Tempol prevents genotoxicity induced by vorinostat: role of oxidative DNA damage

Karem H. Alzoubi · Omar F. Khabour ·
Aya G. Jaber · Sayer I. Al-azzam ·
Nizar M. Mhaidat · Majed M. Masadeh

Received: 15 February 2013 / Accepted: 3 June 2013 / Published online: 13 June 2013
© Springer Science+Business Media Dordrecht 2013

Abstract Vorinostat is a member of histone deacetylase inhibitors, which represents a new class of anticancer agents for the treatment of solid and hematological malignancies. Studies have shown that these drugs induce DNA damage in blood lymphocytes, which is proposed to be due to the generation of oxidative lesions. The increase in DNA damage is sometimes associated with risk of developing secondary cancer. Thus, finding a treatment that limits DNA damage caused by anticancer drugs would be beneficial. Tempol is a potent antioxidant that was shown to prevent DNA damage induced by radiation. In this study, we aimed to investigate the harmful effects of vorinostat on DNA damage, and the possible protective effects of tempol against this damage. For that, the spontaneous frequency of sister chromatid exchanges

(SCEs), chromosomal aberrations (CAs), and 8-hydroxy-2-deoxy guanosine (8-OHdG) levels were measured in cultured human lymphocytes treated with vorinostat and/or tempol. The results showed that vorinostat significantly increases the frequency of SCEs, CAs and 8-OHdG levels in human lymphocytes as compared to control. These increases were normalized by the treatment of cells with tempol. In conclusion, vorinostat is genotoxic to lymphocytes, and this toxicity is reduced by tempol. Such results could set the stage for future studies investigating the possible usefulness of antioxidants co-treatment in preventing the genotoxicity of vorinostat when used as anticancer in human.

Keywords Sister chromatid exchanges · Chromosomal aberrations · 8-OH-dG · Vorinostat · Tempol

K. H. Alzoubi (✉) · A. G. Jaber · S. I. Al-azzam ·
N. M. Mhaidat
Department of Clinical Pharmacy, Faculty of Pharmacy,
Jordan University of Science and Technology,
Irbid 22110, Jordan
e-mail: khalzoubi@just.edu.jo

O. F. Khabour
Department of Medical Laboratory Sciences, Faculty of
Applied Medical Sciences, Jordan University of Science
and Technology, Irbid, Jordan

M. M. Masadeh
Department of Pharmaceutical Technology, Faculty of
Pharmacy, Jordan University of Science and Technology,
Irbid, Jordan

Introduction

Vorinostat (suberoylanilide hydroxamic acid) is a hydroxamic acid derivative that inhibits both class I and II histone deacetylases (Richon 2010). It has been approved in the United States for the treatment of patients with relapsed and refractory cutaneous T cell lymphoma that has progressive, persistent or recurrent disease on or following two systemic therapies (Prebet and Vey 2011; Mann et al. 2007; Siegel et al. 2009).

The mechanism for the antiproliferative effect of vorinostat is believed to be the result of inhibition of histone deacetylase activity, resulting in the accumulation of acetylated proteins, including histones (Richon 2010; Marks et al. 2004). In addition, studies have shown that vorinostat induces DNA damage, which is proposed to be due to the generation of oxidative lesions (Namdar et al. 2010; Petrucci et al. 2011; Lee et al. 2010). The increase in DNA damage caused by several anticancer drugs has been shown to increase the risk of developing secondary cancer (Madeya 1996; Ezoe 2012). Thus, finding a treatment that limits DNA damage caused by anticancer drugs would be beneficial. Interestingly, the response to vorinostat could be improved by combining it with antioxidants such as vitamin E for the treatment of oxidatively stressed human malignancies that are otherwise resistant to vorinostat (Basu et al. 2011). In this study, we hypothesize that treatment with antioxidants may modulate the genotoxicity of vorinostat.

