Alteration of radiation-sensitive processes associated with cancer and longevity by dietary 2-mercaptoethanol

Robert E. Click [retired]
University Minnesota, Minneapolis, MN

Abstract

Background—Previous results demonstrated dietary 2-mercaptoethanol (2-ME) delayed appearance of cancer in certain murine strains. In addition, it had a benefit not found with other organosulfurs, in that it completely prevented spontaneous development of cancer in BXSB-Yaa over an entire lifespan.

Aims—These benefits raise the question: What, if any, alteration of radiation-induced tumorigenesis would 2-ME impart that may differ from that of other sulfur antioxidants? This is relevant based on the extensive use of radiation in diagnoses and therapy and 2-ME’s superior in vitro and in situ immune enhancement properties.

Materials and Methods—This was addressed by exposing long-lived, B10.A(4R) mice to sublethal, 5.5 Gy ionizing gamma-rays and then tumor development monitored over a lifetime.

Statistical Analysis—Two-tailed P-values were determined using the Fischer’s Exact Test.

Results—The only tumors detected were mammary and only in animals that were both exposed to radiation and not treated with 2-ME. The 43% incidence differed significantly from the absence of tumors in non-irradiated mice that were or were not exposed to 2-ME and in those irradiated and treated daily with 2-ME, irrespective of whether treatment was started prior to or post irradiation. However, quite unexpectedly, radiation shortened longevity 29% from undefined causes, including cancer, in animals pretreated with 2-ME; longevity was not altered in those not pretreated or if treatment was started post-irradiation.

Conclusions—The findings have relevance for cancer prevention and the controversy relative to “long term survival/safety” of currently used antioxidants as free radical scavengers in humans undergoing radiotherapy.

Keywords

2-mercaptoethanol; antioxidant; cancer adjuvant; cancer prevention; longevity; radiation; radiosensitizer
Introduction

It has been known for many years that numerous factors influence radiation-induced carcinogenesis in animals. Commonly used agents,\textsuperscript{[1,2]} plus numerous botanicals,\textsuperscript{[3]} that enhance or suppress underlying processes were recently reviewed. It is also known that of those processes protected by antioxidants, organosulfur compounds are especially effective. A review of the literature on organosulfur radioprotectors indicates that a single or a few injections of WR-2721 (amifostine), cysteamine, lipoic acid, N-acetyl-cysteine or piroxicam resulted in both short-term protection\textsuperscript{[4-7]} and a lower cancer incidence.\textsuperscript{[7-14]} However, none of these sulfur chemicals prevented development or progression of cancer. Moreover, in those few cases in which the incidence was longitudinally determined, it increased in both treated and non-treated animals, eventually becoming equivalent. This suggests that the lower incidences found at a single time point (all at ages much earlier than that considered, “a normal survival time”) in treated animals are underestimates and instead, represent consequences of delayed initiation and/or slowed progression. Thus, limited injections of these organosulfur compounds are clearly an inadequate preventive modality for radiation-induced cancer. The initial descriptions that both cell mediated and humoral murine immune responses \textit{in vitro} were dramatically enhanced by four different structurally unrelated, xenobiotic sulfhydryl compounds\textsuperscript{[15-18]} started an onslaught of investigations (presently >1000 cited in PubMed just on 2-ME) on exogenous, xenobiotic and natural (plant) organosulfur chemicals for benefits of multitudes of cellular processes \textit{in vitro}. Subsequently, this led to investigations on alteration of diseases by administering 2-ME directly to animals. The first results\textsuperscript{[19-21]} demonstrated that 2-ME could reverse the age-associated decline in immune responsiveness by either a single or limited (<5) injections of 2-ME or by culturing the cells in the presence of 2-ME. Later, others found that when fed daily as a dietary supplement, spontaneous arising cancers were either delayed\textsuperscript{[22-24]} or completely prevented.\textsuperscript{[24]} A composite of these findings raise the question: What, if any, alteration of radiation-induced tumorigenesis would 2-ME impart that may, or may not, be superior to that of other sulfur antioxidants? With limited numbers of animals, the results herein indicate that mammary tumorigenesis induced by sublethal, total body radiation was completely prevented over an entire lifetime by 2-ME. And equally important, and quite unexpected, 2-ME treatment started prior to radiation accelerated the aging process (longevity was significantly shortened 29%); no alteration occurred when supplementation was initiated post radiation. The latter results have relevance for two areas of cancer: The “long term survival” controversy\textsuperscript{[25,26]} on safety of antioxidants currently used for radiation protection in humans, and the potential of organosulfurs as adjuvants for localized cancer radiotherapy.

