BREAST

A METABOLISM-BASED METHOD TO DETECT CIRCULATING TUMOR CELLS: EXPLORATORY CLINICAL STUDY AND DIRECT COMPARISON WITH CELLSEARCH® ON METASTATIC BREAST CANCER.

F. Del Ben 1, M. Turetta 2, E. Biscontin 1, G. Brisotto 1, G. Celetti 3, A. Piruska 3, M. Bulfoni 2, E. Rossi 4, D. Cesselli 2, W.T.S. Huck 3, R. Zamarchi 4, G. Scoles 2, A. Colombatti 1, A. Steffan 1.

1 Dept. of Translational Research, Centro di Riferimento Oncologico CRO Aviano, I.R.C.C.S. Italy; 2 Institute of Anatomic Pathology, Dept. of Medical and Biological Sciences, University of Udine, Italy; 3 Dept. of Physical Chemistry, Radboud University, Nijmegen, The Netherlands; 4 Istituto Oncologico Veneto IOV, I.R.C.C.S., Padova, Italia.

The number of circulating tumor cells (CTC) is a validated biomarker as an aid in monitoring of metastatic breast cancer. CTC are also an excellent substrate for liquid biopsy, enabling serial monitoring of disease evolution. At present, CTC detection technology are significantly hampered by reduced sensitivity, high cost, and limited harvesting, thus preventing widespread use of CTC. We developed an innovative method to detect and harvest CTC (Del Ben, Turetta, et al. Angew.Chem.Int.Ed.2016). The method exploits the increased extracellular acidification rate of cancer cells compared to normal cells. In order to do this, microfluidics is used to encapsulate single cells individually in microdroplets, together with a pH-dependent fluorescence dye. After a short incubation, the pH of every single drop is screened with laser-induced fluorescence. First, we validated the method in spiking assays using cancer cell lines. Then, we started an exploratory case-control trial in highly metastatic breast cancer patients (N=16) and healthy controls (N=13) finding significantly different medians (179 vs 6 events/7.5mL, respectively. p=0.001). ROC analysis showed 87.5% sensitivity and 85% specificity at set threshold. Then, we started a clinical validation study analyzing samples before the start of a new therapy and after 3-4 weeks. A total of 28 samples from 14 patients were analyzed in parallel with CellSearch® platform. Linearity correlation analysis yielded R2=0.95, suggesting a good correlation. Notably, there were a significant subset of patients with a positive count in our method, but negative count with CellSearch, suggesting an increased sensitivity of our method. Further validation is needed to establish the nature of detected population and direct correlation with clinical parameters.

BREAST

DISSECTING THE MOLECULAR INTERPLAY AMONG MIR155, RAD51 AND C/EBPβ IN THE PROGRESSION AND METASTASIS OF BREAST CANCER.

Barbara Pasculli, Raffaela Barbano, Michelina Rendina, Michelina Coco, Paolo Graziano, Andrea Fontana, Massimiliano Copetti, Roberto Murgo, Vanna Maria Valori, Evaristo Maiello, Tommaso Mazza, Vito Michele Fazio, Paola Parrella.

IRCCS Casa Sollievo della Sofferenza.

BACKGROUND:
RAD51 is a central protein in homologous recombination (HR), playing the critical role of catalyzing the transfer of the strand between a broken sequence and its undamaged homolog to resynthesize the damaged region. Beside its central role in HR repair, RAD51 is currently emerging as novel putative promoter of metastatic pathways through co-regulatory mechanisms involving the c/EBPβ transcription factor. Interestingly, recent evidences potentially allocate miR-155 within such intricate cellular network, as suggested by both inverse correlation between miR155 and RAD51 mRNA and protein expression levels, and miR155 direct targeting of c/EBPβ mRNA in breast cancer (BC).

AIM:
This study is aimed to characterize the miR-155-RAD51-c/EBPβ interplay and its role in breast cancer progression and metastases, to ultimately identify novel molecular biomarkers of clinical use.