Tempol (4-hydroxy-2, 2, 6, 6-tetramethylpiperidine-1-oxyl) is a member of a family of nitroxide compounds that has been extensively studied for its effects on hypertension and endothelial function, in animal models of increased ROS generation (Wilcox 2010; Carroll et al. 2000). Tempol has been shown to preserve mitochondria against oxidative damage and improve tissue oxygenation (Wilcox 2010). It protects normal cells from radiation while maintaining radiation sensitivity of tumor cells. The paradoxical pro-oxidant action of tempol has accounted for a reduction in spontaneous tumor formation in tumor cells (Wilcox 2010). Tempol has been effective in preventing several of the adverse consequences of oxidative stress and inflammation that underlie radiation damage (Mitchell et al. 2012), and aging (Fleenor et al. 2012). Few studies investigated the protective effect of tempol on substances-induced genotoxicity in human lymphocytes. An *in vitro* study found a significant ability of Tempol to diminish the genotoxic effects of cadmium and chromium, which depends, at least in part, on formation of reactive oxygen species (Lewinska et al. 2008). Another *in vitro* study showed that tempol protects human lymphocytes against genotoxicity induced by gamma-radiation (Ramachandran and Nair 2012). In the current study, we investigated the potential genotoxic and oxidative DNA damaging effect of vorinostat and the possible protective effect of tempol against these damages. This is achieved by examining the SCEs, CAs and 8-OHdG in human blood

lymphocytes. Results of this study could provide the base for further clinical studies on tempol, or similar antioxidants, as potential protective agents against genotoxicity associated with vorinostat.

Materials and methods

Subjects

Whole blood samples were obtained from 4 donors under sterile conditions. The donors were healthy males with age range of 23–30 years. Subjects who were using alcohol, drugs, vitamins, tobacco smokers and those who had cancer and hereditary diseases were excluded from the study. Informed consent was obtained from each volunteer according to the Jordan University of Science and Technology-Institutional Review Board (JUST-IRB). Whole blood cultures were prepared within 1 h of sampling.

Lymphocytes culture and drug treatment

Blood cultures were set up by inoculating 1 ml of freshly withdrawn blood into 75 ml tissue-culture flask containing 9 ml of PB-Max medium (Gibco-Invitrogen, Paisley, UK; complete medium that is composed of RPMI 1640 medium with 15 % fetal bovine serum, 1 % penicillin–streptomycin and 3 % of phytohaemagglutinin) in order to stimulate lymphocyte culture (M'Bemba-Meka et al. 2007).

Vorinostat (ABO Swiss Co., Ltd., Fujian, China) was weighed as aliquot to be used fresh each time in culture, the aliquot was dissolved in DMSO to make a stock of 50 mM. The final concentration in culture medium was 10 μ M. Tempol (Sigma-Aldrich, St. Louis, MO, USA) was prepared in a similar way to that of vorinostat and was used in a final concentration of 10 μ M. Tempol was added at the time of culture, while vorinostat was added 16 h prior to harvesting (Petrucci et al. 2011; Lee et al. 2010). To evaluate the effect of vorinostat on DNA and how this effect is modulated by tempol, four groups were used: control non-treated (Control), tempol-treated (Temp), vorinostat-treated (Vori), and tempol and vorinostat treated (Temp + Vori) groups. Two hours prior to cell harvesting, cultures were treated with 100 μ l of 10 μ g/ml colcemid (Gibco-Invitrogen, Paisley, UK), a spindle inhibitor to increase the mitotic index.

Harvesting and metaphases slides preparation

The cells of each blood culture were transferred to a 15 ml polypropylene screw-capped tube and centrifuged at $1,000\times g$ for 5 min. The pellet was resuspended in pre-warmed hypotonic solution (0.56 % KCl) and incubated for 30 min at 37 °C. The swollen lymphocytes were collected by centrifugation at $1,000\times g$ for 5 min, fixed by drop-wise addition of freshly prepared fixative [absolute methanol: acetic acid glacial 3:1 (v:v)] and incubated at room temperature for 30 min. Cells were centrifuged at $1,000\times g$ for 5 min, washed three times with the same fixative. Finally, cells were resuspended in a small amount (approximately 2 ml) of the fixative. The cellular suspension was then dropped on pre-chilled microscope slides to obtain metaphase spreads. The slides were allowed to air dry to be ready for staining with black Giemsa (Sigma-Aldrich, St. Louis, MO, USA) for chromosomal aberration test and with fluorescence-plus Giemsa (Sigma-Aldrich, St. Louis, MO, USA) for sister chromatid exchange test (M'Bemba-Meka et al. 2007).

