the Varroa destructor honeybee mite. The challenging problem still researched by us is how to translate these preliminary results to an economically feasible "in the field" application.

Solving extraction, purification and analytical problems of natural compounds for other ENEA research units

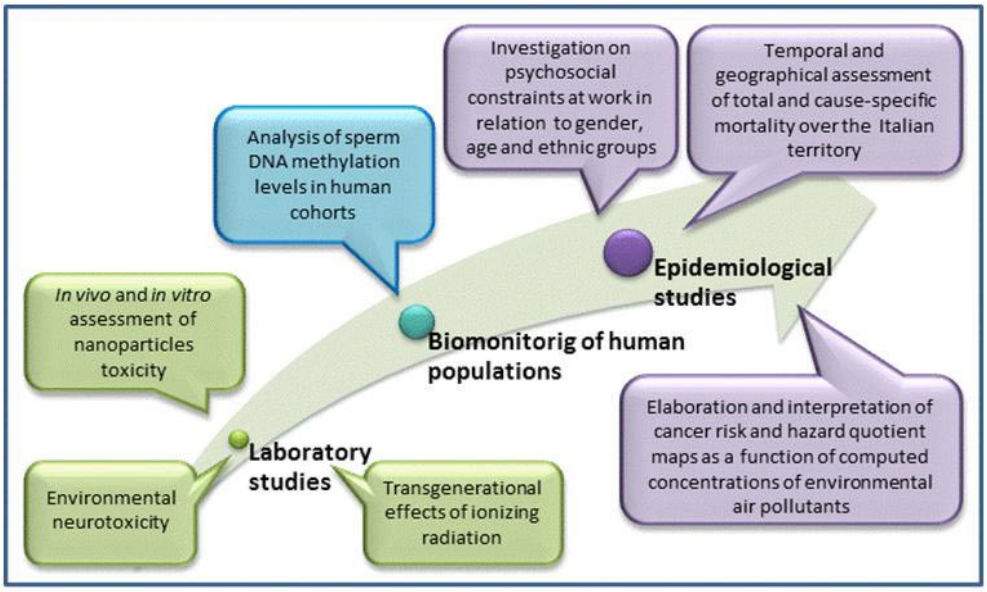
In our 25 years long research on Neem and its constituent compounds (both in cell culture as from the whole plant) our expertise in natural compound extraction, purification, identification and formulation is precious to other units within our organization. One collaboration is on the measurement of inulin content in hydroponic grown artichoke roots while a second collaboration is on the measurement (analytics) of fermentable sugars in hay biomass of triticale for bio-ethanol production

4. UTBIORAD TOSS

In the Laboratory of Toxicology, Technical Unit Radiation Biology and Human Health of ENEA, in vitro and in vivo experimental methods and models are developed, standardized and applied for characterization of health hazards of environmental, occupational or therapeutic exposures.

oxidative stress are assessed, following treatment of cultured cells or experimental rodents with potentially noxious agents.

Ionizing and non-ionizing radiation, foodborne toxins, endocrine disrupting chemicals, nanoparticles are among the agents for which dose-effect relationships are defined under various treatment conditions, to contribute to their risk assessment.



Laboratory of Toxicology: diagram of main research activities

Quantitative, single-cell, analyses of toxicity biomarkers are carried out by microscopy- and flow cytometry-based analytical cytology methods. Molecular biology techniques are used to elucidate mechanisms of toxicity. Cell death, DNA damage, gene mutations, chromosome alterations, epigenetic changes potentially impacting on gene expression, activation of damage-induced response pathways,

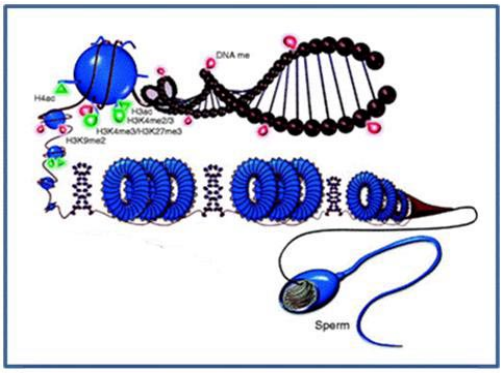
A specific focus is on the assessment of adverse effects on the reproductive system, (epi)genetic changes in male gametes and mechanisms of epigenetic inheritance. In collaboration with other laboratories in Europe and non-European countries, biomonitoring studies in human populations are conducted, measuring markers of (epi)genetic alterations in human semen that are related with individual exposure biomarkers like serum contaminant levels.

Increasing efforts are invested in the recently emerging field of nanotoxicology. In the frame of European funded projects, and national and inter-Unit collaborations, cellular uptake and translocation, mechanisms of damage and effects of nanoparticles, well characterized at a physico-chemical level, are assessed.

Environmental risk factors of neurodegenerative processes are investigated in primary cultures, established cell lines and mice, with emphasis on Parkinson's Disease. Possible effects of electromagnetic fields on the physiology of brain cells are explored to evaluate both their potential toxicity and clinical applications.

Finally, in order to characterize the health status of human populations living in polluted areas, to formulate hypotheses on specific risk factors, to monitor temporal trends of total and cause specific mortality and to pinpoint sites with excess of rare diseases, epidemiological studies are carried out by means of the ENEA Epidemiological Database, shared with the Technical Unit Environmental Technologies. It includes all the Italian mortality data, at municipal level from 1980 and at provincial level from 1969, recorded by the National Institute of Statistics (ISTAT), the VIII, IX and X International Classifications of Diseases and the decennial census populations, used as denominators to calculate some epidemiological indexes.

In the area of occupational health, psychosocial constraints at work are investigated in relation to age, gender and ethnic group.



Epigenetic landscape in sperm (modified from: Denomme and Mann. Reproduction 2012, 144: 393-409)

Reproductive toxicology and germ cell mutagenesis

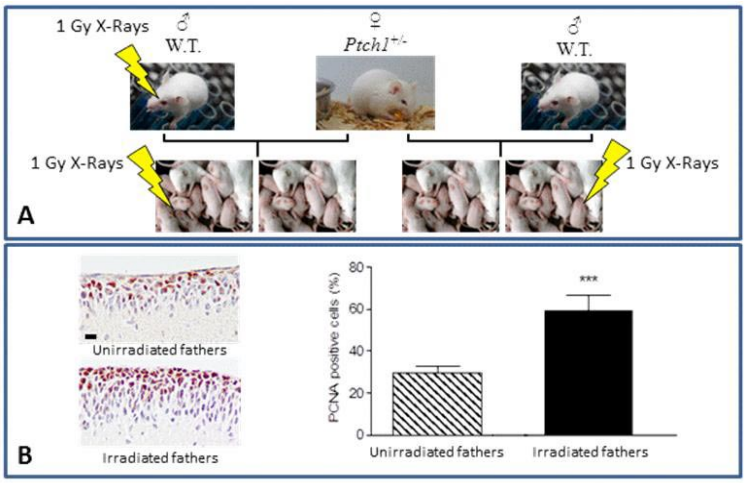
In the framework of the recently concluded European project "Climate change, environmental contaminants and reproductive health" (CLEAR), the Laboratory carried out an analysis of sperm DNA methylation levels in a cohort of 269 healthy men of proven fertility from Greenland, Warsaw, and Kharkiv. DNA methylation was quantified in repetitive DNA sequences (LINE-1, Satα and Alu) by PCR pyrosequencing after bisulfite conversion, and, at a global genome level, by flow cytometric detection of immunolabelled 5-methylcytosine. Possible correlations of individual measurements with body mass index, semen quality parameters, sperm chromatin integrity, biomarkers of accessory gland function, plasma concentrations of reproductive hormones, plasma levels of perfluoroalkyl substances (PFASs), were tested. After multivariate linear regression analysis, the geographical location emerged as the main determinant of the

methylation level in repetitive sequences, whereas no major consistent associations between PFASs exposure and sperm DNA global methylation endpoints could be detected. While there are published data showing effects of chemical exposure and metabolic alterations on sperm DNA methylation in experimental animals, at present, this is the only study that has been conducted in human populations to investigate possible lifestyle and environmental influence on sperm DNA methylation.

A more detailed characterization of the potential environmental signatures on the sperm epigenome of Greenland samples will be carried out within the framework of the international project "Father's lasting influence: Molecular foundations of intergenerational transmission of the paternal environment'' coordinated by Laval University (Quebec City, Canada) and funded by Canadian Institutes of Health Research.

