The Biology Behind

Histone Deacetylase Inhibitors: A New Class of Potential Therapeutic Agents for Cancer Treatment

Commentary re: V. Sandor *et al.*, Phase I Trial of the Histone Deacetylase Inhibitor, Depsipeptide (FR901228, NSC 630176), in Patients with Refractory Neoplasms. Clin. Cancer Res., *8:* 718–728, 2002.

Victoria M. Richon,[1] and James P. O'Brien
Aton Pharma, Inc., Tarrytown, New York 10591

HDAC[2] inhibitors are members of a new class of agents that modulate the expression of genes by causing an increase in histone acetylation, thereby regulating chromatin structure and transcription (1, 2). In this issue, Sandor *et al.* (3) describe the results of a Phase I study with the HDAC inhibitor depsipeptide in patients with refractory neoplasms. This study identifies a dosing regimen in which depsipeptide can be administered safely to patients that causes an increase in histone acetylation in peripheral mononuclear cells. An earlier report by Piekarz *et al.* (4) showed that three patients with cutaneous T-cell lymphoma had a partial response, and one patient with peripheral T-cell lymphoma had a complete response in a Phase I trial of depsipeptide.

Depsipeptide (also known as FR901228 or FK228) is a member of the cyclic peptide class of HDAC inhibitors. Currently, there are three additional classes of HDAC inhibitors in clinical development. These classes are the short chain fatty acids (*e.g.*, phenylbutyrate, Ref. 5 and valproic acid, Ref. 6), the benzamides (*e.g.*, CI-994, Ref. 7 and MS-27–275, Ref. 8), and the hydroxamic acids (*e.g.*, suberoylanilide hydroxamic acid, Ref. 9). These agents are currently undergoing Phase I and II evaluation as monotherapy as well as in combination with cytotoxics (CI-994) and differentiation therapies (phenylbutyrate). The finding that these structurally diverse molecules inhibit HDAC activity supports a model in which HDAC is the critical cellular target causing the antitumor activity of these agents.

HDACs. HDACs are enzymes that catalyze the removal of the acetyl modification on lysine residues of proteins, including the core nucleosomal histones H2A, H2B, H3, and H4. Together with HATs, HDACs regulate the level of acetylation of the histones. The balance of acetylation of nucleosomal histones plays an important regulatory role in the transcription of many genes. Hypoacetylation of histones is associated with a condensed chromatin structure resulting in the repression of gene transcription, whereas acetylated histones are associated with a more open chromatin structure and activation of transcription. In addition to histones, other acetylated proteins have also been shown to be substrates for the HDACs. These include p53, NF-YA, and GATA-1 (10–12).

Ten structurally related HDACs have been described and fall into two classes (2, 13). Class I HDACs consist of HDAC1, 2, 3, and 8; whereas class II HDACs consist of HDAC4, 5, 6, 7, 9, and 10. Members of a third class of HDACs (class III) are structurally unrelated to the human class I and class II HDACs, and consist of homologues of the yeast Sir2 proteins (14). The activity of class I and class II HDACs is inhibited by short chain fatty acids and hydroxamic acids, but class III HDACs are not inhibited by these agents.

Identification of HDAC Inhibitors. The HDAC inhibitors that are currently in clinical trials were not discovered based on their ability to inhibit HDAC activity, rather they were identified based on their ability to change the behavior of transformed cells in culture. For example, depsipeptide is a fermentation product isolated from *Chromobacterium violaceum* that was identified during a screening project for agents that reverse the malignant phenotype of H-ras-transformed NIH 3T3 cells (15). Additional studies showed that depsipeptide inhibited proliferation of human tumor cell lines and inhibited growth of human and murine tumor xenografts *in vivo* (16, 17). Likewise, hydroxamic acids such as suberoylanilide hydroxamic acid (18) and Trichostatin A (19) were identified as potential new cancer therapeutic agents in screens for agents that induce differentiation of murine erythroleukemia cells. All of the HDAC inhibitors currently in clinical development inhibit the proliferation of transformed cells in culture by inducing cell cycle arrest, differentiation, and/or apoptosis, and inhibit tumor growth in animal models. It was only after their identification as potential anticancer agents that these compounds were identified as HDAC inhibitors (9, 20). The identification of HDAC as the target for these agents led to the establishment of screening programs for HDAC inhibitors. One of the future challenges will be to identify specific inhibitors for each of the HDACs.

