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Using Epigenetic Therapy to Overcome Chemotherapy Resistance

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Abstract

It has been known for decades that as cancer progresses, tumors develop genetic alterations, making them highly prone to developing resistance to therapies. Classically, it has been thought that these acquired genetic changes are fixed. This has led to the paradigm of moving from one cancer therapy to the next while avoiding past therapies. However, emerging data on epigenetic changes during tumor progression and use of epigenetic therapies have shown that epigenetic modifications leading to chemotherapy resistance have the potential to be reversible with epigenetic therapy. In fact, promising clinical data exist that treatment with epigenetic agents can diminish chemotherapy resistance in a number of tumor types including chronic myelogenous leukemia, colorectal, ovarian, lung and breast cancer. The potential for epigenetic-modifying drugs to allow for treatment of resistant disease is exciting and clinical trials have just begun to evaluate this area.

Keywords

Epigenetic therapy; chemotherapy resistance; review

It has been known for decades that as cancer progresses, tumors also develop genetic alterations, making them highly prone to developing resistance to therapies. Classically, it has been thought that these acquired genetic changes and subsequent resistance patterns are fixed and unalterable. This has led to the paradigm of moving from one cancer therapy to the next while avoiding past therapies. However, growing data on epigenetic changes during tumor progression and the use of epigenetic therapies may transform this paradigm.

Epigenetics describes a variety of strategies cells use to regulate the transcription of their DNA. Although numerous mechanisms are used, two of the best characterized and most studied are DNA methylation and histone modification. Histone modification may take place through a number of means, including phosphorylation, ubiquitination and methylation, but the most commonly seen changes occur through acetylation. DNA methylation is controlled by DNA methyltransferases (DNMTs) and histone acetylation is controlled by histone acetyltransferases (HATs) and histone deacetyltransferases (HDACs). Data on a myriad of

tumor types including chronic myelogenous leukemia, lung, gastric, colorectal, breast, bladder, and ovarian cancer (1–10) have demonstrated that just as DNA mutations contribute to tumor progression, so do epigenetic alterations participate in tumor growth and chemotherapy resistance. In fact, treatment with DNMT inhibitors azacitidine or decitabine has been shown to produce responses in 13–18% of patients with myelodysplastic syndrome (MDS) lasting a median of 280–330 days (11, 12). In addition, a large randomized phase III trial comparing azacitidine to best conventional care for patients with MDS showed a 9.5-month survival benefit with the addition of azacitidine (13). Beyond the benefit seen with DNMT inhibitors for MDS, the HDAC inhibitors vorinostat and romidepsin have also shown clinical benefit in the treatment of cutaneous T-cell lymphoma (CTCL). In large randomized trials, these two agents produced responses lasting a median of more than 6 and up to 15 months in 29–35% of patients with CTCL (14,15). Taken together, these data led to Federal Drug Administration approval of these four agents, azacitidine and decitabine for MDS and vorinostat and romidepsin for CTCL.

While these agents have shown direct anti-neoplastic effects in MDS and CTCL, perhaps a greater role for epigenetic agents is as chemotherapy sensitizers to help overcome chemotherapy resistance in a wide array of malignancies. Although DNA mutations which lead to chemotherapy resistance may be irreversible, epigenetic alterations which lead to resistance have been found to be reversible with epigenetic therapy (10, 16). In fact, there exist recent data across a multitude of tumor types that epigenetic therapy has the potential to overcome chemotherapy resistance and re-sensitize cancer cells to previously ineffective therapy.

For example, RRx-001 is an epigenetic modifying agent belonging to a new class of drugs known as dinitroazetidines. It functions as an epigenetic agent by increasing the generation of reactive oxygen and nitrogen species under hypoxic conditions. These reactive species result in oxidation of critical cysteine residues at the catalytic sites of a number of epigenetic-modifying enzymes, including DNMTs and HDACs. Oxidation of these cysteine residues results in inhibition of these enzymes and an altered epigenetic profile in cancer cells. In a phase I trial of RRx-001 in patients with advanced refractory solid malignancies, activity was observed in some patients (one partial response and 14 out of 25 having stable disease) (17). In addition and more notably, four patients (three with colorectal and one with lung cancer) who had initially responded to chemotherapy regimens but whose disease then progressed went on to develop responses to these same regimens after receiving RRx-001.

