pipeline, based on fitted beta distributions, to accurately quantify and compare alternative splicing across different types of senescent cells, relying both on public and in-house RNA-seq datasets.

**Results and discussions** Our analyses reproducibly identified, at a transcriptome-wide level, the alternative splicing changes specifically related with replicative and different types of induced senescence in multiple types of cells. For instance, Ras-induced senescence appears to associate with alterations in the splicing of genes involved in the secretory pathway and intracellular trafficking.

**Conclusion** Differential splicing analyses based on beta distribution modelling contribute to elucidate the specific alternative splicing signatures of different types of senescent cells, providing insights for targeting senescence in cancer therapies.

### Cancer Cell Metabolism

**PO-212 MORPHOLOGICAL HETEROGENEITY OF HEPATOSPLENIC GAMMA/DELTA T-CELL LYMPHOMA**

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**Introduction** Hepatosplenic gamma/delta T-cell Lymphoma (HSTL) is a systemic extra-nodal lymphoma originating from cytotoxic T-cells expressing the gd receptor. We report clinical, morphologic and phenotypic characteristics of 3 patients with HSTL.

**Material and methods** All patients were male and presented at a mean age of 23 years. One was treated with azathioprine for inflammatory bowel disease, the two others had no history of immunosuppression. All patients were admitted to the hospital with high fever and hepatosplenomegaly. Two presented severe abdominal pain and lymphadenopathy.

Laboratory studies of the three patients were different:

- **Case 1**: thrombocytopenia and bone marrow infiltration with 69% of large lymphomatous blast cells and minimal peripheral blood infiltration.
- **Case 2**: pancytopenia without excess of blasts in the peripheral blood and moderate bone marrow infiltration in cytometry. Morphological and immune-phenotypical studies of the spleen (after splenectomy) showed a massive infiltration by lymphocytic small cells.
- **Case 3**: pancytopenia with 25% and 45% of blast cells in the peripheral blood and bone marrow aspirate respectively.

Immunophenotyping showed a T lymphocytic population double negative (CD3pos CD4neg CD8neg to dim) with gamma-delta expression, CD1a− CD2 +CD5 CD7+CD56 +CD57 in all 3 patients.

Two patients underwent an initial treatment with Cyclophosphamide, Doxorubicin, Vincristine and Prednisone: the first died after 4 months, and there is no response to treatment in the second patient. Treatment was recently initiated in the third patient.

**Results and discussions** In our series, HSTL confirmed a predilection to develop most often in young men with hepatosplenomegaly. Variable degrees of hematologic abnormalities were observed. Thrombocytopenia was the most striking finding in all. Bone marrow involvement is described in approximately two thirds of patients but was observed by immunophenotyping in our 3 cases. We show that immunophenotyping seems to be the best method for the rapid characterisation of the lymphoma cells morphologically heterogeneous and difficult to identify.

**Conclusion** HSTL is an infrequent, rare aggressive tumour. The diagnosis is difficult. There is no treatment consensus and the prognosis remains poor.

**PO-213 HIGH GLUCOSE AFFECTS ER +BREAST CANCER CELL METABOLISM**

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**Introduction** In vitro high glucose (HG) level, mimicking hyperglycemia condition in vivo, has been reported to influence breast cancer cells growth, proliferation and survival suggesting glucose as being crucial for breast cancer progression and response to therapy in diabetic patients. Diabetes, in turn, might have a direct effect on breast cancer prognosis. This study investigated the impact of HG on MCF-7 breast cancer cell metabolism and phenotype.

**Material and methods** MCF-7 breast cancer cell line were cultured in DMEM with high glucose (HG 25 mM) and low glucose (LG 5 mM). Metabolic fluxes analyses were performed with Seahorse Bioanalyzer. Live cell imaging for reactive oxygen species (ROS) content was performed by confocal microscopy by using DCF-DA as selective probe. Mitochondrial DNA (mtDNA) and protein expression were evaluated by qPCR and western blotting respectively.