Sister chromatid exchange assay

Bromodeoxyuridine (10 µg/ml final concentration; BrdU, Sigma-Aldrich, St. Louis, MO, USA), was added to the culture media prior to incubation and throughout the experiment. All cultures were maintained in total darkness to minimize photolysis of BrdU at 37 °C for 72 h. The culture initiation and slides preparation were as described above. Air dried slides were differentially stained by 10 µg/ml Hoechst 33342 dye solution (Sigma-Aldrich, St. Louis, MO, USA) for 20 min, followed by rinsing in water and mounting in McIlvaine buffer (pH 8.0). The slides were then irradiated with UV lamp (350 nm) using two (15 W tubes) lamps at a distance of 7 cm for 35 min at 50 °C. Slides were then rinsed with distilled water, restained for 6–8 min with 5 % Giemsa stain in phosphate buffer (pH 7.4), and then air dried (Azab et al. 2009; Khabour et al. 2013).

Chromosomal aberration assay

Air dried slides prepared from cultures without BrdU were stained with 5 % Giemsa stain. Structural and numerical CAs were evaluated in 100 well-spread

metaphases per donor (400 metaphases total). CAs were divided into gaps (including both chromatid and chromosomal gaps), breaks (including both chromatid breaks and chromosomal breaks) and exchanges.

Cell kinetics analysis

The mitotic index (MI) values reflect the degree of cytotoxicity of the drugs used. The MI was calculated by analyzing at least 1,000 cells from each donor and scoring the cells that were in metaphase as described by Alsatari et al. (2012). For the cell proliferation index, 100 metaphase cells from each donor were scored. The proliferation index was calculated using the following formula = $(1 \times [M1] + 2 \times [M2] + 3 \times [\geq M3]) / 100$, where M1, M2 and M3 are the number of cells at the first, second and third metaphase, respectively (Khabour et al. 2011; Ivett and Tice 1982). Depending on the proliferation index, the average generation time was calculated as the number of hours for the cells in BrdU, divided by proliferation index (Kaya and Topaktas 2007).

8-Hydroxy-2-deoxy guanosine (8-OHdG) assay

The 8-OHdG assay was performed as previously described (Al-Sweedan et al. 2012; Alzoubi et al. 2012). In brief, blood cultures were set up by inoculating 0.5 ml of freshly withdrawn blood, into 50 ml culture flasks containing 4.5 ml of PB-Max medium. Then, the cultures were incubated for 72 h at 37 °C and treated with drugs as described above. Competitive ELISA assays for 8-OH-dG were performed according to the manufactures protocol (8-hydroxy 2 deoxyguanosine ELISA Kit; Abcam, Cambridge, UK). Samples were assayed in duplicates using 100 µl of supernatant of each. ELISA plate was read at 405 nm using an automated reader (ELx800, Bio-tek instruments, Highland Park, Winooski, USA). Levels of 8-OH-dG were calculated from the blotted standard curve.

Statistical analysis

Statistical analysis was performed using Graphpad Prism Statistical Software (version 5, La Jolla, CA, USA). Data were expressed as mean $\pm$ standard error (SE). The comparison of parameters was performed with Newman-Keuls Multiple Comparison Test.

Differences were regarded as significant at $P < 0.05$ (by using ANOVA test). All experiments were done once and in duplicates. Chi square test was performed using SPSS version 17.

Results and discussion

The results show that vorinostat (10 μM) induced significant aberrations in whole blood lymphocytes and that tempol (10 μM) significantly reduced the number of vorinostat induced aberrations (Table 1). Treatment of cultures with tempol alone did not affect spontaneous levels of CAs in the control group (Table 1). When gaps were included in the analysis, similar data, to that of “CAs without gaps”, were obtained (Table 1).

Results of chromosomal aberrations were confirmed by SCEs assay. The SCEs frequencies were increased in vorinostat treated human lymphocytes compared to other groups ($P < 0.01$, Fig. 1). When treated with tempol, cells were protected from the genotoxic effect of vorinostat ($P < 0.05$). These results indicate that vorinostat induced significant SCEs in normal blood cells. Tempol, on the other hand, significantly reduced the SCEs frequency. In addition, treatment of cultures with tempol alone did not affect the spontaneous frequency of SCEs in lymphocytes ($P > 0.05$).

