COTARGETING OF PIM, PI3K AND mTOR IN MANTLE CELL LYMPHOMA

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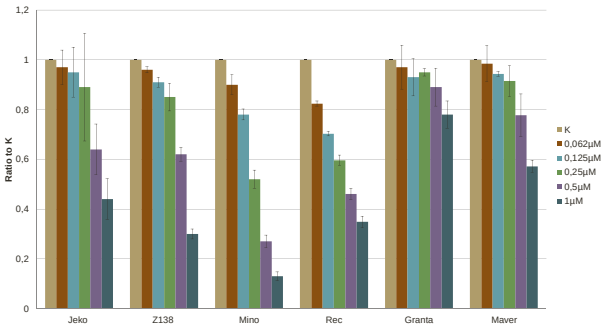
Background

Mantle cell lymphoma (MCL) comprises about 6% of all non-Hodgkin's lymphoma with a median survival of 3-5 years. The proviral insertion in murine (PIM) lymphoma proteins are serine/threonine kinases which play an important role in cell survival and proliferation. They are overexpressed in different human cancers, however mainly in haematological malignancies. In this study we evaluated the efficiency and mode of action of a dual PIM/PI3K and a triple PIM/PI3K/mTOR-Inhibitor (IBL301) in MCL cell lines.

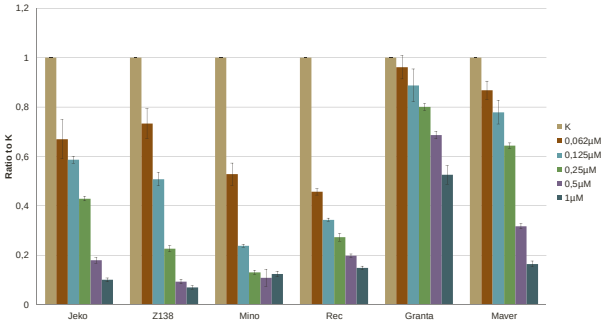
Methods

MCL cell lines (Granta 519, Jeko-1, Rec-1 and Mino), as well as primary cells were exposed to a combined PIM-kinase/PI3K inhibitor (IBL202) and a combined PIM-kinase/PI3K/mTOR Inhibitor (IBL301). Cell proliferation (trypanblue staining), cell death induction (Annexin V PE/7-AAD staining) and cell cycle (FACS) were investigated. Protein expression and phosphorylation status of different downstream proteins (Akt, GSK-3β, 4EBP1) as well as markers of apoptosis (PARP, Caspase 9) were analysed after 1h, 4h, 8h and 24h. CellTiter-Glo® reagent was obtained by Promega and performed after 48h according to manufacturer's instruction.

