GIORNATA A_IATRIS:

Progetti, Collaborazioni e Servizi

LIBRO DEGLI ABSTRACT

16 NOVEMBRE 2022
09:00 / 16:00

Nobile Collegio Chimico Farmaceutico
Via in Miranda 10, Roma

A cura di Chiara De Nuccio

PROGRAMME

8.30 Registration

9.00 Welcome and opening address
S. Brusaferro, President of the Istituto Superiore di Sanità
G. Guglielmi, Ministry of Health
M. Bertelletti, Ministry of University and Research

1st SESSION

EATRIS, A_IATRIS AND THE NATIONAL NODES: OPPORTUNITIES TO PROMOTE EUROPEAN TRANSLATIONAL MEDICINE

Chairpersons: F. Moretti, D. Morrow

9.15 EATRIS and the opportunities it can offer to researchers
D. Morrow

9.35 Organization of the Spanish node
M.L. García Bermejo

9.55 Organization of the Portuguese node
C.M. C. d. Faria

10.15 A success story: REMEDI4ALL
A. Budillon

10.30 **Coffee break**

2nd SESSION

ROUND TABLE: THE A_IATRIS NATIONAL NODE AND RESEARCH IN ITALY

Chairpersons: F. Moretti, A. Budillon

11.00 **Participants: L. Minghetti** (ISS), **M. Racaniello** (Farmindustria), **D. Ederle** (Alisei), **L. Aurisicchio** (Takis), **L. Gabriele** (ISS)

12.00 **Lunch break &** **POSTER SESSION**

3rd SESSION

ORAL COMMUNICATIONS & DISCUSSION

Chairpersons: L. Castiello, R. Canese, E. Iorio, P. Pichierri, L. Gabriele

14.00 R-ache-L Score: A nomogram model to predict the prognosis of neuroendocrine lung tumors according to the tumor side

A. La Salvia

14.10 Expanded NK cells for cancer immunotherapy: manufacturing and quality control results of a phase I clinical trial

L. Castiello

14.20 Towards a pipeline for the evaluation of small molecule compounds targeting the replication stress response and the exploitation of organoids for effective personalized medicine

E. Malacaria

14.30 Questions & answers

14.50 Resting state functional MRI and DTI reveal alteration in brain connectivity in a transgenic mouse which over-express human hydrolase hMTH1 following an oxidative stimulus

T. Singh

15.00 Preliminary results on the lipid signal in cervical cancer

R. Canese

15.10 Research studies with a fully-integrated PET/MRI system at San Raffaele Hospital in Milan

P. Scifo

15.20 Questions & answers

15.40 Conclusions and end of the day

POSTER SESSION

1.

Dissecting the roles of cannabidiol and cannabidiolic acid in triple negative breast cancer cells

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Introduction: Breast cancer is the most frequently diagnosed cancer in women worldwide. Currently 3 subtypes of this disease have been identified estrogen receptor (ER)-positive, which is positive for the biomarker ER alpha. HER2, which is positive for Human Epidermal Growth Factor Two (HER2) and generally negative for ER and the progesterone receptor (PR) and triple-negative breast cancer (TNBC), which is negative for ER, HER2 and PR. Triple negative tumors are deficient for well-defined molecular targets making chemotherapy, which is non-specific and cytotoxic, the most common treatment option. Patients with TNBC tend to develop metastasis and recurrence after treatment as well as lower survival compared to patients with other subtypes of breast cancer. Hence, there is a need for innovative therapeutic interventions to help women with breast tumors. Cannabinoids are products of Cannabis sativa L. They were first introduced as palliative medicinal products, aiding in reducing emesis resulting from chemotherapy for cancer patients. Cannabinoids possesses anti-tumoral activity in breast cancer cell lines. Aims: In this study we tested the effects of CBD and CBDA, natural fitocomplexes extracted from Cannabis sativa, on the growth of triple negative breast cancer cells, MDA-MB-231. Methods: In vitro assays were performed on triple-negative MDA-MB-231 cells treated with cannabidiol (CBD) (1, 5, 10, 20, 40 and 80 µg/mL) and cannabidiolic acid (CBDA) (1, 10, 20, 40, 80, 160 µmol/L), alone and in combination. The effects of CBD and CBDA on viability were determined by wound healing and MTT assays, while cell migration was assessed by transwell migration. Results: Cell proliferation, viability and apoptosis of MDA-MB-231 cells were impaired by CBD and CBDA. Specifically, our data show that CBD and CBDA reduced the proliferation of MDA-MB-231 cells by impairing cell-cycle progression ($p<0.05$). These findings suggest that the combination of these cannabinoids may represent a new strategy for the treatment of patients suffering from triple-negative breast cancer. Conclusions: These findings suggest that the combination of CBD and CBDA may represent a new strategy for the treatment of patients suffering from TNBC and TNBC related pain.

