

## ABSTRACTS

### 1. Katerina Jedlickova

Empirical antibiotic (ATB) therapy is a standard treatment used in intensive care units. Rational antibiotic administration may include two strategies: either antibiotic de-escalation (e.g. initial empirical treatment of antibiotics with a very broad spectrum and subsequent targeted antibiotic treatment with a narrower spectrum), or antibiotic escalation (an initial choice of fundamental broad-spectrum antibiotics, typically aminopenicillins with beta-lactamase inhibitors, which are changed to antibiotic with a broad spectrum of activity, when found ineffective).

In this retrospective study we analyzed all patients hospitalized in the Dept. of Anesthesia and Intensive Care of St. Anna University Hospital Brno (AIC) in the first quarter of 2013. Patients who underwent antibiotic treatment of longer than 24 hours and who were hospitalized for more than 48 hours were included in the study. These patients were divided into two groups: the first group was initially treated with aminopenicillins with beta-lactamase inhibitors alone, or in combination with another antibiotic ("Amino" group). The second group was treated with any other antibiotic as the first choice ("Other ATB" group). The following variables were compared: age, Acute Physiology and Chronic Health Evaluation score II (APACHE II), C-reactive protein (CRP) on admission to ICU, CRP at discharge, length of stay and survival.

## 2. Vaclav Seda

### ZAP-70 directly affects BCR signalling and migration of malignant B cells via GAB-1

High expression of ZAP-70 (70-kDa zeta-associated protein) in chronic lymphocytic leukemia (CLL) is associated with enhanced B-cell receptor signalling, migration of CLL cells and lower levels of patient survival. However, the mechanism underlying its function in CLL has not yet been well established. For this purpose, we investigated whether ZAP-70 can directly affect any proteins involved in BCR activation and AKT phosphorylation and if any of these proteins can alter migration of B cells. A total of 37 CLL samples were analysed by Western blot for expression of ZAP-70 and other proteins involved in B cell receptor signalling pathway. Two samples with ZAP-70 higher expression were stimulated with anti-IgM antibody and they were co-immunoprecipitated. We established that ZAP-70 directly affects expression and function of GAB-1, which is the docking protein involved in B cell receptor signalling. We have observed that ZAP-70 high expression in CLL cells positively correlates with the amount of GAB-1 protein ( $P=0,0003$ ). This aberrant expression of GAB-1 may be caused by the protection provided by ZAP-70, which binds GAB-1 protein, thus preventing its down-regulation. Furthermore, the dependence of AKT activation on the amount of GAB-1 protein is significant ( $P=0,0204$ ). In addition, our results indicate that migration potential of MEC-1 cells is significantly reduced after GAB-1 silencing ( $P=0,0168$ ). Taken together, our data indicate that enhanced B cell receptor signalling and AKT activation is caused by aberrant expression of GAB-1 protein in the case of ZAP-70 positive CLL cells. We conclude that the migration of malignant B cells into the supportive microenvironment of stroma cells is affected by changes in expression of GAB-1.

### 3. Katerina Cerna

MicroRNAs in DNA damage response and BCR signaling in malignant B cells.

The biology of B cell Non-Hodgkin lymphomas (NHLs) is largely influenced by (de)regulation of B cell receptor signaling and DNA damage response pathway (DDR). We and others have previously shown that miRNAs are crucial for regulation of these pathways. To investigate the DDR-related miRNAs, primary chronic lymphocytic leukemia (CLL) cells were treated with fludarabine in vitro and paired samples (before/after treatment, n=20) were analyzed for miRNAs' profile by NGS technology (HiSeq). This identified 6 differentially expressed miRNAs (FDR<0.1). To investigate these miRNAs in vivo we profiled the expression of 3 up-regulated miRNAs (miR-34a, miR-1246, miR-1248) in samples obtained from 50 CLL patients before and during the therapy containing fludarabine (FCR regiment). This confirmed up-regulation of all identified miRNAs (fold change>1.5, P<0.05). Importantly, the miR-34a level was a significant predictor (P<0.05) of patients' response to FCR therapy and progression free survival (19.9 vs. 26.4 months; HR: 2.29). A similar trend was observed for miR-1246, however, this was not statistically significant (P=0.11). Additionally, low miR-34a was an independent predictor of a shorter overall survival in a multivariate analysis (16.7 months vs. not reached; P=0.0002; HR: 3.30). This suggests that CLL cells with low levels of miR-34a fail to down-modulate genes that are crucial for DDR. Several pro-survival genes targeted by miR-34a (BIRC3, BCL2, FOXP1) were recently identified in various cell types. These proteins were down-modulated in CLL cells that up-regulate miR-34a during DDR, but not in cells that failed to induce it. Surprisingly, miR-34a, miR-1246 and miR-1248 share numerous validated targets with miR-150, a known negative regulator of BCR signaling (Mraz et al., 2014). This suggests possible convergence in the mechanism of action of DNA damaging drugs and BCR inhibitors recently approved for treatment of NHLs (e.g. ibrutinib). To compare the effects of the FCR with the ibrutinib, we analyzed miRNA/gene expression in samples from ibrutinib treated CLL patients (n=9) before and during the therapy. The convergence of pathways targeted by DDR and BCR inhibition through changes in miRNAs' expression are currently being interrogated.

