REVIEW

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Review

Mitigating the risk of radiation-induced cancers: limitations and paradigms in drug development

Stephen S Yoo[1,10], Timothy J Jorgensen[2], Ann R Kennedy[3], John D Boice Jr[4,5], Alla Shapiro[6], Tom C-C Hu[7], Brian R Moyer[7], Marcy B Grace[7], Gary J Kelloff[8], Michael Fenech[9], Pataje G S Prasanna[1,10] and C Norman Coleman[1,10,11]

[1] Radiation Research Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA
[2] Department of Radiation Medicine, Georgetown University School of Medicine, Washington DC, USA
[3] Department of Radiation Oncology, University of Pennsylvania, Philadelphia, PA, USA
[4] Department of Medicine, Division of Epidemiology, Vanderbilt-Ingram Cancer Center, Vanderbilt School of Medicine, Nashville, TN, USA
[5] National Council on Radiation Protection and Measurements, Bethesda, MD, USA
[6] Office of Counter-Terrorism and Emergency Coordination, Center for Drug Evaluation and Research, Food and Drug Administration, Silver Spring, MD, USA
[7] Division of CBRN Countermeasures, Biomedical Advanced Research and Development Authority (BARDA), Office of the Assistant Secretary for Preparedness and Response, Office of the Secretary, Department of Health and Human Services, Washington, DC, USA
[8] Cancer Imaging Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, Bethesda, MD, USA
[9] Commonwealth Scientific and Industrial Research Organisation, Adelaide, Australia

E-mail: ccoleman@mail.nih.gov

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Abstract

The United States radiation medical countermeasures (MCM) programme for radiological and nuclear incidents has been focusing on developing mitigators

[10] SSY, PGSP and CNC had equal contribution in the preparation of this manuscript.
[11] Address for correspondence: Radiation Research Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, National Institutes of Health, 9609 Medical Center Dr. 3W102, Bethesda, MD 20892-9727, USA.

for the acute radiation syndrome (ARS) and delayed effects of acute radiation exposure (DEARE), and biodosimetry technologies to provide radiation dose assessments for guiding treatment. Because a nuclear accident or terrorist incident could potentially expose a large number of people to low to moderate doses of ionising radiation, and thus increase their excess lifetime cancer risk, there is an interest in developing mitigators for this purpose. This article discusses the current status, issues, and challenges regarding development of mitigators against radiation-induced cancers. The challenges of developing mitigators for ARS include: the long latency between exposure and cancer manifestation, limitations of animal models, potential side effects of the mitigator itself, potential need for long-term use, the complexity of human trials to demonstrate effectiveness, and statistical power constraints for measuring health risks (and reduction of health risks after mitigation) following relatively low radiation doses (<0.75 Gy). Nevertheless, progress in the understanding of the molecular mechanisms resulting in radiation injury, along with parallel progress in dose assessment technologies, make this an opportune, if not critical, time to invest in research strategies that result in the development of agents to lower the risk of radiation-induced cancers for populations that survive a significant radiation exposure incident.

Keywords: radiation-induced cancer, radiation mitigator, radioprotector, radiation risk

List of abbreviations

Abbreviation	*Term*
ARS	Acute radiation syndrome
ASPR	Office of the Assistant Secretary for Preparedness and Response, DHHS
CBMN	Cytokinesis-Block Micronucleus assay
CDC	Centers for Disease Control and Prevention
COX-2	Cyclooxygenase 2
DCA	Dicentric chromosome assay
DCP	Division of Cancer Prevention, National Cancer Institute, NIH
DEARE	Delayed effects of acute radiation exposure
DHHS	Department of Health and Human Services
FAP	Familial adenomatous polyposis
FDA	Food and Drug Administration
HPBL	Human peripheral blood lymphocytes
hprt	Hypoxanthine-guanine phosphoribosyltransferase
γ-H2AX	Phosphorylation of the histone, H2AX in response to DNA double strand breaks
ICRP	International Commission on Radiological Protection
IAEA	International Atomic Energy Agency
IEN	Intraepithelial neoplasia
KI	Potassium iodide
MCM	Medical countermeasure
MN	Micronucleus or Micronuclei

mSV	Milli-sievert
MTAP	Methylthioadenosine phosphorylase
NCI	National Cancer Institute
NIAID	National Institutes of Allergy and Infectious Diseases, NIH
NIH	National Institute of Health
NSABP	National Surgical Adjuvant Breast and Bowel Project
NSAID	Nonsteroidal anti-inflammatory drug
RCT	Randomised clinical trial
RDA	Recommended daily allowances
RERF	Radiation Effects Research Foundation
TI	Therapeutic Index; ratio of toxicity to efficacy doses

1. Introduction

The possibility of exposure of a large number of people to radiation following a nuclear incident has raised the public's concerns about the increases in the chances of developing cancers (Knebel *et al* 2011). Further, the earthquake/tsunami off the coast of Japan in March 2011 renewed attention to the debate on radiation-induced health risks from nuclear power industry mishaps and other accidental releases of radioactivity into the environment (Boice 2012, González *et al* 2013, Normile 2011). The primary defences against such mishaps are prevention, sheltering in place with delayed evacuation, and restricting the consumption of contaminated foodstuffs. Following a release of radioactivity from a nuclear power plant accident or due to an explosion of a radiological dispersal device (RDD), there is the potential need for agents that block radionuclide uptake and/or enhance excretion of ingested and inhaled radioactive elements. Since preventive strategies can never be 100% effective, it is important for public health officials, radiation researchers and professionals to consider developing a second tier medical intervention strategy to mitigate the health risks, i.e., cancer, from accidental radiation exposure. This article focuses on the potential for assessing and reducing the future cancer risk to large populations following a major nuclear incident.

In considering a strategy of risk reduction, it is critical for the general public and public health officials to understand that lifetime risk for developing cancer for an individual is not exclusively related to accidental exposure to radiation but is dependent on several factors including age at the time of radiation exposure and other variables including genetics, dietary habits, voluntary and involuntary exposures to carcinogens, such as cigarette smoke, and so forth. For example, in the US, the lifetime risk of developing cancer in the absence of radiation exposure is already quite high, i.e. 42% of the population is likely to develop cancer during their lifetimes from factors other than radiation exposure (ACS 2013, NA/NRC 2006). This high baseline rate of developing cancer in the absence of radiation exposure poses a challenge in the determination of probability of causation of cancer in an individual as well as for the development of other late effects. In general, the guidelines for determining the probability of causation under the DHHS (Department of Health and Human Services) Energy Employees Occupational Illness Compensation Program Act of 2000 (DHHS 2002), states that a determination is required that an individual's cancer in question is due to an accidental or occupational exposure to radiation with a probability of a 50% or greater.

The shape of the dose–response curve for radiation-induced cancer remains a subject of much debate. While there are exceptions, for the purposes of radiation protection it is assumed that there is a proportional relationship between exposure and future cancer risk. A linear,

Table 1. Challenges for developing mitigators for radiation-induced cancer.

