Conclusion Our data support the advantage of targeting HBP for therapeutic purpose and encourage further investigation about the use of this small-molecule as promising compound for breast cancer therapy.

**PO-260** ANTI-LYMPHOMA ACTIVITY OF NOVEL SELECTIVE GLUCOCORTICOID RECEPTOR AGONISTS (SEGRAS) IN VITRO AND IN VIVO

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Introduction Glucocorticoids (GCs) are widely used in blood cancer treatment; although, they cause metabolic disorders. Biological response to GCs is mediated by glucocorticoid receptor (GR) regulating gene expression via transactivation (TA), which requires GR binding to GC-responsive elements in gene promoters, and transrepression (TR), negative interaction between GR and transcription factors. TR mediates anticancer effects of GR, while side effects are associated with GR TA. Selective GR agonists (SEGRAs) that preferentially activate GR TR could be a better option for cancer treatment. One of well characterised SEGRAs is 2-(4-acetoxyphenyl)-2-chloro-N-methylethylammonium-chloride, or CpdA, isolated from Namibian shrub Salsola SEGRAs is 2-(4-acetoxyphenyl)-2-chloro-N-methylethylammonium-chloride, or CpdA, isolated from Namibian shrub Salsola tuberculatiformis. CpdA demonstrated anticancer activity in vitro and in vivo. We extended SEGRA list by synthesis of CpdA enantiomers and its chemical derivatives.

Material and methods Synthesis of (S) and (R)-CpdA was based on Sharpless asymmetric dihydroxylation. Chemical analogues of CpdA, CpdA01-08, were designed by appending of bulky substituent into benzene ring and to nitrogen atom or alkylation of carbon atom adjacent to chlorine atom. All experiments in vitro were carried out on Granta (lymphoma) and CEM (leukaemia) cells. Cells were treated with Dex, CpdA, (R) and (S)-CpdA, CpdA01-08. Effects on cell growth were evaluated by cell counting and flow cytometry. Affinity to GR was measured by competitive binding assay. Gene expression was measured by qPCR and Western blotting. GR and NF-kB activity was evaluated using Luciferase reporter analysis. Anti-cancer effect in vivo was determined using the model of murine lymphoma P388.

Results and discussions The most cytotoxic compounds among 10 newly synthesised, CpdA03 and CpdA05, demonstrated the highest affinity to GR. They induced GR TR but not TA and proved their SEGRA properties. Effect of CpdA enantiomers on cell growth and survival was not significantly different from Dex and CpdA. CpdA03, cytotoxic SEGRA with the highest affinity to GR, comparable with DEX and CpdA, was tested in vivo for evaluation its anti-lymphoma activity, and demonstrated 3-fold decrease of tumour size in comparison with 2-2.5-fold decrease after Dex or CpdA treatment.

Conclusion The design of synthesis and evaluation of anticancer properties of new SEGRA are provided. According to our data one novel SEGRA, CpdA03, is perspective for further investigation as anti-lymphoma drug with reduced side effects.

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**PO-261** SUBCELLULAR LOCALIZATION AND EXPRESSION OF NME6, A MEMBER OF THE NME/NM23/NDPK FAMILY, IN HUMAN TUMOUR CELLS

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Introduction Nucleoside diphosphate kinases (NDPKs) catalyse the exchange of the terminal phosphate from trinucleotides to dinucleotides through a high-energy phosphohistidine intermediate. They are encoded by NME genes and have been found, with a few exceptions, in all living beings. Besides their well-known function as key regulators of the cellular nucleotide homeostasis, they have been appointed numerous additional biochemical and biological functions. The family consists of ten members. NME1-NME4, have been extensively studied, especially NME1 in the context of metastasis formation. The NME1-NME4 are highly homologous among themselves and they all possess the NDP kinase activity in their hexameric form. The information on NME5-NME9 members is rather scarce but it is known that they display a lower level of mutual homology and apparently do not possess the NDP kinase activity. Their multimeric structure has not yet been revealed. The goal of our recent studies is revealing the subcellular localization, structure and function of NME6.

Material and methods Subcellular localization has been approached by using a specific anti-NME6 antibody and transfection of an NME6-GFP vector followed by confocal microscopy. The results have been confirmed by cellular fractionation. The expression of NME6 in human tumour cells was revealed by Western blotting with specific anti-NME6 antibodies.