2.

The role for endogenous type I Interferon in the metabolic reprogramming of spontaneous mammary tumors in HER2/neu transgenic mice

Rossella Canese[1], Egidio Iorio[2]*, Mattea Chirico[2], Maria Elena Pisanu[2], Taljinder Singh[1,3], Gianmauro Palombelli[1], Francesca Spadaro[4], Serena Cecchetti[4], Anna Maria Pacca[5], Massimo Spada[5], Daniele Macchia[5], Maria Teresa D'Urso[5], Luciano Castiello[6] and Eleonora Aricò[6]*

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*equal contribution

Our study aims to identify, by in vivo MRS and ex vivo high resolution (HR)-MRS, the metabolic changes involved in spontaneous carcinogenesis occurring in Her2/neu transgenic mice, with particular focus on the role of endogenous type I IFN (IFN-I). Our group reported that the lack of endogenous IFN-I system significantly affects Her2/neu carcinogenesis. Since this phenomenon was not related to the known immunomodulatory properties of these cytokines, we investigated whether the possible reshaping of metabolic pathways was involved. Both in vivo MRS and ex vivo high resolution (HR)-MRS revealed that non-tumoral mammary glands of mice lacking a functional endogenous IFN-I (IFNARI-/-) had increased fatty acids concentration with respect to the wild type counterpart. This result paralleled the observation of specific trascriptomic profiles in IFNARI-/- normal mammary glands, that exhibited decreased expression of genes involved in mitochondrial activity and increased expression of Sterol regulatory element-binding proteins, a known regulator of fatty acids biosynthesis and an independent prognostic marker in breast cancer. HR-MRS analyses of aqueous metabolites revealed that the metabolic fingerprint of Her2+ tumors lesions was significantly different from normal mammary glands in both IFNARI+/+ and IFNARI-/- mice. The concentration of myo-inositol and glutathione was significantly reduced in IFNARI-/- tumors, suggesting that endogenous IFN-I may exert its antitumor activity by affecting key metabolic regulators of tumor cells proliferation. Our study provide the first evidence that endogenous IFN-I is involved in metabolic pathways in both normal mammary glands and early phase breast cancer.

3.

Metabolomic approaches using 1H NMR spectroscopy detect multiple metabolic targets for the antitumor action of metformin on human glioma cells

Mattea Chirico[1], Marco Rinaudo[1], Agnese Sacchetti[1], Maria Elena Pisanu[1], Rossella Canese[1], Serena Cecchetti[1], Mariachiara Buccarelli[2], Lucia Ricci-Vitiani[2], Marica Pinazza[3], Stefano Indraccolo[4,5], Franca Podo[1], Egidio Iorio[1]

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Glioblastoma (GBM), the most aggressive malignant brain tumor, is typically refractory to conventional treatments. There is an urgent need to develop novel therapeutic strategies. Metabolomic approaches using nuclear magnetic resonance (NMR) spectroscopy may identify new molecular targets for an anticancer strategy. The anomalous phosphatidylcholine (PC) metabolic profile detected in cancer by NMR spectroscopy and spectroscopic imaging provides molecular signatures of tumor progression and response to therapy (Iorio et al Cancer Res 2010; Iorio et al Eur Radiol Exp 2021). Purposes of this study were: 1) to investigate the alterations induced on the cell metabolome by metformin (an anti-diabetic drug held to also act as anticancer agent); 2) to evaluate the possibility to enhance the antiproliferative effects of this drug in a human glioma cell line and in glioma stem cells (GSC). NMR-based metabolomics analyses were performed on the exo- and endo–metabolome of either untreated or metformin-treated U87MG cells and GSC. Cell proliferation and mortality assays were then conducted on cells exposed to a combination of metformin with D609, a competitive inhibitor of phosphatidylcholine (PC)-specific phospholipase C (PC-PLC), an enzyme implicated in cell signaling, cell cycle regulation and cell proliferation [Podo et al Front Oncol 2016]. Metformin alone induced, in both U87MG cells and GSC, cell growth arrest but not cell death, along with accumulation of phosphocholine (PCho), a metabolite involved in the PC cycle. Exposure to metformin combined with D609 resulted instead in a reduced PCho content, associated with significantly decreased cell viability and increased cell death. We provided the first evidence in support of a) activation of PC-PLC and PCho accumulation in metformin-treated human glioma cells and GSC; b) induction of cell death in human glioma cells treated with a combination of metformin and D609. These results provide the grounds for the possible development of a new multi-targeted approach against this malignancy.