In 2014, a study was completed aimed at evaluating possible epigenetically mediated transgenerational effects of ionizing radiation. The study was conducted in collaboration with the Laboratory of Radiation Biology and Biomedicine. Tumour-susceptible mice, heterozygous for a null mutation of the *Ptch1* gene, were sired by fathers exposed to an acute dose of ionizing radiation during the stage of spermatogonial proliferation, and were tested for the spontaneous incidence of medulloblastoma and for the incidence of tumours induced by neonatal irradiation. It was shown that paternal irradiation did not influence the spontaneous tumour incidence, but induced in the F1 progeny an increased susceptibility to radiation-induced medulloblastoma. An alteration of the switch between the proliferation and differentiation pathways in the neonatal cerebellum of the progeny of irradiated mice was shown that was consistent with a significantly accelerated progression of preneoplastic lesions. Conversely, no effect of paternal irradiation was evident on DNA damage induction and repair in cerebellar cells. Although the effect on cell proliferation appeared insufficient to trigger spontaneous tumour formation, synergistically with a postnatal radiation stress it was presumably responsible of the increased incidence of induced medulloblastoma. Further studies are in progress to characterize possible modifications of the epigenetic profile of cerebellar cells in the progeny of irradiated male mice and to unravel the transgenerational molecular messengers in sperm.

In the frame of an OECD sponsored initiative aimed at drawing up scientifically based Adverse Outcome Pathways (AOP) as novel tools in toxicology and human risk assessment, in collaboration with the Environmental Health Science and Research Bureau of Health Canada, the Department of Pharmaceutical Sciences, Università degli Studi del Piemonte Orientale, Novara, and the Department of Biology, Università di Padova, the laboratory expertise in germ cell mutagenesis is being spent in the preparation of an AOP on aneuploidy induction in mammalian oocytes by microtubule depolymerizing agents.

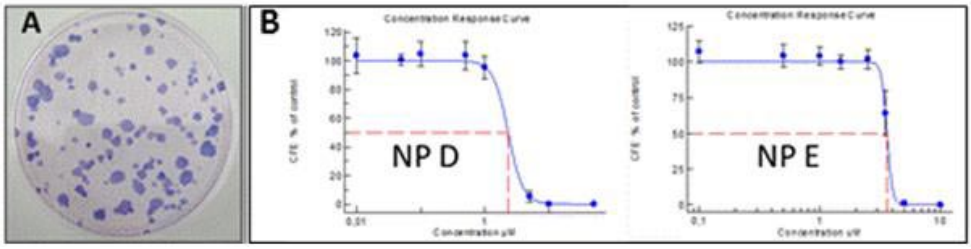


Transgenerational radiation effects on spontaneous and radiation-induced cancer susceptibility. A) Experimental design. B) Higher percentage of proliferating (PCNA positive) cells in cerebellum of the progeny of irradiated fathers. Study in collaboration with the Laboratory of Radiation Biology and Biomedicine

Nanotoxicology

During 2014, the Laboratory of Toxicology participated to an interlaboratory comparison study of the Colony Forming Efficiency (CFE) cytotoxicity assay performed under the auspices of the OECD Working Party on Manufactured Nanomaterials, and coordinated by the European Commission's Joint Research Centre (JRC). The project, started in 2012, was concluded in 2014. A total of twelve laboratories from Europe, Japan, South Korea and South Africa participated in this work. A positive control (sodium chromate) and 9 nanomaterials of different size and/or chemical composition were tested.

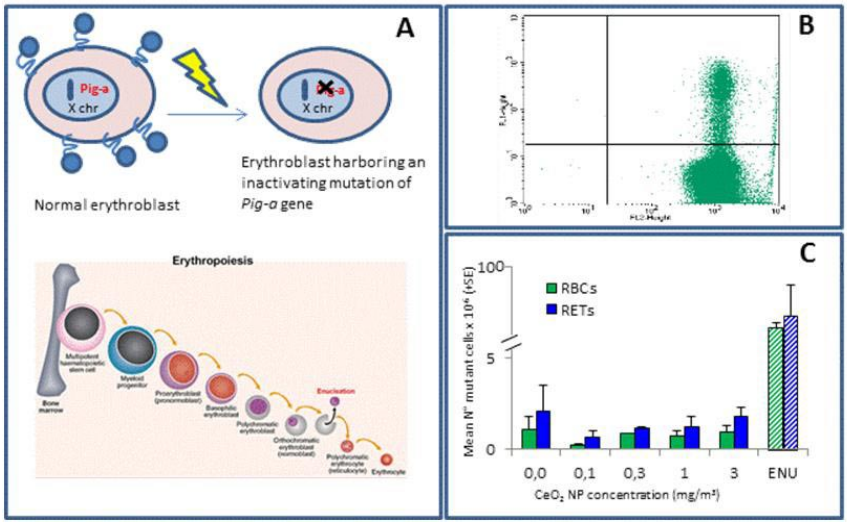
The study demonstrated that the CFE assay protocol is reliably transferable across laboratories. The intra-and inter-laboratory reproducibility of the CFE assay was good. It was concluded that the CFE assay is a suitable early screening method to assess cytotoxicity of nanomaterials, with several advantages over the conventional cytotoxicity assays, as it avoids test interferences caused by the physical presence of insoluble nanoparticles. It could be also used in combination with other in vitro assays (e.g. genotoxicity in vitro assays) to define the subtoxic doses.



The Colony Forming Efficiency assay. A) A typical dish. B) Concentration response curves obtained in a canine kidney cell line with 2 Ag NPs of different nominal sizes (D: 30 nm; E: 20 nm)

In the framework of the European funded Project "A common European approach to the regulatory testing of nanomaterials" (NANoREG), the Laboratory performed single-cell based analyses on rat blood cells, to assess possible systemic genotoxic effects of long-term inhalation exposures to CeO_2 nanoparticles and 90-day oral administrations of SiO_2 nanoparticles.

Three complementary tests, the comet assay, the *Pig-a* assay and the micronucleus test, respectively detecting DNA damage, gene mutations and chromosome aberrations, were used. The animals were exposed to CeO_2 nanoparticles at the BASF laboratories in Germany and to SiO_2 nanoparticles at the National Institute of Health in Rome.



Pig-a assay to assess nanoparticles genotoxicity in rodent blood cells. A) Pig-a as a reporter gene of in vivo somatic cell mutation. B) Representative flow cytometric bivariate plot; C) results obtained after chronic inhalation exposure to different concentrations of CeO_2 nanoparticles or to ethylnitrosourea used as a positive control.

Blood samples were then delivered to the ENEA laboratory for the subsequent analyses. All tests reliably detected the effects of ethylnitrosourea used to demonstrate the laboratory proficiency. Conversely, the results collected so far in the blood samples from nanoparticle-exposed animals did not show any evidence of genotoxicity. Assessment of the biodistribution of nanoparticles to distal organs is in progress in the partner laboratories, which will be instrumental for the interpretation of genotoxicity data.

The activities proceeded within the ECOFIBAR research project aimed at testing the biological safety of a novel basalt fiber-based composite material.

Scanning Electron Microscope images of basalt fibers. A) Whole basalt fiber. B) Fine sized basalt fibers after grinding process. C) Ultra-fine sized basalt fibers after grinding process

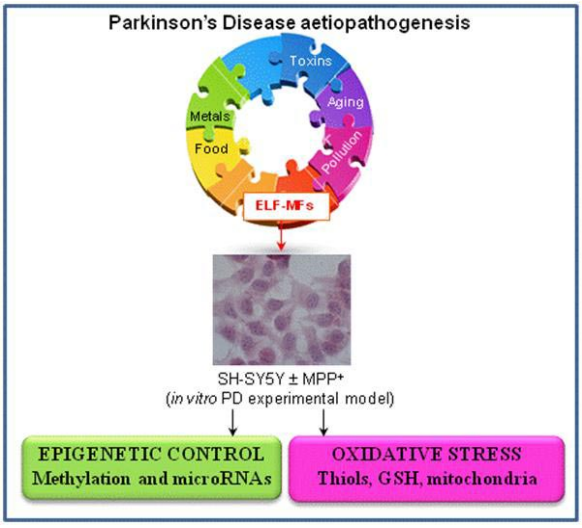
The project is carried out in collaboration with the Technical Unit for Technologies Development, and is funded by the National Operative Programme for Research and Competitiveness of the Italian Ministry of Education, Universities and Research. In particular, the methodologies for material characterization and sample preparation for biological testing were set up, in collaboration with the analytical chemistry expertise of the Quality of Chemical and Biological Measurements Group of the Technical Unit for Sustainable Development and Innovation of Agro-Industrial System

Environmental neurotoxicology

An association has been suggested between Extremely Low Frequency Electromagnetic fields (ELF-MF) exposure and neurodegenerative diseases, particularly Amyothrophic Lateral Sclerosis and Alzheimer's Disease (AD), whereas for Parkinson's Disease (PD) the evidence is less convincing. In the Laboratory of Toxicology, a project is carried out in collaboration with Dr. Giuseppe Filomeni (University of Rome Tor Vergata, Danish Cancer Research Center, Copenhagen) aiming at assessing the effect of prolonged exposure to ELF-MF (50 Hz, 1 mT) on an in vitro PD cellular model. The model is that of SH-SY5Y neuroblastoma cells treated with MPP+, a neurotoxin which, when administered in vivo, is able to reproduce the nigro-striatal lesion typical of PD. In SH-SY5Y cells, ELF-MF exposure was not cytotoxic, but significantly impaired redox homeostasis and thiol content, triggering an increase of protein carbonylation. This effect led to an enhancement of MPP+ induced cell death, which suggests that

ELF-MF might represent a risk co-factor in PD aetiology.