EXPERIMENTAL DESIGN:
We are currently analysing a retrospective cohort of 302 BC cases with at least a 5-years follow-up. miR-155, and RAD51 and c/EBPβ mRNA levels are measured by RT-qPCR, whereas RAD51 and c/ EBPβ proteins levels are being evaluated by IHC on an "in house" Tissue Microarray. Results from these analyses will be correlated with clinicopathological data and patients' outcome. In vitro functional studies are ongoing to evaluate the effects on cell viability, apoptosis and migration upon miR-155, RAD51 and c/ EBPβ induced overexpression/downregulation in a panel of BC cell lines mimicking the molecular breast cancer subtypes. The putative effects of miR-155, RAD51 and c/EBPβ on the sensitivity of cancer cells to chemo-radiotherapy will be also evaluated. Both wild type and corresponding transfected cells will be treated with epirubicin chloride and PARP inhibitors and/or exposed to g-irradiation. The effects on chemo-radiosensitivity will be evaluated by standard procedures.

IMPACT ON CANCER:
We hope that this study will support the development of novel methodological tools for innovative diagnostic and therapeutic interventions in breast cancer.

BREAST

QUERYING THE "PYKNON CODE" TO ELUCIDATE THE MECHANISMS OF METASTASES AND IDENTIFY NOVEL BIOMARKERS IN BREAST CANCER.

Barbara Pasculli 1, Raffaela Barbano 1, Orazio Palumbo 1, Michelina Rendina 1, Raffaella Stallone 1, Michelina Coco 1, Andrea Fontana 1, Massimiliano Copetti 1, Paolo Graziano 1, Roberto Murgo 1, Vanna Maria Valori 1, Evaristo Maiello 1, Tommaso Mazza 1, Massimo Carella 1, Vito Michele Fazio 1, Isidore Rigoutsous 2, George A. Calin 3, Paola Parrella 1.

1 IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo, FG, Italy; 2 Computational Medicine Center, Sidney Kimmel Medical College at Thomas Jefferson University, Philadelphia, PA, USA; 3 Department of Experimental Therapeutics, The University of Texas MD Anderson Cancer Center, Houston, TX, USA.

BACKGROUND:
Breast Cancer (BC) represents the second cause of cancer-related mortality in women mainly due to metastases. Pyknons are short, repeated DNA elements located into non genic and genic regions of the human genome, with a propensity to localize at the 3′ UTRs of genes that suggests their functionality within post-transcriptional regulatory events. Recent findings have demonstrated that pyknons are active sources of non-coding RNAs (pyk-ncRNAs) whose expression levels exhibit tissue- and disease-specificity of prognostic relevance.

AIM:
We aim to identify a pyk-ncRNAs signature of metastases in BC to improve our understanding of BC pathology and provide novel prognostic biomarkers.

EXPERIMENTAL DESIGN:
We performed a global miRNA expression profiling by using the Affymetrix GeneChip® miRNA 4.0 Arrays in a small cohort of 34 BC cases: 17 were non-metastatic (M0) after 10-years follow-up, and 17 developed metastatic disease within 5-years follow-up (M1). As controls, 3 Normal Breast Tissues and 3 in situ carcinomas were also analysed. This analysis identified 8 differentially expressed miRNAs between M1 and M0 cases. Then, we performed a global gene expression analysis in the same patients' set by using the Affymetrix GeneChip® HTAs 2.0. A list of 340 differentially expressed genes between M0 and M1 samples was generated. Based on these data, we will assess the active transcription across 1292 pyknon genomic loci in the same cohort by using the MDACCv5 chip array, in order to identify metastasis-specific pyk-ncRNAs. These results will be integrated with miRNA and gene expression data to uncover potential networks of post-transcriptional regulation in metastases-leading processes. Next, top-3 metastases-specific pyk-ncRNAs will undergo in vitro systematic characterization in a panel of BC cell lines. Last, the prognostic performance of metastasis-specific pyk-ncRNAs and associated miRNAs and genes will be validated in a retrospective cohort of 302 BC cases with at least a 5-years follow-up.

BREAST

BREMIR: MICRORNAS METHYLATION AND EXPRESSION PROFILING FOR IDENTIFICATION OF BREAST CANCER PATIENTS AT HIGH RISK TO DEVELOP DISTANT METASTASES.

Raffaela Barbano 1, Barbara Pasculli 1, Orazio Palumbo 2, Michelina Rendina 3, Raffaella Stallone 2, Michelina Coco 1, Andrea Fontana 3, Tommaso Mazza 4, Massimiliano Copetti 3, Vanna Maria Valori 5, Paolo Graziano 6, Roberto Murgo 7, Evaristo Maiello 5, Massimo Carella 2, Vito Michele Fazio 8, Ella Evron 9, Manel Esteller 10, Paola Parrella 1.