Figure 2 shows the level of 8-OHdG in the different groups. Levels of 8-OHdG in vorinostat treated cultures was higher than in the control ($P < 0.05$). Tempol, on the other hand, prevented vorinostat induced increase in 8-OHdG level. Tempol alone did not affect basal levels of 8-OHdG ($P > 0.05$). Thus, similar to CAs and SCEs data, tempol normalized

Fig. 1 The average of SCEs/cell in control, tempol, vorinostat, and a combination of tempol and vorinostat groups. In the tempol group, tempol (10 μM) was added at zero time for the whole incubation period (72 h). In the vorinostat group, vorinostat (10 μM) was added to the medium 16 h before harvesting. For the last group, a combination of tempol and vorinostat group, tempol was added to the medium at the onset of the culture and vorinostat was added to the medium 16 h before harvesting. Significant differences were detected among groups (control vs. Vori: $P < 0.001$, Temp vs. Vori: $P < 0.001$ and Temp and Vori vs. Vori: $P < 0.01$). These results indicated that vorinostat significantly increased the SCEs frequency in healthy blood lymphocytes and tempol significantly reduced the SCEs frequency ($P < 0.01$)

oxidative DNA damage induced by vorinostat without affecting spontaneous damage levels in the control.

Mitotic and proliferative indices reflect the degree of cytotoxicity of chemical and physical agents. Both, the mitotic and the proliferative indices, were not affected by either vorinostat and/or tempol (Table 2, $P > 0.05$).

Thus, the major finding in this study is that tempol might prevent vorinostat induced DNA damage as indicated by the SCEs and CAs assays. This protective effect of tempol was associated with normalization of

Table 1 Frequencies of CAs in control, tempol, vorinostat, and a combination of tempol and vorinostat groups. Vorinostat (10 μM) treatment increased the number of CAs (gaps, breaks, and interchanges), which was prevented by pre-treatment with tempol (10 μM)

Groups	Total no. scored cells	Frequency of gaps aberrations per cell. (Mean $\pm$ SE)	Frequency of breaks and interchanges aberrations per cell. (Mean $\pm$ SE)	Total frequency of aberrations with gaps per cell (mean $\pm$ SE)
Controls	400	0.174 $\pm$ 0.007	0.004 $\pm$ 0.002	0.178 $\pm$ 0.010
Temp.	400	0.194 $\pm$ 0.014	0.006 $\pm$ 0.002	0.200 $\pm$ 0.015
Vori.	400	0.312 $\pm$ 0.008	0.034 $\pm$ 0.004	0.346 $\pm$ 0.012
Temp + Vori	400	0.194 $\pm$ 0.012	0.010 $\pm$ 0.003	0.204 $\pm$ 0.014
<i>P</i> value*		<0.0001	<0.0001	<0.0001

* P value < 0.05 between: (Control vs. Vori., Temp. vs. Vori, and Temp. + Vori. vs. Vori.)

Fig. 2 Levels of 8-OHdG in control, tempol, vorinostat, and a combination of tempol and vorinostat groups. Vorinostat (10 μ M) significantly increased the levels of 8-OHdG. Tempol (10 μ M), on the other hand, prevented this increase. Value are Mean $\pm$ SE. * indicates significant difference ($P < 0.05$)

vorinostat-induced oxidative DNA damage biomarker 8-OHdG. Results of this study showed that vorinostat, the histone deacetylase inhibitor, is genotoxic to human lymphocytes, as indicated by the increased frequencies of SCEs and CAs in vorinostat-treated cells. This is consistent with previous studies showing that vorinostat induces oxidative DNA damage through the course of its action (Namdar et al. 2010; Petruccielli et al. 2011; Lee et al. 2010), though it was shown to be more effective on cells with lower oxidative stress status or cells pre-treated with an antioxidant (Basu et al. 2011).

Tempol was shown to significantly reduce the effect of vorinostat-induced increase in CAs and SCEs, indicating the ability of tempol to lower DNA damage induced by vorinostat. The SCEs and CAs are sensitive indicators of genotoxicity. They are widely used especially in genotoxicity assessment in human subjects (Kao-Shan et al. 1987). It is generally thought that, the induction of chromosomal aberrations, along with an increase in SCE, indicate higher levels of genomic instability, which could ultimately lead to increase cancer risk (Oh et al. 2007). Few studies

investigated the effect of tempol on substances induced cyto- and geno-toxicity in human lymphocytes in vitro. One study found a significant ability of tempol to diminish genotoxic effects of cadmium and chromium, which depends, at least in part, on prevention of formation of reactive oxygen species (Lewinska et al. 2008). Another study showed that tempol prevents radiation induced genotoxicity (Ramachandran and Nair 2012). Thus, our results and those of others (Lewinska et al. 2008; Ramachandran and Nair 2012) suggest the potential use of tempol as anti-genotoxic agent.