The ELF-MF sensitization effect was linked to the ELF-MF pro-oxidant activity, with increased intracellular Reactive Oxygen Species levels, potentiation of oxidative damage and induction of caspase-dependent apoptosis. This interpretation was supported by experiments showing a protective effect of thiol antioxidants against ELF-MF/MPP+ induced cell death.



An in-depth investigation has been started on the mechanism of MPP+ toxicity in SH-SY5Y cells, with experiments conducted, by one of our scientist, at the Danish Cancer Research Center in Copenhagen. SH-SY5Y cells were silenced for the activity of S-Nitrosoglutathione Reductase (GSNOR), a class III alcohol dehydrogenase that regulates Nitric Oxide (NO) cell level and modulates NO-mediated signaling pathways. Then, the cellular response to MPP+ was assessed.

Preliminary results show that GSNOR-inactivated cells are more sensitive to MPP+ induced toxicity, suggesting a role for NO-mediated signaling pathways in PD neurodegeneration

Extremely low frequency magnetic fields (ELF-MFs) affect the in vitro response of SH-SY5Y neuronal dopaminergic cells to the pro-Parkinson's toxin MPP$^+$, by modulating the epigenetic machinery and the redox pathway

Environmental epidemiology and occupational health

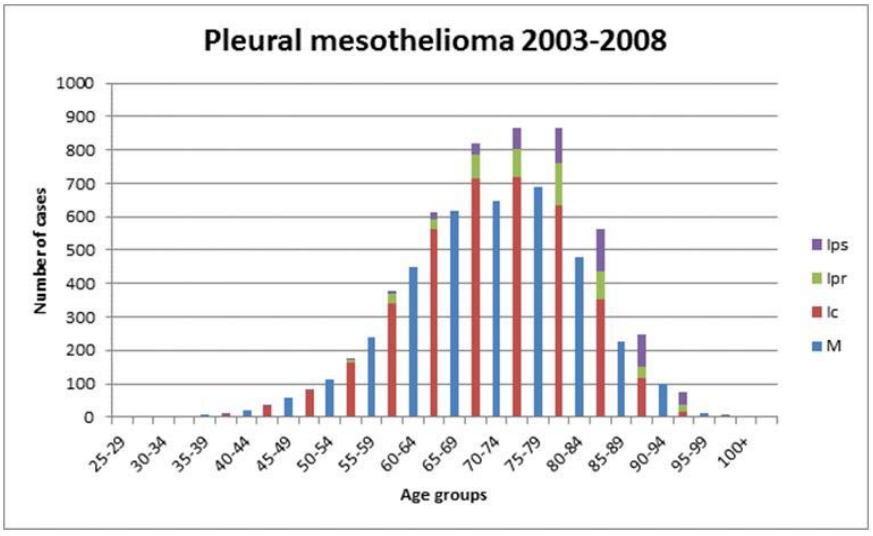
In collaboration with the Technical Unit for Models, Methods and Technologies for Environmental Assessments, the Laboratory has implemented the methodology of risk assessment based on the elaboration and interpretation of cancer risk and hazard quotient (for non-cancer pathologies) maps as a function of computed concentrations of environmental air pollutants.

A thorough comparative search of the scientific literature has been conducted, in order to select the internationally most accredited human physiological parameters and the reference concentrations and unit

risks for each pollutant, which need to be introduced into the algorithms for the calculation of human inhalation risks. The methodology has been validated by a practical exercise.

A collaborative study with the Technical Unit for Environmental Technologies and the Italian Workers' Compensation Authority (INAIL), aimed at performing an integrated analysis of the Italian current data on mesothelioma, was carried out.

Such analysis included the mortality data by the Italian Institute of Statistics (ISTAT), gathered in the ENEA Database, and the incidence data from the Italian Register of Mesothelioma (ReNaM) by INAIL. Comparisons between available mortality and incidence data for mesothelioma were carried out. Mortality data well overlapped with incidence data when considering mesothelioma incident cases with a certain diagnosis. Interestingly, data on the incidence of disease based on a possible/probable diagnosis overlapped with mortality for pleural cancer other than mesothelioma, suggesting that the space-temporal distribution of such deaths should be carefully analysed before excluding they could be due to mesothelioma. The importance of epidemiological surveillance is confirmed as an effective tool for public health and welfare policies.



Number of pleural mesothelioma deaths (M) and incidence cases divided for diagnostic certainty level (Ic=certain, Ipr=probable, Ips=possible) for all the regions and periods of complete overlap between the mortality (ENEA database, ISTAT source data) and the incidence (ReNaM) archives (2003, 2006-2008). (Elaborated in collaboration with the Technical Unit for Environmental Technologies

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UTBIORAD STG:

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FACILITIES

- Animal House for small rodents
- Containment Greenhouse
- X-rays machine for *in vitro* and *in vivo* exposure

RESEARCH PRODUCTS

Peer Reviewed Papers

Amendola R, Innocenzi P, Marcelli A. “Cosmic-Rad”. *Space Magazine 2014; 6: 52-54*.

Alisi A, Nobili V, Ceccarelli S, Panera N, De Stefanis C, De Vito R, Vitali R, Bedogni G, Balsano C, Cucchiara S, Stronati L. “Plasma high mobility group box 1 protein reflects fibrosis in pediatric nonalcoholic fatty liver disease”. *Expert Rev Mol Diagn. Jul 2014;14(6):763-71. PMID: 24927058*

Alleva E, Amendola R., Innocenzi P and Marcelli A. “Cosmic rays and radiobiology in a Sino-Italian network strategy: first bilateral workshop COSMIC-RAD”. *Rendiconti Lincei 2014, doi 10.1007/s12210-013-0284-7*

Aloi M, Nuti F, Stronati L, Cucchiara S. “Advances in the medical management of paediatric IBD”. *Nat Rev Gastroenterol Hepatol 2014;11:99-108. PMID: 23958601*

Amendola R, Cervelli M, Tempera G, Fratini E, Varesio L, Mariottini P, Agostinelli E. “Spermine metabolism and radiation-derived reactive oxygen species for future therapeutic implications in cancer: an additive or adaptive response”. *Amino Acids. 2014 Mar;46(3):487-98 Review. PMID: 23999645*

Arcangeli C, Circelli P, Donini M, Aljabali AA, Benvenuto E, Lomonossoff GP, Marusic C. “Structure-based design and experimental engineering of a plant virus nanoparticle for the presentation of immunogenic epitopes and as a drug carrier”. *J Biomol Struct Dyn. 2014 Apr;32(4):630-47. PMID: 2367234.*

Bellusci M, La Barbera A, Padella F, Mancuso M, Pasquo A, Grollino MG, Leter G, Nardi E, Cremisini C, Giardullo P, Pacchierotti F. “Biodistribution and acute toxicity of a nanofluid containing manganese iron oxide nanoparticles produced by a mechanochemical process”. *Int J Nanomedicine. 2014 Apr 17;9:1919-29. PMID: 24790434; PMC4000180*

Benassi B and Consales C "Environmental Impact on the Etiology of Alzheimer’s Disease: Mechanistic Insights from Oxidative Stress and Epigenetic Perspective”, *Frontiers in Clinical Drug Research - Alzheimer Disorders, Chapter 5, Vol. 2, 2014. Chapter DOI: 10.2174/978160805870911402000*7

Bertuccini L, Costanzo M, Iosi F, Tinari A, Terruzzi F, Stronati L, Aloi M, Cucchiara S, Superti F. “Lactoferrin prevents invasion and inflammatory response following E. coli strain LF82 infection in experimental model of Crohn's disease”. *Dig Liver Dis 2014;46:496-504. PMID: 24631031*

Brokken LJS, Lundberg JP, Spanò M, Bizzaro D, Manicardi GC, Pedersen HS, Struciński, Góralczyk PK, Zviezdai V, Jönsson BA, Bonde JP, Toft G, Lundberg Giwercman Y,

Giwercman A. “Interactions between polymorphisms in the aryl hydrocarbon signalling pathway and exposure to persistent organochlorine pollutants affect human semen quality”. *Reprod Toxicol 2014;49C: 65-73. PMID: 25084496*

Carnevali F, Argentieri M, Ippedico G, Minniti CA, Amodio L, Mellano L, van der Esch S A. "Managing horse wounds either presenting or not with exuberant granulation tissue using an innovative wound dressing: A retrospective non-controlled study". *J Anim and Vet Sci, 2014; 1(2), 6-16*

Cavagnaro M, Amabile C, Cassarino S, Tosoratti N, Pinto R and Lopresto V. “Influence of the target tissue size on the shape of ex vivo microwave ablation zones”. *International Journal of Hyperthermia, in press. PMID: 25677838*