4.

A pilot study on integration of multiomics-based approaches to discover novel biological traits associated with chemotherapy respons

Claudio Tabolacci[1], Egidio Iorio[2], Francesco Facchiano[1], Alessandro Giuliani[3], Mattea Chirico[2], Stefania Rossi[1], Rossella Canese[4], Marina Bagnoli[5], Delia Mezzanzanica[5], Antonella Tomassetti[5], Maria Elena Pisanu[2]

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Despite progress in the development of new therapies, high-grade serous epithelial ovarian cancer (HGSOC) remains the most lethal gynecological cancer mainly due to late diagnosis and development of incurable chemo-resistant relapse. Primary debulking surgery and platinum-based chemotherapy remain the standard treatment for advanced-stage epithelial ovarian cancer (EOC). However, despite the high response-rate to initial therapy, most of the patients experience disease relapse ultimately developing drug resistance. Stage and residual disease, although helpful in the management of patients, are not sufficiently accurate to guide therapeutic strategies for EOC. Thus, to improve patients' prognosis it is mandatory to refine our knowledge about the biology characterizing response to therapy. The aim of the study is to provide a multi-parametric novel tool to discover biological features and likely potential biomarkers of EOC chemotherapy responses. An integrated multi-omics approach based on NMR-based metabolomics and secretomes by Luminex xMAP multiplex technologies has been applied on ascites from HGSOC patients with disease recurrence within 6 months from the end of treatment (Platinum (Pt)-resistant) and 6 patients with disease recurrence after 18 months from the end of treatment (Pt-sensitive). Multivariate analyses (PLS-DA) identified a panel of metabolites responsible for the difference between the resistant and sensible groups, particularly higher contents of alanine, isoleucine, acetoacetate, and unsaturated fat acids, and lower levels of lactic acid, histidine, fumaric acid, phosphatidylcholine plus its lyso-derivates and triacylglicerols in ascites from resistant patients. Preliminary secretome analyses relevead a significant higher level of IL-β, Serpin E1, TNF-a, VCAM-1, and a decreased level of MMP-12 in resistant patients. Finally, correlation analysis between the metabolic and immune profiles highlighted the significant correlation between the most discriminant metabolites (VIP>1 in PLS-DA) and the immune secretome profile. The PBEF/visfatin, adiponectin, resistin, and INFγ molecules are strongly correlated to metabolites involved in glucose and lipid metabolism, suggesting a potential immune-metabolic signature in resistant and sensitive patient ascites.

5.

A New Comprehensive Workflow for CircRNA Analysis

Enrico Cabri[1], Maddalena Fratelli[1]

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Literature demonstrated the great potential of precision medicine when it comes to analyze genomic data. The availability of a plethora of field-specific tools grants the ability to develop in a quicker fashion new fast and versatile dedicated software for discoveries in biology. These new implementations significantly hasten the analysis of big amounts of data, leading to the establishment of novel solutions mainly for clinics and diagnostics. In recent years circular RNAs, showed the potential to become biomarkers for critical diseases such as Alzheimer's disease and cancer, metastatic especially. While the succession of numerous scientific papers led to the development of a high number of circRNAs identification tools, there still is a lack of software focused on quantification and differential analysis for this newly interesting biomolecule. Also, the intrinsic complexity of the available tools, thus the poor accessibility by less experienced computer users hinders the path towards new discoveries. Therefore, circular RNAs research is still in its infancy and the functional role of these molecules in disease aetiology is just starting to be determined. However, conclusions reached so far confirmed its potential as a valuable biomarker for cancer, while also uncovering the possibility of exploiting them as targets or drugs in cancer therapy. Due to this potential, we overviewed a large number of tools available for quality control, alignment and those specifically developed for circular RNAs identification. The wide range of choice in software underlined the necessity for a thorough workflow, starting from primary all the way to tertiary analysis, thought in a way to fit the needs both of bioinformaticians and less experienced users. The development of a workflow also helps boosting the analysis speed thanks to the optimization of the tools it uses and the reduction of the time related to the researchers' effort in deciding what tools to be used and their comprehension. These considerations led to the development of the presented workflow which identified in TopHat2 and CIRCExplorer2 the fittest tools to match these needs when it comes to secondary analysis, while developing a whole new approach to circRNAs tertiary analysis with the creation of CircRNAPartner a R-Shiny Web Application which delivers fast and comprehensible circRNA quantification, differential analysis and the relative data visualization and storage.

