Health Protection Agency

Human Radiosensitivity

Report of the independent Advisory Group on Ionising Radiation

RCE-21

Human Radiosensitivity

Report of the independent Advisory Group on Ionising Radiation

Documents of the Health Protection Agency
Radiation, Chemical and Environmental Hazards
March 2013

ISBN 978-0-85951-740-9

Contents

Foreword

There is understandable concern about the risks to health of ionising radiation arising from natural and man-made sources. In 1995 the Director of the National Radiological Protection Board (now part of the Health Protection Agency) set up an Advisory Group on Ionising Radiation (AGIR):

> 'to review work on the biological and medical effects of ionising radiation relevant to human health in the occupational, public health, medical and environmental fields and advise on research priorities'

The Health Protection Agency (HPA) has a statutory responsibility for advising UK government departments and others on health effects of radiation and appropriate standards of protection. The AGIR is an independent body reporting to the HPA Centre for Radiation, Chemical and Environmental Hazards. Full details of past and current AGIR work can be seen at www.hpa.org.uk.

This report is the tenth full report prepared by the AGIR and its subgroups set up to focus on specific topics. It considers variation in human radiosensitivity and how this might impact on approaches to radiological protection. It updates the first published report of the AGIR, *Genetic Heterogenecity in the Population and its Implications for Radiation Risk* (*Doc NRPB,* **10**(3), 1–47, 1999).

This report reviews evidence for variation in human radiosensitivity from epidemiological, clinical and experimental studies, considers mechanisms of radiosensitivity and ethical implications of current and potential future knowledge on the range of radiosensitivity in the human population. It concludes that there is growing evidence from a range of sources for variation in radiosensitivity that can affect the risk of radiation-induced cancer or, at higher doses, tissue damage. A proportion of this range is likely to have a genetic origin but there is also substantial evidence for lifestyle factors, and particularly tobacco smoking, affecting individual risk. Currently there is no adequate test predictive of individual radiation health risk, but knowledge is accumulating and therefore consideration of how this new knowledge might be used in radiological protection is important and timely.

Human Radiosensitivity

HAS BEEN PREPARED BY THE

Subgroup on Human Radiosensitivity of the Advisory Group on Ionising Radiation

CHAIRMAN

Professor AMR Taylor, University of Birmingham

MEMBERS

Professor F Balkwill OBE, Institute of Cancer Studies at St Bartholomew's

Dr G Barnett, University of Cambridge

Professor BA Bridges OBE, University of Sussex

Professor AJ Dawson, University of Birmingham

Professor J Hendry, University of Manchester

Dr L Izatt, Guy's and St Thomas' NHS Foundation Trust

Professor PA Jeggo, University of Sussex

Dr P Pharoah, University of Cambridge

Dr R Wakeford, University of Manchester

Professor C West, University of Manchester

SECRETARIAT

Dr C Badie, HPA Chilton

Dr SD Bouffler, HPA Chilton

OBSERVER

Dr S Conney, Department of Health, London

Advisory Group on Ionising Radiation

CHAIRMAN

Professor BA Bridges OBE, University of Sussex

MEMBERS

Professor DT Goodhead OBE, MRC Harwell

Professor P Hoskin, University College London Hospital

Dr MP Little, National Cancer Institute, USA

Professor TJ McMillan, Lancaster University

Professor AMR Taylor, University of Birmingham

SECRETARIAT

Dr SD Bouffler, HPA Chilton

OBSERVERS

Dr S Conney, Department of Health, London

Dr H Walker, Department of Health, London

Acknowledgements

The authors of this report would like to thank Dr Roy Shore (Radiation Effects Research Foundation, Japan) for reviewing the text and providing constructive comments.

Human Radiosensitivity

Report prepared by the Subgroup on Human Radiosensitivity of the independent Advisory Group on Ionising Radiation

Chairman of the Subgroup: Professor A M R Taylor

Chairman of the Advisory Group: Professor B A Bridges OBE

This report from the independent Advisory Group on Ionising Radiation reflects understanding and evaluation of the current scientific evidence as presented and referenced in this document.

1 Introduction

Exposure to ionising radiation is unavoidable. The average dose received by a UK citizen is 2.7 mSv per year (Watson et al, 2005, see Box 1.1 for information on radiation dose quantities), the majority (around 84%) of which is from natural background sources such as radon gas of geological origin and cosmic radiation from outer space. Man-made sources contribute to exposure as well and these include medical (eg diagnostic X-rays) and industrial sources (eg nuclear power plants and industrial radiography) and a very small component (around 0.2%) from fallout from nuclear weapons explosions and incidents and accidents around the world.

Most human exposures to radiation are to low doses over a lifetime. There are situations where doses are elevated. Registered radiation workers in the nuclear industry and elsewhere are permitted to receive up to 20 mSv per year (averaged over a five-year period with no single-year dose greater than 50 mSv). However, occupational exposures tend to be well below dose limits so again are chronic low dose exposures.

BOX 1.1

Radiation dose quantities used to assess radiation exposures and effects

The primary quantity is **absorbed dose**, defined as the amount of energy deposited per unit mass. One joule of energy deposited in a kilogram of matter is referred to as 1 gray (1 Gy).

To account for the different biological effectiveness of different types of radiation – for example, X-rays, beta particles and alpha particles – so-called **radiation weighting factors** (w_R) are applied to absorbed doses to provide **equivalent doses**, expressed in sievert (Sv), for the estimation of stochastic health effects (cancer and hereditary effects).

w_R values are approximations based on judgements of available scientific data, not exact quantities, and are recommended by the International Commission on Radiological Protection (ICRP) (ICRP, 2007). The w_R values for X- and gamma rays are both 1, so a 1 Gy absorbed dose equates to 1 Sv equivalent dose. By contrast, the w_R for alpha particles is 20 and so 1 Gy absorbed alpha-particle dose represents 20 Sv equivalent dose.

As the radiation-associated risk for stochastic diseases varies between organs and tissues, the ICRP recommends the use of **tissue weighting factors**, w_T, to allow for the different effects on different organs. In some cases radiation exposures are not uniform, particularly when considering internally ingested or inhaled radiation sources. The use of w_T values allows the summation of risks to different organs to provide the health-risk-related quantity, **effective dose**, to be calculated. w_T values are applied to equivalent doses to provide effective doses, also expressed in sievert (Sv).

It should be noted that equivalent dose and effective dose are for use in radiological protection, they are not directly measurable quantities – mechanistic and experimental work should use absorbed dose (Gy) as the main measure of radiation dose.

Radiation incidents and accidents, notably the atomic bombings in Japan and the Chernobyl nuclear plant accident, have led to significantly higher exposures. In the case of the Japanese atomic bombings, many acute high dose exposures were experienced. For the general public acute radiation exposure should only occur in the context of clinical radiotherapy for cancer where doses of several tens of gray are delivered to the target cancer site in fractions typically of around 2 Gy. Broadly speaking, three modes of radiation exposures might be experienced by humans:

a Acute high dose exposures (1 Sv and above) – in cancer radiotherapy (which is precisely targeted to the cancer site) or total body irradiation therapy.

b Exposure to moderate to high doses (100 mSv and above) over a working life at low dose rate (below 5 mGy h^{-1}), which can be the case for registered radiation workers (for example, medical radiographers, industrial radiographers and nuclear industry workers).

c Exposure to low doses (below 100 mSv) delivered in a short time such as encountered in diagnostic medical examinations such as computed tomography (CT) scans. Some modern scans deliver tens of millisievert per examination.

It should be noted that any or all of these modes of exposure could occur as a consequence of radiation incidents or accidents.

The primary source of information on the health risks of radiation exposure is epidemiological studies of exposed human populations. Most important of these are the studies of the survivors of the Japanese atomic bombings, but studies of working populations and those exposed medically are also of importance. The two health effects of most importance following the low dose exposures most commonly encountered are cancers and heritable genetic effects. These are usually referred to as stochastic effects as their incidence, but not severity, is determined by the exposure dose. Some evidence of non-cancer diseases at lower doses has also been obtained, in particular circulatory diseases and cataracts (AGIR, 2010; Ainsbury et al, 2009), but risks of these are not sufficiently well established at low doses to be considered in estimates of low dose radiation risk. A range of tissue damaging effects or deterministic effects can be observed after higher dose radiation exposure (AGIR, 2009; ICRP, 2012). The internationally recognised system of radiological protection (ICRP, 2007) aims to 'manage and control exposures to ionising radiation so that deterministic effects are prevented and the risks of stochastic effects are reduced to the extent reasonably achievable'.

The term 'radiosensitivity' is used to describe many different phenomena. This report is limited to consideration of sensitivity to ionising radiation; some references to ultraviolet radiosensitivity occur but this is not the main focus. In general, the term radiosensitivity should always be used in conjunction with a defined endpoint. The endpoint might be defined clinically, as in the case of normal tissue reactions to radiotherapy or in relationship to specific disease endpoints such as cancer. Alternatively, radiosensitivity can relate to cellular phenomena such as cell killing or the induction of chromosomal damage. Radiosensitivity is also defined by the cell, tissue or organism to which it relates. In particular, there are many endpoints of cellular radiosensitivity described. Classically, cellular radiosensitivity is defined by clonal cell survival assays (see below). Radiosensitivity to cell killing can be assessed in many different assays now (eg apoptosis, trypan blue dye exclusion and resazurin dye tests). There are additionally several measures of chromosomal radiosensitivity and cell cycle checkpoint radiosensitivity. More recently, measures based

on the assessment of nuclear foci of DNA damage response related proteins such as γH2AX, 53BP1 and BRCA1 have been developed. These complement more traditional DNA damage/repair assays such as pulsed field gel electrophoretic assessment of DNA double strand breaks and comet assays. Cellular radiosensitivity is also used in relation to the induction of mutations. Full definitions can become cumbersome – for example, murine T-lymphocyte apoptotic radiosensitivity or human normal skin erythemal radiosensitivity – and so contractions are frequently used. Throughout this report when a specific radiosensitivity is being described a full definition of the endpoint and cell, tissue or organism will be given initially. General terms such as normal tissue radiosensitivity, cellular radiosensitivity or radiosensitive carcinogenesis will be used once the specific radiosensitivity has been adequately described. Box 1.2 summarises the main forms of radiosensitivity recognised and the assays used to assess radiosensitivity. As will become apparent, there is only very fragmentary understanding of the relationships between the various measures of radiosensitivity. For the purposes of this report, it is radiosensitivity as it relates to human health that is of most importance.