The antioxidant activity of tempol is well documented (Wilcox 2010), and given the strong connection between DNA damage and oxidative stress level of the cell (Vera-Ramirez et al. 2012; Murata et al. 2012), oxidative DNA damage represents an obvious possible mechanism for the protective effect of tempol against genotoxicity. In accordance with that, results of the current study show that vorinostat increases the levels of 8-OHdG, a known marker of oxidative DNA damage. This increase in 8-OHdG was normalized by treatment of cultured lymphocytes with tempol. Thus, it is likely that tempol protects against vorinostat induced genotoxicity through its normalization of oxidative DNA damage. A recent study reported that tempol increases the rate of DNA repair as indicated by elevated DNA repair index in cells exposed to tempol, immediately after exposure to gamma-radiation that was shown to induce genotoxicity in the absence of tempol (Ramachandran and Nair 2012). This could represent another possible mechanism for protective effect of tempol against vorinostat genotoxicity. Future work could examine this point.

It is worth mentioning that the response to vorinostat could be improved by combining it with other antioxidants such as vitamin E for the treatment of oxidatively stressed human malignancies that are otherwise resistant to vorinostat (Basu et al. 2011). Similar to vitamin E, tempol is approved medication

Table 2 Mean values of studied cytotoxicity parameters in control, tempol, vorinostat and a combination of tempol and vorinostat groups

Parameters	Control (Mean $\pm$ SE)	Tempol (Mean $\pm$ SE)	Vorinostat (Mean $\pm$ SE)	Tempol and Vorinostat (Mean $\pm$ SE)	P value
MI	8.64 $\pm$ 1.72	9.36 $\pm$ 1.33	5.34 $\pm$ 1.07	6.82 $\pm$ 0.96	0.165
PI	1.47 $\pm$ 0.09	1.41 $\pm$ 0.09	1.39 $\pm$ 0.05	1.28 $\pm$ 0.13	0.5534

No significant change was observed among studied groups in both the MI and PI

with strong antioxidant effect. Thus, tempol protects against genotoxicity of vorinostat and at the same time it may enhance vorinostat activity against malignant cells. This requires further investigation, which might be a subject of future studies.

In this study, we examined the potential protective effect of tempol on genotoxicity of vorinostat in whole blood lymphocytes using SCEs and CAs assays. SCEs and CAs are sensitive indicators of genotoxicity. They are widely used especially in genotoxicity assessment in human subjects (Kao-Shan et al. 1987). It is generally thought that, the induction of chromosomal aberrations, along with an increase in SCE, indicate higher levels of genomic instability, which could ultimately lead to cancer (Oh et al. 2007). Confirming the effect of tempol on the cells using other assays such as cytokinesis-block micronucleus (CBMN) assay to measure micronucleus (MN) formation, the nuclear division index (NDI) and the percentage of apoptotic and necrotic cells is recommended for future study.

Collectively, vorinostat is a genotoxic drug to whole blood lymphocytes. It also induces oxidative DNA damage. Tempol, on the other hand, has a protective effect against vorinostat-induced genotoxicity and oxidative DNA damage.

Acknowledgments This project was supported by a grant (No 12/2012) from the Deanship of Research at the Jordan University of Science and Technology.

