

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.

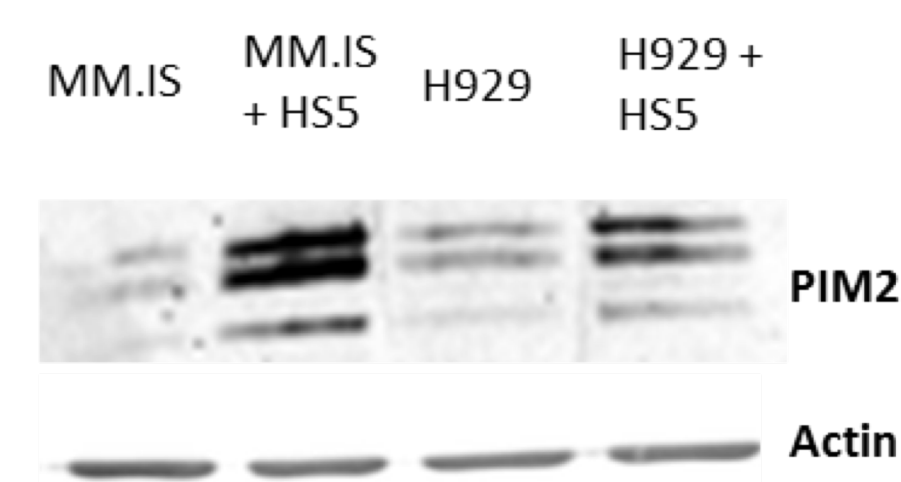
BM microenvironmental signalling along with other factors such as treatment with proteasome inhibitors (PI) can contribute to activation of the PI3K/AKT survival pathway. The redundancy of signalling pathways provides back-up mechanisms allowing escape from targeted inhibition. One such compensatory pathway is that driven by PIM kinases, which produce parallel oncogenic signals to AKT and mTOR sharing several downstream molecular targets.

As with PI3K/AKT, the BM microenvironment plays a major role in PIM activation. PIM1 and particularly PIM2 are known to be highly expressed in MM and play important roles in regulating MYC-driven transcription, apoptosis, cytokine signalling, cell proliferation and protein translation. Combinations of separate PI3K and PIM inhibitors have shown evidence of synergy in MM cell lines and animal models and a PIM kinase inhibitor has recently shown activity in relapsed/refractory MM.

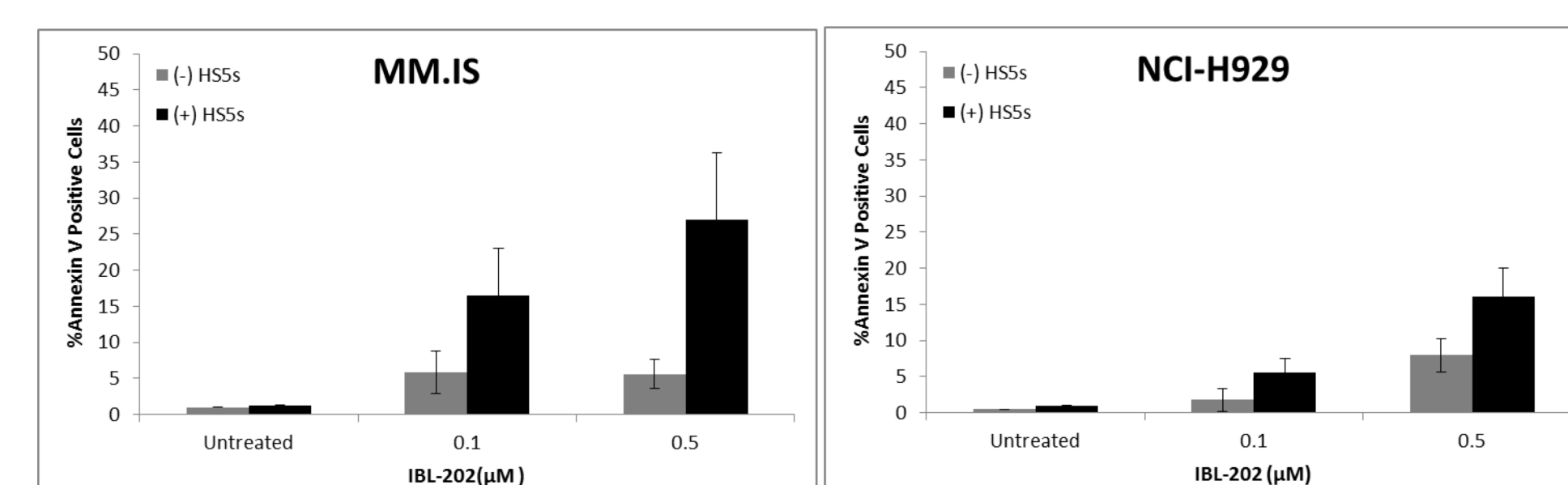
Our objective is to evaluate the activity of a novel family of kinase inhibitors capable of inhibiting not only PIM kinases (pPIMi) but also PI3K (dual inhibitors (IBL-202)) and PI3K/mTOR (triple inhibitors (IBL-301)).

