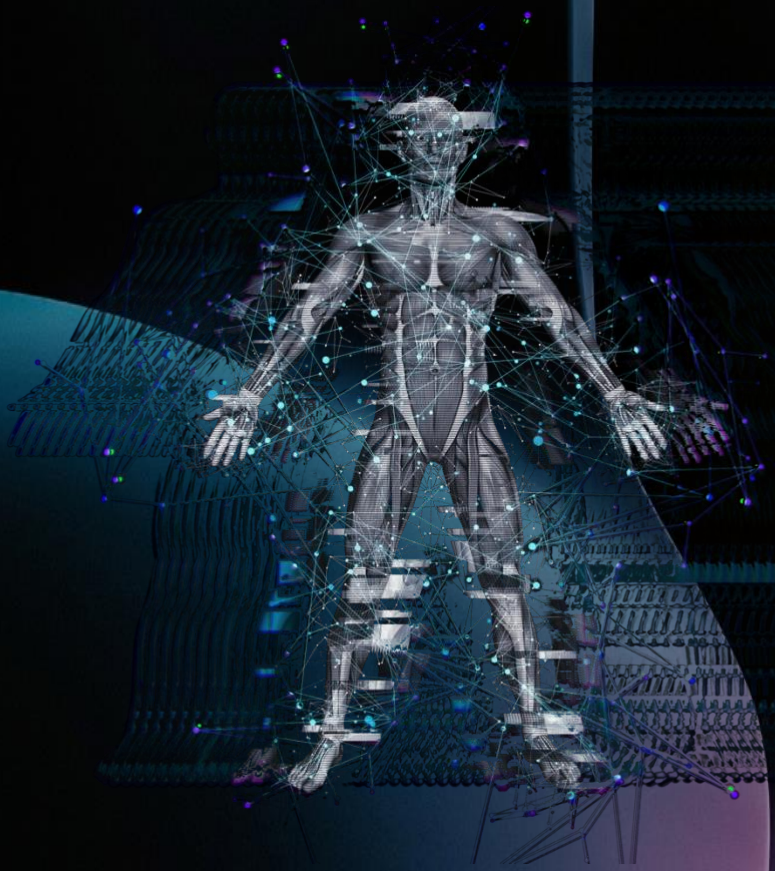
RETREAT 2023

MESSINA 20 – 22 GIUGNO



BOOK OF ABSTRACTS

Consiglio Nazionale delle Ricerche
ISTITUTO PER LA RICERCA E L'INNOVAZIONE BIOMEDICA

Il **Retreat** è un evento dedicato alla promozione della condivisione e dell'interazione tra **i colleghi di CNR-IRIB**. Durante l'evento, i partecipanti hanno l'opportunità di presentare e discutere le loro attività di ricerca, con la possibilità di esplorare nuove idee e stabilire connessioni **all'interno della comunità scientifica**.

Il Retreat di CNR-IRIB è un momento privilegiato per ampliare le prospettive, **ispirarsi a nuove idee e creare reti di collaborazione durature**. Oltre alla divulgazione delle attività di ricerca, l'evento **promuove lo sviluppo personale e professionale** dei partecipanti attraverso o scambio di esperienze, conoscenze e *best-practices*.

Le sessioni di discussione, i poster, i progetti e gli incontri di networking favoriscono un clima collaborativo, stimolante e fertile per **l'interazione tra colleghi**.

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ISBN 9788894 33 70744

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Sommario

PRESENTAZIONI ORALI

RELATORE: Irene Deidda

The sea urchin, an old yet emerging in vivo model organism for neurotoxicity studies: preliminary results

Irene Deidda; Roberta Russo; Francesca Zito; Nadia Lampiasi; Rosa Bonaventura

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Background: Environmental pollution is recognized as one of the major risks for human health. Some pollutants of anthropogenic origin called "emerging contaminants" (ECs), such as heavy metals, pharmaceutical drugs, pesticides, are harmful to the marine ecosystem. Due to their persistence in the environment, some ECs accumulate in marine organisms through the food chains, with potential toxicity also on human neurodevelopment or mature nervous system (NS), causing structural or behavioural neurological alterations. To date, the neurotoxicity mechanisms of many ECs remain limited. Several marine organisms are used as in vivo animal models for ecotoxicological studies, alternative to traditional, ethically questionable, mammalian models. For over a century, the sea urchin with its peculiar characteristics has been considered a crucial model organism in the developmental biology field. Recently, its simple NS has been studied showing significant similarities with chordates, being composed by different neuronal subtypes, glial cells and neurotransmitters.
Material and Methods: The sea urchin is proposed as in vivo model to assess neurotoxic effects of different ECs. High-throughput technology, along with immune-histological and molecular methodologies, have been conducted to evaluate developmental neurotoxicity in embryos exposed to heavy metals and UVB.
Results: Initial studies focused on the identification of neuronal and glial markers in the P. lividus embryo. Using the newly identified biomarkers, we found that exposure of sea urchin embryos to UVB and heavy metals determined molecular and structural alterations in its NS. Preliminary studies have also been conducted on adult NS.
Conclusion: The new neuronal/glial markers appear related to neurotoxicity. All data support the sea urchin as potential in vivo model for developmental neurotoxicity studies. Further analyses will be conducted in other phases of sea urchin life cycle, i.e. from larval stage to adult, thus providing a valuable approach for a comprehensive screening of neurotoxic ECs and for safeguarding human health.

RELATORE: Giovanna Barbieri

The HLA-DR mediated signalling in melanoma cells

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Background: The metastatic progression of melanoma, one of the most widespread cancers in the Western population, is associated to the expression of Major Histocompatibility Complex (MHC) class II molecules. Indeed, the MHC class II molecules are signalling receptors whose engagement leads to the activation of several signalling pathways and are constitutively expressed in almost 50% of melanoma. Melanoma cells secrete in their microenvironment extracellular vesicles, that circulating in advancing tumour front could interact with immune cells and different cell types as mediators of metastasis.
Material and Methods: The HLA-DR signalling was studied in class II constitutive expressing melanoma cell lines through western blot experiments of cell extracts, lipid raft compartments and extracellular vesicles. The HLA-DR signalling mediated migration, extracellular matrix proteins adhesion and cell-cell adhesion was also analysed in melanoma cells. Dynamic Light Scattering (DLS) analysis was performed to measure the size distribution of extracellular vesicles and through flow cytometry analysis we studied the apoptotic PBMCs treated with extracellular vesicles secreted by melanoma cells.
Results: HLA-DR mediated signalling increases the expression and the lipid raft localisation of HLA-DRα, PD-L1, Integrin and CAM adhesion receptors, FAK, AKT and STAT3 signalling proteins and their activation. The HLA-DR mediated signalling increases in extracellular vesicles the expression of HLA-DR, adhesion receptors, PDL1 and STAT3. Furthermore, through co-culture experiments of PBMCs and extracellular vesicles, we showed that HLA-DR mediated signalling enhance the cytotoxic effects of extracellular vesicles on PBMCs.
Conclusion: The results showed suggest a new model in which HLA-DR stimulation activates a signalling in melanoma cells that provides a platform useful to frustrate an effective anti-tumour response and to increase melanoma migration and metastatic dissemination regulating cell adhesion, motility and immune escape. Furthermore, our results suggest that HLA-DR mediated signalling promotes melanoma progression enhancing, through the extracellular vesicles secreted, the inhibition of immune response.

RELATORE: Valeria Longo

Effects of environmental pollutants on immune response

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Background: The exponential growth of industrial production caused a transfer of contaminants in the environment with multiple impacts on health. Several studies highlighted the relationship between environmental pollutants and immunological diseases such as allergy, asthma, immunosuppression and autoimmunity. Recently, it has been suggested that pollutants can damage epithelial barriers and induce a low chronic inflammation status as well as molecular oxidative stress. These molecular mechanisms involving the immune system alter the cell's physiology and the intercellular communications and have a pivotal role in the onset of Non-communicable diseases. In these perspectives, we focused our interest on macrophage cell to cell communication, particularly on: 1) the epigenetic alterations induced by pollutants exposure on macrophage EV's miRNA cargo; 2) the effects of the signal mediated by macrophage-derived EVs on different target cells.
Material and Methods: PBDE-treated macrophages-derived EVs were purified by dUC method. The EV's miRNA cargo was analyzed by microarrays. Resting THP-1 M(0) cells and ALI cultured A549 cells were cultured with PBDE-treated macrophages EVs. MiRNA microarray and flow cytometry analyses were performed on resting THP-1 M(0) cells. The expression of genes involved in epithelial integrity were evaluated by qPCR analysis in lung epithelial cells.
Results: PBDE 47 modifies the miRNA profile of macrophages-derived EVs. The signal mediated by these sEVs induces epigenetic alteration in resting THP-1 M(0) macrophage-like cells and might impair the macrophage's ability to make immunological synapses and present antigens, down-regulating the expression of HLA-DR and CD209 antigens. Furthermore, the macrophage-derived EVs affect the mRNA expression of TJs, adhesion molecules, cytokines and EMT markers damaging the normal function of the lung epithelium.
Conclusion: Our study supports the model that perturbation of miRNA cargo by PBDE-47 treatment contributes to the rewiring of cellular regulatory pathways capable of inducing perturbation in target cells.