Materials and Methods

Mice and their husbandry

Long-lived, B10.A(4R) male mice were chosen for the present study because they have a very low incidence of spontaneous cancer (<1%) and were readily available in the author’s breeding colony. They were housed within a facility that maintained a 12-hour light-dark cycle in Plexiglas, hood-ventilated shoe-boxes (3-4 animals/box) that were cleaned,
sterilized and rebedded weekly. The animals were fed ad libitum Harlan Teklad mouse/rat diet (6% fat). Deionized, autoclaved water (changed biweekly) was supplied in glass bottles, with or without 2-ME. Because of both, the ease for continuous delivery and quantifiable consumption, 2-ME was added on the day the bottles were changed to obtain a concentration of $10^{-2}$ M. No effort was taken to maintain it in a reduced or oxidized form, although it most likely was oxidized within hours. Initiation of treatment was started at a mature age of 90 days. Supplementation was well-tolerated as it did not significantly alter the consumption of food or water and did not result in altered weight loss/gain compared with age-matched controls - these data are not included since they are similar to those reported previously for many other strains.[22-24] Average daily consumption of 2-ME fluctuated over a lifetime from 70-80 ugm/gm body wt. Survival and visual monitoring for palpable tumors (<1 mm$^3$) and other cancers were done biweekly. The palpable tumors were characteristic of MMTV-induced mammary tumors in C3H strains in that they originated at a single, subcutaneous site and progressed in size until the animal succumbed (or was euthanized) at 50–75 days after initial detection; they were not characterized further. It should be emphasized that these tumors were only detected in animals that were both irradiated and never treated with 2-ME. Because the goal of the project was to determine the effect of 2-ME on radiation-induced cancer and longevity, none of the animals were killed for biochemical, molecular, or necropsy analysis. Animals found recumbent or incapacitated were euthanized by cervical dislocation. Further, because of autolysis prior to discovery, necropsies done on the first few animals were uninformative, therefore they were discontinued. No part of the research inflicted pain or suffering, except that which may have occurred naturally with age. All experiments approved by the local Animal Research Committee were performed in strict accordance with recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institute of Health.

**Sublethal irradiation**

Animals were started on 2-ME treatment at 90 days of age. They and age-matched untreated controls were further divided at an age of 287 days in that some in each group were irradiated. Even though the results are presented as a single experiment, since all animals were not born on the same day, multiple radiation runs over 30 days (each a mini-experiment) were necessary to accumulate sufficient animals for the different groups. At most, six mice per run were exposed to gamma rays in a 6-plex, rotating hotel from a cesium radiator at 80 rads/min for a total dose of 5.5 Gy. Radiation was performed by personnel in the University Radiology Laboratory. After being irradiated, they were randomly assigned to one of four groups - in no case were all of a given run assigned to a single group. They were then housed and cared for under the same conventional conditions as those not radiated. Since the goal of the present study was to evaluate 2-ME benefits for radiation-induced disease, details regarding numerous radiation parameters were not deemed relevant and were not pursued.

**Statistics**

Two-tailed $P$-values for differences in tumor incidence and survival/longevity of treated versus not treated animals were determined using the Fischer's Exact Test. $P$-values less than 0.05 were considered significant.
Results

For ease of presentation, the median survival times, percent changes, and statistical significances are summarized in Table 1.