Cervelli M, Pietropaoli S, Signore F, Amendola R, Mariottini P. “Polyamines metabolism and breast cancer: state of the art and perspectives”. *Breast Cancer Res Treat. 2014; 148(2):233-48. PMID: 25292420*

Cervelli M, Angelucci E, Germani F, Amendola R, Mariottini P. “Inflammation, carcinogenesis and neurodegeneration studies in transgenic animal models for polyamine research”. *Amino Acids. 2014;46(3):521-30. Review. PMID: 23933909*

Chen GB, Lee SH, Brion MJ, Montgomery GW, Wray NR, Radford-Smith GL, Visscher PM; International IBD Genetics Consortium. “Estimation and partitioning of (co)heritability of inflammatory bowel disease from GWAS and immunochip data”. *Hum Mol Genet 2014;23:4710-20. PMID: 24728037*

Ciucci A, Meco D, De Stefano I, Travaglia D, Zannoni GF, Scambia G, Riccardi R, Saran A, Mancuso M, Gallo D. “Gender effect in experimental models of human medulloblastoma: does the estrogen receptor β signaling play a role?” *PLoS One, 2014 Jul 7;9(7):e101623. PMID: 25000562*

Colamartino M, Santoro M, Duranti G, Sabatini S, Ceci R, Testa A, Padua L, Cozzi R. “Evaluation of Levodopa and Carbidopa Antioxidant Activity in Normal Human Lymphocytes In Vitro: Implication for Oxidative Stress in Parkinson's Disease”. *Neurotox Res. 2014 in press. PMID: 25355370*

Consales C, Leter G, Bonde JPE, Toft G, Eleuteri P, Moccia T, Budillon A, Jönsson BAG, Giwercman A, Pedersen HS, Ludwicki JK, Zviezdai V, Heederik D, Spanò M. “Indices of methylation in sperm DNA from fertile men differ between distinct geographical regions”. *Hum Reprod 2014;29: 2065-72. PMID: 25035434*

Cordeiro MN, Paolini F, Massa S, Curzio G, Illiano E, Duarte Silva AJ, Franconi R, Bissa M, Morghen CD, de Freitas AC, Venuti A. “Anti-tumor effects of genetic vaccines against HPV major oncogenes”. *Hum Vaccin Immunother. 2014 Aug 6;11(1). [Epub ahead of print] PMID: 25115152*

Dassano A, Mancuso M, Giardullo P, Cecco LD, Ciuffreda P, Santaniello E, Saran A, Dragani TA, Colombo F. “N(6)-isopentenyladenosine and analogs activate the NRF2-mediated antioxidant response”. *Redox Biol. 2014 Mar 6;2:580-89. PMID: 24688894*

De Stefano I, Tanno B, Giardullo P, Leonardi S, Pasquali E, Antonelli F, Tanori M, Casciati A, Pazzaglia S, Saran A, Mancuso M. “The Patched 1 Tumor-Suppressor Gene Protects the Mouse Lens from Spontaneous and Radiation-Induced Cataract”. *Am J Pathol. 2014 Nov 15. PMID: 25452120. [Epub ahead of print]*

Di Carli M, Tanno B, Capodicasa C, Villani ME, Salzano AM, Scaloni A, Raschellà G, Benvenuto E, Donini M. “Proteome changes induced by c-myb silencing in human chronic myeloid leukemia cells suggest molecular mechanisms and putative biomarkers of hematopoietic malignancies”. *J Proteomics. 2014 Jan 16;96:200-22. PMID: 24220303.*

Di Nardo G, Barbara G, Cucchiara S, Cremon C, Shulman RJ, Isoldi S, Zecchi L, Drago L, Oliva S, Saulle R, Barbaro MR, Stronati L. “Neuroimmune interactions at different intestinal sites are related to abdominal pain symptoms in children with IBS”. *Neurogastroenterol Motil 2014;26:196-204. PMID: 24304324*

Di Nardo G, Oliva S, Menichella A, Pistelli R, De Biase RV, Patriarchi F, Cucchiara S, Stronati L. “Lactobacillus reuteri ATCC55730 in cystic fibrosis”. *J Pediatr Gastroenterol Nutr 2014;58:81-6. PMID: 24121143*

Farina L, Weiss N, Nissenbaum Y, Cavagnaro M, Lopresto V, Pinto R, Tosoratti N, Amabile C, Cassarino S and Goldberg SN. “Characterization of tissue shrinkage during microwave thermal ablation”. *International Journal of Hyperthermia 2014; 30(7), 419–428, 2014. PMID: 25323026*

Fratini E and Amendola R. “Caves and other subsurface environments in the future exploration of Mars: the absence of natural background radiation as biology concern”. *Rendiconti Lincei 2014, doi 10.1007/s12210-013-0270-0*

Giusti N, Bufalieri F, Licursi V, Castrignanò T, D'Antonio M, Amendola R, Negri R.. “General features of the transcriptional response of mammalian cells to low- and high-irradiation”. *Rendiconti Lincei 2014, doi 10.1007/s12210-013-0274-9*

Grasso F, Ruggieri V, De Luca G, Leopardi P, Mancuso M, Casorelli I, Pichierri P, Karran P, Bignami M. “MUTYH mediates the toxicity of combined DNA 6-thioguanine and UVA radiation”. *Oncotarget. 2014 Dec 2. PMID: 25638157 [Epub ahead of print]*

Hehle VK, Lombardi R, van Dolleweerd CJ, Paul MJ, Di Micco P, Morea V, Benvenuto E, Donini M, Ma JK. “Site-specific proteolytic degradation of IgG monoclonal antibodies expressed in tobacco plants”. *Plant Biotechnol J. 2014 Oct 4. PMID: 25283551. [Epub ahead of print]*

Ieranò C, Santagata S, Napolitano M, Guardia F, Grimaldi A, Antignani E, Botti G, Consales C, Riccio A, Nanayakkara M, Barone MV, Caraglia M, Scala S. "CXCR4 and CXCR7 transduce through mTOR in human renal cancer cells". *Cell Death Dis. 2014 Jul 3;5:e1310. PMID: 24991762*

Jahan Z, Cerreto M, Aversa G, Rufini S, Desideri A, Giovanetti A "Chapter: Role of Topoisomerase IB in repairing the radio-induced DNA damage in tumor cell lines. Book: Radiation Exposure: Monitoring Systems, Long-Term Health Effects and Methods for Protection". *Ed Prof Kaushala Prasad Mishra in publication by Nova Scientific Publishers, New York, USA. Invited from Editor*

Kempf SJ, Casciati A, Buratovic S, Janik D, von Toerne C, Ueffing M, Neff F, Moertl S, Stenerlöw B, Saran A, Atkinson MJ, Eriksson P, Pazzaglia S, Tapio S. "The cognitive defects of neonatally irradiated mice are accompanied by changed synaptic plasticity, adult neurogenesis and neuroinflammation". *Mol Neurodegener. 2014 Dec 16;9:57. PMID: 25515237*

Kulka U, Ainsbury L, Atkinson M, Barnard S, Smith R, Barquinero JF, Barrios L, Bassinet C, Beinke C, et al. "Realising the European Network of Biodosimetry: Reneb-Status Quo". *Radiat Prot Dosimetry. 2014 Sep 9. PMID: 25205835 [Epub ahead of print]*

Lenters V, Portengen L, Smit LAM, Jönsson BAG, Giwercman A, Rylander L, Lindh CH, Spanò M, Pedersen HS, Ludwicki JK, Chumak L, Piersma AH, Toft G, Bonde JP, Heederik D, Vermeulen R. "Phthalates, Perfluoroalkyl Acids, Metals and Organochlorines and Male Reproductive Function: A Multi-pollutant Assessment in Greenlandic, Polish and Ukrainian Cohorts". *Occup Environ Med 2014 Sep 10. PMID: 25209848 [Epub ahead of print]*

Leter G, Consales C, Eleuteri P, Uccelli R, Specht IO, Toft G, Moccia T, Budillon A, Jönsson BAG, Lindh CH, Giwercman A, Pedersen HS, Ludwicki JK, Zviezdai V, Heederik D, Bonde JPE, Spanò M. "Exposure to perfluoroalkylsubstances (PFASs) and sperm DNA global methylation in Arctic and European populations". *Environ Mol Mutagen 2014; 55: 591-600. PMID: 24889506*

Licursi V, Fratini E, Benassi B, Cestelli Guidi M, Consales C, Marcelli A, Mirri C, Negri R, Amendola R. "A proposed integrated systems approach to the radiation biology of cosmic interest: biophysics and molecular characterization of tissues irradiated with 14 MeV neutrons". *Rend. Fis. Accad. Lincei. 2014; 25:23-27. DOI: 10.1007/s12210-013-0272-y*

Lodato R, Lopresto V, Pinto R and Marrocco G. "Numerical and experimental characterization of through-the-body UHF-RFID links for passive tags implanted into human limbs". *IEEE Transactions on Antennas and Propagation, 62(10), 5298 – 5306, 2014.DOI 10.1109/TAP.2014.2345586*

