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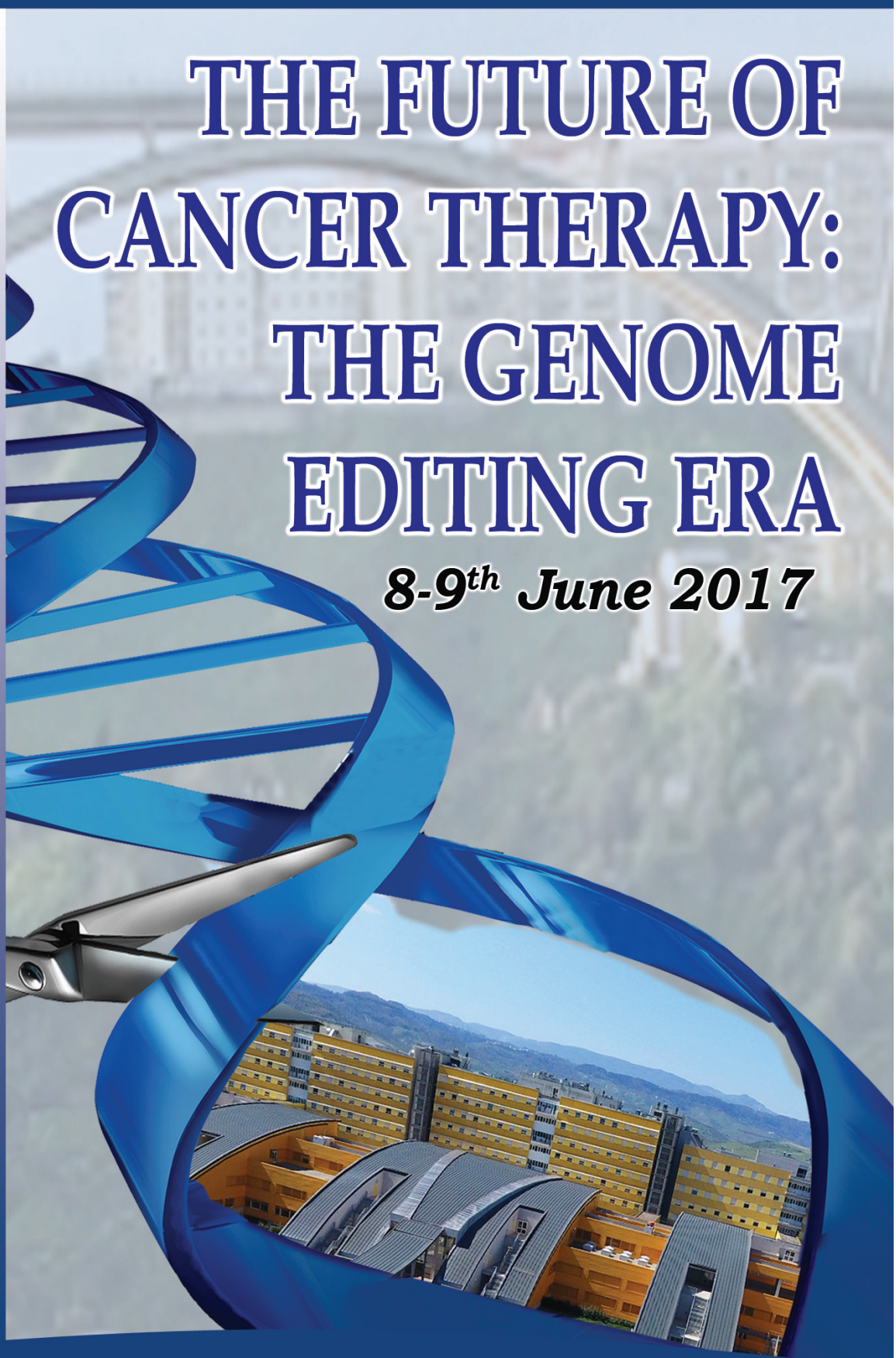
THE FUTURE OF CANCER THERAPY: THE GENOME EDITING ERA

8-9th June 2017



PROGRAM & ABSTRACT BOOK

University Magna Græcia, Catanzaro (Italy)

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1° Level Building G

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Ordine Nazionale Biologi

SOCIETÀ ITALIANA DI BIOCHIMICA E BIOLOGIA MOLECOLARE - 1951 - SIB

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PRESENTATION

In June 2017, the Italian Association for Cell Cultures (AICC) have organized focused meeting dedicated to "Genome Editing". During the meeting several national and international experts in the field will discuss this interesting technical innovation and its possible applications in basic, translational research and design of novel anti-cancer therapy. Genome editing is a technology that induces specific changes in the DNA of a cell or organism that allows studying their influence in the determination of the phenotypic characteristics. The meeting aims to transfer to the participants the know-how on the currently available methods to induce changes in the DNA of a cell system and how to use them to accurately study the function of different genes, to understand how they work and their definitive biological effects. The meeting will also illustrate several cell and animal models, and will end with a practical demonstration of advanced models for the study of different human diseases in order to design innovative therapeutic approaches. With these specific objectives, the meeting has been approved by Italian Ministry of Health for Continuous Education and Training in medical, biological, pharmaceutical and technological areas.

Maria Teresa Di Martino

THE FUTURE OF CANCER THERAPY: THE GENOME EDITING ERA

JUNE 8th 2017

10.00 Registration

10.30 Introduction: **Pierosandro Tagliaferri, Pierfrancesco Tassone**
Greetings from the Chancellor and local authorities

Pre-meeting: "Hot topics in Medical Oncology: From genomics to molecular therapies of human cancer"

Chairs: **Vito Barbieri** *(Catanzaro, Italy)* - **Marco Rossi** *(Catanzaro, Italy)*

11.00 PDL1 analysis and beyond in lung cancer immunotherapy
Umberto Malapelle (*Naples, Italy)*

11.20 New generation of kinase inhibitor in the continuum of care of thyroid and renal-cell cancer
Camillo Porta *(Pavia, Italy)*

11.40 CDK inhibitors: a new perspectives in breast cancer
Enrico Ricevuto *(L'Aquila, Italy)*

12.00 Liquid Biopsy in novel cancer treatment scenario
Marta Castiglia *(Palermo, Italy)*

12.20 Genomics of ovarian cancer in predictive oncology: the PARP inhibitors case
Ettore Capoluongo *(Rome, Italy)*

12.40 Discussion and closing remarks

13.00 Light lunch*

14.00 Opening ceremony
Greeting from AICC President **Katia Scotlandi** *(Bologna, Italy)*
Welcome and Meeting introduction **Maria Teresa Di Martino** *(Catanzaro, Italy)*

Session 1: CRISPR-Cas9-based therapeutic strategies against human diseases

Chairs: **Stefania Meschini** *(Rome, Italy)* - **Carlo Leonetti** *(Rome, Italy)*

14.30 TALEN gene edited cells for Allogeneic Adoptive Immunotherapy: from clean room to off-the-shelf
Roman Galetto *(Cellectis SA, France)*

14.50 Genome editing of T lymphocytes for cancer immunotherapy
Chiara Bonini *(Milan, Italy)*

15.10 CRISPR/Cas9 mediated knock-out of RUNX2 in melanoma cells
Maria Teresa Valenti *(Verona, Italy)*

15.30 CRISPR/Cas9 rescue of gene dosage in patient-specific models of neurodevelopmental disorders caused by large CNV
Giuseppe Testa *(Milan, Italy)*

15.50 Functional genomic in cancer research
Simona Lamba *(Turin, Italy)*

16.10 Ultra-sensitive Quantification of Genome Editing Events by Droplet Digital PCR (ddPCR) (NOT for ECM evaluation)
Letizia Ciccone *(Bio-Rad, Italy)*

16.25 Coffee Break*

16.45 CRISPR/Cas9 system on zebrafish to generate human disease model
Gianluca Deflorian *(Milan, Italy)*

17.05 The use of CRISPR/Cas9 knock-out technology in non-mammalian animal models
Thomas Vaccari *(Milan, Italy)*

17.25 CRISPR Gene Editing: Resources for High-Throughput Screening and Disease Models
Livio Loffarelli (*Thermo Fisher Scientific, Italy)*

17.45 Novel Technologies For Rapid Generation Of Custom Animal Models And Streamlined Cloning Workflows (NOT for ECM evaluation)
Matthew Wheeler, Kristofer Müssar *(Cyagen Biosciences GmbH, Europe)*

Close session and concluding remarks

JUNE 9th 2017

Session 2: Insights in Genome Editing Technologies

Chairs: **Francesca Zazzeroni** *(L'Aquila, Italy)* - **Massimo Donadelli** *(Verona, Italy)*

9.00 Construction of sgRNA-Cas9 complexes and identification of single-cell mutations to determine editing frequency
Franco Martinez *(EuroClone, Italy)*

9.20 How to improve your CRISPR genome editing experiments
Mirko Vanetti *(Integrated DNA Technologies, Italy)*

9.40 Rapid and tunable method to temporally control gene editing based on conditional Cas9 stabilization
Vincenzo Corbo *(Verona, Italy)*

10.00 CRISPR/Cas9 for the study of the interactions between viral and cellular proteins
Donato Zipeto *(Verona, Italy)*

10.20 Targeted Genome Editing using CRISPR Technology (NOT for ECM evaluation)
Silvia Di Meglio *(Merck Sigma-Aldrich srl, Italy)*

10.35 Revealing genome editing induced metabolimic swithching using Agilent technologies solutions (NOT for ECM evaluation)
Emma Nora Di Capua *(Agilent, Italy/Spain)*

Close session and concluding remarks

10.50 Coffee Break*

Session 3: CRISPR-Cas9 perspectives in genome editing

Chairs: **Marcello Maggiolini** *(Cosenza, Italy)* - **Michele Caraglia** *(Naples, Italy)*

11.10 A non-integrative strategy to streamline the derivation of isogenic iPSC lines through the Cas9/sgRNAribonucleoproteic complex
Sebastiano Trattaro *(Milan, Italy)*

11.30 miR-194-5p/BCLAF1 deregulation is a hallmark of AML tumorigenesis
Carmela Dell'Aversana *(Naples, Italy)*

11.50 Genome editing in human repopulating hematopoietic stem cells
Pietro Genovese *(Milan, Italy)*

12.10 Genome editing and generation of physiologically relevant model of leukemogenesis
Giovanni Morrone *(Catanzaro, Italy)*

12.30 High efficiency Genome Editing using EnGenCRISPR/Cas9 Ribonucleoprotein Complexes (NOT for ECM evaluation)
Karl Von Laer *(New England Biolabs, Germany)*

12.45 Efficient delivery of Cas9 and gRNA for in vitro use of CRISPR/Cas9 technology (NOT for ECM evaluation)
Jelena Vjetrovic *(Polyplus Transfection, France)*

Close session and concluding remarks

13.00 Light lunch*

Session 4: Young investigators meeting and round table on genome editing

Poster Discussant: **Giuseppe Viglietto** *(Catanzaro, Italy)*

14.10 **Selected oral presentations**
Functionally validating the significance of BCL11A-CHD8 interaction by CRISPR-Cas9 (P20)
Santolla MF *(University of Cambridge, UK)*

14.20 CRISPR/CAS9-mediated knockout of Bim antagonizes apoptosis induced by miR-17-92 inhibitor in multiple myeloma cells (P16)
Morelli E *(Catanzaro, Italy)*

14.30 Gene panel mutation screening for a better molecular stratification of colorectal cancer patients (P03)
Belardinilli F *(Rome, Italy)*

14.40 Definition of miRNA signatures as a potential therapeutic target of nodal involvement in laryngeal cancer patients (P11)
Hiromichi Kawasaki *(Naples , Italy)*

14.50 **Round table**
Award Ceremony

POSTER INDEX

P11 **Apoptosis of breast cancer cells induced by lauric acid occurs through p21Cip/WAF1 in a p53 independent manner**
Lappano R, Sebastiani A, Cirillo F, Vivacqua A, Rigiracciolo D, Santolla MF, De Marco P, Curcio R, Cappello AR, Maggiolini M.

