Introduction: Recent studies confirmed that a moderate amount of wine may have health benefits. Experimental evidence suggested that these beneficial effects could be due to the polyphenol content of red wine.
Objectives: In this study, we evaluated the effect of red wine polyphenols (RWP) in J774, a cellular model widely used for studies on oxidative stress and redox capacity of plant extracts and molecules.
Results: In the presence of RWP, J774 cells showed an opposite effect on cell viability. Increased cell growth of about 20% was measured at low concentration (4 μg/ml GAE [gallic acid equivalents]), while 50% cytotoxicity was observed at higher concentrations (40 μg/ml GAE). We also detected a rapid (15 min) and dose-dependent increase of superoxide anions (15%—and 180% at 4 and 40 μg/ml GAE, respectively compared to untreated controls). However, while at the lower concentration applied GSH and H_2O_2 production did not change significantly, at the higher RWP value, GSH and H2O2 contents decreased significantly of 38% and 15%, respectively. To counteract the intracellular ROS production, cells triggered a rapid dose-dependent activation of all the antioxidant enzymes investigated at 4 μg/ml GAE (41% SOD; 18% GSH peroxidase; 24% GSH reductase; 23% G6PD) and 40 μg/ml GAE (150% SOD; 97% GSH peroxidase; 100% GSH reductase and G6PD).
Conclusions: Our preliminary results indicate a pro-oxidant activity of RWP, which may promote cell proliferation at low doses and cell death at higher ones.

A5
VITAMIN D ANALOGUE EB1089 SENSITIZES HUMAN TRANSFORMED CELL LINES TO GAMMA RADIATIONS BY INDUCING LETHAL AUTOPHAGY AND APOPTOSIS

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Introduction: The gold standard for unresectable solid tumours and advanced forms of leukemia is represented by chemo-radiation. However, therapeutic options are limited and patients undergo systemic cytotoxicity. Vitamin D insufficiency is a widespread problem and its low serum levels are linked to higher cancer incidence. Previous studies showed a strong correlation between serum concentration of vitamin D and time of first treatment in chronic lymphocytic leukemia (CLL). Other studies demonstrated that vitamin D and its ipocalcemic analogue EB1089 are able to bypass radio-resistance in breast and lung cancer cell lines by activating cytostatic/cytotoxic forms of autophagy. However, only few data are available on the intracellular and molecular effects of vitamin D in osteosarcoma (OS) and CLL-derived cells and on its association with gamma-radiation in sensitizing these cancer types.
Objectives: We studied the antiproliferative effects of an active form of vitamin D, the analogue EB1089, in two cells lines U2Os and HG3 derived from a human OS and a CLL, respectively, and its efficacy after treatment with gamma-radiations in terms of cytotoxicity, autophagic and apoptotic effects.
Results: EB1089, used at physiological concentration (100 ng/ml), is able to bypass gamma-radiations resistance in U2Os and HG3 cell lines by activating cytotoxic autophagy and apoptosis. The co-treatment resulted highly synergic in terms of combination index (C.I. <1) inducing 85% of cell death at higher doses of radiation after 24h of co-treatment.
Conclusions: The results obtained will be discussed at the light of the cytostatic/cytotoxic function of autophagy mediated by vitamin D and involving MAPK/ERK and AMPK pathways in enhancing the therapeutic response to gamma-radiations.

A6
AUTOPHAGY FLUX MODULATION BY A CAROTENOID-ENRICHED EXTRACT FROM THE PUMPKIN CUCURBITA MOSCHATA ON HUMAN CHRONIC LYMPHOCYTIC LEUKEMIA CELL LINE

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Introduction: Chronic lymphocytic leukemia (CLL) is the most frequent form of leukemia in adult population and chemotherapy resistance occurs in 15—30% of patients with elevated genomic complexity. Apoptosis resistance and induction of a protective form of autophagy are possible explanations of the poor responsivity of CLL to conventional and novel therapeutic drugs. Given the difficulties to maintain in culture B-CLL lymphocytes, the HG3 cell line represents an interesting preclinical model to study the effects of natural bioactive molecules or extracts derived from food matrix as potential, chemo-sensitizers in CLL.
Objective: A previous study demonstrated the induction of "not-protective" autophagy on osteosarcoma and colon adenocarcinoma cell lines after prolonged treatment with a carotenoid-enriched extract (CE) obtained from the pumpkin Cucurbita moschata, variety "long of Naples". To extend and confirm these data, the present communication focuses on the anti-proliferative effect of the same extract in HG3 cell line derived from EBV immortalization of B-CLL cells.
Results: CE was obtained from pumpkin by supercritical CO2 extraction and delivered to HG3 cells in combination with foetal bovine serum. After 96 h, we detected a 40% delay in cell proliferation compared to untreated cells, without signs of cytotoxicity. This delay was due to p27/KIP1 over-expression and modulation of autophagic flux, measured by different autophagy markers (LC3II; p62) and 30% autophagosome intracellular increase.
Conclusions: The results obtained will be discussed at the light of the functional cross-talk between the modulation of the autophagy flux by the CE extract and the retard in cell growth observed in HG3 cells, as an opportunity to prolong the asymptomatic phase of CLL before disease occurrence.