Shown in Figure 1a is the survival of non-radiated (control) mice and mice that were irradiated at 287 days; none of these animals were ever exposed to 2-ME. Surprisingly, longevity of animals in each group was indistinguishable (median survival of 895 vs. 913 days). Three of the seven that were irradiated succumbed with massive, subcutaneous tumors, compared to none of the 22 that were not radiated ($P = 0.0096$). In contrast, even though tumors did not develop in mice started on 2-ME prior to and continued post-irradiation (Panel C), this group had a statistically shorter mean survival ($P = 0.0043$) than those treated with 2-ME, but not irradiated (median of 729 vs. 933 days), as well as shorter than those shown in Figure 1a.

To assess the time, prior to or post radiation, at which 2-ME imparted this life-shortening, radiation-sensitive event, survival was determined after reversing the treatment protocols 24 h post-radiation. Shown in Figure 1b is the survival of animals that were not treated up until they were all switched to 2-ME at 288 days; six were not radiated and five were irradiated the day before the switch. As shown, even with small numbers of animals, no tumors were detected and survival times were indistinguishable (965 vs. 946 days). Shown in Panel D is the survival of animals initially on 2-ME that were all switched to untreated water at 288 days of age. Survival was significantly ($P = 0.0011$) shortened by radiation (median of 658 vs. 845 days), similar to the shortened survival shown in Figure 1c. Again no tumors were evident in any animals in Figure 1d. It should be emphasized that over an entire unabated lifetime, mammary tumors were only detected in animals that were both irradiated and never exposed to 2-ME shown in Figure 1a. Furthermore, gross postmortem exams did not reveal other cancer types in any of the eight groups.

Discussion

The results herein suggest three potential areas in which 2-ME may be beneficial in disease intervention: (a) a radiation protective agent, (b) a safe, stand-alone preventive modality for radiation-induced tumorigenesis when applied post-radiation; and (c) a potential pre-radiation adjuvant to enhance effectiveness of localized radiotherapy.

Based on the results of others,\textsuperscript{[27]} it was anticipated that free radicals formed by sublethal ionizing radiation would induce cancer in the long-lived strain of mice used for the present studies. Further, the finding that 2-ME altered tumorigenesis is in agreement with that reported for the xenobiotic non-organosulfur antioxidant, Tempol,\textsuperscript{[27]} for other organosulfur antioxidants - cysteamine, amifostine, piroxicam, lipoic acid, and N-acetyl cysteine,\textsuperscript{[7-14]} and for organosulfurs present in Allium (garlic) and Brassica plant families,\textsuperscript{[28-31]} An important difference, however, is that none of these antioxidants completely prevented tumor development over a normal lifetime; all delayed initiation and/or slowed progression only. The 43% incidence of mammary tumors found herein was higher than 0% in 44 animals not irradiated - 22 treated with 2-ME ($P = 0.0096$) and 22 not treated ($P = 0.0096$) -
and the 0% in the 50 treated with 2-ME (P = 0.0012). This lifetime curtailment adds another 2-ME anticancer benefit to those previously described for other strains: (a) lower incidence of Dunn tumors in CBA/Ca,\textsuperscript{23} (b) delay of liver carcinomas associated with aging in CBA/Ca and BC3F1\textsuperscript{22,23} and mammary tumors induced by exogenous milk-bourne virus in C3H/He,\textsuperscript{24} and (c) complete prevention of the 100% spontaneously occurring, age associated, tumors in BXSB-Yaa\textsuperscript{+}.\textsuperscript{24} It should be noted that in the two models in which complete prevention of cancer was achieved, the latency was extremely long - median time from radiation until death due to cancer was 620 days, a time that is essentially identical to the median age of 650 days at which death occurred due to spontaneously arising tumors in untreated BXSB-Yaa\textsuperscript{+}.\textsuperscript{24} Complete prevention verses delaying/slowing progression raises an interesting question: Is the variability in incidence found for different organosulfurs associated with (a) their structure, (b) the length of the latency period, (c) treatment duration/dose, and/or (d) different etiologic agents?