HDAC Inhibitor Mechanism of Action. An attractive model for the antitumor action of HDAC inhibitors is that the increase in histone acetylation leads to the activation of transcription of a few genes of which the expression causes the inhibition of tumor growth. This model is based on the finding that the expression of a finite number of genes (<2% of the expressed genes) is regulated after exposure to HDAC inhibitors (21). The mechanism of selectivity of gene expression is currently not understood but is an area of intense study. One of the

Received 1/30/02; accepted 2/4/02.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] To whom requests for reprints should be addressed, at Aton Pharma, Inc., 777 Old Saw Mill River Road, Tarrytown, New York 10591. Phone: (914) 347-2900; Fax: (914) 347-2002; E-mail: vrichon@atonpharma.com.
[2] The abbreviations used are: HDAC, histone deacetylase; HAT, histone acetyltransferase.

most commonly induced genes is the cell cycle kinase inhibitor $p21^{WAF1}$ (22–25). Other commonly induced genes include gelsolin, $p16^{ink4a}$, and $p27^{Kip}$ (26–28). An interesting study by Kitazono *et al.* (29) shows that depsipeptide induces the expression of the sodium/iodide symporter in thyroid carcinoma cells. An increase in expression of the sodium/iodide symporter is associated with an increase in ^{125}I accumulation. HDAC inhibitors in conjunction with radioiodine therapy may, therefore, be an effective treatment for thyroid carcinomas that have reduced ability to trap iodine.

Not only is gene expression induced, but the expression of an equal or greater number of genes are repressed, including cyclin D1 (26). The mechanism of gene repression after an increase in histone acetylation may be the consequence of several effects, including both direct and indirect effects. A gene could be repressed because of the activation of a transcriptional repressor. Alternatively, acetylation of histones could lead to a chromatin conformation that directly recruits a repressor protein rather than a transcriptional activator. Another possibility is that rather than histone being the substrate for the HDAC, another protein such as a transcription factor could become acetylated. Several transcription factors have been shown to be substrates for HDACs, including GATA-1 (10), p53 (11), and NF-YA (12). The acetylation could either repress or activate the transcription factor leading to either the repression or activation of transcription, respectively. These findings that additional proteins are substrates for the HDACs raise important questions regarding the use of histone acetylation as a surrogate marker of drug activity in clinical trials.

Aberrant HDACs and/or HAT Activity Associated with Cancer. There is increasing evidence that alterations in HAT and HDAC activity occur in cancer (30, 31). HAT activity has been found to be disrupted by translocation, amplification, overexpression, or mutation in a variety of cancers, including those of both hematological and epithelial origin. HDACs have been found to be associated with aberrant transcription factors. HDACs mediate the function of oncogenic translocation products in specific forms of leukemia (31) and lymphoma (32). For example, aberrant recruitment of HDAC activity has been reported in cell lines derived from patients with acute promyelocytic leukemia (33–35). The oncoprotein encoded by the translocation-generated fusion gene in acute promyelocytic leukemia (PML-RARα) represses transcription by association with a corepressor complex containing HDAC activity. In these examples, transcriptional repression appears to be mediated by the recruitment of HDACs and provides a mechanistic rationale for the treatment of these leukemias with inhibitors of HDAC activity.

Therefore, a defect in the acetylation machinery appears to lead to alterations in acetylation and perhaps the development of cancer. An imbalance in histone acetylation may lead to changes in chromatin structure and transcriptional dysregulation of genes involved in the control of cell cycle progression, differentiation, and apoptosis. HDAC inhibitors may block tumor cell proliferation by restoring the balance of histone acetylation resulting in the proper expression of genes.

The findings that HDAC inhibitors have antitumor activity in a wide variety of cells in culture and tumor xenograft models and that defects in the acetylation machinery occur in epithelial and hematological tumors suggest that HDAC inhibitors may have broad antitumor activity in patients. Sandor *et al.* (3) show that depsipeptide can be administered to patients at doses that cause an increase in acetylated histones in peripheral mononuclear cells. The Phase I trials with depsipeptide have shown responses in patients with both renal cell carcinoma (3) and T cell lymphomas (4).

References

1. Cress, W. D., and Seto, E. Histone deacetylases, transcriptional control, and cancer. J. Cell. Physiol., *184:* 1–16, 2000.

2. Marks, P. A., Rifkind, R. A., Richon, V. M., Breslow, R., Miller, T., and Kelly, W. K. Histone deacetylases and cancer: causes and therapies. Nature Rev. Cancer, *1:* 194–202, 2001.

3. Sandor, V., Bakke, S., Robey, R. W., Kang, M. H., Blagosklonny, M. V., Bender, J., Brooks, R., Piekarz, R. L., Tucker, E., Figg, W. D., Chan, K. K., Goldspiel, B., Fojo, A. T., Balcerzak, S. P., and Bates, S. E. Phase I trial of the histone deacetylase inhibitor, depsipeptide (FR901228, NSC 630176), in patients with refractory neoplasms. Clin. Cancer Res., *8:* 718–728, 2002.

4. Piekarz, R. L., Robey, R., Sandor, V., Bakke, S., Wilson, W. H., Dahmoush, L., Kingma, D. M., Turner, M. L., Altemus, R., and Bates, S. E. Inhibitor of histone deacetylation, depsipeptide (FR901228), in the treatment of peripheral and cutaneous T-cell lymphoma: a case report. Blood, *98:* 2865–2868, 2001.