Lombardi CC, Uccelli R. "Caccia alla cicca". *L'Astrolabio. 2014 Nov 13.*

Lopresto V, Pinto R and Cavagnaro M. “Experimental characterisation of the thermal lesion induced by microwave thermal ablation”. *International Journal of Hyperthermia 2014; 30(2), 110–18. PMID: 24571174*

Lori C, Pasquo A, Montanari R, Capelli D, Consalvi V, Chiaraluce R, Cervoni L, Loiodice F, Laghezza A, Aschi M, Giorgi A, Pochetti G. “Structural basis of the transactivation deficiency of the human PPARγ F360L mutant associated with familial partial lipodystrophy”. *Acta Crystallogr D Biol Crystallogr. 2014 Jul;70(Pt 7):1965-76. PMID: 25004973.*

Marusic C and Donini M.. “Plant-made HIV Vaccines and Neutralizing Antibodies”. *In book: Plant-Derived Pharmaceuticals. Edited by Kathleen Laura Hefferon, CABI Publishing ISBN: 2014 978-1-78064-343-4; 137-54.*

Moretti M, Grollino MG, Pavanello S, Bonfiglioli R, Villarini M, Appolloni M, Carrieri M, Sabatini L, Dominici L, Stronati L, *et al.* “Micronuclei and chromosome aberrations in subjects occupationally exposed to antineoplastic drugs: a multicentric approach”. *Int Arch Occup Environ Health. 2014 Nov 2. PMID: 25362515 [Epub ahead of print]*.

Negroni A, Prete E, Vitali R, Cesi V, Aloi M, Civitelli F, Cucchiara S, Stronati L. “Endoplasmic reticulum stress and unfolded protein response are involved in paediatric inflammatory bowel disease”. *Dig Liver Dis 2014;.46:788-94. PMID: 24953208*

Ning Y., Multari C., Luo X., Cheng X., Hwang J.C.M., Denzi A., Merla C., Apollonio F., Liberti M. “Broadband Electrical Detection of Individual Biological Cells”. *IEEE Transaction on Microwave Theory and Technique 2014; 62(9), 1905-11, DOI: 10.1109/TMTT.2014.2342660*

Palone F, Vitali R, Cucchiara S, Pierdomenico M, Negroni A, Aloi M, Nuti F, Felice C, Armuzzi A, Stronati L. “Role of HMGB1 as a suitable biomarker of subclinical intestinal inflammation and mucosal healing in patients with inflammatory bowel disease”. *Inflamm Bowel Dis 2014;20:1448-57. PMID: 24983978*

Paoli D, Gallo M, Rizzo F, Spanò M, Leter G, Lombardo F, Lenzi A, Gandini L.”Testicular cancer and sperm DNA damage: short- and long-term effects of antineoplastic treatment”. *Andrology 2014 Sep 2. PMID: 25180491 [Epub ahead of print]*

Patrono C, Monteiro Gil O, Giesen U, Langner F, Pinto M, Rabus H., Testa A. “BioQuaRT” project: Design of a novel in situ protocol for the simultaneous visualization of chromosomal aberrations and micronuclei after irradiation at microbeam facilities”. *Radiat. Prot. Dosim. (accepted for publication)*

Patrono C, Sterpone S, Testa A, Cozzi R. “Polymorphisms in base excision repair genes: Breast cancer risk and individual radiosensitivity”. *World J Clin Oncol 2014; 5(5): 874-882. PMID: 25493225*

Pierdomenico M, Negroni A, Stronati L, Vitali R, Prete E, Bertin J, Gough PJ, Aloi M, Cucchiara S. “Necroptosis is active in children with inflammatory bowel disease and contributes to heighten intestinal inflammation”. *Am J Gastroenteroly 2014;109:279-87. PMID: 24322838*

Rosado MM, Nasta F, Prisco MG, Lovisolo GA, Marino C, Pioli C.”Effects of GSM-modulated 900 MHz radiofrequency electromagnetic fields on the hematopoietic potential of mouse bone marrow cells”. *Bioelectromagnetics 2014;35(8):559-67. PMID: 25256206*

Salerno S and Draicchio F. “Change of the name of the Italian Ergonomics Society to Italian Society of Ergonomics and Human Factors”. *Med Lav. 2014; 105(2) :157. PMID: 24909049*

Salerno S. “Capitolo 3. Donna, lavoro e salute: il genere negato dove, come, quando, perchè”. *In: Salute e sicurezza di genere (a cura di Felice Paolo Arcuri, Cinzia Ciacia). Collana Questioni di Genere. Edizioni Palinsesto, Rome 2014, 57-69*

Salerno S. “Women, work and health between the nineteenth and twentieth centuries from a national and international perspective”. *Med Lav 2014; 105 : 435-45. PMID: 25431982*

Spera R., Petralito S., Liberti M., Merla C., d'Inzeo G., Pinto R. and Apollonio F. “Controlled release from magnetoliposomes aqueous suspensions exposed to a low intensity magnetic field”. *Bioelectromagnetics 2014; 35(4), 309-12. PMID: 24482311*

Strigari L, Mancuso M, Ubertini V, Soriani A, Giardullo P, Benassi M, D'Alessio D, Leonardi S, Soddu S, Bossi G. “Abscopal effect of radiation therapy: Interplay between radiation dose and p53 status”. *Int J Radiat Biol. 2014 Mar;90(3):248-55. PMID: 24350918*

Toft G, Lenters V, Vermeulen R, Heederik D, Thomsen C, Becher G, Giwercman A, Bizzaro D, Manicardi GC, Spanò M, Rylander L, Pedersen HS, Struciński P, Zviezdai V, Bonde JP. “Exposure to Polybrominated Diphenyl Ethers and male reproductive function in Arctic and European populations”. *Reprod Toxicol 2014;43: 1-7. PMID: 24513925*

Tremante E, Ginebri A, Lo Monaco E, Benassi B, Frascione P, Grammatico P, Cappellacci S, Catricalà C, Arcelli D, Natali PG, Di Filippo F, Mottolese M, Visca P, Benevolo M, Giacomini P. “A melanoma immune response signature including Human Leukocyte Antigen-E”. *Pigment Cell Melanoma Res. 2014; 27:103-12. PMID: 24011128*

Conference presentations and proceedings

Ambrosini F., Ampollini A., Marracino F., Nenzi P., Picardi L., Piccinini M., Ronsivalle C., Surrenti V., Vadrucci M., Balduzzi M., Marino C., Esposito G., De Angelis C., Tabocchini A. “Experimental activity in the enea-frascati irradiation facility with 3-7 MeV protons”. *5th*

International Particle Accelerator Conference IPAC'14 , Dresden, Germany, June 15 – 20, 2014

Amendola R. "Topical review 11: The leptin modulation in response to irradiation: time to move from cell to tissues interplay". *60th Radiation Research Conference, Las Vegas (NV),USA, 22-24 September 2014* . Invited Lecture

Andreoli C, Leter G, De Berardis B, Degan P, De Angelis I, Pacchierotti F, Crebelli R, Barone F, Zijno A. "Genotoxicity detection and flow cytometric evaluation of TiO2 nanoparticles in human PBMC". *NANOTOX 2014. Antalya, Turkey, 23-26 Apri, 2014.*

Andreoli C, Leter G, De Berardis B, Degan P, De Angelis I, Pacchierotti F, Crebelli R, Barone F, Zijno A. "Genotoxicity detection and flow cytometric evaluation of TiO2 nanoparticles in human PBMC*". XIII Congress of the Italian Federation of Life Sciences (FISV). Pisa, Italy, 24-27 September 2014.*

Antonelli F, Casciati A, Tanori M, Mancuso M, Leonardi S, Giardullo P, Pasquali E, De Stefano I, Tanno B, Saran A, Pazzaglia S. "Role of Shh signaling in the control of adult hippocampal neurogenesis". *Keystone Symposia meeting on Adult neurogenesis, Stockholm, Sweden,12-17 May 2014.*

Antonelli F. "Effetto delle radiazioni ionizzanti sulla neurogenesi dell'ippocampo. *XVI National Congress of Italian Society for Research on Radiation (SIRR), Pavia, Italy, 7-8 November 2014.*

Casciati A., Antonelli F., Dobos K., Tanori M., Heredia L., Bellés M., Sáfrány G., Saran A., Linares-Vidal L.M.V, Lumniczky K., Pazzaglia S. "Age-related effects of x-rays irradiation on adult hippocampal neurogenesis". *The Sixth International MELODI Workshop, Barcelona, Spain, 7- 9 October 2014.*

Casciati A., Antonelli F., Dobos K., Tanori M., Sáfrány G., Saran A., Lumniczky K., Pazzaglia S.. "Age-related effects of X-rays irradiation on adult hippocampal neurogenesis". *Keystone Symposia meeting on Adult neurogenesis", Stockholm, Sweden, 12-17 May 2014.*