1 Laboratory of Oncology IRCCS "Casa Sollievo della Sofferenza", San Giovanni Rotondo, Italy; 2 Medical Genetics, IRCCS "Casa Sollievo della Sofferenza", San Giovanni Rotondo, Italy, 3 Unit of Biostatistics IRCCS "Casa Sollievo della Sofferenza", San Giovanni Rotondo, Italy; 4 Unit of Bioinformatics IRCCS "Casa Sollievo della Sofferenza", San Giovanni Rotondo, Italy; 5 Oncology Department IRCCS "Casa Sollievo della Sofferenza", San Giovanni Rotondo, Italy; 6 Pathology Unit IRCCS "Casa Sollievo della Sofferenza", San Giovanni Rotondo, Italy; 7 Breast Unit, IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo, Italy; 8 Laboratory of Genetic and Clinical Pathology, University Campus Bio-Medico of Rome, Italy; 9 Assaf Harofeh Medical Center Zerifin, Affiliated with Tel Aviv University, Sakler School of Medicine, Israel; 10 Cancer Epigenetics and Biology Program (PEBC), Bellvitge Biomedical Research Institute (IDIBELL), L'Hospitalet de Llobregat, Barcelona, Spain.

BACKGROUND:
The mortality of breast cancer (BC) is primarily caused by metastatic spread to distant organs, rendering the ability to predict, detect and eliminate metastases one of the most important challenges in patients' management. AIM: This study is aimed to evaluate the potential role of several candidate miRNAs as predictive markers of the metastatic processes.

EXPERIMENTAL DESIGN:
The correlation of miRNAs expression with distant metastasis free survival will be evaluated in a large retrospective cohort (n=302). The best candidate markers will be validated in two independent prospective cohorts (n=500 and n=300). Additionally, miRNA potentialities as circulating biomarkers for treatment monitoring and early metastases detection will be evaluated in plasma samples.

RESULTS:
To date, the analysis of 129 cases from our retrospective cohort has identified miR-10b, miR-10a, mIR-30a, miR-155, miR-9-3p and miR-9-5p as crucially involved in BC progression. For miR-9-5p and miR-9-3p we also found a strong inverse correlation with ER and PgR expression, suggesting that they may be directly involved in hormone regulated pathways. To identify novel miRNAs associated with tumour metastases potentials, we also executed a global miRNA profiling on a selected number of breast cancer cases by using the Affymetrix GeneChip® miRNA 4.0. In particular, we analysed 34 BC cases characterized by different outcomes: 17 non metastatic after 10-years follow-up and 17 who developed metastatic disease within 5-years follow-up. As controls, 3 Normal Breast Tissues from reductive mammoplasty, 3 in situ carcinomas and 3 breast cancer cases with synchronous metastases were also evaluated. This analysis identified 8 miRNAs differentially expressed between metastatic and non metastatic cases. The association with study endpoints is currently under evaluation on the entire retrospective cohort.

IMPACT ON CANCER:
Our hope is that this study will lead to the identification of novel biomarkers allowing a better management of breast cancer patients.

BREAST

BREAST

NANOMETRONOMIC TREATMENT OF TRIPLE NEGATIVE BREAST CANCER WITH DOXORUBICIN NANOCAGES: NEW PERSPECTIVES FOR THE METRONOMIC APPROACH.

Mazzucchelli S., Truffi M., Monieri M., Bonizzi A., Sorrentino L., Corsi F.

University of Milan, Department of Biomedical and Clinical Sciences "L. Sacco", via G. B. Grassi 74, 20157 Milan (MI), Italy.