RELATORI: Gaspare Drago e Silvia Ruggieri

Environmental epidemiology: from field research to public health interventions

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Background: The exposome paradigm is an attempt to draw attention to the several environmental factors possibly affecting individual health. Humans are exposed to multiple simultaneous environmental stressors including pollutants, nutrition, lifestyles and sociodemographic factors. The main goal of environmental epidemiology is to identify environmental determinants of disease in order to improve public health actions. In this context, pregnancy, childhood and developmental age are highly sensitive life stages representing the best opportunities for primary prevention interventions aimed at reducing environmental influences on life health trajectories.
Material and Methods: Data on possible exposures are collected through numerous data sources including face-to-face and web questionnaires, xenobiotic concentration data in environmental matrices, geospatial data and human biomonitoring. Health outcome data and possible confounding/effect modifier factors are obtained by means of questionnaires, hospital discharge record data, disease registries (i.e., Congenital Anomalies [CA]) and clinical evaluations. Appropriate statistical frameworks are used to describe the relationship between environmental risk factors and health outcomes or to associate bio-molecular data with exposures.
Results: Biomonitoring data from mother-infant pairs of the NEHO birth cohort were used to identify the main routes of maternal exposure during pregnancy and early molecular signatures of exposure. Different dietary patterns were identified and their association with lifestyles during pregnancy and risk perception was investigated. The Sicilian CA surveillance system was implemented through hospital discharge records and used to describe CA incidence in the Sicilian risk areas. A survey was also launched among Sicilian students to determine the prevalence of eating disorders.
Conclusion: The obtained results allow us the participation to national and European research networks primarily aimed at identifying actions for reducing the burden of environment-related non-communicable diseases.

RELATORE: Monica Salamone

Inhibition of Hyaluronan synthesis in INS1E pseudoislet increase their insulin secretion and adhesiveness

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Background: In the context of new findings in type 1 diabetes mellitus, there has been an observed increase in the presence of hyaluronic acid (HA) within the islets of Langerhans. Recent studies have demonstrated that inhibiting HA in mice can modify the stiffness of the islets and have an anti-diabetic effect. However, the specific cellular source of this elevated HA remains largely unknown. 4-methylumbelliferone (4MU) is known to decrease the availability of HA substrates and inhibit the activity of different HA synthases. Nevertheless, it has been observed that 4MU may have also an independent effect. Controlling HA synthesis could potentially impact the functionality of beta cells. In this study, we utilize the rat insulinoma beta cell line (INS1E) as an in vitro model to investigate the potential role of hyaluronan in diabetes.
Material and Methods: To mimic type 1 diabetes mellitus, we utilize the INS-1E cultured in both two-dimensional (2D) and three-dimensional (3D) experimental settings (pseudo islets). Additionally, we selectively eliminate pancreatic β-cells by employing streptozotocin (STZ). Our assessments include measuring insulin secretion, evaluating adhesion capability, and quantifying the levels of hyaluronic acid (HA) and extracellular vesicles (EVs) secretion, under various conditions, including the presence of 4MU.
Results: For the first time, we observed that treating INS1E cells with 4MU results in improved insulin secretion in both 2D and 3D experimental settings. Moreover, we found that 4MU treatment induced distinctive morphological changes in both 2D and 3D settings. These experiments aim to provide a better understanding of HA's role in beta cells.
Conclusion: These findings highlight the potential therapeutic value of 4MU in treatments for early-stage diabetes or for post-islet transplantation. In the future, we will use the INS1E model to investigate new therapeutic strategies, including the use of EVs in regenerative medicine.

RELATORE: Roberta Russo

Evaluation of immunomodulatory and antioxidant effects of bioactive molecules

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Background: Bioactive molecules are compounds derived from plants, animals, and microbes, with positive effects on human health by influencing gene expression and protein metabolic pathways. They are often employed as dietary supplements. Among plants, Ginger (Zingiber officinale) is commonly used as spice or herbal medicine with various pharmacological properties. Moreover, the reduced side effects, its availability and accessibility, and the reduced production costs are further attractive features. Ginger is rich in many bioactive constituents, mainly gingerols and shogaols. Although the bioactive constituents have been identified, the molecular mechanisms of ginger action are still limited, and the related signalling pathways not completely defined.
Material and Methods: By an alcoholic extraction method, we obtained a new ginger extract (GE), chemically characterised by HPLC assay. The antioxidant ability was tested by Folin-ciocalteu and ORAC methods. Cell viability tests verified the non-toxic doses of GE and commercial [6]-gingerol. Anti-inflammatory/antioxidant properties of GE/[6]-gingerol were assessed at cellular and molecular levels in RAW264.7 murine macrophages pre-treated with GE or [6]-gingerol and stimulated with LPS 2h later. The differential expression of various genes was evaluated by qPCR. The genes belonged to different categories: immune signalling, pro/anti-inflammatory cytokines, pro/anti-antioxidant enzymes, hallmarks of macrophage polarization.
Results: GE and [6]-gingerol pre-treatments reduced the LPS-induced expression of interleukins and genes of the TLR4 signalling pathway, as well as promoted the expression of specific markers of macrophage polarization, activating an anti-inflammatory M2 phenotype. Commercial [6]-gingerol tested on sea urchin immune cells provided similar molecular effects.
Conclusion: This study highlighted anti-inflammatory/antioxidant properties and effects on M1/M2 polarization of a new GE. Further analyses are needed to investigate the dosages, bioavailability, metabolism, and role of individual GE components in immunomodulation. In the long term, the aim would be to provide a pharmacological basis for treatment of inflammatory diseases using new mixture of bioactive molecules.

RELATORE: Walter Arancio

Transcripts derived from AmnSINE1 repetitive sequences are depleted in the cortex of autism spectrum disorder patients

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Background: DNA repetitive sequences (RS) represent about half of the human genome. Despite since few years ago RS have been considered slightly more than "junk DNA", now RS have a recognized role in almost every aspect of human biology, from embryonic development to infectious diseases. Indeed, it is widely reported that RS possess specific activities in the developing human brain. Autism spectrum disorder (ASD) is a brain developmental disability with a not-fully clarified etiogenesis. In order to test if RS may play a role in ASD, global RS transcription was investigated in ASD specimens.
Material and Methods: Global RS transcription was investigated in postmortem dorsolateral prefrontal cortex of 13 ASD patients and 39 matched controls retrieved from public transcriptomic datasets. A custom pipeline of analysis was adopted to quantify the expression of RS together with canonical coding genes. Competing endogenous RNA analysis was adopted to investigate the miRNA regulatory landscape in which selected genes are involved. Gene Ontology (GO) enrichment analysis was performed by using the analysis tools from the PANTHER Classification System.
Results: AmnSINE1 transcription is significantly and specifically downregulated in ASD specimens in comparison with controls. The investigation on the 986 human genetic loci that contain AmnSINE1 highlighted that the genes of those loci are associated with nervous system development and autism susceptibility. Looking for a possible direct role of AmnSINE1 non-coding transcripts in ASD, we report that AmnSINE1 transcripts can alter the miRNA regulatory landscape for genes involved in neurogenesis.
Conclusion: AmnSINE1 is a novel interesting candidate player in the development of ASD.

RELATORE: Sabrina Sanzone

Innovations in IRIB administration

Sabrina Sanzone

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Background: IRIB was created on June 1st, 2019 from the fusion of the Institute of Biomedicine and Molecular Immunology of Palermo, the Institute of Neurological Sciences with offices in Mangone (CS), Roccelletta di Borgia (CZ) and Catania, and the Research Unit of the Institute of Applied Sciences and Intelligent Systems located in Messina.
Material and Methods: Following the guidelines for the procurement of goods and services and the elaboration of the documents relevant to negotiated procedures above and below threshold, as well as for direct procurement issued by the CNR Headquarters concerning the PNRR projects, changes were made to the SIGEO management software already in use at IRIB. Moreover, considering the need to correctly manage the processing of the Timesheets for the reporting of all the existing projects, an IT procedure is currently developed to be used for this purpose. In addition, following a Director's request, a KPI panel has been developed for the evaluation of administrative activities. The innovations have been implemented by means of IT procedures.
Results: An improved management of orders, timesheets and administrative practices has been obtained.
Conclusion: Institute Administration ha achieved an increase in effectiveness and efficiency of its functioning along with an optimization of resources.

RELATORE: Flavia Marino

Digit@l Therapeutics lab: an overview

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Background: A new type of therapy called "digital therapy" (DTx) uses digital devices including smartphones, tablets, robotics, and virtual reality as therapeutic treatments for treating medical disorders.
Material and Methods: In order to satisfy the unique needs of patients, we present a critical assessment of the justification for employing these new modalities from the standpoint of translational medicine, which can both increase interventional efficacy and lower current healthcare expenditures.
Results: In the domains of neurodevelopmental problem, eating disorder, stress, and cardiovascular diseases, we also discuss innovative treatment trajectories using digital therapy. We end by giving a few current illustrations of digital therapeutics that suggest potential directions for the near future.
Conclusion: DTx is a group of newly developed therapy modalities prepared to handle chronic and other challenging-to-treat illnesses. DTx is anticipated to have a big impact on how healthcare is provided and used globally.

