Initial Evaluation of Novel Dual PIM/PI3K and Triple PIM/PI3K/mTOR Inhibitors in Multiple Myeloma.

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Introduction

Multiple myeloma (MM) is characterised by clonal expansion of malignant plasma cells in the bone marrow (BM). Despite significant advances in treatment it remains incurable in part due to the supportive role the BM environment plays in migration, survival, proliferation and drug resistance. BM microenvironmental signalling along with other factors such as treatment with proteasome inhibitors (PI) can contribute to activation of the PI3K/AKT survival pathway. The redundancy of signalling pathways provides back-up mechanisms allowing escape from targeted inhibition. One such compensatory pathway is that driven by PI3K enzymes, which produce parallel oncogenic signals to AKT and mTOR sharing several downstream molecular targets. As with PI3K/AKT, the BMI microenvironment plays a major role in PI3 inhibition. PMI1 and particularly PMI2 are known to be highly expressed in MM and play important roles in regulating MTG/Mdr-driven transcription, apoptosis, cytokine signalling, cell proliferation and protein translation. Combinations of separate POM and PMI inhibitors have shown evidence of synergy in MM cell lines and animal models and a PI3 kinase inhibitor has recently shown activity in relapsed/refractory MM.

Our objective is to evaluate the activity of a novel family of kinase inhibitors capable of inhibiting not only PI3K enzymes (pPI3K) but also PI3K (dual inhibitors (IBL-202) and PIM/PI3K/mTOR (triple inhibitors (IBL-301)).

IBL-202 and IBL-301 are active in multiple myeloma.

IBL-202 and IBL-301 caused a loss in viability in multiple myeloma cell lines. For these experiments cell lines were treated for 24h and 48h with a number of compounds including a pan PI3K inhibitor (pPI3), a dual inhibitor of PI3K and PKC (IBL-202), a triple inhibitor of PI3K and PKC/IKB (IBL-301), a commercially available inhibitor of PI3, AK2, AZD1228 and GDC-0061, respectively. Viability was assessed using a CellTiter-Glo assay. IC50 values (μM) for each multiple myeloma cell line are depicted as a bar graph for both 24h and 48h.

The apoptotic effect of IBL-202 is enhanced in the presence of stromal cells.

We next examined the effect of IBL-202 on the expression levels of proteins which are important downstream targets of PI3K and/or PKC enzymes. These included Akt, Bad and the antionormal protein Bcl-2. MM cell lines were treated with 1μM of IBL-202 or a timecourse of 0h and harvested for analysis by western blot. There is a notable reduction in pAkt, pBAD (PI3K target) and pBcl (PKC target).

PIM/PI3K inhibition induces apoptosis and inhibits cell cycle progression.

Since the PIM/PI3K pathways can mediate survival and growth of multiple myeloma cells we next examined if IBL-202 could induce apoptosis in MM cell lines. NC-H929 and MM1S cells were treated with varying concentrations of IBL-202 and apoptosis was measured by AnnexinV and flow cytometry analysis at 24h. We observed a marked decrease in the percentage of live cells (AnnexinV negative). AZD1228 and GDC0061 were less effective at inducing apoptosis.

Conclusion

Dual inhibition of PIM/PI3K and triple inhibition of PIM/PI3K/mTOR using the novel compounds IBL-202 and 3-301 respectively are active in multiple myeloma.

IBL-202 can induce apoptosis and cause a block in cell cycle.

Reappraisal of the bone marrow microenvironment through the addition of stromal cells on maintaining the cells in a hypoxic environment serves to further enhance the apoptotic effect of IBL-202. Hypoxic conditions can upregulate levels of PIM1 which is reported to regulate CXCR4. IBL-202 has an inhibitory effect on surface levels of CXCR4 which needs further investigation.

References

Decker, S., J. Fricke et al. (2014) 'PIM Kinase as Essential for Chromophobes Lacteal Cell Survival (PIM2) and CXCR4-Mediated Microenvironmental Interactions (PIM3). Molecular Cancer Therapeutics 13(8): 1251-1260.


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