#### 4. Zuzana Jašková

##### Mutated p53 Protein Elimination and Induction of p53 Downstream Genes in Chronic Lymphocytic Leukemia (CLL) Cells Using PRIMA-1MET

In CLL poor prognosis is associated with missense mutations located in p53 DNA-binding motifs, leading to accumulation of mutated p53 protein with oncogenic properties. Thus, the affected patients could benefit from therapy targeted to p53-mut protein. We tested the possibility of subtle molecular manipulation with accumulated mutated p53 in CLL cells using PRIMA-1MET declared to change p53-mut to p53-wt conformation with rescue of its activity. CLL patients' B-cells were chosen from the cohort characterized for TP53 status. Viability was determined by WST-1 assay. Impact of PRIMA-1MET on p53 protein level was analyzed by WB. The p53 downstream genes' induction was assessed by qRT-PCR. The impact of PRIMA-1MET on CLL cells' viability was assessed to determine concentration range for reactivation. We observed concentration-dependent viability curves in all samples (n=5) between 0.5-4 $\mu$ M. Therefore, 2 $\mu$ M and 4 $\mu$ M were applied for reactivation testing at molecular level. Five out of eleven mutated samples showed diminished or absent p53 protein after 4 $\mu$ M treatment and four samples manifested partially reduced level. The complete p53 protein elimination at 2 $\mu$ M was observed for mutations p.Y205H, p.R248W, and p.C275R. The p53 downstream genes' induction was pronounced for p21 and GADD45, which were induced 118 and 45-fold, respectively, compared to control. Concerning the specificity of observed effects, we noted the following: (i) in line with other studies, the impact of PRIMA-1MET on p53-wt and p53-mut samples' viability was similar, and (ii) p53 target genes' induction was also evident in p53-wt samples. Additionally, we analyzed 10 TP53-mut samples without p53 protein stabilization for downstream genes' induction. Again, the effects of PRIMA-1MET were apparent, especially for p21 and GADD45. We demonstrated that PRIMA-1MET can effectively manipulate p53 protein accumulated due to the high-risk TP53 mutations. What remains to be elucidated is the specificity of the p53 pathway activation with respect to TP53 status.

## 5. Veronika Olejnickova

Voltage sensitive dye (VSD) represents an important tool for the study of cardiac electrophysiology. This touchless method allows simultaneous monitoring action potential of the cardiac tissue with high space and temporal resolution. The VSD mapping is based on excitation of the dye molecules bound to the cell membrane under fluorescent light. Consequently, the excitation of the molecules results in emission of light and shifting of the emitted light spectrum. The spectrum is proportional to the transmembrane potential of the cells. Nevertheless, there is a concern about interference with measured parameters due to bounding of the dye to the cell membranes. The slowing of cardiac impulse propagation in the presence of the VSD was shown. There is no evidence about impact of VSD to the conduction system of the heart, however, slowing of the impulse propagation may subsequently resulted in disturbances in generation of heart rhythm or to its block, which, in turn may trigger arrhythmias. The aim of this study was therefore to evaluate the influence of the most employed VSD di-4-ANNEPS to the cardiac conduction system in isolated rabbit hearts. The study was performed in Langendorff isolated rabbit hearts. The electrograms (EGs) were recorded by three orthogonal touch-less system. The effect on the cardiac conduction system was assessed by change in the heart rate (HR), EG morphology and arrhythmogenicity scoring. Perfusion of the di-4-ANNEPS (1 $\mu$ M) leads to the slowing of the HR to 70%. QT and QTc interval and EG morphology were not changed. Moderate arrhythmias were observed, mainly AV blocks and single ventricular complexes. The arrhythmogenicity score according to Lambeth was 0,4. We can conclude that 1 $\mu$ M di-4-ANNEPS slows heart rate and leads to the disturbance of heart rhythm in isolated rabbit hearts. The findings strongly suggest direct impact of the di-4-ANNEPS to the conduction system of the heart.

