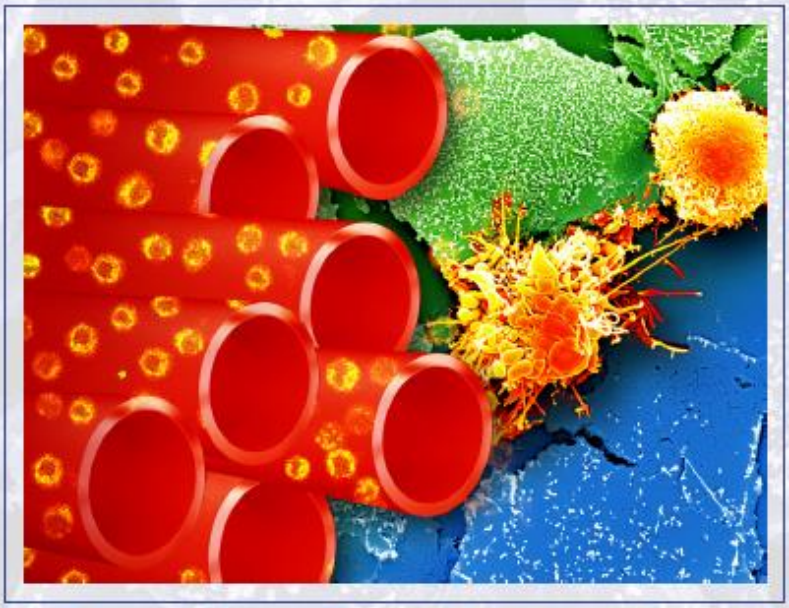

Therapeutic Nanoproducts:
from Biology to Innovative Technology

June 19th - 20th, 2019

Aula Pocchiari
Istituto Superiore di Sanità, Rome

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ISTITUTO SUPERIORE DI SANITÀ
and
Associazione Italiana di Colture Cellulari - AICC

Drug resistance in metastatic melanoma: development of nanoparticles for therapeutic microRNA tumor delivery.

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Emerging data support the rationale of combined therapies in advanced melanoma. The association of drugs with different mechanisms of action can reduce the frequency of resistant clone selection. By screening a library of 349 anticancer compounds, currently in clinical use or trials, we selected PIK-75, an inhibitor of the PI3K/AKT pathway. PIK-75 was then utilized alone or in combination with Vemurafenib, the first BRAF inhibitor approved for patients with melanoma harboring $BRAF^{V600E}$ mutation. In the last decade, accumulating evidences suggested that non coding RNAs (ncRNAs) (e.g. microRNAs and long ncRNAs) play important roles in all the key cellular processes. In our hands, the presence of microRNA126 (miR126), already proved as tumor suppressor in metastatic melanoma, significantly increased PIK-75. The effectiveness of miR126 to boost up PIK-75 action was demonstrated in patient-derived cells, in resistant cell lines and in *in vivo* models (Pedini F. *et al*, 2019).

In view of the important role of miR126 as tumour suppressor, our project aims to develop a system able to deliver this miR into metastatic melanoma cells in combination with PIK-75 and/or Vemurafenib. For this purpose, we intend to produce different types of nanoparticles containing miR126, possibly conjugated with antibodies specific for metastatic melanoma cells. As second approach, we propose to use miR126 enriched exosomes (EXO) as vectors for delivery system. Nano- and EXO-miR126 will be tested both *in vitro* and *in vivo*. Moreover, we will study the tumour micro-environment cross-talk between resistant and sensitive metastatic melanoma cells, with particular attention to exosome-mediated transfer of ncRNAs.

"Progetto di Gruppo di Ricerca finanziato ai sensi della L.R. Lazio 13/08"

Urotensin II-targeted liposomes as a new drug delivery system towards prostate and colon cancer cells.

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Urotensin II (UT-II) and its receptor (UTR) are involved in the occurrence of different epithelial cancers. UTR was found over-expressed on colon, bladder and prostate cancer cells. The conjugation of ligands, able to specifically bind receptors that are over-expressed on cancer cells, to liposome surface represents an efficient active targeting strategy to enhance selectivity and efficiency of drug delivery systems. The aim of this