- Biomarkers for cancer
  - Radiation—biomarker for dose
  - Radiation risk—biomarker for radiation damage
  - Cancer biomarker—is there one to assess person's risk of cancer in general or of a specific cancer and efficacy of an intervention?
- Individuals at risk
  - Based on underlying genetic susceptibility
  - Family history (no known genetic marker)
  - Individual exposure history to genotoxic injury including lifestyle choices (e.g., smoking)
  - Children? Pregnant women?
  - Threshold dose at which to consider mitigation
- Operative molecular and biological mechanisms during latency
  - Are any subject to 'chemo-modification'?
  - What mechanisms can be affected by an individual's effort and what are beyond intervention?
- Drug development
  - Develop a drug for this purpose?
  - Utilise an existing drug—repurposing?
  - Assess safety
  - Assess efficacy of common dietary foods or nutraceuticals
- Cost versus benefit
  - At what excess lifetime risk is intervention appropriate?
  - Toxicity, expense, time and excess stress from an intervention

no-threshold model is used to assess risk at doses below 100 mSv for these purposes, recognising that epidemiological information in the low-dose domain, i.e. below 100 mSv, cannot unambiguously support or refute the model assumptions (ICRP 2007). For example, recent data from the Radiation Effects Research Foundation (RERF, Japan) seems to be more in line with a linear relationship between radiation dose and cancer risk (Ozasa *et al* 2012). How the increased lifetime risk may be considered for using a mitigator is discussed later in this paper. Development of radiation medical countermeasures (MCM) for specific organ system damage, i.e. acute radiation syndromes (ARS), in the immediate aftermath of a radiation event is discussed in detail elsewhere (DiCarlo *et al* 2011, Grace *et al* 2011). The challenges in developing agents for health risks that may not be apparent until years after exposure are in table 1. Given the substantial public concerns about radiation exposure on their health and radiation-related cancer risks, this paper brings together opinions of the experts on issues of radiation risk and the experimental difficulties in the development of mitigators. This paper is intended to benefit scientists in this field of research and development, the public at large, and the public health policy makers to better understand ways to address the late occurring health risks.

2. Radiation concerns and public perception

The general public is known to have a perceived 'fear of radiation' (Dauer *et al* 2011) that is felt to be well beyond the actual risk (Ropeik 2013). This fear was enhanced as a consequence of the Fukushima disaster (Brumfiel 2013). Public concerns have raised more interest for agents to mitigate the cancer risks associated with radiation, including exposures from diagnostic imaging (Mettler *et al* 2011). While there are, at present no such radiomitigants, the problem of finding treatments for reducing cancer risks arising from accidental radiation exposures is different from that arising from medical radiation exposures. Clinically directed radiation exposures are relatively well characterised with respect to several factors (e.g. dose of radiation, age, gender, organ sites, etc) that will not be well defined in a radiation accident. Regarding

diagnostic imaging, Kuefner *et al* (2012) have reported that a mixture of antioxidants and glutathione-elevating compounds in human peripheral blood lymphocyte (HPBL) cultures when exposed *in vitro* to a radiological dose of 10 mGy, reduces the number of γ-H2AX foci, a surrogate marker for DNA damage. The observed reduction in γ-H2AX foci suggests that antioxidant pretreatment may provide protection from radiation-induced DNA damage in a diagnostic setting. Since DNA damage is thought to be a precursor to radiation-induced cancer, these and related compounds may provide protection against cancer induction and thus reduce the risk of cancer. These findings need to be validated (Brink and Boice 2012) but even if confirmed it is unlikely that any strategy that relies on treatment of people prior to their radiation exposure would be useful in an accidental exposure situation, except perhaps for first responders. Some first responders may benefit from pre-exposure prophylaxis if they anticipate exposure up to 0.25 Gy (or a very few possibly up to 0.5–0.75 Gy for 'lifesaving or protection of large populations') (see table 2.2 in the PAG (protective action guides) Manual, US EPA 2013). Recently, Mettler *et al* (2011) reviewed pharmacological strategies for minimising the cancer risk from diagnostic radiation doses and concluded that there are no proven chemical compounds that could be administered after diagnostic radiation to reduce cancer risk. Since, at present, there are no drugs currently available from clinical practice that can be used for the post-exposure treatment, specific research and development efforts that are needed to address the issues provided in table 1. A first consideration is what radiation dose may be a reasonable threshold to consider as a trigger for monitoring and commencing potential mitigation activities (e.g. $>$0.75 Gy) and in what special populations this threshold may need a reassignment, e.g. children, pregnant women, and special populations with genetic susceptibilities, to afford ARS, DEARE and cancer risk–benefit.

Radiation-inducible cancer is a stochastic event in which probability of occurrence increases with dose with no threshold (assuming the linear no-threshold model) and severity of the cancer is independent of radiation dose. The risk of excess cancers and potential heritable effects varies with age, sex, dose rate, radiation type, smoking status, underlying genetic susceptibility, and nutritional and other factors. A reasonable summary risk for an exposed general population used in this paper is 6% per Sv that is based on an increased excess lifetime cancer risk (ICRP 2007), which is likely to be used by the decision makers as is provided in the PAG manual (US EPA 2013).

The number of people at risk in an incident will depend on many factors (e.g. type of device, height of burst, natural or structural shielding, effectiveness of sheltering-in-place, and weather conditions). Looking at a range of scenarios, the number of people exposed to $>$0.75 Gy, where the increased lifetime risk is at least an additional 4%, could be in the tens to hundreds of thousands (Knebel *et al* 2011, Murrain-Hill *et al* 2011). There will be hundreds of thousands of concerned citizens who have a lower dose, and potentially a million unexposed people who are still concerned, and people who have some degree of post-traumatic stress, which is known to be a key consequence of any major disaster (Bromet 2012, 2014, Brumfiel 2013). Thus, we feel that the potential benefit from an efficacious 'non-toxic chemopreventive agent'—should one exist—would be of benefit to overall individual and society recovery and resilience in the long-term.

3. Challenges to measuring and developing matrices for medical countermeasure (MCM) efficacy at low doses ($<$0.75 Gy)

Radiation epidemiological studies from diverse populations (atomic-bomb survivors, populations from therapeutic and diagnostic exposures, communities exposed to environmental and accidentally released sources of radiation) have indeed shown an increase in cancers occurring

many years post-exposure. However, risk–benefit analysis and risk communication are keys to how the public will respond. Even for people who survive ARS-causing radiation doses, their long-term risk of fatal cancer is still predominately due to their baseline risk of dying from a fatal cancer because cancer is a common cause of death. With the average lifetime risk of dying from cancer in the United States being approximately 21% (ACS 2013, Siegel *et al* 2012) a whole body radiation dose sufficient to cause symptoms (approximately 0.75 Gy) would add only another 4% to the lifetime risk of cancer death bringing the risk to 25%. Furthermore, for the suggested maximum dose for first-responders of 0.25 Gy, the additional 1.5% brings their lifetime risk to approximately 22.5% (also see PAG Manual, US EPA 2013). This is not meant to minimise the concern but to point out that for the vast majority of concerned citizens and individuals exposed to doses below those requiring treatment for ARS symptoms (∼0.75 Gy) or even ARS haematology syndrome (∼2 Gy), the added lifetime risk must be balanced against any toxicity related to the use of a post-exposure mitigant (Jacob *et al* 1999, 2009, Thompson *et al* 1994). Although lowering of exposure is expected to decrease risk, greater is the difficulty in detecting any increase in the number of cancers possibly attributable specifically to the individual's radiation exposure from the incident (Boice 2012), and in turn, demonstrating any mitigation of this risk.

Conducting various chemo-preventative and nutritional intervention studies is possible for various cancer risks, but it is not possible to directly measure radiation-inducible cancer resulting from whole body low-dose exposures, even in a controlled epidemiological study or a clinical trial to assess the efficacy of a mitigation strategy, as an exposed population of the size needed to accurately address these issues, does not exist. Therefore, MCM development requires assumptions and extrapolation from knowledge from experimental systems and animal models of radiation injury.

4. Mechanistic approaches for developing MCM drugs

It is generally agreed that carcinogenesis is a multistep process involving initiation, promotion, and progression, although it is not proven for all forms of cancer. This is a useful framework and as the understanding of the underlying molecular mechanisms of carcinogenesis increases, these steps and new potential areas of mitigation will be better defined (Gillies *et al* 2012, McCormick 1999, Vogelstein *et al* 2013). It is known that cancer is caused by the stepwise accumulation of mutations that affect growth control, differentiation, and survival pathways that intersect and overlap (McCormick 1999). Thus, arguably it is possible to develop interventional strategies at each of these steps. Figure 1 illustrates a multistep carcinogenesis model encompassing 'initiation', 'promotion' and 'progression', and possible risk assessment and mitigation strategies applicable to a radiation mass casualty incident.

