OncoImmunology 2:11, e27157; November 2013; © 2013 Landes Bioscience

Immune effectors required for the therapeutic activity of vorinostat

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Keywords: B lymphocytes, histone deacetylase inhibitors, immunogenic cell death, interferon γ, natural killer cells, SAHA

Suberanilohydroxamic acid, a pan-histone deacetylase inhibitor (HDACi) best known as vorinostat (marketed under the name Zolinza® by Merck and Co., Inc.), was approved by the US Food and Drug Administration (FDA) for use in patients with cutaneous T-cell lymphoma (CTCL) in 2006.[1-3] Similar to other targeted anticancer agents, vorinostat has been developed based on the assumption that it would mediate direct cytotoxic effects on malignant cells. Indeed, vorinostat has been shown to trigger the apoptotic demise of cancer cells in vivo, in syngeneic models of murine adenocarcinoma and lymphoma[4] as well as in mice bearing human acute lymphoblastic leukemia.[5]

As for many other successful chemotherapeutics,[6,7] the antineoplastic activity of vorinostat does not originate from purely cancer cell-autonomous mechanisms, but is (at least partially) mediated by the immune system, as indicated by multiple observations. First, the antitumor effects of vorinostat can be improved by the co-administration of immunostimulatory antibodies targeting tumor necrosis factor receptor superfamily, member 4 (TNFRSF4, best known as OX40) or TNFRSF9 (best known as CD137),[8-10] as well as with α-galactosylceramide, a natural killer T (NKT) cell-activating agent that stimulates the production of interferon γ (IFNγ).[11] Second, the antineoplastic activity of vorinostat against MC38 colon adenocarcinomas is lost in mice lacking immune effectors due to the combined knockout of recombination activating gene 2 (*Rag2*) and interleukin 2 receptor, γ chain (*Il2rg*, also known as γ*c*).[11] Along similar lines, the antitumor effects of vorinostat are abolished in *Ifng*$^{-/-}$ hosts (which lack IFNγ) and in mice treated with IFNγ-neutralizing antibodies.[11] Finally, malignant cells expressing a dominant-negative form of the IFNγ receptor also fail to respond to vorinostat.[11]

Reminiscent of anthracyclines and oxaliplatin, which are immunogenic cell death (ICD) inducers,[12-15] vorinostat can stimulate a variant of apoptosis that is accompanied by all the major hallmarks of ICD, namely the exposure of calreticulin on the cell surface, the active secretion of ATP and the release of the non-histone chromatin-binding protein high mobility group box 1 (HMGB1).[11] Accordingly, cancer cells succumbing to vorinostat are engulfed by dendritic cells in vitro.[11] If injected subcutaneously into immunocompetent mice, vorinostat-treated cancer cells can induce an immune response that protects the animals against the inoculation of living cells of the same type.[16]

In a transplantable mouse tumor model, namely, MC38 colon adenocarcinoma, the anticancer effects of vorinostat are abolished upon the injection of antibodies that deplete CD8$^+$ T cells. Moreover, when tumors are implanted into mice lacking the gene coding for granzyme B, which is essential for the cytotoxic activity of T and NK cells, they fail to respond to vorinostat treatment.[8] These observations suggest that the immunogenic demise of cancer cells induced by vorinostat elicits an antitumor immune response that is mediated by cytotoxic T lymphocytes. Thus, vorinostat resembles in its mode of action other ICD inducers, including anthracyclines and digoxin, which induce T cell-dependent but B cell-independent anticancer immune responses.[17-21]

However, vorinostat recently turned out to mediate therapeutic effects against B-cell lymphomas driven by the transgenic expression of *v-myc* avian myelocytomatosis viral oncogene homolog (*Myc*) in an unsuspected fashion. In this setting, the knockout of *Rag2* or *Ifng* does abolish the therapeutic effects of vorinostat, yet the depletion of CD8$^+$ T cells alone, CD4$^+$ and CD8$^+$ cells, NK cells alone, or all cytotoxic lymphocytes (NK and CD8$^+$ T cells) fails to do so.[11] Unexpectedly, in this model, the absence of B cells (due to the targeted disruption of the immunoglobulin μ chain-coding gene) fully abrogates the antineoplastic activity of vorinostat. These findings demonstrate that B cells (but neither T nor NK cells) play an essential role in the therapeutic response of lymphoma to vorinostat.[11] At this stage, it is unknown whether tumor-specific antibodies and/or the production of IFNγ by B cells contribute to the therapeutic effects of vorinostat in this setting.