RELATORE: Liliana Ruta

Disentangling heterogeneity in autism under the lens of early motor behavioral markers

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Background: Autism is a heterogeneous neurodevelopmental condition characterized by deficits in social communication and restrictive and repetitive patterns of interests and behavior. Autism manifests itself from very early childhood with either delayed or atypical behaviors in social orienting and interests, gestural communication, basic motor repertoire and language development, with different individual trajectories and response to early interventions. The aim of the study is to explore autism heterogeneity and early developmental trajectories in relation to early motor, gestural and communicative individual profiles.
Material and Methods: A total sample of n=105 young children with autism, aged between 17 and 42 months, both males and females (19% females) were evaluated longitudinally throughout the course of high or low intensity Early Start Denver Model (ESDM-higher, n=29; ESDM-lower, n=18) or an as usual treatment (TAU, n=58) program, implemented in the context of the autism territory service of Catania. Children were assessed at treatment start and after 6 months of intervention using the Griffiths Mental Development Scale (GMDS) to have a measure of individual trajectories in the different developmental domains. A detailed micro-analytic moment-by-moment coding strategy was applied in a subgroup to capture the amount and types of actions, gestures and speech, from video footage of naturalistic mother-child interactions. +F11:I11
Results: A main effect of both intervention style and intensity on individual early developmental trajectories was found. Furthermore, we found two different clusters within the autism group based on early motor and gestural markers, related to developmental trajectories.
Conclusion: These findings have an impact for their clinical translation, supporting tailored interventions for autism, since early stages of development.

RELATORE: Carmela Zizzo

Study of genetic and biochemical alterations in lysosomal storage disorders: focus on Gaucher disease and Acid Sphingomyelinase Deficiency

Carmela Zizzo; Daniele Francofonte; Marcomaria Zora; Monia Anania; Annalisa D'errico; Irene Giacalone; Miriam Giacomarra; Alessia Lo Curto; Emanuela Maria Marsana; Elisa Messina; Marta Moschetti; Giovanni Duro and Paolo Colomba

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Background: Our research project aims to deepen knowledge on the pathophysiology of lysosomal storage disorders (at date more than 50 Lysosomal Storage Disorders described), using some of ones representative of the whole group as a model. In Particular, we deal with Fabry disease, Gaucher disease, Acid Sphingomyelinase Deficiency, Pompe disease and Mucopolysaccharidosis type I – MPS.
Material and Methods: Study of enzymatic and genetic alterations in patients with suspected lysosomal storage disease.
Results: Since 2005 at the Center for Research and Diagnosis of Lysosomal Storage Disorders of IRIB-CNR in Palermo, we have studied over 45,000 patients with suspected lysosomal disease, coming from several hospitals throughout the country. Specifically, since 2017 we have studied the biochemical and genetic alterations in patients with symptoms referable to Gaucher disease: the study analyzed over 9,000 patients and confirmed the clinical diagnosis in 2% of them. Main collateral projects aimed the study of Gaucher disease in patients with Multiple Myeloma and Monoclonal Gammopathy of Uncertain Significance (MGUS) and the study of GBA1, responsible for Gaucher disease, as main risk factor for Parkinson's, given the correlation between mutations in the gene and this syndrome. Moreover, because of the overlapping of clinical picture between Gaucher disease and Acid Sphingomyelinase Deficiency, the study of activity of both enzymes responsible for these diseases in all patients who came to our observation with initial clinical suspicion of Gaucher disease, made it possible to diagnose Acid Sphingomyelinase Deficiency in patients who have received a misdiagnosis of Gaucher disease.
Conclusion: The study of rare diseases, such as all metabolic pathologies, is a very delicate and complex process which requires a synthesis between clinical picture and molecular analysis. In last years our project has aimed at a better understanding of pathophysiology of lysosomal diseases and the diagnosis of affected patients.

RELATORE: Maria Vincenza Catania

Neurobiological aspects of neurodevelopmental disorders associated with intellectual disability and autism

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Background: Intellectual disability (ID) and autism spectrum disorder (ASD) are the most frequent neurodevelopmental disorders. Although they are considered distinct nosological entities, ID and ASD are frequently associated, especially in syndromic forms. ID is defined by a disturbance of intellectual and adaptive functioning with onset before the age of 18, whilst ASD is characterized by deficits in social communication and restricted, repetitive interests with an onset before the age of 3 years. Altered synaptic plasticity and unbalanced excitatory/inhibitory transmission are common mechanisms underlying the pathophysiology of ID/ASD. Emerging evidence also suggests that dysfunction of glial cells contributes to ID/ASD pathogenesis. In this context, our group is interested in dissecting molecular and cellular mechanisms that involve excitatory amino acid receptors in neurons and glial cells, with the final aim of identifying biomarkers and pharmacological targets.
Material and Methods: We use several rodent models of syndromic (Fmr1-/- and Ube3Am-/p+ knock-out mice modeling Fragile X [FXS] and Angelman syndrome [AS], respectively) and non-syndromic ID/ASD (neurolighin-3R451C knockin mice and rats prenatally exposed to valproate) and perform a combination of biochemistry, imaging, electrophysiology, and behavioral studies in in vitro and in vivo experiments.
Results: We observed: 1) an abnormal expression of metabotropic glutamate (mGlu) receptors in all these animal models; 2) downregulation of the canonical transduction pathway activated by mGlu5 receptors in different brain regions of mice modeling FXS and AS; 3) changes in RNA metabolism, response to stress, senescent phenotypes and calcium dynamics in astrocytes of FXS mice.
Conclusion: Our findings expand the current knowledge of the pathophysiological mechanisms of ASD and may have important implications for future therapeutic strategies potentially useful for diseases with ID and autism.

RELATORE: Sabrina Picciotto

A nature designed drug delivery platform: extracellular vesicles for therapeutic and cosmetics applications

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Background: Extracellular Vesicles (EVs) are membrane-enclosed bio-nanoparticles secreted by cells for long-distance shuttling of proteins and RNAs. They are considered as promising bio-nanovehicles for the delivery of bioactive compounds and have recently gained attention in the field of nanotechnology.
Material and Methods: A novel platform was developed and industrially validated for the production, characterization, and bioengineering of microalgae-derived EVs, named algosomes. This platform aimed to address the limitations of current EV-based nano-delivery systems. The approach involved using the growth of microalgal cells instead of commonly used chemical synthesis for liposomal formulations.
Results: The methodology is built upon the results of the H2020 FET-Open VES4US and BOW projects and the associated patented bioprocesses for producing and bioengineering EVs from microalgae. The goal is to exploit the natural properties of EVs in sustainable and scalable manners. The research laid the foundations for:
1) Preclinical Evaluation of the Bioactivity of Microalgal-derived Extracellular Vesicles.
2) Development of an innovative methodology for EV quality check. Potential applications: EV potency quality control. Italian patent proposal (n. 102023000004503): March 10, 2023 (Inventors: Bongiovanni, Adamo, Picciotto - CNR).
3) commercial exploitation of algosomes. The EVEBiofactory start-up company (https://www.evebiofactory.com/), was launched in 2022 to exploit algosomes for novel pharmaceutical and cosmetic formulations.
Conclusion: The study established a novel platform for producing and bioengineering algosomes, based on microalgal cell growth. This approach is a fundamental shift in nanomaterial manufacturing for delivering bioactive compounds. The research aimed to harness EV properties and lay the foundation for commercializing algosomes. Additionally, a novel enzymatic assay predicted EV potency effectively, providing a valuable tool for quality control assessment.