Cordelli E, Eleuteri P, Villani P, Maranghi F, Tassinari R, Narciso L, Cubadda F, Aureli F, D'Amato M, Martinelli A, Di Virgilio A, Pacchierotti F. "Assessment of SiO2 nanoparticle genotoxicity in blood cells of rats after sub-chronic exposure". *Annual Conference of the European Environmental Mutagen Society, Lancaster, UK, 6-10 July 2014.*

Cordelli E, Eleuteri P, Villani P, Pacchierotti F. "Evaluation of genotoxic effects in peripheral blood cells of rats exposed to 6-month inhalation of CeO2 nanoparticles". *NANOTOX 2014, Antalya, Turkey, 23-26 April 2014.*

De Stefano I, Tanno B, Giardullo P, Leonardi S, Pasquali E, Antonelli F, Tanori M, Casciati A, Pazzaglia S, Saran A and Mancuso M. "Il cross-talk tra Shh e TGF-ß promuove lo

sviluppo di cataratta radio-indotta in topi Ptch1+/-". *XVI National Congress of Italian Society for Research on Radiation (SIRR), Pavia, Italy, 7-8 November 2014.*

De Stefano I. "Effetto del sesso in modelli sperimentali di medulloblastoma umano: ruolo di ERβ". *La collaborazione tra Unimarconi ed Enea: aspetti multidisciplinari. University of Guglielmo Marconi, Rome. 18 November 2014.*

Di Carli M, Tanno B, Capodicasa C, Villani ME, Salzano AM, Scaloni A, Raschellà G, Benvenuto E, Donini M. "2D-Dige Proteome Changes Induced By C-Myb Silencing In Human Chronic Myeloid Leukemia Cells". *9th Congress of the Italian Proteomic Association, Naples, Italy, 24-27 June 2014.*

Dini V, Pecchia I, Esposito G, Anello P, Balduzzi M, Fratini E, Ricci-Vitiani L, Biffoni M, RunciD, Pallini R, Strigari L, De Andrea M, Tabocchini MA. "Does novel technology need novel radiobiology?" *41st Annual Meeting of the European Radiation Research Society Rhodes, Greece, Sept 14, 2014*

Dini V, Pecchia I, Ricci-Vitiani L, Biffoni M, Pelacchi F, Pallini R, Balduzzi M, Fratini E, Belli M, Campa A, Esposito G, Cirrone G, Romano F, Stancampiano C, Tabocchini MA. "Biological effects in glioblastoma stem cells after charge-particle irradiation: hadrontherapy as a new therapeutic opportunity?" *41st Annual Meeting of the European Radiation Research Society, Rhodes, Greece, Sept 14, 2014*

Dini V, Pecchia I, Esposito G, Anello P, Balduzzi M, Fratini E, Ricci-Vitiani L, Biffoni M, RunciD, Pallini R, Strigari L,De Andrea M, Tabocchini MA. "Una nuova tecnologia richiede una nuova tecnologia?" *XVI National Congress of Italian Society for Research on Radiation (SIRR), Pavia, Italy, 7-8 November 2014*

Franconi R. "Vaccini del futuro". *Workshop 'Promoción de Programas de Prevención y Tratamiento de los Tumores del Cuello del Útero' (Programa de Cooperación Internacional Italia-América Latina- Promozione di programmi di prevenzione e cura del cancro del collo dell'utero). Istituto Italo Latino Americano (IILA) e Red de Institutos Nacionales de Cancer (RINC), Rome 13-14 October 2014.*

Giardullo P, De Stefano I, Tanno B, Leonardi S, Pasquali E, Antonelli F, Tanori M, Casciati A, Pazzaglia S, Saran A, Mancuso M. "Ptch1 heterozygous mice as a model for radiation cataractogenesis". *60th Annual Meeting of the Radiation Research Society, Las Vegas, USA, 21-24 September 2014.*

Giardullo P. "Biodistribuzione e tossicità organo specifica in vivo dopo somministrazione endovenosa di nanoparticelle in topo". *La collaborazione tra Unimarconi ed Enea: aspetti multidisciplinari. University of Guglielmo Marconi, Rome, 18 November 2014.*

Giliberti C, Figà Talamanca I, Giordano F, and Salerno S. "Cell Phones Exposure and Children's Health". *EHE2014 - 5th International Conference on Electromagnetic Fields, Health and Environment, Oporto, Portugal, 24-26 May 2014.*

Giliberti C, Figà Talamanca I, Salerno S. "Ergonomic design for young users of mobile phones". *Proceedings of the 5th International Conference on Applied Human Factors and Ergonomics, AHFE 2014, Kraków, Poland, 19-23 July 2014.*

Lico C, Blandino A, Barberini L, Cirotto C, Benvenuto E, Mancuso M, Giardullo P, Blasi P, Santi L, Baschieri S. "Plant virus nanoparticles: toxicity and teratogenicity evaluation using in vitro and in vivo models". *The first conference of the "International Society for Molecular Farming", Berlin-Dahlem, Germany, 17-19 June 2014.*

Lico C, Blandino A, Barberini L, Cirotto C, Mancuso M, Giardullo P, Blasi P, Santi L, Benvenuto E, Baschieri S. "Plant virus nanoparticles: toxicity and teratogenicity evaluation using in vitro and in vivo models". *1st Parma Nanoday, Parma, Italy, 28 November 2014.*

Mancuso M, Pasquali E, Braga-Tanaka I, Tanaka S, Gulay KCM, Pannicelli A, Giardullo P, Pazzaglia S, Atkinson M J and Saran A. "Development of atherosclerotic plaques after acute and chronic low-dose irradiation of ApoE-/- mice". *60th Annual Meeting of the Radiation Research Society, Las Vegas, USA, 21-24 September 2014.*

Mancuso M, Pasquali E, Braga-Tanaka I, Tanaka S, Gulay KCM, Pannicelli A, Giardullo P, Pazzaglia S, Atkinson M J and Saran A. "Development of atherosclerotic plaques after acute and chronic low-dose irradiation of ApoE-/- mice". *The Sixth International MELODI Workshop, Barcelona,* Spain, *7- 9 October 2014.*

Mancuso M. "Abscopal effect: much more than a conceptual dichotomy". *60th Annual Meeting of the Radiation Research Society (RRS), Las Vegas*, USA, *21-24 September 2014.* Invited Lecture.

Marino C, Pinto R, Lopresto V, Pioli C. "Exposure to radiofrequencies during embryo life, childhood or adulthood: developmental and functional effects on the immune system". *5th International Conference on Electromagnetic Fields Health and Environment (EHE), Porto, Portugal, 24-25 April 2014.*

Marusic C, Novelli F, Salzano AM, Scaloni A, Benvenuto E, Pioli C and Donini M. "Production of an active anti-CD20-hIL-2 immunocytokine in N. benthamiana". *1st conference of the International Society for Plant Molecular Farming, Berlin-Dahlem, Germany , 17-19 June 2014.*

Massa S, Paolini F, De Carolis D, Venuti A, Franconi R. "Developments in plant-derived therapeutic vaccines for the immunotherapy of Human Paillomavirus-related cancers". *Ninth World Congress on Vaccines, Immunization and Immunotherapy, Genoa, Italy, 29-30 April 2014*

Massa S, Paolini F, Venuti A, Franconi R. "HPV Therapeutic Vaccines from Plant Production Platforms". *12th National Congress of the Italian Society for Virology, Orvieto (TR), Italy, 22 - 24 September 2014.*

Massa S, Paolini F, Venuti A, Franconi R. "Plant-Based Production Platforms for HPV Vaccines. *The 14th International Conference on Progress in Vaccination Against Cancer (PIVAC-14), Rome, Italy, 24-26 September 2014.*

Massa S, Rodriguez Hernandez M, Illiano E, Paolini F, Pasquo A, De Carolis D, Di Micco P, Morea V, De Giuli Morghen C, Radaelli A, Dionisi-Vici C, Venuti A, Franconi R. "Plant-derived therapeutics for the treatment of a rare disease (glycogen storage disease type III, GSDIII) and of HPV-related tumors". *First International Meeting of The Society for Plant Molecular Farming, Berlin, Germany.17-19 June 2014.*

Monteiro Gil O, Patrono C, Testa A, Antunes AC, Giesen U, Langner F, Rabus H. "Detection of micronuclei after irradiation with alpha particles at the PTB microbeam facility within the BioQuaRT project". *6th International MELODI Workshop, Barcelona, Spain, 7 - 9 October 2014*

Novelli F, Piscitelli M, Rozenblum N., Pasquali E., Mancuso M., Lopresto V., Pinto R., Merla C., Marino C., Goldberg S.N., Pioli C.. "Effects of PARP-1 gene knockdown in RFA-induced inflammatory response". *29th Annual Meeting of European Society for Hyperthermic Oncology, Turin, Italy, 11-14August 2014.*

Pacchierotti F. "Germline transmission of enhanced susceptibility to radiation-induced brain tumors", *Annual Conference of the European Environmental Mutagen Society, Lancaster, UK, 6-10 July 2014*. Invited lecture

Paolini F, Massa S, Cordeiro MN, Curzio G, Illiano E, De Carolis D, Campos Coimbra E, De Giuli Morghen C, de Freitas AC, Radaelli A, Franconi R, Venuti A. "Development of Plant-Derived Anti-HPV Therapeutic Vaccines by Targeting the E5, E6 and E7 Oncogenes". *29th International Papillomavirus Conference and Public Health & Clinical Workshops, Seattle, Washington, USA. Basic Science , 21– 25, August 2014*.

