15P The analgesic compound palmitoylethanolamide reduces inflammation in human cardiomyocytes and vascular endothelial cells exposed to doxorubicin and anti-HER2 monoclonal antibody through PPAR-α and NLRP3-related pathways

S. Buccolo[1], V. Quagliariello[1], C. Maurea[2], M. Berretta[3], A. Paccone[1], M. De Laurentiis[1], N. Maurea[1]

[1]Division of Cardiology, Istituto Nazionale Tumori IRCCS - Fondazione G. Pascale, Naples, Italy; [2]Medical Oncology, University of Salerno, Fisciano, Italy; [3]Medical Oncology, University of Messina, Messina, Italy

Background: Palmitoylethanolamide (PEA) is an endogenous fatty acid mediator that is synthetized from membrane phospholipids by N-acyl phosphatidylethanolamine phospholipase D. It is a new analgesic drug with anti-inflammatory effects through the induction of PPAR-related pathways. We aimed to assess whether palmitoylethanolamide co-incubated during doxorubicin and trastuzumab, reduces anticancer drugs-related cardiotoxicity in cellular models.

Methods: Human vascular endothelial cells and cardiomyocytes were exposed to subclinical concentration of doxorubicin (at 100 and 200 nM) combined to trastuzumab (at 100 and 200 nM) alone or in combination with a formulation composed by palmitoylethanolamide 500 nM for 48h. After the incubation period, we performed the following tests: determination of cell viability, through analysis of mitochondrial dehydrogenase activity, study of lipid peroxidation (quantifying cellular Malondialdehyde and 4-hydroxynonenal), intracellular Ca2+ homeostasis. Moreover, pro-inflammatory studied were also performed (activation of NLRP3 inflammasome; expression of peroxisome proliferator-activated receptor-α; mTORC1 Fox01/3a; p65/NF-κB and secretion of cytokines involved in cardiotoxicity (Interleukins 1β, 8, 6).

Results: Palmitoylethanolamide co-incubated with doxorubicin exerts vasculoprotective and cardioprotective effects, enhancing cell viability of 56.3-78.7 % compared to untreated cells ($p<0,001$ for all). Notably, PEA reduced significantly the cardiotoxicity through peroxisome proliferator-activated receptor-α −related pathways and NLRP3 inflammasome but without the involvement of calcium homeostasis. Several cytokines and chemokines were also reduced confirming its anti-inflammatory effect.

Conclusions: The present study demonstrates that palmitoylethanolamide protects against vasculotoxicity and cardiotoxicity of doxorubicin and trastuzumab by promoting an anti-inflammatory phenotype, representing a new therapeutic approach to resolve doxorubicin-induced vasculo-cardio toxicity and inflammation.

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16P Development of a 3D lung cancer model and evaluation of the efficiency of a human antibody directed against a novel target

E. Hatzidaki[1], P. Apostolou[1], I. Papasotiriou[2]

[1]Research & Development, RGCC - Research Genetic Cancer Centre S.A., Florina, Greece; [2]Research & Development, RGCC - Research Genetic Cancer Centre GmbH, Zug, Switzerland

Background: In-vitro cell models have been employed for the study of lung cancer progression and metastasis. Most often, these models are 2D cell cultures and are not representative of cancer complexity and cell interactions in vivo. Immune therapy, alone or in combination with chemo- or radio treatment is an attractive alternative for lung cancer management. We have designed a 3D model of lung cancer composed of bronchial epithelial, fibroblasts, epithelial, lung adenocarcinoma and immune cells grown in different layers in a matrigel.

Methods: Genes upregulated in the 3D culture were evaluated and an antibody against a specific over-expressed protein produced. The biological efficacy of the produced antibody on the expression of gene and various cell markers was evaluated. Expression of cell markers was determined using flow cytometry. Antibody was produced using immune cells cultured ex-vivo and activated against an immunogenic epitope of the protein-target. Antibody efficiency was calculated using gene and flow cytometry analysis.

Results: By genetic analysis, BMPR2 was one of the genes found over-expressed and was chosen as the antibody target. Lung cancer model was found to have increased expression of markers like Epcam (over expressed in lung cancer) and Notch (promotes tumor initiation). Analysis of the immune cell population only, showed an increased in the expression of CD25 (activated T and B cells), CD80/86 (APC), CD206 (macrophages), and also CTLA4 (down regulation of immune system). Incubation of the lung model with anti-BMPR2 antibody, decreased cell growth, increased immune marker expression (CD206) and decreased expression of genes involved in cancer progression (CD44, FOS, NRAS, ARAF).