5. Gore, S. D., and Carducci, M. A. Modifying histones to tame cancer: clinical development of sodium phenylbutyrate and other histone deacetylase inhibitors. Expert Opin. Investig. Drugs, *9:* 2923–2934, 2000.

6. Phiel, C. J., Zhang, F., Huang, E. Y., Guenther, M. G., Lazar, M. A., and Klein, P. S. Histone deacetylase is a direct target of valproic acid, a potent anticonvulsant, mood stabilizer, and teratogen. J. Biol. Chem., *276:* 36734–36741, 2001.

7. Prakash, S., Foster, B. J., Meyer, M., Wozniak, A., Heilbrun, L. K., Flaherty, L., Zalupski, M., Radulovic, L., Valdivieso, M., and LoRusso, P. M. Chronic oral administration of CI-994: a phase 1 study. Investig. New Drugs, *19:* 1–11, 2001.

8. Saito, A., Yamashita, T., Mariko, Y., Nosaka, Y., Tsuchiya, K., Ando, T., Suzuki, T., Tsuruo, T., and Nakanishi, O. A synthetic inhibitor of histone deacetylase, MS-27–275, with marked *in vivo* antitumor activity against human tumors. Proc. Natl. Acad. Sci. USA, *96:* 4592–4597, 1999.

9. Richon, V. M., Emiliani, S., Verdin, E., Webb, Y., Breslow, R., Rifkind, R. A., and Marks, P. A. A class of hybrid polar inducers of transformed cell differentiation inhibits histone deacetylases. Proc. Natl. Acad. Sci. USA, *95:* 3003–3007, 1998.

10. Boyes, J., Byfield, P., Nakatani, Y., and Ogryzko, V. Regulation of activity of the transcription factor GATA-1 by acetylation. Nature (Lond.), *396:* 594–598, 1998.

11. Gu, W., and Roeder, R. G. Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain. Cell, *90:* 595–606, 1997.

12. Jin, S., and Scotto, K. W. Transcriptional regulation of the MDR1 gene by histone acetyltransferase and deacetylase is mediated by NF-Y. Mol. Cell Biol., *18:* 4377–4384, 1998.

13. Kao, H. Y., Lee, C. H., Komarov, A., Han, C. C., and Evans, R. M. Isolation and characterization of mammalian HDAC10, a novel histone deacetylase. J. Biol. Chem., *277:* 187–193, 2002.

14. Landry, J., Sutton, A., Tafrov, S. T., Heller, R. C., Stebbins, J., Pillus, L., and Sternglanz, R. The silencing protein SIR2 and its homologs are NAD-dependent protein deacetylases. Proc. Natl. Acad. Sci. USA, *97:* 5807–5811, 2000.

15. Ueda, H., Nakajima, H., Hori, Y., Goto, T., and Okuhara, M. Action of FR901228, a novel antitumor bicyclic depsipeptide produced by Chromobacterium violaceum no. 968, on Ha-ras transformed NIH3T3 cells. Biosci. Biotechnol. Biochem., *58:* 1579–1583, 1994.

16. Ueda, H., Manda, T., Matsumoto, S., Mukumoto, S., Nishigaki, F., Kawamura, I., and Shimomura, K. FR901228, a novel antitumor bicyclic depsipeptide produced by Chromobacterium violaceum No. 968. III. Antitumor activities on experimental tumors in mice. J. Antibiot. (Tokyo), *47:* 315–323, 1994.

17. Ueda, H., Nakajima, H., Hori, Y., Fujita, T., Nishimura, M., Goto, T., and Okuhara, M. FR901228, a novel antitumor bicyclic depsipeptide produced by Chromobacterium violaceum No. 968. I. Taxonomy, fermentation, isolation, physico-chemical and biological properties, and antitumor activity. J. Antibiot. (Tokyo), *47:* 301–310, 1994.

18. Richon, V. M., Webb, Y., Merger, R., Sheppard, T., Jursic, B., Ngo, L., Civoli, F., Breslow, R., Rifkind, R. A., and Marks, P. A. Second generation hybrid polar compounds are potent inducers of transformed cell differentiation. Proc. Natl. Acad. Sci. USA, *93:* 5705–5708, 1996.

19. Yoshida, M., Nomura, S., and Beppu, T. Effects of trichostatins on differentiation of murine erythroleukemia cells. Cancer Res., *47:* 3688–3691, 1987.

20. Nakajima, H., Kim, Y. B., Terano, H., Yoshida, M., and Horinouchi, S. FR901228, a potent antitumor antibiotic, is a novel histone deacetylase inhibitor. Exp. Cell Res., *241:* 126–133, 1998.