study was to develop liposomes conjugated with UT-II (LipoUT) for efficient targeting of cancer cells that overexpress UTR. We evaluated UTR expression on prostate (DU145, PC3 and LNCaP) and colon (WIDR and LoVo) cancer cells by FACS and western blotting analysis. UTR protein was expressed in all tested cell lines; the expression levels were higher in WIDR, PC3 and LNCaP cells compared with LoVo and DU145. MTT assay showed that LipoUT-Doxo was more active than Lipo-Doxo on the growth inhibition of cells that overexpressed UTR (PC3, LNCaP and WIDR) while in LoVo and DU145 cell lines the activity was similar or lower than that one of Lipo-Doxo, respectively. Moreover, cell uptake of Bodipy-labelled liposomes in PC3 and DU145 was higher for LipoUT than the not armed counterparts but at higher extent in UTR overexpressing PC3 cells (about 2-fold higher) as evaluated by both confocal and FACS. In conclusion, the encapsulation of Doxo in UII-targeted liposomes potentiated its delivery in UTR overexpressing cells and could represent a new tool for the targeting of prostate and colon cancer.

Assessment of Activin A effect on B-Cell precursor acute lymphoblastic leukemia (BCP-ALL) cell vesiculation.

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Despite cure rate of B-Cell Precursor Acute Lymphoblastic Leukemia (BCP-ALL) exceeds 85%, it remains the leading cause of childhood cancer-related death. In the bone marrow, stromal and leukemic cells dialogue through soluble factors and extracellular vesicles (EVs) which reprogram the stroma to become leukemia-supporting and chemoprotective. In the context of BCP-ALL, we showed that ActivinA positively regulates cytosolic calcium, associated to EVs formation. Therefore, we investigated the effect of ActivinA on leukemic cell vesiculation. We optimized a method to isolate and characterize EVs from BCP-ALL cells by using the 697 cell line cultured for 24-48h in presence or not of ActivinA (50 ng/mL) (n=8). Culture media were analyzed with NanoSight software to measure EVs concentration (particle/mL) and size distribution, discriminating between exosomes (30-150 nm) and microvesicles (151-700 nm). Notably, ActivinA increased the mean concentration of 697-derived exosomes of about 60% ($p<0,008$) and 30% ($p<0,016$) at 24h and 48h, respectively. The microvesicle mean concentration was slightly increased after 24h of ActivinA stimulation ($p=0,055$) and this difference became statistically significant after 48h ($p<0,016$). EVs samples, obtained by ultracentrifugation, were stained with CFSE to evaluate their integrity and with specific antibodies to discriminate between microvesicles and exosomes by flow cytometry. Microvesicles were identified by CD19 antibody and exosomes by CD9/CD63 antibodies. Overall, we demonstrated that ActivinA stimulates leukemic cells to release both exosomes and microvesicles populations. A better comprehension of the EVs' role in the context of leukemic bone marrow niche may provide new therapeutic targets to eradicate BCP-ALL without affecting healthy hematopoiesis.

Microvesicles in celiac disease: possible biomarkers and players in gut inflammation.

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Celiac disease (CeD) is an immune-mediated enteropathy triggered by gluten ingestion in genetically susceptible individuals (HLA-DQ2/DQ8). Recent studies have highlighted the possible role of luminal extracellular vesicles (exosomes, microvesicles and apoptotic bodies) in inflammatory bowel diseases, and their possible use as biomarkers of disease progression and response to treatment. However, their role still remains unexplored in celiac disease.

The aim of this study is to evaluate the role of microvesicles (MVs), purified from plasma and intestinal cultured biopsies (ICBs) from CeD patients, as a source of biomarkers and pathogenic players in gut inflammation.

The specific objectives are:

1)To identify biomarkers associated with active CeD by proteomic analysis of MVs purified from plasma of patients.

2)To evaluate the role of MVs purified from ICBs in gut inflammation spreading.

The comparison of proteomic profiles of circulating MVs between CeD patients and healthy subjects showed the presence of desmosomal proteins associated with active celiac disease. To evaluate the MV role in inflammation, 21 days differentiated Caco-2 cells monolayer, as an intestinal *in vitro* model, were treated with purified MVs from the ICB supernatants: MVs from active CeD patients induced a rearrangement of actin filaments, an increase in tissue transglutaminase 2, a decrease in ZO1 expression and an increase of the pro-inflammatory cytokine IL-8, respect to controls.

These preliminary results suggest that MVs, produced during chronic active CeD, may have a role in inflammation spreading and constitute a novel source of circulating biomarkers of the disease.

A nanoencapsulated fenretinide formulation targets cancer stem cells from solid tumors.

