(PF881) HDAC INHIBITORS SUPPRESS SURVIVAL OF NEOPLASTIC MAST CELLS AND COUNTERACT CYTOKINE-INDUCED UPREGULATION OF IMMUNE CHECKPOINT MOLECULES PD-L1, CD47 AND TIM-3

Topic: 15. Myeloproliferative neoplasms - Biology & translational research

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Background

Systemic mastocytosis (SM) is a clonal hematopoietic stem cell disorder characterized by the expansion and accumulation of neoplastic mast cells (MC) in one or more organs. In most patients, SM is associated with the *KIT* D816V mutation, which confers resistance to multiple tyrosine kinase inhibitors (TKIs), including imatinib. Midostaurin and avapritinib are FDA and EMA approved TKIs that target KIT D816V. However, long-lasting remissions occur only in a subset of patients and many relapse during therapy.

Aims

The aim of this study was to develop novel therapeutic strategies capable of overcoming drug resistance in neoplastic mast cells in advanced systemic mastocytosis.

Methods

A robot-based high-throughput multidrug cell viability screen using eight different MC lines, including the *KIT* D816V+ cell lines HMC-1.2 and ROSA$^{KIT\ D816V}$ was performed. Selected candidates were tested using proliferation assays, apoptosis by flow cytometry assays, qPCR, immunocytochemistry, and Western blot analysis.

Results

We identified several compounds that effectively inhibit survival of *KIT* D816V+ MC lines. Among these, HDAC inhibitors were the most potent agents. The HDAC inhibitors panobinostat and quisinostat were selected for further validation experiments and showed strong anti-proliferative effects in HMC-1.1, HMC-1.2, ROSA$^{KIT\ WT}$, and ROSA$^{KIT\ D816V}$ cell lines, as well as in the multidrug-resistant cell lines MCPV-1.1, MCPV-1.2, MCPV-1.3 and MCPV-1.4. Panobinostat and quisinostat also suppressed proliferation of primary neoplastic MC obtained from patients with advanced SM. Both HDAC inhibitors profoundly reduced cell survival by inducing apoptosis across all tested cell lines, including the multidrug-resistant MCPV-1 subclones. We next assessed HDAC expression in neoplastic mast cells and found that all MC lines as well as primary neoplastic MC from patients with various subtypes of SM express HDAC1 and HDAC2 mRNA. Immunocytochemical analysis confirmed HDAC1 and HDAC2 protein expression in all cell lines. In Western blot experiments, panobinostat and quisinostat induced dose-dependent acetylation of histones H3 and H4 in HMC-1 and ROSA cells, whereas no effects on KIT phosphorylation were observed. In addition, both inhibitors downregulated MYC mRNA expression in these cells. Moreover, combinations of HDAC inhibitors (panobinostat or quisinostat) and KIT-targeting drugs (midostaurin or avapritinib) produced cooperative growth-inhibitory effects in all HMC-1 and ROSA subclones. Finally, we found that panobinostat and quisinostat downregulate cytokine-induced expression and/or constitutive expression of PD-L1, CD47 and TIM-3 in HMC-1 and ROSA cells in a dose-dependent manner.

Summary/Conclusion

HDAC inhibitors are novel potent inhibitors of growth and survival of neoplastic mast cells including multidrug-resistant subclones. Both agents induce histone acetylation and downregulate MYC mRNA expression. Both drugs also exert cooperative growth-inhibitory effects when combined with midostaurin or avapritinib. Furthermore, HDAC inhibitors counteract cytokine-driven upregulation of immune checkpoint molecules. These findings identify HDAC inhibition as a promising therapeutic strategy in advanced SM. Whether these drugs can be successfully applied *in vivo* in patients with advanced SM needs to be determined in clinical studies.