21. Van Lint, C., Emiliani, S., and Verdin, E. The expression of a small fraction of cellular genes is changed in response to histone hyperacetylation. Gene Expr., *5:* 245–253, 1996.

22. Archer, S. Y., Meng, S., Shei, A., and Hodin, R. A. p21(WAF1) is required for butyrate-mediated growth inhibition of human colon cancer cells. Proc. Natl. Acad. Sci. USA, *95:* 6791–6796, 1998.

23. Kim, Y. B., Ki, S. W., Yoshida, M., and Horinouchi, S. Mechanism of cell cycle arrest caused by histone deacetylase inhibitors in human carcinoma cells. J. Antibiot. (Tokyo), *53:* 1191–200, 2000.

24. Sambucetti, L. C., Fischer, D. D., Zabludoff, S., Kwon, P. O., Chamberlin, H., Trogani, N., Xu, H., and Cohen, D. Histone deacetylase inhibition selectively alters the activity and expression of cell cycle proteins leading to specific chromatin acetylation and antiproliferative effects. J. Biol. Chem., *274:* 34940–34947, 1999.

25. Richon, V. M., Sandhoff, T. W., Rifkind, R. A., and Marks, P. A. Histone deacetylase inhibitor selectively induces p21WAF1 expression and gene-associated histone acetylation. Proc. Natl. Acad. Sci. USA, *97:* 10014–10019, 2000.

26. Kim, Y. B., Lee, K. H., Sugita, K., Yoshida, M., and Horinouchi, S. Oxamflatin is a novel antitumor compound that inhibits mammalian histone deacetylase. Oncogene, *18:* 2461–2470, 1999.

27. Hoshikawa, Y., Kwon, H. J., Yoshida, M., Horinouchi, S., and Beppu, T. Trichostatin A induces morphological changes and gelsolin expression by inhibiting histone deacetylase in human carcinoma cell lines. Exp. Cell Res., *214:* 189–197, 1994.

28. Mielnicki, L. M., Ying, A. M., Head, K. L., Asch, H. L., and Asch, B. B. Epigenetic regulation of gelsolin expression in human breast cancer cells. Exp. Cell Res., *249:* 161–176, 1999.

29. Kitazono, M., Robey, R., Zhan, Z., Sarlis, N. J., Skarulis, M. C., Aikou, T., Bates, S., and Fojo, T. Low concentrations of the histone deacetylase inhibitor, depsipeptide (FR901228), increase expression of the Na(+)/I(−) symporter and iodine accumulation in poorly differentiated thyroid carcinoma cells. J. Clin. Endocrinol. Metab., *86:* 3430–3435, 2001.

30. Timmermann, S., Lehrmann, H., Polesskaya, A., and Harel-Bellan, A. Histone acetylation and disease. Cell. Mol. Life Sci., *58:* 728–736, 2001.

31. Pandolfi, P. P. Transcription therapy for cancer. Oncogene, *20:* 3116–3127, 2001.

32. Dhordain, P., Lin, R. J., Quief, S., Lantoine, D., Kerckaert, J. P., Evans, R. M., and Albagli, O. The LAZ3(BCL-6) oncoprotein recruits a SMRT/mSIN3A/histone deacetylase containing complex to mediate transcriptional repression. Nucleic Acids Res., *26:* 4645–4651, 1998.

33. Lin, R. J., Nagy, L., Inoue, S., Shao, W., Miller, W. H., Jr., and Evans, R. M. Role of the histone deacetylase complex in acute promyelocytic leukaemia. Nature (Lond.), *391:* 811–814, 1998.

34. He, L. Z., Guidez, F., Tribioli, C., Peruzzi, D., Ruthardt, M., Zelent, A., and Pandolfi, P. P. Distinct interactions of PML-RARα and PLZF-RARα with co-repressors determine differential responses to RA in APL. Nat. Genet., *18:* 126–135, 1998.

35. Grignani, F., De Matteis, S., Nervi, C., Tomassoni, L., Gelmetti, V., Cioce, M., Fanelli, M., Ruthardt, M., Ferrara, F. F., Zamir, I., Seiser, C., Lazar, M. A., Minucci, S., and Pelicci, P. G. Fusion proteins of the retinoic acid receptor-α recruit histone deacetylase in promyelocytic leukaemia. Nature (Lond.), *391:* 815–818, 1998.

Clinical Cancer Research

Histone Deacetylase Inhibitors: A New Class of Potential Therapeutic Agents for Cancer Treatment: Commentary re: V. Sandor et al., Phase I Trial of the Histone Deacetylase Inhibitor, Depsipeptide (FR901228, NSC 630176), in Patients with R efractory Neoplasms. Clin. Cancer Res., 8: 718–728, 2002.

Victoria M. Richon and James P. O'Brien

Clin Cancer Res 2002;8:662-664.