A7
ANTI-INFLAMMATORY EFFECTS OF BLUEBERRY EXTRACT IN MICROGLIAL CELLS

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Background: Microglia (MG), the immunocompetent cells of the CNS, respond to brain injury activating and modifying their morphology. Microglia can exist broadly between two different activation states, namely the classical (M1) and the alternative activated (M2) phenotype. The first one is characterized by the production of pro-inflammatory cytokines, in contrast, the latter is characterized by the production of anti-inflammatory cytokines (Kettenmann et al., Neuron. 2013; 77:10—18). Blueberry is involved in the control of the redox state of the cell, cooperating with antioxidant mechanisms, whereas its anti-inflammatory activity is still poorly understood (Businaro et al., Curr. Alzheimer Res. 2018; 15: 363— 380). The aim of the present study is to determine the effect of blueberry extract in resting form or lipopolysaccharide (LPS)-stimulated BV-2 murine MG cells.
Methods: The hydroalcoholic extract obtained from fresh blueberries was analyzed by UHPLC/MS. The cellular viability was evaluated by MTT test and Trypan blue assay. Cellular migration was determined by Boyden chamber and Scratch assay. Cytokines mRNA levels were determined by qPCR. Actin cytoskeletal organization and M1/M2 marker expression were analyzed by immunofluorescence.
Results: Isomers of the chlorogenic acid, a powerful antioxidant, were detected in the blueberry extract, which, added to the cultures, had no cytotoxic effect, but induced increased cell viability and reduced LPS-driven migration. mRNA expression of pro-inflammatory cytokines IL-1β, IL-6 and TNF-α and that of iNOS (M1 marker) was decreased, whereas Arg-1 expression (M2 marker) was increased.
Conclusion: Our results suggest that blueberry may promote MG polarization towards the M2 phenotype, and therefore may be used as a nutraceutical in the treatment of neuroinflammatory diseases.

A8
INTESTINAL EPITHELIUM RESPONSES TO TITANIUM DIOXIDE NANOPARTICLES

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Introduction: Humans are exposed to nanoparticles from a variety of sources through a broad range of exposure ways since nanomaterials are increasingly used in different productive sectors. Titanium dioxide (TiO2) is enclosed in many consumer products including pharmaceuticals, cosmetics, and foods. TiO2 (E171) is daily ingested as mixed nano- and submicron-sized particles since it is approved as a white pigment in Europe in a variety of food products. Noteworthy, the relevant risk assessment has never been satisfactorily concluded and growing alarms for human hazards deriving from TiO2 exposure are incrementally reported.
Objectives: The objective of the present study was to establish conceivable mechanisms by which nano-sized TiO2 particles affect physiological function of the intestinal epithelium layer. The well-established Caco-2 cell line differentiated on permeable supports was used as a predictive model of the intestinal barrier due to its ability to naturally differentiate into polarized cells which resemble the intestinal architecture. The resultant system was adopted to investigate changes triggered by TiO2 nanoparticles in monolayer barrier since intestinal epithelial barrier is crucial for the maintenance of physiological function and the prevention of uncontrolled antigens trafficking.
Results: Exposure to nanoparticles disrupted the tight junctions-permeability barrier with a prompt effect detectable after 4h incubation time and wide effects on barrier integrity at 24h. Transport and ultrastructural localization of TiO2 nanoparticles were determined by ICP-OES, TEM and ESI/EELS analysis, respectively. Nano-sized particles were efficiently internalized and preferentially entrapped by monolayers. Storage of nanoparticles inside the cells affected enterocytes viability and triggered the production of pro-inflammatory cytokines, including TNF-alpha and IL-8.
Conclusion: Taken together these data indicate that nano-sized TiO2 particles exert detrimental effects on the intestinal epithelium layer.