An equally and perhaps more important finding was the unexpected creation by 2-ME of a radiation-sensitive process that became manifested later in life by significantly shortening longevity.\textsuperscript{32} The critical 2-ME exposure period was limited to that prior to, or at the time of, radiation; no alteration of longevity occurred when 2-ME treatment was initiated 24 h post-radiation. Since the cause was by some process not obvious by gross necropsy (including other cancers), its identification will likely require histopathology - a procedure not undertaken because of extensive autolysis prior to discovery. Processes of considerable interest are those in which sulfur moieties play a role in DNA damage, DNA repair or misrepair, and extrinsic factors of the microenvironmental niche of stem cells\textsuperscript{33-35} that rescue acute marrow suppression post radiation. As one example, deficiency/damage in the repair proteins, breast cancer type 1 or 2 (each with many cysteines involved in their functions,\textsuperscript{36}), leads to defective DNA repair. These two proteins also play a critical role in cancer therapies in that they reduce the efficacy of therapeutic agents that are based on double strand breaks (DSBs). For example, a slight elevation in temperature induced degradation of BRCA2; a consequence that potentiated DSBs in tumors.\textsuperscript{37} Similarly, BRCA1 expressed in normal cells of breast tissue, when damaged, did not properly repair DNA and resulted in an increased risk for cancer development.\textsuperscript{38} Irrespective of what radio-sensitive process (es) shortened longevity,\textsuperscript{32} the similarities of biological events (tumor radio-resistance, residual bone marrow injury, acute bone marrow suppression) - with an emphasis on DNA strand breaks - that occur during normal aging (hematopoietic cells) and radiotherapy of tumors raise two important points. First, is there a process in cancer cells and/or their microenvironmental niches that could be altered by 2-ME such that they are rendered more vulnerable to radiotherapy? If so, use as an adjuvant pretreatment could be advantageous for treating tumors amenable to localized radiation. And second, the shortened longevity has implications for the dialogue\textsuperscript{25,26} regarding “long-term survival” of irradiated patients that are “protected” with various antioxidants, many of which possess potential active/activatable sulfur. This controversy seems to hinge on the belief that in vitro molecular changes and short-term, antioxidant benefits\textsuperscript{27,39-41} are predictive of long-term benefits/safety. Based on results herein, such assumptions need to be considered with caution. Instead, safe use of antioxidants for radiation protection should consider timing of exposure relative to irradiation and structure of the antioxidant (sulfur vs. non-sulfur).
Conclusions

The 2-ME radiation model described herein seems perfectly suited to test long-term benefits of organosulfurs for (a) radioprotection, (b) autonomous surveillance processes that normally control cancer, and (c) tumorigenesis per se. One specific area for such opportunities and challenges was recently discussed; i.e., targeting allosteric disulphide bonds as a potential therapeutic strategy for cancer. In addition, the radiation and the age-associated, BXSB-Yaa models are ideal for directly comparing any xenobiotic or nature’s organosulfur for anticancer benefits in the absence of any ambivalent interpretation of results - they either prevent development or they do not. Most interestingly, of all the organosulfurs with anticancer benefits, the only one not approved for human noncancerous diseases is 2-ME. Perhaps the stigma that it is a poison should be reassessed and its status upgraded to that of BoTox!

Acknowledgments

This work was supported by the National Institutes of Health (Grant numbers R01CA023678 and R01AI019643) prior to retirement from the Univ. Minnesota, Minneapolis, MN.

Source of Support: NIH.

