# SYMPOSIUM ON ADVANCES IN MOLECULAR HEMATOLOGY 1: LYMPHOMA PATHOGENESIS

## 2915. ABILITY TO DOWNREGULATE THE LEVEL OF CYCLIN-DEPENDENT KINASE INHIBITOR P27KIP1 AFTER DNA DAMAGE IS RETAINED IN CHRONIC LYMPHOCYTIC LEUKEMIA CELLS WITH FUNCTIONAL ATM/P53 SIGNALING PATHWAY

**Rašková Kafková L., Jarošová M., Navrkalová V., Loja T., Chovancová J., Fialová Kučerová J., Šimková D., Procházka V., Pospíšilová Š., Divoký V.** (***Department of Biology, Faculty of Medicine and Dentistry, Palacky University, Olomouc; Department of Hemato-oncology, Faculty of Medicine and Dentistry, Palacky University and University Hospital, Olomouc; Center of Molecular Medicine, CEITEC - Central European Institute of Technology, Masaryk University, Brno***)

DNA-damage response (DDR) plays a key role in sensitivity of CLL to chemotherapy. The activity of the key DDR kinases, ATR and ATM, is largely compromised in CLL cells. Non-cycling CLL cells lack ATR protein expression, and ATM is commonly targeted for inactivation by genetic abnormalities of the ATM gene, such as deletion of 11q22.3-q23.1 chromosome bands involving ATM. Biallelic inactivation of ATM (often a mutation on one allele and a deletion of the other allele, mut/del ATM) results in impaired DDR. In this study, we combined genetic screening of ATM and TP53 with functional assay of DDR and searched for a marker that would enable a rapid evaluation of sensitivity of CLL samples carrying 11q22-q23 deletion to DNA damage. Since decreased p27Kip1 level by caspase cleavage represents a key step in chemotherapy-induced apoptosis in CLL cells, we hypothesized that p27Kip1 function may represent a marker link between functionality of the canonical DDR pathway and non-DDR cell cycle regulatory proteins in CLL, and therefore, can serve as a surrogate marker for ATM/p53 deficiency. We analyzed 18 CLL samples carrying 11q22-q23 deletion for the presence of mutations on the residual ATM allele using next-generation deep sequencing. Based on the results we divided our samples into two groups – a group with samples carrying wild type ATM allele (wt/del ATM samples) and the second group with samples carrying mutated form of ATM allele (mut/del ATM samples). Next we aimed to unravel the functionality of ATM by analysis of phosphorylation of p53 on Ser15, an ATM target site, in response to ionizing radiation (IR). This screening divided our CLL samples into two groups - with ATM/p53 functional and ATM/p53 non-functional pathway. We found apparent functionality of ATM/p53 pathway in some mut/del ATM samples and on the contrary non-functionality of ATM in some wt/del ATM samples. Apparently, some ATM mutations do not completely abolish ATM activity. On the other hand, we assume the involvement of other factors impacting on the expression and activity of ATM in the wt/del ATM samples with lack of p53-Ser15 phosphorylation. Next we characterized changes in p27Kip levels in response to IR in our groups with ATM/p53 functional and ATM/p53 non-functional pathway. To assess degradation or accumulation of p27Kip after DNA damage, we used western blotting and flow cytometry (FACS) analyses. Our comparative experiments suggest that retention of ATM kinase activity in CLL cells is essential for downregulation of the level of p27Kip1 after DNA damage, or, in reverse, our data suggest a close association between the non functionality of ATM/p53 pathway and accumulation of p27Kip1 in CLL cells in response to DNA damage. In conclusion, we propose that accumulation of p27Kip1 after DNA damage is a suitable marker for characterization of non-functionality of ATM in CLL cells, i.e. it is associated with lack of ATM/p53-Ser15 activity. Our experiments also highlight importance of functional test assessing ATM activity in CLL as some wt/del ATM CLL B-cell samples lack phosphorylation of p53-Ser15 and accumulate p27Kip1 levels in response to DNA damage, i.e. behave as samples with mut/del ATM genotype. We also provide evidence that multicolor flow cytometry is a powerful tool for p27Kip1 level quantification in B-CLL samples after experimentally induced DNA damage and that such measurement of p27Kip1 degradation or accumulation can serve as a surrogate marker for ATM/p53 functionality in B-CLL cells. Supported by: IGA NT13576 from Ministry of Health, Czech Republic

## 2858. IBRUTINIB INHIBITS CD20 UP-REGULATION ON CLL B CELLS MEDIATED BY THE CXCR4/SDF-1 AXIS

**Pavlasova G., Borsky M., Seda V., Cerna K., Doubek M., Mayer J., Pospisilova S., Davids S. M., Brown R. J., Mraz M.** ***(IHOK FN, LF MU a CEITEC MU, Brno – CZ; FN, Brno – CZ; IHOK, FN, Brno – CZ; IHOK FN a CEITEC MU, Brno – CZ; Dana-Farber Cancer Institute, Boston – USA; FN a CEITEC MU, Brno – CZ)***