MCL cell lines treated with IBL202 for 72h

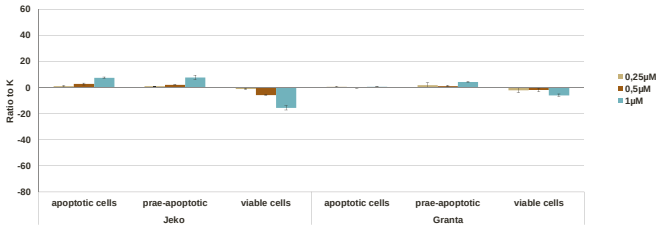


MCL cell lines treated with IBL301 for 72h

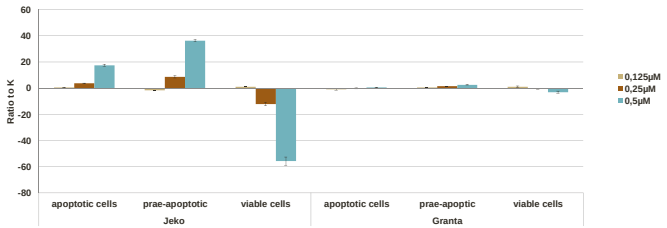


Cell Death Analysis

Jeko and Granta treated with IBL202 for 72h

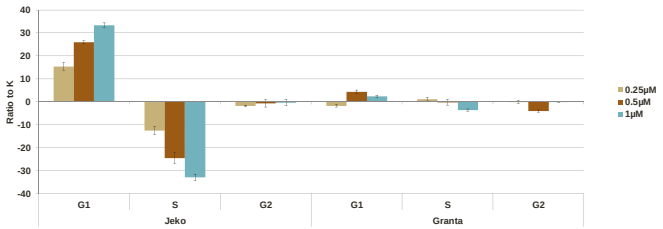


Jeko and Granta treated with IBL301 for 72h

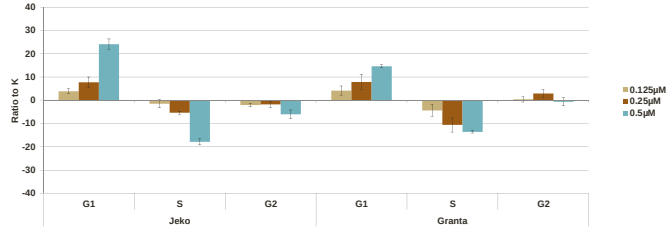


Cell Cycle Analysis

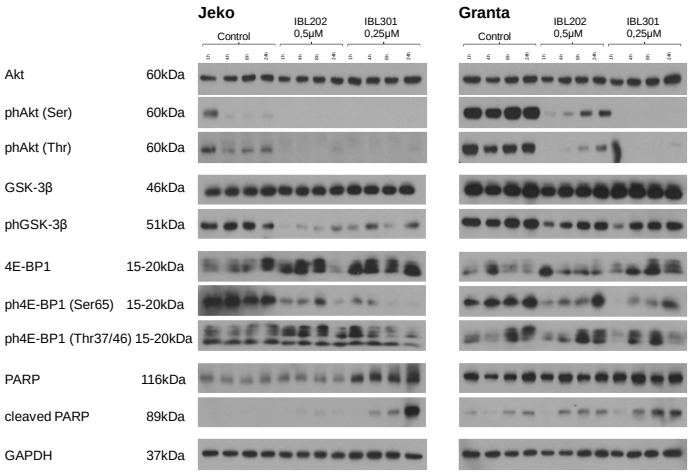
Jeko and Granta treated with IBL202 for 24h



Jeko and Granta treated with IBL301 for 24h



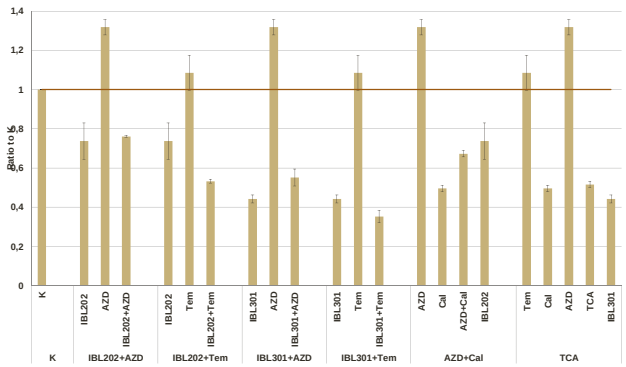
Western Blot Analysis



Both, IBL202 and IBL301 show a significant decrement in phosphorylation of Akt, GSK-3β and 4E-BP1 (Ser65). Levels of unsphosphorylated proteins are not affected. PARP cleavage was more pronounced in IBL301 treated cells.

Combination treatment of MCL primary cells with IBL202, IBL301, AZD, temsirolimus and idelalisib (CellTiter-Glo® Viability Assay):

IBL202 62,5nM, IBL301 31,25nM, AZD 2,5µM, idelalisib 1,25µM, temsirolimus 5nM



Conclusion

Triple inhibition of PIM kinases, PI3K and mTor is very efficient in MCL cell lines as well as in primary cells, exceeding dual inhibition of PIM kinases and PI3K. Cotargeting PIM kinases, PI3K and mTOR is a promising novel approach for MCL treatment.