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Fenretinide is a synthetic retinoid characterized by anticancer activity in preclinical models and favorable toxicological profile, but also by a low bioavailability that hindered its clinical efficacy in former clinical trials. We developed a new formulation of nanoencapsulated fenretinide complexed with 2-hydroxypropyl-β-cyclodextrin (nanofenretinide) characterized by high bioavailability, reduced toxicity and increased therapeutic efficacy. Nanofenretinide showed high inhibitory effect on the metabolism, proliferation and survival of cancer stem cells (CSCs) derived from lung and colorectal tumors, resulting in antitumor action *in vitro* and *in vivo*. A global profiling of pathways activated by nanofenretinide in CSCs was performed by phospho-proteomics and lipid analysis, resulting in a landscape of functional effects ranging from cell death to cell cycle arrest and to a generalized metabolic repression mediated by inhibition of the mTOR pathway and massive production of dihydroceramide. Altogether, these results indicate that nanofenretinide activates a multifactorial program in CSCs composed by signals of apoptosis, autophagy and proliferative/metabolic inhibition, resulting in a widespread and durable antitumor effect in mice. The combined properties of elevated bioavailability, low toxicity and anti-CSCs action indicate nanofenretinide as a potential anticancer agent with broad clinical spectrum.

miR-125b upregulates miR-34a and sequentially activates stress adaption and cell death mechanisms in multiple myeloma.

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miR-125b, ubiquitously expressed and frequently dysregulated in several tumors, has gained special interest in the field of cancer research, displaying either oncogenic or oncosuppressor potential based on tumor type. We have previously demonstrated its tumor-suppressive role in multiple myeloma (MM), but the analysis of molecular mechanisms needs additional investigation. The purpose of this study was to explore the effects of miR-125b and its chemically modified analogs in modulating cell viability and cancer-associated molecular pathways, also focusing on the functional aspects of stress adaptation (autophagy and senescence), as well as programmed cell death (apoptosis). Based on the well-known low microRNA (miRNA) stability in therapeutic application, we designed chemically modified miR-125b mimics, laying the bases for their subsequent investigation in in vivo models. Our study clearly confirmed an oncosuppressive function depending on the repression of multiple targets, and it allowed the identification, for the first time, of miR-125b-dependent miR-34a stimulation as a possible consequence of the inhibitory role on the interleukin-6 receptor (IL-6R)/signal transducer and activator of transcription 3 (STAT3)/miR-34a feedback loop. Moreover, we identified a pattern of miR-125b-co-regulated miRNAs, shedding light on possible new players of anti-MM activity.

Finally, functional studies also revealed a sequential activation of senescence, autophagy, and apoptosis, thus indicating, for the first two processes, an early cytoprotective and inhibitory role from apoptosis activation.

Proteomic analysis of exosome from plasma of patients affected by active or inactive *Echinococcus granulosus* hydatid cysts

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Cystic echinococcosis (CE) is a parasitic zoonosis caused by the cystic larval stage (metacestode) of the dog tapeworm Echinococcus granulosus sensu lato (*E. granulosus)*. The global burden of human CE has been estimated in more than 1 million people infected, with over 1 million DALYs lost every year when accounting for underreporting. US is the reference diagnostic tool, weakly supported by serology when imaging is inconclusive. The availability of circulating biomarkers would considerably improve the diagnosis and much significantly the cyst staging. To identify potential plasma protein-markers, and possible target signalling involved in disease establishment, is one of the main goals and greatest challenges of clinical proteomics. Blood is easily accessible and can be described as the most comprehensive human proteome, potentially informative in regards to almost any disease state; but, its comprehensiveness of is counterbalanced by the complexity and deep dynamic range, with very high abundant proteins interfering with proteomic analysis. In this study we investigate exosomes isolated from human plasma of CE infected patients and uninfected controls. Exosome delivery in bloodstream can be described as sending "bottle messages" in an extremely sensitive and complex "cell language" throughout vesicle trafficking. Proposed to mediate cell/cell communication in patho/physiological conditions, nowadays extracellular vesicles represent sensible biomedical research targets, deeply investigated for biomarker identification, understanding of cell-communication, metastasis process and host-pathogen interplay or as drug delivery vehicles. We set up a suitable method for a right isolation of exosomes from human plasma and we applied this method to analyse plasma samples collected during 2014-2015 HERACLES project in Italy, Turkey and Romania. Proteomics and bioinformatics analyses resulted in a wide list of probably activated pathways underpinning the different patterns elicit by *E. granulosus* to modulate the immune response, inflammation and the Th1/Th2 balance, inducing immunological tolerance. Further, we identified potential biomarkers to be validated as additional tools for diagnosis and cyst viability definition.