Radiosensitivity is a broad term applied to cells, tissues and individuals. The terms 'radiosensitivity' and 'radioresponsiveness' are often used interchangeably. Classically, cellular radiosensitivity is a measure of the degree of response of a cell to radiation, with a large response indicating high sensitivity. In contrast, radioresponsiveness describes the rate of response. Radiosensitivity is characterised commonly by either the dose estimated to produce an average of one event per cell (or its reciprocal as an inactivation constant)

BOX 1.2

Radiosensitivity can be defined at many different levels and through the use of many different assays. A summary of the main forms recognised and assays in common use is given below

Whole organism radiosensitivity is measured by assays such as $LD_{50/30}$, which refers to the radiation dose required to kill 50% of a given population within 30 days of exposure. See Chapter 4.

Normal tissue radiosensitivity or clinical radiosensitivity, generally used in the context of the reaction/damage to non-target tissues as a consequence of radiotherapy for cancer and other conditions. They are assessed by clinical evaluation of tissue damage using a variety of scoring schemes. The main tissues of concern include skin (burning), lung and connective tissue (fibrosis). See Chapter 3.

Susceptibility to radiation carcinogenesis refers to differences in susceptibility amongst individuals (or strains of mice) to radiation-induced cancer in specific tissues. It is measured in epidemiological (human) studies or experimental animal carcinogenesis studies. Generally this is considered in terms of yield of tumours (in a specific tissue) per unit absorbed dose. See Chapter 6.

Tissue radiosensitivity (for cancer) refers to the difference in sensitivity of individual tissues in organisms to radiation-associated carcinogenesis. Most information for this comes from epidemiological studies and is generally considered in terms of yield of tumours (in a specific tissue) per unit absorbed dose. Tissue radiosensitivity can also refer to differences in response to radiotherapy of tissues in terms of their function or structure. See Chapters 2, 3 and 4.

Cellular radiosensitivity refers to a wide range of phenomena measured at the cellular level where responses to radiation can vary between individuals or cell types. Endpoints can be cell killing, chromosomal damage, damage/repair to DNA, cell cycle endpoints, apoptosis, etc. It is measured in yield of the endpoint per unit absorbed dose or parameters derived from dose-response relationships. See Chapter 7.

or by the response to a fixed dose of radiation. Table 1.1 lists the various parameters and Figure 1.1 illustrates some of these. The event is usually taken to refer to the injury resulting in lethality (destruction of reproductive integrity or apoptosis), and hence the term clonogenic radiosensitivity describing the survival of colony forming cells using colonies of 50 cells and above as a minimum size criterion. Other endpoints can be used, such as those describing chromosomal radiosensitivity, and the endpoint should be stated because it could refer, for example, to chromatid aberrations or micronuclei. Different cell types in a single individual vary in radiosensitivity, and there is variation also in the radiosensitivity of a single cell type among different individuals.

Some tissues are radiosensitive because their cell populations have a high propensity to undergo apoptosis, eg salivary glands and some lymphocytes. Some other tissues are more radioresistant and tolerant of radiation because of their structural organisation, eg if a small part of a lung is destroyed by a high dose of radiation, lung function can be maintained by the remaining healthy tissue, but if a small section of spinal cord is damaged it can lead to paralysis. Individuals also vary in radiosensitivity and this can be associated with cellular radiosensitivity and possibly genomic instability. The term 'intrinsic radiosensitivity' has sometimes been used to refer to an individual's genetically determined radiosensitivity – on the understanding that the measured radiosensitivity varies between endpoints and cell types (cellular radiosensitivity) and, in particular, for tissue and individual radiosensitivity, is influenced by confounding variables (eg co-morbid conditions such as some types of collagen vascular disease) and non-genetic modifiers (eg volume irradiated and smoking). However, intrinsic radiosensitivity is a nebulous

TABLE 1.1 Radiation survival curve parameters used to measure radiosensitivity (see also Figure 1.1)

Parameter	Description
α	Inactivation constant for single-hit-type events proportional to dose in a linear-quadratic survival curve ($1/\alpha$ corresponds to ${}_1D_0$ below)
β	Inactivation constant for cumulative-type events proportional to dose-squared in a linear-quadratic survival curve [the tangential slope $1/(\alpha + 2\beta D)$ on the curve at dose D corresponds to D_0 below]
D_{37}	Dose to give 37% survival, ie for a dose giving an average of one event per cell, $e^{-1} = 0.37$ of cells, by chance, have no event and survive (D_{10} or other levels are used occasionally for empirical reasons)
${}_1D_0$	Dose to reduce survival to 37% on the *initial* exponential portion of a multi-target-type survival curve
D_q	'Quasi-threshold' dose on a multi-target survival curve
D_0	Dose to reduce survival to 37% of a value on the *final* exponential portion of the survival curve, called the mean lethal dose
D_{10}	Dose required to give 10% survival. Other subscript values are also used occasionally to indicate different survival levels (may be as percentage or fractional value of 1)
$\overline{D}$	Mean inactivation dose (averaged over all doses in linear coordinates)
SFx	Surviving fraction at x Gy. SF2 is most often used but others, such as SF4, are used occasionally. Measurements of radiosensitivity at a fixed dose do not provide detailed information about dose-response slopes

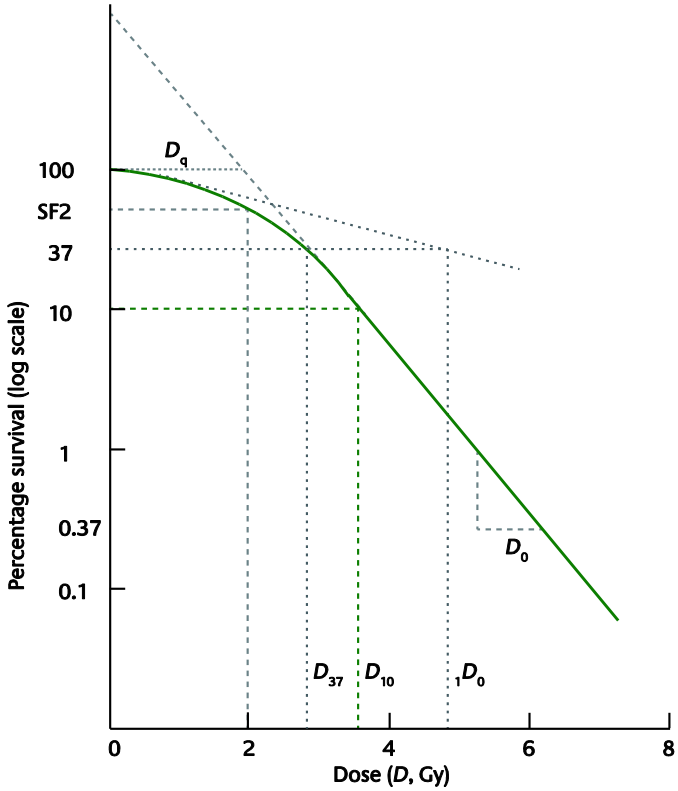


FIGURE 1.1 Clonogenic cell survival curve illustrating some parameters of radiosensitivity, as described in Table 1.1

term and even the term 'genetically determined radiosensitivity' has its limitations. While it may be useful to identify the actual alleles that are present, the expression of these will be modified from the moment of conception to the time of irradiation by all the normal changes that take place during development involving interaction with the outside world (exogenous factors such as lifestyle and environment). In addition, exogenous factors acting post-irradiation will contribute to the final outcome whether they be culture conditions when cells are examined *in vitro*, or lifestyle and environmental factors influencing the response to initial damage and progression towards cancer.

The radiosensitivity of tissues, organs or the whole body can be described by the dose-response curve for specified injuries in a population of individuals. The injuries can be tissue or organ dysfunction levels (morbidity) or mortality after whole-body irradiation. The time of assessment needs to be stated, because there are early reactions as well as late reactions, and for the latter there can be progression and manifestation of injury over many decades. There is often a threshold dose (zero incidence up to that dose, used for protection purposes) or tolerance dose (incidence of about 1% or at most a few per cent, in radiotherapy practices), followed by an increase in incidence with increasing dose to form a sigmoid dose-incidence curve which asymptotes to 100% incidence at high dose. Figure 1.2 provides an example of a

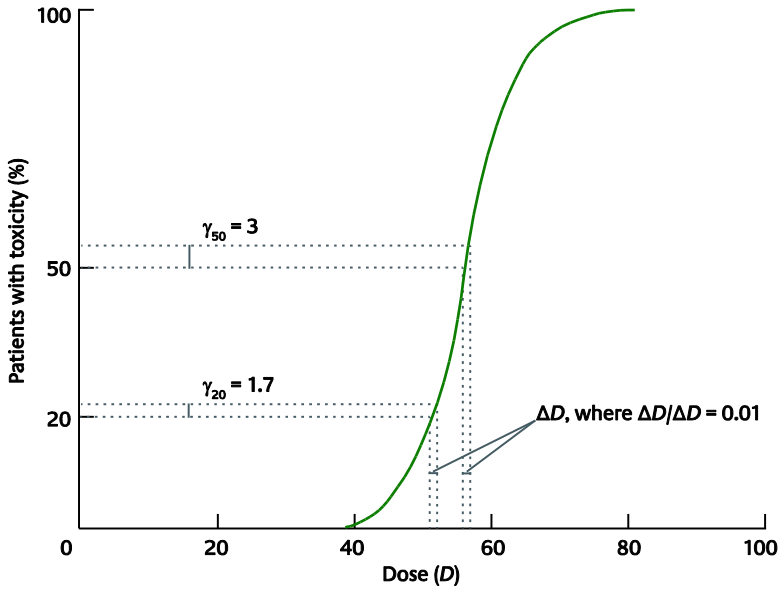


FIGURE 1.2 Example of a dose-incidence curve for radiotherapy-associated normal tissue toxicity