References

1. Kennedy AR. Factors that modify radiation-induced carcinogenesis. Health Phys. 2009; 97:433–45. [PubMed: 19820453]
2. Okunieff P, Swarts S, Keng P, Sun W, Wang W, Kim J, et al. Antioxidants reduce consequences of radiation exposure. Adv Exp Med Biol. 2008; 614:165–78. [PubMed: 18290327]
3. Sagar SM. Can the therapeutic gain of radiotherapy be increased by concurrent administration of Asian botanicals? Integr Cancer Ther. 2010; 9:5–13. [PubMed: 20042406]
4. Neal R, Matthews RH, Lutz P, Ercal N. Antioxidant role of N-acetyl cysteine isomers following high dose irradiation. Free Radic Biol Med. 2003; 34:689–95. [PubMed: 12633746]
5. Kouvaris JR, Kouloulias VE, Vlahos LJ. Amifostine: The first selective-target and broad-spectrum radioprotector. Oncologist. 2007; 12:738–47. [PubMed: 17602063]
6. Wambi C, Sanzari J, Wan XS, Nuth M, Davis J, Ko YH, et al. Dietary antioxidants protect hematopoietic cells and improve animal survival after total-body irradiation. Radiat Res. 2008; 169:384–96. [PubMed: 18363433]
7. Yuhas JM, Spellman JM, Culo F. The role of WR-2721 in radiotherapy and/or chemotherapy. Cancer Clin Trials. 1980; 3:211–6. [PubMed: 6254681]
8. Milas L, Hunter N, Stephens LC, Peters LJ. Inhibition of radiation carcinogenesis in mice by S-2-(3-aminopropylamino)-ethylphosphorothioic acid. Cancer Res. 1984; 44:5567–9. [PubMed: 6093999]
9. Watanabe H, Kamikawa M, Nakagawa Y, Takahashi T, Ito A. The effects of ranitidine and cysteamine on intestinal metaplasia induced by X-irradiation in rats. Acta Pathol Jpn. 1988; 38:1285–96. [PubMed: 3218508]
10. Northway MG, Scobey MW, Cassidy KT, Geisinger KR. Piroxicam decreases postirradiation colonic neoplasia in the rat. Cancer. 1990; 66:2300–5. [PubMed: 2245384]
11. Grdina DJ, Barnes BA, Grahn D, Sigdestad CP. Protection against late effects of radiation by S-2-(3-Aminopropylamino)-ethylphosphorothioic acid. Cancer Res. 1991; 51:4125–30. [PubMed: 1651155]
12. Barnes BA, Grdina DJ. In vivo protection by the aminothiol WR-2721 against neutron-induced carcinogenesis. Int J Radiat Biol. 1992; 61:567–76. [PubMed: 1349621]
13. Inano H, Onoda M, Suzuki K, Kobayashi H, Wakabayashi K. Inhibitory effects of WR-2721 and cysteamine on tumor initiation in mammary glands of pregnant rats by radiation. Radiat Res. 2000; 153:68–74. [PubMed: 10630979]

14. Kennedy AR, Davis JG, Carlton W, Ware JH. Effects of dietary antioxidant supplementation on the development of malignant lymphoma and other neoplastic lesions in mice exposed to proton or iron-ion radiation. Radiat Res. 2008; 169:615–25. [PubMed: 18494549]

15. Click RE, Benck L, Alter BJ. Enhancement of antibody synthesis in vitro by mercaptoethanol. Cell Immunol. 1972; 3:156–60. [PubMed: 5061825]

16. Click RE, Benck L, Alter BJ. Immune responses in vitro. I Culture conditions for antibody synthesis. Cell Immunol. 1972; 3:264–76. [PubMed: 4551691]

17. Heber-Katz E, Click RE. Immune responses in vitro. V Role of mercaptoethanol in the mixed-leukocyte reaction. Cell Immunol. 1972; 5:410–8. [PubMed: 4645593]

18. Peck AB, Katz-Heber E, Click RE. Immune responses in vitro. IV A comparison of the protein-free and mouse serum-supplemented mouse mixed lymphocyte interaction assays. Eur J Immunol. 1973; 3:516–9. [PubMed: 4271076]

19. Makinodan T, Albright JW. Restoration of impaired immune functions in aging animals. II Effect of mercaptoethanol in enhancing the reduced primary antibody responsiveness in vitro. Mech Ageing Dev. 1979; 10:325–40. [PubMed: 384104]

20. Makinodan T, Albright JW. Restoration of impaired immune functions in aging animals. III Effect of mercaptoethanol in enhancing the reduced primary antibody responsiveness in vivo. Mech Ageing Dev. 1979; 11:1–8. [PubMed: 491773]