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

The bone marrow microenvironment, including the presence of stromal cells has been shown to be important for the survival of multiple myeloma cells. In a co-culture setting which includes multiple myeloma cells and stromal cells (HS5s) we see an upregulation in expression levels of PIM2 after 48h.

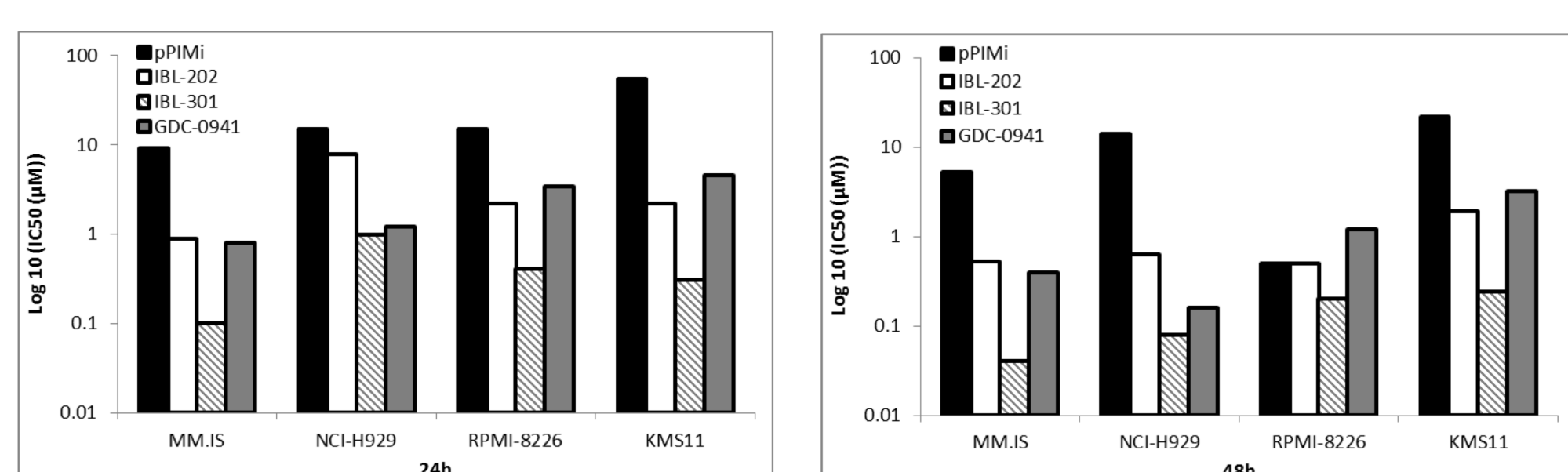


We followed this by examining the apoptotic effect of IBL-202 on MM.IS and NCI-H929 cells in the presence and absence of the stromal cell line, HS5. Cells were treated for 24h and cell death was determined by AnnexinV staining and flow cytometry analysis. In the presence of HS5s we observed a greater induction of apoptosis in MM cells compared to MM cells cultured in the absence of HS5s.