P12 **Different mutations as potential prognostic markers in non-small cell lung cancer**
Lombardi A, Grimaldi A, Morgillo F, Della Corte CM, Ronchi A, Desiderio V, Papaccio G, Ciardiello F and Caraglia M

P13 **Reduction of mitochondrial antioxidant glrx2 in familial oncocytic tumors is associ ted with mtdna mutations-dependent indolence**
Marchio L, De Luise M, Iommarini L, Girolimetti G, Amato LB, Salfi N, Zuntini R, Repaci A, Guarnieri V, Graziano P, Pagotto U, Turchetti D, Kurelac I, Porcelli AM and Gasparre G

P14 ***KRAS* and two rare *PI3KCA* mutations coexisting in a metastatic colorectal cancer patient with aggressive and resistant disease**
Mastroiaco V; Tessitore A; Bruera G; Cannita K; Cortellini A; Cocciolone V, Dal Mas A; Calvisi G; Zazzeroni F; Ficorella C; Ricevuto E; Alesse E

P15 **CRISPR/CAS9-mediated knockout of Bim antagonizes apoptosis induced by miR-17-92 inhibitor in multiple myeloma cells**
Morelli E, Biamonte L, Federico C, Amodio N, Di Martino MT, Gallo Cantafio ME, Scionti F, Gullà A, Stamato A, Pitari MR, Caracciolo D, Rossi M, Tagliaferri P and Tassone P

P16 **CRISPR/Cas9 for the Study of the Interactions between Viruses and Host**
Parolini F, Mutascio S, Serena M, Fochi S, Romanelli MG, Zipeto D

P17 **Targeting systems vulnerabilities in uveal melanoma by CRISPR/Cas9 focal adhesion kinase (FAK) genome editing and therapeutic inhibition**
Rigiracciolo DC, Feng X, Maggiolini M, Gutkind JS

P18 **TCR Sequencing shaping cancer immunotherapy**
Ruggiero E, Magnani Z, Carnevale E, Tassara M, Vago L, Bondanza A, Naldini L , Ciceri F , Bonini C

P19 **Functionally validating the significance of BCL11A-CHD8 interaction by CRISPR-Cas9**
Santolla MF, Lazarus K, Maggiolini M, Liu P, Caroll J, Caldas C, Khaled WT

P20 **Identification of novel estrogen- regulated microRNAs in breast tumor cells and cancer-associated fibroblasts (CAFs)**
Vivacqua A, Santolla MF,De Marco P, Lappano R,Cirillo F, Sebastiani A, Rigiracciolo DC and Maggiolini M

IS01 NEW GENERATION OF KINASE INHIBITOR IN THE CONTINUUM OF CARE OF THYROID AND RENAL-CELL CANCER

Silvia Chiellino, Nicole Gri, Camillo Porta

Medical Oncology, I.R.C.C.S. San Matteo University Hospital Foundation, Pavia, Italy.

A huge amount of preclinical research has demonstrated the role of several tyrosine kinase receptors (TKRs) and their downstream signaling cascades as key drivers in the pathogenesis and progression of thyroid cancer, and expecially of both medullary (MTC) as well as differentiated thyroid cancer (DTC). This ultimately led to the development of a number of small molecules as a whole known as tyrosine kinase inhibitors (TKIs). Four of these agents are now approved by regulatory agencies for use in advanced thyroid cancer. Notably, although some of these TKIs inhibit specific TKRs associated with known genetic lesions evidenced in thyroid cancer, most are multitargeted agents, affecting a variety of TKRs.

Rather than exhibiting a direct cytotoxic activity, the effect of TKRs blockade is to inhibit angiogenesis, as well as tumor growth and survival. Indeed, almost all these agents do target, among the other RTKs, also vascular endothelial growth factor receptors (VEGFRs). Renal cell carcinoma (RCC), and particularly its clear cell histotype (ccRCC), by far the commonest, on the other hand do share pathogenetic similarities with thyroid cancer; indeed, ccRCC is characterized, in about 80% of its sporadic cases, by a genetic alteration (mutation, deletion or hypermethylation) of the Von Hippel Lindau (VHL) tumor suppressor gene. This results in the stabilization of the VHL multiprotein complex, in the accumulation of hypoxia inducible factors (HIFs), in the transcription of the so-called hypoxia-inducible genes, and ultimately in the overproduction of a series of pro-angiogenic cytokines, among which the most prominent is VEGF.

Beyond that other transduction pathways have been proven to be activated in both malignancies, including the MAP kinase pathway, as well as the PI3K/Akt/mTOR pathway.

Going back to thyroid cancer, in 2011, vandetanib, a multitargeted inhibitor of several TKRs including RET, VEGFR-2 and -3, and EGFR, became the first TKI to obtain regulatory approval for the treatment of MTC. Indeed, in the randomized controlled phase III “ZETA” trial involving 331 patients with locally advanced or metastatic MTC, vandetanib demonstrated prolonged projected median progression-free survival (PFS) of 30.5 months compared to 19.3 months with placebo (hazard ratio (HR) 0.46; 95% confidence interval (CI), 0.31–0.69; $p<0.001$); furthermore, nearly all responses in patients randomized to placebo occurred after the cross-over into the open-label treatment phase of the study.

In 2012, cabozantinib, a multitargeted TKI affecting RET kinase, VEGFR-2 and -3, MET and AXL, attained regulatory approval for MTC, following the presentation of the results of the placebo-controlled, randomized phase III “EXAM” trial involving 330 patients with unresectable, locally advanced or metastatic, progressive MTC. In this trial, cabozantinib-treated patients demonstrated median PFS of 11.2 months compared to 4.0 months in the placebo group (HR 0.28; 95% CI 0.19–0.40; $p<0.001$).

In 2013, Sorafenib, another TKI targeting RAF, VEGFR-1-3, PDGF, as well as other kinases became the first agent ever approved for the treatment of radioiodine ablation-resistant DTC (RAIR-DTC). In the “DECISION”, placebo-controlled, phase III trial conducted on 417 patients affected with RAI-DTC, sorafenib yielded a median PFS of 10.8 vs 5.8 months

with placebo, meaning a 41% decrease in the risk of progression or death (HR 0.59; 95% CI 0.45-0.76; p<0.0001).
Finally, in 2015, lenvatinib became the latest TKI to yield regulatory approval for use in RAIR-DTC. Lenvatinib is another multikinase inhibitor targeting VEGFR-1-3, FGFR, PDGFR, RET and KIT. In the phase III, placebo-controlled, randomized phase III "SELECT" trial, conducted on 392 RAIR-DTC patients – 24% of whom were pretreated with a TKI, mainly sorafenib – median PFS with lenvatinib was 18.3 months, compared to 3.6 months fro placebo (HR 0.21; 99%CI 0.14-0.31; p<0.001); moreover, at 18 months, 51.1% of patients treated with lenvatinib had no disease progression, as compared with 3.8% of those treated with placebo. Both in the sorafenib, as well as in the lenvatinib trial, the status of BRAF and RAS mutation, other common (and targetable) genetic alterations often observed in thyroid cancer, was checked, but did not affect the degree of increase of PFS in the patients treated with the two TKIs.
As far as RCC, over the years, a number of TKIs have been registered for the treatment of this once orphan disease: sunitinib, sorafenib, pazopanib, axitinib, cabozantinib and lenvatinib (the latter being used in combination with the mTOR inhibitor everolimus). Although only cabozantinib proved able to improve overall survival (OS) within a randomized controlled, phase III trial conducted against an active comparator, taken together all these agents significantly improved PFS; furthermore, the sequential use of these, as well as of other agents (i.e. bevacizumab plus interferon, everolimus, temsirolimus and nivolumab), yielded an unprecedented improvement in patients' survival, which was independant of known prognostic features.
Another important common pathway, i.e. the PI3K/Akt/mTOR one, already targeted with success in RCC, is presently under active evaluation as a therapeutic target also in thyroid cancer.
It is however clear that, differently from RCC, thyroid cancer shows many other exclusive molecular pathways which play a key pathogenetic role, such as the already mentioned BRAF and RAS ones, which are clearly and easily targetable.
In conclusion, RCC and thyroid cancer, besides being biologically and epidemiologically very different, share common pathogenetic pathways, which lead to the development of targeted agents which – at a certain extent surprisingly – proved to be active against both malignancies.
Further biological insights could contribute to elucidate other putative common pathways, even though those presently under study for therapeutic purposes seem finally to diverge from one disease to the other.

KEY REFERENCES

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IS02 CDK Inhibitors: a new perspective in breast cancer

Enrico Ricevuto
Oncology Territorial Care, Oncology Network ASL1 Abruzzo
San Salvatore Hospital L'Aquila
Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila, Italy

More than 20 years ago, it has been demonstrated that cyclin D-cyclin-dependent kinase (cdk) 4/6-p16-retinoblastoma pathway is commonly disrupted in cancer, leading to abnormal cell proliferation. Inactivation of genes (MTS1, or WAF-1, or p16) controlling cell cycle progression at the G1/S checkpoint by cyclins (cdk4,6) inhibition is one of the most important way contributing to the development and biologic progression of cancer cells. The mechanism of the inactivation of the cdk4,6 inhibitor p16, consisting in methylation of CpG islands highly represented in p16 promoter, transcriptional silencing and homozygous deletion represented a new paradigma.

Subsequent studies demonstrated that p16-methylation represents one of the early steps of transformation in different cancers, and specifically is prevalent in breast cancer cells; molecular diagnostic assays of p16 methylation, methylation density and methylated genes were developed to better evaluate the prevalence and the clinical implications of such a new model of cancer biomarker.

More recently, cdk4,6 inhibitors, such as palbociclib, ribociclib, and abemaciclib were developed, accessed the pathway of clinical trials, demonstrated efficacy in improving clinical outcome in hormone-resistant ER-positive (ERBB2-negative) Breast Cancer patients. In PALOMA-2 trial, the addition of palbociclib to letrozole prolonged progression-free survival from 14.5 to 24.8 months resulting in the longest PFS data ever reported in the first-line setting, thus obtaining therapeutic indication. P16 gene inactivation is early and prevalent in breat cancer and it probably justifyes the absence of a predictive biomarker to better select breast cancer patients for treatment with cdk4,6 inhibitors, aiming to restore cell cycle control.

Thus, this innovative perspective of genetic alterations characterizing cancer cells landed the real world of cancer treatment. Further translational and clinical researches would evaluate the efficacy of cdk4,6 inhibitors in different molecular subgroups of breast cancer patients and various tumor types, such as lung, gastric, pancreatic cancer harboring p16 inactivation, potentially by companion molecular diagnostic assays, and seek to identify optimal CDK4/6-based targeted combination regimens to advance the field of precision therapeutics in oncology.

IS03 Liquid Biopsy in novel cancer treatment scenario

Marta Castiglia *(Palermo, Italy)*

In the last decades the approach to cancer patient management has been deeply revolutionized. We are moving from “one-fits-all” strategy to “precision medicine” based on the molecular characterization of the tumor. In this new era it is becoming more and more clear that the monitoring of the disease is fundamental for a successful treatment. More precisely in the last years the scientific community has started to use the term “liquid biopsy”. A liquid biopsy is a liquid biomarker that can be easily isolated from many body fluids (blood, saliva, urine, ascites, pleural effusion, etc.) and, as well as a tissue biopsy, is representative of the tissue from which it is spread. The term liquid biopsy was originally introduced to define circulating tumor cells (CTCs). Currently it is also used for circulating tumor DNA (ctDNA) as well as for exosomes. The most important feature of ctDNA is that it harbors somatic mutations that are exclusively found in the tumor. This juxtaposition assures ctDNA exquisite biologic specificity as a biomarker.

Liquid biopsy, and specifically ctDNA, has been already shown to be a reliable tool for response monitoring in NSCLC patients treated with TKI. NSCLC is characterized by several oncogenic driver mutations that are actionable. Indeed EGFR-mutated patients can benefit from TKI drugs that competitively inhibit ATP binding to the catalytic site of EGF receptor. In this specific case liquid biopsy is currently used to monitor treatment response over time and to promptly detect the T790M mutation that causes nearly 60% of TKI resistance onset.

The presentation will provide an overview of the main innovations regarding the clinical utility of liquid biopsy, mainly ctDNA, in different tumor types (colorectal, breast and lung cancer) focusing on its role as a new predictive and surrogate biomarker for treatment monitoring.