Hyaluronated liposomes for the targeted delivery of H_2S-releasing doxorubicin to osteosarcoma cells.

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Osteosarcoma is the most frequent type of bone cancer, with 110-125 new cases/year in Italy. Anthracycline-based regimes (including doxorubicin, Dox) are used as neo-adjuvant and adjuvant treatment, but this therapy is successful in 55%-60% of patients only. The main drawbacks of Dox are the onset of drug resistance for the presence of the drug efflux transporter P-glycoprotein (Pgp), and the onset of cardiotoxicity. In a previous work we found that Doxs conjugated with a H_2S-releasing moiety (Sdox) were less cardiotoxic and more effective than Dox against Pgp-overexpressing osteosarcoma cells [Chegaev et al. 2016, doi: 10.1021/acs.jmedchem.6b00184; Buondonno et al. 2019, doi: 10.1007/s00018-018-2967-9]. To improve the tumor active delivery of SDox, we encapsulated the drug in hyaluronic acid (HA)-conjugated liposomes (HA-Lsdox), since the HA receptor CD44 is abundant in osteosarcoma. HA-Lsdox was more accumulated in tumor cells and showed a higher toxicity in vitro and in vivo with respect to Dox or Caelyx® (the FDA-approved liposomal Dox), and maintained the same cardiotoxicity profile of Caelyx®. HA-Lsdox delivered the drug within the endoplasmic reticulum (ER), where it induced protein sulfhydration, increased protein misfolding and ubiquitination, thus activating a ER stress response inducing cell apoptosis. Beside its ER stress-dependent effects, HA-Lsdox increased tumor cell death by reducing the efflux of Sdox by Pgp that was sulfhydrated within the ER and ubiquitinated.
In conclusion we propose HA-Lsdox as an effective new therapeutic tool that may represent a significant advancement in the treatment of Pgp-positive tumors.

Non-viral gene delivery using biodegradable PGA NPs.

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Gene therapy is described as the direct transfer of genetic material to cells or tissue for the treatment of inherited disorders and acquired diseases, such as cancer. Nanoparticle (NP)-based therapeutic systems developed in recent years have shown efficient delivery of nucleic acids with low toxicity and sustained cargo release. NP-based systems overcome safety problems and limitations of viral vectors. FDA-approved polymers are particularly attractive for *in vivo* drug/gene delivery applications. An attractive polymer for gene delivery applications is polyglycolic acid (PGA), which has been approved by the FDA. Our PGA NPs are obtained by nanoprecipitation and desolvation method, and are composed of a core loaded with the acid nucleic molecules and a PGA shell. Prior to particle assembly, active agent is complexed with a pH (as chitosan) or enzymatic (as protamine)-responsive polymer. By combining the sensitivity of the core polymer with the

slow degradation of surface PGA, we obtained a simple and easy way to control the release of an active agent and improve its therapeutic efficiency.
The mean size of PGA nanoparticles is 100 nm, with a negative ζ-potential of -12 mV. No cytotoxicity using our PGA NPs is observed on different cell lines tested.
In addition, our PGA NPs have showed an efficient delivery of cDNA, observing relative numbers and mean fluorescence intensity of transfected GFP-positive cells comparable to those achieved with standard reagents used to promote transfection.

Satureja montana L. essential oils and nanoemulsions: evaluation of physical-chemical and antimicrobial properties.

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Satureja montana L. Essential Oil (SEO) presents a wide range of biological activity due to the high content of active phytochemicals which are strictly dependent by the environmental and growth conditions. The application of an untargeted metabolomic approach has allowed to obtain the chemical fingerprints of four different SEOs, by covering a wide range of polar and semi-polar metabolites. The composition of the complex metabolic mixtures, differing for the biological sample harvesting and distillation time, have been determined by means of high resolution Fourier-transform ion cyclotron resonance (FTICR) mass spectrometry (MS) coupled to electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) sources. Terpenes, terpenoids and low- and medium chain fatty acids are the most abundant components. Subsequently, the antimicrobial properties against different Gram-negative and Gram-positive bacterial strains have been assessed in order to determine the minimum inhibitory (MIC) and the minimum bactericidal (MBC) concentration. All the sampled SEOs have shown significant MIC and MBC values. In only one case, an additional antibiofilm activity has been found, alone or in combination with the antibiotic gentamicin. In order to obtain a useful therapeutic nanoproduct, Oil in Water (O/W) Nanoemulsions (NEs) composed by different amount of SEO were prepared. All formulations were analyzed in terms of hydrodynamic diameter (HD), ζ-potential (ζ-Pot.) and polydispersity index (PDI). Furthermore, stability studies on NEs were performed. The obtained results confirmed the formation of stable NEs characterized by homogeneous dimensions. Future studies will be conducted to compare the antimicrobial activity of SEO alone and structured as NEs.