Results and discussions The results of immunofluorescent techniques revealed that NME6 colocalizes with mitochondria. The cellular fractioning confirmed these results. The endogenous NME6 was not detected in the nuclear or cytoplasmic fraction. NME6 has a fairly strong expression in a panel of human melanoma cell lines and is HeLa cells, as well.

Conclusion The mitochondrial localization of NME6 has been confirmed by three different methods although it is known that it does not possess the mitochondrial signalling sequence. The expression of NME6 has been proven to be quite prominent. Since it has been shown that NME6 is an evolutionary very old gene it is obvious it has an important role in homeostasis of every living cell. Our future studies will be focused on revealing the precise localization of NME6 in the mitochondria and its role in cellular bioenergetics, the quaternary structure of the protein, its potential NDPK activity, its function in basic cellular processes and potential role in cancer onset and progression.

**PO-262** DIFFERENTIAL EXPRESSION OF SPHINGOSINE-1-PHOSPHATE METABOLISING ENZYMES IN ORAL SQUAMOUS CELL CARCINOMA

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EPIGENETIC MODULATION OF CELL METABOLISM AND ITS EFFECTS ON CELL SURVIVAL IN MELANOMA

Introduction Sphingosine-1-phosphate (S1P), a potent signalling lipid. It mediates its actions by binding to a family of G-protein coupled receptors (GPCRs), known as S1P receptors. It has been implicated in the several processes integral to carcinogenesis. S1P signalling promotes cancer by inhibiting apoptosis and by enhancing proliferation, transformation, angiogenesis and inflammation. S1P levels are regulated by eight enzymes i.e. sphingosine kinases (SphK1 and SphK2), five lipid phosphatases and a S1P lyase. The role of S1P metabolising enzymes in oral squamous cell carcinoma (OSCC) has not been fully understood.

Material and methods Here, we determined the mRNA expression profile of eight S1P metabolising genes (SphK1, SphK2, SGPL1, SGPP1, SGPP2, LPP1, LPP2 and LPP3) quantitative real-time PCR in tumour tissues of 50 OSCC patients compared with adjacent normal tissue of the same patient. We also performed immunohistochemistry for Sphk1, Sphk2, SGPP1 and LPP3 in the paraffin-embedded sections of tumour tissues of OSCC patients and normal mucosa.

Results and discussions In this study, we demonstrate that the expression of four out of eight major enzymes that regulate S1P levels were altered significantly in OSCC. Expression levels of SphK1 and SGPP1 genes were upregulated significantly in 70% and 75% OSCC tumours, respectively. Importantly, expression levels of SphK2 and LPP3 (PPAB2B) were downregulated in tumour tissue of 70% of OSCC patients. Cytosolic positivity for SphK1 was observed in majority of tumour cases in malignant squamous epithelial cells. Most of these cases showed high positivity. However, low IRS score was observed SphK2 in the tumours. Amongst the non-epithelial tissues, cytoplasmic positivity was also noted for SphK1 and SphK2 in the skeletal muscle fibres.

Conclusion Our data shows that S1P metabolising enzymes are expressed differentially in OSCC tumours, further studies are warranted to determine their role in carcinogenesis of OSCC.

MITOCHONDRIAL METABOLISM: A KEY FACTOR OF MYELOID LEUKEMIC CELL RESPONSE UPON EXPOSURE TO TYROSINE KINASE INHIBITORS

Introduction Tyrosine kinase inhibitors (TKI) have dramatically changed the prognosis of patients with leukaemia. Unfortunately, they fail to achieve durable remissions for many patients with advanced BCR-Abl leukaemia or acute myelogenous leukaemia. Myeloid leukemic cells depend on mitochondrial oxidative phosphorylation (OxPhos) and/or glycolysis to produce ATP and maintain a multi-resistant phenotype. Particularly, leukemic cells driven by oncogenes, like BCR-Abl or FLT3ITD, present high glycolysis rate. Here we investigated metabolic modifications induced by several TKI in leukaemic cells and we propose new strategies combining TKI and inhibitors of metabolism for patients with BCR-Abl and FLT3ITD leukemias.