IS04 Genomics of ovarian cancer in predictive oncology: the PARP inhibitors case

Ettore Capoluongo (*Rome, Italy)*

In the last decades, several efforts have been done in order to clarify the role of genes involved in ovarian cancer. The most studied are BRCA1 and BRCA2 (responsible for hereditary breast and ovarian cancer; HBOC), along with genes involved in genome stability. Mutational status in women with advanced ovarian cancer is demonstrating its role in both cancer development and the prognostic significance of BRCA genotype. Moreover, the eligibility to PARP-inhibitor treatments is currently based on evaluation of BRCA1/2 status.

Next-generation sequencing (NGS) has enabled new approaches for detection of mutations in the BRCA1 and BRCA2 genes. The search for germline mutations in the BRCA1 and BRCA2 genes is of importance with respect to oncogenetic and surgical (bilateral mastectomy, ovariectomy) counselling. The evaluation of BRCA1/2 status is now suggested also at tumor level in order to identify more women who could benefit from PARPi-inhibitor treatment. Molecular genetics laboratories should develop reliable and sensitive techniques for the complete analysis of both BRCA1/2 and BRCA-related genes. This is a challenge due to the size of the coding sequence of the BRCA1/2 genes, the absence of hot spot mutations, and particularly by the lower DNA quality obtained from Formalin-Fixed Paraffin-Embedded (FFPE) tissue.
New markers are now under investigation, such as copy number variation, large rearrangements, dynamics of ctDNA and mutation reversion in order to define what is the tumor pattern more responsive to PARP-inhibitor therapies.
The present talk will show the main challenges and literature evidences regarding molecular approach to ovarian cancer anti-PARP therapies, along with issues and pitfalls regarding NGS technologies and pipelines.

IS05 TALEN gene edited cells for Allogeneic Adoptive Immunotherapy: from clean room to off-the-shelf

Roman Galetto *(Cellectis SA, France)*

Autologous T-cells engineered to express chimeric antigen receptors (CARs) targeting specific tumor antigens are known to be of high potential in treating different kinds of cancer. However, a limitation of this approach is that they must be generated on a “per patient” basis, imposing not only significant logistical constraints, but also excluding its application to certain type of patients (as immune-deficient patients). To circumvent this issue, Cellectis has developed a platform for the production of “off-the-shelf” CAR T-cells via application of Transcription Activation-Like Effector Nucleases (TALEN) gene-editing technology. Nucleases targeting the TCR alpha constant (TRAC) gene are used to generate TCR-deficient T-cells with minimal potential to induce graft-versus-host disease (GvHD) in recipient patients. Additional gene inactivation events can be performed to generate allogeneic T-cells resistant to different lymphodepleting regimens, less sensitive to checkpoint inhibitory pathways, or even to bypass the constraint of targeting tumor antigens that can also be expressed in normal T-cells.

We will present data illustrating these approaches, using CAR T-cells targeting different tumor antigens, and discuss how they can be produced in a GMP compatible manufacturing process.

IS06 CRISPR/Cas9 mediated knock-out of RUNX2 in melanoma cells

Valenti MT[1], Zipeto D[2], Deiana M[1], Serena M[2], Parolini F[2], Cheri S[1], Gandini A[3], Mina M[4], Mutascio S[2], Viser A[1], Dalle Carbonare L[1].

[1]Department of Medicine, Internal Medicine, section D, University of Verona
[2]Dep. of Neurosciences, Biomedicine and Movement Sciences, - University of Verona
[3]Department of Surgery, Dentistry, Paediatrics and Gynaecology
[4]Department of Medicine, Medical Oncology Section, University of Verona.

Incidence of MM has increased considerably as a consequence of lifestyle and environmental changes. The mortality rate for MM is very high as it is highly invasive and also genetically resistant to chemotherapeutic treatments. It has been reported that mutation rate and gene modulation in melanoma are higher than in other solid malignancies. In addition, transcription factors by acting on gene expression can affect cellular processes. In particular a higher expression of RUNX2 in melanoma than in normal melanocytes has been shown.

RUNX2 is overexpressed in several tumor tissues, including pancreatic cancer , breast cancer, ovarian epithelial cancer , prostate cancer , lung cancer and osteosarcoma.

As no direct RUNX2 inhibitor is available and experiments performed with RNA interference were scarcely reproducible we applied CRISPR/Cas 9 technology to knockout the RUNT domain of RUNX2 in melanoma cell line.

CRISPR/Cas 9 tecnology was able to delete, partially, the RUNT domain. The deleted clone showed a reduced proliferation, reduced EMT features and reduced migration ability, suggesting the involvement of RUNT in different pathways of MM. In addition, the deleted clone showed a reduction of genes involved in migration ability and an increased expression of SSBP1 gene suggesting RUNT as oncotarget in MM.

IS07 Functional genomic in cancer research

Simona Lamba *(Turin, Italy)*

Cancer is a multistep, polygenic disease, caused by accumulation of genetic alterations in oncogenes and/or tumor suppressor genes resulting in neoplastic transformation. Molecular alterations in genes involved in DNA mismatch repair (MMR) promote cancer initiation and foster tumor progression. Mutations in DNA mismatch repair genes (such as MLH1, MSH2 or MSH6) result in failure to repair errors in repetitive sequences, leading to microsatellite instability (MSI) of the tumours. MSI can occur in tumours of different organs, including colorectal cancers.

MMR deficient cancers frequently show favorable prognosis and indolent progression, but the functional basis of the clinical outcome of patients with MMR deficient tumors is not clear.

Recent evidence that MSI tumors respond prominently to anti-immune checkpoint blockade (based on the anti PD-1 antibody pembrolizumab) led to the hypothesis that elevated mutation loads (high mutation burdens) are required for immunotherapy to be effective.

However, the association between mutation levels (number of mutations) and response to immuno-checkpoint blockade is not complete, as a large fraction of hyper-mutated tumors do not respond to immune-modulators.

The main objective of this project is to study MSI colorectal cancer using CRISPR/Cas9 technology to genetically inactivate genes involved in MMR such as MutL homolog 1 (MLH1) in colorectal mouse cancer cells and to test new therapeutic options provided by the development of new drugs that target the immune response to cancer cells.

IS08 "CRISPR/Cas9 system on zebrafish to generate human disease models"

Gianluca Deflorian (*Milan, Italy)*

In the last decade, zebrafish (Danio rerio) has become widely used in a large number of laboratories and facilities as small animal model system to study in vivo the human diseases. In particular, the use of this organism has convincingly demonstrated the potential of fish for improving our understanding of the molecular and cellular mechanisms leading to pathological conditions and for the development of new diagnostic and therapeutic tools. Moreover, in the recent years, CRISPR/Cas9 technology emerged as powerful, simple and robust method for targeted mutagenesis also in the zebrafish.

The alternative splicing (AS) is a molecular process that generates multiple, distinct mature mRNAs from a primary transcript (pre-mRNA), leading to the production of protein isoforms with different structural and functional properties. More than 90% of human multi-exonic genes undergo AS and this process represents a major mechanism underlying the expansion of the proteome from a limited repertoire of genes. AS deregulation is causally related to tumor progression and about 15'000 AS events are cancer-specific. Using CRISPR/Cas9, we generated in our lab mutant fish for the AS regulator Nova2. The analysis of these mutants allowed us to describe a novel role for Nova2, which was previously considered to be neural cell-specific, in the process of angiogenesis, a process that in the adult is strictly correlated with cancer progression.

IS09 The use of CRISPR/Cas9 knock-out technology in non-mammalian animal models

Thomas Vaccari *(Milan, Italy)*

Gene editing approaches based on CRISPR/Cas9 are revolutionizing model system biology. *Drosophila melanogaster* is a premier system for *in vivo* studies, thanks to the superior genetic tractability. Such advantages have been invaluable in modeling a number of human diseases. However, while flies have been used for forward countless genetic screening, mapping of mutations have been lengthy. In addition, the ability to target specific genes by reverse genetics has been limited by the inefficiency of homologous recombination. The recent application of CRISPR/Cas9 mutagenesis in *Drosophila* is rapidly overcoming such limitations. We will present at the meeting the generation of 3 *Drosophila* models of rare diseases generated by CRISPR/Cas9. In all cases, we have inactivated the an uncharacterized targeted gene, whose function is lost in patients of the corresponding human disease, and we have analyzed the phenotype in tissues and organs, or in the entire organism. We will briefly discuss our findings to illustrate how editing in *Drosophila* could be helpful to accelerate the study of new genes and of pathogenic mechanisms that might be relevant to rare diseases.

IS10 CRISPR gene editing: Resources for high-throughput screening and Disease Model

Livio Loffarelli (*Thermo Fisher Scientific, Italy)*

The transformative CRISPR-Cas9 technology is able to achieve highly flexible and specific targeting. CRISPR-Cas9 system can be modified and redirected to become a powerful tool for genome editing in broad applications such as *stem cell engineering,* gene therapy, tissue and animal disease models, and engineering disease-resistant transgenic plants. We've put together a *collection of resources* that we hope will give you the confidence to get started and to continuously improve your research. CRISPR-Cas9 provides an efficient method for specific, complete, and permanent gene knockout, making it also a potent tool for new discoveries about gene function. The power of the CRISPR-Cas9 system is also applied to screening applications for new capabilities and discoveries. CRISPR libraries are designed to provide you with a flexible system that adapts to your needs and your screening goals. Find out how CRISPR libraries can expand your screening capabilities and help you make your next big discovery.

IS11 Construction of sgRNA-Cas9 complexes and identification of single-cell mutations to determine editing frequency

Franco Martinez *(EuroClone, Italy)*

Genome editing is a technology used to make precise, targeted changes to the genome of living cells. Recentlya new tool based on a bacterial CRISPR-associated protein-9 nuclease (Cas9) from Streptocaccus pyogenes has generated considerable excitement. This follows several attempts over the years to manipulate gene function, including homologous recombination and RNA interference.

RNAi, in particular, became a laboratory sta pie enabling inexpensive and high-throughput interrogation of gene function, but is hampered by unpredictable off-target effects. The simplicity ofthe CRISPR nuclease system, with only three components (Cas9, crRNA and trRNA) makes this system amenable to adaptation for genome editing. By combining the crRNA and trRNA into a single synthetic guide RNA (sgRNA), a further sim plified two component system can be used to introduce a targeted double stranded break. This break activates repair through error prone Non-Homologous End Joining (NHEJ) or Homology Directed Repair (H DR). In the presence of a donor template with homology to the targeted locus, the HDR pathway operates allowing for precise mutations to be made. In the absence of a template, NHEJ is activated resulting in insertions and/or deletions which disrupt the target locus.

For a successful experiment you need Cas9 protein, obtained as recombinant form or transcribed by vectors, and sgRNAs. A new innovative method combines an S. pyagenes Cas9-specific Scaffold partially complementary to target-specific oligos designed against the sequence/gene of interest. The two oligos anneal at the overlapping region and are filled in by the DNA polymerase, creating a double-stranded DNA (dsDNA) template for transcription. Synthesis ofthe dsDNA template and transcription of RNA occur in a single reaction, resulting in the generation of a functional sgRNA. The sgRNA-Cas9 complexes can be quite easily delivered to cells by transfection or nucleofection and ce lls are screened for CRISPR induced mutations.

A widely used method to identify mutations is the T7 Endonuclease I mutation detection assay. This assay detects heteroduplex DNA that results from the annealing of a DNA strand, including desired mutations. with a wild-type DNA strand. Here is presented a method for manual and high-throughput screening of samples to detect CRISPR induced mutations.