Paolini F, Massa S, Illiano E, Cordeiro MN, Campos Coimbra E, De Freitas AC, Radaelli A, De Giuli Morghen C, Franconi R, Venuti A. "Anti-tumour effects of genetic vaccines against HPV major oncogenes". *Ninth World Congress on Vaccines, Immunization and Immunotherapy, Genoa, Italy, 29-30 April 2014.*

Pasquali E, Pannicelli A, Braga-Tanaka I, Tanaka S, Gulay KCM, Giardullo P, Pazzaglia S, Atkinson MJ, Saran A and Mancuso M. "Sviluppo di placche aterosclerotiche in topi ApoE-/- dopo esposizione acuta e cronica alle radiazioni ionizzanti". *XVI National Congress of Italian Society for Research on Radiation (SIRR), Pavia,* Italy, *7-8 November 2014.*

Patrono C, Monteiro Gil O, Giesen U, Langner F, Palma V, Rabus H, Testa A. "Progetto BioQuaRT (Biologically weighted Quantities in RadioTherapy): valutazione del danno cromosomico indotto da particelle alfa dopo irraggiamento con microbeam". *XVI National Congress of Italian Society for Research on Radiation (SIRR), Pavia, Italy, 7 - 8 November 2014*

Pazzaglia S. "Effetti non cancerogeni". *La collaborazione tra Unimarconi ed Enea: aspetti multidisciplinari. University of Guglielmo Marconi, Rome, 18 November 2014.*

Pazzaglia S. "Investigating non-cancer effects of radiation in mouse models". *41st Annual Meeting of the European Radiation Research Society (ERR2014), Rhodes, Greece, 14-19 September 2014.* Invited lecture.

Pecchia I, Dini V, Esposito G, Anello P, Balduzzi M, Fratini E, Ricci-Vitiani L, Biffoni M, Runci D, Pallini R, Molinelli S, Facoetti A, Ciocca M, Tabocchini MA. "Effetti biologici in cellule staminali di glioblastoma irradiate con ioni carbonio presso la facility di adroterapia del CNAO". *XVI National Congress of Italian Society for Research on Radiation (SIRR), Pavia, Italy, 7-8 November 2014*

Pierdomenico M, Cesi V, Stronati L, Costanzo M, Aloi M, Oliva S, Rossi P and Cucchiara S. Mir320 family regulates nod2/card15: a new mechanism for controlling inflammation? *Digestive Disease Week, Chicago, IL,USA, 3-6 May 2014.*

Prete E., Negroni A., Cesi V., Stronati L., Oliva S., Aloi M., Di Nardo G., Cucchiara S.. Endoplasmic reticulum stress and unfolded protein response are involved in pediatric inflammatory bowel disease. *47th Annual Meeting of European Society of Paediatric Gastroenetrrology, Hepatology and Nutrition (ESPGHAN), Jerusalem, Israel, 9 - 12 June 2014.*

Rodriguez M, Demurtas OC, Illiano E, Giuliano G, Pasquo A, Amato F, Di Micco P, Morea V, Marino C, Dionisi-Vici C, Franconi R, Massa S. "Novel approaches for the treatment of glycogen storage disease type III (GSDIII)". *Jornada de Enfermedades Raras. ICBP Victoria De Girón, La Habana, Cuba,21, 22, 28 February, 2014.*

Salerno S. "Donne, lavoro e salute: cambia lo stile di vita, cambiano le condizioni di salute". *Atti del Convegno "Il cuore una questione di genere", in occasione della giornata internazionale della donna, Rome, 6 March 2014.*

Salerno S. "Gender and ergonomics: the recognition of women's occupational diseases". *Proceedings of the 5th International Conference on Applied Human Factors and Ergonomics, AHFE 2014, Kraków, Poland, 19-23 July 2014.*

Salerno S. "Riconoscimento delle malattie muscolo-scheletriche da lavoro in Italia: esiste una disuguaglianza per il genere femminile?" *77th Congress of Società Italiana di Medicina*

del Lavoro ed Igiene Industriale, Bologna, 15-17 October 2014. Giornale Italiano di Medicina del lavoro e Ergonomia. Vol. XXXV Oct. Dec. 2014 Supplemento al n. 4.

Salerno S. Women occupational diseases: Italian women pioneers (medical doctors, trade unionists, philantropists) in an international view of their first publications". *In 5th International Conference on the History of Occupational and Environmental Health "Framing occupational diseases", Rotterdam, The Netherlands, 24-25 April 2014.*

Saran A.and Mancuso M. "Effetti abscopali delle radiazioni ionizzanti". *La collaborazione tra Unimarconi ed Enea: aspetti multidisciplinari. University of Guglielmo Marconi, Rome, 18 November 2014.*

Snels C, Ambrosini F, Ampollini A., Balduzzi M, Bonfigli F, et al. "Attivita' sperimentale di dosimetria e radiobiologia con il fascio di protoni di bassa energia dell'acceleratore top-implart". *XVI National Congress of Italian Society for Research on Radiation (SIRR), Pavia, Italy, 7-8 November 2014*

Specht IO, Bonde JPE, Toft G, Giwercman A, Spanò M, Bizzaro D, Manicardi GC, Jönsson BOAG, Robbins WA. "Environmental Hexachlorobenzene exposure and human male reproductive function". *XXVI Conference of the International Society for Environmental Epidemiology (ISEE), Seattle, Washington, USA, 24-28 August 2014.*

Tanno B, Leonardi S, Giardullo P, De Stefano I, Pasquali E, Pazzaglia S, Atkinson M, Saran A, Mancuso M. "Identification of non-coding miRNA involved in the development of radio-induced medulloblastoma". *60th Annual Meeting of the Radiation Research Society (RRS), Las Vegas, USA, 21-24 September 2014.*

Tanno B, Leonardi S, Giardullo P, De Stefano I, Pasquali E, Pazzaglia S, Atkinson MJ, Saran A and Mancuso M. "Identification of non-coding miRNAs involved in the development of radio-induced medulloblastoma". *The Sixth International MELODI Workshop, Barcelona, Spain, 7- 9 October 2014.*

Tanori M, Leonardi S, Pasquali E, Casciati A, Giardullo P, Antonelli F, De Stefano I, Tanno B, Mancuso M, Saran A, Pazzaglia S. "Synthetic interactions in DNA Repair Pathways in development and oncogenesis". *41st Annual Meeting of the European Radiation Research Society (ERR2014), Rhodes*, Greece, *14-19 September 2014.*

Tassinari R, Narciso L, Cubadda F, Aureli F, D'Amato M, Raggi A, Di Virgilio A, Martelli A, Eusepi A, Di Felice G, Barletta B, Butteroni C, Corinti F, Cordelli E, Villani P, Eleuteri P, Pacchierotti F, Campagnolo L, Maranghi F, "Identificazione e caratterizzazione del pericolo della silice amorfa sintetica, additivo alimentare E551, mediante uno studio di tossicità orale a 90 giorni nel ratto". *I Congresso Nazionale della Società Italiana di Nanotossicologia. Naples, Italy, 27-28 June 2014.*

Testa A, Patrono C, Monteiro Gil O, Giesen U, Langner F, Palma V, Rabus H. "Analysis of radiation-induced chromosome damage on a cell-by-cell basis: alpha-particles microbeam irradiation within the BioQuaRT project" *ERR 2014 - 41st Annual Meeting of the European Radiation Research Society, Rhodes, Greece, 14 - 19 September 2014*

Toft G, Jørgensen KT, Lenters V, Høyer BB, Specht IO, Mocevic E, Heederik D, Giwercman A, Bizzaro D, Manicardi GC, Spanò M, Rylander L, Pedersen HS, Struciński P, Zviezdai V, Jønsson B, Bonde JP. "Are high levels of environmental contaminants in the Arctic impairing human fertility: studies of semen quality and time to pregnancy". *8th Arctic Frontiers Annual Conference "Humans in the Arctic", Tromsø, Norway, 19-24 January 2014.*

Chairmanships, Committees, Teaching and other activities

Amendola R. Editor of "Plos One" - Editor of "Rendiconti dei Lincei, Cosmic rays and radiobiology in a Sino-Italy network strategy: first bilateral workshop COSMIC-RAD". Springer Ed., vol. 25, Suppl. 1, March 2014.

Benvenuto E. Associated Editor of "Plant Cell Reports" (Springer)

Cordelli E. Member of OECD Expert Group on "In vivo Comet assay"

Galloni P. Lecture on "Effetti biologici dei campi elettromagnetici". FASE (European Health and Environmental Physics) Project, High School "Istituto Tecnico Industriale Leonardo da Vinci" - Carpi (MO).