4.1. Targeting stages of carcinogenesis

Because radiation interacts with each of the three stages, MCM drug development approaches for radiation-induced cancer have sought to discover and develop inhibitors of each stage. Furthermore, numerous drugs have already been shown to either enhance or suppress the stages of carcinogenesis in both *in vitro* and *in vivo* systems (Kennedy 2009). This suggests that identification of a drug that targets one or more stages of the carcinogenic process is possible.

4.1.1. Initiation. Examples of drugs capable of inhibiting the initiation stage of radiation-induced carcinogenesis in preclinical studies include amifostine, cysteine, and tempol

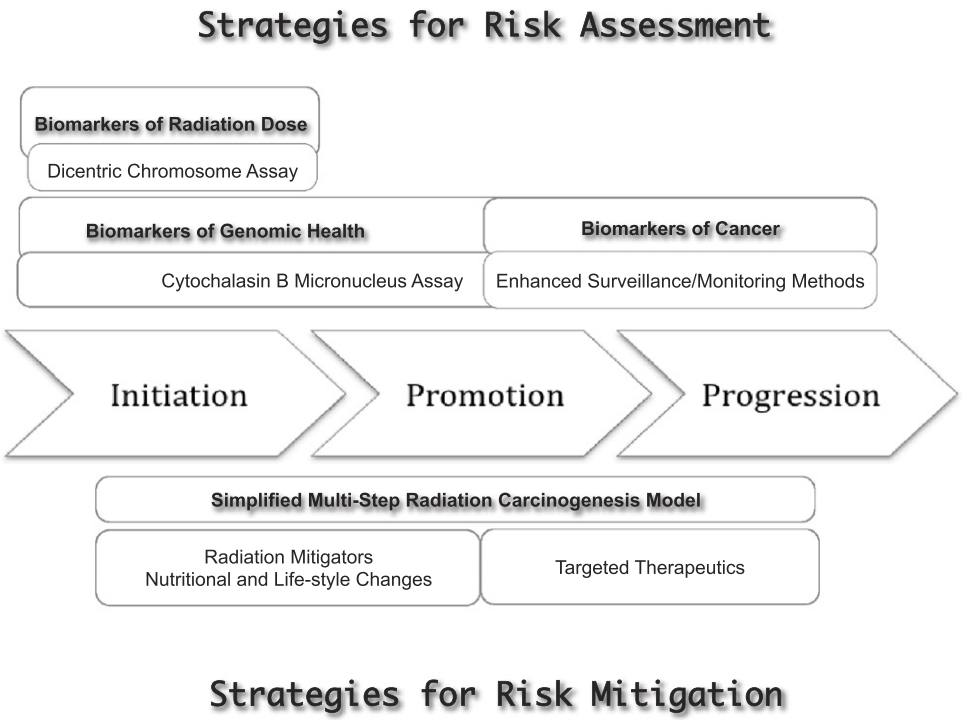

Figure 1. Illustration of a generalised multistep carcinogenesis model involving 'initiation', 'promotion', and 'progression', and possible risk assessment and risk mitigation strategies as applicable to radiation mass casualties. Current understanding of the process of carcinogenesis implicate sequential accumulation of various mutations in specific genes and overcoming distinct microenvironmental proliferation barriers, eventually leading to distinct signalling pathways that regulate cell fate, cell survival, and genome maintenance that intersect and overlap (McCormick 1999, Vogelstein*et al* 2013). Radiation exposure will interact with all stages of carcinogenesis: 'initiation', 'promotion', and 'progression'. 'Initiation' is due to genotoxic insults (e.g. mutations), 'promotion' is due to non-genotoxic mechanisms with altered cellular regulatory processes providing selective growth advantage to 'initiated cells' eventually leading to 'progression' where complex genetic alterations and uncontrolled cell proliferation become evident. Thus, strategies for radiation risk assessment will require biomarkers of radiation dose (e.g. Dicentric Chromosome Assay), biomarkers of genomic health (e.g. Cytokinesis-block micronucleus assay) and biomarkers of cancer (e.g. utilisation of enhanced cancer surveillance/monitoring methods). Similarly, strategies for risk mitigation will involve appropriate changes to lifestyle and cautious use radiation mitigators as well as nutritional changes, and targeted therapeutics with risk versus benefit ratio in view.

(reviewed in Mettler *et al* 2011, Weiss and Landauer 2009) and Bowman–Birk inhibitor (Dittman *et al* 2003). In studies where animals were treated with such compounds prior to irradiation, it has been shown to either increase the latency period and/or reduce the incidence of tumour formation (Grdina *et al* 1991, Milas *et al* 1984). Still, no studies to date have evaluated the effectiveness of these drugs as anti-carcinogens when administered after radiation exposure. WR-1065, the active metabolite of amifostine has been demonstrated to be effective in reducing

radiation-induced mutations at the hypoxanthine-guanine phosphoribosyltransferase (*hprt*) locus in V79 Chinese hamster cells when administered up to three hours following radiation exposure (Grdina *et al* 1985). Amifostine also reduced neutron-induced mutations at the *hprt* locus in mouse splenocytes when administered both prior to and following irradiation (Grdina *et al* 1992). Since mutagenesis in these *in vitro* cellular assays is highly correlated with carcinogenesis, these two drugs may have promise (Compton *et al* 1991, Grdina *et al* 2000, Liu *et al* 1997).

4.1.2. Promotion. The use of drugs targeting the promotion stage of carcinogenesis builds on data from the chemoprevention literature. Since promotion occurs subsequent to initiation, these drugs might be effectively administered at some time after exposure, but the trade-off is that these drugs would likely need to be taken consistently for the long-term since the promotion stage of carcinogenesis can last for years. This raises the issue of long-term compliance and support by both the exposed individuals and healthcare providers. Additionally, prolonged use of such drugs may reveal toxicities that are not apparent under short-term drug testing regimens, and therefore, development of non-toxic and efficacious mitigator(s), or dietary, and/or lifestyle modifications are advocated. This, of course, speaks to the whole issue of risk versus benefit for using a mitigator(s), thus logically requiring the use of predictive biomarkers or other forms of screening to identify those individuals at a higher genomic risk for cancer. These points are discussed in detail below in the biomarkers section.

4.1.3. Progression. Progression, which occurs after promotion, can last for decades and is, therefore, the least likely stage of the carcinogenic process to benefit from drug intervention. Therefore, during the progression stage an enhanced cancer surveillance programme may be preferred over drug treatment to individuals at 'a higher genomic risk' (similar to the monitoring of progression of cardiovascular disease). Furthermore, the screening would likely be targeted to those who may already be at elevated risk for cancer due to either increased genetic susceptibility or familial predisposition to cancer, because of other carcinogenic exposures (e.g. smoking) or because of a persistent high level of chromosomal instability in apparently normal somatic tissues following the radiation exposure incident. Although genetic testing to identify high cancer risk individuals is still in its infancy and there are no genetic tests to specifically identify individuals at high radiation risk in the general population, even a known family history of cancer might be sufficient to identify genetically based cancer susceptibility that warrants increased surveillance (Boice 2007). It may be likely that such individuals might already be under an appropriate surveillance programme (e.g., breast, lung, or colon cancer screenings).

4.2. Other mechanistic targets for mitigation of cancer risk

In addition to targeting the three stages of carcinogenesis, there are also established mechanistic determinants of cancer risk that might be amenable to intervention. For example, use of pro-apoptotic drugs may enhance the quiescence or removal of DNA-damaged cells and thus preclude their carcinogenic transformation, may be an effective intervention to lower cancer risk. Caution is warranted, however, as clinical use of such drugs might be only theoretically beneficial and even may be ineffective, or even detrimental, in practice. For example, a drug that enhances apoptotic killing of irradiated cells to cull potential transformation may prove deadly to individuals who have sustained a near-lethal radiation dose since the drug may lower the threshold for the haematopoietic or gastrointestinal radiation syndrome.