Similar responses to previously ineffective therapy have also been found following treatment with other epigenetic-modifying agents. Hydralazine, most commonly known for its role as an anti-hypertensive medication, has also been found to act as a weak epigenetic agent by inhibiting nonnucleoside DNA methylation (18–20). In addition valproic acid, commonly used as a mood stabilizer and anti-epileptic, has been found to be a non-specific HDAC inhibitor (21,22). Similarly to RRx-001, valproic acid has been found to alter epigenetics through oxidative stress by generating reactive oxygen species that modulate and inhibit HDACs (23, 24). A phase II trial was conducted to evaluate epigenetic therapy with hydralazine and magnesium valproate in an effort to overcome chemotherapy resistance in patients with refractory solid tumors (25). Fifteen patients were evaluated who had cervical,

breast, lung, testicular or ovarian cancer which was progressing on chemotherapy. These patients were treated with hydralazine and magnesium valproate daily for one week followed by addition of hydralazine and magnesium valproate daily to the same chemotherapy regimen their disease previously progressed on. Out of these 15 patients, four (three ovarian, one cervical) had a partial response and eight (four ovarian, and one each of cervical, lung, testicular and breast) were noted to have a stable disease confirmed 4 weeks after completing another one to two cycles of chemotherapy. In a separately published series, eight patients with imatinib-refractory chronic myelogenous leukemia (two in blast phase, five in accelerated phase, and one in the chronic phase) who had no access to second-line tyrosine kinase inhibitors, and for whom transplant was not an option, were given hydralazine and magnesium valproate daily in addition to their previous imatinib regimens (26). Of these eight patients, two had a complete hematological response, one had a complete cytogenetic response, one had a major cytogenetic response and three patients had stable disease after a median of 18 months of follow-up. This further supports the notion that epigenetic agents, including agents which induce oxidative stress, have the potential to re-sensitize refractory tumors to chemotherapy and possibly even targeted therapies.

Furthermore, in a phase I/II trial evaluating the epigenetic agents azacytadine and entinostat in patients with recurrent metastatic non small cell lung cancer, four out of 19 patients (21%) who had discontinued the study went on to have major responses to subsequent therapy (27). This occurred in spite of the fact that these patients' tumors were refractory to multiple standard therapies (median of three previous therapies). Moreover, the partial responses seen in these patients with subsequent therapy were dramatic, ranging from 45–85 %. Of these four patients, three received chemotherapy as subsequent therapy (carboplatin/etoposide, cisplatin/docetaxel/bevacizumab, alimta) and one received an monoclonal antibody to PD1 (moAb). The response to these wide array of subsequent regimens was also noteworthy, suggesting that these epigenetic agents may have the potential to sensitize cancer cells to a wide array of chemotherapy regimens and possibly immunotherapies.

Very promising response data were also seen in a phase II trial evaluating low-dose (10 mg/m²) decitabine, a DNA methyltransferase inhibitor, in 17 women with heavily pre-treated and platinum-resistant ovarian cancer (28). Patients received low-dose decitabine on days 1 through 5 with carboplatin (area under the curve of 5) on day 8 of each 28-day cycle. Out of these patients, six experienced an objective response. Progression-free survival for this group was 10.2 months and nine patients (53%) were free of progression after 6 months. The response to carboplatin in that study was dramatically better than the historically reported response rates to carboplatin in similar patients with platinum-resistant ovarian cancer (<10%) (29).

In addition, another phase II trial also provides intriguing data that suggest that treatment with an epigenetic agent has the potential to improve tumor sensitivity to subsequent chemotherapy. A randomized phase II trial was conducted which evaluated exemestane with or without entinostat, a known HDAC inhibitor, in 130 post-menopausal women with locally recurrent or metastatic estrogen receptor-positive breast cancer previously progressing on non-steroidal aromatase inhibitors (30). The trial found that the addition of entinostat to exemestane significantly improved overall survival by 8.3 months (28.1 *versus* 19.8 months;

hazard ratio=0.59; 95 % confidence interval=0.36 to 0.97; $p=0.036$). However, despite this promising result, they also demonstrated that the progression-free survival was not significantly improved, nor was the response rate different with the addition of entinostat. Assessment of the post study treatments of both arms also showed that there were no significant differences. Based on these data, the authors hypothesized that the survival benefit seen may have been due to a sensitizing effect entinostat had on post study chemotherapies.