6.

HDACi and Interferon combination treatment blocks the oncogenic phenotype and increases the immunogenicity of BRAF-mutated and BRAFi-resistant metastatic melanoma cells.

Alessandra Fragale[1], Emilia Stellacci[1], Giulia Romagnoli[1], Valerio Licursi[2], Stefania Parlato[1], Irene Canini[1], Giacomo Remedi[1], Maria Buoncervello[3], Paola Matarrese[4], Lucia Pedace[5], Barbara Ascione[4], Simone Pizzi[6], Marco Tartaglia[6], Stefania D'Atri[7], Carlo Presutti [8], Imerio Capone[1] and Lucia Gabriele[1]*

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Melanoma is characterized by diverse genetic alterations and dysregulated cellular signals that drive tumor growth and metastasis. The aminoacid substitutions at codon 600 of the BRAF kinase (BRAFV600) are the most common somatic mutations driving tumor development and progression. On this basis, the advent of targeted therapies based on the use of BRAF inhibitors (BRAFi) has changes the clinical management of BRAF-mutated melanomas. However, the success of these revolutionary treatments is hampered by the emergence of drug resistance in the vast majority of patients. Understanding and blocking the cellular signals associated to BRAFi resistance is the unmet need for those patients who do not respond to treatments. Here, we provide evidence that combined treatment of the histone deacetylases inhibitor (HDACi) romidepsin and the immunomodulatory agent IFNα-2b (IFN) exerts a strong antitumor and immunomodulating activity. RNA-seq results of six of metastatic melanoma (MM) cells treated with romidepsin/IFN vs untreated MM cells reveal a sharp drug-driven transcriptional modulation highly linked to the IFN-α and IFN-γ signals and at the same time, the downregulation of hallmark genes related to the cell cycle block and MYC pathway. Noteworthy, treatment with the romidepsin/IFN combination was capable to limit the proliferation, migration, and longterm survival of primary BRAFV600 MM and BRAFi-resistant (MM-R) cells. Moreover, romidepsin alone or even more in combination with IFN abrogated BRAF-activated MAPK or AKT signaling pathways in both MM and MM-R along with AXL and MITF phenotype reshaping. The functional cell death analysis, by using specific death inhibitors, showed that drug-treated MM and MM-R undergo apoptosis/necroptotic cell death capable of stimulated the phagocytosis of dendritic cells. Overall, epigenetic-immune treatment drives reprogramming of oncogenic phenotype toward an immunogenic signature, overcoming BRAF metastatic potential and BRAFi-acquired resistance, thus warranting further clinical exploitation to control metastatic melanomas.

7.

Epigenetic drugs modulate dendritic cells behaviour towards melanoma cells

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Epigenetic dysregulations, including histone modifications, are hallmarks of cancer, including melanoma. On this basis, the histone deacetylase inhibitors (HDACi) have been widely considered very promising antitumor agents. We have previously reported the antitumor and immunomodulatory effects of class I-II HDCAi romidepsin combined with IFNalpha2b on colon-rectal cancer (CRC). Herein, we focus on the antitumor effects of the HDACi romidepsin, tubastatin A, valproic acid (VPA) and mocetinostat on Sk-Mel-28 melanoma cells and on their capability to induce an immune response to cancer. Our results indicate that all HDACi considered in this study reduce the viability of Sk-Mel-28 in a dose-dependent manner. 72hr-treatment with HDACi induce classic apoptosis of Sk-Mel-28. In particular romidepsin and VPA were found to induce 73% and 44% of early/late apoptosis, respectively, and to reduce melanoma invasiveness, as demonstrated by wound healing closure assay. In addition, HDACi-treated Sk-Mel-28 cells exhibited the capability to produce an array of chemokines (e.g. CCL19, CCL20, CCL21, CXCL12, CXCL10) enhancing the capability of dendritic cells (IFN-DC) to migrate towards and phagocyte cancer cells. Overall, our data indicate that romidepsin, VPA, tubastatin A and mocetinostat own a significant antitumor potential against melanoma cells targeting both cancer cells and the innate arm of the antitumor immune response, thus representing possible candidates in melanoma therapy.

8.