Conclusions: We have developed a 3D cell culture model for lung cancer. We have identified a potential target for immunotherapy and developed and antibody against the specific target. It was found that addition of anti-BMPR2 antibody in our lung cancer model increased the expression of genes involved in immune activation and decreased genes involved in tumor progression. Our antibody could potentially be a novel therapeutic antibody for lung cancer treatment.

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17P Preclinical mouse model of palbociclib/fulvestrant resistance in hormone-receptor-positive breast cancer

Y.W. Moon[1], K. Pandey[2]

[1]Hematology and Oncology Dept., CHA Bundang Medical Center, Seongnam, Republic of Korea; [2]Radiation Oncology, UT Southwestern Medical Center, Dallas, TX, USA

Background: Breast cancer is a leading cancer in global women cancer incidence. Although cyclin-dependent kinase 4 and 6 (CDK4/6) inhibitor combined with endocrine therapy is a highly effective therapy for hormone receptor (HR)-positive breast cancer, acquired resistance ultimately occurs in almost all cases. Preclinical in vivo model is essential to study mechanisms and to develop overcoming strategies of CDK4/6 inhibitor resistance.

Methods: We performed xenografting with HR-positive breast cancer cell line in nude mice. When the tumor size reached 50-100 mm^3, palbociclib ± fulvestrant treatment was initiated. This resulted in an initial dose-dependent decrease in xenograft tumor size and subsequently acquired resistance occurred (>25% regrowth from maximal reduction) after 8-9 months. Mice were sacrificed and xenograft tumors were harvested. Using xenograft tumors, RNA microarray and whole exome sequencing (WES) were performed to find out resistance mechanisms of fulvestrant/palbociclib.

Results: We successfully established preclinical mouse model of palbociclib/fulvestrant resistance. RNA microarray revealed SNORA14B (-3.09X), TPT1(-2.33X), SNORA74A (-2.32X), SYNPO2 (2.12X), S100A7A (-2.10X), SNORD10(-1.64X), KIR2DL2 (-1.61X), PGM5 (-1.55X) as palbociclib-resistance genes. In addition, WES revealed ACLY, PRB4, and SMPD1 genes as palbociclib/fulvestrant combination-resistance genes. We also found fulvestrant resistance genes such as AGR3, ELOVL2, GFRA1, GREB1, IGF1R, IGKV1-17, NRIP1, PPM1K, TM4SF1 by WES. Those resistance genes are under further validation.

Conclusions: We established preclinical mouse model of palbociclib/fulvestrant resistance in HR-positive breast cancer. In addition we found out palbociclib/fulvestrant resistance-associated genes using this model. Our palbociclib/fulvestrant-resistance mouse model could be used for drug development to overcome CDK4/6 inhibitor and/or fulvestrant resistance.

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18P Adoptive cell therapy (ACT) with bispecific gamma delta TCR+ invariant TCR+ NKT-like cells for multiple myeloma (MM): Comparison of preconditioning (PC) on outcome

T.A. Deisher[1], P. Jarzyna[2], S.H. Sawas[2], M. Pathak[2], S. Limasi[2]

[1]Research/Administration, AVM Biotechnology, Seattle, WA, USA; [2]Research and Development, AVM Biotechnology, Seattle, WA, USA

Background: AVM0703 (AVM) is the subject of an actively enrolling US trial in relapsed/refractory (R/R) no-option Non-Hodgkin's Lymphoma (NHL), with drug related adverse events limited to grades 1-3 and durable complete (CR) and partial responses (PR) to date. Acute supra-pharmacologic doses (>6 mg/kg) of AVM0703 mobilized endogenous bispecific gdTCR+ invTCR+ Natural Killer T-like cells (AVM_NKT) (PCT/US21/19773) in mice, humanized mice and clinical trial patients1. An ACT model was conducted to verify the direct tumor killing activity of AVM0703 induced novel immune cells (AVM_NKT) and to determine the effect of different PC on tumor killing of the ACT.

Methods: MOPC315 (Balb/c) MM cells were inoculated into the flank as single cell suspensions. When tumor volume reached ~400mm^3 well-established tumors, mice were PC'd with human equivalent dose (HED) cyclophosphamide-fludarabine (CyFlu HED 500/30 mg/mm^2), with Placebo (PL) or with AVM0703 HED 18 mg/kg. ACT splenocytes (3.3M) from mice dosed with PL or AVM were iv injected 48 hours later (PL_ACT had 8,240 AVM_NKT and AVM_ACT had 150,000). The next day (13-16 hours later) the mice were euthanized and remaining live MOPC cells were measured in the tumor, spleen, bone marrow, thymus and blood by flow cytometry.