Given all of these emerging data, it appears that epigenetic agents may have the potential to help overcome chemotherapy resistance and improve sensitivity to chemotherapy, with promising clinical data on a number of malignancies, including chronic leukemia, and colorectal, ovarian, lung and breast cancer. This hypothesis is continuing to be evaluated in ongoing trials using multiple epigenetic agents against various tumor types.

Finally, while the above data are promising, it is worth noting that many of the trials mentioned above only enrolled patients with minimal residual toxicities from prior therapies. Unfortunately, in general practice, many patients come off prior chemotherapy not just due to progression but also to intolerance. While a possible role of epigenetic agents in overcoming chemotherapy resistance may not offer much benefit to patients intolerant to additional chemotherapy, these patients may still see a benefit from these therapies if these drugs are found to induce tumor-specific chemosensitivity, allowing for reduced doses and overall lower toxicities. Additionally, future studies should work to identify those patients who will benefit from these therapies by identifying and validating predictive biomarkers (such as by methylation or epigenomic profiling). All in all, we are excited about the direction the epigenetic field is exploring and hopeful that these avenues will alter the treatment armamentarium we can offer our patients.

References

1. Jelinek J, Gharibyan V, Estecio MRH, Kondo K, Rong H, Woonbok C, Lu Y, Zhang N, Liang S, Kantarjian HM, Cortes JE and Issa JPJ: Aberrant DNA methylation is associated with disease progression, resistance to imatinib and shortened survival in chronic myelogenous leukemia. *PLoS ONE* 6(7): e22110, 2011. [PubMed: 21760961]
2. Lee SM, Bae JH, Kim MJ, Lee HS, Lee MK, Chung BS, Kim DW, Kang CD and Kim SH: Bcr–Abl independent imatinib-resistant K562 cells show aberrant protein acetylation and increased sensitivity to histone deacetylase inhibitors. *J Pharmacol Exp Ther* 322: 1084–1092, 2007.
3. Zhang YW, Zheng Y, Wang JZ, Lu XX, Wang Z, Chen LB, Guan XX and Tong JD: Integrated analysis of DNA methylation and mRNA expression profiling reveals candidate genes associated with cisplatin resistance in non-small cell lung cancer. *Epigenetics* 9(6): 896–909, 2014. [PubMed: 24699858]
4. Ooki A, Yamashita K, Yamaguchi K, Mondai A, Nishimiya H and Watanabe M: DNA damage-inducible gene, repressin functions as a tumor suppressor and is suppressed by promoter methylation in gastric cancer. *Mol Cancer Res* 11: 1362–1374, 2013. [PubMed: 23982217]
5. Van Engeland M, Derks S, Smits KM, Meijer GA and Herman JG: Colorectal cancer epigenetics: complex simplicity. *J Clin Oncol* 29: 1382–1391, 2011. [PubMed: 21220596]
6. Giessrigl B, Schmidt WM, Kalipciyan M, Jeitler M, Bilban M, Gollinger M, Krieger S, Jäger W, Mader RM and Krupitza G: Fulvestrant induces resistance by modulating GPER and CDK6 expression: implication of methyltransferases, deacetylases and the hSWI/SNF chromatin remodelling complex. *Br J Cancer* 109: 2751–2762, 2013. [PubMed: 24169358]