**Results and discussions** Using a metabolic fluxes analyses, we showed a significant reduction of the mitochondrial oxygen consumption rate (OCR) and glycolysis-related extracellular acidification rate (ECAR) in MCF-7 cultured in HG- as compared in LG-medium. According with these results, MCF-7 in HG displayed lower mtDNA amount and increased ROS level. Furthermore, the analysis of stemness markers revealed a significant upregulation of Nanog, Lin28 and Myc thus suggesting an increased stem-like phenotype due to growth in HG.

**Conclusion** Overall our results indicate that glucose may foster breast cancer progression promoting stem cell-like phenotype strongly affecting the metabolic profile in MCF-7 cell line. Further investigations are ongoing to define the mechanism underlying the switch towards an undifferentiated state to be exploited as therapeutic target.

**PO-214 COMBINATION OF MOLECULAR HYDROGEN (H2) AND 5-FUOROURACIL (5-FU) IN CANCER TREATMENT**

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**Introduction** Oxidative stress is clearly recognised as involved in cancer development, as H2 is clearly recognised as a patent
antioxidant and anti-inflammatory and potentially anticancer like activities (anti-apoptotic effect in health or injury cells). There are many factors and variables that contribute to the pathogenesis of cancers such as oxidative stress, DNA damage and mutation, ionising radiations, carcinogenic chemicals.

**Material and methods** H₂ has significant potential to reduce somatic mutation through the reduction of excessive reactive oxygen species (ROS).

Formation of ROS is strongly related to the emergence of several human pathologic conditions such as atherosclerosis, neurodegenerative diseases, and ageing as well as certain types of human cancers including lung, breast and colon.

ROS are generated in organisms by γ, X, and UV radiation, bio transformation of dietary chemicals and some diet components. Somatic mutation is a genetic alteration acquired by a cell that can be passed to another mutated cell in the course of cell division.

Moreover, it has shown through medical studies that H₂ has a protective effect against chemotherapy drugs.

**Results and discussions** To illustrate that, it is recognised a potential effect of H₂ for improving the quality of life of patients during chemotherapy by efficiently mitigating the side effects of cisplatin. That is well demonstrated that H₂ water consumption might mitigate the side effects of anticancer drugs by decreasing oxidative stress, ameliorating metamorphosis due to decreased apoptosis.

H₂ also exhibits radio-protective action by protecting the immune system.

Furthermore, H₂ may alleviate the haematological injury induced by radiation through the suppression of radiation-induced caspase 3 activation, in addition to rescuing the radiation-induced depletion of white blood cells and platelets.

Although anticancer properties of H₂ have been suggested, the mechanism(s) and efficiency by which H₂ act at the cellular level remained to be established.

It has demonstrated recently that H₂ water enhances the cancer cell apoptotic effect of 5-FU.

**Conclusion** In this study, the obtained data suggest that hydrogen water enhances the therapeutic response to retinoid administration is undoubtedly an important aspect of their use in clinical practice. Several putative biomarkers indicating sensitivity or resistance of NBL cells to retinoids were reported in recent studies. The main aim of our study was to analyse the expression of five candidate proteins (PBX1, HOXC9, HMGAl, HMGAl2 and DDX39A) in one experimental cohort (NBL cell lines; relevant FFPE tumour samples).

**Material and methods** In this study, 20 patient-derived NBL cell lines were used for the experiments. Sensitivity or resistance to natural (all-trans retinoic acid, 13-cis retinoic acid, 9-cis retinoic acid) and synthetic (fenretinide, bexarotene) retinoids was determined by MTT assay. Endogenous expression of the candidate biomarkers was analysed both on mRNA (RT-PCR) and protein (immunoblotting) levels in cell lines and on protein level (immunohistochemistry) in FFPE tumour samples. Changes in expression of these markers after treatment with retinoids were also analysed.

