**Maternal Embryonic Leucine Zipper Kinase (MELK) Drives a High-Risk Gene Network and Represents an attractive Novel Drug Target in Multiple Myeloma and its associated Bone Disease.**

Roy Heusschen\*, Arnold Bolomsky\*, Karin Schlangen, Kathrin Schönfelder, Josephine Muller, Wolfgang Schreiner, Niklas Zojer, Jo Caers#, Heinz Ludwig#

Recent advances in therapy improved the outcome for multiple myeloma (MM) patients. However, effective treatment options are still lacking for high-risk patients. A better understanding of high-risk disease is therefore needed for the development of novel therapeutic options. Here, we report maternal embryonic leucine zipper kinase (MELK) is a promising novel drug target in this setting.

Analysis of primary MM cell gene expression profiling data (n=551) revealed that MELK expression was elevated in the high-risk proliferation (PR) associated subgroup compared to healthy donor plasma cells, but barely detectable in other MM subgroups. In accordance, high MELK expression negatively impacted overall survival in the TT2, TT3 and apex trial protocols. In addition, MELK expression was increased in relapsed patients compared to baseline, suggesting a role in drug resistance.

MELK expression was detected in 8/8 human MM cell lines (HMCLs). Treatment with MELK inhibitor OTSSP167 downregulated MELK protein levels and dose-dependently reduced both primary and HMCL cell viability. OTSSP167 induced apoptosis in HMCLs as assessed by several approaches. This was accompanied by a downregulation of IRF4 and MCL-1 expression. In addition, OTSSP167 induced G2/M cell cycle arrest linked to downregulation of CCNB1, AURKA and PLK-1. Importantly, we observed reduced clonogenic growth of MM cells treated with OTSSP167 and the activity of OTSSP167 was upheld in the presence of bone marrow stromal cells. Finally, OTSSP167 reduced the protein levels of other factors that are elevated in the PR subgroup, including FOXM1, EZH2 and DEPDC1. Knockdown of MELK in HMCLs confirmed a relationship between MELK and these factors. In vivo, MELK inhibition led to a reduction in tumor load in the 5TGM.1 model as determined by bone marrow infiltration, spleen weight and serum IgG2b levels. MELK inhibition also prevented bone disease in these mice, as assessed by micro-computed tomography, and direct effects on bone cells have been observed.

The current study identifies high expression of MELK in the PR subgroup of MM patients and our results suggest a central role for MELK in the proliferative signaling network that characterizes this subgroup. We report preclinical results that support initiating clinical testing of MELK inhibition.