Inflammatory microenvironment of HPV-positive cells.

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Human papillomaviruses (HPVs) include more than 100 small DNA viruses, divided into mucosal or cutaneous HPVs genotypes. Based on their capability to induce Squamous Cell Carcinomas, a group of them is classified as High-Risk HPVs (HR-HPVs). The E6 and E7 oncoproteins of HR-HPVs are the viral mediators that affect the cellular transformation by deregulating several cellular processes. HR-HPVs might be the interplay between infection, inflammation and malignant transformation. Our recent evidence highlights an active role of Extracellular vesicles (EVs) in HPV-induced tumorigenesis.
Here, the role of inflammatory microenvironment in the HPV-induced carcinogenesis is addressed. Inflammatory cyto-/chemokines has been analyzed in primary human foreskin keratinocytes (HFK) and in keratinocytes transduced by E6 and E7 from mucosal HPV-16 (K16) or cutaneous HPV-38 (K38). A broad downmodulation in the expression levels of these cytokines in K16 and K38 cells has been found.
EVs from HFK, K16 and K38 cells have been isolated to analyze carried cyto-/chemokines mRNAs. The expression profile in EVs cargo was superimposable to those of the corresponding producer cells. By analyzing the microRNAs expression in K16 and E6/E7 silenced K16, different levels have been observed not only between K16- and K16 silenced-derived EVs but also between producer cells and the corresponding EVs.
Our results suggest that E6 and E7 affect the HPV^+ cell micronviroment by deregulation of inflammatory cyto-/chemokines expression levels thereby modifying the inflammatory profile of the secreted mediators; they also indicate a possible role of EVs delivery involvement in the settlement of the inflammatory microenvironment.

Nanotech revolution for miRNA delivery in glioblastoma: a new strategy to overcome temozolomide resistance by targeting O6-methylguanine methyl transferase (MGMT).

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Glioblastoma (GBM) is a highly aggressive brain cancer with poor clinical outcome. Unfortunately, temozolomide (TMZ) has a very limited efficacy due to the occurrence of chemoresistance mainly attributed to O6-methylguanine methyl transferase (MGMT) activity. Recently, a microRNA, namely miR-603, has been identified to target MGMT. These findings suggest the possibility to use microRNAs as powerful tools to enhance the efficacy of TMZ in the treatment of GBM.
On these bases, we aim to test a new delivery strategy using self-assembling nanoparticles (SANPs) based on cationic lipids encapsulating miR-603 in combination with TMZ. In the first stage, we investigated the influence of the lipid composition on the physical characteristics of the SANPs, plain and following complexation with miRNA, as wild type or O-methylated by detecting size, polydispersity index and **ζ** potential. All the

formulations were characterized by a miRNA encapsulation efficiency very high, frequently close to 100%.
In vitro studies were carried out on GBM cells (LN229, U87MG) to evaluate cytotoxicity of SANPs using MTT assay. Formulations showing on both cell lines 80% of cell viability at the highest concentration were considered not toxic in our experimental conditions and used for the following experiments.
Further investigations were based on the analysis of miR-603 expression by quantitative real-time PCR after treatment with SANPs on LN229 and U87MG. We found that SANPs induced a higher uptake of miR603 compared to conventional lipofection.
Collectively, these results provided new insights for using SANPs to deliver microRNAs as a new therapeutic strategy for GBM patients.

Polydatin incorporated in polycaprolactone nanofibers improves osteogenic differentiation of human osteosarcoma and mesenchymal stem cells.

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Polymer nanofibers are applied in the field of tissue engineering as scaffolds for tissue regeneration or local delivery of active substances. Polycaprolactone (PCL) is a synthetic polymer approved by FDA for medical use because biologically safe. Moreover, the incorporation of molecules with osteoinductive activities into PCL nanofibers allows to create biomimetic scaffolds able to promote the differentiation of mesenchymal stem cells (MSCs) into the osteoblast-lineage. Polydatin is a molecule isolated from Polygonum cuspidatum plant known for its antioxidant, anti-inflammatory and antitumor effects. On these bases, we tested nanofibrous scaffolds incorporating polydatin to improve the osteogenic differentiation of human osteosarcoma and mesenchymal stem cells.
Firstly, we evaluated the biological effects of polydatin on osteosarcoma cells. Polydatin induced osteogenic differentiation in SAOS-2 cells as shown by the increase of bone alkaline phosphatase (ALP) activity and intracellular accumulation of calcium. Furthermore, when incorporated in nanofibers, the polydatin improved cell adhesion to scaffold compared to control. Then, we investigated metabolic activity, cell proliferation, and ALP activity of MSCs to evaluate the biocompatibility of nanofibers. After 7 days of cultivation, cell viability and proliferation on scaffolds containing polydatin were very similar to control fibers. On the other hand, on day 14, ALP activity was higher in cells cultivated on coated nanofibers than on empty scaffolds. These results were confirmed by the extensive cell spreading and calcium deposits assessed via SEM.