Precise replication of the genome and continuous surveillance of its integrity ensuring 'error-free' repair of the damage is critical not only for survival but also for avoidance of

carcinogenesis (Aziz *et al* 2012). Thus, another mechanistically based approach might be to utilise drugs that facilitate an enhanced fidelity of DNA repair; for example, strategies for promoting homologous recombinational error-free repair and/or suppressing error-prone non-homologous end joining. The rationale is that 'error-prone' DNA repair pathways are thought to promote cell survival at the expense of introducing mutations into the primary DNA sequence (Bunting and Nussenzweig 2013) because the DNA polymerases involved in error-prone DNA repair pathways tend to have lower fidelity than the DNA polymerases dedicated to replication. Radiomitigators might work by stabilising the DNA enzymatic repair complexes or by shuttling repair to one of the 'error-free' repair pathways that are known to introduce fewer mutations. It might also be possible to reduce mutations by prolonging radiation-induced cell cycle arrest thereby allowing more time for 'error-free repair' mechanisms to operate. Unfortunately, all approaches based on enhancing DNA repair would likely have a limited time window for use since DNA repair processes typically run their course soon after irradiation (Jeggo *et al* 2011). Several micronutrients are required for DNA replication and repair to occur accurately (a) either as cofactors for DNA polymerases (e.g. magnesium and zinc) or for the synthesis of nucleotides required for DNA repair (e.g. folate), (b) as an integral component of a DNA repair enzyme (e.g. Zn in hOGG1 (human 8-oxoguanine glycosylase), which is required to remove oxidised guanine), or (c) a precursor of substrates to generate NAD (nicotinamide adenine dinucleotide) for poly-adenosine diphosphate ribosylation (e.g. niacin), which is essential in the detection of strand breaks in DNA and recruitment of the required DNA machinery (Fenech 2010a, Ferguson and Fenech 2012, Hageman and Stierum 2001, Sharif *et al* 2012). Micronutrient strategies are discussed below in section 5.

5. Chemoprevention products

As mentioned above, products that target the tumour promotion stage of a multistep carcinogenesis model have largely come from the field of chemoprevention. Some cancer preventive products that can modify radiation transformation within *in vitro* systems have been reported. These include the following classes of compounds: (a) vitamins (or vitamin-like compounds), their precursors (e.g., vitamin A, its precursor β-carotene and other carotenoids), and derivatives (e.g. retinoids), (b) protease inhibitors (e.g. the soybean-derived protease inhibitor, Bowman–Birk inhibitor), (c) hormones (e.g. glucocorticoid hormones, testosterone, dihydrotestosterone), (d) modifiers of arachidonic acid metabolism (eicosanoids), (e) inhibitors of protein kinase C, and (f) antioxidants, including numerous products that scavenge free radicals.

Retinoids, which have been evaluated extensively as cancer preventive products, have been shown to prevent radiation transformation *in vitro* (Borek 1981, Harisiadis *et al* 1978). Antioxidants have also been shown to decrease radiation-induced carcinogenesis in both *in vivo* and *in vitro* systems (Kennedy 2009). Combinations of antioxidants have been used to inhibit radiation-induced carcinogenesis (Kennedy 2009). Numerous constituents of vitamin pills (i.e., vitamins, minerals and non-vitamin micronutrients) have been shown to prevent DNA damage (Ames 2001). In the nutrition intervention trials in Linxian, China, supplementation of the diet with beta-carotene, vitamin E and selenium together was associated with a statistically significant lower total mortality rate in a nutritionally deficient population with low dietary vitamin intake (Blot *et al* 1993). In this population, the reduction was mainly due to lower cancer rates; the reduced risk became apparent in about 1–2 years after the start of supplementation with vitamins and minerals. As cancer was the leading cause of death in

this population, these results indicate that dietary antioxidant vitamin supplementation was effective at suppressing mortality, primarily from cancer. A recent report indicates a reduction in the radiation-induced formation of γ-H2AX foci in HPBL cultures *in vitro* by the addition of a mixture of antioxidants and glutathione-elevating compounds (Kuefner *et al* 2012) suggesting that prior administration of micronutrients might mitigate radiation risks, although evidence is not entirely convincing (Brink and Boice 2012).

Some of the chemoprevention products have shown activity when treatments began after the exposure to a carcinogenic agent. In rats treatment with a soy isoflavone mixture and soy derivative protease inhibitor (Bowman–Birk Inhibitor Concentrate; BBIC), started one week after the end of treatment with a carcinogen, effectively prevented prostate carcinogenesis (McCormick *et al* 2007). There is also evidence from *in vitro* and *in vivo* models that some cancer preventive products may reduce cancer risk even when administered after radiation exposure (Kennedy *et al* 2011). Many cancer preventive products recognised by Division of Cancer Prevention (DCP, National Cancer Institute) also may be also useful as mitigators of carcinogenesis after exposures to carcinogens implying their potential to intervene in radiation-induced carcinogenesis at the promotion stage as exemplified by a curcumin study in which dietary administration significantly reduced the incidence of mammary tumours in rats (Inano *et al* 1999).

6. Intervention of radiation-induced thyroid cancer

Relevant to a nuclear power plant accident the release of a complex mixture of radionuclides consisting of radioactive iodine (^{131}I) and caesium (^{137}Cs) can pose a significant health hazard through ingestion and inhalation. While radioactive caesium poses a risk to the lungs by inhalation and to the whole body through ingestion, radioiodine presents a unique radiation hazard since iodine is readily concentrated in the thyroid gland. Upon accumulation from consuming contaminated food and water, ^{131}I can deliver a substantial local tissue dose, significantly increasing the risk of thyroid cancer among people who are exposed when young. ^{131}I has a short half-life (8 days) and usually presents an appreciable health risk only from ingestion of contaminated food or water. It may also pose a more significant threat in individuals who happen to be iodine deficient. While interdiction of potentially contaminated food and water is the primary and preferred means of protection, for those who potentially internalise radioactive iodine by continued consumption of contaminated food and water, the approach to reduce radioactive iodine uptake by thyroid is to saturate it promptly with non-radioactive (i.e. 'cold') iodine by ingesting potassium iodide (KI) tablets prior to or within a few hours of ^{131}I exposure (CDC 2013).

It has been reported that providing KI in the form of dietary supplements (as antistrumin, multivitamins containing iodine and iodised salt) at several months to years after the Chernobyl accident to the children from exposed areas reduced the risk of thyroid cancer by a factor of approximately three (Cardis *et al* 2005). In these studies, the KI could not have reduced the uptake of ^{131}I into the thyroid gland since the ^{131}I had decayed to insignificant doses by the time of drug treatment. It is more likely that the dietary supplements acted by a mechanism other than blocking uptake of ^{131}I by thyroid. Iodide could have been the active chemopreventive drug in the KI supplements, but the vitamins and minerals in which KI was administered to at least some of the children also could have contributed to the observed cancer preventive activity of the dietary supplements (Cardis *et al* 2005). An alternative explanation could be that KI supplementation may have also corrected local iodine deficiency, which may independently influence the risk of thyroid cancer initiation or its progression (Boice 2005, Shakhtarin *et al* 2003).

7. Possible medical countermeasures

There are several drugs that are used as human cancer chemopreventive or 'risk reduction' products, such as exemestane for breast cancer (Goss *et al* 2011) and aspirin in several common cancers (Rothwell *et al* 2011). It is possible that some of these products may also have the ability to prevent radiation-induced human cancer. NCI's DCP has funded numerous studies to determine the effectiveness of these products in chemoprevention. Several of these drugs have been tested in clinical trials, which are reviewed elsewhere (Greenwald 2002, Kelloff *et al* 2006, O'Shaughnessy *et al* 2002). The findings show that in addition to anti-cancer vaccines there are four major classes of compounds that show promise as chemopreventive products in randomised controlled trials (RCT), which are discussed below.