RELATORE: Elena Lo Presti

Exploring novel immunotherapeutic interventions through ex-vivo and in vitro analysis: the challenge for pancreatic cancer treatment

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Background: Amongst the solid tumor, PDAC shows delayed diagnosis caused by nonspecific symptoms and lack of early diagnostic markers with 4-6 months survival after diagnosis. The main reason for this poor prognosis is low immunogenicity of tumor cells and robust tumor immunosuppressive mechanisms that results in resistance to therapies, including current modalities of immune checkpoint blockade. Therefore, identification of new biomarkers is urgently needed to identify the most suitable target to develop further drug treatment strategies.
Material and Methods: Peripheral blood and tumor biopsies were obtained from drug-naïve patients enrolled to "Endoscopy and Gastroenterology Unit" at ARNAS Civico Hospital. Flow cytometry (FACS Canto) and Immunohistochemistry were used to characterize infiltrating and circulating leukocytes.
Results: Although PDAC is a poorly infiltrated tumor, through a specific protocol, it is possible immunocharacterize infiltrating cells from naïve patients starting from fresh material obtained from tumor biopsies. γδ T lymphocyte represents a small proportion of infiltrating T cells approximately 2.8% (SEM ± 0.9) among infiltrating leukocytes (47.38% ± SEM 12.4). In fact, we found an abundant infiltration of Neutrophils which represent 68% of CD45+ cells. γδ T cells are considered a positive prognostic factor in many solid tumors while in pancreatic cancer their presence in tumor tissue is associated with worse patient outcome. Anyway, in our analysis, γδ T lymphocytes are present in the early stages of tumor development (stage II) and few in advanced or metastatic tumors. Their presence did not correlate with N2 lymph node infiltration. Our experiments using pancreatic tumor organoids have shown a significant change in many commonly expressed cellular markers upon cytokine stimulation likely promoting a change in the role of these cells during tumor development.
Conclusion: Based on our results, the tumor microenvironment dysregulates γδ T cells losing their antitumor function which could be restored by appropriate immunotherapeutic strategies

RELATORE: Giuseppa Biddeci

New p65 isoforms, protein of NF-kB complex: regulation of inflammatory response and their potential role in Fabry disease

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Background: Inflammation is a physiological process whose deregulation is the basis of multiple diseases. Nuclear Factor kB (NF-kB) is a family of ubiquitous and inducible transcription factors that play critical roles in inflammation. The p65/p50 heterodimer is the most abundant complex. Glucocorticoid Receptor (GR) is a ligand-activated transcription factor and acts as an anti-inflammatory agent and immunosuppressant. Thus, NF-kB and GR are physiological antagonists in the inflammation process. A new spliced variant of p65, called p65 iso5, has been identified in our laboratory. This new isoform can bind the corticosteroid hormone dexamethasone amplifying the effect of GR, therefore possessing biochemical characteristics diametrically opposed to p65. Moreover, another p65 isoform, called p65 iso6, has been recently identified. Fabry disease (FD) is an X-linked lysosomal storage disease (LSD), affecting glycosphingolipid metabolism. FD is caused by several mutations in the GLA gene on the X chromosome, resulting in a deficiency of the lysosomal enzyme alpha-galactosidase A (aGAL). This leads to the progressive accumulation of globotriaosylceramide (Gb3) in cells, causing multi-systemic effects. FD, and other LSDs, have been classified as a subgroup of autoinflammatory diseases. Our goal is to study the role of these new p65 isoforms in the regulation of the inflammatory response and in FD.
Material and Methods: Therefore, we evaluated in peripheral blood mononuclear cells (PBMCs) of more than one hundred FD patients, through Real Time PCR, the mRNA expression level of new isoforms.
Results: The patients affected by FD had significantly increased p65 iso6 mRNA expression levels compared to controls. Moreover, we identified new transcripts never detected before.
Conclusion: The results, along with the ability of p65 iso5 to bind dexamethasone and the glucocorticoid's regulation response in the opposite way of the wild type, strongly suggest a role for these isoforms as new therapeutic targets to control inflammation related diseases.

RELATORI: Roberta Bruschetta e Gennaro Tartarisco

Artificial intelligence and wearable technologies to investigate socio-relational competences in children with autism

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Background: In recent years, in-depth studies on caregiver-child interactions in children with autism spectrum disorders (ASD) have revealed a range of socio-related deficits including motor atypicalities, difficulties in emotional comprehension and a lack of spontaneous attention towards social stimuli. Early impairments in gestural communication, particularly in deictic gestures as well as specific eye-gaze and simpatho-vagal patterns are strongly associated to autism risk. The main goal of this project is the use of artificial intelligence (AI) and wearable technologies to examine heterogeneous behavioural and physiological biomarkers, for early detection and treatment of autism.
Material and Methods: We developed an automatic digital coding approach based on a deep-learning transformer model to recognize four main deictic gestures (pointing, giving, showing, and requesting) from videos of naturalistic caregiver-child interactions. Furthermore, we investigated children engagement during therapies with social robot using wearable sensors. Finally, a linear mixed-effects model and Markov chains, were employed for the examination of gaze patterns in social imitation scenarios.
Results: Training, validation, and testing of the digital coding approach achieved promising performance evidencing its potentiality for the automatic recognition of deictic gestures from naturalistic videos. The analysis of simpatho-vagal parameters revealad the presence of physiological biomarkers of socio-emotional engagement in ASD children during interaction with QTrobot. Furthermore, analysis of eye gaze temporal series demonstrated ASD children's visual attention preference towards non-social stimuli compared to social ones.
Conclusion: Future developments involve applying the proposed transformer-based architecture to a larger sample set to improve the automatic identification of deictic gestures. Additionally, ongoing research aims to further investigate the correlation between robot-interactive tasks and children's emotional state. Lastly, additional analyses are currently underway to identify specific gaze patterns that differentiate ASD children from TDs.

RELATORE: Marcello Tagliavia

Engineered probiotic bacteria to produce recombinant anticancer immunotherapeutics

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Background: Immune checkpoint inhibitors (ICIs) act by mitigating the immunosuppression exerted by the tumor microenvironment, which unleashes immune cells to kill malignant cells. Their intratumoral (IT) administration, expected to result in improved responses without the -even life-threatening- side effects associated with systemic therapy, is unreliable in most cases. The IT delivery of therapeutic cargos by engineered, clinically-approved bacteria like E. coli Nissle 1917 (EcN), capable of colonizing tumors and stimulating immune anticancer activity, is an emerging strategy that merges the benefits of bacteriotherapy and immunotherapy. Moreover, it may overcome the unresponsiveness of many patients to intravenous ICIs therapy, due to gut microbiota issues. The IT production of nanobody-based ICIs (blocking the key anti-phagocytic CD47/SIRPα signal) by engineered bacteria resulted in tumor regression and durable immunity in preclinical models. Recently, synthetic peptidic ICIs (pICIs) have been proposed as an alternative to antibody-based ones. Their unique features make them a potential cutting-edge technology, suitable for the expression in engineered bacteria, which might help overcome limits associated with the expression of therapeutic proteins in microorganisms. No pICI-expressing microorganism has been reported to date. The project aims at engineering EcN for the production of secretory recombinant immunotherapeutics, including selected aCD47 and aSIRPα pICIs.
Material and Methods: Plasmidic constructions were obtained in E. coli Top10F' using standard cloning techniques. Chromosomal integrations in EcN were achieved by recombineering.
Results: Plasmids encoding pICIs as cleavable fusions, transcribed under the control of either inducible or constitutive promoters, were successfully obtained. As these constructions showed high instability in EcN, single-copy chromosomal integration of the expression cassettes was performed. The genetic stability of these strains is being assessed, as well as the efficiency of ICIs expression.
Conclusion: Stable strains expressing pICIs will be tested in a preclinical model for anticancer activity. This expression platform shall be implemented for the IT delivery of multiple therapeutics.

RELATORE: Luigi Citrigno

Genomics landscape of Mitochondrial DNA variations in patients from South Italy affected by mitochondriopathies

Luigi Citrigno; Antonio Qualtieri; Annamaria Cerantonio; Selene de Benedittis; Olivier Gallo; Gemma Di Palma; Patrizia Spadafora; Francesca Cavalcanti

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Background: Mitochondrial DNA (mtDNA) is a 16.6 kb, double-stranded, circular molecule that contains 37 genes coding for 13 subunits of the respiratory chain plus two rRNAs and 22 tRNAs. Mutations in these genes have been identified in patients with a variety of disorders affecting every system in the body. The advent of next generation sequencing technologies has provided the whole mtDNA sequencing, allowing the identification of disease-causing pathogenic variants in a single platform.
Material and Methods: In this study, we developed an NGS strategy by using an amplicon-based method to sequence the entire mitogenome, in order to identify disease-causing pathogenic variants in patients affected by mitochondrial diseases. The whole mtDNA coming from 100 patients was analyzed using an amplicon-based approach. The enriched libraries were sequenced on the Personal Genome Machine (PGM) from ThermoFisher Scientific. The Genome analysis tool kit (GATK) was used in order to perform the bioinformatic analysis. After the alignment and the base calling, the founded variants were annotated and filtered considering the type of pathogenic variation, the population frequencies, the presence in database (Mitomap, MitImpact2) and an heteroplasmy >5%.
Results: We were able to find a total of 27 novel non synonymous variants with a MAF <1% that are associated with the different phenotypes present in the patients.
Conclusion: The NGS approach, compared to the standard methods, is a more reliable and time-cost reducing strategy to detect all the variants present in the mitogenome associated with mitochondrial diseases. Further molecular simulation studies such as AI DeepMind's Alphafold system can be used to predict the 3D protein structures with great precision, to understand how the change of structure determining the importance of structural changes in mitochondrial proteins and genes will open new ways to develop possible future precision therapies.