IS12 Rapid and tunable method to temporally control gene editing based on conditional Cas9 stabilization

Vincenzo Corbo *(Verona, Italy)*

Genome-sequencing studies have often failed to identify a clear causative relation between genetic alterations and cancer. The “functionalization” of the genome is therefore needed to evaluate the impact of genetic variants on tumor phenotypes, which is essential to create novel opportunities for therapeutic intervention. When evaluating gene’s function, the identification of complex spatial and temporal interactions with co-occurring alterations is vital for the successful use of the proposed biomarker into a clinical setting. An unprecedented opportunity to address these questions comes from advances both in genome-editing technologies and in approaches to the development of patients-specific models of the disease. The advent of the genome-editing technology of Crispr/Cas9 has indeed made possible to easily and rapidly manipulate genes, while novel three-dimensional organoid cultures has shown potentials to be better models than traditional in vitro systems to test gene functions and compounds efficacy. However, an ideal system to study gene function should also include the possibility of controlling gene activation/inactivation in timely fashion as well as reducing the risk of off-target effects. Constitutive Cas9 expression has been demonstrated to increase the likelihood of having off-target effects and to trigger DNA damage response. Here, we devise a method to temporally control Cas9 nuclease expression by fusing the protein to a destabilizing domain (DD) derived from a mutant FKBP12. The presence of the DD induces the rapid degradation of the fused protein by the proteasome system, which can be rapidly prevented by the addition of a cell-permeable synthetic ligand (termed Shield-1). We further demonstrate that the “shield” effect is reversible following few hours of ligand withdrawal. The system can be easily adapted to co-express from the same promoter any other gene of interest, including fluorescent reporters to create a traceable vector. By using organoid cultures of pancreatic cancer, we also demonstrate that Crispr/Cas9 can be used to target specific genes in more complex in vitro system. Finally, the system can be adapted to incorporate an inducible Cre recombinase to allow parallel genetic manipulation, which enables interrogation of gene functional interactions.

IS13 CRISPR/Cas9 for the study of the interactions between viral and cellular proteins

Donato Zipeto *(Verona, Italy)*

The CRISPR/Cas9 system has many applications in virology: it has been used to achieve viral DNA inactivation from latently infected cells, allowing viral eradication, or to inactivate specific proteins involved in virus-host cell interaction. Herein we applied the CRISPR/Cas9 technique to generate knock-out cell lines useful for the study of cellular determinants critical for HIV-1 infection. As a preliminary screening, the editing efficiency was evaluated by T7 endonuclease I assay, and then confirmed by western blot and flow cytometry analyses. We targeted β2microglobulin (β2m), human thioesterase 8 (ACOT8) and histone deacetylase 6 (HDAC6) genes. β2microglobulin is required for the membrane translocation of HLA molecules where HLA-C interacts with HIV-1 Env and modulates viral infectivity (Zipeto & Beretta, Retrovirology 2012). We edited β2m in 293T, HeLa-Lai (expressing HIV-1 Env), TZM-bl (CD4 and CCR5 expressing HeLa, highly sensitive to HIV-1 infection) and parental HeLa cells. We showed in 293T cells that HIV-1 proteins transfection did not translocate HLA-C at the cell surface in absence of β2m. We obtained similar result in β2m negative HeLa-Lai cells, showing that HIV-1 Env interacts with HLA-C at the plasma membrane after its surface translocation. Besides, we demonstrated that HIV-1 pseudoviruses produced in 2m negative 293T cells were significantly less infectious than those produced in parental ones (Serena et al., Scientific Reports, 2017). ACOT8 thioesterase interacts with HIV-1 Nef protein preventing its degradation (Serena et al, Scientific Reports 2016). To better understand the role of ACOT8 in HIV-1 infectivity, we developed ACOT8 knock out 293T and TZM-bl cell lines. We observed in TZM-bl cells, susceptible to HIV-1 infection, that ACOT8 absence did not affect the infectivity. The role of ACOT8 in pseudoviruses production is being tested using 293T edited cells. HDAC6 is an important regulator of membrane dynamics involved in HIV-1 infection (Valenzuela-Fernandez et al, Molecular biology of the cell, 2005). We inactivated the HDAC6 gene in 293T cells. These cells will be used to test the HIV-1 infectivity and syncytia formation. In conclusion, the CRISPR/Cas9 system represents a new, powerful tool in basic and applied research in virology.

IS14 A non-integrative strategy to streamline the derivation of isogenic iPSC lines through the Cas9/sgRNA ribonucleoproteic complex

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7dup autism spectrum disorder (7dupASD) and Weaver syndrome are two multisystemic diseases characterized by intellectual disability caused by the duplication of the region 7q11.23 and PRC2 complex components mutations, respectively. The neurocognitive phenotype of 7dupASD patients is strongly linked to the dosage of the general transcription factor-2I (GTF2I) and about 20% of the dysregulation observed in human pluripotent stem cell lines (hiPSC) derived from patients with 7dupASD and William Syndrome, the symmetrical opposite disease caused by the deletion of the same genomic region, was attributed to this gene (Adamo et al., 2015). In the same study, GTF2I was found to regulate several targets by associating with the lysine (K)-specific demethylase 1A (LSD1), that is known to regulate the decommissioning of bivalent domain genes' enhancers bound by the PRC2 complex in mouse embryonic stem cells. Interestingly, GTF2I is mutated in a subset of thymic epithelial tumors and characterization of this mutation reveals an increase in the TFII-I protein levels, partially mimicking the molecular phenotype observed in 7dupASD patients. This T>A substitution leads to the disruption of a non-canonical destruction box, seemingly without causing conformational changes. We used two different genome editing strategies based on CRISPR/Cas9 in order to generate 1) a hiPSC line containing the T>A substitution in heterozygosis together with a tag at the C-terminus of the gene to model the dosage imbalance of GTF2I in the context of 7dupASD and 2) isogenic lines where the mutations in the PRC2 complex are restored in our cohort of Weaver patients. Regarding the second point, we developed a non-integrative strategy to enrich the population of clones in recombinant cells using a reporter system and the Cas9/gRNA ribonucleoproteic complex. This approach facilitates the screening of clones upon genome editing increasing efficiency and without leaving any unwanted genomic DNA modification.

IS15 miR-194-5p/BCLAF1 deregulation is a hallmark of AML tumorigenesis

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Deregulation of epigenetic mechanisms, including miRNA, contributes to leukaemogenesis and drug resistance by interfering with cancer-specific molecular pathways. Here, we show that the balance between miR-194-5p and its newly discovered target BCL2-associated transcription factor 1 (BCLAF1) regulates differentiation and survival of normal haematopoietic progenitors. In acute myeloid leukaemias (AMLs) this balance is perturbed, locking cells into an immature, potentially 'immortal' state. Enhanced expression of miR-194-5p by treatment with the HDAC inhibitor SAHA or by exogenous miR-194-5p expression re-sensitizes cells to differentiation and apoptosis by inducing BCLAF1 to shuttle between nucleus and cytosol. miR-194-5p/BCLAF1 balance was found commonly deregulated in 60 primary AML patients and was largely restored by ex vivo SAHA treatment. Our findings link treatment responsiveness to re-instatement of miR-194-5p/BCLAF1 balance.

IS16 Genome editing in human repopulating hematopoietic stem cells

Pietro Genovese *(Milan, Italy)*

Targeted genome editing by artificial nucleases has brought the goal of site-specific transgene integration and gene correction within the reach of gene therapy. However, its application to long-term repopulating Hematopoietic Stem/Progenitor Cells (HSPCs) has remained for long time only elusive. By tailoring delivery platforms and culture conditions we overcame the barriers that specifically constrain homology directed DNA repair in the primitive HSPC subset and provide stringent evidence of targeted integration in human HSCs by long-term multilineage repopulation of transplanted mice. We demonstrate the therapeutic potential of our strategy by targeting a corrective cDNA into the IL2RG gene of HSCs from healthy donors and a subject with X-linked Severe Combined Immunodeficiency (SCID-X1). To support the rationale and explore the safety of gene correction we took advantage of a suitable preclinical mouse model of the disease that allows defining i) the threshold level of gene editing necessary to provide therapeutic benefit, ii) the proper choice of conditioning regimen and iii) validate the functionality of the edited cells in the disease host. These results established safety and robustness of HSPC gene editing for SCID-X1 and open new avenues for the treatment of other hematologic disorders.

IS17 Genome editing and generation of physiologically relevant model of leukemogenesis

Giovanni Morrone *(Catanzaro, Italy)*

Over the past two decades, the accumulation of a wealth of detailed information on leukemia-associated genetic aberrations and the development of efficient methods for gene transfer into hematopoietic stem and progenitor cells (HPSCs) have enabled the generation of *in vitro* and *in vivo* experimental models that recapitulate key features of acute myelogenous leukemias (AML) and may greatly help the design of novel and more effective therapeutic strategies. Using retro- or lentiviral-mediated transduction, we and others have demonstrated that enforced expression of candidate AML-associated oncogenes in primary mouse or human HPSCs confers on these cells a significant proliferative advantage accompanied by altered differentiation. Frank leukemic transformation is not usually observed, suggesting that multiple hits are required for AML development (Kelly and Giliiland, *Annu Rev Genomics Hum Genet*. 2002). One notable exception is represented by fusions of the *mixed lineage leukemia (MLL)* gene, in particular *MLL-AF9*. Enforced overexpression of MLL-AF9 protein in early myeloid progenitors transforms these cells with high efficiency, resulting in the development of ALLs or AMLs when they are inoculated in syngeneic or immunocompromised hosts. However, owing to the integration of multiple viral genomes into the target cells' DNA and to the fact that transgene expression is driven by potent ubiquitous promoters rather than the endogenous regulatory regions, viral-mediated transduction results in strong transgene overexpression that is likely to produce a phenotype that does not faithfully mimic the conditions underlying leukemia development. Indeed, attempts to produce a genomic scenario more reminiscent of the *"physiological"* AML development by generating MLL-AF9 knock-in mice showed that the nature of the AML cells-of-origin, as well as the frequency of leukemia-initiating cells in this model significantly diverged from those observed in a MLL-AF9 transduction-mediated model. A similar attempt was recently carried out in human HSPCs by exploiting state-of-the-art genome editing technology to generate two distinct endogenous MLL oncogenes in cord blood-derived $CD34^+$ cells. This produced a phenotype that more accurately recapitulated the morphological and biological features of MLL cells. It is expected that the application of such approaches, combined to the use of experimental animals bearing a humanised hematopoietic microenvironment, will offer in the near future unique opportunities to establish highly physiologically-relevant models to finely dissect the molecular mechanisms leading to the development of AMLs, and to discover and validate novel molecular targets for innovative therapeutics.

P01 Th17 cells targeting by miR-21 inhibitor impairs Multiple Myeloma related bone disease

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Multiple myeloma (MM) is a malignant B-cell disorder characterized by plasma cell infiltration in the bone marrow (BM), and monoclonal immunoglobulins in the serum and/or urine [1]. MM patients suffer from destructive bone lesions, leading to pain, osteopenia, osteolytic lesions and fractures, a condition known as MM related bone disease, MMBD [2]. The interactions among MM cells, osteoclasts (OCLs), osteoblasts and other bone effector cells of the BM microenvironment (BMM) support MMBD [3]. Among the multiple factors underlying OCL differentiation and function, the RANKL-RANK signal pathway plays a central role. BMM derived RANKL drives OCL generation from BM monocytes and promotes OCL mediated bone resorption. Interestingly, T helper 17 lymphocytes (Th17) secrete pro OCL cytokines such as RANKL, TNF, and IL-17, contributing to MMBD development. Univariate analyses in MMBD patients showed that the extent of BD is tightly correlated to Th17 development and maintenance [4]. Recent findings showed that microRNAs (miRNAs) are involved in MMBD [5 - 6]. MiRNAs are short non-coding RNAs that regulate gene expression at a post transcriptional level. Interestingly, miR-21 promotes Th17 differentiation by targeting SMAD-7, a negative regulator of TGF-β signaling, promotes the activation of SMAD-2/3, suppresses IL-2 expression, and ultimately enhances the activity of the TGF-β signaling pathway [7]. Taken together, these data support a therapeutic strategy of miR-21 targeting to ameliorate MMBD through Th17 inhibition. On these bases, we differentiated freshly isolated naïve $CD4^+$ T cells from peripheral blood mononuclear cells into Th17 by polarizing cytokines (IL-1β, IL-23, IL-6 and TGF-β). To prevent Th1/Th2 differentiation, anti-IFNγ and anti-IL4 antibodies were added to the culture. Cultures were replated every 5 days with cytokine/antibodies. Based on IL-17 expression, as a marker of fully mature Th17, cultures were stopped on days 18-22. As a model of MMBD *in vitro*, OCL resorptive activity was evaluated on dentine discs with/without Th17. To counteract miR-21 activity, we electroporated naïve $CD4^+$ T cells with miR-21 inhibitor (miR21i) before starting Th17 differentiation culture. MiR-21 expression levels were assessed by real time pcr before and at the end of differentiation culture to ascertain its effective inhibition. In the second set of experiments, OCLs were plated with dentine discs and exposed to either Th17 alone, Th17 carrying miR-21i, or RANKL/MCSF cytokine alone as positive control. OCL resorptive activity was represented by the number of pits generated by OCLs on dentine discs. Dentine pits were significantly lower in the presence of Th17 carrying miR-21i as compared to Th17 alone and positive control. Overall, these data indicated that miR-21 antagonism negatively regulates Th17 mediated OCL activity. To further define the underlying

mechanisms of Th17 impairment, we performed western blot analyses of several proteins involved in Th17 generation and maintenance. Retinoid-related orphan receptor-γ (RORC) encodes a transcription factor that drives Th17 differentiation and activity. Intriguingly, Th17 carrying miR-21i showed lower RORC levels as compared to control. Besides, Protein Inhibitor Of Activated STAT Protein 3 (PIAS3), a validated target of miR-21 and involved as a negative regulator of IL6/STAT3 dependent Th17 generation [8] was significantly upregulated in the presence of mir-21 antagonism. Therefore, miR-21i could affect Th17 differentiation at the level of master transcriptional regulators. Taken together, these preliminary findings strongly support the development of miR-21 antagonism based therapies in the treatment of Th17 mediated MMBD.