7.1. Vitamins and other essential micronutrients

Vitamin A, its precursor beta-carotene, and vitamin E have been used in clinical trials as chemoprevention products based on laboratory rationale (reviewed in Gallicchio *et al* 2008 and Huang *et al* 2006). Paradoxically alcohol drinkers showed an increase in lung cancer risk (Ratnasinghe *et al* 2000) despite increased serum carotenoid levels. Vitamin E, an antioxidant, also showed a slightly increased risk for developing prostate cancer (Klein *et al* 2011). These findings are counterintuitive and warrant caution when recommending interventions of 'off the shelf' food supplements or vitamins. While there may be a theoretical reason to consider using such an approach, there may be a dose–effect and/or other mechanisms by which antioxidants could actually increase cancer risk suggesting that we may not yet fully understand the clinical biological activities of antioxidants. Several retinoids have shown activity in clinical studies in treatment of pre-cancers and prevention of second primary cancers, but are less effective in early carcinogenesis (Kitareewan 2004).

An example of a non-vitamin essential micronutrient that may influence cancer risk is methionine. The amino acid, methionine is a precursor required for polyamine synthesis which accelerates cell division (Cavuoto and Fenech 2012). Several cancers are methionine-dependent for their growth; frequent co-deletion of the methylthioadenosine phosphorylase (MTAP) gene with p16, a tumour suppressor that is silenced or deleted, is observed in irradiated cells (Belinsky *et al* 2004, Yamada *et al* 2010). MTAP codes for a key enzyme in the methionine salvage pathway (methylthioadenosine phosphorylase), which if deleted or silenced, makes cells unable to regenerate methionine (Cavuoto and Fenech 2012). Methionine restriction has been shown to prevent cancer and prolong lifespan in rodents (Komninou *et al* 2006, Orentreich *et al* 1993, Richie *et al* 1994). Methionine restriction may therefore help to control radiation-induced methionine-dependent cancers and could prove to be one of the possible strategies of preventing progression of such cancers. Similarly, long-term dietary supplementation with the thiol-containing antioxidant, N-acetyl-L-cysteine (NAC) appears to suppress carcinogenesis-associated biomarkers, such as DNA deletions and oxidative DNA damage in an ATM-deficient mouse model (Relience *et al* 2004). Further NAC significantly increased the lifespan and reduced both the incidence and multiplicity of lymphoma in this model (Relience and Schiestl 2006). Thus, understanding which nutrition-relevant genes may be commonly deleted by ionising radiation exposure could inform dietary restriction strategies for prevention of the growth of such cancers.

7.2. Inhibitors of hormone actions

Tamoxifen citrate (tamoxifen) was the first drug to receive US FDA approval as a cancer preventive drug for breast cancer in 1998. It was shown to prevent cancer recurrence in a

randomised, double blind breast cancer prevention trial known as the breast cancer prevention P-1 trial (Fisher *et al* 1998). While widely acknowledged to be a human breast cancer preventive drug, it has not been widely used in non-cancer populations for the prevention of breast cancer primarily due to its potential side effects (e.g. increased rates of endometrial cancer and thrombotic events, as shown in the original National Surgical Adjuvant Breast and Bowel Project). A subsequent trial of tamoxifen and raloxifene hydrochloride (raloxifene) indicated that raloxifene was as effective as tamoxifen for breast cancer prevention in postmenopausal women but with less toxicity (Vogel *et al* 2006); the FDA for breast cancer risk reduction has also approved raloxifene. An important aspect of these trials is that reduction of recurrence persisted after discontinuation of the drug (Jordan *et al* 2011).

Proscar (finasteride)—an α-reductase inhibitor—has been shown to prevent or delay the development of human prostate cancer (Thompson 2003) but there was evidence in this initial study that finasteride treatment led to sexual side effects and an increased risk for the development of high-grade prostate cancer. Findings with finasteride and a related drug, Avodart (dutasteride) (Andriole *et al* 2010), revealed an increase in high-grade tumours despite the major overall decreased incidence in cancer and led the FDA (see product labels at http://www.accessdata.fda.gov/scripts/cder/drugsatfda/) not to approve α-reductase inhibitors for prostate cancer prevention (Hamilton *et al* 2010, Logothetis and Schellhammer 2008, Lucia *et al* 2007, Redman *et al* 2008, Theoret *et al* 2011).

7.3. Cyclooxygenase-2 (COX-2) inhibitors and other nonsteroidal anti-inflammatory drugs (NSAIDs)

Celecoxib (Celebrex), a COX-2 selective inhibitor, and other NSAIDs have received FDA approval as cancer prevention products and/or drugs used for treating advanced premalignancy. The US FDA approved Celebrex in December 1999 for a reduction of incidence rate in polyps in familial adenomatous polyposis (FAP). However, this indication was removed from the approved labelling in 2011 due to a want of a confirmatory trial. COX-2 inhibitors were widely used in numerous human cancer prevention trials but many of these trials were stopped when results from the Adenomatous Polyp Prevention on Vioxx (APPROVe) (Bresalier *et al* 2005) and Adenoma Prevention with Celecoxib (Bertagnolli *et al* 2006) trials indicated that COX-2 inhibitors were associated with serious cardiovascular adverse events. This information is publicly available in the approval letters and the drug labels, which can be accessed at the FDA website (http://www.fda.gov/Drugs/default.htm—accessed 18 October 2013). Some of the NCI/DCP studies on the use of celecoxib for prevention and treatment for human cancer were continued and have shown positive results (Meyskens *et al* 2011). It has been proposed that COX-2 inhibitors could be used to prevent human cancer without cardiovascular adverse effects under certain conditions such as using low to moderate doses of the drug, reduced frequency of drug administration, and/or use in combination with other cancer risk reduction drugs (Meyskens and McLaren 2010).

Thus, there are several drugs that have already been shown to be useful for human cancer prevention and which might, in theory, be useful to prevent radiation-induced cancer in human populations. The key issue of balancing of benefits versus risks remains; therefore, the drugs should best be targeted to those who could benefit the most. As noted earlier, this might include those who are already at intrinsically high risk of developing cancer either due to their genetics or susceptibility or undue exposure to other carcinogens. One approach could be to identify people with premalignant lesions (e.g., IEN or benign breast disease) who may be already at increased cancer risk that might be further increased due to a 'significant dose' of radiation (Meyskens and McLaren 2010, Meyskens *et al* 2008). What is considered a 'significant dose'

would be somewhat subjective (perhaps >500 or 750 mSv). Consequently, a role for active chemoprevention is likely to apply to only a very small proportion of people exposed in a radiological or nuclear incident (Meyskens and Gerner 2011) and identification of this cohort is imperative.

7.4. Dietary-related compounds

There are a wide variety of dietary compounds that have been shown to prevent carcinogenesis induced by chemical carcinogens (Wattenberg 1985, 1990, 1992, Wattenberg *et al* 1985) but only a few classes of agents that have been shown to prevent radiation-induced cancers in animal models. The dietary agents such as protease inhibitors (Kennedy 1998a, 1998b), Vitamin A (Burns *et al* 2007), a retinoid (Burns *et al* 2002), other various dietary antioxidant vitamins (Kennedy *et al* 2008, 2011), and a soybean diet (Troll *et al* 1980) have been shown to inhibit radiation-induced carcinogenesis *in vivo*.

Protease inhibitors such as the soybean-derived Bowman–Birk inhibitor have been shown to inhibit radiation-induced cancer in animals and to have anti-carcinogenic effects in humans (Kennedy 2006). Protease inhibitors have been observed to suppress carcinogenesis when administered to cells or animals at long periods of time after exposure to the carcinogenic agent(s) (Kennedy 1998a,b). This phenomenon has also been observed for drugs that prevent radiation-induced cancer in animals (e.g. cortisone suppresses radiation-induced leukaemogenesis when administered to animals months after the radiation exposure; Kaplan *et al* 1951).