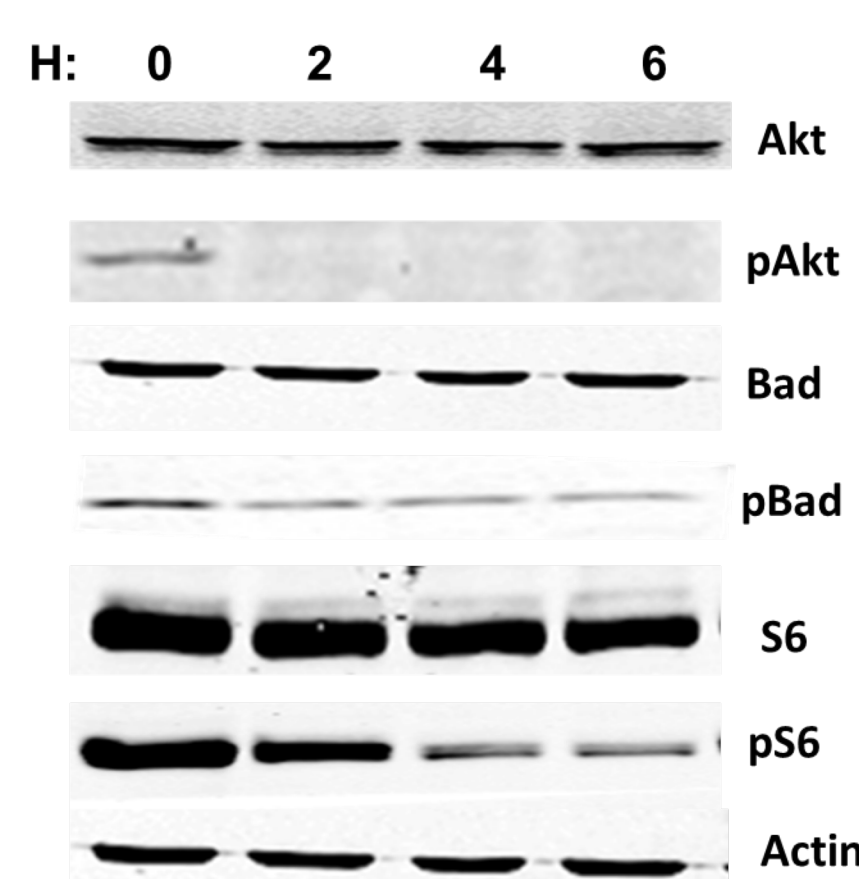


IBL-202 and IBL-301 are active in multiple myeloma.

IBL-202 and IBL-301 caused a loss in viability in multiple myeloma cell lines. For these experiments cells were treated for 24h and 48h with a number of compounds including a pan PIM inhibitor **pPIMi**, a dual inhibitor of PIM kinases and PI3K (**IBL-202**), a triple inhibitor of PIM kinases and PI3K/mTOR (**IBL-301**), a commercially available inhibitor of Pim and PI3K, **AZD1208** and **GDC-0941**, respectively. Viability was assayed 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.