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P02 Identification of polymorphic variants in ADME genes related to the risk of developing Multiple Myeloma

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OBJECTIVE: Multiple myeloma (MM) is a malignant plasma cell disorder characterized by complex heterogeneous cytogenetic abnormalities and marked genetic instability. Inherited genetic variants play a role in MM susceptibility. Carcinogenic metabolism is mediated by the activity of multiple absorption, distribution, metabolism and excretion (ADME) polymorphic gene products which may contribute to cancer risk. The aim of our study was to investigate the relationship between MM predisposition and xenobiotic metabolism by the drug-metabolizing enzyme and transporter (DMET) microarray Affymetrix platform. We genotyped1936 genetic variants across 231 ADME genes in 65 MM patients matched with a dataset of 59 Caucasian HapMap-related healthy controls.

METHODS: One µg of genomic DNA, extracted from peripheral blood cells, was genotyped by Affymetrix DMET Plus microarray. SNP markers with a call rate minor than 98% were excluded from the subsequent analysis. The genotyping profiles were generated by DMET Console software®, based on the BRLMM (Bayesan Robust Linear Model with Mahalanobis distance classifier) algorithm. Genotype frequencies were analyzed by DMET-Analyzer software and the association analysis was performed by two-tailed Fisher exact test. Results of potential interest were limited to those in which the p-value was ≤0.05.

RESULTS: Among 21 selected genes potentially associated ($p<0.05$) to susceptibility to MM we focused on genetic variants in GSTP1, CYP2B6, CYP2D6, NR1I2, GSTA4 genes, already proposed in independently published studies as risk factor for MM. The polymorphic variants in Cytochrome P4502B6 (*CYP2B6*, rs8192709 and rs2279341),and D6 (CYP2D6, rs72549356), nuclear receptor subfamily 1 group I member 2 (*NR1I2*, rs2276707) and glutathione S-transferaseA4 (*GSTA4,* rs7496)genes were related to MM susceptibility in our training set.

CONCLUSIONS: Our exploratory study identified 6 polymorphic variants in GSTP1, CYP2B6, CYP2D6, NR1I2, GSTA4 genes involved in ADME genes metabolism of xenobiotic and carcinogenic agents as predictive for MM susceptibility. The findings from our pharmacogenomic microarray study need validation in independent series of patients. DMET platform confirms as a robust tool for genetic biomarkers discovery.

P03 Gene panel mutation screening for a better molecular stratification of colorectal cancer patients

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Colorectal carcinoma (CRC) is one of the most commonly diagnosed cancers worldwide. The metastatic disease contributes to the high mortality rate reported for such tumors. Significant benefit on overall survival was brought about the introduction of monoclonal antibodies (anti-EGFR and anti-VEGF) used in combination with chemotherapy. While anti-VEGF treatment does not require biomarker-based selection criteria, the potential efficacy of anti-EGFR antibodies is neglected to patients with activating mutations in KRAS and NRAS (RAS) genes, whose molecular analysis became a clinical routine.

The advent of third-generation sequencing instruments, able to reach quick testing of multiple clinically-relevant hotspots, yet maintaining precision and low costs, allows the simultaneous determination of the mutation status of an expanding number of genes. Despite only few of these molecular biomarkers have gained clinical utility in the routine oncological practice, the acquisition of more complex cancer mutational patterns might provide more efficient tumor characterization for prognostic and predictive purposes and highlight actionable targets.

Here we report the Next Generation Sequencing of defined hotspots and targeted regions of 22 genes (including RAS) of 781 CRCs reaching the lab for the routine determination of RAS mutation status. We identified recurrent mutations (≥1%) in 12/22 genes, being KRAS, TP53 and PIK3CA the most frequently mutated ones. Statistical analysis, indicated that the mutation associations follow a non-random distribution. Categorization of the cases on the base of KRAS and p53 mutation status led us the definition of four Mutation Association Patterns (MAP1-4) characterized by specific mutation associations. Analysis of the clinicopathological data available for 89 out of 781 cases indicate interesting trends for the associations of MAP1-4 with specific parameters, some of which reached statistical significance.

Thus, application of gene panel NGS as a routine for the characterization of RAS/BRAF status required for predictive purposes in CRC patients, may provide additional prognostic/predictive information, with no significant extra-costs.

P04 Physiological role of membrane citrate transporters in prostate tumor

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The normal human prostate is a highly specialized mammalian organ that accumulates and secretes extraordinarily high citrate levels into prostatic fluid that acts mainly as an energy substrate for sperm. In 1959, Cooper and Imfeld [1] first reported that citrate is also important pathophysiologically, as in malignant prostate tissue the citrate levels were significantly lower than the benign prostate levels. Normally citrate is synthetized in mitochondria where it is utilized via its entry into the Krebs cycle or alternatively, the mitochondrial citrate is transported into cytoplasm by the mitochondrial citrate carrier (CiC), where it is metabolized to acetylCoA for lipid biosynthesis [2]. Recently, two isoforms of human CiC, carried out by alternative splicing of human SLC25A1 gene, have been identified in human prostate epithelial cells (3). Ýespite the importance the the citrate transport across the mitochondrial and plasma membrane plays in the progression of prostate cancer until now the two proteins are not been biochemical characterised and the citrate release mechanism is not known.Wn order to achieve this goal the two proteins encoded by SLC25A1 and designated as mCIC and pCIC respectively, have been overexpressed in Escherichia coli and reconstituted into liposomes. The transport properties demonstrate that mCIC catalysed the transport of citrate, cis-aconitate, L-isocitrate, phosphenolpyruvate and L-malate. The tissue distribution of mRNAs for mCIC and pmCIC showed that mCIC gene was expressed ubiquitously whereas pmCIC was detected in very low amount only in liver. Furthermore prostate, breast and hepatic carcinoma cell lines were tested for the presence of the two isoforms of human CiC. An increased expression level of mCIC mRNA, to respect the non tumorigenic MCF10A cells, was detected in all cell lines tested with the exception of PC-3 cell line. Immunohistochemical analisys revealed a high expression level in normal human prostate to respect the neoplastic samples.

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P05 GPER is involved in the regulation of CYP1B1 expression in breast cancer cells and CAFs

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17 β-estradiol (E2) may stimulate the development and progression of hormone-dependent cancers through multiple mechanisms including the oxidative estrogen metabolism (Gajjar K et al. 2012). For instance, cytochrome P450 1B1 (CYP1B1) is a major E2 hydroxylase involved in estrogen biosynthesis and metabolism, generation of DNA damaging procarcinogens and, at least in part, in the failure of anti-estrogen therapies (Blackburn HL et al. 2015). Higher CYP1B1 expression has been reported in tumors respect to normal tissues, especially in breast, endometrial and ovary cancers (Kwon Y et al. 2016). CYP1B1 catalyzes the 4-hydroxylation of E2 that subsequently generates free radicals from the reductive-oxidative cycling with the corresponding semiquinone and quinone forms, leading to DNA damage and altered expression of genes involved in cell cycle regulation (Go R et al. 2015; Sissung TM et al. 2006). In this context, it has been demonstrated that CYP1B1 may be regulated by E2 through the classical estrogen receptor (ER) (Tsuchiya Y et al. 2005), therefore we aimed to ascertain whether the G protein estrogen receptor (GPER) (Lappano R et al. 2014; Maggiolini M. and Picard D. 2010) may be also involved in E2-stimulated expression of CYP1B1 in breast cancer cells and cancer-associated fibroblasts (CAFs) obtained from breast cancer patients. Our results show that estrogenic GPER signalling triggers CYP1B1 expression through the EGFR/ERK/c-fos transduction pathway in the aforementioned model system, as determined using specific pharmacological inhibitors, gene-silencing experiments and ChIP assays. As a biological counterpart, E2 induced proliferative effects in breast cancer cells and CAFs in a GPER and CYP1B1-dependent fashion. Altogether, our data suggest that GPER may be included among the transduction mediators involved by E2 in the regulation of CYP1B1 toward the development of breast cancer.

P06 Angiocrine engagement of GPER by IGF1/IGF1R signaling triggers the activation of HIF-1α/VEGF transduction pathway and breast tumor angiogenesis

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The G-protein estrogen receptor GPER acts as a receptor for estrogens in diver physio-pathological conditions, including breast cancer (1). In this regard, GPER has been shown to unlock access to estrogens in those tumor cells devoid of the classic Estrogen Receptor (ER), thus allowing the stimulatory actions of these mitogens (1). Previously, we demonstrated that GPER signaling facilitates the reciprocal communications between cancer cells and the surrounding microenvironment, particularly in hypoxic conditions, by stimulating the angiogenic switch through the HIF-1 α/VEGF transduction pathway (2). In addition, the cross-signaling between GPER and growth factor receptors like IGF1R integrates complex biological responses in ER positive breast cancer, like cell proliferation and migration (3). Here, we sought to evaluate the role of GPER in promoting breast tumor angiogenesis in response to IGF1, using ER negative experimental models. We found that in SkBr3 and Cancer Associated Fibroblasts (CAFs), IGF1/IGF1R signaling activates the HIF-1α/VEGF transduction pathway, as evaluated by RT-PCR, western blotting, immunofluorescence and reporter assays. A time-dependent increase of HIF-1 α, GPER and VEGF mRNA and protein expression was detected treating both SkBr3 and CAFs with 100 ng/mL IGF1. Reporter assays performed in SkBr3 cells showed that IGF1 triggers a nearly 2-fold increase in the luciferase activities of a HIF-1α, GPER and VEGF reporter constructs. Biologically, the increase in endothelial tube formation induced by IGF1 is prevented silencing the expression of HIF-1α and GPER. Of note, data showing gene expression from 17 published Affymetrix microarray datasets of 2999 breast cancer patients indicate that GPER is significantly co-expressed with IGF1R, as well as with the micro-vessel density marker CD34. A similar co-expression pattern is observed analyzing datasets from Metabric studies on 1904 breast tumor samples, thus strengthening that GPER is included in an angiocrine network leaded by IGF1/IGF1R signaling in breast cancer. Our data indicate that the functional interaction between GPER and IGF1/IGF1R transduction pathway may be regarded as a novel target to inhibit angiogenesis in breast tumors.