A 1% increase in dose (ΔD) from a reference dose D results in an increase in response equal to γ percentage points. At the steepest part of the dose-response curve, ie the 50% response level, $\gamma_{50} = 3$, which corresponds to a 3% increase in patients experiencing toxicity for a 1% increase in dose, a typical value for late normal tissue effects. The γ-value at the 20% response level (γ_{20}) is 1.7. Redrawn from Barnett et al (2012a), with permission

dose-incidence curve. The steepness of the curves reflects the degree of heterogeneity in response among individuals, with flatter curves indicating greater heterogeneity. This is as a result of variations in dose received, volume irradiated, genetic differences in radiosensitivity and other confounding factors. Of several models used to describe the sigmoid shape, the logistic model is often favoured. This has the form:

$$\text{NTCP} = \frac{1}{1 + \exp\left[4\gamma_{50}\left(1 - {}^{D}/_{D_{50}}\right)\right]}$$

where NTCP is the normal-tissue complication probability after a dose D, D_{50} is the dose resulting in the endpoint in 50% of individuals, and γ_{50} is the (absolute) change in percentage points of incidence for a 1% *relative* dose change at the 50% incidence level. For a variety of endpoints measured in series of radiotherapy patients receiving schedules of 2 Gy daily fractions, values of γ_{50} for different endpoints have ranged from around 1 through to values as high as 5.5 for subcutaneous fibrosis and 6.6 for frozen shoulder in irradiated breast cancer patients (Bentzen, 1994; Bentzen and Overgaard, 1996).

The steepness of the dose-incidence curve (a measure of radiosensitivity) will be highest at the 50% incidence level, and can be described by the slope $1/(\alpha + 2\beta D)$ at a single dose $D = \text{ED}_{50}$ (dose for 50% incidence of a specified effect). If doses are fractionated, the slope is $1/(\alpha + 2\beta d)$ if the number of fractions is kept constant and the dose per fraction (d) is increased to generate the curve, or is $1/(\alpha + \beta d)$ if the dose per fraction is kept constant and the number of fractions is increased to generate the curve.

Another parameter used to characterise the slope is the γ-factor, defined as the absolute change in percentage points of incidence for a 1% relative dose change. For a logistic dose-response curve the γ-value is specified at the 50% response level, γ_{50}, and this is the point where the logistic curve attains its maximum steepness. For a Poisson-based dose-response curve the γ-value is specified at the 37% response level, γ_{37}, and this is the point where the Poisson curve attains its maximum steepness. The maximum slope varies between tissue and organ types, it is dependent on dose fractionation, and it is influenced by heterogeneity in the population. Greater heterogeneity gives a lower threshold or tolerance dose and a lower maximum slope.

Figures 1.3 and 1.4 provide examples of the range of cellular and clinical radiosensitivity, respectively, obtained in human population studies.

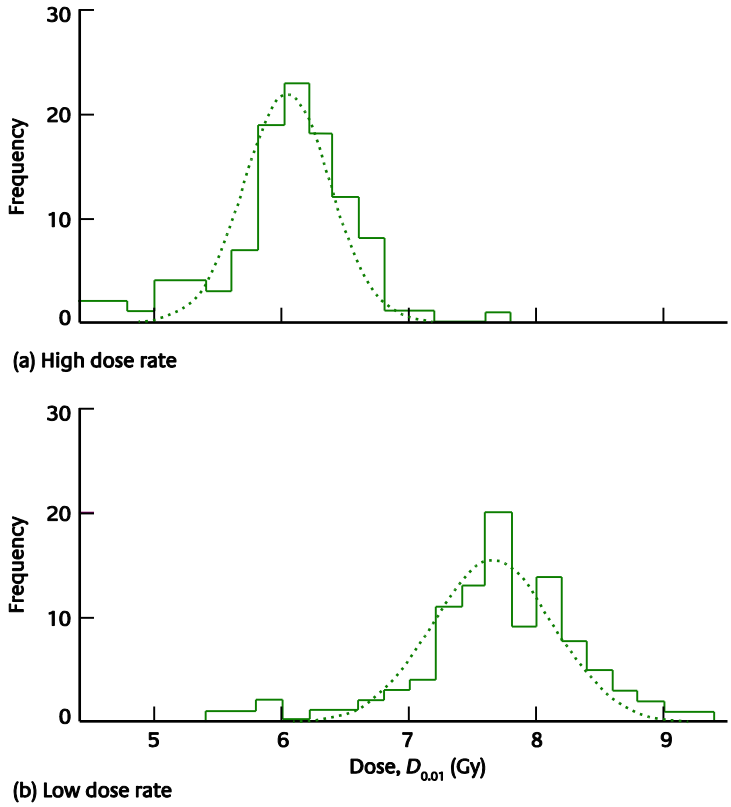


FIGURE 1.3 Range of human fibroblast radiosensitivity at: (a) high dose rate, 1.6 Gy min^{-1} ^{60}Co gamma radiation, and (b) low dose rate, 0.01 Gy min^{-1} ^{60}Co gamma radiation, in 104 skin fibroblast strains from breast cancer patients, 39 with clinical over-reaction to therapy and 65 with normal reaction to therapy

The measure of radiosensitivity, $D_{0.01}$, is the dose required to reduce survival to 1%. The more sensitive cellular responders fall to the left-hand part of each curve. It should be noted that the low dose rate irradiation leads to a broader range of sensitivity and therefore potentially increases the sensitivity of the assay. Redrawn from Peacock et al (2000), with permission

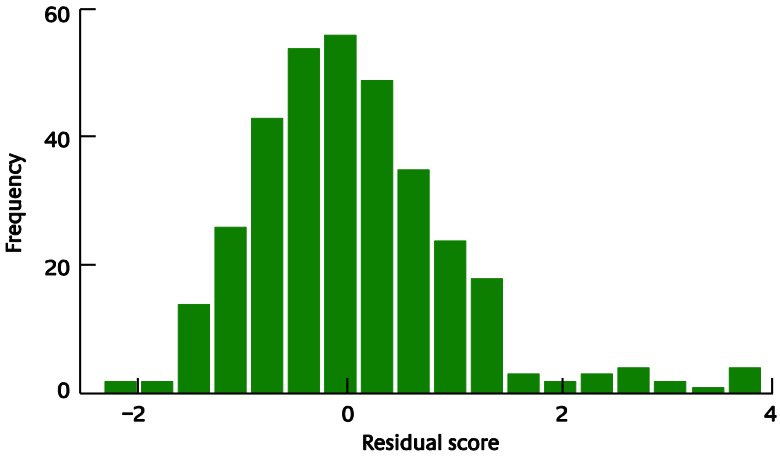


FIGURE 1.4 Example of the range of human clinical radiosensitivity in a sample of 1010 breast cancer patients treated with intensity modulated radiotherapy

The residual score is a measure of clinical radiosensitivity (toxicity) that standardises several clinical indicators into a single score having accounted for patient- and treatment-related factors (Barnett et al, 2012b). A score of 0 indicates the expected level of sensitivity, positive scores indicate higher-than-expected radiosensitivity, while negative scores indicate relative radioresistance. Redrawn from Barnett et al (2012b), with permission

Radiosensitivity with respect to cancer induction is usually expressed as a risk (incidence) per gray or per sievert, assuming no threshold dose. This forms a linear relationship, and there can be a quadratic term as well. Because there is often a baseline natural rate of cancer incidence, risk can be expressed in two different ways. The rate of cancer incidence in an exposed population minus the corresponding cancer rate in an unexposed population is known as the excess absolute risk (EAR). Alternatively, risk may be expressed as relative risk (RR) and excess relative risk (ERR, where ERR = RR − 1), where RR is the ratio of cancer risk in exposed and unexposed populations. High values of EAR, RR or ERR per gray indicate high radiosensitivity for cancer induction. It should be noted that these terms are also used for radiation-induced non-cancer diseases (eg cardiovascular disease) in a radiological protection context. For both cancer and non-cancer diseases, the time of assessment needs to be stated along with the parameter values, because there can be progression and manifestation of injury over long periods of time.

It is recognised that age and sex can affect the risk of radiation-associated cancer but, in general, the system of protection is designed to protect a notional average individual in the population. Nonetheless, several strands of evidence indicate that individuals in the population vary in their sensitivity to radiation and in some cases this can be quite extreme, for example:

a Differences between age and sex groups in epidemiological studies of cancer incidence.

b Cancer-prone genetic conditions observed in the human population such as Gorlin syndrome and Li-Fraumeni syndrome.

c Studies of variation in human cellular radiosensitivity in the normal population.

d The existence of human radiosensitivity syndromes such as ataxia telangiectasia.

e Observations of human variation on severity of normal tissue damage following cancer radiotherapy.

f Animal models displaying variation in acute radiosensitivity and susceptibility to radiation-induced disease.

Many aspects of human radiosensitivity were considered by the AGIR in its 1999 report, *Genetic Heterogeneity in the Population and its Implications for Radiation Risk* (AGIR, 1999). The report concluded: 'There are considerable advances needed, both technical and in the understanding of cancer genetics, before genetic testing for individual hypersensitivity to the carcinogenic action of radiation is feasible'. The report did nonetheless anticipate that the identification of susceptible individuals was likely to be possible in the future and acknowledged that the ability to identify sensitive individuals could raise ethical problems.

