

## 1 ABSTRACT

B-cell non-Hodgkin lymphomas (B-NHL) represent the most common mature lymphoproliferative diseases. B-NHL arise at different stages of B-cell development and represent their malignant counterpart. Diffuse large B-cell lymphoma (DLBCL) and mantle cell lymphoma (MCL) are aggressive types of B-NHLs. Deregulation of cell cycle control, inhibition of apoptosis or abnormal DNA damage response play a key role in the pathogenesis of DLBCL and MCL. Aberrant activation of several signaling pathways that further promote survival, cell proliferation or affect the tumor microenvironment have been recently recognized. Increased understanding of the oncogenic mechanisms implicated in pathogenesis of B-NHL lead to development of novel agents that target the oncogenic drivers of distinct lymphoma subtypes.

MCL is an aggressive subtype of B-NHL associated with poor prognosis. *In vivo* models of human MCL for experimental therapy are however scarce. We established and characterized several mouse models of human MCL by xenotransplantation of either primary cells or established cell lines into immunodeficient mice (publication no 1). We demonstrated that engrafted MCL cells displayed complex changes of gene expression profile, phenotype and sensitivity to cytotoxic agents compared to the original *in vitro* growing control cell lines. These results can contribute to the preclinical research.

The randomized clinical trial of European MCL network demonstrated that the alternation of R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone) with R-DHAP (rituximab, dexamethasone, cisplatin, HDAC) significantly improves the overall survival of MCL patients compared to R-CHOP alone. So far it has not been elucidated which component of the DHAP regimen (cisplatin, cytarabine, or the combination of both agents) is the key contributor of improved efficacy of the regimen. Recently published results of the Nordic Lymphoma Group MCL trial 5 showed, that single HDAC (in combination with rituximab) is insufficient for the treatment of younger patients with aggressive MCL and the study was prematurely terminated. Using our mouse models of human MCL we experimentally showed, that cisplatin, alone or with cytarabine, is significantly superior to single-agent cytarabine in eliminating lymphoma cells (publication no 2). Our data confirmed the results of the Nordic Lymphoma Group MCL trial 5 concluding that single-agent cytarabine is not appropriate treatment for biologically aggressive MCL.

*BCL2* (B-cell lymphoma 2) gene is deregulated in subsets of DLBCL and overexpression of BCL2 protein is associated with adverse prognosis. The expression status as well as the role of other two key antiapoptotic BCL2 proteins such as MCL1 (myeloid-cell leukemia sequence 1) and BCL-XL (B-cell lymphoma-extra large) for the survival of DLBCL is less clear. By targeting of BCL2 proteins we demonstrated that DLBCL can be divided into biologically distinctive subgroups: BCL2 and/or MCL1 dependent, with minor role left for BCL-XL. BCL2-positive subgroup comprises both BCL2- and MCL1-dependent cells that might be pharmacologically targeted by specific BCL2 inhibitor, drug entitled venetoclax (ABT-199 / GDC0199), plant alkaloid homoharringtonin (HHT), or the combination of both agents (with a marked synergistic efficacy). BCL2-negative DLBCL subgroup appears to be predominantly MCL1-dependent (publication no 3). Our data might have direct implications for novel concepts of experimental therapy of DLBCL targeted at BCL2 and/or MCL1 using ABT-199 and HHT, single agent, or in combination

Implementation of high-dose cytarabine (HDAC) into induction therapy became standard-of-care for all newly diagnosed younger MCL patients. However, many patients relapse even after HDAC-based regimens. Molecular mechanisms responsible for cytarabine resistance in MCL (in contrast to acute leukemias) are unknown and optimal treatment strategy for relapsed/refractory MCL patients remains elusive. In order to study the mechanisms responsible for cytarabine resistance we derived several cytarabine-resistant clones from established MCL cell lines (see publication no 3). We demonstrated that acquired resistance of MCL cells to cytarabine is associated with marked downregulation of deoxycytidinkinase (DCK), an enzyme responsible for the first phosphorylation and thus activation of nucleoside analogs following their entry to the cell. The downregulation of DCK results not only in cytarabine resistance but leads to cross resistance to other nucleoside analogs used in the therapy of MCL (i.e. fludarabine, cladribine, gemcitabine). These results were confirmed in vivo using our established mouse models of human MCL (publication no 4). Our data suggest that nucleoside analogs (namely fludarabine, cladribine and gemcitabine) should not be used for the second-line therapy of MCL patients, who fail after cytarabine-based regimen.