## 6. Gabriela Pavlasova

Bone marrow stromal cells protect CLL cells from rituximab effects, but do not induce down-modulation of CD20 expression

Rituximab, a monoclonal antibody against CD20, is widely used in treatment of B-cell non-Hodgkin lymphomas and chronic lymphocytic leukaemia (CLL). However, it has been shown that microenvironmental interactions protect mature B cell malignancies from rituximab induced apoptosis and complement-dependent cytotoxicity. Therefore, we tested whether bone marrow (BM) stromal cells (HS-5) cause down-modulation of CD20 on CLL surface.

We isolated CLL cells from peripheral blood (PB) and cultured them with HS-5, or on plastic alone as a control, for 24, 48, 72 hrs and 1 week. Flowcytometric analysis of CD20 expression was performed only on Annexin-V neg/CD105 neg cells. We found that CLL cells spontaneously down-modulate CD20 expression when cultured on plastic in vitro, and the co-culture with HS-5 up-regulates CD20 ( $P=0.03$ ). Additionally, isolated CXCR4dim/CD5bright cells, representing CLL cells that had been recently released from the lymph node (LN) microenvironment, expressed higher surface levels of CD20 when compared to CXCR4bright/CD5dim from the same patient. This finding suggests that the in vivo contact with immune niches also up-regulates CD20. Finally, to further investigate the effect of microenvironment on CLL cells in vivo, gene expression profiling of CLL cells purified from PB, BM and LN was performed. A statistically significant change in CD20 expression was observed in only 1 of 4 analyses (using different probes), when comparing PB and BM or LN. In this case, the expression of CD20 was slightly higher in BM/LN than PB ( $P=0.0482$ ;  $P=0.0495$ ).

In summary, these data indicate that down-modulation of CD20 by bone marrow stromal cells is not likely to be the major mechanism of stroma mediated rituximab resistance of CLL cells.

## 7. Katerina Musilova

Low-level expression of miR-150 in follicular lymphoma is related to higher B cell proliferation rate and unfavourable disease prognosis

We and others have shown that deregulation of microRNAs (miRNAs) is associated with the pathogenesis and the progression of B-cell malignancies. Moreover, miRNAs can be used as strong predictors of prognosis in chronic lymphocytic leukaemia (CLL), diffuse large B-cell lymphoma (DLBCL), and mantle cell lymphoma (MCL). However, their role as prognostic markers in follicular lymphoma (FL) has not been investigated yet.

To address this issue we selected three miRNAs (miR-155, miR-181b, miR-150) which play critical roles in B-cell development and their aberrant expression was shown to affect the prognosis of CLL, DLBCL, and MCL patients. We analysed the expression of these miRNAs in 89 FL samples (fresh frozen and formalin-fixed paraffin-embedded [FFPE] tissues). miR-150 expression levels were significantly lower in the patients with high Ki67 positivity (>20%;  $P=0.003$ ) and high FLIPI score (3-5;  $P=0.03$ ). We also observed significantly reduced miR-150 levels in FLs that transformed to DLBCL compared to the samples before transformation ( $P=0.01$ ), and miR-150 was significantly less expressed in DLBCL than FL (4-fold change,  $P<0.001$ ). Furthermore, FL patients with low miR-150 levels (<median) had significantly shorter survival levels (6.2 years vs. not reached;  $P=0.007$ ; HR 3.0 [CI: 1.3-6.8]). In contrast, miR-181b and miR-155 did not associate with the clinical course or the survival of FL patients.

In conclusion, our results suggest that miR-150 plays an important role in the clinical course of FL and that it could be used as a prognostic marker. This is, according to our knowledge, the first miRNA-based prognostic marker in FL, and one of the few available RNA markers that can be used both for FFPE and fresh tissue.