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P07 Estrogenic GPER signaling triggers RERG expression in breast cancer cells and cancer-associated fibroblasts

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Breast cancer is the most frequent cancer in women and the most common cause of cancer-related death among females. It has been extensively reported that an over-expression and/or activation of diverse members of the Ras superfamily are often associated with an increased aggressiveness of breast tumors. In this regard, it has been demonstrated that Ras proteins activated by various stimulatory factors including hormones and growth factors, trigger multiple transduction pathways as PI3K/AKT, MAPKs and STAT3, then leading to cell proliferation, migration and invasion.
Previous studies performed by gene microarray analysis of primary breast tumors and breast cancer cells identified the RAS member named RERG as a 17β estradiol (E2) responsive gene, which may be regulated through the classical estrogen receptor (ER) toward the stimulation of tumor cell growth.
Recently, the G protein estrogen receptor, namely GPER, has been involved in estrogenic signaling in both normal and malignant cells, including breast cancer. In order to provide novel evidence on the molecular mechanisms involved in the expression and function of RERG as well as on its biological action, we performed gene expression studies, immunoblotting, ChIP analysis, gene silencing experiments and 3D culture assays in SkBr3 and MCF7 breast cancer cells and in cancer-associated fibroblasts (CAFs) derived from breast tumor patients. We found that E2 and the selective GPER ligand G-1 stimulate through the GPER/EGFR/MAPK signaling the mRNA and protein levels of RERG in breast cancer cells used and in CAFs. Of note, 3D culture assays revealed that GPER and RERG are required for the growth response of SkBr3 and MCF7 breast cancer cells upon exposure to E2 and G1.
Overall, our data provide novel insights on the molecular mechanisms involved in the regulation of RERG by estrogens regardless of ER. In addition, our findings shed new light on estrogenic GPER signaling toward the RERG-mediated growth of breast cancer cells.

P08 The importance of NGS platform for the evaluation of mutational status of genes in CLL patients

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Chronic lymphocytic leukemia (CLL), a highly genetically heterogeneous disease, is characterized by clonal proliferation and accumulation of neoplastic B lymphocytes in blood, bone marrow, lymph nodes, and spleen. The clinical course of CLL ranges from a very indolent condition, with a nearly normal life expectancy, to rapidly progressive disease leading to early death. Genetic alterations are commonly observed in CLL patients, and some f these are associated with aggressive disease progression and poor prognosis. Notably, Tumor-suppressor p53 gene (TP53) maps to chromosome 17 and is pivotal for genome integrity. Aberrant p53 function, due to 17p deletion and/or TP53 mutation, is associated with poor prognosis in CLL patients. Over 80 % of cases harboring deletion also carry TP53 mutations in the remaining allele. The frequency of mutations lacking del(17p) varies among different studies, but in general it accounts for about 30% of all TP53 defects, while 17p deletions, without the TP53 mutation, are less frequent, representing about 10 % of all TP53 alterations. Combination chemo-immunotherapy with fludarabine, cyclophosphamide and rituximab (FCR) is the most effective regimen for young and fit patients, as it yields excellent long-term results. As CLL often affects elderly individuals, more tolerable therapeutic approaches have been successfully applied, such as chemoimmunotherapy combining chlorambucil with an anti-CD20 antibody (rituximab, obinutuzumab, ofatumumab), and bendamustine-based regimens. Most recently, therapies targeting the B-cell-receptor (BCR) signalling pathway, such as ibrutinib and idelalisib, have shown high-response rates and tolerability. Next Generation Sequencing (NGS) is a powerful technology, allowing the detection of many low-rate mutations in every type of disease and sample including CLL. We performed an extensive biologic characterization of newly diagnosed CLL. The genomic DNA from peripheral blood samples of 27 CLL patients was analyzed using Ion Torrent Personal Genome Machine, a NGS platform that uses semiconductor sequencing technology. Most of the sequenced patients, with a panel containing the major genes mutated in LLC, have genetic alterations. Almost all patients express SNP mutations of the TP53 gene. Particularly, a total of 6 different missense SNP mutations (Pro72Arg; Pro72Ala; Tyr163Cys; Gly244Asp; Arg 248Trp; Arg337Cys), 1 nonsense SNP mutation (Arg306Ter) and 1 frameshift insertion (Arg209fs) of TP53 gene were found. Patients with TP53 mutated and positive to FISH showed a more fast course of disease and resistance to conventional chemotherapy. Sequenced patients analysis explained some cases of particular importance. Here we report two cases of patients. Patient n. 6, after confirming the diagnosis of CLL showing the classic symptoms of the disease, was initially treated with fludarabine. This treatment was ineffective and replaced with bendamustine-rituximab therapy. This patient has 3 different mutations of TP53 gene that inactivate p53 protein; therefore, he is eligible to continue the treatment with the first BTK (Bruton's tyrosine kinase) inhibitor named ibrutinib.

Patient n.7 was negative to FISH and was IGHV mutated. After NGS we found only one SNP mutation (Arg248Trp) of the TP53 gene. This patient showed a very slow course of the disease, so the strategy of observing and monitoring the disease is currently used without drug administration. In our series of patients we have also found other gene mutations including missense mutation (Phe386Leu) of FGFR gene in 3 patients that occurred always in combination with other mutations: in patient n. 5 it was associated with the SNP mutation (Met441Ile) of the DDR2 gene; in patient n. 17 with the SNP mutation (Gly469Ala) of the BRAF gene and in patient n.21 with SNP mutation (Gly244Asp) of gene TP53. Presently, we are studying the structure and function of mutated FGFR and DDR2.

P09 A CRISPR/Cas9 based approach to study the implication of HTLV regulatory proteins in the NF-κB modulation

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Human T-cell leukemia virus type 1 (HTLV-1) infects approximately 20 million people worldwide and 5% of them may develop adult T-cell leukemia (ATL), a fatal T-cell malignancy with no effective treatment currently available. The homologous HTLV-2 does not cause ATL, but is associated with milder neurologic disorders. Both viruses encode a potent viral oncoprotein, termed Tax, which deregulates several cellular pathways, including NF-κB. In addition to Tax, the HTLV-1 proviral genome encodes from the antisense strand, a basic leucin zipper factor, HBZ, which plays an essential role in the oncogenic process leading to ATL. Comparative studies of the functional activity of Tax-1 and HBZ, and the HTLV-2 homologous, Tax-2 and APH-2 (HTLV-2 antisense protein), may provide clues to explain the dissimilar pathobiology of HTLVs. Herein, we compared the effect of the viral regulatory proteins HBZ and APH-2 on Tax-modulated NF-κB cell signaling. Our data demonstrated that APH-2 suppressed, more efficiently than HBZ, the Tax-dependent NF-κB activation. By confocal microscopy, we observed that, differently from HBZ, the APH-2 protein is recruited into cytoplasmic structures where co-localized with Tax. The co-expression of APH-2 and Tax impaired the degradation of the NF-κB inhibitor IκB-α, restraining the transcriptional factor p65 into the cytoplasm. APH-2, but not HBZ, was present in complex containing the TRAF3 protein, an upstream inhibitor of the alternative NF-κB pathway. Applying the CRISPR/Cas9 technique, we generated TRAF3 knock-out cell lines. Several TRAF3-/- clones were selected and NF-κB promoter activity was analyzed by luciferase assays. The results showed that, in absence of induction, the NF-κB promoter is slightly activated, in the TRAF3-/- cell line compared to the parental cell line. The absence of TRAF3 adaptor factor did not inhibit the Tax-mediated NF-κB activation. Ongoing studies using TRAF3-/- clones will allow to clarify the effect of the HTLV antisense protein on the alternative NF-κB pathway activation.

P10 Definition of miRNA signatures as a potential therapeutic target of nodal involvement in laryngeal cancer patients

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Introduction

Laryngeal cancer (LCa) is one of the most common types of head and neck malignancies. Conventional therapeutic options, including surgery, radiation, and combination therapies, are treatable with an early stage of LCa. However, the treatments for advanced ones still need further improvement despite improved therapeutic approaches in the past decades. Metastases are often responsible for cancer patient's outcome. LCa may initially spread to regional cervical lymph nodes by metastasis, resulting in poor prognosis. Thus, metastatic nodal involvement represents a key prognostic factor of malignancies at the larynx. However, there is still a lack of information on effective treatment strategies for LCa patients with lymph nodes invasion. Therefore we need to set up promising new methods for more reliable clinical management of LCa to reduce disease mortality. MicroRNAs (miRNAs) are a category of short, highly conserved non-protein coding RNAs that negatively and post-transcriptionally regulate multiple messenger RNAs (mRNAs). MiRNAs may act as either oncogenes or tumor suppressor genes, depending on their target mRNAs and contexts involved. Numerous studies have demonstrated that specific miRNA expression patterns are aberrantly altered in different types of diseases, including LCa. Owing to these findings, miRNA deregulated signatures are emerging as attractive therapeutic targets. Many earlier studies have revealed that several miRNAs deregulated are strongly associated with pathological events of LCa; however, abnormally expressed miRNAs in LCa with metastases remain to be largely unknown. Hence, in this present study, we investigated miRNA deregulated signatures in LCa patients suffering from lymph node metastasis to discover candidate miRNAs which are available as a new potential therapeutic target of LCa with metastatic events.

Methods

A comprehensive miRNA expression profiling was performed with a microarray assay, which allows simultaneous analysis of 377 miRNAs sequences. Expressions of candidate miRNAs were then validated using quantitative RT-PCR analysis.

Results

Both tissue and serum specimens were collected from LCa patients either with lymph node metastasis (N+) or no metastasis (N-). Non-pathological serum samples were taken from healthy individuals as a control. We first conducted the microarray screening assay to

explore candidate miRNAs deregulated in the tissues. Our screening analysis showed that miR-449 and miR-652 were the most deregulated in N+ when comparing to N-, respectively. Besides the miR-133b and miR-223 were relatively highly altered.
For further validation study, these miRNAs were chosen as tissue candidate miRNAs. We subsequently performed the validation of miRNA expression level. Of the 4 miRNAs, miR-449 was significantly deregulated in N+ if compared to N-. Although the microarray assay revealed remarkably different expression levels, miR-652 was not definitely deregulated. Additionally, miR-133b and miR-223 expressions were, respectively, not much altered, whereas both miRNAs were dramatically differentially expressed in the tumor against normal laryngeal tissues. We also performed a serum miRNA profiling. The analysis showed that miR-224 and miR-423-5p were significantly changed in N+ when comparing to N-. In addition, relatively significant deregulation of miR-223 expression was also observed.

Conclusion

The results indicate that miRNA-449 could contribute to new treatment of LCa with metastases as a great potential target. Serum miRNAs, miR-224 and miR-423-5p, are also remarkable candidate targets for the treatment approaches.

P11 Apoptosis of breast cancer cells induced by lauric acid occurs through p21Cip/WAF1 in a p53 independent manner

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Breast cancer is the most common malignancy and the leading cause of cancer-related death in Women worldwide (Ferlay et al. 2015). In recent years, the use of natural products for preventing and Wreating breast cancer has received renewed attention (Gullett et al. 2010). Fatty acids, classified as Whort, medium (MCFA) or long-chain, have been considered as promising adjunctive Chemotherapeutic agents for the treatment of diverse types of tumors (Laviano et al. 2013; Fauser Þt al. 2011). In this regard, it has been reported that the cytotoxicity of diverse fatty acids may Ýepend on the carbon chain length and the number of double bonds (Lima et al 2002). The Antineoplastic potential of the saturated MCFA lauric acid, which is a major component of coconut oil WDayrit et al. 2015), remains to be further ascertained especially in breast cancer. In order to provide Wovel insights into the biological responses to lauric acid, we performed Ó-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and TUNEL assays in SkBr3 breast Cancer cells. Additionally, gene expression studies and immunoblotting analysis were performed to Þlucidate the mechanisms that may be involved into the anticancer property of lauric acid. We found What lauric acid inhibits in a dose-dependent manner the viability of SkBr3 breast cancer cells without Affecting the proliferation of normal MCF-10A breast epithelial cells. On the contrary, the MCFA Capric acid did not show any effect on the growth of both cell types, suggesting that the Anti-proliferative response is triggered specifically by lauric acid. Moreover, we determined that Wauric acid enhances the expression of cyclin-dependent kinase inhibitor p21Cip/WAF1 at both mRNA And protein levels in a p53 independent manner. Of note, the involvement of p21Cip/WAF1 in cell Apoptosis by lauric acid was ascertained using the selective p21 inhibitor UC288, which is a Cell-permeable phenylcyclohexyl-urea chemical that selectively lowers p21 expression. Overall, our ßindings shed light on novel molecular mechanisms through which lauric acid may induce Antiproliferative and pro-apoptotic responses, therefore suggesting its possible use as anticancer Agent in mammary tumors.