21. Chang MP, Tanaka JL, Stosic-Grujicic S, Yamamoto EK, Perkins EH, Strehler BL, et al. Restoration of impaired immune functions in aging animals. VI Differential potentiating effect of 2-mercaptoethanol on young and old murine spleen cells. Int J Immunopharmacol. 1982; 4:429–36. [PubMed: 6982245]

22. Heidrick ML, Hendricks LC, Cook DE. Effect of dietary 2-mercaptoethanol on the lifespan, immune system, tumor incidence and lipid peroxidation damage in spleen lymphocytes of aging BC3F1 mice. Mech Ageing Dev. 1984; 27:341–58. [PubMed: 6334792]

23. Beregi E, Regius O, Rajczy K, Boross M, Péntez L. Effect of cigarette smoke and 2-mercaptoethanol administration on age-related alterations and immunological parameters. Gerontology. 1991; 37:326–34. [PubMed: 1662659]

24. Click RE. Dietary supplemented 2-mercaptoethanol prevents spontaneous and delays virally-induced murine mammary tumorigenesis. Cancer Biol Ther. 2013; 14:521–6. [PubMed: 23760494]

25. Lawenda BD, Kelly KM, Ladas EJ, Sagar SM, Vickers A, Blumberg JB. Should supplemental antioxidant administration be avoided during chemotherapy and radiation therapy? J Natl Cancer Inst. 2008; 100:773–83. [PubMed: 18505970]

26. Bhutani M, Pathak AK. Re: Should supplemental antioxidant administration be avoided during chemotherapy and radiation therapy? J Natl Cancer Inst. 2008; 100:1334. [PubMed: 18780866]

27. Mitchell JB, Anver MR, Sowers AL, Rosenberg PS, Figueroa M, Thetford A, et al. The antioxidant Tempol reduces carcinogenesis and enhances survival in mice when administered after nonlethal total body radiation. Cancer Res. 2012; 72:4846–55. [PubMed: 22805306]

28. Wattenberg, LW. Chemoprevention of cancer by naturally occurring and synthetic compounds. In: Wattenberg, LW.; Lipkin, M.; Boone, CW.; Kelloff, GJ., editors. Cancer Chemoprevention. Boca Raton, FL: CRC Press; 1992. p. 19-39.

29. Milner JA. Preclinical perspectives on garlic and cancer. J Nutr. 2006; 136(Suppl 3):827S–31S. [PubMed: 16484574]

30. El-Bayoumy K, Sinha R, Pinto JT, Rivlin RS. Cancer chemoprevention by garlic and garlic-containing sulfur and selenium compounds. J Nutr. 2006; 136(Suppl 3):864S–9S. [PubMed: 16484582]

31. Clarke JD, Dashwood RH, Ho E. Multi-targeted prevention of cancer by sulforaphane. Cancer Lett. 2008; 269:291–304. [PubMed: 18504070]

32. Niedernhofer LJ, Robbins PD. Signaling mechanisms involved in the response to genotoxic stress and regulating lifespan. Int J Biochem Cell Biol. 2008; 40:176–80. [PubMed: 18023240]
33. Cao X, Wu X, Frassica D, Yu B, Pang L, Xian L, et al. Irradiation induces bone injury by damaging bone marrow microenvironment for stem cells. Proc Natl Acad Sci USA. 2011; 108:1609–14. [PubMed: 21220327]

34. Nguyen DH, Oketch-Rabah HA, Illa-Bochaca I, Geyer FC, Reis-Filho JS, Mao JH, et al. Radiation acts on the microenvironment to affect breast carcinogenesis by distinct mechanisms that decrease cancer latency and affect tumor type. Cancer Cell. 2011; 19:640–51. [PubMed: 21575864]

35. Tieu KS, Tieu RS, Martinez-Agosto JA, Sehl ME. Stem cell niche dynamics: From homeostasis to carcinogenesis. Stem Cells Int. 2012; 2012:367567. [PubMed: 22448171]