References

- Alsatari ES, Azab M, Khabour OF, Alzoubi KH, Sadiq MF (2012) Assessment of DNA damage using chromosomal aberrations assay in lymphocytes of waterpipe smokers. *Int J Occup Med Environ Health* 25:218–224. doi:[10.2478/S13382-012-0027-5](https://doi.org/10.2478/S13382-012-0027-5)
- Al-Sweedan SA, Khabour O, Isam R (2012) Genotoxicity assessment in patients with thalassemia minor. *Mutat Res* 744:167–171. doi:[10.1016/j.mrgentox.2012.02.010](https://doi.org/10.1016/j.mrgentox.2012.02.010)
- Alzoubi K, Khabour O, Hussain N, Al-azzam S, Mhaidat N (2012) Evaluation of vitamin B12 effects on DNA damage induced by pioglitazone. *Mutat Res* 748:48–51. doi:[10.1016/j.mrgentox.2012.06.009](https://doi.org/10.1016/j.mrgentox.2012.06.009)
- Azab M, Khabour OF, Al-Omari L, Alzubi MA, Alzoubi K (2009) Effect of every-other-day fasting on spontaneous chromosomal damage in rat's bone-marrow cells. *J Toxicol Environ Health A* 72:295–300. doi:[10.1080/15287390802526357](https://doi.org/10.1080/15287390802526357)
- Basu HS, Mahlum A, Mehraein-Ghomi F, Kegel SJ, Guo S, Peters NR, Wilding G (2011) Pretreatment with anti-oxidants sensitizes oxidatively stressed human cancer cells to growth inhibitory effect of suberoylanilide hydroxamic acid (SAHA). *Cancer Chemother Pharmacol* 67:705–715. doi:[10.1007/s00280-010-1364-3](https://doi.org/10.1007/s00280-010-1364-3)
- Carroll RT, Galatsis P, Borosky S, Kopec KK, Kumar V, Althaus JS, Hall ED (2000) 4-Hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (Tempol) inhibits peroxynitrite-mediated phenol nitration. *Chem Res Toxicol* 13:294–300
- Ezoe S (2012) Secondary leukemia associated with the anti-cancer agent, etoposide, a topoisomerase II inhibitor. *Int J Environ Res Public Health* 9:2444–2453. doi:[10.3390/ijerph9072444](https://doi.org/10.3390/ijerph9072444)
- Fleenor BS, Seals DR, Zigler ML, Sindler AL (2012) Superoxide-lowering therapy with TEMPOL reverses arterial dysfunction with aging in mice. *Aging Cell* 11:269–276. doi:[10.1111/j.1474-9726.2011.00783.x](https://doi.org/10.1111/j.1474-9726.2011.00783.x)
- Ivett JL, Tice RR (1982) Average generation time: a new method of analysis and quantitation of cellular proliferation kinetics. *Environ Mutagen* 4:358
- Kao-Shan CS, Fine RL, Whang-Peng J, Lee EC, Chabner BA (1987) Increased fragile sites and sister chromatid exchanges in bone marrow and peripheral blood of young cigarette smokers. *Cancer Res* 47:6278–6282
- Kaya FF, Topaktas M (2007) Genotoxic effects of potassium bromate on human peripheral lymphocytes in vitro. *Mutat Res* 626:48–52. doi:[10.1016/j.mrgentox.2006.08.006](https://doi.org/10.1016/j.mrgentox.2006.08.006)
- Khabour OF, Alsatari ES, Azab M, Alzoubi KH, Sadiq MF (2011) Assessment of genotoxicity of waterpipe and cigarette smoking in lymphocytes using the sister-chromatid exchange assay: a comparative study. *Environ Mol Mutagen* 52:224–228. doi:[10.1002/em.20601](https://doi.org/10.1002/em.20601)
- Khabour OF, Soudah OA, Aaysh MH (2013) Genotoxicity assessment in iron deficiency anemia patients using sister chromatid exchanges and chromosomal aberrations assays. *Mutat Res* 750:72–76. doi:[10.1016/j.mrgentox.2012.09.006](https://doi.org/10.1016/j.mrgentox.2012.09.006)
- Lee JH, Choy ML, Ngo L, Foster SS, Marks PA (2010) Histone deacetylase inhibitor induces DNA damage, which normal but not transformed cells can repair. *Proc Natl Acad Sci U S A* 107:14639–14644. doi:[10.1073/pnas.1008522107](https://doi.org/10.1073/pnas.1008522107)
- Lewinska A, Wnuk M, Slota E, Bartosz G (2008) The nitroxide antioxidant Tempol affects metal-induced cyto- and genotoxicity in human lymphocytes in vitro. *Mutat Res* 649:7–14. doi:[10.1016/j.mrgentox.2007.07.014](https://doi.org/10.1016/j.mrgentox.2007.07.014)
- Madeya ML (1996) Oral complications from cancer therapy: part 1—pathophysiology and secondary complications. *Oncol Nurs Forum* 23:801–807
- Mann BS, Johnson JR, He K, Sridhara R, Abraham S, Booth BP, Verbois L, Morse DE, Jee JM, Pope S, Harapanhalli RS, Dagher R, Farrell A, Justice R, Pazdur R (2007) Vorinostat for treatment of cutaneous manifestations of advanced primary cutaneous T-cell lymphoma. *Clin Cancer Res* 13:2318–2322. doi:[10.1158/1078-0432.CCR-06-2672](https://doi.org/10.1158/1078-0432.CCR-06-2672)
- Marks PA, Richon VM, Miller T, Kelly WK (2004) Histone deacetylase inhibitors. *Adv Cancer Res* 91:137–168. doi:[10.1016/S0065-230X\(04\)91004-4](https://doi.org/10.1016/S0065-230X(04)91004-4)
- M'Bemba-Meka P, Lemieux N, Chakrabarti SK (2007) Role of oxidative stress and intracellular calcium in nickel carbonate hydroxide-induced sister-chromatid exchange, and alterations in replication index and mitotic index in cultured human peripheral blood lymphocytes. *Arch Toxicol* 81:89–99. doi:[10.1007/s00204-006-0128-7](https://doi.org/10.1007/s00204-006-0128-7)