Vorinostat appears to exert immunomodulatory effects by acting on both cancer and immune cells. Schmudde and colleagues demonstrated that vorinostat sensitizes cancer cells to the cytotoxic effects of NK cells by promoting the expression of the NK-cell receptors killer cell lectin-like receptor subfamily K, member 1 (KLRK1, best known as NKG2D) and CD226 (also known as

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Submitted: 11/01/2013; Accepted: 11/01/2013
Citation: Kepp O, Galluzzi L, Kroemer G. Immune effectors required for the therapeutic activity of vorinostat. OncoImmunology 2013; 2:e27157; http://dx.doi.org/10.4161/onci.27157

DNAM1).[22] Conversely, Fiegler and collaborators showed that vorinostat limits the recognition of malignant cells by NK cells through the downregulation of natural killer cell cytotoxicity receptor 3 ligand 1 (NCR3LG1), a ligand for natural cytotoxicity triggering receptor 3 (NCR3) best known as B7-H6.[23] Along similar lines, several groups reported that vorinostat exerts immunosuppressive side effects. In particular, vorinostat has been reported to stimulate the function of regulatory T cells,[24-27] to prevent the activation of tumor-specific NK and T cells,[28,29] and to stimulate the expression of the immunosuppressive enzyme indoleamine 2,3-dioxygenase 1 (IDO1) by dendritic cells.[30] Although vorinostat can interfere with acute graft-vs.-host disease, it does not inhibit the graft-vs.-leukemia effects in mouse models, underscoring the complexity of its immunological effects.[31,32] In the context of its clinical indication, CTCL, vorinostat has been found to inhibit the expression of the immunosuppressive cytokine interleukin-10[33] and to suppress the cytotoxic action of NK cells.[34]

Which are the conclusions that we can draw from the aforementioned studies? First, vorinostat seems to differ from other anticancer agents that operate in a purely cancer cell-autonomous fashion, since its capacity to limit tumor growth fully depends on the immune system.[11,35,36] Second, it appears that, at least in some tumor types (exemplified by *Myc*-driven lymphomas), B lymphocytes play a major positive role in the antineoplastic activity of vorinostat.[11] This is in line with the fact that the accumulation of B cells within neoplastic lesions has a positive prognostic impact, at least in some cancers.[37-42]