Cancer genetics is now much better understood than it was in 1999, although this understanding remains incomplete. Many more advanced, high throughput technologies potentially suitable for identifying radiosensitive individuals are now available and in routine use in research laboratories. Individual genetic profiling and even whole genome sequencing are now much more realistic expectations.

In the light of these advances in understanding and technology, the AGIR established a subgroup in 2010 to review and investigate human radiosensitivity with a somewhat broader remit than that used for the 1999 report. The terms of reference for the Subgroup on Human Radiosensitivity were as follows.

1 **To review the evidence for variation in human radiosensitivity**
This will include both genetic and non-genetic sources of variation. Evidence will likely be drawn from studies of human syndromes, cellular radiosensitivity assays, radiotherapy reaction studies, experimental animal radiosensitivity and cancer susceptibility studies, epidemiological analyses including age- and sex-specific aspects, and studies of interaction between radiation and other agents (eg radon and smoking) or processes such as inflammatory reactions.

2 **To consider the likely impact of this variation on the main late developing health effect of concern, cancer induction at low doses, and medical aspects of radiological protection. Variation in radiosensitivity likely exists of other diseases of possible concern – for example, circulatory diseases**
It is acknowledged that much of the available evidence will be based on relatively high dose studies. However, the Subgroup aims to use this understanding to anticipate effects of variation on situations that arise in practical radiological protection. This will require understanding of the mechanisms that underlie radiosensitivity.

3 **To explore the possible ethical implications for radiological protection of differences in response to radiation within the population in the light of current knowledge**
The current system of protection is based on population-averaged estimates of risk. A greater appreciation of the human variation in sensitivity to adverse health effects is anticipated and ways are likely to be identified to assess the sensitivity of individuals or subpopulations. The implications of these on the radiological protection system will need to be considered not only from a scientific viewpoint but also in terms of ethical implications. It would appear most likely that application of knowledge of variation in radiosensitivity will first come in a clinical setting in, for example, tailoring of radiotherapy.

These issues are reviewed in the chapters that follow. On the basis of the review, a number of conclusions and recommendations are provided (Chapter 9).

1.1 References

AGIR (1999). Genetic Heterogeneity in the Population and its Implications for Radiation Risk. Report of the Advisory Group on Ionising Radiation. *Doc NRPB,* **10**(3), 1–4.

AGIR (2009). High Dose Radiation Effects and Tissue Injury. Report of the independent Advisory Group on Ionising Radiation. *Doc HPA,* RCE-10, 1–94. Available at www.hpa.org.uk

AGIR (2010). Circulatory Disease Risk. Report of the independent Advisory Group on Ionising Radiation. *Doc HPA,* RCE-16, 1–116. Available at www.hpa.org.uk

Ainsbury EA, Bouffler SD, Dörr W, Graw J, Muirhead CR, Edwards AA and Cooper JR (2009). Radiation cataractogenesis: a review of recent studies. *Radiat Res,* **172,** 1–9.

Barnett GC, Coles CE, Elliott RM, Baynes C, Luccarini C, Conroy D, et al (2012a). Independent validation of genes and polymorphisms reported to be associated with radiation toxicity: a prospective analysis study. *Lancet Oncol,* **13**(1), 65–77.

Barnett GC, West CM, Coles CE, Pharoah PD, Talbot CJ, Elliott RM, et al (2012b). Standardized total average toxicity score: a scale- and grade-independent measure of late radiotherapy toxicity to facilitate pooling of data from different studies. *Int J Radiat Oncol Biol Phys,* **82**(3), 1065–74.

ICRP (2007). The 2007 Recommendations of the International Commission on Radiological Protection. ICRP Publication 103. *Ann ICRP,* **37**(2–4).

ICRP (2012). ICRP Statement on Tissue Reactions/Early and Late Effects of Radiation in Normal Tissues and Organs – Threshold Doses for Tissue Reactions in a Radiation Protection Context. ICRP Publication 118. *Ann ICRP,* **41**(1/2).

Peacock J, Ashton A, Bliss J, Bush C, Eady J, Jackson C, et al (2000). Cellular radiosensitivity and complication risk after curative radiotherapy. *Radiother Oncol,* **55,**173–8.

Symonds RP, Wilkinson JS, Dunning AM, Burnet NG and Bentzen SM (2012). Standardized total average toxicity score: a scale- and grade-independent measure of late radiotherapy toxicity to facilitate pooling of data from different studies. *Int J Radiat Oncol Biol Phys,* **82,**1065–74.

Watson SJ, Jones AL, Oatway WB and Hughes JS (2005). Ionising Radiation Exposure of the UK Population: 2005 Review. Chilton, HPA-RPD-001. Available at www.hpa.org.uk

2 Variation in Radiosensitivity from Epidemiological Studies

Epidemiological investigations remain the major source of quantitative information on health risks to humans following radiation exposure. This chapter will consider the approaches taken in radiation epidemiology and the use of its findings in risk estimation, and how these relate to radiosensitivity. Information is available from epidemiological studies of particular groups of people exposed to radiation for a variety of reasons. These will also be considered here. The majority of studies relate to cancers associated with radiation exposure; very few reports on human variation in sensitivity to the acute effects of radiation exposure are available*. The nominal risk coefficients for stochastic health effects (the nominal excess risks of such effects per unit radiation dose received) employed for the purposes of radiological protection are derived from epidemiological studies in order that dose limits and constraints may be set to control the adverse health effects arising from exposures to ionising radiation involving low doses or low dose rates, ie the exposure circumstances mainly of relevance to everyday radiological protection. The health effects associated with low doses or low dose rates of ionising radiation are termed stochastic effects because, in contrast to tissue reactions (also termed deterministic health effects) that are characterised by threshold doses below which the effects do not occur, the probability of the increased occurrence of stochastic effects, but not their severity, is related to the dose (and dose rate) of radiation received.

The present understanding is that there is no threshold dose for stochastic health effects, so that some (small) risk exists even after the receipt of low doses. However, it is very difficult to reliably distinguish the predicted level of effects produced by low level exposure to ionising radiation from statistical fluctuations in the background risk or from relatively small effects produced by biases or confounding factors, so the level of risk actually posed by low doses is inevitably uncertain (although it must be small or it would have

---

* Although most of the information is unavailable in English translation, a review of Russian work on variation in human acute radiosensitivity in English is available (AFRRI, 1996). It is clear that variation in human radiosensitivity is recognised, but there seems to be little that can be used in connection with the present report. A number of studies are cited in which patients have been exposed to whole-body radiation with doses ranging from 15 to 20 roentgen, from which it was concluded that 8–10% were radiosensitive and around 14% were radioresistant. The criteria by which this was judged are not clear; moreover, these were patients and their underlying condition is not clear from the review. Studies on Chernobyl clean-up crew have also apparently indicated differences in radiosensitivity.

Perhaps the most interesting data in the AFRRI report come from the study cited on page 162 of the report:

> "Fifty healthy individuals were exposed to single 15-roentgen (an obsolete unit of radiation dose, 15 roentgen equates to 0.144 Gy in soft tissue) doses for the purpose of excluding those considered unsuitable for work in hazardous radiation conditions. They were considered radiosensitive if the leukocyte count decreased from the initial level by 33% or more. Decreases in the leukocyte count by 33–50% were only noted in 15 individuals who showed a higher radiosensitivity than the other 35 people. No symptoms or other signs of general response manifestation were observed."

While merely anecdotal, the study is mentioned here because it is unique and unlikely to be repeated.

been obvious in low dose studies). Currently, radiation-related stochastic health effects are considered to be cancer in the exposed individual and hereditary disease in the exposed individual's descendants, and the overall risk of stochastic health effects is dominated by the excess risk of future cancer in the exposed individual.

The summary risk coefficients for cancer are expressed usually as either the excess relative risk (ERR) or the excess absolute risk (EAR, see Chapter 1 for definitions) experienced over a lifetime (or some other specified period) as a result of receiving a unit effective dose of radiation. These nominal risk coefficients are derived for the purposes of applying to a world-averaged population of both sexes and all ages to obtain quantities for international application in the context of radiological protection (eg for the derivation of common dose limits and dose constraints that may be applied around the world), and therefore are composed of a range of component risk coefficients that represent a variation in the sensitivity to radiation-induced cancer due to a number of factors. These factors include race, sex, age at exposure, time since exposure and attained age, and the variation in sensitivity to radiation-induced cancer will differ between the site of the cancer, eg between most leukaemias, breast and stomach cancers that are known to be particularly sensitive to induction by radiation, and rectal, uterine and prostate cancers that are known not to be particularly sensitive. Indeed, there are some types of cancer for which there is little evidence of their being sensitive to radiation induction at all, eg chronic lymphocytic leukaemia, Hodgkin's lymphoma and malignant melanoma of the skin. As will be discussed further below, certain other factors can increase the susceptibility of an individual to radiation-induced cancer, an example being the increased risk of radiation-induced lung cancer among cigarette smokers relative to non-smokers: tobacco smoke and ionising radiation interact such that the overall risk of lung cancer is greater than the sum of the risks from tobacco and radiation alone.

2.1 Japanese atomic-bomb survivor data and their implications for radiosensitivity and risk transfer

In the absence of sufficiently detailed knowledge of radiobiology to quantify from fundamental principles the risk of stochastic effects in humans arising from a given radiation dose, radiation risk estimates are obtained from the epidemiological study of exposed groups of people. Many such epidemiological studies have been conducted, but the primary source of information remains the experience of the Japanese survivors of the atomic bombings of Hiroshima and Nagasaki in 1945 (the Life Span Study cohort), and substantial effort has been devoted to studies of these survivors. Nonetheless, studies of the Japanese atomic-bomb survivors have been complemented by many other epidemiological studies, such as of patients exposed to radiation for therapeutic or diagnostic purposes, those exposed in the workplace and people exposed environmentally. Indeed, for some exposure circumstances, such as the risk of lung cancer consequent to the inhalation of radon and its radioactive decay products, the evidence from groups of underground hard-rock miners is the primary source of risk estimates (supported by evidence from those residentially exposed to radon and its decay progeny).