36. Brzovic PS, Rajagopal P, Hoyt DW, King MC, Klevit RE. Structure of a BRCA1-BARD1 heterodimeric RING-RING complex. Nat Struct Biol. 2001; 8:833–7. [PubMed: 11573085]

37. Krawczyk PM, Eppink B, Essers J, Stap J, Rodermund H, Odijk H, et al. Mild hyperthermia inhibits homologous recombination, induces BRCA2 degradation, and sensitizes cancer cells to poly (ADP-ribose) polymerase-1 inhibition. Proc Natl Acad Sci USA. 2011; 108:9851–6. [PubMed: 21555554]

38. Friedenson B. The BRCA1/2 pathway prevents hematologic cancers in addition to breast and ovarian cancers. BMC Cancer. 2007; 7:152. [PubMed: 17683622]

39. Grdina DJ, Nagy B. The effect of 2-[(aminopropyl)amino] ethanethiol (WR1065) on radiation-induced DNA damage and repair and cell progression in V79 cells. Br J Cancer. 1986; 54:933–41. [PubMed: 3801289]

40. Stanton J, Taucher-Scholz G, Schneider M, Heilmann J, Kraft G. Protection of DNA from high LET radiation by two OH radical scavengers, tris (hydroxymethyl) aminomethane and 2-mercaptoethanol. Radiat Environ Biophys. 1993; 32:21–32. [PubMed: 8384725]

41. Ayene IS, Koch CJ, Krisch RE. Modification of radiation-induced strand breaks by glutathione: Comparison of single- and double-strand breaks in SV40 DNA. Radiat Res. 1995; 144:1–8. [PubMed: 7568762]

42. Hogg PJ. Targeting allosteric disulphide bonds in cancer. Nat Rev Cancer. 2013; 13:425–31. [PubMed: 23660784]
Figure 1. Survival of control and gamma-radiated B10.A(4R) mice on and off dietary supplemented 2-Me compared to those that had their 2-Me treatment reversed at 288 days

Panel A. Mice in this panel were never exposed to 2-Me at any time of their life. At 287 days of age, one group was irradiated (○----○); the other group was not (○ - ○). (+) designates animals with mammary tumor. Panel B. Mice in this panel were on water devoid of 2-Me for 288 days, at which time they were all switched to 2-Me; one group was also irradiated at 287 days of age (○----○). Panel C. Mice in this panel were started on 2-Me at 90 days of age, and then continued for the remained of their lives. At 287 days of age, one group was irradiated (○----○); the other group was not (○ - ○). Panel D. Mice in this panel were on 2-Me for 288 days, at which time they were all switched to water devoid of 2-Me; one group was irradiated at 287 days of age. (○----○); the other was not (○ - ○)
| Radiation Exposure | Never Exposed to 2-ME | | Exposed to 2-ME starting on day 90 | | | Percent change vs not radiated | Percent change vs never exposed to 2-ME |
|--------------------|-----------------------|---|-------------------|---|-------------------|---|-------------------|
|                    | Figure panel | Total # animals - # with tumors | Median survival (days) | Percent change vs not radiated | Figure panel | Total # animals - # with tumors | Median survival (days) | Percent change vs not radiated | Percent change vs never exposed to 2-ME |
| Not radiated       | A | 22 - 0 | 895 | – | C | 22 - 0 | 933 | – | +4.2 |
| Radiated at day 287| A | 7 - 3 | 913 | +2 | C | 6 - 0 | 729<sup>a</sup> | -21.9<sup>a</sup> | -20.2<sup>a</sup> |
| Exposed to 2-ME starting on day 288 | | | Exposed to 2-ME only from day 90 through day 287 | | | | |
| Not radiated       | B | 6 - 0 | 965 | – | D | 5 - 0 | 845 | – | -5.6 |
| Radiated at day 287| B | 5 - 0 | 946 | -2 | D | 6 - 0 | 658<sup>a</sup> | -18.9<sup>a</sup> | -27.9<sup>a</sup> |

<sup>a</sup> Statistically significant from both those not radiated and those not on 2-Me