**Results and discussions** NBL cell lines resistant to retinoids showed either presence of HMGAl2 or increased expression of HMGA1 together with PBX1. Cell lines without a detectable expression of HOXC9 is on both mRNA and protein level are resistant to retinoids. Increase of expression of HOXC9 protein after retinoid treatment was detected in sensitive cell lines only. Very strong expression of PBX1 protein was found in tumour samples taken from patients showing resistance or poor clinical outcome after treatment with retinoids.

**Conclusion** Our experimental study confirmed the usefulness of selected putative markers indicating sensitivity or resistance of NBL cells to retinoids in one experimental cohort consisting of patient-derived cell lines and respective tumour samples.

This study was supported by the project AZV MZCR 15-34621A.

**Abstracts**

**PO-215** RESISTANCE TO RETINOIDS – ANALYSIS OF PUTATIVE BIOMARKERS IN NEUROBLASTOMA CELLS AND TUMOUR TISSUE SAMPLES

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**Introduction** Retinoids represent a popular group of differentiation inducers that are successfully used in oncology for treatment of neuroblastoma (NBL) in children. However, differentiation therapy has some limitations including toxicity and intrinsic or acquired resistance to retinoids observed in many patients. Therefore, seeking for molecular markers able to predict therapeutic response to retinoid administration is undoubtedly an important aspect of their use in clinical practice. Several putative biomarkers indicating sensitivity or resistance of NBL cells to retinoids were reported in recent studies. The main aim of our study was to analyse the expression of five candidate proteins (PBX1, HOXC9, HMGAl, HMGAl2 and DDX39A) in one experimental cohort (NBL cell lines; relevant FFPE tumour samples).

**Material and methods** In this study, 20 patient-derived NBL cell lines were used for the experiments. Sensitivity or resistance to natural (all-trans retinoic acid, 13-cis retinoic acid, 9-cis retinoic acid) and synthetic (fenretinide, bexarotene) retinoids was determined by MTT assay. Endogenous expression of the candidate biomarkers was analysed both on mRNA (RT-PCR) and protein (immunoblotting) levels in cell lines and on protein level (immunohistochemistry) in FFPE tumour samples. Changes in expression of these markers after treatment with retinoids were also analysed.

**Results and discussions** NBL cell lines resistant to retinoids showed either presence of HMGAl2 or increased expression of HMGA1 together with PBX1. Cell lines without a detectable expression of HOXC9 is on both mRNA and protein level are resistant to retinoids. Increase of expression of HOXC9 protein after retinoid treatment was detected in sensitive cell lines only. Very strong expression of PBX1 protein was found in tumour samples taken from patients showing resistance or poor clinical outcome after treatment with retinoids.

**Conclusion** Our experimental study confirmed the usefulness of selected putative markers indicating sensitivity or resistance of NBL cells to retinoids in one experimental cohort consisting of patient-derived cell lines and respective tumour samples.

This study was supported by the project AZV MZCR 15-34621A.

**PO-216** METABOLIC AND MOLECULAR PROGRAMMING INDUCED DUE TO HYPERGLYCEMIA IN BREAST CANCER

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**Introduction** Cancer and diabetes are the leading cause of mortality worldwide. Recent literature suggests alarming association between hyperglycemia and various cancers. But the complications induced due to hyperglycemia associated risk factors in breast cancer are not well established. In this study, an in depth analysis is done to understand the metabolic and molecular programming induced due to hyperglycemia in breast cancer.

**Material and methods** The effect of hyperglycemia in breast cancer was studied via various techniques. Proliferation, long term survival and molecular events were analysed using doubling time, colony formation, microarray, immunoblotting and immunofluorescence assays. Cell cycle analysis was performed via FACS PI staining. TRANSFAC approach was employed for gene sequence analysis. Pharmacological and siRNA mediated knockdown studies were used for studying targeted molecular events.

**Results and discussions** In silico screening for molecules with TFBS on cell-cycle regulating agents which were enhanced due to hyperglycemia in breast cancer cells was carried out via