## 8. Lucie Poppova

Chronic lymphocytic leukaemia (CLL) is an incurable, slowly growing malignancy, affecting mostly the elderly population. Although slowly growing, the clinical outcome of CLL differs considerably among individual patients, from indolent disease with no requirement for treatment, to aggressive disease with urgent requirement for treatment. Recently, it was reported that components of the noncanonical WNT/PCP pathway are overexpressed in CLL and WNT pathway is implicated in disease pathogenesis via changes in cell migration.

In this study, we contribute to clarifying the role of the WNT3 ligand in CLL. Using quantitative real-time PCR, we assessed WNT3 expression in peripheral blood (PB) B cells from 137 previously untreated CLL patients and compared the results with the clinical data of the patients and with WNT3 expression in PB and tonsillar non-malignant B-cells. WNT3 was significantly upregulated over non-malignant control and we detected significant correlations between low WNT3 levels and shorter treatment free survival of the patients. The strongest correlation was observed within patients with mutated IGHV (generally good prognosis). Furthermore, we revealed the relation of low WNT3 levels to mutation in SF3B1 and NOTCH1 genes, which were previously published in connection with worse prognosis of CLL patients.

To conclude, low WNT3 expression is a useful marker defining rapidly progressing patients within the IGHV-mutated subgroup.

## 9. Michal Jez

Cell adhesion belongs among interactions that take place in the microenvironment of lymph nodes and bone marrow and which are responsible for progression of chronic lymphocytic leukaemia (CLL). In particular, the cell adhesion is considered to be a key event supporting therapy resistance of the CLL cells with its crucial regulator focal adhesion kinase (FAK). In order to understand its function in CLL biology, we examined expression of PTK2 gene (encoding FAK) in CLL samples. A cohort of 64 randomly chosen and well-characterised CLL patients was analysed using qRT-PCR, and the expression data were analysed with routinely used parameters in CLL diagnostics. P-values were calculated using the Mann-Whitney test. Generally, the expression levels of PTK2 varied from values at the detection limit to samples with 1000-fold amounts. Comparing stages of the disease, patients at lower stage (RAI 0-II; n=34) had significantly higher expression of PTK2 than patients at higher stage (RAI III-IV; n=27;  $P < 0.05$ ). For survival prognosis (counted from the date of diagnosis), we divided the patients into two groups according to PTK2 expression median. The group with lower expression (n=29) had a median survival of 108 months, whereas the group with higher expression (n=34) had a median survival of 173 months ( $P = 0.09$ ; HR=2.126). Evaluating other parameters (p53, IgHV, cytogenetics, age and sex) showed no significant difference in PTK2 expression. In conclusion, FAK expression plays a significant role in CLL and ongoing analyses will help with its deciphering.

## 10. Vlasta Stepanova

Plasma treatment of glass enhances surface wettability, adhesion and cleanness. Surface properties of glass play a critical role in applications (thin film coating, glass gluing, glass production, etc.). The aim of this study is to compare plasma treatment of soda-lime glass with dielectric barrier discharge (DBD) and with gliding arc. Soda-lime glass was chosen for its homogeneity and small initial roughness. Both plasma discharges operate at atmospheric pressure and air is used as a carrier gas. The benefit of dielectric barrier discharge is the large discharge area and plasma homogeneity. Glow discharge has a higher temperature of produced plasma. The DBD plasma source is a home-made device from our research group, whereas the gliding arc is a commercial plasma source from Diener Instruments. The duration of plasma treatment was the same for both plasma sources, specifically 15 s and 30 s. The observed parameters were contact angle, roughness and change of chemical bonds on the surface. Contact angle and surface energy were measured using Surface energy evaluation System. Atomic force microscopy was used for evaluation of surface tomography and calculation of roughness. Change of surface chemistry was studied using X-ray photoelectron spectroscopy. The results indicate that DBD plasma is more efficient for improvement of hydrophilicity than gliding arc. Roughness after DBD plasma treatment stays stable. Plasma treated glass with glow discharge was also hydrophilised after plasma treatment, however, the roughness of plasma treated glass with glow discharge increased. The increase of roughness is an undesirable effect because of applications. Shorter time of plasma treatment (15 s) was sufficient for wettability change, while longer plasma treatment time (30 s) was more stable in time. More significant chemical changes on the surface between untreated and plasma treated glass were observed after DBD treatment. The conclusion is that DBD discharge is a more suitable device for plasma treatment of glass than gliding arc.