Triple Negative Breast Cancer (TNBC) is the main clinical challenge in breast cancer treatment due to its heterogeneity, biological behaviour, invasiveness and poor prognosis. The only clinical option is represented by high-dosed combinatorial chemotherapy (MTD), which is quite effective on cancer cells and prolongs disease-free survival (DFS), but enhances oncogenic response of tumor microenvironment increasing the risk of tumor recurrence and metastases. Overcoming the oncogenic response of tumor stroma is the goal achievable taking advantage of a powerful tumor-targeted drug delivery system, which combines the positive effects of MTD in killing cancer cells with the advantages of metronomic (LDM) scheduling. Indeed, LDM displays a positive impact on cancer stroma, along with reduced neoangiogenesis and restored anticancer immunity, but it has low direct effectiveness on cancer cell. Treatment of TNBC with doxorubicin (DOX)-loaded H-ferritin nanocages (HFn) in nanometronomic (LDNM) scheduling has been shown to improve DOX antitumor efficacy, reduce tumor angiogenesis and chemoresistance and abolish DOX cardiotoxicity. In view of these results, the study of the molecular basis of LDNM efficacy on TNBC treatment by targeting specific crucial pathways for cancer progression and invasiveness, could be essential to focus subsequent oncological research and to optimize the next therapeutic nanostrategies. Comparing LDNM treatment with HFn-DOX with NMTD setting, we will elucidate the impact of LDNM on cancer cell killing (1), on cross-talk between cancer cells and tumor stroma (2), on tumor angiogenesis (3) and anticancer immunity (4), to discover new molecular mechanisms involved in LDMN response by transcriptomic analysis of LDNM and NMTD-treated tumors (5). This novel and promising approach, which exploits the natural tumor homing capability of HFn to mediate a specific targeted delivery of DOX with subcellular precision, promises to increase drug effectiveness on cancer cells and prolong DFS, without enhancing the oncogenic response of tumor microenvironment.

BREAST

BREAST

CHARACTERIZATION OF A NEW PATHWAY REGULATING METASTASIS IN TRIPLE-NEGATIVE BREAST CANCER.

Letizia Campanini, Giuseppina Messa, Manfredi Ponente, Stefania Zambelli, Luca Gianni, Claudio Doglioni, Rosa Bernardi.

Division of Experimental Oncology, Department of Medical Oncology, San Raffaele Scientific Institute Milano Italy.

Metastasis is the leading cause of cancer-associated mortality. Triple-negative breast cancers (TNBCs) are aggressive tumors characterized by high metastasis rates, recurrence to therapy, and poor overall survival. Due to elevated genetic complexity and lack of expression of hormone and HER2 receptors, patients with TNBC currently lack tailored therapeutic options. For this reason, a deeper understanding of the molecular circuitry regulating the formation and metastatic dissemination of TNBC is urgently needed to develop new therapies for these patients. It was recently suggested that one of the defining features of TNBC is activation of the hypoxia-inducible HIF-1a transcription factor, which is expressed in an oxygen-independent manner and predominantly promotes metastasis in this tumor context. We have recently found that the promyelocytic leukemia gene PML is a novel HIF-1a-target gene upregulated in TNBC. Importantly, in TNBC PML takes part to a positive feedback loop where it acts as the co-regulator of a set of HIF-1a-target genes that promote metastatic dissemination. Like HIF-1a, PML binds the regulatory regions of these genes, and promotes their expression specifically in TNBC, and not in other breast cancer sub-types. Starting from this observation, we have accumulated solid evidence that PML is a novel and tumor-specific regulator of metastasis, as PML silencing prevent metastasis only in the triple-negative sub-type of breast cancer. Importantly, because PML is targeted by arsenic trioxide, a compound that is currently used to treat patients with acute promyelocytic leukemia, we propose that inhibiting PML may provide a new therapeutic option for TNBC.

BREAST

BREAST

INDUCTION OF EPIGENETIC BRCANESS IN HOMOLOGOUS RECOMBINATION–PROFICIENT TRIPLE NEGATIVE BREAST CANCER: BET INHIBITION AS A NEW THERAPEUTIC APPROACH.

Gerratana Lorenzo 1; Mio Catia 1; Franzoni Alessandra 2; Basile Debora 1; Damante Giuseppe 1,2; Puglisi Fabio 1,3.

1 Department of Medicine (DAME), The University of Udine, Udine (UD); Italy 2 Institute of Genetics, ASUIUD - University Hospital of Udine, Udine (UD); Italy 3 Department of Clinical Oncology, CRO Aviano National Cancer Institute, Aviano (PN); Italy.

BACKGROUND:
Triple-negative breast cancer (TNBC) represents a challenge because of its heterogeneity and the absence of a well-defined molecular target, which restricts treatment options to chemotherapy. Bromodomains (BRD) are promising new epigenetic targets for cancer treatment, thanks to their interaction with histone acetyl-lysines through a tandem bromodomain at the N-terminal region. Aim of this study was to explore a novel therapeutic strategy based on the combination of a BET bromodomain inhibitor (GSK525762) and an alkylating agent (CDDP).