It is known that there are compounds with strong cancer preventive activities in the normal human diet(s) (Wattenberg *et al* 1985, Bode and Dong 2009, Wattenberg 1985) but their role in human cancer prevention by taking higher than the normal dietary amount is uncertain. However, both in human populations and animal studies, vitamin and micronutrient deficiencies are known to increase cancer incidence and mortality rates (Blot *et al* 1993, Fairfield and Fletcher 2002, Li *et al* 1993, NA/NRC 1982, Nelson 1987, Newberne and Rogers 1985). Americans do, in fact, have significant dietary deficiencies (Ames 2001) that may put them at an increased risk of cancer. Dietary deficiencies of agents that have a Recommended Dietary Allowances (RDAs; generally those with a maximum allowances per day) should be avoided as therapeutics due to dose-limiting toxicities and potentially narrow therapeutic index (TI), but this should be measured and patient-specific as taking amounts beyond the RDA could be beneficial to some. The American Medical Association (AMA) recommended that everyone take a daily multivitamin pill for the prevention of numerous adverse health conditions, which includes carcinogenesis (Fletcher and Fairfield 2002), although there are examples of adverse effects from dietary supplements (Mursu *et al* 2011). There have been some studies suggesting a negative health impact from dietary supplements that utilise analytical methods that are controversial (Kennedy and Wan 2011). A very recent report by the US Preventive Services Task Force Evidence Syntheses (formerly Systematic Evidence Reviews), found no evidence confirming a health benefit resulting from vitamin, mineral, or multivitamin supplements (Fortmann *et al* 2013). A recommendation (editorial) was published recently supporting this position in the annals of internal medicine (Guallar *et al* 2013). Moreover, recent *in vitro* studies with HPBL show that both deficiency or excess of certain micronutrients (e.g. zinc) may increase chromosomal DNA damage and moderate folate deficiency can increase micronuclei counts to a similar extent as an exposure of 0.2 Gy from low-LET radiation (Fenech 2010a, Ferguson and Fenech 2012, Sharif *et al* 2011). As a general conservative approach, deficiencies should be avoided but use of supplementation beyond the RDA remains debatable. Given such constraints, there is a pressing need to define dietary reference values based on DNA damage

prevention and optimal DNA repair. Defining a generalised 'optimal nutrition' status may likely be a useful recommendation to minimise both 'spontaneous' and 'radiation-induced' genome mutations.

8. Drug development programmes

Development of drugs and biologics as MCMs for treatment of radiation injuries has been largely the purview of the United States government (BARDA 2013). As such, to date, research and development has focused on developing MCMs against ARS and also against DEARE such as lung fibrosis. MCMs used for ARS are discussed here because of their discovery, development, regulatory and translational challenges share many features as those agents which may potentially be developed as mitigators of DEARE, and health risks, specifically cancer. However, similarities with molecular pathways of damage are to be explored, as many exist.

The MCM programmes include basic radiation biology studies supported through NCI, scientific studies supported by the National Institute of Allergy and Infectious Disease (NIAID; www.niaid.nih.gov—accessed December 29, 2013) and the Armed Forces Radiobiology Research Institute (AFRRI; www.afrri.usuhs.mil—accessed 29 December 2013), advanced drug development and biodosimetry studies by Biomedical Advanced Research and Development Authority (BARDA; www.phe.gov/about/barda/Pages/default.aspx—accessed 20 January 2014), and drug development by Department of Defense Chemical Biologic Medical Systems—Medical Identification Treatment Systems (CBMS/MITS; www.jpeocbd.osd.mil—accessed 29 December 2013).

These above government programmes have identified several MCM drugs with promise of preventing the acute and intermediate health consequences of high-dose exposures. It is not known whether these agents can also prevent late health effects among those exposed to below threshold doses for ARS development as well as those who survive ARS. The focus of ARS studies is primarily on the haematopoietic depression, gastrointestinal tract pathologies, pneumonitis and delayed fibrosis, and cutaneous damage that follow shortly after total body acute exposures in the 2–10 Gy range as well as combined injuries (operationally defined as trauma plus radiation of >2 Gy; DiCarlo *et al* 2011). The delayed fibrosis seen in the lung and other tissues following doses of >6–8 Gy are considered to be in the DEARE category as they arise months to years post-exposure. Countering ARS will require administration of MCM drugs within hours or possibly within days of an incident to be effective since ARS can occur quickly (days to weeks). By mitigating an acute injury, or by other mechanisms of action, these drugs might also mitigate DEARE pathologies that manifests months or years later. Conceivably, mitigation of cancer risks of radiation might follow a similar treatment paradigm in that early intervention might affect initial underlying mechanisms that may mitigate late event risks. The intervention could be targeted against cellular damage, tissue loss and/or the inflammatory responses as well as many signalling networks that are involved in carcinogenesis.

Testing MCM drugs for effectiveness against any of the above radiation-induced health conditions is problematic since human studies to induce ARS or DEARE are unethical. The animal rule provides a regulatory mechanism to test MCM's efficacy in animal models when testing in human subjects is neither ethical nor feasible. MCM drug developers can conduct their experiments in animal models with the assumption that the effect seen in the animal models will be predictive of the response in humans. Thus, the Animal Rule provides the opportunity to evaluate the efficacy of candidate radiation protectors/mitigators in animals.

Neupogen® (Filgrastim) a granulocyte colony stimulating factor (G-CSF), has been approved by the FDA 'to decrease the incidence of infection, as manifested by febrile neutropenia, in patients with nonmyeloid malignancies receiving myelosuppressive anti-cancer drugs associated with a significant incidence of severe neutropenia with fever' (www.accessdata.fda.gov/scripts/cder/drugsatfda/—accessed 20 January 2014). Neupogen may be used in the event of a radiation emergency for treatment of radiation-induced neutropenia. The FDA has not approved the indication of radiation-induced neutropenia. In a joint meeting in May 2013, the Medical Imaging Advisory Committee and Oncologic Drug Advisory Committee reviewed and discussed animal data and clinical experience with Neupogen, and were supportive of the use of Neupogen in radiation-induced neutropenia. (http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/MedicalImagingDrugsAdvisoryCommittee/UCM363898.pdf—accessed December 29, 2013).

DEARE are deterministic effects and, therefore, distinct from the sporadic health effects typified by radiation-induced cancer. Nevertheless, the appearances of both DEARE and radiation-induced cancers are significantly delayed after exposure. Whether or not there are mechanisms in common, such as inflammation, tissue healing, immunological effects, and others needs to be explored. While speculative, given the current state of knowledge, assessment of MCM drugs for efficacy against DEARE may thus allow assessment of the concomitant long-term endpoint of cancer as well. It is not clear what aspects of the animal models appropriate to both ARS and DEARE endpoints and would be equally appropriate for cancer endpoints, but it would be desirable to employ such animal models whenever possible since the potential exists to concurrently evaluate the efficacy of MCM drugs for all early and DEARE, including cancer, endpoints.

8.1. Is a prospective clinical trial even possible for MCM drugs?

A short answer at present is 'no'. To test an MCM for reducing cancer risk from radiation for a general population would be a challenge based on the heterogeneity of risk factors (stochastic in nature) and the length of study needed. For a '*time-to-event*' analysis (e.g. Cox proportional hazard model or Kaplan–Meier model) of a clinical response to a hypothetical mitigator/protector, the '*event*' is a confirmed cancer diagnosis. This is problematic in a clinical trial as cancer *per se* is not radiation-specific and because long periods (decades) of observation are required to accumulate a sufficient number of '*events*'. There may be certain populations who were treated with radiation therapy and are at elevated risk of developing cancer, and for which such studies could be conducted regarding mechanisms and possible biomarkers of radiation injury. The mechanisms and biomarkers remain to be explored but availability of some populations at increased risk of treatment-induced cancer may facilitate the design and approach to define their roles in cancer. For example, patients treated with total body irradiation for bone marrow transplants are at high risk of developing cancer (Rizzo *et al* 2009). Large numbers of survivors of childhood cancer, currently under study, are at higher risk of radiation-induced cancers of the thyroid, breast, bone and brain as well as heart disease (Armstrong *et al* 2010). Patients treated with radiotherapy for Hodgkin's lymphoma are at higher risk of radiation-induced cancers of the breast and lung (Boice 2007, Gilbert *et al* 2003, Travis *et al* 2003). Young women given radiotherapy to treat breast cancer are at two-fold risk of developing a second breast cancer (Boice *et al* 1992, Darby *et al* 2010, Stovall *et al* 2008). Persons irradiated as children for enlarged thymus glands are at an elevated risk of radiation-induced cancers of the breast and thyroid (Adams *et al* 2010a, 2010b). Occupational

studies of underground uranium miners exposed to radon and its decay products have found high risk (>5-fold) increases in lung cancer (Lubin *et al* 2005).