- Mitchell JB, Anver MR, Sowers AL, Rosenberg PS, Figueroa M, Thetford A, Krishna MC, Albert PS, Cook JA (2012) The antioxidant tempol reduces carcinogenesis and enhances survival in mice when administered after nonlethal total body radiation. *Cancer Res* 72:4846–4855. doi: [10.1158/0008-5472.CAN-12-1879](https://doi.org/10.1158/0008-5472.CAN-12-1879)
- Murata M, Thanan R, Ma N, Kawanishi S (2012) Role of nitrate and oxidative DNA damage in inflammation-related carcinogenesis. *J Biomed Biotechnol* 2012:623019. doi: [10.1155/2012/623019](https://doi.org/10.1155/2012/623019)
- Namdar M, Perez G, Ngo L, Marks PA (2010) Selective inhibition of histone deacetylase 6 (HDAC6) induces DNA damage and sensitizes transformed cells to anticancer agents. *Proc Natl Acad Sci U S A* 107:20003–20008. doi: [10.1073/pnas.1013754107](https://doi.org/10.1073/pnas.1013754107)
- Oh SD, Lao JP, Hwang PY, Taylor AF, Smith GR, Hunter N (2007) BLM ortholog, Sgs1, prevents aberrant crossing-over by suppressing formation of multichromatid joint molecules. *Cell* 130:259–272. doi: [10.1016/j.cell.2007.05.035](https://doi.org/10.1016/j.cell.2007.05.035)
- Petrucelli LA, Dupere-Richer D, Pettersson F, Retrouvey H, Skoulikas S, Miller WH Jr (2011) Vorinostat induces reactive oxygen species and DNA damage in acute myeloid leukemia cells. *PLoS ONE* 6:e20987. doi: [10.1371/journal.pone.0020987](https://doi.org/10.1371/journal.pone.0020987)
- Prebet T, Vey N (2011) Vorinostat in acute myeloid leukemia and myelodysplastic syndromes. *Expert Opin Investig Drugs* 20:287–295. doi: [10.1517/13543784.2011.542750](https://doi.org/10.1517/13543784.2011.542750)
- Ramachandran L, Nair CK (2012) Prevention of gamma-radiation induced cellular genotoxicity by tempol: protection of hematopoietic system. *Environ Toxicol Pharmacol* 34:253–262. doi: [10.1016/j.etap.2012.04.008](https://doi.org/10.1016/j.etap.2012.04.008)
- Richon VM (2010) Targeting histone deacetylases: development of vorinostat for the treatment of cancer. *Epigenomics* 2:457–465. doi: [10.2217/epi.10.20](https://doi.org/10.2217/epi.10.20)
- Siegel D, Hussein M, Belani C, Robert F, Galanis E, Richon VM, Garcia-Vargas J, Sanz-Rodriguez C, Rizvi S (2009) Vorinostat in solid and hematologic malignancies. *J Hematol Oncol* 2:31. doi: [10.1186/1756-8722-2-31](https://doi.org/10.1186/1756-8722-2-31)
- Vera-Ramirez L, Ramirez-Tortosa M, Perez-Lopez P, Grana-dos-Principal S, Battino M, Quiles JL (2012) Long-term effects of systemic cancer treatment on DNA oxidative damage: the potential for targeted therapies. *Cancer Lett* 327:134–141. doi: [10.1016/j.canlet.2011.12.029](https://doi.org/10.1016/j.canlet.2011.12.029)
- Wilcox CS (2010) Effects of tempol and redox-cycling nitroxides in models of oxidative stress. *Pharmacol Ther* 126:119–145. doi: [10.1016/j.pharmthera.2010.01.003](https://doi.org/10.1016/j.pharmthera.2010.01.003)