MATERIALS AND METHODS:
Two TNBC cell lines (MDA-MB-157 and MDA-MB-231) were exposed to 0.5 μM GSK525762 and 2 μM CDDP, alone or in combination. After 72h treatment, cell viability was analyzed through MTT assay and DNA damage induction was investigate through immunofluorescence assay using an anti-phosphorylated H2AX (Ser139) (γH2AX) antibody.

RESULTS:
GSK525762 and CDDP combination is able to trigger synergism, leading to a strong reduction in TNBC cell viability ($P<0.0001$). Moreover, an exponential DNA damage was observed, though a marked increase in γH2AX mean fluorescence intensity ($P<0.0001$).

CONCLUSION:
Our data clearly show that BET inhibition can sensitize BRCA1 wild-type TNBC cells to genotoxic agents, suggesting a novel combination strategy for this subtype. Future perspectives: Based on the above results, two main developments are planned. The proposed cellular model will be applied to primary cell cultures in order to further refine our knowledge on epigenetic induced BRCAness. On parallel, this approach will be tested in the clinic through a phase I/II trial. The ALTEA trial will be focused on assessing the activity and tolerability of GSK525762 in combination with carboplatin as first line treatment of women with metastatic TNBC. Patients will be enrolled after a multimodal assessment of both genomic and epigenomic BRCAness to ensure homologous recombination proficiency. Endpoints will be Objective Response Rate (CR + PR), progression-free survival and overall survival.

BREAST

BREAST

IS ANDROGEN RECEPTOR USEFUL TO PREDICT THE RESPONSE TO ANTIESTROGEN THERAPY IN ADVANCED BREAST CANCER?

S. Bravaccini 1, S. Ravaioli 1, G. Bronte 1, M. Puccetti 2, M.M. Tumedei 1, E. Scarpi 1, R. Maltoni 1, S. Sarti 1, L. Cecconetto 1, L. Bedei 3, A. Fedeli 1, D. Andreis 1, E. Pietri 1, D. Calistri 1, D. Amadori 1, A. Rocca 1.

1 Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS – Meldola, FC, (Italy), 2 S. Maria delle Croci Hospital - Ravenna (Italy), 3 Cancer Prevention Unit, Morgagni-Pierantoni Hospital, Forlì, FC, (Italy).

BACKGROUND:
The androgen receptor (AR) is widely expressed in breast cancers (BC) but its role in estrogen receptor (ER)-positive tumors is still unclear. The AR/ER ratio may impact prognosis and the response to antiestrogen endocrine therapy (ET).

METHODS:
We determined whether AR in primary tumors and/or matched metastases is a predictor of efficacy of first-line antiestrogen ET in advanced BC (ABC).We evaluated 102 patients treated with firstline ET (2002–2011, 93% with an aromatase inhibitor), excluding those receiving concomitant chemotherapy or trastuzumab or pretreated with >2 lines of chemotherapy.ER, progesterone receptor (PgR), Ki67 and AR expression was assessed by immunohistochemistry. A cut-off of ≥1% immunostained cells was used to determine AR positivity.AR expression was analyzed in relation to the other conventional biomarkers (ER, PgR, HER2 and Ki67), best response (CR, PR, SD, PD), and time to progression (TTP) (months). Hazard ratios and their 95% confidence intervals (95% CI) were estimated using the Cox regression model.

RESULTS:
Biomarkers were determined in primary tumors in 70 cases, in metastases in 49 and in 17 in both. Median TTP was 17 months (95% CI 14- 21.5, median follow-up 75 months).The overall concordance rate for AR expression between primary tumors and metastases was 64.7% (95% CI 42%-87.4%). Differences in TTP according to AR status were not statistically significant.AR/PgR ≥0.96 was associated with a significantly shorter TTP (HR = 1.65, 95% CI 1.05-2.61, p = 0.028). AR status in primary tumors or metastases was not associated with PD as best response. Using a cut off of <10% for AR expression, results did not change. In contrast, Ki67 ≥20% and PgR<10 showed a significant association with PD as best response.

CONCLUSIONS:
AR expression does not appear to be useful to predict the efficacy of ET in ABC. Ki67 and PgR exert a greater impact than AR.