A small molecule that targets the serotonin receptor 7 as a novel therapeutic strategy for Rett syndrome

Chiara De Nuccio[1], Chiara Urbinati[2], Livia Cosentino[2], Antonietta Bernardo[3], Marcello Belfiore[4], Anita Greco[3], Sergio Visentin[3], Bianca De Filippis[2]

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Rett syndrome (RTT) is a rare and severe neurodevelopmental disorder for which there is no cure. Mutations in the methyl CpG binding protein 2 gene (MECP2) cause most RTT cases. Based on compelling preliminary data demonstrating that a small molecule that targets the serotonin receptor 7 substantially rescues the neurobehavioural phenotype in a mouse model carrying a truncated mutation of the MeCP2 gene, we are currently testing the generalization of the outcomes of this treatment strategy on representative RTT-causing mutations in murine models and in human cells from patients. The molecular mechanisms underlying the beneficial effects of the systemic treatment are also under active investigation. These studies will pave the way to the clinical testing of an innovative therapeutic strategy for RTT.

9.

Chlorpromazine affects glioblastoma bioenergetics by interfering with pyruvate kinase M2

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Background. Glioblastoma (GBM) is characterized by high morbidity and mortality, thus making new effective therapeutic approaches highly desirable. In our attempts to identify repositionable drugs in GBM therapy, we identified chlorpromazine (CPZ) as a very promising compound. Here we aimed at further unveiling the mode of action of this drug. Methods. We performed a supervised recognition of the signal transduction pathways potentially influenced by CPZ via Reverse-Phase Protein microArrays (RPPA) and carried out an Activity-Based Protein Profiling (ABPP) followed by Mass Spectrometry (MS) analysis to possibly identify cellular factors targeted by the drug. In addition, using the Seahorse platform, we analyzed the bioenergetics changes induced by the drug. Results. CPZ was able to hinder GBM anabolic pathways and stimulate autophagy. We also identified the glycolytic enzyme PKM2 as a major target of the drug, a result concordant with the profound interference of CPZ in GBM cells bioenergetics, attributable to the ability of the drug to induce tetramerization of PKM2 and inhibit its oncogenic properties. RPE-1 non-cancer neuroepithelial cells appeared resistant to the effects of the drug. PKM2 silencing significantly reduced the effects of CPZ in GBM. 3D modeling showed that CPZ interacted with PKM2 tetramer in the same region involved in the binding with other known activators. Conclusions. Overall, the effect of CPZ can be epitomized as an inhibition of the Warburg effect and thus malignancy in GBM cells, while sparing RPE-1 cells. The present preclinical data enforce the bases that allowed us to add CPZ to the standard therapeutic treatment for GBM in an ongoing multicenter Phase II clinical trial supervised by our group.

10.

Prediction and study of the immunogenicity of new SARS-CoV-2 T HLA-A*02:01 restricted epitopes

Fabiola Mancini[1], Iole Macchia[2], Valentina La Sorsa[3], Martina Borghi[1], Irene Ruspantini[4], Valentina Durastanti [5], Maria Concetta Altavista [5], Silvia Taglieri [2], Alessandra Ciervo[1] and Francesca Urbani[2]**

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COVID-19 recovered and anti-SARS-CoV-2 vaccinated individuals develop specific antibodies and show persisting memory T-cells for at least 6 months.
Currently, vaccines are based on the SARS-CoV-2 Spike protein. However, both structural and non-structural proteins should be considered as potential targets for vaccine design. In fact, non-structural proteins are produced at an early stage of the viral infection, eliciting the immune system and contributing to the inhibition of virus replication and its systemic spreading.
In this view, it is crucial to assess the levels of protection generated by natural infection or vaccine immunization, by measuring T-cell responses that may complement currently in use antibody testing to find correlates of protection. Moreover, to better understand the immune response against SARS-CoV-2, it would be helpful to identify immunogenic epitopes capable of activating cell-mediated immunity in the host.
The aim of the present work was the identification of new potential epitopes, specific for cytotoxic T-lymphocytes, derived from the Spike protein and from four Non-structural proteins (Nsp-1, Nsp-2, Nsp-3 and Nsp-16), focusing on HLA-A*02:01 restricted peptide. For this purpose, we first predicted potential HLA-A*02:01-restricted CD8+ T-cell epitopes of SARS-CoV-2 by means of suitable computer prediction tools and selected 13 peptides for the Spike protein (including four pairs of peptides according to the Wuhan/Delta/Omicron variants) as well as 2 peptides for each Nsp (-1, -2, -3, -16). We excluded peptides that have been already described as immunogenic by other authors. The second step of the study consisted in testing peptide immunogenicity on cryopreserved PBMCs derived from 14 recovered patients' blood. We choose the ELISPOT immunoassay as the more appropriate and sensitive approach compared to other two methods (activation-induced markers assay and intracellular cytokine staining after Ag-specific stimulation of PBMCs).
Our results indicated that most of the selected peptides showed low and variable ability to induce an IFN-g production in subjects' PBMCs (ranging from 0/14 to 5/14 positive responses). Two of them (named FV10 and LA9, both belonging to the SPIKE protein) showed an acceptable rate of immunogenicity (6/14 and 7/14 positive responses, respectively) and, for this reason, they could be considered worthy of further investigation.