A9
INTERPLAY BETWEEN THE TOXIC ALPHA-GLIADIN PEPTIDE 31-43 AND TYPE 2 TRANSGLUTAMINASE ENZYME IN CELIAC DISEASE

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Introduction: Celiac disease (CD) is a widespread enteropathy triggered by a diet containing cereals with gliadins in genetically predisposed individuals. Alpha-gliadin peptide 31–43 (p31-43) is considered the main responsible of the innate immune response in CD patients and type 2 transglutaminase (TG2) enzyme is involved in CD by enhancing gliadin immunogenicity. Evidence has been reported on a role of TG2 in modulate p31-43 uptake by intestinal cells; indeed, antibodies to TG2 specifically reduced both p31-43 uptake by cells and its biological activity. However, little is known about molecular mechanism underlying p31-43 uptake. We aim to investigate the effect of p31-43 on TG2 expression and activity into a model of skin-derived CD fibroblasts; furthermore we investigate whether cell surfaceTG2 could be directly responsible of p31-43 translocation into intestinal cells.
Methods: We analysed TG2 levels by PCR and western blot analysis and we monitored TG2 activity by a microplate assay using the pentylamin-biotine as substrate in skin-derived CD fibroblasts. To visualize probable complex between cell surface TG2 or membrane proteins and p31-43 we chemical cross-linked of p31-43 on intestinal cell surface proteins and next, pulled-down peptide-proteins complexes using antibodies raised against p31-43.
Results: We found that p31-43 stimulation induced TG2 activity more in skin-derived control fibroblasts than in CD cells. On the contrary, TG2 expression was more markedly induced in celiac cells than in control ones. We also found that that cell surface TG2 was not necessary for p31-43 internalization, even if it had a regulating role in the process.
Conclusions: We demonstrated that p31-43 did not behave as a classical ligand; indeed, membrane composition and organization, instead of a specific receptor protein, may have a major role in p31-43 internalization by cells. The interplay between p31-43 and TG2 has an important role in CD pathogenesis.

A10
CELLULAR AND SYSTEMIC ANALYSIS BASED ON POLYUNSATURATED FATTY ACIDS PROTECTIVE EFFECTS AGAINST INSULIN- RESISTANCE CONDITION

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Introduction: ω3 Poly-unsaturated Fatty Acids (PUFA-ω3) have a protective and therapeutic role to prevent insulin – resistance (IR). In this study, the protective effect was evaluated through: 1) serum parameters related to IR (HOMA index and apelin serum levels); 2) hepatic insulin signaling pathway markers (phosphorylated protein kinase B, p-Ser473-AKT/PKB); 3) endoplasmic reticulum (ER) stress marker (phosphorylated transcription factor p-eIF2α); 4) mitochondrial dynamics marker (Mitofusin 2, Mfn2).
Methods: These parameters were evaluated into 3 Wistar rats groups, so treated for 6 weeks: 1- N rats, treated with a standard diet (10.6% fats J/J); 2- L rats, treated with a high fat diet, rich in lard (40% fats J/J); 3- F rats, treated with a high fat diet rich in fish oil, major PUFA-ω3 source (40% fats J/J). Standard methods were used to analyse glucose and insulin serum levels and to determine HOMA index. ELISA assay was utilized for serum apelin levels. Hepatic p-Ser473-AKT/PKB, p-eIF2α and Mfn 2 levels were determined by western blot.
Results and conclusions: L group exhibited systemic and hepatic IR (as showed by increased HOMA index and p-Ser473-AKT content, respectively) associated with ER stress (as showed by increased p-eIF2α content). At the systemic level, F group showed reduced HOMA index associated with increased apelin serum level compared to L group. Furthermore, we observed increased hepatic insulin sensitivity (as showed by reduced p-Ser473-AKT content) associated to ER stress reduction (reduced p-eIF2α content) in F group compared to L group. A fundamental role seems to be played by Mfn2, that increased in F vs L group, preventing not only mitochondrial integrity, but also eIF2α phosphorylation. In this way, fish oil may have positive effect in the prevention of ER stress and IR onset.

A11
EFFECTS OF SOME NUTRACEUTICALS ON THE TPC1 THYROID CELL LINE

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The majority of thyroid carcinomas come from follicular cells and are defined as differentiated thyroid tumors (DTC) and the two histological subtypes are papillary CT and follicular CT. Curcumin has a wide variety of biological functions, currently, in the literature has considerable attention. The present work defines the role of curcumin on the modulation of gene expression of different cell markers and cell cycle modulation. The study was carried out using CURCUMA NATUREX and adding other nutraceuticals such as piperine and vit. And, in order to define the role of these in the modulation of gene expression of cell and tumor markers.
TPC-1 cells were the cellular model. Initially treated with the different turmeric extracts and examined the expression levels of markers (proliferative, inflammatory, antioxidant, apoptotic). Thereafter TPC-1 cells were treated with MIX of turmeric, piperine and vitamin E to understand its efficacy and biomodulation on thyroid papillary carcinoma. Treatment with the three different curcumin extracts shows