In conclusion, we provided evidence that PCL nanofibers coated with polydatin are particularly effective in promoting bone differentiation program in osteosarcoma and mesenchymal stem cells.

An exosome-based vaccine platform for CTL immunity against tumors and infectious diseases.

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Exosomes are small vesicles secreted by all cells and are involved in the cell-to-cell intercellular communication by delivering DNAs, RNAs, proteins, and lipids. They find potential applications as diagnostic biomarkers as well as drug/vaccine delivery vehicles, Despite high expectations, clinical trials have not yet confirmed therapeutic application of exosomes.
In particular, exosome-based immunization strategies face with huge technical difficulties including industrial manufacturing, cost of production, and storage. To overcome these hurdles, we designed an original exosome-based immunization strategy relying on the delivery by intramuscular (im) inoculation of a DNA vector that expresses antigens of choice fused to the exosome-anchoring protein Nef^{mut}. The strong efficiency of Nef^{mut} to accumulate in multivesicular bodies (MVBs) results in the production of exosomes/extracellular vesicles (EVs) incorporating huge amounts of the desired antigen. When translated in animals, the injection of Nef^{mut}-based DNA vectors generates engineered exosomes whose internalization in antigen-presenting cells induces cross-priming and antigen-specific CTL immunity. We demonstrated that the expression of the Nef^{mut}-derived vectors in muscle cells results in a continuous source of endogenous (ie, produced by the inoculated host) engineered exosomes able to induce an antigen-specific CTL immune response.
In sum, we established a novel method to generate immunogenic exosomes *in vivo* consisting in the im inoculation of DNA vectors expressing the exosome-anchoring protein Nef^{mut} and its derivatives.

Uptake and cytotoxicity of ZnO-NR in different mammalian cell lines.

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In recent years, many methods have been used to synthesize ZnO nano and micro materials with different morphologies. ZnO-nanomaterials are used in a wide range of products ranging from cosmetics to fabric and electronic devices.
For their increased exposure, their potential harm to human health needs to be carefully investigated. In addition, some ZnO derived nano and microparticles have been demonstrated to exert antibacterial and antitumoral properties.
In this study, we used ZnO nanorods (ZnONR) obtained through the thermal decomposition method, to investigate their uptake, increase of intracellular Zn++ and ROS production in HaCat cells, a human immortalized keratinocyte cell line, MCF7 cells, a breast cancer cell line and SK-N-BE(2) cells, a human neuroblastoma cell line.
Zn++ released from ZnO NRs, measured by fluorimetry was found differently elevated in the different cell lines even at the same time and concentration exposure.
Based on our previous results that both ZnO NR and ZnO MR affected growth and induced ROS in subtoxic concentrations in HaCat and MCF7 cells, we found that exposure to ZnO NR at the same concentration and time, induces increased growth arrest and ROS productions in SK N BE (2) cells.
The differences in cytotoxicity may be due to the different intracellular pH triggered release of ionic Zn++ and the different uptake may be due to the different NP contact with cell surface. We are reporting here that uptake of NP is related to cytotoxicity and this is related to the different metabolic state and morphology of cells.

GO nanosheets: a new promising nano carrier for the S29: 1-(2-chloro-2-(4-chlorophenyl)ethyl)-N-(4-fluorobenzyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine, therapeutic agent in a neuroblastoma cell line.