BACKGROUND: It was shown that BCR inhibitors such as ibrutinib interrupt microenvironmental interactions and mobilize CLL cells from lymph node niches and bone marrow into the blood stream. Therefore, it has been suggested that a combinatorial therapy of BCR-inhibitors with anti-CD20 or other antibodies might be an effective therapeutic combination. AIMS: The aim of this study was to test for the effect of ibrutinib on the expression of selected CLL cell-surface molecules that could be potentially targeted by available monoclonal antibodies. RESULTS: We performed gene expression profiling in samples obtained from CLL patients treated with ibrutinib as a single agent (pre-ibrutinib vs. day 15 and/or week 5/12) and analyzed changes in >20 cell-surface molecules that could be potentially targeted by different available therapeutic antibodies. Surprisingly, we observed that CD20 mRNA had the most significantly changed expression (down-modulation of 3.4-fold; P<0.0001). This suggested that CD20 expression might be regulated by a yet unknown mechanism in the context of microenvironmental interactions impaired by ibrutinib. The co-culture of primary CLL cells with stromal cell line HS-5 induced higher CD20 surface levels on CLL cells, and ibrutinib inhibited this CD20 up-regulation. Then we assessed the CD20 expression on CLL cell populations defined according to CXCR4 and CD5 levels. CLL cells that have recently exited the lymph node microenvironment to the peripheral blood express lower levels of chemokine receptor CXCR4 and higher levels of activation marker CD5 (CXCR4dimCD5bright cells) than those cells circulating in the blood stream for a long time (CXCR4brightCD5dim cells). The CXCR4dimCD5bright cells had 2-times higher CD20 surface as well as mR-NA expression (P<0.01) suggesting that changes of CD20 levels within immune niches reflect the changes in its transcription. Moreover, CD20 expression gradually decreased with the transition of CLL cells from CXCR4dimCD5bright to CXCR4brightCD5dim (P<0.01). This led us to hypothesize that CXCR4/SDF-1 is directly implicated in CD20 regulation. Indeed, in vitro treatment of CLL cells with SDF-1α (ligand for CXCR4 produced by stromal cells) up-regulated CD20 expression (P<0.01). The application of plerixafor (CXCR4 inhibitor) or ibrutinib abolished the SDF-1 mediated CD20 up-regulation (P<0.01). CONCLUSION: We have described the first known mechanism of CD20 regulation in CLL cells. The CXCR4/SDF-1 axis up-regulates CD20 expression in CLL B cells, this is inhibited by ibrutinib. Supported by the MSMT project CEITEC 2020 (LQ1601); GACR (GA16-13334Y); Ministry of Health of the Czech Rep, grant nr. 16-29622A; TACR (TEO2000058/2014-2019); MUNI/A/1028/2015; MSMT COST CZ (LD15144); Horizon 2020 (No. 692298); and G.P. is a city of Ostrava scholarship holder. This publication reflects only the author's views and the Union is not liable for any use that may be made of the information contained therein.

## 2976. MICROENVIRONMENT-DEPENDENT DRUG RESISTANCE THROUGH BCL2 FAMILY UNBALANCE IN MANTLE CELL LYMPHOMA

**Chiron D., Bellanger C., Dousset C., Maiga S., Touzeau C., Le Gouill S., Amiot M., Pellat--Deceunynck C.** ***(INSERMU892 CNRS6299, Université de Nantes, Nantes – F)***

Extrinsic signaling from soluble and cellular surrounding environment supports the progression of most cancers, including B-cell lymphomas. Mantle cell lymphoma (MCL), which initially accumulates in lymphoid organs, disseminates early on in extranodal tissues but the nature of pro-tumoral signals resulting from these various microenvironments is still unclear. In the present study, we observed that both survival and cell-cycle progression are strongly dependent on extrinsic signals in MCL. The evaluation of cytokine receptors expressed in situ and comparison of mesenchymal or lymphoid-like interactions allowed us to develop a unique ex-vivo model that supported long-term expansion of primary malignant cells. To identify microenvironment-dependent molecular modulations we have initiated the transcriptomic analysis of primary MCL in coculture. Preliminary results confirmed that our model efficiently mimicked molecular modulations observed in lymphoid tissues, such as proliferation and NFkB signatures and unbalanced regulation of the Bcl2 family towards survival. The latter led to a decrease in mitochondrial priming and a consequent selective drug resistance to BH3-mimetics or alkylating agents. Integration of extrinsic signals from the multiple components of the microenvironment increases our understanding in drug resistance and

will offer opportunities to develop novel mechanism-based therapeutic strategies in incurable lymphomas.