Giovanetti A. Participation to the organization of the V FIRR Interdisciplinary Workshop: Radon monitoraggio, normativa e rischi per la salute umana, Seconda Università degli Studi di Napoli, 28/11/2014. - Member of the Board and Scientific Committee of FIRR. - Member of the Scientific Committee of "Scuola superiore di studi in radioprotezione Carlo Polvani". - University "Tor Vergata" (Rome) A:A. 2013-14, Teacher of the course in "Diagnostica per immagini e radioterapia: uso ed effetti delle radiazioni ionizzanti", Degree in "Tecniche della Prevenzione nell'Ambiente e nei Luoghi di Lavoro". - "Biological research on low doses", 6th International Summer School, Operational Issues in Radioactive Waste Management and Nuclear Decommissioning, Ispra, Italy, 8th -12th September 2014, Invited lecture

Leter G. Scuola nazionale di citometria Corsi teorico-pratici residenziali di formazione e aggiornamento. "Metodi citofluorimetrici in nano tossicologia". Scientific Campus "Enrico Mattei", "Carlo Bo" University of Urbino, 23rd-26th September 2014

Mancuso M. Chairman of the Session "S15: Origin and Mechanism of Radiation Carcinogenesis", 60th Annual Meeting of the Radiation Research Society (RRS), 21st-24th September 2014, Las Vegas. - Member of the Scientific Committee of XVI National Congress of Italian Society for Research on Radiation (SIRR), 7th-8th November 2014, Pavia

Marino C. member of the Main Commission ICNIRP (International Committee of Non Ionizing Radiation Protection); National Italian Delegate in the COST BM 1309 Action (20014-2018) "European network for innovative uses of EMFs in biomedical applications (EMF-MED)". - Member of the Scientific Committee of XVI National Congress of Italian Society for Research on Radiation (SIRR), 7th-8th November 2014, Pavia - Chairman of the Session "S6: Non-Ionizing radiation-EMR-UV", 41st Annual Meeting of the European Radiation Research Society (ERR2014), 14th-19th September 2014, Rhodes, Greece

Pacchierotti F. Seminar: Stato dell'arte e prospettive della valutazione tossicologica di nanomateriali ingegnerizzati. In the frame work of Webinar "Attività regolatoria sui nano materiali: punto della situazione e prospettive", organized by ENEA UTTAMB, Rome, January 28th, 2014.

Pazzaglia S. Chairman of the Session "S10: Non Cancer Effects", 41st Annual Meeting of the European Radiation Research Society (ERR2014); 14-19 September 2014, Rhodes, Greece - Member of the Scientific Committee of The Sixth International MELODI Workshop", 7th- 9th October 2014, Barcelona.

Pioli C. Member of the International Scientific Committee of the 5th International Conference on Electromagnetic Fields Health and Environment (EHE2014) 24th-25th April 2014, Porto, Portugal. - Contract Professor, Molecular Immunology, University of Rome Tor Vergata. - Faculty board of professors for the PhD course in Immunology, Molecular Medicine and Applied Biotechnology, University of Rome Tor Vergata

Salerno S. Contract Professor, Course on Psychosocial Risks, University Degree in Environment and Workplace Prevention Techniques, Tor Vergata University of Rome. - Contract Professor, Course on Environmental Hygiene, University Degree in Geography for Environment and Health, Sapienza University of Rome.

Spanò M. International Editorial Board of the "Annals of the National Institute of Hygiene" of Poland.

Uccelli R. ENEA's delegate for collaboration with the Italian Association of Doctors of Environment (ISDE) concerning coordination and collaboration in epidemiological and toxicological research and training activities. - Reviewer of progress reports of projects funded by NATO through the Science for Peace and Security (SPS) Programme approved during the membership in the Chemistry/Biology/Physics Advisory Panel and in the Independent Scientific Evaluation Group (2009-2012).

Villani P. Member of OECD Expert Group on "In vivo Comet assay"

Projects and Grants

- BioQuaRT Biologically weighted quantities in radiotherapy. (1/06/2012-31/05/2015). EMRP.
- BIOxTREME, Research project “BIO-fabbriche vegetali per la formulazione di molecole bioattive ad attività microbicida, immunostimulatoria e antiossidante per la vita in condizioni esTREME, funded by Italian Space Agency (2014 – 2017): coordinator ENEA (UT BIORAD).
- CEREBRAD, ‘Cognitive and Cerebrovascular Effects Induced by Low Dose of Ionizing Radiation’. PI Dr. S. Pazzaglia; (01/10/2011 - 31/03/2015).
- DARK.RISK, ‘Studies on a cohort of Serbian children exposed to x-irradiation to determine the contribution of the non-coding genome to susceptibility at low doses’. PI Dr. M. Mancuso; (01/10/12 - 30/09/15).
- Defence against CBRN Agents. A Panel of Biomarkers as Novel Tool for Early Detection of Radiation Exposure. NATO SPS call, topic 1.d.
- Development of innovative bio-pharmaceuticals for anti-metastatic therapy. Funded by Attività di Ricerca per la finalità dell’Articolo 2, comma 44 L. 23 Dicembre 2009, N.191, Extended to June 2015
- DoReMi, ‘Dose/Dose-rate Radiation Effects in Brain Cancer Risk-DDRE-BrainCancer’. PI Dr. A. Saran; (01/07/2011 - 30/06/2015).
- ECOFIBAR, Composti cementizi ecocompatibili realizzati con fibra di basalto e con aggregati di riciclo. Project MIUR PON 01-01522.
- Improvement of Energy Efficiency in e-mobility coming under the "Electric System Research" project. Research contract between ENEA and the Department of Industrial Engineering, University of Padua, funded by Italian National Regulatory Authority for Electricity and Gas (AEEG) and implemented under Program Agreements between Italian Ministry of Economic Development and ENEA, CNR and RSE Ltd (2013 – 2014).
- In vitro and ex vivo studies of Electromagnetic Fields' effects on stem cells and risk assessment of health care workers. Finalized Research funded by Italian Ministry of Health. (2011 – 2015): coordinator Bambino Gesù Children Hospital; Operative Units: Catholic University of Sacro Cuore in Rome, INAIL, ENEA.
- Mechanism of action of Glycyrrhizin in the mucosal healing process in Dextran Sulfate Sodium-induced colitis. Avvio alla Ricerca Anno 2014 (Sapienza University, Rome) – N. Prot. 0042358 (2014-2015)
- Molecular Study of Pathogenetic Mechanisms Underlying Inflammatory Bowel Diseases. FIRB - N.Prot: RBAP104JYK (2012-2015)
- NANoREG Project. A common European approach to the regulatory testing of manufactured nanomaterials - EU FP7 Programme, NMP Area, contract no 310584,

- Nuove acquisizioni sui meccanismi delle malattie infiammatorie intestinali e identificazione di nuovi target terapeutici. PRIN - N. Prot. 2010K34C45 (2011-2014)
- PROCARDIO, 'Cardiovascular Risk from Exposure to Low-dose and Low-dose-rate Ionizing Radiation'. PI Dr. A. Saran; (01/10/2011 - 31/03/2015).
- RENEB Realizing the European Network in Biodosimetry. FP7 - EURATOM.. (01/01/2012 – 31/12/2015)
- Sicurezza e Tecnologie Sanitarie: Rischi diretti e indiretti per la salute e la sicurezza di lavoratori e pazienti derivanti dall'utilizzo di tecnologie emergenti basate sui campi elettromagnetici. Research Project part of the National Strategic Program, funded by Italian Ministry of Health (2010 – 2013): coordinator Italian National Health Institute (ISS); Operative Units: ISS, INAIL, CNR-IFAC, ENEA, Fondazione IRCCS Policlinico San Matteo, Pavia.
- Studio e messa a punto di una nuova miscela di probiotici e molecole anti-infiammatorie da utilizzare nel trattamento delle malattie infiammatorie intestinali. MIUR art.11 D.M. 593/2000 N.10/12, N. Prot. 1831/12 (2012-2015)
- Studio, validazione e realizzazione di un nuovo kit per la diagnosi precoce delle infiammazioni intestinali tramite utilizzo della proteina HMGB1 come marcatore fecale - FILAS - N. Prot. FILAS-CR-2011-1162 (2012-2014)

Prizes

Giardullo P. Scholars-in-Training (SIT) Travel Award to the 60th Radiation Research Society Annual Meeting, Las Vegas, Nevada.
Raffaella Uccelli, Inclusion in the Marquis Who's Who in the World bibliographical profiles 2014.

Patents

Franconi R, Spanò L, Venuti A, Massa S. 'Vaccines based on genetic chimera of viral and/or tumoral antigens and plant proteins'. Italian Patent n.° 0001394887; PCT/IT2010/00324, EP10747517.0 (Submitted 04 September 2012). European patent n. 2456785 (European Patent Bulletin 14/47 of 19.11.14). Validation request in Italy, France and Germany.

Collaborations

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