It is of interest to review the process by which the International Commission on Radiological Protection (ICRP) has arrived at the nominal risk coefficients that form the basis of its latest recommendations on radiological protection, since this sheds light on some basic issues involving radiosensitivity (ICRP, 2007).

The ICRP developed radiation-related cancer risk models using data generated by the substantial studies of the Japanese survivors of the atomic bombings of Hiroshima and Nagasaki in 1945 – recently updated cancer incidence data for solid tumours (cancers other than leukaemia, lymphoma and multiple myeloma) and older incidence data for leukaemia (because an update of incidence data for leukaemia, lymphoma and multiple myeloma was not available at the time the models were developed). Separate risk models were derived for leukaemia (excluding chronic lymphocytic leukaemia, CLL, which has a much lower sensitivity to radiation induction than other types of leukaemia), and cancers of the oesophagus, stomach, colon, liver, lung, breast, ovary, bladder and thyroid, and nominal risk coefficients (rather than specific models, because of insufficient data) were assigned to cancers of the bone and skin. Other types of cancer were considered as a single group in a category 'other solid tumours', because the data for specific types of cancer within this grouping were insufficient to permit reliable risk models to be derived.

It is of note that about the same time as the ICRP was developing its cancer risk models, similar exercises were underway by the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR, 2008) and the Seventh Committee on the Biological Effects of Ionizing Radiations (BEIR VII Committee, 2006) in the USA, using a similar database as the ICRP (ie cancer incidence data for solid cancers, but mortality data for leukaemia, from the Life Span Study of the Japanese atomic-bomb survivors). UNSCEAR developed separate risk models for non-melanoma skin cancer and cancers of the bone and brain/central nervous system (CNS), while including cancer of the ovary in the other solid tumours model. The BEIR VII Committee derived separate risk models for cancers of the prostate and uterus, while including cancers of the oesophagus, bone and skin in its other solid tumours risk model. Even though these expert panels employed essentially the same database (derived from the experience of the Japanese atomic-bomb survivors), they arrived at somewhat different cancer risk model structures, which illustrates one aspect of uncertainty in cancer risk modelling.

The cancer risk models developed by these committees were expressed in terms of either the ERR or the EAR. For sufficiently sophisticated models, whether the radiation-related excess risk is expressed as the ERR or the EAR in the risk model should make very little difference to the derivation, under particular circumstances, of the excess risk experienced by the population that generated the data from which the risk model was developed. However, whether the model is defined in terms of the ERR or the EAR, it is of importance to take into account when a model that has been developed from data relating to the exposure of one specific population, say part of the Japanese population at the end of the Second World War, is applied to other populations, say a Western workforce. Why this is so may be appreciated from the consideration of two types of cancer: stomach and breast. In the Japanese population the background incidence of stomach cancer is notably greater than in a Western population, and the situation is reversed for breast cancer.

An ERR model for stomach cancer derived from a Japanese population if applied to a Western population would apply the same proportional increase in risk to a substantially lower background incidence rate of stomach cancer, leading to a smaller EAR than in the Japanese population. In contrast, if the EAR model for stomach cancer derived from the Japanese population was applied to a Western population then a much larger EAR would be obtained than that using the ERR model. This principle is illustrated in Figure 2.1(a), where the disease under consideration is stomach cancer, 'population 1' is a Japanese population and 'population 2' is a Western population.

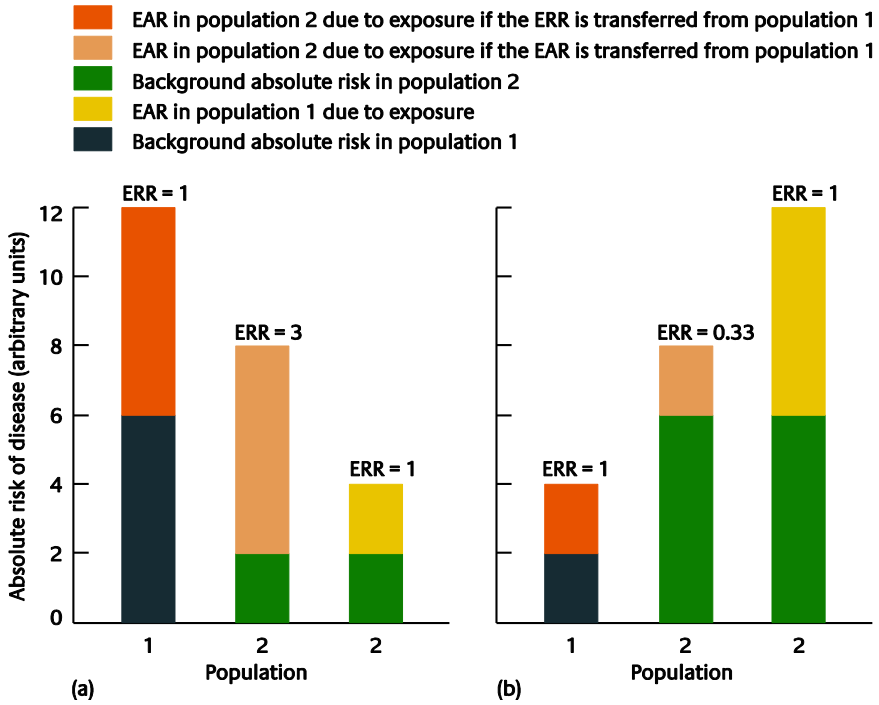


FIGURE 2.1 Transfer of radiation-related risk between populations 1 and 2 with different background absolute risks of a disease, where the background incidence is: (a) greater and (b) smaller in population 1 than in population 2

The reverse situation occurs for breast cancer: in transferring the excess risk from the Japanese population to a Western population, using the ERR model gives a much larger EAR than that obtained by transferring the excess risk using the EAR model, because the background breast cancer incidence rate in Japan is much lower than that in the West. This is illustrated in Figure 2.1(b), where the disease in question is breast cancer, 'population 1' is a Japanese population and 'population 2' is a Western population.

Consideration of the nature of the transfer of radiation-related risk between populations illustrates a particular aspect of radiosensitivity that is apparent when the background risk of specific types of cancer varies non-trivially between populations. Under these circumstances, the magnitude of the excess risk of a particular type of cancer arising from exposure to radiation will depend on how the background risk of that cancer influences the effect of radiation (if at all). If the transfer of the EAR is assumed to be appropriate, then for stomach cancer the EAR above background is the same for the Japanese and Western populations, but the consequent ERR (ie the proportional increase over background) for the Western population is much greater given the lower background absolute risk (Figure 2.1(a)). For breast cancer the situation is reversed (Figure 2.1(b)), in that the same EAR is now a smaller proportion of the background risk in a Western population, producing a smaller ERR. Transfer of the EAR assumes the absolute level of additional risk is the same in different populations, regardless of the background absolute risk, but that the proportional increase relative to the background risk differs. In this instance of the

transfer of the EAR, when it is assumed that the radiation-related risk in the exposed population from which the risk is derived is additive to the background absolute risk, the radiation-related EAR in equivalent individuals in different populations (ie individuals having the same major risk-modifying factors, such as age and sex) is the same, and it is only the size of the EAR in comparison to the background absolute risk that differs (ie the ERR – see Figure 2.1).

However, the transfer of the ERR between populations having a different background risk of a particular cancer implies that radiation interacts with those risk factors that produce this difference in background incidence so that, for a higher background risk in the population to which the ERR model is being applied, the radiation-related EAR is greater than that in the population from which the risk model was derived. The implication of the ERR transfer is that radiation is interacting multiplicatively with the background risk factors, and since these background risk factors are unlikely to be distributed uniformly within a population, the EAR that is experienced by an individual as a consequence of exposure to radiation is dependent upon the levels of background risk factors relevant to that individual. To illustrate by way of an example, if the transfer of the EAR of lung cancer between populations provides an accurate reflection of the radiation-related excess risk of lung cancer in the population to which the EAR is transferred, then it does not matter whether an individual smokes cigarettes or not, since the radiation-related risk is independent of the background risk and therefore the risk from radiation exposure does not interact with the risk from exposure to tobacco smoke. On the other hand, if the transfer of the ERR reflects reality, then the radiation-related excess risk very much depends on whether an individual smokes or not since the radiation-related risk is proportional to the background risk of lung cancer, which is dominated by the risk from tobacco smoke – under these circumstances, the risk from radiation interacts multiplicatively with the risk from cigarette smoke and the overall risk of lung cancer is greater than the sum of the two independent risks (radiation and tobacco).

So, the effect of radiation upon the risk of cancer in different populations (with different background incidence rates) is indicative of the influence of background factors upon the radiation-related risk and therefore of the sensitivity of an individual to radiation-induced cancer (since individuals will be exposed to different levels of background risk factors in any particular population). However, this will only be the case if the transfer of radiation-related risk from one population to another is more than additive (ie some interaction with background factors exists) and there is a meaningful difference between background rates of a particular type of cancer. Ideally, a comparison needs to be made between the risk coefficients (excess risks per unit radiation dose) for a specific type of cancer in one population with that in another population having a substantially different background rate of incidence – a similar ERR or EAR will indicate which mode of risk transfer is likely to be the more correct (although the actual transfer may be somewhere between the two). Unfortunately, the opportunities to carry out such a comparison exercise are not great, although they have been conducted for some of the commoner types of cancer: Preston et al (2002a) examined breast cancer in eight groups, Ron et al (1995) studied thyroid cancer in five groups, and Little et al (1999) considered leukaemia in three major exposed groups.