RELATORE: Maria Guarnaccia

Analysis of copy-number variants in sporadic ALS cases

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Background: Amyotrophic lateral sclerosis (ALS) is an age-related neurodegenerative disease characterized by rapid and progressive loss of motor neurons at different levels: bulbar, cervical, thoracic, and lumbar. Several genetic factors have been associated to ALS, ranging from causal genes to potential risk factors and disease modifiers. The search for pathogenic variants in these genes has been mostly focused on single nucleotide variants (SNVs) while relatively understudied and not fully elucidated is the contribution of structural variants, such as copy number variants (CNVs). To date, only a handful of ALS genes have been known to vary in copy number, and using standard diagnostic tools, it is not generally appreciated that a wide range of genes may be affected by CNVs.
Material and Methods: Here, we applied an exon-centric aCGH method to investigate in patients with sporadic ALS, the type and frequency of CNVs in several ALS-related genes whose variants may increase the risk or modify the clinical phenotype of ALS.
Results: Results revealed that more that 80% of patients harbored multiple CNVs in causative genes or linked to a different clinical phenotype of ALS.
Conclusion: The precise delineation of multiple chromosomal aberrations in ALS-relevant genes provided by our approach may help define the genomic architecture of ALS and enable a genotype-first approach.

RELATORE: Noemi Vetrano

Positive Technology 2.0: Mindfulness-Based Interventions mediated by Virtual Reality (VR) and transcranial Direct Current Stimulation (tDCS) for Fear Modulation

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Background: Treatments based on extinction have been shown to be effective for fear modulation (Meulders, 2020). These treatments act on the ventromedial prefrontal cortex (vmPFC) and can be supported by transcranial Direct Current Stimulation (tDCS; Vicario et al., 2020). The classical fear conditioning paradigm represents a valid clinical model for analyzing the mechanisms of fear acquisition and extinction (Marković et al., 2021) and is applicable in Virtual Reality (VR; Lucifora et al., 2022). Mindfulness can also activate fear extinction processes (Tang et al., 2016; Holzel, 2016). This experimental study aims to compare the effect of mindfulness in VR and tDCS neuromodulation in fear extinction processes, using a classical fear conditioning paradigm in VR on healthy subjects.
The study evaluates the effect on fear extinction processes of:
1. tDCS neuromodulation,
2. a mindfulness-based intervention delivered through VR,
3. tDCS neuromodulation and a mindfulness-based intervention delivered through VR.
Additionally, the study tests fear extinction recall.
Material and Methods: A classical Pavlovian fear conditioning/extinction paradigm is applied in an ecologically mediated VR context to modulate conditioned fear response in participants. Fear extinction is modulated through the application of tDCS and/or a mindfulness-based intervention in VR. Outcome measures include the evaluation of psychological variables through the administration of neuropsychological tests (PCL-5, Trait Fear Evaluation Questionnaire, STAI-Y/S, Maia-2, VAS) and physiological measures using a sensor (eSense Skin Response) which measures galvanic skin response.
Results: The expected outcome of combining tDCS neuromodulation and mindfulness in VR is to enhance the impact on fear extinction processes, leading to more significant results.
Conclusion: The primary objective of this study is to propose interventions that are innovative, highly effective, and tailored to individuals for fear modulation.
By focusing on fear modulation, our goal is to facilitate substantial improvements in the health status and quality of life of individuals.

RELATORE: Chiara Failla

ARCADIA: Assistance and Rehabilitation of Eating Behavior through Devices Based on Artificial Intelligence and Virtual Reality

Chiara Failla[1]; Paola Chilà[1]; Germana Doria[1]; Roberta Minutoli[1]; Ileana Scarcella[1]; Noemi Vetrano[1]; Giuseppe Riva[2]; Renato Tino[3]; Antonio Cerasa[1]; Flavia Marino[1]; Giovanni Pioggia[1]

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Background: Eating disorders (ED) are a public health concern due to their prevalence, early onset in younger populations, and complex etiology, receiving scientific and media attention. The ARCADIA VR project aims to create an innovative system for the treatment of ED using Artificial Intelligence, Virtual Reality, and biosensors. ARCADIA has four objectives: 1) to develop and test VR-based tools, biosensors, and AI for diagnosing and treating ED 2) to implement studies to test and validate tools in clinical setting 3) to analyze the impact of the disorder on caregivers and their quality of work 4) to transfer the developed methods to two new ED treatment centers in Lecce and Messina.
Material and Methods: The project includes three tools for the treatment: 1) Enhanced Body Swap for Anorexia Nervosa, which uses virtual reality to reconstruct the patient's body and record its physiological information through wearable biosensors 2) Enhanced Virtual Exposure for Bulimia Nervosa, which uses virtual reality to reproduce virtual environments and foods that induce patients to binge episodes 3) Artificial Intelligence Diagnostics for eating disorders which uses a machine learning model to predict symptom severity and disease course from information available at patient admission. A total of 40 subjects with ED aged between 15 and 40 years will be recruited as follows: N=20 (10 M; 10 F) will be randomly assigned to the intervention group for AN; N=20 (10 M;10 F) will be randomly assigned to the intervention group for BN; N=40 (20 M; 20 F) will be randomly assigned to the control group.
Results: The system combines cognitive-behavioral treatment and considers various variables, including pathophysiological, family, and contextual factors, to provide assistance, rehabilitation, and tele-rehabilitation.
Conclusion: The ARCADIA VR project aims to develop an innovative system for ED diagnosis, evaluation, and treatment using virtual reality, biosensors, and AI.

RELATORE: Paola Chilà

INTER PARES PROJECT: Inclusion, Technology and Networking: a Project for Autism between Research, E-health and Social

Paola Chilà, Maria Luisa Scattoni, Carmelo Caporlingua, Carmelo Cutrufello, Luca Donato, Flavia Marino, Giovanni Pioggia

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Background: Autism spectrum disorders affect the behavioural, social, communicative, perceptual sensory and emotional regulation levels from the earliest stages of development, and clinical research has provided considerable evidence to support early detection of risk signs, early diagnosis and intervention models with empirical evidence of effectiveness, based on evolutionary-behavioural and naturalistic principles. Autism it directly and dynamically affects the entire life trajectory of the individual and the family. It is within this theoretical framework that the Interpares Project, financed by the Municipality of Messina, is set. Its aim is to contribute to an ecological model of autism that envisages following the individuals involved and their families through all developmental stages.
Material and Methods: The specific objectives of INTER PARES follow the following project actions: training and information, research and innovation and inclusion trajectories with integration innovative learning methods. The research and innovation methodologies of INTER PARES focus on the scientific application of methodologies and technologies that can help develop and integrate learning and support paths in the territory through tele-health technologies and a new generation of intelligent devices.
Results: The expected results will be to foster the integration, development and implementation of innovative assessment, intervention and social inclusion paths for people with Autism Spectrum Disorder, through new and innovative technological methodologies.
Conclusion: This project support, through innovative interventions and research, families of individuals with autism for their general well-being

RELATORE: Roberta Minutoli

AREA Project: Assistance and Rehabilitation through Evolutionary behavioral intervention models for Autism

Roberta Minutoli; Chiara Failla; Paola Chilà; Germana Doria; Ileana Scarcella; Noemi Vetrano; Gaspare Cusimano; Maddalena Virone; Davide Pinio; Nicola Corleo; Francesca Bova, Flavia Marino; Giovanni Pioggia

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Background: The problems that people with autism spectrum disorders and their families are forced to face on have assumed a particularly important health and social significance in recent years. AREA is a multidisciplinary applied research pilot project, proposed as a synergistic collaboration between public entities, which has the specific objective of introducing new eHealth methodologies and technologies to support effective and sustainable care and rehabilitation models in the context of the Trapani ASP area.
Material and Methods: AREA project aims to activate research paths aimed at implementing evidence-based protocols for the diagnosis and intervention of communication, social and cognitive deficits of preschool, school and adolescent children with autism spectrum disorders.
The implementation goals of the AREA project are:
to develop and systematize diagnosis and follow-up pathways that allow early intake and periodic monitoring of the child's developmental and habilitative path at different stages of development;
to create personalized treatment pathways supported by new methodologies and digital technologies that allow the enhancement of socio-adaptive skills according to an eco-relational model. In the different age groups (preschool, school and adolescent) the AREA project will integrate treatment programs based on scientific evidence of effectiveness such as the Early Start Denver Model (ESDM) and Applied Behavior Analysis (ABA) supported by the use of smart devices such as tablets and smartphones, social robots and serious games. A total number of 40 families per year will be recruited.
Results: The expected results will promote the development and implementation of experimental assessment and treatment pathways supported by the latest digital methodologies and technologies.
Conclusion: AREA project will give an answer to the requests and needs of the territory by taking care of children with a clinical classification of risk or diagnosis of autism spectrum disorder from the Trapani ASP services.

RELATORI: Cristina Carrozza e Elisa Leonardi

Characteristics of gesture communication in young children with autism during naturalistic play interactions

Elisa Leonardi[1] & Cristina Carrozza[1]; Marilina Mastrogiuseppe[2]; Stefania Aiello[1]; Francesca Isabella Fama[1]; Agrippina Campisi[1]; Carla Blandino[1]; Roberta Bruschetta[1]; Early Change Team[1*]; Flavia Marino[1]; Gennaro Tartarisco[1]; Olga Capirci[3]; Giovanni Pioggia[1] & Liliana Ruta[1]

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*Early Change team: Boccafoschi I., Boncoddo M., Bruschetta M., Crimi I., Di Bella F., Lazzaro G., Martines S., Rosano M.G.