P12 Different mutations as potential prognostic markers in non-small cell lung cancer

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In recent years, somatic mutations within the epidermal growth factor receptor (EGFR) gene were discovered as driver mutations in lung cancer, thus dramatically changing the therapeutic strategy for unresectable lung cancer. Driver mutations are predictive factors of the effect of EGFR tyrosine kinase inhibitors (TKI), which are molecular-targeted agents (such as gefitinib, erlotinib, and afatinib). The response rate of these drugs against cancers harboring driver mutations is >70% in many studies. Activating mutations in the MAPK pathway, which includes RAS, RAF, MEK, and ERK, result in constitutive signalling, leading to oncogenic cell proliferation and escape from apoptosis; therefore, this pathway is a focus of crucial interest for the development of cancer drugs. The frequency of V600E BRAF mutation in lung adenocarcinoma is 1.5% to 2.8%. Treatment of V600E BRAF-mutant lung adenocarcinomas with dabrafenib is under evaluation in a phase 2 trial, and could represent another milestone in individualized therapy for lung cancer patients. Moreover, rearrangement of the anaplastic lymphoma receptor tyrosine kinase (ALK) gene was also found to predict the response to ALK inhibitors (crizotinib and alectinib). Thereafter, rearrangements of ret proto-oncogene (RET) and ROS proto-oncogene 1, receptor tyrosine kinase (ROS1) genes were revealed as predictors for NSCLC. The molecular-targeted therapies crizotinib and vandetanib were used to target cancers harboring these two rearrangements. The predictive and prognostic value of KRAS mutation and its type of mutations in NSCLC are controversial. Some reports demonstrated increased overall survival (OS) in KRAS mutants compared with wild type, but others have failed to demonstrate consistent results. Subgroup analysis done by location of amino acid substitution, codon 12 and codon 13, also failed to show significant difference in OS and response to chemotherapy. In human studies, results were also controversial in retrospective studies comparing pemetrexed sensitivity by type of KRAS mutation. Although all studies have been reported in median OS, same tendency of poor respond to pemetrexed in subjects with G12C were demonstrated in a report but another showed the opposite result. In this study, we have investigated the predictive value of each type of KRAS mutation to response to chemotherapy. We have analyzed 30 patients with AdCa from October 2016 in the Diagnostica Citometrica e Molecolare Unit of the Azienda Ospedaliera Universitaria "L. Vanvitelli" of Naples and we have found 23% of patient with mutations in EGFR, 10% in KRAS e 3% either in BRAF or PIK3Ca. For the patients with K-RAS mutations, 1 patient had a mutation in KRAS p.Gly12Val, one in

p.Gly12Cys exon 2 and one in p.Ala146Ser exon 4. The first and second were also EGFR wild type but the third has an EGFR mutation in p.Leu858Arg exon 21 (activating mutation). The first patient is in treatment with AVELUMAB (anti-PDL1) because expressing >25% PDL-1. The second patient is in treatment with Cisplatin and Pemetrexed. The third patient is in treatment with Erlotinib and Bevacizumab. We are studying the interaction between therapy and mutations in KRAS. At the moment, the patients have clinical improves but the schedule of therapy is in initial phase. Interestingly, another patient had a mutation in BRAF p. Val600G and we are studying the association between the mutation in BRAF and the resistance mechanisms to chemotherapy and we are eventually programming to use BRAF inhibitor in the case of progression of disease. The molecular study of the mutations in NSCLC could be useful not only to predict the response to targeted therapies but also for the prediction of response to conventional cytotoxic drugs.

P13 Reduction of mitochondrial antioxidant glrx2 in familial oncocytic tumors is associated with mtdna mutations-dependent indolence

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Oncocytic transformation is responsible for mitochondrial hyperplasia but likely not for cancer initiation and severe respiratory dysfunction caused by homoplasmic mitochondrial DNA (mtDNA) mutations is a distinct hallmark of benign oncocytic tumors. We here report a family affected by Hyperparathyroidism-Jaw Tumor (HPT-JT) syndrome due to a large germline deletion in CDC73, with an unusual hereditary predisposition to develop oncocytic neoplasms. CDC73 somatic loss of heterozygosity (LOH) has been associated with parathyroid carcinomas/adenomas but rarely with the oncocytic phenotype. All tumors in the family accumulated different somatic mtDNA mutations at high heteroplasmy, with an evident inverse correlation between the severity of the phenotype and the mutations load. Additionally, a rare inherited heteroplasmic mtDNA mutation affecting the mitochondrial ribosomal subunit peculiarly shifted towards either mutated or wild-type homoplasmy in benign or malignant tumors, respectively. Characterization of cybrids, i.e. osteosarcoma cells carrying the ribosomal RNA mutation, showed that cells carrying the homoplasmic version indeed displayed a delayed mitochondrial protein synthesis, suggesting its potential contribution to the bioenergetic defect and tumor indolence. To delve into the cause for the predisposition to accumulate mtDNA mutations particularly in this family, we investigated the region where the CDC73 deletion occurred. We revealed that loss of regulatory elements within the deleted region causes half-dose expression of GLRX2, a protein involved in mitochondrial ROS detoxification and CI glutathionylation. Decrease of this protein confers a higher sensitivity to ROS in EBV transformed peripheral blood lymphocytes from patients, therefore we will use CRISPR-Cas9 technology on a parathyroid normal cell line to obtain a CDC73-/- GLRX2-/- model. This will allow us to investigate whether a complete loss of GLRX2, following the tumorigenic hit, confers a higher sensitivity to ROS, accounting for a ‘protective’ effect by favoring mtDNA mutations accumulation and conferring an indolent oncocytic phenotype. Studies are underway to understand the potential role of detox proteins in oncocytic sporadic tumors, that may be translated into therapeutic strategies.

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P14 *KRAS* and two rare *PI3KCA* mutations coexisting in a metastatic colorectal cancer patient with aggressive and resistant disease

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Today, KRAS and NRAS genotype addresses medical treatment of metastatic colorectal cancer (mCRC) patients, including anti-epidermal growth factor receptor or anti-vascular endothelial growth factor targeted agents. Other genes activating downstream RAS-RAF-MAPK and PI3K-AKT pathways, such as BRAF and PIK3CA, are under investigation to assess their prognostic and predictive clinical implication.We describe an early-onset mCRC patient with familial history and synchronous liver, lung and lymph-nodes metastases. The patient was treated with first-line intensive triplet chemotherapy plus bevacizumab, according to FIr-B/FOx schedule. KRAS, NRAS (exons 2, 3, 4), BRAF (exon 15), PI3KCA (exons 9, 20) were analyzed. A KRAS exon 2 mutation (c.34 G>A, GGT>AGT, Gly12Ser) was detected in primary tumor and confirmed in liver metastasis, where two PI3K exon 9 mutations (c.1633 G>C, GAG>CAG, E545Q; c.1645 G>C, GAT>CAT, D549H) were furthermore detected. The c.1633G>C somatic mutation was already described in CRC, whereas the second one c.1645G>C was reported with very low frequency just in hepatocellular and cervical carcinoma. On the basis of in silico analysis, this variant might have pathological significance. Akt/p-Akt(Ser473), PTEN, p53, MMR, EGFR proteins' expression was evaluated by immunohistochemistry. Both primary tumor and liver metastasis expressed Akt; conversely, the activated form p-AktSer473 was detectable in liver metastasis, but not in primary tumor. Liver metastasis expressed PTEN and p53. No abnormal MLH1, MSH2, MSH6 and EGFR protein expression was detected. Patient showed an aggressive and resistant disease, with 7 months progression-free survival and 15 months overall survival, lower than that reported in overall KRAS exon 2 mutant patients treated with the same FIr-B/FOx regimen.

The analysis highlighted the presence of a KRAS (c.34 G>A) and two very rare PI3KCA (c.1633 G>C, c.1645 G>C) mutations coexisting in liver metastasis from a mCRC patient with aggressive disease, resistant to one of the most effective medical treatment regimens. Interestingly, the c.1645 G>C PI3KCA substitution was never reported in CRC. Cells showing p-AktSer473 expression were detected just in liver metastasis, despite PTEN expression, leaving to hypothesize a role of the double PI3KCA mutant genotype, which might contribute to further enhance the aberrant activity of PI3KCA-AKT pathway.

P15 CRISPR/CAS9-mediated knockout of Bim antagonizes apoptosis induced by miR-17-92 inhibitor in multiple myeloma cells

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Mature microRNA (miRNA) members of miR-17-92 cluster cooperate to mantain cellular homeostasis in malignant cells, including c-Myc (Myc)-driven lymphomas. Here we describe the development of a first-in-class inhibitor of this oncogenic cluster, named MIR17PTi (MIR17 Primary Transcript inhibitor). This is a locked nucleic acid (LNA) gapmeR which induces RNase H-mediated degradation of miR-17-92 primary transcript, resulting in a one shot downregulation of all six cluster members. We show that MIR17PTi antagonizes proliferation of several cancer cell lines and induces apoptosis via the impairment of pro-survival Myc/miR-17-92 feedforward loops (FFLs) in patient-derived multiple myeloma (MM) cells. Moreover, in MM as well as in other Myc-driven cellular models (P493-6 and Myc-ER HMECs), MIR17PTi triggered Myc-dependent synthetic lethality. In this scenario, the disruption of a Bim-centred FFLs played a key role: indeed, MIR17PTi-resistant MM cell lines (n=2) did not express detectable levels of Bim and, in MIR17PTi-sensitive MM cells, CRISPR/CAS9-mediated genomic knockout of Bim significantly antagonized MIR17PTi-mediated apoptosis. Finally, treatment with MIR17PTi inhibited in vivo MM growth at mice- and monkeys-safe doses. Pharmacokinetic profile in cynomolgus monkeys indicated early plasmatic clearence via tissue uptake. Altogether these findings support the clinical development of MIR17PTi against Myc-driven malignancies.

P16 CRISPR/Cas9 for the Study of the Interactions between Viruses and Host

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The CRISPR/Cas9 system has many applications in virology: it has been used to achieve viral DNA inactivation from latently infected cells, allowing viral eradication, or to inactivate specific proteins involved in virus-host cell interaction. Herein we applied the CRISPR/Cas9 technique to generate knock-out cell lines useful for the study of cellular determinants critical for HIV-1 infection. As a preliminary screening, the editing efficiency was evaluated by T7 endonuclease I assay, and then confirmed by western blot and flow cytometry analyses. We targeted β2microglobulin (β2m), human thioesterase 8 (ACOT8) and histone deacetylase 6 (HDAC6) genes. β2microglobulin is required for the membrane translocation of HLA molecules where HLA-C interacts with HIV-1 Env and modulates viral infectivity (Zipeto & Beretta, Retrovirology 2012). We edited β2m in 293T, HeLa-Lai (expressing HIV-1 Env), TZM-bl (CD4 and CCR5 expressing HeLa, highly sensitive to HIV-1 infection) and parental HeLa cells. We showed in 293T cells that HIV-1 proteins transfection did not translocate HLA-C at the cell surface in absence of β2m. We obtained similar result in β2m negative HeLa-Lai cells, showing that HIV-1 Env interacts with HLA-C at the plasma membrane after its surface translocation. Besides, we demonstrated that HIV-1 pseudoviruses produced in β2m negative 293T cells were significantly less infectious than those produced in parental ones (Serena et al., Scientific Reports, 2017). ACOT8 thioesterase interacts with HIV-1 Nef protein preventing its degradation (Serena et al, Scientific Reports 2016). To better understand the role of ACOT8 in HIV-1 infectivity, we developed ACOT8 knock out 293T and TZM-bl cell lines. We observed in TZM-bl cells, susceptible to HIV-1 infection, that ACOT8 absence did not affect the infectivity. The role of ACOT8 in pseudoviruses production is being tested using 293T edited cells. HDAC6 is an important regulator of membrane dynamics involved in HIV-1 infection (Valenzuela-Fernandez et al, Molecular biology of the cell, 2005). We inactivated the HDAC6 gene in 293T cells. These cells will be used to test the HIV-1 infectivity and syncytia formation. In conclusion, the CRISPR/Cas9 system represents a new, powerful tool in basic and applied research in virology.