11.

Heterogeneous and unpredictable profiles of cytokines in COVID-19 patients

Stefania Rossi[1], Imerio Capone[1], Enrico Cabri[2], Mirko Scordio[3], Cinzia Marcantonio4, Roberto Giuseppetti[4], Giulia Romagnoli[1], Stefano Santini[1], Federica Frasca[3], Alessandra Pierangeli[3], Matteo Fracella[3], Gabriella d'Ettorre[5], Giancarlo Ceccarelli[5], Claudio Mastroianni[5], Roberto Bruni[4], Anna Rita Ciccaglione[4], Guido Antonelli[3], Maddalena Fratelli[2], Carolina Scagnolari[3], Lucia Gabriele[1]

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Coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections that results in a broad-spectrum of clinical manifestations, ranging from asymptomatic infection to severe respiratory failure. High levels of proinflammatory cytokines are believed to associate with disease severity in ill COVID-19 patients. Here, by performing a comprehensive evaluation of an array of immune molecules, including cytokines, chemokines, immune factors and antibodies (Abs), in the sera of a cohort of COVID-19 on hospital admission, we report that (SARS-CoV-2) infection causesthe dysregulation of different patterns of serum immune factors. Specifically, unsupervised machine learning analysis revealed that COVID-19 patients maybe characterized by three different serum expression clusters of immune molecules that did not uniquely associate with disease outcome. Importantly, patients with death events were gathered mainly within two different immune clusters of severe COVID-19. Taken together, our study underscores the absence of a unique pattern of inflammatory immune factors linked with worst disease outcome and points out to the major relevance of the immune molecule balance.

ORAL COMMUNICATIONS

R-ache-L Score: A nomogram model to predict the prognosis of neuroendocrine lung tumors according to the tumor side

Anna La Salvia[1,2], Benedetta Marcozzi[3], Chiara Manai[2], Rossella Mazzilli[4], Matteo Pallocca[3], Gennaro Ciliberto[5], Lorenza Landi[2], Federico Cappuzzo[2], Antongiulio Faggiano[4]

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Background: The 2021 WHO classification of lung neuroendocrine neoplasms is based on morphology and proliferative activity. For well-differentiated forms (carcinoids or Lung neuroendocrine tumors, Lu-NETs), the histotype is the main prognostic factor (being the prognosis of atypical carcinoids, AC, worse than typical carcinoids, TC). The stage (according to the international TNM system) also has prognostic value, but insufficient to stratify Lu-NETs. Recent molecular analyses have suggested that the ACs constitute a more heterogeneous entity. Hence the need to integrate the stage with clinical-pathological and molecular characteristics. In a previous study by our group, for the first time the laterality of the primary tumor (right vs left lung) emerged as a potential relevant prognostic marker. Materials & Methods: Overall survival (OS) was estimated using the Kaplan-Meier method. Significant p values <0.05. Univariate & multivariate Cox proportional hazards model were used to determine the impact of individual variables on prognosis. Therefore, we developed a nomogram that integrates prognostic factors to predict OS of Lu-NETs. Each of the prognostic markers was assigned a score, the sum of which gives a value from 0 to 280. Wilcoxon's non-parametric statistical test was applied for significant differences between the parameters. Statistical analyses were performed using the SPSS biostatistic software, IBM-SPSS 25 version and the R v.4.2.0 software. Results: we included 300 Lu-NETs. Age, sex, nodal status, laterality of the primary tumor (right vs left) and diagnosis (TC vs AC) as parameter of the nomogram. We were able to identify three prognostic groups, associated with three ranges of scores (I: 0-100; II: 101-150; III: 151-280) obtained through the nomogram, significant in predicting outcome. According to the Rachel score, patients were stratified into three risk groups (high, medium and low) based on the score obtained with the nomogram. The Kaplan-Meier curves compare OS of the three groups, with significant differences (p <0.0001). Conclusions: A specific prognostic nomogram was developed that incorporates variables with a significant impact on Lu-NETs survival. The nomogram showed a satisfactory ability to predict the OS in this population, confirming the heterogeneity beyond the histopathological diagnosis of TC vs AC.