Background: One of the earliest signs of autism is the absence or the delayed onset of non-verbal communication, such as gesture behaviors. Very limited studies explored gesture behaviors during naturalistic play interactions in young autistic children. For this reason, we investigated early socio-communicative development in very young children with autism under the lens of gesture behaviors, using a fine-grained microanalytic coding system. The main goal is to detect whether possible differences in gesture behavior could be expressed not only quantitatively but also qualitatively.
Material and Methods: The study was conducted on a group of 30 infants and young children with and without autism (N=15 per group, 8 F, mean age = 23 months). We used a detailed micro-analytic moment-by-moment coding strategy specifically developed to capture the amount and types of gestures, from video footage of naturalistic mother-child interactions.
Results: Results showed that, compared to the TD group, the autism group: (i) produced a significantly lower number of total gestures ($Z = 100$, $p = 0.003$); (ii) tended to produce a lower proportion of conventional gestures with a trend towards statistical significance ($Z = 50$, $p = 0.052$); (iii) produced a significantly lower proportion of showing gestures ($Z = 69$, $p = 0.013$); (iv) produced gestures mainly for expressing the pragmatic function of request ($Z = -73$, $p = 0.007$) while TD children used significantly more gestures for declarative purposes ($Z = 93$, $p = 0.006$). Interestingly, among different gesture types, only children with autism produced instrumental gestures.
Conclusion: Overall these preliminary findings suggest a specific deficit in social sharing and declarative functions (i.e. lower production of conventional gestures and showing) in autism, with a preferential use of gestures to direct others behavior and address their requests (exclusive use of instrumental gestures and greater use of requestive function).

POSTER

PRESENTATORE: Noemi Aloi

Immunotoxicity of innovative nanomaterials within the FETPROACT BOW project

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Background: Extracellular vesicles are recognized universal shuttles of intercellular communication, transferring lipids, proteins and nucleic acids, mediating physiological and pathological processes. The main goal of the BOW project is to explore and consolidate the technology able to impart biological surface precision, circulation and targeting abilities of extracellular vesicle nanoparticles to superparamagnetic nanodevices (Magnetic Bead Devices, MBDs) by "dressing" them with EV membranes. Superparamagnetic iron oxide nanoparticles (SPIONs) have gained increasing interest in nanomedicine suggesting their use as a platform for magnetic treatment in vivo. In this view, we performed the study of their biocompatibility performance by an ad hoc thorough in vitro nanotoxicological methodology.
Material and Methods: First of all, the potential presence of endotoxins in the sample preparations was assessed using LAL test. Furthermore, SPIONs were added to human erythrocyte solution to study haemolysis. Allergenicity was studied in whole blood detecting CCR3 and CD63 markers by flow cytometry. Then, immunotoxicology was studied using a PMA differentiated THP-1 macrophage-like cell line. Cell viability was determined in vitro by the MTS assay in THP-1 M0. M1 polarization was studied determining the TNF-α production in M0 macrophages after incubation with SPIONs at different concentrations for 24 and 48h by ELISA.
Results: LAL test showed a small amount of endotoxins' contamination in the range of concentrations used in the biological assays. Indeed, SPIONs did not induce significant lysis of erythrocytes and did not display the ability to activate human basophil cells. SPIONs did not affect cell viability after 24 and 48h, but a mild effect following 72h exposures was observed. Finally, after 24h exposure, SPIONs induced a dose response release of TNF-α only at the higher concentration.
Conclusion: SPIONs proved to be biocompatible in vitro and ex vivo paving the way for their implementation for theranostic applications.

PRESENTATORE: Mariamena Arbitrio

Genetic biomarkers for predictive response to sorafenib in patients with hepatocellular carcinoma

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# These authors contributed equally to this work.

Background: Sorafenib is a multi-target tyrosine-kinase inhibitor with a strong antiangiogenic effect which improves outcomes in advanced hepatocellular carcinoma (HCC) patients. However, sorafenib resistance reduces the number of those benefiting from treatment. The identification of biomarkers for predicting inter-individual sorafenib response variability and facilitating patient stratification could improve its effectiveness.
Material and Methods: Five known single nucleotide polymorphisms (SNPs) in angiogenesis-related genes, including rs2010963 (VEGF-A), rs4604006 (VEGF-C), rs12434438 (HIF-1a), rs55633437 (ANGPT2) and rs2070744 (NOS3), were investigated in 34 HCC patients (9 sorafenib responders and 25 non-responders). A subgroup (n = 23) was genotyped for 1931 SNPs and 5 copy number variations in 231 drug absorption, distribution, metabolism, and excretion (ADME) genes. A genetic signature of predictive response was generated by a learning method for classification rules which permits non-responder/responder patient stratification for tailored approaches using genotype data.
Results: Among the known predictive biomarkers, we found that only the VEGF-A (rs2010963) C allele and CC genotype were significantly associated with sorafenib response. ADME-related gene analysis identified 10 polymorphic variants in ADH1A (rs6811453), ADH6 (rs10008281), SULT1A2/CCDC101 (rs11401), CYP26A1 (rs7905939), DPYD (rs2297595 and rs1801265) FMO2 (rs2020863) and SLC22A14 (rs149738, rs171248 and rs183574) significantly associated with sorafenib response. The correlation between angiogenesis- and ADME-related genes was confirmed by cumulative genetic risk score and network and pathway enrichment analysis, demonstrating the association of 8/12 identified genes in key common biological pathways correlated to HCC and sorafenib.
Conclusion: Our findings could have relevance for personalized approaches in patient stratification for sorafenib prescription and are a “proof of concept” to be further validated in follow-up studies to improve personalized options for HCC patients.

PRESENTATORE: Francesca Cavalcanti

Fast and accurate SNVs and CNVs screening in Parkinson's Disease patients using Next-Generation Sequencing approach

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Background: Parkinson's disease (PD) is the second most common neurodegenerative disorder, affecting millions of people. Genome-wide association studies have found >25 genetic risk factors and at least 15 loci PD-associated. Recent advances in Next-Generation Sequencing technologies, such as the semiconductor-based Ion Torrent platform, make multigene sequencing cheaper, faster, and more reliable. Our objective was to test the power of this technology to analyze large cohort samples of PD patients by screening the most relevant PD-related genes known for single and compound mutations.
Material and Methods: To achive a rapid and robust genetic analysis of a PD cohort, we designed an amplicon based panel made by 42 PD-associated genes. We conducted parallel sequencing using the Ion Torrent Personal Genome Machine to detect mutations in 42 DNA samples from PD patients. Ion Torrent Suite software v. 5.10 was used to process data. The annotation was made using annovar and the variants where priorized using a standard filtering pipeline.
Results: After bioinformatics analysis and filtering, 98% coverage of the targeted regions was obtained with at least >200-fold mean depth. We detected 50 coding nonsynonymous variants (indels, single-nucleotide variations SNVs and frameshift variants with a MAF<0.01. Of these 50 variations, 9 were identified in PARK2, 12 in LRRK2 and 1 in PINK1. The remaining variants were found in the other genes sequenced, most of which are involved in PD pathogenesis. A total of 154 copy number variations CNVs (52 amplifications and 102 deletions) were revealed. Among these CNVs, we detected 1 amplification in SNCA, 1 deletion in PARK2, 1 deletion in PARK7, 1 amplification and 7 deletions in LRRK2.
Conclusion: Next-generation sequencing is a powerful method for PD genetic screening. Our results indicated that it yielded a high frequency of discovery of SNV e CNV variants in carriers from an enriched PD sample.

PRESENTATORE: Antonella Cusimano

Extra-Virgin Olive Oil phenols attenuate lipid accumulation in in vitro hepatic steatosis models

Antonella Cusimano[1]; Vincenzo Cucinella[1]; Alessandra Cusimano[2]; Antonina Azzolina[1]; Giuseppa Augello[1]; Lydia Giannitrapani[1,2]; Melchiorre Cervello[1]

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Background: Non-alcoholic fatty liver disease (NAFLD) is an emerging issue for global health with increasing incidence and is becoming a main risk factor for the development of hepatocellular carcinoma (HCC). Lifestyle modifications are effective in the treatment of NAFLD; however, long-term compliance is low. Therefore, the development of therapeutic treatments is required. Recently, the healthy properties of extra-virgin olive oil (EVOO) have been highlighted. Oleocanthal (OC), oleacein (OL) and hydroxytyrosol (HT) are minor phenolic compounds present in EVOO. They are strong antioxidants and radical scavengers, with proved anti-inflammatory, neuroprotective, immunomodulatory, and anticancer activities.
Material and Methods: Hepatocytic cells (HepG2 and Huh7) were exposed to a 3:2 oleate/palmitate fatty acid (FA) mixture, as this ratio recapitulates the amount of dietary fat involved in the development of steatosis in humans. Lipids accumulation in HepG2 and Huh7 cells was analysed in the presence/absence of the selected phenols. Lipid droplets accumulation was evaluated by Oil Red O and Bodipy staining. Western blotting analysis was conducted to investigate the molecular mechanisms involved in steatosis induction/protection.
Results: Treatment of HepG2 and Huh7 cells with FA mixture in the presence of OC, OL and HT reduced the number of intracellular lipid droplets after FA exposure. At the molecular level, in Huh7 cells, FA treatment in the presence of HT reduced the expression levels of fatty acid synthase (FASN) and acetyl-CoA carboxylase alpha (ACACA), two key enzymes responsible for de novo lipogenesis (DNL). In HepG2 cells, HT treatment increased phosphorylation levels of AMP-activated protein kinase (AMPK), a kinase involved in the inhibition of DNL, and increased the expression of peroxisome proliferator-activated receptor α (PPARα), a protein implicated in fatty acid oxidation processes.
Conclusion: These findings highlighted the ability of OC, OL and HT to interfere with lipid metabolism, supporting the importance of EVOO and its phenols in a healthy diet.