Altogether, these results point to a surprising heterogeneity in the immune effectors mobilized by successful anticancer agents. After T and NK lymphocytes,[43] B cells now enter the stage, asking for their share in the limelight of anticancer immunosurveillance.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Mann BS, Johnson JR, Cohen MH, Justice R, Pazdur R. FDA approval summary: vorinostat for treatment of advanced primary cutaneous T-cell lymphoma. Oncologist 2007; 12:1247-52; PMID:17962618; http://dx.doi.org/10.1634/theoncologist.12-10-1247
2. Duvic M, Talpur R, Ni X, Zhang C, Hazarika P, Kelly C, Chiao JH, Reilly JF, Ricker JL, Richon VM, et al. Phase 2 trial of oral vorinostat (suberoylanilide hydroxamic acid, SAHA) for refractory cutaneous T-cell lymphoma (CTCL). Blood 2007; 109:31-9; PMID:16960145; http://dx.doi.org/10.1182/blood-2006-06-025999
3. Olsen EA, Kim YH, Kuzel TM, Pacheco TR, Foss FM, Parker S, Frankel SR, Chen C, Ricker JL, Arduino JM, et al. Phase IIb multicenter trial of vorinostat in patients with persistent, progressive, or treatment refractory cutaneous T-cell lymphoma. J Clin Oncol 2007; 25:3109-15; PMID:17577020; http://dx.doi.org/10.1200/JCO.2006.10.2434
4. Lindemann RK, Newbold A, Whitecross KF, Cluse LA, Frew AJ, Ellis L, Williams S, Wiegmans AP, Dear AE, Scott CL, et al. Analysis of the apoptotic and therapeutic activities of histone deacetylase inhibitors by using a mouse model of B cell lymphoma. Proc Natl Acad Sci U S A 2007; 104:8071-6; PMID:17470784; http://dx.doi.org/10.1073/pnas.0702294104
5. Bachmann PS, Piazza RG, Janes ME, Wong NC, Davies C, Mogavero A, Bhadri VA, Szymanska B, Geninson G, Magistroni V, et al. Epigenetic silencing of BIM in glucocorticoid poor-responsive pediatric acute lymphoblastic leukemia, and its reversal by histone deacetylase inhibition. Blood 2010; 116:3013-22; PMID:20647567; http://dx.doi.org/10.1182/blood-2010-05-284968
6. Zitvogel L, Galluzzi L, Smyth MJ, Kroemer G. Mechanism of action of conventional and targeted anticancer therapies: reinstating immunosurveillance. Immunity 2013; 39:74-88; PMID:23890065; http://dx.doi.org/10.1016/j.immuni.2013.06.014
7. Galluzzi L, Senovilla L, Zitvogel L, Kroemer G. The secret ally: immunostimulation by anticancer drugs. Nat Rev Drug Discov 2012; 11:215-33; PMID:22301798; http://dx.doi.org/10.1038/nrd3626
8. Christiansen AJ, West A, Banks KM, Haynes NM, Teng MW, Smyth MJ, Johnstone RW. Eradication of solid tumors using histone deacetylase inhibitors combined with immune-stimulating antibodies. Proc Natl Acad Sci U S A 2011; 108:4141-6; PMID:21368108; http://dx.doi.org/10.1073/pnas.1011037108
9. West AC, Christiansen AJ, Smyth MJ, Johnstone RW. The combination of histone deacetylase inhibitors with immune-stimulating antibodies has potent anti-cancer effects. Oncoimmunology 2012; 1:377-9; PMID:22737621; http://dx.doi.org/10.4161/onci.18804
10. Verbrugge I, Galli M, Smyth MJ, Johnstone RW, Haynes NM. Enhancing the antitumor effects of radiotherapy with combinations of immunostimulatory antibodies. Oncoimmunology 2012; 1:1629-31; PMID:23264917; http://dx.doi.org/10.4161/onci.21652