### 3001. CRISPR/CAS9-MEDIATED MUTAGENESIS OF MIR-155 REPRESENTS POTENTIALLY EFFICIENT TOOL IN SEARCHING FOR NOVEL MIR-155 PROTEIN TARGETS IN CHRONIC LYMPHOCYTIC LEUKEMIA

**Vargová K., Simerský R., Lenobel R., Vargová J., Zikmund T., Savvulidi F., Šebela M., Stopka T.** (***Depts. Biocev and Pathophysiology, 1st Faculty of Medicine, Charles University, Praha; Centre of the Region Hana for Biotechnological and Agricultural research, Faculty of Science, Palacký University, Olomouc***)

Chronic lymphocytic leukemia (CLL) is most prevalent B-cell leukemia with lymph node involvement, leukemization and relatively heterogeneous outcome. The CLL pathogenesis involves distinct cytogenetic changes and somatic mutations, however, the understanding of the disease heterogeneity and its aggressiveness is not complete. Therapy of CLL is usually initiated when malignant clone accelerate proliferation or infiltrates bone marrow (to suppress myelopoiesis) or causes un-tolerated enlargement of liver, spleen or lymph nodes. Therapy of CLL is oriented to suppress viability of B-cells using immuno-chemotherapy. MicroRNAs are ~22-nt long hairpin like structures that are posttranscriptional inhibitors of gene expression via associating with mRNAs within the miRNA-induced silencing complex. miRNAs are transcribed similarly as mRNAs from genes or gene clusters. Among over thousands of miRNAs, there exist miR-155, which has been repeatedly found overexpressed in CLL and other hematologic malignancies. Mouse model of miR-155 overexpression confirmed its role as oncogene. Number of target mRNAs was reported including transcription factor PU.1 or Src homology-2 domain-containing inositol 5-phosphatase 1 (SHIP1). To advance our understanding of CLL pathogenesis we utilized genome-editing technology of clustered regularly-interspaced short palindromic repeats (CRISPR) and introduced mutations of the miR-155 sequence that is known to inhibit expression of PU.1 and SHIP1 and utilized for this the MEC-1 (CLL-derived) cell line. Individual clones were isolated and the miR-155 level as well as levels of its targets was determined. We asked what other targets than PU.1 and SHIP1 were upregulated (or alternatively downregulated) upon loss of miR-155 and performed a quantitative proteomic analysis. Our data suggest that hundreds of protein targets are differentially expressed upon miR-155 mutagenesis, some of them being not yet predicted on mRNA level using tools such as miRBase or TargetScan. We can conclude that nucleotide-specific CRISPR edited mutations of miR-155 in CLL cells revealed a candidate set of proteins that are potentially important therapeutic targets for developing CLL-specific therapies.

# KONFERENCE OŠETŘOVATELSTVÍ 1

### 2971. NEPŘÍBUZENSKÉ DOBROVOLNÉ DÁRCOVSTVÍ KOSTNÍ DŘENĚ JE ALTRUISMEM V PRAXI. AKTUÁLNÍ STAV VE SVĚTĚ I V ČR SE ZAMĚŘENÍM NA ČESKÝ NÁRODNÍ REGISTR DÁRCŮ DŘENĚ

**Jindra P., Navrátilová J., Pagáč D.** (***Český Národní Registr Dárců Dřeně, Plzeň***)

Program registrů nepříbuzných dobrovolných dárců krvetvorných buněk (KB) vznikl před více než 40 lety v Anglii (v roce 2014) jako výraz snahy maminky nemocného syna bez dárce v rodině nalézt pro něj nějakého dárce. V následujících letech došlo etablování tohoto programu prakticky ve všech vyspělých zemích, takže aktuálně je pro všechny pacienty potřebující transplantaci KB bez dárce v rodině k dispozici téměř 28 miliónů dárců v 53 zemích. V současnosti tento program zajišťuje 70-80 % celosvětově prováděných alogenních transplantací a ročně je provedeno více než 16 000 nepříbuzných transplantací, což představuje 46 výkonů denně. Přibližně 50% nepříbuzných daruje pro pacienta v zahraničí, který nenalezne dárce v domácím registru, což znamená, že denně 23 štěpů KB překračuje hranice států. Celý program registrů a procedura darování včetně transportů štěpů k pacientovi jsou velmi striktně regulovány a standardizovány tak, aby byla zaručena kvalita KB a zaručena bezpečnost jak dárců, tak transplantovaných pacientů. Tyto standardy jsou zpracovány organizací sdružující všechny registry - tzv. World Marrow Donor Association (WMDA). Registry splňující všechna přísná kritéria a standardy jsou touto organizací akreditovány a je úspěchem, že Český národní registr dárců dřeně tuto akreditaci získal jako 4. registr na světě již v roce 2005 a následně ji 2x úspěšně