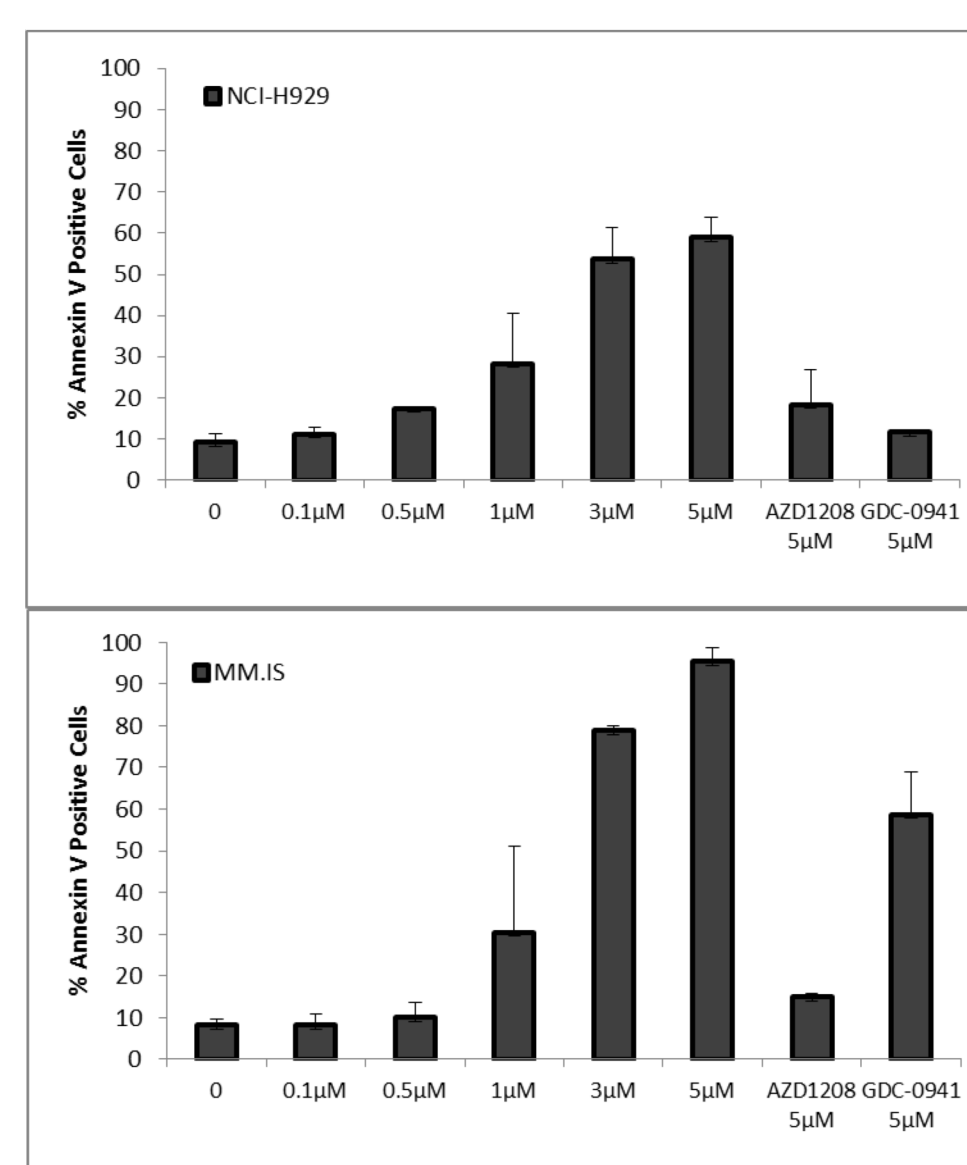


We next examined the effect of IBL-202 on the expression levels of proteins which are important downstream targets of PI3K and/or PIM kinases. These included Akt, Bad and the ribosomal protein S6. MM.IS cells were treated with 1μM of IBL-202 over a timecourse of 6h and harvested for analysis by western blot. There is a notable reduction in pS6, pAkt (PI3K target) and pBad (PIM target).