Thus, at present the need to explore a range of potential strategies and endpoints, the lack of complete knowledge of the radiobiological mechanisms of radiation-induced cancer, the resultant lack of a biomarker (see below), the heterogeneity of risk factors and uncertainty of lifetime exposure to ionising radiation of individuals, and the likely need for a defined mechanism for an effective MCM, makes it almost impossible to conduct a prospective efficacy study of an MCM using cancer incidence as an endpoint.

9. Biomarkers

Mitigating the risk of radiation-induced cancers will require the use of three categories of biomarkers: (i) radiation dose assessment (biodosimeters), (ii) genomic health prediction, and (iii) cancer surveillance. We have provided a rationale in preceding sections for using and/or developing suitable diagnostic biomarkers for determining the radiation dose of individuals and assigning a population at risk for developing delayed health risks including cancer. In so doing, we seek to identify individuals from the at-risk pool who may benefit from a reduction in their cancer risk from a mitigator-based therapy. Similarly, there is also a need for assessing the overall 'genomic health risk' of individuals in such an exposed population and also to monitor the efficacy of mitigator intervention by possibly using appropriate biomarkers. Further, we have also argued for a need for using an enhanced cancer surveillance programme for selected high-risk radiation-exposed cohort following a radiological mass casualty, specifically for radiogenic cancers such as breast, respiratory, digestive system, thyroid and other solid cancers, and leukaemia among children. The Early Detection Research Network of Division of Cancer Prevention (EDRN, NCI, http://edrn.nci.nih.gov—accessed 29 December 2013), a consortium of laboratories, is focused on identifying, testing, and validating methods to noninvasively and accurately detect cancers at their earliest stages. Biomarker-based treatment decisions may eventually drive personalised medicine (Hamburg and Collins 2010), however, the definitive utility of these biomarkers requires conducting large RCTs for validation. Issues related to designing such RCTs with biomarkers have been discussed elsewhere (Freidlin *et al* 2010).

There are many sources of genotoxic damage but our focus is on radiological and nuclear incidents, primarily a nuclear detonation and mass casualty response. In this context, how we can distinguish the sources of carcinogenic insults and detect added health risks due to such exposure to radiation and develop biomarker-based approaches to assess a reduction of such risks due to intervention—pharmacological, nutritional or other?Comprehensive strategies for determining 'total genomic health risk' assessment and companion radiation dose diagnostic tools to predict the radiation component of such genomic risks becomes a critical need. Such tools could serve the dual purpose of assessing MCM efficacy (table 1) for use at doses below the assigned threshold doses for ARS and also for monitoring survivors of ARS. Furthermore, if it is possible to distinguish the source of 'genomic insult' between radiogenic or not, that would be useful in determining the 'probability of cause of late effects' and help improve resolution of medico/legal compensation issues (DHHS 2002).

Identifying an at-risk population cohort for increased health risk due to radiation exposure is critical in addressing risk–benefit issues and the potential need for a prolonged mitigator treatment and/or enhanced cancer screening. In this context, consideration is given to two well-established/validated cytogenetic assays performed using HPBL, cytokinesis-block micronucleus (CBMN) for assessing 'total genomic health risk' and the dicentric chromosome assay (DCA) to determine the risk related to radiation component. We recognise the need for

both validated assays and logistics/resources to conduct such an analysis on an appropriate at-risk population, which could be defined based on their exposure during an incident above a certain dose (e.g., >0.5 Gy). Biodosimetry techniques, their capability, window of utility, and the concept of operations (CONOPs) needed for deployment of various tests are under consideration by the Office of the Assistant Secretary for Preparedness and Response (ASPR) (Sullivan *et al* 2013).

9.1. Cytokinesis-block micronucleus (CBMN) assay

The CBMN assay is well validated as a biomarker for radiation biodosimetry and inter-laboratory studies have shown it to be highly reproducible both when scored visually or automatically (Fenech 2010b, IAEA 2011, Romm *et al* 2013). It is useful for rapidly triaging individuals exposed to doses above 1 Gy of radiation with high degree of accuracy and specificity (McNamee *et al* 2009, Romm *et al* 2013). However, at doses less than 0.5 Gy, a substantial proportion of assessed damage may not be very specific to ionising radiation due to a variety of other contributors to genomic damage such as age, gender, smoking, diet, genetic predisposition, etc, but it, nonetheless, reflects 'total genomic damage' (Bonassi *et al* 2003, Fenech 1999, Fenech and Bonassi 2011, Fenech and Morley 1985). The CBMN assay performed using HPBL obtained from children from the Chernobyl radiation accident cohort showed an increase in their 'total genomic risk' (Fenech *et al* 1997, Zotti-Martelli *et al* 1999). However, because nutritional deficiency or excess can also cause chromosomal aberrations and micronuclei in children or adults (Fenech and Bonassi 2011) it is not possible to exclude malnutrition as a contributing variable affecting MN frequency at exposure doses less than 0.5 Gy. Furthermore, the observation that MN frequency can be reduced by nutritional supplementation (e.g. folic acid and vitamin B12) (Thomas *et al* 2011) indicates the possibility of using nutritional strategies for DNA damage prevention after a radiation incident that could complement and further enhance countermeasures against radiation-induced DNA damage.

Evidence of a direct link between increased genomic damage and elevated risks for adverse health outcome is becoming stronger. For example, an increase in chromosomal damage as determined by the CBMN assay is predictive of cancer risk, cardiovascular disease mortality and pregnancy complications such as pre-eclampsia and intrauterine growth restriction (Bonassi *et al* 2007, Federici *et al* 2008, Furness *et al* 2010). Furthermore a high MN frequency is associated with the pathogenesis of neurodegenerative disorders such as Parkinson's and Alzheimer's disease, metabolic syndrome, and cardiovascular diseases (Andreassi *et al* 2011, Migliore *et al* 2011). Using a biomarker of chromosomal instability it has also been shown that folate is an important determinant of chromosomal stability and a modifying factor of cellular sensitivity to radiation (Beetstra *et al* 2005).

Mechanistic, theoretical, and empirical evidence accumulated over the last several decades supports the role of chromosomal abnormalities in the etiology of human cancer and the CBMN assay has emerged as a powerful tool for measuring chromosomal damage and total genomic health, especially in selected populations. A recent large international study assembling data from over 6000 individuals from 10 countries, showed a significant association between MN frequency in healthy subjects and cancer risk (Bonassi *et al* 2011). This study supports the idea that MN frequency in HPBL is predictive of cancer risk suggesting that it is associated with early events in carcinogenesis and potential for prospective use of the CBMN assay in cancer screening programmes. To investigate the translational feasibility of the above observations, Fenech and colleagues have begun a Genome Health Clinic aimed at diagnosis and mitigation against DNA damage using dietary and lifestyle interventions (Fenech 2005, 2013).

Given that this paper aims to stimulate potential new approaches, one speculative idea is to use the CBMN assay as a low cost minimally invasive predictive biomarker that is also responsive to various other genomic health determinants in the aftermath of a nuclear event. In theory, the lymphocyte MN frequency index could become a very useful medical test result available in each person's medical record so that a recent baseline value is available, which could, in theory, be used to obtain a more accurate assessment of radiation exposure particularly at doses less than 0.5 Gy rather than resorting to a statistical estimate as done in current practice (Tucker *et al* 2013). That a CBMN assay should become a routine medical test is not implausible because the use of MN in haematological clinical practice is already accepted via the measurement of Howell–Jolly bodies (also MN) in erythrocytes as a biomarker of folate and/or vitamin B12 deficiency (Dawson and Bury 1961, Jolly 1905, Howell 1890−1891). It was in fact this development that eventually led to the use of micronuclei in erythrocytes as an *in vivo* biomarker of DNA damage in rodents (mouse micronucleus test, MMT; an *in vivo* standardised genetic test used in drug development for drug-induced micronuclei) and then eventually to the measurement of MN in lymphocytes in humans (Heddle *et al* 2011).