Recently, both the BEIR VII Committee and the ICRP have made judgements about the likely nature of the transfer of risks between populations for various types of cancer, based on the available evidence – UNSCEAR did not conduct such an exercise. The BEIR VII Committee concluded that for most types of cancer a transfer of risk between the Japanese population (that group exposed to radiation from the

atomic bomb explosions in 1945) to the US population is best described by a mixture of 70% ERR and 30% EAR. The exceptions are thyroid cancer (for which a pure ERR model was derived), breast cancer (for which a pure EAR model was derived) and lung cancer (for which a mixture of 30% ERR and 70% EAR was employed, reflecting the importance of different smoking patterns in the two populations). An interesting comparison may be made with the conclusions of previous BEIR committees: in 1980, the BEIR III Committee had concluded that EAR transport of risk between Japan and the USA was appropriate for all types of cancer, whereas, in 1990, the BEIR V Committee had concluded that the ERR transport of risk between the two populations was appropriate for all types of cancer (BEIR III Committee, 1980; BEIR V Committee, 1990). This divergence illustrates the difficulties of drawing reliable inferences from rather limited epidemiological data in the absence of sound biological evidence.

For its latest (2007) recommendations, the ICRP has inferred the nature of the transport of risks between populations. The ICRP concluded that insufficient information existed for most types of cancer to assume anything other than a 50 : 50 mixture of the transfer of the ERR and EAR between populations. The exceptions are breast and leukaemia (for which pure EAR models are used), thyroid and skin (for which pure ERR models are used) and lung (for which a 30% ERR and 70% EAR model mixture was employed). The differences between the conclusions of the ICRP and the BEIR VII Committee illustrate that, even using recent sources of evidence, there remain substantial uncertainties in the understanding of the nature of the transport of radiation-related risk between populations having different background levels of risk. Nonetheless, both the BEIR VII Committee and the ICRP judge that, for most types of cancer, a mixture of ERR and EAR models is appropriate for the transfer of radiation-related excess risk between populations with different levels of background risks.

2.1.1 Implications of risk transfer models for human radiosensitivity

What does emerge from these reviews of the transfer of cancer risk between populations is that, with the possible exception of breast cancer, the excess cancer risks per unit dose of radiation received are dependent, to a greater or lesser extent, upon the background risk of cancer. The implication for individual radiosensitivity is that, to a greater or lesser extent, the excess risk experienced by an individual following a given exposure to radiation is dependent upon the level of (at least some of) the background cancer risk factors associated with the individual. For example, the radiation-related excess risk of lung cancer will be dependent upon the degree to which the individual inhales tobacco smoke, the primary risk factor for lung cancer. The interaction of cigarette smoke and radiation in the context of lung cancer is an example of an interaction with an exogenous risk factor, which will vary substantially within a population. This will generally be the case with other major known exogenous risk factors, such as alcohol consumption or chemical exposures, in relation to certain types of cancer. Therefore, under the assumption of a supra-additive combination of radiation-related risk and risk from other exposures of relevance to a certain type of cancer, the individual risk arising from a given exposure to radiation will depend upon the level of background risk from exposures to other substances.

These considerations will apply not only to exogenous risk factors, but also to endogenous risk factors. Should the difference in the background incidence rate of a particular cancer between two populations be due, in a non-trivial way, to inherent racial differences between the populations (rather than being

entirely due to environmental factors) then the cancer risk from an exposure to radiation will depend upon the genetic make-up of the individual as influenced by their race. Additionally, it is most likely that there will be genetic variations within a particular race that will further influence, to some extent, the risk resulting from a unit dose of radiation. Indeed, it is known that certain rare genetic conditions (eg Gorlin syndrome) markedly increase the risk per unit dose, and that other genetic conditions (eg the retinoblastoma *Rb* gene) also increase the risk of radiation-induced cancer, but to a lesser extent.

So, the inference that, with the possible exception of breast cancer, the transfer of radiation-related excess cancer risk between populations has at least a component of ERR (multiplicative) transfer, and that the radiation-related risk is more than purely additive to the background cancer risk, implies that the effect of radiation upon the risk experienced by an individual is not independent of the level of their background risk factors. Given the international variation in the incidence rates of site-specific cancers (see Parkin, 2004), the expectation is that the radiation-related EAR of cancer resulting from a particular dose of radiation will also vary significantly for both populations and individuals. This would appear to be the inevitable consequence of the conclusion that the transfer of radiation-related excess cancer risk between populations is likely to possess at least some component of ERR (multiplicative) transfer. Just how much variation of radiation-induced EAR of cancer per unit dose there is between populations and individuals will depend upon the strength of the ERR component of transfer compared to the EAR component of transfer, the difference in background risk between populations and the variation of background risk factors within a population. However, it will be seen that implicit within the inference of expert panels of a transfer of radiation-related excess risk between populations that is greater than additive to the background risk is the assumption that there will be, to a greater or lesser extent depending on the particular circumstances, a variation in individual sensitivity to radiation-induced cancer.

Those cancer risk models that have been developed by various expert bodies provide an indication of the major risk-modifying factors that generate heterogeneity of radiation-related risk within an exposed population. Within a given population, the radiation-related risk of a particular type of cancer will, in general, depend on the sex and age of the exposed individual and the time since exposure, with the risk experienced by females and those at a young age at exposure being greater, overall, than for the general population. Indeed, the cancer risk coefficients derived by the ICRP for the purposes of its 2007 recommendations are higher for the general population than for the working population because the working population does not include children, who have higher cancer risk coefficients because of their young age at exposure.

2.2 Analysis of risk in breast cancer patients and the possibility of sensitive subgroups

The atomic-bomb survivors show female breast cancer as a late effect of exposure and the dose-related excess is highest, relative to population rates, in those exposed before 20 years of age (Land et al, 1993). These findings were generally confirmed in a cohort-based study, except that the estimated relative risk (RR) at 1 Sv was especially high for early onset breast cancer (defined by diagnosis before the age of 35 years). Early-onset cases occurred almost exclusively among women exposed before the age of

20 years. In that group the RR at 1 Sv was 14.6 (95% CI 4.5–108) compared with 3.0 (95% CI 2.2–4.2) in cases diagnosed at older ages (Land et al, 1993). The authors speculated that there might be a small, genetically susceptible subgroup with a large excess of radiation-induced breast cancer. Alternatively, there might be a subgroup with increased sensitivity to (solely) early-onset radiation-induced breast cancer. These results were extended and discussed more fully in a later paper (Land et al, 2003). The 'early-onset' phenomenon was not observed among women exposed as adolescents to multiple chest fluoroscopies in Massachusetts (Little and Boice, 1999), but something similar was seen among female Hodgkin's disease survivors in two Dutch cancer centres (van Leeuwen et al, 2000). In this study the RR among women treated at 20 years of age and younger was 61.5 (95% CI 25–127) for diagnosis before the age of 40 years, compared to an RR of 5.4 (95% CI 0.7–19.5) for diagnosis in the age range 40–49 years. The authors discussed whether the effect was because the early-onset cases showed a high RR due to exposure at more sensitive ages or because they were observed for risk at younger ages. On their isotonic regression approach, the early-onset contrast describes virtually all of the variability of ERR by attained age. Preston et al (2007), using a further 13 years of breast cancer ascertainment in the atomic-bomb survivor Life Span Study cohort, found that the magnitude and statistical significance of the early-onset effect was highly dependent upon the nature of the baseline risk model and the estimated baseline rates for young women. When baseline rates for women under 35 years of age were allowed to be lower than the predictions of the simple model, there appeared to be an early-onset effect. In particular, the excess risk for radiation-associated breast cancer prior to the age of 35 years was estimated to be about 4.5 times greater ($p = 0.01$) than predicted by the standard ERR model and 3.5 times higher ($p = 0.08$) than in the EAR model. It remains unclear whether there is a breast-cancer-sensitive subgroup among the Japanese atomic-bomb survivors.

2.3 Meningioma families

Most meningiomas are sporadic and genetic conditions with high penetrance genes account for only a small fraction of cases. Familial aggregation is rare and usually associated with type-2 neurofibromatosis. However, there are aggregations in families without neurofibromatosis, suggesting that additional unknown genes have a role in predisposing to meningioma. In a Swedish study of 1845 cases, 19 had parents with meningiomas and 10 of these had siblings with meningiomas (Hemminki and Li, 2003). Radiation has been shown unequivocally to be a causal factor in the aetiology of the disease (Bondy and Ligon, 1996; Longstreth et al, 1993; Modan et al, 1974; Preston et al, 2002b; Sadetzki et al, 2005). Much of the information has come from the follow-up of patients who had received radiation treatment for tinea capitis in the 1950s and who proved to have a significantly increased risk for meningiomas (Ron et al, 1988; Sadetzki et al, 2005). A subsequent study examined families which included both irradiated and unirradiated members. All meningiomas in these families were in irradiated individuals (Flint-Richter and Sadetzki, 2007) and significant familial aggregation of cases was observed. However, this was only just significant at the 0.04 level. Since the participants in these studies were initially selected by having a fungal infection of the scalp and the number of irradiated individuals developing meningioma approached 1% – the relative risk being 9.5 (CI 3.5–25.7) – the predisposing genes must be present in a significant proportion of the population.

2.4 Lifestyle and environmental factors

2.4.1 Tobacco smoking and radiosensitivity

Probably the best example of population heterogeneity for radiation-induced cancer comes from three studies of pooled data on lung cancer and domestic radon exposure (American, European and Chinese). Both the American and European studies indicate that the rate of radon-induced lung cancer is linearly related to dose, with no threshold below which the risk is zero. Both also show that the *relative* risks are similar for tobacco smokers and non-smokers. On the basis of the European pooling study, the AGIR (2009) has estimated the cumulative *absolute* risks of lung cancer to the age of 75 years due to residential radon exposure (Table 2.1). From these figures it can be seen that the *additional* absolute cumulative risk to age 75 years arising from radon exposure is more than 30 times greater in smokers than in non-smokers (Table 2.2).