P17 Targeting systems vulnerabilities in uveal melanoma by CRISPR/Cas9 focal adhesion kinase (FAK) genome editing and therapeutic inhibition

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Uveal melanoma (UM) is the most common primary cancer of the eye in adults. It is diagnosed in about 2,500 adults in USA every year and approximately 50% of UM patients develop liver metastasis mostly within 5 years after diagnosis, independently of the successful treatment of the primary lesions. The survival of metastatic UM patients is often only few (2-6) months. To date, there are not effective options to treat or prevent UM metastasis. UM is genetically characterized by mutually exclusive activating mutations in the *GNAQ* and *GNA11* oncogenes, which encode heterotrimeric Gαq family members. To date, these mutations have been identified in about 92% and 4% of uveal and skin melanomas, respectively. Here, we have focused on a cytoplasmic protein-tyrosine kinase associated with integrins named focal adhesion kinase (FAK), which modulates important cell processes such as growth, survival, and migration. By the analysis of UM genomic alterations (TCGA), we found that the gene encoding FAK (*PTK2*) is amplified or overexpressed in >56% of all UM lesions. UM represents the human cancer harboring the highest levels of FAK, which we confirmed by immune histochemical analysis of a large collection of UM lesions. We found that Gq-GPCRs triggers a rapid phosphorylation of FAK at position Y397, which reflects its activation, through a guanine nucleotide exchange factor (TRIO)/Rho-A signaling circuitry. This promotes the assembly of focal adhesions, independently of the PLCβ/Ca2+ second messenger system and the canonical PKC pathway. Next, we have assessed whether Gαq promotes the FAK-dependent proliferation of uveal melanoma cells. Both, CRISPR/Cas9 genome editing of FAK as well as the use of FAK inhibitors under clinical evaluation for other diseases resulted in reduction of UM tumor growth *in vivo*. The impact of these FAK inhibiting strategies in UM metastasis is under current evaluation. Collectively, our findings support the potential clinical benefit of targeting FAK as a precision therapy approach for UM and other *GNAQ*-driven malignancies and pathological conditions.

P18 TCR Sequencing shaping cancer immunotherapy

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T cells are key components of the adaptive branch of the immune system. Their function relies on the recognition of an antigenic peptide by their T cell receptor (TCR), that varies for each T cell. The naïve TCR repertoire is assembled in the thymus and formed by somatic rearrangements of non-contiguous variable, diversity and joining genes. Further random insertion and deletion of nucleotides at the rearrangement loci create junctional diversity of the highly variable CDR3 region. As this region is unique for every T cell clone and encodes the receptor portion which makes the majority of TCR contacts with antigenic peptides bound by the major histocompatibility complex (MHC), a dissection of the CDR3 sequence at the nucleotide level is fundamental to characterize the abundance of T cell clones and the functionality of a T cell population. The advent of next generation sequencing technologies has rendered feasible the comprehensive analysis of such a complex target.

Deep sequencing of the CDR3 region provides a new high resolution view of cellular immunology and can unequivocally identify the clonality of the TCR repertoire. In particular, immunorepertoire profiling has yielded new insights into the properties of normal T-cell repertoires in healthy individual and has led to the detection of reactive and pathogenetic T cell clonotypes in disease conditions.

In the context of cancer immunotherapy, TCR sequencing has been widely used to track with high sensitivity clonal expansion and contraction of T cells in response to tumors/infectious diseases or treatment and to determine clonality changes in patients undergoing hematopoietic stem cell transplantation. Here, we show the exploitation of TCR sequencing for the identification of novel tumor-specific TCRs. In particular, we have focused our study on antigens expressed by acute leukemia, a heterogeneous group of diseases that still represent major unmet medical needs. Final aim of this study is to build a TCR library that encompass diverse antigens and HLA restrictions and that can be used in the TCR gene editing strategy for the treatment of leukemia.

P19 Functionally validating the significance of BCL11A-CHD8 interaction by CRISPR-Cas9

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Triple-negative breast cancer (TNBC) is an aggressive form of breast cancer, which accounts for around 15-20% of all breast cancer cases (1). TNBC patients have a higher risk of developing secondary and metastatic disease and hence have lower rates of survival.
The molecular and cellular mechanisms underpinning TNBC pathology are not fully understood. We analysed both METABRIC and TCGA data and found that the transcription factor *BCL11A* is overexpressed in TNBC and its genomic locus is amplified in up to 38% of these tumours. (2). Furthermore, we reported that BCL11A overexpression promotes tumour formation, whereas its knockdown in TNBC cell lines suppresses tumour growth. Mechanistically, proteomic studies have revealed that BCL11A interacts with chromatin modifier chromodomain helicase DNA binding protein 8 (CHD8) (3, 4) in tumour cells but not in normal cells. To further explore this interaction we perform immunoprecipitation studies in two different cell lines and TNBC PDX models and demonstrated BCL11A and CHD8 binding. We next showed that BCL11A and CHD8 protein levels are high in TNBC PDX samples, with no signal detected in luminal PDX samples. However this correlation was not detected at the RNA level. Furthermore, we confirmed this by analysing mammary glands from mice overexpressing BCL11A, and found an upregulation of CHD8 at the protein but not RNA level. These results indicate stabilisation of CHD8 protein by BCL11A. We then investigated the impact of CHD8 loss on tumour formation. We used a lentiviral CRISPR/Cas9 vector to knockout CHD8 in TNBC cell lines. We observed a significant reduction in tumour growth and colony numbers when CHD8 is reduced. To explore the functional significance of this interaction, we analysed, by performing ChIP-seq experiment, the global CHD8 binding pattern in BCL11A knockdown cells and found a significant decrease in genomic binding. Furthermore, in control cells BCL11A and CHD8 both bind to ~1000 genes. These studies highlight the potential for targeting BCL11A as an approach for TNBC-targeted therapy. To this end we are investigating the functional significance of the interaction between BCL11A and CHD8, thereby pinpointing a mechanistic pathway through which BCL11A elicits its action in TNBC.

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P20 Identification of novel estrogen- regulated microRNAs in breast tumor cells and cancer-associated fibroblasts (CAFs)

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Estrogens are involved in diverse patho-physiological processes mainly binding to and activating the estrogen receptor (ER)α and ERβ. In recent years, the G protein estrogen receptor, namely GPER/GPR30, has been also involved in the action of estrogens in numerous types of normal and tumor cells as well as in cancer-associated fibroblasts (CAFs), which are main components of the tumor microenvironment. microRNAs (miRNAs) are non-coding RNA playing an important role in various conditions due to their ability to regulate the stability and/or translation of target mRNAs. In malignant cells, many miRNAs have been shown to act as oncogenes, while others have been demonstrated to behave like tumor suppressors. In recent years, an increasing interest has been addressed to estrogen-induced gene regulation mediated by certain miRNAs. In this regard, estrogens were reported to modulate the expression of diverse miRNAs through both ER and GPER, therefore leading to relevant biological responses in different cell contexts. In order to better define the role of miRNAs as breast cancer biomarkers, we evaluated the expression profile of 754 miRNAs upon 17β-estradiol treatment in three different cell types: CAFs (ER-negative and GPER-positive) obtained from breast cancer tissues, MCF7 (both ER and GPER-positive) and SkBr3 (ER-negative and GPER-positive) breast cancer cells. Using TaqMan^{R} Low Density Arrays and considering a threshold value of 1.5 fold induction and 50 % reduction respect to vehicle-treated cells, we identified 117, 74 and 95 estrogen-regulated miRNAs in CAFs, MCF7 and SkBr3 cells, respectively. Moreover, MCF7 and SkBr3 cells shared 30 miRNAs, CAFs and SkBr3 shared 11 miRNAs, while CAFs and MCF7 shared 3 miRNAs (Fig.1). Next, CAFs, SkBr3 and MCF7 shared 3 miRNAs (Fig.1). The *in silico* analysis performed by available bioinformatics algorithms, indicated that all these miRNAs may regulate the expression of genes involved in cell differentiation, adhesion, migration and proliferation. Current studies are ongoing in order to corroborate the aforementioned results toward a better comprehension of the mechanisms by which estrogens trigger miRNA modulation.Collectively, our data identified novel estrogen-regulated miRNAs toward the assessment of their role as potential biomarkers in breast cancer.

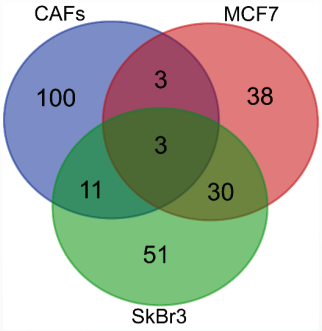

Figure 1. Venn Diagram summarizing the estrogen-responsive miRNAs found in CAFs, MCF7 and SkBr3 cells.

GENERAL INFORMATION

Conference Venue

Magna Graecia University - Campus S. Venuta - Aula Magna C, 1° Level, Building G
Viale Europa - 88100 Catanzaro, Italy

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REGISTRATION FEES (VAT included)

AICC Member € 120,00
Non-Member € 170,00 (€ 120,00+50,00 mandatory membership fee)
Student € 50,00 (membership fee)

The registration fee includes:

- Conference kit
- Participation in the Scientific Sessions
- Attendance Certificate
- Admission to the Opening Ceremony, coffee break and lunch.

* *Please indicate to the Secretariat any food intolerances*.

ADDITIONAL GENERAL INFORMATION

LANGUAGE: The official language of the Meeting is English.
SLIDES: Slides must be in English. Simultaneous translation will not be provided.
Computer videoprojection will be available in Power Point.
Slides should be given to the slide center one hour before the beginning of the session.
ATTENDANCE CERTIFICATE: A certificate will be sent to all participants at the end of the Meeting.

Poster Award

During the Meeting, AICC will assign "Poster Award" to the first author of the best posters who is under 35 years of of age at the time of the Meeting. As prescribed by law, as income tax will be applied. The awards will be delivered during during the Poster Awards Ceremony directly to the winners (delegation for collection is not allowed-the possible absence will cause disqualification from the contest).

HOW TO REACH THE MEETING VENUE

By air: From International Airport Lamezia Terme, the Campus can be reached by car in about 35 min. Proceed along the SS280 direction Catanzaro, take the exit for Germaneto, continue along Viale Europa.

By bus: from Catanzaro railway station take local public transport, routes 47 or 48 and from Catanzaro Lido railway station route 46 (for timetables visit the website www.amcspa.it).

By car: from Catanzaro 15 minutes roughly: proceed along the SS280 direction Lamezia Terme, take the exit to Germaneto, continue along Viale Europa. From A3 motorway, exit Lamezia Terme - Catanzaro, the Campus can be reached in about 35 min.: proceed along the SS280 direction Catanzaro, take the exit to Germaneto, continue along Viale Europa. From Soverato 25 min. roughly: proceed along the Ionian highway 106 northwards up to the intersection of Catanzaro Lido, turn to direction Lamezia Terme, continue along Viale Europa.

COME RAGGIUNGERE LA SEDE DEL CONVEGNO

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In auto:
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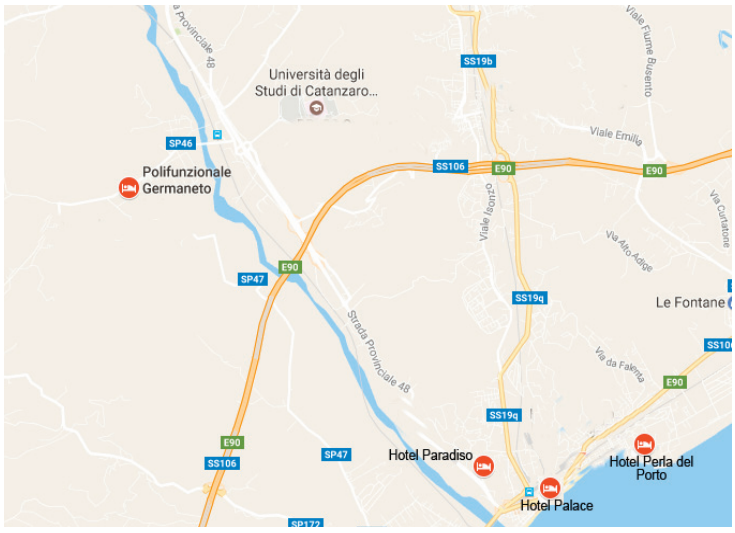

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