9.2. Dicentric chromosome assay (DCA)

The DCA is the current 'gold standard' method for radiation dose assessment. For many years, the DCA performed using HPBL has been used as a biological dosimeter for specifically assessing radiation dose to individuals. DCA shows a high degree of radiation specificity, high sensitivity, and robust dose dependency in the range 0.15–5.0 Gy. Furthermore, a sampling delay of up to six weeks after radiation exposure does not interfere with dose assessment and the effects of many confounders have already been well characterised. Dose estimation can be made independent of radiation type and dose rate (IAEA 2011). Well-defined laboratory protocols and quality control standards are already available (IAEA 2011, ISO 2004, 2008). Estimated doses using the DCA correlate well with the severity of ARS. The assay can diagnose partial-body exposures and indicate the presence of surviving bone marrow, as this may be helpful in managing partial-body exposures (Lloyd 1997). The DCA is validated in several inter-laboratory comparison studies (Beinke *et al* 2013, Rothkamm *et al* 2013, Wilkins *et al* 2008, 2011). While precise dose estimation for health risk assessment requires analysis of 500 or more metaphase spreads, analysis of approximately 50 metaphase spreads per person is adequate for initial mass casualty triage and management of people at risk for the ARS (Prasanna *et al* 2010, Romm *et al* 2011). Since predicted doses by different laboratories were in good agreement a network of laboratories can improve throughput during a mass casualty incident. Several national and international collaborative networks are already in place (e.g. WHO BioDoseNet, Canadian Network, European MULTIDose and Japanese Networks). Both sample preparation and dicentric analysis are time-consuming and laborious. Laboratory automation is essential to further increase throughput and to enhance DCA's practical applications in mass casualties. Serving as a radiation-specific diagnostic tool to assess risks of developing ARS, DCA can also serve as a means to estimate dose and help identify a populations at high risk for delayed health risks.

Several other new biodosimetry technologies are also currently under development including biomarkers based on DNA damage while others are based on metabolomics, gene and protein expression changes (Sullivan *et al* 2013) as well as biophysical methods (Brink *et al* 1980). The biomarker development and MCM drug development efforts complement one another, similar to companion diagnostics in treatment for a given disease setting. Commercial development and use of radiation risk mitigators will benefit from predictive biomarkers for radiation dose and genomic health concurrently and these would also help in the understanding of the impact of nutrients, repurposed drugs or other interventions.

Having a unique set of biomarkers that show an MCM drug is preventing or delaying cancer development will be extraordinarily important for physicians to assess and medically manage at-risk populations. The challenge will be in developing the marker, its validation, and regulatory considerations.

A major caveat in the clinical utility of a predictive biomarker for cancer is inter-individual differences in genetics and radiation responses. The biology underlying these variable responses among individuals to radiation remains poorly understood. Indeed, significant variation in response to radiation-induced chromosome damage is evident even when studying just ten or more randomly chosen healthy individuals (Fenech 2010a, Rothfuss *et al* 2000). These differences may be partly due to genetic defects in key genes involved in homologous recombinational DNA repair pathways such as the ATM, BRCA1 and BRCA2 genes, and, in fact, the CBMN assay and DCA can be used successfully to identify individuals with such defects (Claes *et al* 2013, Rothfuss *et al* 2000, Scott 2000, Vral *et al* 1996).

There is much to be learned from biomarker development applied to cancer research, as biomarkers can be prognostic (estimate overall disease prognosis) and/or predictive (who may respond to treatment or develop disease). The lack of standardised technologies and assays for verifying and validating candidate biomarkers, as well as a plethora of parameters to consider for validation studies, complicates the development pipeline from biomarker discovery through clinical use. Nonetheless, the impact of radiation on DNA damage and the radiation-induced stress response may enable biomarkers to be developed for early triage in predicting late effects and potentially identifying who might be suitable candidates for an MCM drug trial.

10. Conclusion

In the event of a nuclear detonation or major radiological incident the potential number of people at risk (Knebel *et al* 2011) for radiation-induced cancers is large. It is important to identify people who are at significant risk and for whom treatment with mitigating agents is worth consideration. While a nuclear detonation is catastrophic, the size of the device modelled for terrorist-related incidents leaves much of the local and regional infrastructures intact so that a response is both possible and mandatory to save lives (US EPA 2013). The adverse psychological impact of prolonged fear of radiation-induced cancer also would be a major public health concern (Bromet 2012, 2014) and psychological stress itself may be related to chromosomal DNA damage (Fischman and Kelly 1999, Fischman *et al* 1996, Ingel *et al* 2001, York *et al* 2013). While managing those with immediate and critical medical needs is the first priority, the large number of people potentially with lower radiation doses deserve, and will ask for, attention. The vast majority of people following a nuclear incident will have very low or no exposure so that the use of any MCM may produce little benefit and could involve the risk of side effects. Furthermore, the additional burden and expense of employing a drug that requires long-term use must be considered. Nevertheless, the availability of an MCM drug that could be used after a radiation incident to lower the risk of radiation-induced cancer is considered desirable. At present, there is no MCM drug for such use.

A logical strategy to identify MCMs that reduce the risk of cancer might be to first establish and validate biomarkers of radiation injury that relate to increased cancer risk and then evaluate potential efficacy in an appropriate laboratory model. Well-known chemopreventive agents, antioxidants, and nutritional supplements might be the prime or at least initial candidates, since many have already been studied in clinical trials and some are already US FDA approved for indications other than radiation-induced cancers. The testing of entirely novel MCM drugs in humans is problematic (or not possible) for scientific, statistical, ethical and logistical reasons,

so that MCM development would likely require the use of FDA's Animal Rule and early discussion with the FDA in project design.

An alternative or complement to MCM drug treatment might be increased surveillance of radiation-exposed people known to be a high risk for cancer and other radiation-associated health effects. Surveillance is already in use for people known to be at high genetic risk for certain types of cancers (e.g., breast cancer in young women with known mutations in breast cancer genes such as BRCA1, or a positive family history of cancer), and an approach would be to ensure that they maintain appropriate surveillance. Surveillance could also be an option for radiation victims if the doses that they received put them at 'significant' additional lifetime cancer risk, which would need to be determined but likely at a dose above 0.75 Gy given the high background rate of cancer.

Since cancers associated with behavioural choices typically entail much greater risk than radiation exposure, sound medical advice for victims might be to modify lifestyle activities to reduce general cancer risk—healthy diet and weight, no smoking, exercise and good general health practices. This could be particularly beneficial if the postulated synergistic interaction between some of these and cancer risks and radiation are validated (e.g. smoking and radiation interaction for lung cancer).

In conclusion, there is ongoing individual and societal concern about radiation-induced cancer from accidental exposures as evidenced by the recent nuclear power plant accident in Japan. Addressing this concern requires credible dose information following an incident, risk assessment and mitigation strategies, a better understanding of the impact of radiation and the competing risks for lifetime cancer in general and effective risk communication with the public. For some subset of those exposed to 'significant' doses, increased disease surveillance is logical and effective MCM that can reduce cancer risk after radiation exposure is needed. Progress in understanding the molecular mechanisms of cancer and radiation injury, along with progress in establishing biomarkers of cancer risk; make this an opportune time to consider investing in research and development into mitigating the risks of radiation-induced cancers. While solutions will be challenging, the widespread concern and potential positive impact of even a mildly effective compound or cocktail of compounds (polypharmacy approaches) given the large number of people potentially affected by a radiation exposure incident would make a careful investment in this research area important for both science and society. While minimising and mitigating risks of the occurrence of a nuclear incident are critical, preparation and planning are key to response and societal resilience should one occur. Research and development can benefit cancer care so dual-utility drugs, or drug repurposing makes investment in this area applicable to the general public health.

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