This is a hugely significant finding for two reasons. First, it means that a very large fraction of the population responds differently to internal alpha-particle (high LET*) irradiation to the lung from radon and its decay products. Second, this difference is not primarily genetic in origin, but 'environmental' in the sense that it is the result of a lifestyle choice.

The mechanism of the effect is unclear. The number of energy loss events must be similar in the two subpopulations so the difference in cancer incidence must be attributable to subsequent processes, either involving DNA repair or cell cycle and other precancerous responses. In-house work at the HPA Centre for Radiation, Chemical and Environmental Hazards on cultured cells exposed chronically to various chemical genotoxins before irradiation has provided no evidence for any interaction greater than additive (Nuta et al, 2009). If this also applies to the chemicals to which the lung is subjected in smokers, it might suggest that the effect of smoking occurs after the establishment of damage in the chromosome. Such processes might be loosely termed 'promotional'. It may be significant that the lungs of smokers are in a continual state of chronic inflammation.

A more complex interaction between radiation and smoking has been found in the Japanese atomic-bomb survivors (Furukawa, 2010). Light/moderate smokers developed more radiation-induced lung cancer than non/never-smokers, but this was not apparent above consumption of a pack (ie 20 cigarettes) per day where there was little indication of any radiation-associated excess risk. The authors suggest that there may be a certain pool of people who are genetically susceptible to lung cancer and that high levels of smoking have saturated that pool so that there is little room for an additional radiation effect. Direct comparison with radon exposure is not easy, since the atomic-bomb survivors' cancer incidence is dominated by acute whole-body exposure to an external source of penetrating low LET radiation, as compared with chronic exposure of (predominantly) the lung at specific sites to high LET radiation in the case of radon exposure.

* LET is an abbreviation of linear energy transfer, one of the basic physical characteristics of ionising radiations. It is defined as the amount of energy deposited per unit length of a radiation track traversal, and measured in electronvolts per micrometre. Low LET radiations include X-rays and gamma radiation; high LET radiations include alpha particles.

TABLE 2.1 Cumulative absolute risk of death from lung cancer to age 75 years in the UK by smoking history and long-term average residential radon concentration (AGIR, 2009)

Long-term average radon concentration (Bq m^{-3})	Cumulative risk of death from lung cancer to age 75 years (%) [b,c]			
	Lifelong non-smoker	Ex-cigarette smoker: stopped at age 30	Ex-cigarette smoker: stopped at age 50	Continuing cigarette smoker
0	0.41	1.57	5.5	14.7
21 [a]	0.42	1.62	5.7	15.2
100	0.47	1.8	6.4	16.9
200	0.53	2.1	7.2	19.0
400	0.66	2.6	8.9	23.0
800	0.92	3.5	12.2	30.5

Notes

(a) Mean UK long-term average residential radon concentration.

(b) Radon risk estimated directly from the European pooling study (Darby et al, 2005, 2006, and AGIR, 2009: Appendix I). Lung cancer death rate in male lifelong non-smokers from the American Cancer Society CPS-II (Thun et al, 2006). Lung cancer risks in continuing and ex-cigarette smokers from males in the UK case–control study of lung cancer (Peto et al, 2000) and the UK 2006 national lung cancer death rate (Cancer Research UK, 2008).

(c) Cumulative risks ignore the risk of deaths from other causes. If, for one particular category, the lung cancer rates per 100,000 people in all the five-year age groups before age 75 years add up to c, then the cumulative risk by age 75 is $1 - \exp(-5c/100{,}000)$. Thus, cumulative risks depend only on age-specific lung cancer rates and not on competing causes of death.

TABLE 2.2 Additional cumulative absolute risk of radon-induced lung cancer per 100,000 people (to age 75 years)

Long-term average radon exposure (Bq m^{-3})	Non-smokers *A*	Continuing smokers *B*	*B/A*
100	0.06	2.2	36.7
200	0.12	4.3	35.8
400	0.25	8.3	33.2
800	0.51	15.8	31.6

Smoking is known to be associated with cancers in organs other than the lung and this raises the question as to whether smokers might also be more sensitive to the carcinogenic action of penetrating low LET radiation in these other organs. However, the available studies are unlikely to have the power to address this.

Smoking is recognised as a risk factor for increased normal tissue toxicity in radiotherapy patients (see Section 3.1.5). In peripheral blood lymphocytes of 441 healthy subjects, Wang et al (2000) found that irradiation induced more aberrations in the G2 assay in smokers than in non-smokers, an effect more noticeable in men than in women.

2.4.2 Hepatitis patients

Besides variations in the factors that influence inherent sensitivity to radiation-induced cancer that affect everyone in the population (eg race, sex and age), the specific circumstances experienced by particular individuals will also affect their radiation-related risk. The importance of the influence of tobacco smoke has been discussed above, particularly (but not only) with respect to lung cancer risk, but certain other risk factors have been identified by epidemiological studies. For example, radiation exposure and hepatitis B and C virus infections would appear to interact in the overall risk of liver cancer (Ohishi et al, 2011), although the exact nature of this interaction has yet to be determined.

2.5 Summary

Nominal risk coefficients for cancer are expressed usually as the population average of either the excess relative risk (ERR) or the excess absolute risk (EAR) experienced over a lifetime (or some other specified period) as a result of receiving a unit effective dose of radiation. This population-averaged risk is composed of components of the population (eg sex and age at exposure) that are known to experience different radiation-induced excess risks.

The implication of some component of ERR transfer between populations is that radiation is, to some extent, interacting with the background of (non-radiation) risk factors, and since these background risk factors are unlikely to be distributed uniformly within a population, the EAR that is experienced by an individual as a consequence of exposure to radiation is dependent upon the levels of background risk factors relevant to that individual.

Given the international variation in the incidence rates of site-specific cancers, the expectation is that the radiation-related EAR of cancer resulting from a particular dose of radiation will also vary significantly for both populations and individuals.

For its latest (2007) recommendations, the ICRP has concluded that (with the exception of leukaemia, and breast, lung, thyroid and skin cancers) insufficient information exists for most types of cancer to assume anything other than a 50 : 50 mixture of the transfer of the ERR and EAR between populations.

This implies that, with the exception of breast cancer (and possibly leukaemia), for which a pure EAR transfer is assumed, the excess cancer risks per unit dose of radiation received are dependent, to a greater or lesser extent, upon the background risk of cancer. The implication for individual radiosensitivity is that, to a greater or lesser extent, the excess risk experienced by an individual following a given exposure to radiation is dependent upon the level of (at least some of) the background cancer risk factors (both genetic and non-genetic) associated with the individual. It should be noted, however, that there is good evidence for genetic factors affecting breast cancer risk in humans.

The existence of a sensitive subpopulation for the induction of breast cancer among the atomic-bomb survivors has been proposed but remains controversial.

There is genetic predisposition to radiation-induced meningioma and possibly tumours at other sites (see also Chapter 7) in some members of the general population. The size of this radiosensitive fraction is currently unknown.

Smokers are more sensitive to radiation-induced lung cancer than non-smokers; for radon alpha-particle irradiation the factor may be more than 30-fold. The effect in smokers is important evidence that lifestyle and environmental factors may be important in determining susceptibility to radiation-induced cancer. The possibility that the existing data allow the identification of an excess of smoking-related tumours at sites other than the lung among those induced by radiation deserves to be further explored.

2.6 References

AGIR (2009). Radon and Public Health. Report of the independent Advisory Group on Ionising Radiation. *Doc HPA,* RCE-11, 1–240. Available at www.hpa.org.uk

AFRRI (Armed Forces Radiobiological Research Institute) (1996). Estimation of Radiation Risk Based on the Concept of Individual Variability in Radiosensitivity. AFRRI Contract Report 96-1, June 1996. Bethesda MD, USA.

BEIR III Committee (Committee on the Biological Effects of Ionizing Radiations) (1980). The Effects on Populations of Exposure to Low Levels of Ionizing Radiation. US National Academy of Sciences, National Research Council. Washington DC, National Academy Press.

BEIR V Committee (Committee on the Biological Effects of Ionizing Radiations) (1990). Health Effects of Exposure of Low Levels of Ionizing Radiation. US National Academy of Sciences, National Research Council. Washington DC, National Academy Press.

BEIR VII Committee (Committee on the Biological Effects of Ionizing Radiations) (2006). Health Risks from Exposure to Low Levels of Ionizing Radiation. US National Academy of Sciences, National Research Council. Washington DC, National Academy Press.

Bondy M and Ligon BL (1996). Epidemiology and aetiology of intracranial meningiomas:a review. *J Neurooncol,* **29,** 197–205.

Cancer Research UK (2008). Cancer statistics. Available at http://info.cancerresearch.uk/org/cancerstats/2008. Accessed September 2008.

Darby S, Hill D, Auvinen A, Barros-Dios JM, Baysson H, Bochicchio F, et al (2005). Radon in homes and risk of lung cancer: collaborative analysis of individual data from 13 European case–control studies. *BMJ,* **330,** 223–7.

Darby S, Hill D, Auvinen A, Barros-Dios JM, Baysson H, Bochicchio F, et al (2006). Residential radon and lung cancer: detailed results of a collaborative analysis of individual data on 7,148 subjects with lung cancer and 14,208 subjects without lung cancer from 13 epidemiological studies in Europe. *Scand J Work Environ Health,* **32**(Suppl 1), 1–83.

Flint-Richter P and Sadetzki S (2007). Genetic predisposition for the development of radiation-associated meningioma: an epidemiological study. *Lancet Oncol,* **8,** 403–10.

Furukawa K, Preston DL, Lönn S, Funamoto S, Yonehara S, Matsuo T, et al (2010). Radiation and smoking effects on lung cancer incidence among atomic bomb survivors. *Radiat Res,* **174,** 72–82.

Hemminki K and Li X (2003). Familial risks in nervous system tumours. *Cancer Epidemiol Biomarkers Prev,* **12**(11 Part 1), 1137–42.