11. West AC, Mattarollo SR, Shortt J, Cluse LA, Christiansen AJ, Smyth MJ, Johnstone RW. An intact immune system is required for the anti-cancer activities of histone deacetylase inhibitors. Cancer Res 2013; Forthcoming; PMID:24158093; http://dx.doi.org/10.1158/0008-5472.CAN-13-0890
12. Zitvogel L, Kepp O, Kroemer G. Immune parameters affecting the efficacy of chemotherapeutic regimens. Nat Rev Clin Oncol 2011; 8:151-60; PMID:21364688; http://dx.doi.org/10.1038/nrclinonc.2010.223
13. Vacchelli E, Galluzzi L, Rousseau V, Rigoni A, Tesniere A, Delahaye N, Schlemmer FD, Menger L, Sukkurwala AQ, Adjemian S, et al. Loss-of-function alleles of P2RX7 and TLR4 fail to affect the response to chemotherapy in non-small cell lung cancer. Oncoimmunology 2012; 1:271-8; PMID:22737602; http://dx.doi.org/10.4161/onci.18684
14. Gupta A, Sharma A, von Boehmer L, Surace L, Knuth A, van den Broek M. Radiotherapy supports protective tumor-specific immunity. Oncoimmunology 2012; 1:1610-1; PMID:23264910; http://dx.doi.org/10.4161/onci.21478
15. Kroemer G, Galluzzi L, Kepp O, Zitvogel L. Immunogenic cell death in cancer therapy. Annu Rev Immunol 2013; 31:51-72; PMID:23157435; http://dx.doi.org/10.1146/annurev-immunol-032712-100008
16. Guillot F, Boutin B, Blanquart C, Fonteneau JF, Robard M, Grégoire M, Pouliquen D. Vaccination with epigenetically treated mesothelioma cells induces immunisation and blocks tumour growth. Vaccine 2011; 29:5534-43; PMID:21619908; http://dx.doi.org/10.1016/j.vaccine.2011.05.029
17. Kepp O, Menger L, Vacchelli E, Adjemian S, Martins I, Ma Y, Sukkurwala AQ, Michaud M, Galluzzi L, Zitvogel L, et al. Anticancer activity of cardiac glycosides: At the frontier between cell-autonomous and immunological effects. Oncoimmunology 2012; 1:1640-2; PMID:23264921; http://dx.doi.org/10.4161/onci.21684
18. Menger L, Vacchelli E, Adjemian S, Martins I, Ma Y, Shen S, Yamazaki T, Sukkurwala AQ, Michaud M, Mignot G, et al. Cardiac glycosides exert anticancer effects by inducing immunogenic cell death. Sci Transl Med 2012; 4:143ra99; PMID:22814852; http://dx.doi.org/10.1126/scitranslmed.3003807
19. Hannani D, Locher C, Yamazaki T, Colin-Minard V, Vetizou M, Aymeric L, Viaud S, Sanchez D, Smyth MJ, Bruhns P, et al. Contribution of humoral immune responses to the antitumor effects mediated by anthracyclines. Cell Death Differ 2013; Forthcoming; PMID:23744294; http://dx.doi.org/10.1038/cdd.2013.60
20. Michaud M, Sukkurwala AQ, Martins I, Shen S, Zitvogel L, Kroemer G. Subversion of the chemotherapy-induced anticancer immune response by the ecto-ATPase CD39. Oncoimmunology 2012; 1:393-5; PMID:22737627; http://dx.doi.org/10.4161/onci.19070
21. Vacchelli E, Galluzzi L, Fridman WH, Galon J, Sautès-Fridman C, Tartour E, Kroemer G. Trial watch: Chemotherapy with immunogenic cell death inducers. Oncoimmunology 2012; 1:179-88; PMID:22720239; http://dx.doi.org/10.4161/onci.1.2.19026
22. Schmudde M, Braun A, Pende D, Sonnemann J, Klier U, Beck JF, Moretta L, Bröker BM. Histone deacetylase inhibitors sensitize tumour cells for cytotoxic effects of natural killer cells. Cancer Lett 2008; 272:110-21; PMID:18718708; http://dx.doi.org/10.1016/j.canlet.2008.06.027