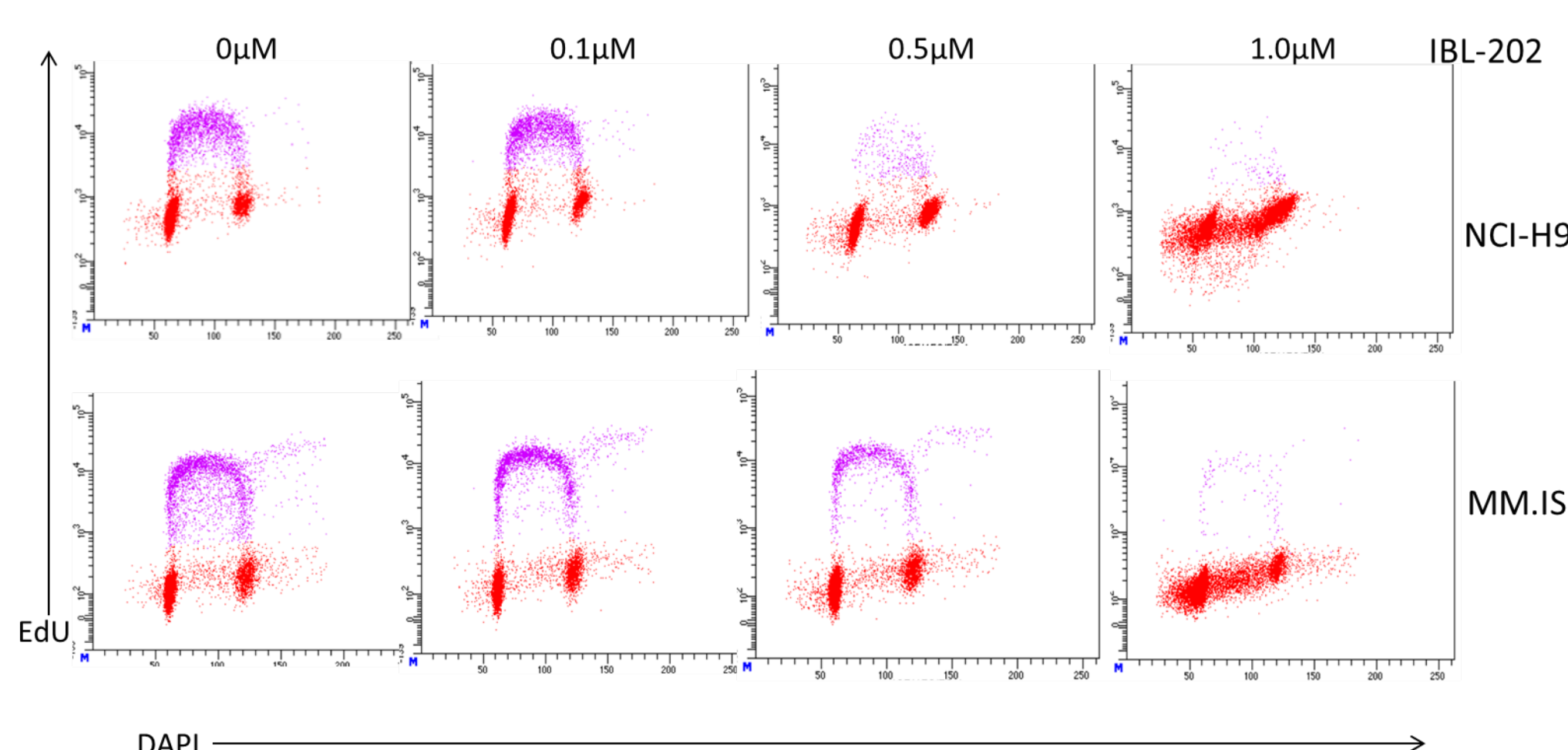


PIM/PI3K inhibition induces apoptosis and inhibits cell cycle progression.

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. NCI-H929s and MM.IS 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). AZD1208 and GDC0941 were less effective at inducing apoptosis.