Expanded NK cells for cancer immunotherapy: manufacturing and quality control results of a phase I clinical trial

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Natural killer (NK) cells are cytotoxic lymphocytes of the innate immune system that can kill virally infected and/or cancerous cells in a non-antigen-restricted manner and as such represent a promising immunotherapy platform for cancer treatment. We developed a good manufacturing practice (GMP) compliant protocol for the ex vivo expansion of autologous NK cells in the presence of autologous plasma, interleukin (IL)-2, IL-15, and irradiated autologous feeder cells. A multicenter, phase 1 study designed to assess the feasibility and maximum tolerated dose (range: 1-50x10^6 cells/kg) of immunotherapy based on our expanded NK cells in patients with Philadelphia (Ph)+ acute lymphoblastic leukemia (ALL) in complete hematologic remission (CHR) but with persistent/recurrent MRD was successfully conducted (EudraCT 2013-002547-29). Here, we report the analysis of manufacturing and quality control data collected on NK batches generated for the clinical trial, including not-for-release data. For manufacturing we evaluated: the total number of PBMC and NK in the apheretic product, the number of NK cells collected after CD3 depletion/CD56 selection, the number and viability of plated NK cells, the number and viability of day-14 expanded NK cells, post-thaw viability, post-thaw purity (in terms of CD3-/CD56+ cells), post thaw functional activity (in terms of CD107+ upon unspecific and specific stimulations). Ten batches were manufactured from six Ph+ ALL patients, since for four patients two apheresis were needed to reach highest dose-levels. All batches fulfilled specifications and were released. NK cell expansion showed high variability, that was not patient-specific, and that did not guarantee the generation of doses 50x10^6 cells/kg from one single apheresis. NK cell reactivity against unspecific and specific stimulations did not correlate with each other, but both correlated with relevant manufacturing parameters, suggesting the need to address the biological significance of both parameters in large trials for more specific potency evaluation. In conclusion, in order to sustain more advanced clinical trials aiming at evaluating the efficacy of the expanded NK cells at clinically relevant dosages, our GMP manufacturing process would benefit of additional optimization in terms of cell expansion capacity and automating some laborious steps.

Towards a pipeline for the evaluation of small molecule compounds targeting the replication stress response and the exploitation of organoids for effective personalized medicine

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The majority of novel promising therapeutic approaches in cancer exploit concepts of personalized medicine. Effective personalized medicine stems from two converging pieces of information: molecular characterisation of individual tumors combined with identification of specific vulnerabilities. However, the adoption of effective model platforms for mechanistic studies and biomarkers analysis is critical for identification of actionable therapeutic targets. Blocked or delayed progression of DNA replication can result in stalling and potentially collapse of replication forks. This condition is referred to as DNA replication stress (RS) and, if not promptly or properly handled, impacts on cell fate and genome integrity. RS is a feature of cancer cells and contributes to neurodegeneration and developmental abnormalities. Since different types of cancer display signs of RS, its exploitation for a targeted therapy is becoming more and more popular. One such approach relies on inhibition of cancer-specific or cancer-relevant RS-responding pathways to increase RS over the tolerated threshold leading to selective cancer cell death. In our institute, we decided to take advantage of the long-standing expertise in RS and DNA damage, imaging, cancer organoid models and signaling pathway analysis to build a pipeline tailored to the identification and characterization of RS-targeting compounds. This pipeline, which is still under development, includes i) high-content automated quantitative image-based cytometry (QIBC) to analyze accumulation of RS and DNA damage-related endpoints, ii) automated evaluation of protein-protein and DNA-protein interactions by proximity-labeling in situ and iii) high-content signaling pathway investigation by reverse-phase protein arrays. This pipeline is based on the use of a wide spectrum of 2D and 3D models and is aimed at screening small molecule compounds targeting RS-related factors and pathways as well as for biomarker identification/validation. Standard cell cultures or patient-derived organoids will allow for ad-hoc genome editing and generation of disease-specific models. As a proof of principle, here, we present results on the development and characterization of novel inhibitors targeting the DNA repair and RS-related factor RAD52, a protein that is synthetic lethal with loss of BRCA2, and WRN, an helicase implicated in fork recovery, which is synthetic lethal in MSI-positive colorectal cancer.