PRESENTATORE: Simona D'Antoni

METABOTROPIC GLUTAMATE RECEPTORS: POTENTIAL PHARMACOLOGICAL TARGETS FOR AUTISM SPECTRUM DISORDERS

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Background: Autism spectrum disorder (ASD) are a group of clinically and genetically heterogeneous neurodevelopmental disorders characterized by impaired social interaction and stereotyped behaviors. Different mechanisms are involved in ASDs pathophysiology; nevertheless, growing evidence suggests that a disrupted glutamatergic signaling can be common to different ASDs. Many autistic cases have an environmental origin and embryonic exposition to valproic acid (VPA) a common anti-epileptic drug, is associated with ASD in humans and autistic-like behaviors in rodents. A potential involvement of glutamate receptors in autism-like phenotypes observed in VPA-exposed rats has been suggested; however, few studies were carried out on metabotropic glutamate (mGlu) receptors. To this aim, we evaluated protein expression levels of group I (mGlu1 and mGlu5) and group II (mGlu2/3) mGlu receptors in rats prenatally exposed to VPA.
Material and Methods: Western blotting analysis was performed to study mGlu receptors expression in synaptosomes obtained from forebrain of control and VPA rats at different ages (post-natal day, 13, 35, 90). Ultrasound vocalization emission (USVs) test was carried out to evaluate the effect of mGlu receptors modulation on an early autism-like phenotype present in VPA rats.
Results: We found an enhancement of both group I and group II mGlu receptors expression in infant rats prenatally exposed to VPA and a correction of the reduced USVs, after a treatment with an antagonist of the mGlu2/3 receptor.
Conclusion: Our results provide the first evidence of an up-regulation of both group I and group II mGlu receptors at synapses in infant rats prenatally exposed to VPA and suggest that mGlu2/3 receptor modulation may have a therapeutic potential in ASDs. Future research will be directed to study mGlu2/3 receptor in other animal models of ASDs to evaluate its possible role as therapeutic target in ASDs and related conditions.

PRESENTATORE: Paola Dell'Albani

Effects of Quercetin Derivatives on O6-MethylGuanine-DNA MethylTransferase (MGMT) in primary human gliomas cultures

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Background: Gliomas are malignant glial tumors with poor prognoses. The current therapy utilizes Temozolamide (TMZ), an alkylating drug. The addition of Alkyl groups at specific DNA regions can damage cancer cells triggering the apoptotic process. However, this effect is sometimes thwarted by the activity of the MGMT enzyme. MGMT removes alkyl groups from damaged DNA, thereby interfering with the therapeutic effects of alkylating agents (1). Therefore, there is a critical need of novel therapeutic strategies. Quercetin, a natural flavonoid, has anticancer properties (2). However, the clinical application of quercetin is hindered by its low bioavailability and stability. In recent years, derivatives of Quercetin (QDs) with enhanced efficacy have been developed (3). This study aimed to highlight the possible therapeutic effects of QDs on glioma survival with a focus on MGMT promoter epigenetic changes.
Material and Methods: Primary glioma cell cultures were prepared from human GBM biopsies as in Dell'Albani et al. (2014) (4). Cultures were treated with 50 mM QDs (Q-3-Dec, diBr-Q-3-Palm) for 24 hours. Western Blotting analyses were used to evaluate the expression of MGMT; DNA extraction, Bisulphite conversion and end-point PCR were used to analyze the Methylation of MGMT gene promoter. MTT analysis was used to test glioma survival.
Results: Results obtained highlight a significant increase in the methylation levels of the MGMT gene promoter of glioma cells treated with QDs and a corresponding decrease in the un-methylated DNA. These results also correlate with decreased expression levels of MGMT after 24 hours of treatment.
Conclusion: QDs treatment of primary glioma can reduce the expression of the MGMT, through the introduction of epigenetic modifications, such as promoter methylation of the CpG islands. Then, the QDs favoring the inactivation of MGMT, with consequently decreased DNA repair, might yield a favorable prognosis in GBM patients receiving TMZ and other alkylating agents.

PRESENTATORE: Giacoma Galizzi

Circulating mtDNA in Fabry disease

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Background: Fabry disease is a rare genetic disorder due to the deficiency of the lysosomal enzyme α-galactosidase A (α-Gal A). It is caused by mutations in the GLA gene. The diagnosis of Fabry disease can be done through enzymatic test, which measure the activity of the α-Gal A enzyme in the blood. Furthermore, the dosage of Lyso-Gb3, a fatty substance that accumulates in the blood or in the urine as a result of α-Gal A deficiency, can also help to achieve a Fabry diagnosis and indicate the severity of the disease.
Material and Methods: Mitochondrial DNA (mtDNA) is an intrinsically inflammatory nucleic acid released from mitochondria to extracellular space during cell death and involved in the pathogenesis of various diseases. Patients with different types of mutations for atypical and classic phenotype were selected, based on the enzymatic activity of α–Gal and accumulation of Lyso-GB3. With the aim to look for a correlation between lysosomal dysfunction with mitochondrial damage, we collected cell-free plasma and we evaluated the presence of circulating mtDNA by quantitative PCR using the primers for the ND1 and 16S mitochondrial genes.
Results: Our preliminary data show that in all Fabry samples the amount of circulating mtDNA is higher than in the controls. Interestingly, the «Atypical» phenotype shows a slight increase in mtDNA while the «Classic» phenotype shows a significant increase in mtDNA. Enzyme replacement therapy (ERT) is a long-term treatment that may reduce the risk for cardiac, cerebrovascular and kidney complications. We also examined a patient with classic phenotype before and after 5 months of ERT. We found that ERT increases α-Gal A enzyme activity and decreases Lyso-Gb3 accumulation and circulating mtDNA.
Conclusion: These results show that circulating mtDNA levels may be correlated with different phenotypes of Fabry disease and can be an indicator of the disease's severity.

PRESENTATORE: Maria Lucibello

Targeting TCTP in breast cancer: an opportunity for personalized medicine

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Background: Chemotherapy remains the mainstay of standard therapy for early-stage and advanced TNBC. Unfortunately, many patients become resistant and/or poorly tolerant to chemotherapy. Despite the efforts to improve cancer therapy we need to do more. Recently, we have shown that TCTP in the phosphorylated form (phospho-TCTP) is an independent marker of poor prognosis in breast cancer and a critical molecular target of dihydroartemisinin (DHA), a natural product. DHA, by reducing phospho-TCTP activities, increased sensitivity to chemotherapy in TNBC cells. Based on these premises, we propose to validate TCTP as a negative prognostic factor for identifying tumors at high risk of progression at an early stage, and as a potential new clinically relevant biomarker for more aggressive TNBC tumors.
Material and Methods: A pilot study on BC specimens from aggressive TNBC carcinoma was performed to investigate the expression of phospho-TCTP levels. As in vitro model for anticancer drug screening, we established a collection of 3D culture systems. 3D cultures are enriched with cells with stem cell-related characteristics that can persist after chemotherapy and are better predictors of in vivo drug responses.
Results: Our preliminary data show a high number of phospho-TCTPpos cells in patients with triple-negative breast cancer (TNBC) with a bad prognosis. Moreover, we have observed that knockdown of TCTP in MDA-MB-231 cells (with a basal-like phenotype, ER−, Pr−, Her2−) increased the sensitivity to chemotherapy. In addition, TCTP levels were increased in 3D spheroids generated from MDA-MB-231 cells compared to the corresponding 2D monolayer. Notably, we also found that DHA induced a reduction of the spheres size (diameter) and ATP as a marker of cell viability.
Conclusion: Altogether, these findings suggest that phospho-TCTP may be a critical target for a therapy based on DHA, and for identifying tumors at high risk of progression at an early stage.