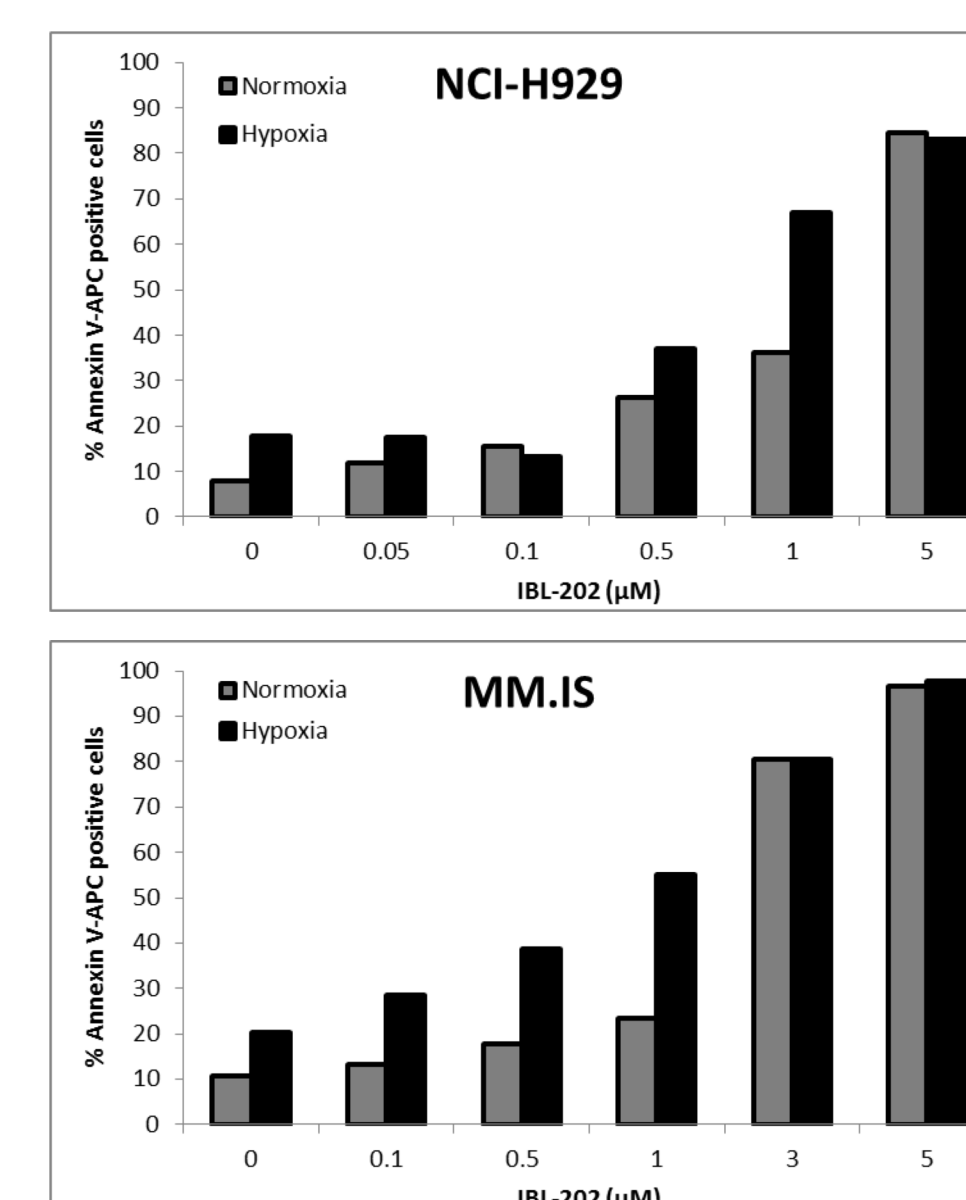


The PIM kinases can phosphorylate cyclin dependent kinases inhibitors p21 and p27. In addition the PI3K/Akt pathway is a strong regulator of cyclin D1, a key player in cell cycle progression. Thus we sought to investigate the impact of IBL-202 on cell cycle progression in both MM.IS and NCI-H929 cells. Cell proliferation was measured by assessing the incorporation of DAPI and EdU. Treatment of cells with IBL-202 over 24h resulted in a prominent reduction in the S-phase (EdU) cells.