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GO derivatives are recently reported as a valid alternative to the conventional carrier of therapeutic agents, because they exhibit a large surface area (i.e., 3000 m^2/g), an excellent electrical and thermal conductivity and a great capacity to selective binding of drugs and therapeutics.
In this work graphene oxide (GO) nanosheets, synthesized by electrochemical exfoliation of graphite (patent N 102015000023739, Tor Vergata University), have been selected as a possible carrier of an anticancer drug, the S29, inhibitor of a cytoplasmic tyrosine kinase (c-SRC). S29 is able to reduce tumour mass in neuroblastoma but it shows an unfavorable pharmacokinetic profile. Nanostructured innovative materials seem to be able to improve and to increase the pharmacokinetic and pharmacodynamic properties of compounds, especially in terms of biodistribution, stability and bioavailability. We are reporting on cytotoxicity, growth and migration assay of neuroblastoma cell lines SK-N-BE(2), treated with GO and S29. Treatment of cells with 10 μM S29 and 2 μg/mL GO started to inhibit cell growth after 24 h of exposure and became significantly different from GO alone or S29

alone at 48h and 72 h. ROS production in cells treated with 2mg of GO was very low and it increased slightly when S29 was combined with GO. GO-S29 reduced cell migration, that plays a central role in methastatic processes. This was investigated with the wound healing assay. In conclusion, GO used at the lowest concentration needed to carry the minimum effective dose of S29 is a promising nanomaterial for the treatment of neuroblastoma.

Resveratrol enriched gold nanoparticles strengthen breast cancer cell susceptibility to chemotherapeutic agent.

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The mainstay treatments of estrogen-sensitive breast cancer result in remarkable side effects especially for pre-menopausal women in which the incidence of estrogen-sensitive breast cancer is increasing in these latter years. Our previous data demonstrated that the pathway 17β-estradiol (E2)/estrogen receptor (ERα)/neuroglobin (NGB) is one of vital mechanisms triggered by E2 to increase the survival of E2-related cancer cells even in the presence of chemotherapy. Thus, we hypothesize that the reduction of NGB levels could render breast cancer cells more prone to death. The plant derived polyphenol resveratrol, is a good candidate due its ability to bind to ERα, modulating receptor activities. We showed that resveratrol treatment increased the susceptibility of breast cancer cells to the chemotherapeutic agent paclitaxel (Pacl) by affecting E2/ERα/NGB pathway. In MCF-7 and T47D (ERα-positive), but not in MDA-MB 231 (ERα-negative), Res decreases NGB levels interfering with E2/ERα-induced NGB upregulation. Although Res treatment does not reduce cell viability by itself, this compound potentiates Pacl pro-apoptotic effects. Unfortunately, resveratrol displays a low bioavailability in human beings, principally due to its high metabolism in both gut and liver. Here we enriched functionalized gold nanoparticles with resveratrol. MCF-7 cells have been treated with the enriched nanoparticles and their effect on NGB levels and on breast cancer cell susceptibility to Pacl have been evaluated. Results showed that enriched nanoparticles are more efficient than resveratrol to reduce NGB levels increasing Pacl-dependent apoptotic effects.

Acknowledgements: The Grant of Excellence Departments, MIUR (ARTICOLO 1, COMMI 314 – 337 LEGGE 232/2016), is gratefully acknowledged by authors of Roma Tre University.

Facing Alzheimer's disease: new strategies for delivery of a β-sheet breaker peptide against β-amyloid aggregation.

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One of the key pathogenic events in the onset of Alzheimer's disease (AD) is the aggregation of beta-amyloid (Aβ) peptides into toxic aggregates. Molecules that interfere with this process might act as therapeutic agents for the treatment of AD. The peptide KLVFF (part of Aβ aminoacidic sequence) is known to be essential for the formation of these toxic aggregates. It was also shown that KLVFF binds to the homologous sequence in Aβ and prevents its aggregation. However, KLVFF peptide suffers from poor bioavailability and inability to cross the blood brain barrier (BBB). In this work, we study the possibility to adopt nanomedicine to overcome the above-mentioned limitations. A tailored nanoprecipitation procedure was set up by using mixture of organic solvents (DMSO/Acetone) to dissolve the polymer and the peptide and to create KLVFF loaded NPs (K-NPs) able to deliver a therapeutic dose of KLVFF peptide.
The K-NPs demonstrated to be safe and to restore cell health with a similar efficacy as free KLVFF peptide, significantly reducing the damage caused by Aβ aggregation (e.g. dendritic fragmentation and synaptic density). The K-NP exerted a strong disaggregation effect in the presence of existing Aβ aggregates, suggesting the possibility to exploit these NPs also during the late stage AD and not only in the initial phase of the pathology. Overall, these results indicate that K-NPs possess full therapeutic potential to advance KVLFF treatment as a therapeutic option for AD.

New decorated nanocarriers for biomedical applications.