Resting state functional MRI and DTI reveal alteration in brain connectivity in a transgenic mouse which over-express human hydrolase hMTH1 following an oxidative stimulus

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Introduction The role of the oxidative stress in the pathogenesis of neurodegeneration is well known. The Human MutT homologue (hMTH1) is a hydrolase able to protect nucleic acids from oxidative damage. Interestingly, transgenic mice which overexpress the human MTH1 gene (hMTH1-Tg) are protected from neurodegeneration and motor impairment. Aims To understand if the over-expression of hMTH1 is able to counteract the effects of a chronic exposure to an oxidant, like Paraquat. Methods Male C57bl6 mice, wild-type and hMTH1-Tg, were analysed by rs-fMRI and DTI before and after a chronic treatment with Paraquat. Experiments were performed on a Pharmascan Bruker (Ettlingen, DE) system operating at 7T equipped with a cryo-probe. Mouse brain resting state fMRI was studied with seed-based analysis. In the DTI study an EPI sequence with 30 direction gradient was used. The diffusivity values (fractional anisotropy, FA and mean diffusivity, MD) were derived from the tensor. Results Our preliminary seed-based analysis shows differences in the functional networks of the wt and the hMTH1-Tg mice after exposure to the oxidant compound. DTI analysis reveals region specific alterations of FA and MD which differ in the two group of animals after Paraquat exposure. Analysis in other relevant brain areas are in progress. Discussion and Conclusion Taken together these results show a diverse brain vulnerability of the transgenic mice to the Paraquat exposure, compared to wt and help to deepen insights into the possible mechanisms of action of the hMTH1 over-expression on brain function and structure.

Preliminary results on the lipid signal in cervical cancer

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Purpose The purpose of our study was to investigate the role of the lipid peak derived from 1H-MRS in assessing cervical cancer (CC) prognosis, particularly in assessing response to neoadjuvant chemotherapy (NACT) of locally advanced cervical cancer (LACC). Methods We enrolled 17 patients with histologically proven CC who underwent 3T-MR at baseline. In addition to conventional imaging sequences, the quantitative protocol included a PRESS sequence (TR/TE=1500/28 and 144 ms). Spectra were analysed using the LCModel fitting routine, thus extracting multiple metabolites, including lipids (Lip) and total choline (tCho). Patients with LACC were treated with NACT and reassessed by MRI at term. Based on tumor volume reduction, patients were classified as good responder (GR; tumour volume reduction > 50%) and poor responder or nonresponder (PR-or-NR; tumour volume reduction ≤ 50%). The short echo time (TE=28 ms) was chosen for metabolite quantification, while the longest TE for the detection of lactate to avoid overestimation of lipid signal. Results Of 17 patients, 11 were LACC. Of these 11, only 6 had both completed NACT and had good-quality 1H-MR spectra; 3 GR and 3 PR-or-NR. A significant difference in lipid values was observed in the two groups of patients, particularly with higher Lip values and higher Lip/tCho ratio in PR-NR patients (p=0.040). A significant difference was also observed in choline distribution (tCho), with higher values in GR patients (p=0.040). Conclusion Assessment of lipid peak at 1H-MR-spectroscopy could be a valuable tool in predicting the response to NACT in patients with LACC.

Research Studies with a Fully-Integrated PET/MRI system at San Raffaele Hospital in Milan

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In this work, we present the ongoing PET/MRI research and clinical activity at IRCCS San Raffaele Scientific Institute, Milan, which is partner of the "Imaging and Tracers" Platform in A_IATRIS. The system has been installed in 2018 in the Nuclear Medicine department and it is mostly dedicated to Oncological research protocols. Since 2021, PET/MRI has been also used in oncological clinical practice. The scanner is whole body system SIGNA PET/MRI (General Electric Healthcare, Waukesha, WI, USA), integrating a 3 Tesla MR system with a 25 cm LYSO-SiPM PET ring with TOF capability. The research protocols we are performing include: Neuro-Oncology studies of pediatric and adult patients with 11C-Methionine; 18F-FDG Breast Cancer studies, Prostate studies with 68Ga-PSMA and 68Ga-RM2 (Bombesin), 68Ga-DOTATOC for Neuroendocrine Tumor (NET) studies, 18F-FDG esophagus cancer studies. Some of the ongoing PET/MRI research protocols will be presented. Routine oncological clinical applications include: 18F-FDG Gynecological oncological studies, 18F-PSMA prostate studies, 18F-FDG bladder cancer studies, with whole body PET and diagnostic dedicated MRI study. Some representative cases will be presented. Finally, advantages and disadvantages in the use of this technology for research and clinical activity will be discussed together with future perspectives.