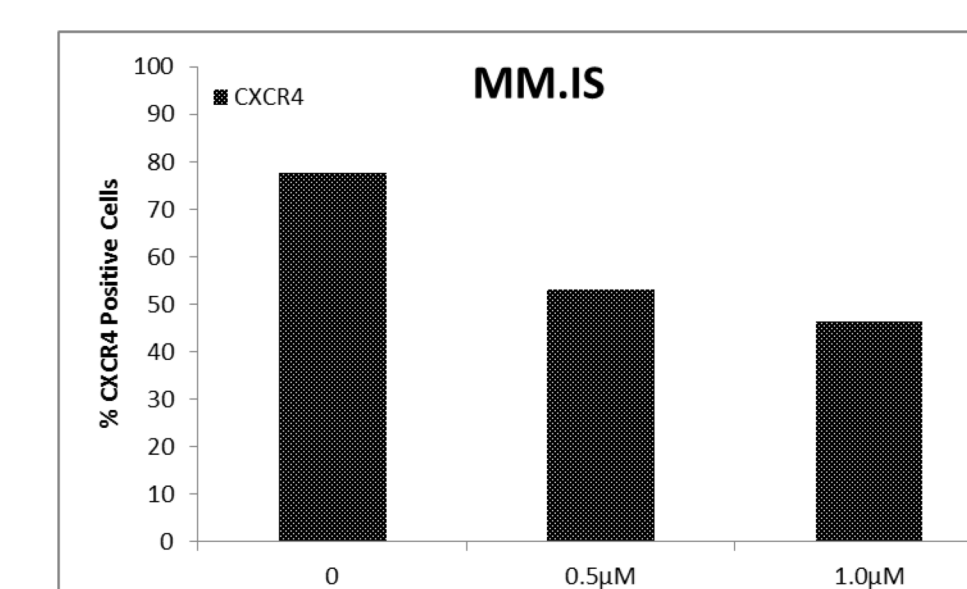
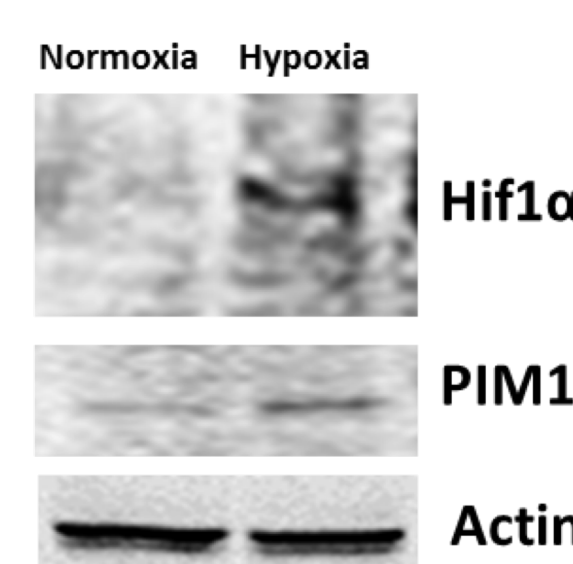
IBL-202 apoptotic effect is enhanced in hypoxia and IBL-202 down-regulates CXCR4 expression levels

In an effort to further recapitulate the bone marrow microenvironment NCI-H929s and MM.IS cells were incubated in a hypoxic chamber at 1% O₂ for 15h. These cells were then treated with IBL-202 for 48h. Apoptosis levels were determined by AnnexinV and flow cytometry analysis. In a hypoxic environment MM cells are more sensitive to IBL-202.



Hypoxia induces protein levels of HIF1α and PIM1. MM.IS samples were harvested for western blot analysis after 15h incubation at 1% O₂.

Similarly it has been reported that hypoxia can induce levels of CXCR4 through regulation of PIM1. For initial examination MM.IS cells were treated for 24h with IBL-202 and samples were analysed for CXCR4 expression with flow cytometry. IBL-202 reduces levels of CXCR4 in a dose dependent manner.



Conclusion

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

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

Recapitulation of the bone marrow microenvironment through the addition of stromal cells or maintaining the cells in a hypoxic environment served 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

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Acknowledgements