23. Fiegler N, Textor S, Arnold A, Rölle A, Oehme I, Breuhahn K, Moldenhauer G, Witzens-Harig M, Cerwenka A. Downregulation of the activating NKp30 ligand B7-H6 by HDAC inhibitors impairs tumor cell recognition by NK cells. Blood 2013; 122:684-93; PMID:23801635; http://dx.doi.org/10.1182/blood-2013-02-482513
24. Lucas JL, Mirshahpanah P, Haas-Stapleton E, Asadullah K, Zollner TM, Numerof RP. Induction of Foxp3+ regulatory T cells with histone deacetylase inhibitors. Cell Immunol 2009; 257:97-104; PMID:19358983; http://dx.doi.org/10.1016/j.cellimm.2009.03.004
25. Akimova T, Ge G, Golovina T, Mikheeva T, Wang L, Riley JL, Hancock WW. Histone/protein deacetylase inhibitors increase suppressive functions of human FOXP3+ Tregs. Clin Immunol 2010; 136:348-63; PMID:20478744; http://dx.doi.org/10.1016/j.clim.2010.04.018
26. de Zoeten EF, Wang L, Sai H, Dillmann WH, Hancock WW. Inhibition of HDAC9 increases T regulatory cell function and prevents colitis in mice. Gastroenterology 2010; 138:583-94; PMID:19879272; http://dx.doi.org/10.1053/j.gastro.2009.10.037
27. Shen L, Pili R. Class I histone deacetylase inhibition is a novel mechanism to target regulatory T cells in immunotherapy. Oncoimmunology 2012; 1:948-50; PMID:23162767; http://dx.doi.org/10.4161/onci.20306
28. Ogbomo H, Michaelis M, Kreuter J, Doerr HW, Cinatl J Jr. Histone deacetylase inhibitors suppress natural killer cell cytolytic activity. FEBS Lett 2007; 581:1317-22; PMID:17349632; http://dx.doi.org/10.1016/j.febslet.2007.02.045
29. Schmudde M, Friebe E, Sonnemann J, Beck JF, Bröker BM. Histone deacetylase inhibitors prevent activation of tumour-reactive NK cells and T cells but do not interfere with their cytolytic effector functions. Cancer Lett 2010; 295:173-81; PMID:20346580; http://dx.doi.org/10.1016/j.canlet.2010.02.024
30. Reddy P, Sun Y, Toubai T, Duran-Struuck R, Clouthier SG, Weisiger E, Maeda Y, Tawara I, Krijanovski O, Gatza E, et al. Histone deacetylase inhibition modulates indoleamine 2,3-dioxygenase-dependent DC functions and regulates experimental graft-versus-host disease in mice. J Clin Invest 2008; 118:2562-73; PMID:18568076
31. Reddy P, Maeda Y, Hotary K, Liu C, Reznikov LL, Dinarello CA, Ferrara JL. Histone deacetylase inhibitor suberoylanilide hydroxamic acid reduces acute graft-versus-host disease and preserves graft-versus-leukemia effect. Proc Natl Acad Sci U S A 2004; 101:3921-6; PMID:15001702; http://dx.doi.org/10.1073/pnas.0400380101
32. Bridle BW, Chen L, Lemay CG, Diallo JS, Pol J, Nguyen A, Capretta A, He R, Bramson JL, Bell JC, et al. HDAC inhibition suppresses primary immune responses, enhances secondary immune responses, and abrogates autoimmunity during tumor immunotherapy. Mol Ther 2013; 21:887-94; PMID:23295947; http://dx.doi.org/10.1038/mt.2012.265
33. Tiffon C, Adams J, van der Fits L, Wen S, Townsend P, Ganesan A, Hodges E, Vermeer M, Packham G. The histone deacetylase inhibitors vorinostat and romidepsin downmodulate IL-10 expression in cutaneous T-cell lymphoma cells. Br J Pharmacol 2011; 162:1590-602; PMID:21198545; http://dx.doi.org/10.1111/j.1476-5381.2010.01188.x
34. Stephen S, Morrissey KA, Benoit BM, Kim EJ, Vittorio CC, Nasta SD, Showe LC, Wysocka M, Rook AH. Inhibition of cell-mediated immunity by the histone deacetylase inhibitor vorinostat: implications for therapy of cutaneous T-cell lymphoma. Am J Hematol 2012; 87:226-8; PMID:22189940; http://dx.doi.org/10.1002/ajh.22231
35. Casares N, Pequignot MO, Tesniere A, Ghiringhelli F, Roux S, Chaput N, Schmitt E, Hamai A, Hervas-Stubbs S, Obeid M, et al. Caspase-dependent immunogenicity of doxorubicin-induced tumor cell death. J Exp Med 2005; 202:1691-701; PMID:16365148; http://dx.doi.org/10.1084/jem.20050915
36. Paget C, Duret H, Ngiow SF, Kansara M, Thomas DM, Smyth MJ. Studying the role of the immune system on the antitumor activity of a Hedgehog inhibitor against murine osteosarcoma. Oncoimmunology 2012; 1:1313-22; PMID:23243595; http://dx.doi.org/10.4161/onci.21680
37. Fridman WH, Galon J, Pagès F, Tartour E, Sautès-Fridman C, Kroemer G. Prognostic and predictive impact of intra- and peritumoral immune infiltrates. Cancer Res 2011; 71:5601-5; PMID:21846822; http://dx.doi.org/10.1158/0008-5472.CAN-11-1316
38. Schmidt M, Micke P, Gehrmann M, Hengstler JG. Immunoglobulin kappa chain as an immunologic biomarker of prognosis and chemotherapy response in solid tumors. Oncoimmunology 2012; 1:1156-8; PMID:23170262; http://dx.doi.org/10.4161/onci.21653
39. Manuel M, Tredan O, Bachelot T, Clapisson G, Courtier A, Parmentier G, Rabeony T, Grives A, Perez S, Mouret JF, et al. Lymphopenia combined with low TCR diversity (divpenia) predicts poor overall survival in metastatic breast cancer patients. Oncoimmunology 2012; 1:432-40; PMID:22754761; http://dx.doi.org/10.4161/onci.19545
40. Nielsen JS, Nelson BH. Tumor-infiltrating B cells and T cells: Working together to promote patient survival. Oncoimmunology 2012; 1:1623-5; PMID:23264915; http://dx.doi.org/10.4161/onci.21650
41. Senovilla L, Vacchelli E, Galon J, Adjemian S, Eggermont A, Fridman WH, Sautès-Fridman C, Ma Y, Tartour E, Zitvogel L, et al. Trial watch: Prognostic and predictive value of the immune infiltrate in cancer. Oncoimmunology 2012; 1:1323-43; PMID:23243596; http://dx.doi.org/10.4161/onci.22009
42. Galon J, Angell HK, Bedognetti D, Marincola FM. The continuum of cancer immunosurveillance: prognostic, predictive, and mechanistic signatures. Immunity 2013; 39:11-26; PMID:23890060; http://dx.doi.org/10.1016/j.immuni.2013.07.008
43. Delahaye NF, Rusakiewicz S, Martins I, Ménard C, Roux S, Lyonnet L, Paul P, Sarabi M, Chaput N, Semeraro M, et al. Alternatively spliced NKp30 isoforms affect the prognosis of gastrointestinal stromal tumors. Nat Med 2011; 17:700-7; PMID:21552268; http://dx.doi.org/10.1038/nm.2366