PRESENTATORE: Domenico Nuzzo

Antiviral and antioxidant effects of Artemisia annua against virus infection

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Background: Natural extracts are a rich source of bioactive with potential pharmacotherapeutic applications. Artemisia annua (A. annua) is a medicinal plant belonging to the Asteraceae family, which is widely distributed throughout temperate areas. Artemisinin derivatives, collectively called artemisinins, include artesunate, dihydro-artemisinin, artemether, arteannuin B, and artemisone. Over the centuries, medicinal herbs have been used as a treatment and preventive strategy for several diseases, including respiratory viral infections. The benefit of these herbs in viral respiratory infections is mainly due to their antiviral and antioxidant effects. The identification of natural molecules able to counteract virus replication, inflammation, and oxidative stress represents a new medical challenge for infections.
Material and Methods: We focused our attention on A. annua enriched extracts which were submitted to in vitro biological evaluation as antiviral and antioxidant agents with the potential application against the COVID-19 infection. Epithelial A549 lung cell line were used as a model of induced oxidative stress. Vero E6 cells were used SARS-CoV-2 replication.
Results: In our first results, the crude extract of A. annua showed antioxidant effect, in concentrations that do not affect cell viability. This extract showed also an antiviral effect when used at a concentration of 100 µg/mL.
Conclusion: Our investigation evidenced the antiviral and antioxidant effects of the A. annua crude extract for the treatment of SARS-CoV-2 infection. Investigation of single main components of the extract brought to light artemisinic acid as a promising natural compound worth of further investigation. Found: This research was supported by EU funding within the NextGenerationEU-MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases (Project no. PE00000007, INF-ACT).

PRESENTATORE: Antonio Qualtieri

MALDI-TOF MS protein profiling of extracellular vesicles from cancer cells in culture: preliminary data

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Background: Extracellular vesicles (EVs) play key roles in extracellular trafficking. In the last years they have emerged as the most promising biomarkers for clinical diagnostics, prognostics and potentially as vehicle for drug transport. Therefore, there is a great need for their efficient collection and complete characterization from biological samples, especially in oncology. We aimed at identify specific EVs protein profiles from cancer cells in culture using MALDI-TOF Mass Spectrometry (MS).
Material and Methods: HeLa Cells and Lymphoblastoid EBV cells were grown in DMEM and RPMI-1640 medium respectively supplemented with 10% FBS depleted of endogenous EVs, 2mM Glutamine, 100ug Penicillin/Streptomicin and at 37° C, 5% CO2. EVs were collected by differential centrifugation of culture medium over 4 step at 500g, 3800g, 10000g, 100000g. Pellet of the ultracentrifugation at 10000g and 100000g were analyzed by MALDI-TOF MS and protein profiling was obtained in linear mode in the range 1-25 kDa and 25kV, 275 ns delay time. Spectra were analyzed using Data Explorer 5.0 applying baseline correction and base peak detection over 2 % Relative Intensity.
Results: Acquired spectra show for both cell lines at least 20 mono charged ion signals well resolved and intense. On the basis of the m/z values we have tentatively identified 4 known small protein, Ac-Thymosin b-4 (4963.7), Ac-Thymosin b-10 (4936.5), b2 microglobulin (11730.4), free Ubiquitin (8564.8). Very interesting, qualitative ion differences were observed between EVs from the two cell lines, in particular at m/z 11386.8 +/- 1.5 and 10091.11 +/- 1.5.
Conclusion: MALDI-TOF MS analysis of whole EVs is a very useful approach to identify differential protein profiles from EVs with relative ease of execution and speed. These preliminary data let us to extend the analysis on EVs derived from other biological samples such as Human serum and urine. Data validation will be pursued through alternative methods.

PRESENTATORE: Patrizia Spadafora

Mutation spectrum and phenotypic variability in Calabrian patients affected by Limb Girdle Muscular Dystrophy

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Background: Limb Girdle Muscular Dystrophy (LGMD) is the fourth most common hereditary muscle disorder. LGMD comprises a genetically heterogeneous group of musculoskeletal disorders characterized by the primary involvement of the shoulder and pelvic girdle muscles. The clinical course can be variable, ranging from severe forms with early onset and rapid progression to milder forms. The overlapping of clinical symptoms and the large inter/intrafamilial variability observed make their genetic determination difficult. Molecular diagnosis remains elusive in about 30% of LGMD patients. Moreover, many genes associated with LGMDs are among the largest in the genome making it impossible to make a molecular diagnosis with classical sequencing techniques. Next-generation sequencing (NGS) has given a strong impulse to the genetic diagnosis of muscular dystrophies. The research activity is focused on the study of patients affected by LGMD orphaned by genetic diagnosis.
Material and Methods: The molecular analysis was performed by NGS with a custom panel that includes all the genes so far associated with LGMD. The identified variants were validated by direct Sanger sequencing.
Results: The study allowed us to identify a large family in which LGMDR2 was associated with a new homozygous variant c.5033G>A; it also showed that in southern Italy the 427C>A variant associated with LGMDR9 was more frequent than the 826C>A variant reported in western and northern Europe.
Conclusion: The study of patients affected by LGMD is part of a larger National project which consists in the molecular subtyping of patients and in the genomic analysis of those without genetic determination. The results generated in-house will be fed back into a network, for the purpose of increase the power of molecular-genetic investigation and define the molecular basis of such complex neurodegenerative pathologies. All this will also allow to identify prognostic and diagnostic markers and potential therapeutic approaches for personalized medicine.

PRESENTATORE: Michela Spatuzza

Stress response and senescence of astrocytes from Fmr1 knockout mice modelling Fragile X syndrome

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Background: Fragile X Syndrome (FXS) is the most frequent form of inherited intellectual disability and a leading monogenic cause of autism, which is caused by lack/reduction of the Fragile X mental retardation protein (FMRP), an RNA binding protein involved in several aspects of RNA metabolism. Abnormal expression of proteins at synapses are believed to underlie brain dysfunction in FXS. FMRP is also implicated in DNA damage response and is a component of stress granules (SGs), cytoplasmatic aggregates that form in response to stress and are protective against apoptosis. Evidence in the mouse modelling FXS suggest an involvement of glia in FXS and the presence of an excess of oxidizing agents in the brain. However, how glial cells cope with chronic stress in the absence of FMRP is presently unknown. Oxidative stress and DNA damage can also trigger cellular senescence. Therefore, we investigated response to stress and astrocytic senescence in FXS mice.
Material and Methods: We examined the appearance of SGs and the cell survival in wild-type (WT) and Fmr1 knockout (KO) cultured astrocytes after exposure to different insults by immunocytochemistry and MTT assay. We revealed the senescent phenotype of astrocytes by using Beta-Galactosidase assay.
Results: We found: 1. an increased number of cells with SGs after exposure to oxidative stress; however, Fmr1 KO astrocytes exhibited a lower number of cells with SGs than WT astrocytes both under basal condition and after exposure to oxidative stress. 2. a lower cell survival in Fmr1 KO cultured astrocytes upon exposure to oxidative stress. 3. increased features of senescent phenotype in Fmr1 KO astrocytes.
Conclusion: These results indicate that the lack of FMRP impairs the formation of SGs and sensitizes to oxidative stress-induced damage in astrocytes, disclosing a contribution of this cell type to brain dysfunction in FXS.

PRESENTATORE: Elisa Eleonora Tavormina

Temporal analysis of the prevalence of congenital hypospadias in the municipality of Gela

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Background: For over 30 years, the epidemiological investigations on Congenital Anomalies (CA) in Gela, a National Priority Contaminated Site (NPCS) characterized until 2014 by the presence of a petrochemical pole, highlighted significant excess of hypospadias in resident newborns compared to any reference data. Therefore, aim of the study is to evaluate the risk profile of hypospadias in Gela.
Material and Methods: Data from the Sicilian CA Register and Hospital Discharge Cards were used. The birth prevalence of hypospadias was calculated through the ratio between the number of births with hypospadias and total births (ISTAT) per year in Gela (per 10,000 live births) and for the respective references. Geographical analyzes were conducted by comparing the prevalence of the Gela municipality to those found in Sicily, in a territorial area bordering Gela (ALG) and in the NPCSsN of Milazzo and Priolo, characterized by similar environmental impact. The geographical comparisons were conducted for the period 2010-2020: the trend within the Gela NPCS was evaluated by comparing the sub-periods 2010-2014 and 2015-2020. Crude Odds Ratios (ORs) and 95% Confidence Intervals [95% CI] were calculated for comparisons.
Results: Excess risk for hypospadias was highlighted in 2010-2020 in Gela vs Sicily (OR 4.45 [3.45-5.75]), vs ALG (OR 3.51 [2.46- 5.01]) and vs both the NPCSs of Milazzo (OR 2.32 [1.32-4.07]) and Priolo (OR 2.37 [1.55-3.62]). The temporal between-period comparisons in Gela didn't show a statistically significant difference between 2010-2014 and 2015-2020 (p=0.22) with a prevalence of 72.4 and 98.9 for 10,000 respectively.
Conclusion: The prevalence study revealed a huge excess of births with hypospadias in Gela. The prevalence of hypospadias in 2015-2020 remains very high even if reduced compared to 2010-2014. Although the refinery was closed, an anomalous situation remains. Indeed, the effect of several risk factors is reasonable and it requires an appropriate analytical investigations.