7. Al-Nakhle H, Smith L, Bell SM, Burns PA, Cummings M, Hanby AM, Lane S, Parker MD, Hughes TA and Speirs V: Regulation of estrogen receptor β 1 expression in breast cancer by epigenetic modification of the 5' regulatory region. *Int J Oncol* 43: 2039–2045, 2013. [PubMed: 24068253]
8. Ferber A, Tan WS, de Winter P, Freeman A, King K and Gurung P: Epigenetic alterations associated with neo-adjuvant chemotherapy resistance in bladder cancer. *Eur Urol Suppl* 12(1): e51, 2013.
9. Zeller C, Dai W, Steele NL, Siddig A, Walley AJ, Wilhelm-Benartzi CS, Rizzo S, van der Zee A, Plumb JA and Brown R: Candidate DNA methylation drivers of acquired cisplatin resistance in ovarian cancer identified by methylome and expression profiling. *Oncogene* 31: 4567–4576, 2012. [PubMed: 22249249]
10. Strathdee G, MacKean MJ, Illand M and Brown R: A role for methylation of the hMLH1 promoter in loss of hMLH1 expression and drug resistance in ovarian cancer. *Oncogene* 18: 2335–2341, 1999. [PubMed: 10327053]
11. Kaminskas E, Farrell A, Abraham S, Baird A, Hsieh LS, Lee SL, Leighton JK, Patel H, Rahman A, Sridhara R, Wang, YC and Pazdur R: Approval summary: azacitidine for treatment of myelodysplastic syndrome subtypes. *Clin Cancer Res* 11(10): 3604–3608, 2005. [PubMed: 15897554]
12. Kantarjian H Issa JP, Rosenfeld CS, Bennett JM, Albitar M, DiPersio J, Jimek V, Slack J, de Castro C, Ravandi F, Helmer R, 3rd, Shen L, Nimer SD, Leavitt R, Raza A and Saba H: Decitabine improves patient outcomes in myelodysplastic syndromes: results of a phase III randomized study. *Cancer* 106(8): 1794–1803, 2006. [PubMed: 16532500]
13. Fenaux P, Mufti GJ, Hellstrom-Lindberg E, Santini V, Finelli C, Giagounidis A, Schoch R, Gattermann N, Sanz G, List A, Gore SD, Seymour JF, Bennett JM, Byrd J, Backstrom J, Zimmerman L, McKenzie D, Beach C and Silverman LR: Efficacy of azacitidine compared with that of conventional care regimens in the treatment of higher-risk myelodysplastic syndromes: a randomised, open-label, phase III study. *Lancet Oncol* 10(3): 223–232, 2009. [PubMed: 19230772]
14. Olsen EA, Kim YH, Kuzel TM, Pacheco TR, Foss FM, Parker S, Franel SR, Chen C, Reker JL, Arduino JM and Duvic M: Phase IIb multicenter trial of vorinostat in patients with persistent, progressive, or treatment refractory cutaneous T-cell lymphoma. *J Clin Oncol* 25(21): 3109–3115, 2007. [PubMed: 17577020]
15. Whittaker SJ, Demierre MF, Kim EJ, Rook AH, Lerner A, Duvic M, Scarisbrick J, Reddy S, Robak T, Becker JC, Samtsov A, McCulloch W and Kim YH: Final results from a multicenter, international, pivotal study of romidepsin in refractory cutaneous T-cell lymphoma. *J Clin Oncol* 28(29): 4485–4491, 2010. [PubMed: 20697094]
16. Plumb JA, Strathdee G, Sludden J, Kaye SB and Brown R: Reversal of drug resistance in human tumor xenografts by 2'-deoxy-5-azacytidine-induced demethylation of the hMLH1 gene promoter. *Cancer Res* 60: 6039–6044, 2000. [PubMed: 11085525]
17. Reid T, Oronsky B, Scicinski J, Scribner CL, Knox SJ, Ning S, Peehl DM, Korn R, Stirn M, Carter CA, Oronsky A, Taylor MJ, Fitch WL, Cabrales P, Kim MM, Burris HA, Lao CD, Abrouk NED, Fanger GR and Infante JR: Safety and activity of RRx-001 in patients with advanced cancer: a first-in-human, open-label, dose-escalation phase I study. *Lancet Oncol* 16(9): 1133–1142, 2015. [PubMed: 26296952]
18. Cornacchia E, Golbus J, Maybaum J, Straler, Hanash S and Richardson B: Hydralazine and procainamide inhibit T-cell DNA methylation and induce autoreactivity. *J Immunol* 140(7): 2197–2200, 1988. [PubMed: 3258330]
19. Segura-Pacheco B, Trejo-Becerri C, Perez-Cardenas E, Taja-Chayeb L, Mariscal I, Chavez A, Acuna C, Salazar AM, Lizano M and Duenas-Gonzalez A: Reactivation of tumor suppressor genes by the cardiovascular drugs hydralazine and procainamide and their potential use in cancer therapy. *Clin Cancer Res* 9(5): 1596–1603, 2003. [PubMed: 12738711]
20. Chuang JC, Yoo CB, Kwan JM, Li TW, Liang G, Yang AS and Jones PA: Comparison of biological effects of non-nucleoside DNA methylation inhibitors versus 5-aza-2'-deoxycytidine. *Mol Cancer Ther* 4(10): 1515–1520, 2005. [PubMed: 16227400]
21. Rocchi P, Tonelli R, Camerin C, Purgato S, Fronza R, Bianucci F, Pession A and Ferrer AM: p21Waf1/Cip1 is a common target induced by short-chain fatty acid HDAC inhibitors (valproic