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Biocompatible and biodegradable nanoparticles (NPs) are widely studied as an effective drug delivery device (1). Among candidates for a drug carrier system, chitosan represents a kind of natural cationic polymer showing nontoxic, biocompatible, biodegradable features. Chitosan represents a kind of natural cationic polymer showing nontoxic, biocompatible and biodegradable features. One of the main prerequisite of a therapeutic drug is to overcome a series of physiological barriers and it to be less toxic for the human. Recently, some peptides have been used to improve the specific targeting and topical absorption of biologically active substances such as peptides and other organic molecules through the epidermis. In the presented work we would like to design peptide decorated-chitosan nanoparticles in which active elements are associated with antifungal and anticancer properties. Therefore, this new decorated delivery system would have a synergistic and/or additive effect decreasing the drug resistance reactions.

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Serum miR-93, miR-223, and miR-532 as potential non-invasive biomarkers for diagnosis of laryngeal cancer.

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Laryngeal cancer (LCa) is the second most frequent head and neck malignancy. Despite the remarkable advancement in both diagnosis and treatment options, the disease morbidity and mortality have not been sufficiently decreased. The early detection is still a challenge because of its asymptomaticity until the advanced stage and the failure to detect micro-metastases by conventional imaging analyses. Consequently, there is an urgent need to identify non-invasive reliable molecular markers and to develop useful detection tools. Recently, microRNAs (miRNAs), small non-coding RNAs regulating mRNA translation, have been considered potential biomarkers and therapeutic targets for various diseases. In the present study, we aimed to characterize the serum miRNAs profile in LCa patients and to identify the biomarker candidates for LCa detection. Preliminary analysis showed 11 up-regulated and 5 down-regulated serum miRNAs in LCa patients, compared to healthy individuals. Then, we focused on three overexpressed candidates (miR-93, miR-223, miR-532) confirming their significant up-modulation ($p<0.0001$) by qRT-PCR validation tests. Moreover, the AUC values coming from ROC analysis (0.75 for miR-93, 0.74 for miR-223, and 0.78 for miR-532) suggested that these serum miRNAs are potential non-invasive biomarkers.
Our perspective is to realise high-sensitivity optical and electrochemical biosensors to improve an easily diagnostic of the LCa. We will bio-functionalise some innovative electrospun nanofibers (such as PAN/PEDOT, PU/P3ANA) by a proper chemistry for the immobilization of the probes. We will follow up the recognition hybridization between the ssDNA and the miRNA by different sensitive and reproducibly methodologies such as fluorescence and impedentiometric spectrometry.

Gold nanoparticles and nanorods in nuclear medicine: new tools in tumors treatment.

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Nowadays, the *in vivo* molecular imaging plays a crucial role in diagnosis and therapy and several kinds of nanoparticles have received significant attention in this field. In particular, gold nanoparticles and nanorods (AuNPs and AuNRs) have advantageous properties including multifunctionality and multivalency effects.[1,2] In fact, they show modulable surface chemistry and little or no cytotoxicity in various cell/animal models.[2,3] Moreover, they can be conjugated with targeting ligands or imaging agents, such as ^{90}Y and ^{125}I, for improved affinity (avidity) and targeting efficiency.[4-5] In this framework, new functionalized AuNPs and AuNRs, suitables for theranostic applications, together with data for *in vitro* evaluation, were presented. [6,7]

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Acknowledgements: The Grant of Excellence Departments, MIUR (ARTICOLO 1, COMMI 314 – 337 LEGGE 232/2016), is gratefully acknowledged by authors of Roma Tre University

Hydrophilic gold nanoparticles and nanorods as drugs vehicles: structural and biological studies for biomedical applications.

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Gold nanoparticles and nanorods (AuNPs, AuNRs) are successfully applied in drug delivery, photothermaltherapy, and biotechnologies.[1-2] Their success is due to unique chemical and physical properties, biocompatibility, and well-established strategies for surface modification. In this framework we present AuNPs and AuNRs synthetized with the aim to obtain strongly hydrophilic materials, suitable for drug delivery. These were used as biocompatible vehicles for commercial drugs, *i.e.* dexamethasone and methotrexate, synthetic drugs, *i.e.* copper(I)-based anti-tumor complexes, and active biological molecules, *i.e.* resveratrol.[3-6] The drug loading efficiency was investigated, with the aim to optimize both bioavailability and controlled release.

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Acknowledgements: The Grant of Excellence Departments, MIUR (ARTICOLO 1, COMMI 314 – 337 LEGGE 232/2016), is gratefully acknowledged by authors of Roma Tre University.