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Targeting Waldenstrom's Macroglobulinemia with HDAC Inhibitors

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Histone deacetylase inhibitors (HDAC inhibitors) represent members of a class of epigenetically-acting agents that modify chromatin structure and by extension, gene expression. In general, HDAC inhibitors, by acetylating histone tails, induce chromatin relaxation, an event that is permissive for the expression of genes encoding proteins associated with cell differentiation or death. However, HDAC inhibitors acetylate diverse substrates, including DNA repair and chaperone proteins, which may also contribute to cell death. Indeed, the lethal effects of HDAC inhibitors toward transformed cells have been attributed to diverse actions i.e., induction of oxidative injury and DNA damage, activation of Bid, up-regulation of death receptors, interference with checkpoint regulation, and down-regulation of Hsp90 chaperone proteins, among numerous others [1]. Despite continuing uncertainty about their precise mechanism(s) of action, HDAC inhibitors such as the hydroxamate pan-HDAC inhibitor vorinostat have been approved for the treatment of certain disorders e.g., cutaneous T-cell lymphoma [2]. However, evidence of activity against other hematologic malignancies, particularly B-cell malignancies, has been more elusive. Nevertheless, preliminary findings suggest a potentially beneficial role for vorinostat in combination with other targeted agents e.g., bortezomib in patients with refractory multiple myeloma [3].

The mitogen-activated protein kinase (MAPK) pathways consist of a group of evolutionarily conserved signaling cascades that regulate cellular response to a variety of stimuli, including cytokines and environmental stresses. These signaling cascades control diverse cellular processes, including cell survival, proliferation, differentiation, apoptosis, motility, and metabolism. In general, the Ras/Raf/MEK1/2/ERK1/2 cascade promotes cell survival, whereas the stress-related p38 and JNK (c-Jun N-terminal kinase) cascades promote apoptosis. In fact, the relative balance between these pro- and anti-apoptotic signaling pathways is believed to represent a critical determinant of cell survival [4]. To date, relatively little attention has been paid to the impact of HDAC inhibitors on stress-related MAPK pathways, although an association between HDAC inhibitor-mediated JNK activation and cell death has been described [5].

Waldenstrom's macroglobulinemia (WM) is a B-cell lymphoproliferative neoplasm associated with dysregulated IgM secretion, which, like its closely related counterpart, multiple myeloma, is in most cases an incurable disorder. In addition to the use of cytotoxic agents such as chlorambucil, fludarabine, and cladribine, attention has recently focused on the use of targeted agents such as rituximab, often in combination with other drugs or regimens i.e., lenalidomide, CHOP, or bendamustine [6]. Furthermore, the proteasome inhibitor bortezomib, which is highly active in multiple myeloma, is effective in WM as well [7]. However, to date, the activity of HDAC inhibitors has been minimally explored in WM at either the preclinical or clinical levels.

This situation has changed with the report by Sun et al., in the current issue of *Leukemia and Lymphoma* [8]. This group observed that vorinostat potently induced apoptosis in a WM

cell line (BCWM.1) as well as in primary WM cells. This phenomenon was associated with down-regulation of anti-apoptotic members of the IAP (inhibitors of apoptosis) family of proteins accompanied by marked activation of multiple caspases, most notably caspase-7, which occurred as an early event. Interestingly, vorinostat treatment induced down-regulation/inactivation of the pro-survival MAPK ERK1/2, while simultaneously activating the pro-apoptotic, stress-related MAPKs p38 and JNK. Although vorinostat interacted synergistically with bortezomib in BCWM.1 cells, this interaction was not apparent in primary WM cells. The authors concluded that vorinostat induces a stress response in WM cells which leads to activation of stress-related members of the MAPK family, while simultaneously disabling the cytoprotective MEK1/2/ERK1/2 pathway, leading to a shift in the net balance away from survival and towards death signaling. They also propose that vorinostat warrants attention as a therapeutic option in patients with WM.

This report provides a plausible basis for attempts to expand the indication for HDAC inhibitors beyond cutaneous T-cell lymphoma to another lymphoid malignancy, WM. Multiple preclinical studies have shown that HDAC inhibitors effectively induce cell death in B-cell malignancies such as multiple myeloma and NHL [9, 10] as well as in AML [11]. However, in the clinic, it has been more difficult to reproduce the success achieved in patients with CTCL in other hematologic malignancies, at least with HDAC inhibitors as single agents. The molecular mechanism(s) underlying the unique susceptibility of CTCL cells to HDAC inhibitors remain(s) to be elucidated, although interactions at the level of the proteasome cargo transporter protein HR23B have been proposed [12]. Whether similar mechanisms are operative in the case of WM cells remains to be determined.

The possibility that HDAC inhibitor-mediated shifts in the balance between pro-survival (e.g., ERK1/2) versus pro-death (e.g., p38, JNK) MAPKs stem from the induction of stress, and particularly ER stress, is certainly plausible, although alternative possibilities exist. For example, in a recent study, interactions between HDAC inhibitors and bortezomib in mantle cell lymphoma cells was attributed to a shift toward the pro-apoptotic arms of the ER stress pathway [13]. Analogous events may occur in WM cells. Another possibility is that HDAC inhibitors may induce oxidative stress (e.g., ROS production) in these cells [14], leading to DNA damage, as has been described in human leukemia cells [15]. Because multiple arms of the MAPK pathway can be triggered by oxidative injury and/or DNA damage, and as these events govern cellular responses to such environmental stresses, the possible contribution of ROS to HDAC inhibitor lethality in WM warrants further investigation. In this context, it has long been recognized that MAPK responses can be integrated at the level of apoptosis through alterations in the function and abundance of pro- and anti-apoptotic proteins e.g., Bim, Bcl-2, and Mcl-1, among numerous others [16]. The ability of HDAC inhibitors to modulate the expression of these proteins directly, as well as indirectly through perturbations in MAPK pathways, adds considerably to the complexity of these networks.

In light of multiple preclinical studies demonstrating synergism between HDAC and proteasome inhibitors in malignant hematopoietic cells [17, 18], and encouraging early preclinical results in myeloma [3], it is noteworthy that vorinostat and bortezomib interacted synergistically in BCWM.1 cells. Multiple mechanisms have been proposed to explain synergism between these agents, including inhibition of NF- κ B activation, disruption of aggresome function, and induction of ER stress [19, 20]. Although results were not recapitulated in primary WM cells, in view of the established activity of bortezomib in WM [7], the preclinical evidence of HDAC inhibitor activity against WM cells reported here, and the activity of the vorinostat/bortezomib in the closely related disorder multiple myeloma [3], the combined use of HDAC and proteasome inhibitors should continue to be considered in WM. Perhaps *in vivo* studies of this combination strategy could provide further evidence either for or against this approach. Regardless of the outcome of such studies, the results of

the report by Sun et al., provide a rational basis for adding HDAC inhibitors, either alone or possibly in combination with other agents, into the therapeutic armamentarium for patients with refractory WM.

Acknowledgments

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