- acid, tributyrin and sodium butyrate) in neuroblastoma cells. *Oncol Rep* 13(6): 1139–1144, 2005. [PubMed: 15870934]
22. Fredly H, Gjertsen BT and Bruserud O: Histone deacetylase inhibition in the treatment of acute myeloid leukemia: the effects of valproic acid on leukemic cells, and the clinical and experimental evidence for combining valproic acid with other antileukemic agents. *Clin Epigenetics* 5(1): 12, 2013. [PubMed: 23898968]
 23. Chang TK and Abbott FS: Oxidative stress as a mechanism of valproic acid-associated hepatotoxicity. *Drug Metab Rev* 38(4): 627–639, 2006. [PubMed: 17145692]
 24. Rossi L, Valle C and Carri MT: Altered gene expression, mitochondrial damage and oxidative stress: converging routes in motor neuron degeneration. *Int J Cell Biol* 2012: 908724, 2012. [PubMed: 22675362]
 25. Candelaria M, Gallardo-Rincon D, Arce C, Cetina L, Aguilar-Ponce JL, Arrieta O, Ganzalez-Fierro A, Chavez-Blanco A, de la Cruz-Hernandez E, Camargo MF, Trejo-Becerril C, Perez-Cardenas E, Perez-Plasencia C, Taja-Chayeb L, Wegmen-Ostrosky T, Revilla-Vazquez A and Duenas-Gonzalez A: A phase II study of epigenetic therapy with hydralazine and magnesium valproate to overcome chemotherapy resistance in refractory solid tumors. *Ann Oncol* 18(9): 1529–1538, 2007. [PubMed: 17761710]
 26. Cervera E, Candelaria M, Lopez-Navarro O, Labardini J, Gonzalez-Fierro A, Taja-Chayeb L, Cortes J, Gordillo-Bastidas D and Duenas-Gonzalez A: Epigenetic therapy with hydralazine and magnesium valproate reverses imatinib resistance in patients with chronic myeloid leukemia. *Clin Lymphoma Myeloma Leuk* 12(3): 207–212, 2012. [PubMed: 22420986]
 27. Juergens RA, Wrangle J, Wendetti FP, Murphy SC, Zhao M, Coleman B, Sebree R, Rodgers K, Hooker CM, Franco N, Lee B, Tsai S, Delgado IE, Rudek MA, Belinsky SA, Herman JG, Baylin SB, Brock MV and Rudin CM: Combination epigenetic therapy has efficacy in patients with refractory advanced non-small cell lung cancer. *Cancer Discov* 1 (7): 598–607, 2011. [PubMed: 22586682]
 28. Matei D, Fang F, Sherr C, Schilder J, Arnold A, Zeng Y, Berry WA, Huang T and Nephew KP: Epigenetic resensitization to platinum in ovarian cancer. *Cancer Res* 72(9): 2197–2205, 2012. [PubMed: 22549947]
 29. See HT, Freedman RS, Kudelka AP, Burke TW, Gershenson DM, Tanqitqamol S and Kavanagh JJ: Retrospective review: retreatment of patients with ovarian cancer with carboplatin after platinum resistance. *Int J Gynecol Cancer* 15(2): 209–216, 2005. [PubMed: 15823101]
 30. Yardley DA, Ismail-Kahn RR, Melichar B, Lichinister M, Munster PN, Klein PM, Cruickshank S, Miller KD, Lee MJ and Trepel JB: Randomized phase II, double-blind, placebo-controlled study of exemestane with or without entinostat in postmenopausal women with locally recurrent or metastatic estrogen receptor-positive breast cancer progressing on treatment with a nonsteroidal aromatase inhibitor. *JCO* 31(17): 2128–2135, 2013.