ICRP (2007). The 2007 Recommendations of the International Commission on Radiological Protection. ICRP Publication 103. *Ann ICRP,* **37**(2–4).

Land CE, Tokunaga M, Tokuoka S and Nakamura N (1993). Early-onset breast cancer in A-bomb survivors. *Lancet,* **342,** 237.

Land CE, Tokunaga M, Koyama K, Soda M, Preston DL, Nishimori I and Tokuoka S (2003). Incidence of female breast cancer among atomic bomb survivors, Hiroshima and Nagasaki, 1950–1990. *Radiat Res,* **160,** 707–17.

Little MP and Boice JD (1999). Comparison of breast cancer incidence in the Massachusetts tuberculosis fluoroscopy cohort and in the Japanese atomic bomb survivors. *Radiat Res,* **151,** 218–24.

Little MP, Weiss HA, Boice JD Jr, Darby SC, Day NE and Muirhead CR (1999). Risks of leukemia in Japanese atomic bomb survivors, in women treated for cervical cancer, and in patients treated for ankylosing spondylitis. *Radiat Res,* **152**(3), 280–92.

Longstreth WT, Dennis LK, McGuire VM, Drangsholt MT and Koepsell TD (1993). Epidemiology of intracranial meningioma. *Cancer Epidemiol Biomarkers Prev,* **72,** 639–48.

Modan B, Baidatz D, Mari H, Steinitz R and Levin SG (1974). Radiation-induced head and neck tumours. *Lancet Oncol,* **1,** 277–9.

Nuta O, Bouffler S, Lloyd D, Sepai O and Rothkamm K (2009). Impact of long term exposure to chemical agents on exposure to ionising radiation. In *Proceedings 37th Annual Meeting of the European Radiation Research Society,* pp 63–65.

Ohishi W, Fujiwara S, Cologne JB, Suzuki G, Akahoshi M, Nishi N, et al (2011). Impact of radiation and hepatitis virus infection on risk of hepatocellular carcinoma. *Hepatology,* **53**(4),1237–45.

Parkin DM (2004). International variation. *Oncogene,* **23**(38), 6329–40. Review.

Peto R, Darby S, Deo H, Silcocks P, Whitley E and Doll R (2000). Smoking, smoking cessation, and lung cancer in the UK since 1950: combination of national statistics with two case–control studies. *BMJ,* **321,** 323–9.

Preston DL, Mattsson A, Holmberg E, Shore R, Hildreth NG and Boice JD Jr (2002a). Radiation effects on breast cancer risk: a pooled analysis of eight cohorts. *Radiat Res,* **158**(2), 220–35. Erratum: *Radiat Res,* 2002, **158**(5), 666.

Preston DL, Ron E, Yonehara S, Kobuke T, Fujii H, Kishikawa M, et al (2002b). Tumours of the nervous system and pituitary gland associated with atomic bomb radiation exposure. *J Natl Cancer Inst,* **94,** 1555–63.

Preston DL, Ron E, Tokuoka S, Nishi N, Soda M, Mabuchi K and Kodama K (2007). Solid cancer incidence in atomic bomb survivors: 1958–1998. *Radiat Res,* **168,** 1–64.

Ron E, Modan B, Boice JD, Alfandary E, Stovall M, Chetrit A and Katz L (1988). Tumours of the brain and nervous system after radiotherapy in childhood. *N Engl J Med,* **319,** 1033–9.

Ron E, Lubin JH, Shore RE, Mabuchi K, Modan B, Pottern LM, et al (1995). Thyroid cancer after exposure to external radiation: a pooled analysis of seven studies. *Radiat Res,* **141**(3), 259–77.

Sadetzki S, Chetrit A, Freedman L, Stovali M, Modan B and Novokov I (2005). Long-term follow-up for brain tumour development following childhood exposure to ionizing radiation for tinea capitis. *Radiat Res,* **163,** 424–32.

Thun MJ, Henley SJ, Burns D, Jemal A, Shanks TG and Calle EE (2006). Lung cancer death rates in lifelong nonsmokers. *J Natl Cancer Inst,* **98**(10), 691–9.

UNSCEAR (United Nations Scientific Committee on the Effects of Atomic Radiation) (2008). Sources and Effects of Ionizing Radiation. Report to the General Assembly with Scientific Annexes. New York, United Nations.

van Leeuwen FE, Klokman WJ, Veer MB, Hagenbeek A, Krol AD, Vetter UA, et al (2000). Long-term risk of second malignancy in survivors of Hodgkin's disease treated during adolescence or young adulthood. *J Clin Oncol,* **18,** 487–97.

Wang LE, Bondy ML, de Andrade M, Strom SS, Wang X, Sigurdson A, et al (2000). Gender difference in smoking effect on chromosome sensitivity to gamma radiation in a healthy population. *Radiat Res,* **154,** 20–27.

3 Clinical Radiosensitivity

Clinical radiosensitivity is defined here as an individual's radiosensitivity measured as the level of toxicity following radiotherapy. There is evidence for variation in clinical radiosensitivity (see Figure1.4). Several studies have shown that inter-patient variability in toxicity (ie variability in toxicity between patients) is greater than intra-patient variability (ie variability in toxicity in an individual patient) and some have suggested that, after accounting for known risk factors, the genetic component could be as high as 80% (Safwat et al, 2002; Turesson et al, 1996). Radiotherapy toxicity is operationally referred to as acute (within six months of starting radiotherapy) or late (after six months of starting radiotherapy), with a second cancer induction considered a very late effect. Clinical radiosensitivity, therefore, represents a spectrum of effects manifest from weeks (acute radiotherapy toxicity) to years (late radiotherapy toxicity and cancer induction). There are numerous types of radiotherapy toxicity, which depend on the site irradiated (West and Barnett, 2011). The underlying molecular and pathological bases are tissue and, therefore, site dependent. Unfortunately, recording toxicity is not routine and there are multiple scoring systems with different grading scores. Summarising data on determinants of clinical radiosensitivity identified from studies of radiotherapy patients is, therefore, hampered by the heterogeneity of recording toxicity. It is also limited by the lack of systematic recording of toxicity not only within clinical practice but also within trials. There is increasing adoption of the Common Toxicity Criteria for Adverse Event (CTCAE) system as the tool to use for recording toxicity. There is also more routine recording of toxicity in some centres and within clinical trials. Recent and future data will, therefore, provide increasingly more useful information.

This chapter summarises the available data on clinical radiosensitivity in terms of determinants of a cancer patient's risk of developing toxicity following radiotherapy. Unfortunately, there is a paucity of data on normal tissue reactions in children, but second cancer induction is particularly relevant for young patients. Cancer patients receiving similar radiotherapy schedules show a range of toxicity varying from minor to severe and in duration from weeks to a lifetime (Bentzen, 2006). Very rare extreme toxicity results in death and is usually associated with an undiagnosed radiosensitive genetic condition. The first documented illustration of variation in toxicity following radiotherapy was reported by Holthusen (1936). The main recognised determinants of clinical radiosensitivity defined as radiotherapy toxicity relate to physics (radiation dose, dose rate, dosimetry, dose inhomogeneity and treatment volume), treatment (interaction with other modalities such as surgery, chemotherapy and hormone therapy), patient factors (age, haemoglobin, smoking and co-morbid conditions such as diabetes and vascular disease) and genetics. The influence of dose is obvious and well reported, particularly in recent studies involving large numbers of patients. Interpretation of the literature should, therefore, focus on recent papers where multivariate analyses have allowed for differences in prescribed dose.

The evidence for individual variation in radiosensitivity and that genetics/patient-related factors can dominate the risk of radiotherapy toxicity led to the development of studies aimed at measuring

radiosensitivity to predict a cancer patient's toxicity. This chapter, therefore, also summarises research aimed at trying to measure a patient's radiosensitivity to predict their risk of radiotherapy toxicity. Radiotherapy is also known to carry a risk of second cancers developing within the treatment area, a factor becoming more important as survival of cancer therapy is improving (see Box 3.1).

3.1 Factors explored as potentially influencing radiotherapy toxicity

3.1.1 Sex

Borgmann et al (2002) carried out a systematic review of sex-specific differences in outcome following cancer therapy. Examples cited in their review along with a search of findings from the more recent literature are given in Table 3.1. The findings are mixed. Unfortunately monitoring and reporting of differences between sexes has rarely been done in a systematic manner and the data are equivocal. Also, the largest studies tend to be on breast and prostate cancers, which are effectively sex specific. It is difficult to draw any firm conclusions. There is a need to improve the recording of toxicity in existing studies to further clarify whether there are differences in clinical radiosensitivity between men and women.

Postulated mechanisms underlying the influence of a patient's sex in determining the risk of radiotherapy toxicity include sex hormone differences, lower female average body mass index, high female average body fat content, and smaller female average organ blood flow (Borgmann et al, 2002). Comparison of human radiosensitivity tends to show more radiation-induced DNA and chromosomal damage in lymphocytes from females (Borgmann et al, 2002).

BOX 3.1

Second cancer risk

Radiotherapy is known to cause a small increased risk of second cancer within the treatment area. The risk of second radiotherapy-induced cancer has been shown to be inversely associated with age and increases with time from diagnosis (Berrington de Gonzalez et al, 2011; Bhatti et al, 2010).

The current evidence from childhood and adult cancer survivors suggests that increasing radiotherapy dose is associated with an increased risk of second cancers (Berrington de Gonzalez et al, 2011; Taylor et al, 2010; Tukenova et al, 2011). However, a meta-analysis of the risk of second cancers after radiotherapy among childhood cancer cases suggests considerable heterogeneity between studies (Doi et al, 2011), probably representing differences in treatment delivery, eg radiotherapy margins used (Dasu et al, 2011). The use of external beam radiotherapy for endometrial carcinoma is associated with a greater risk than use of brachytherapy alone (Lönn et al, 2010).

Studies of possible associations between genetic factors and the development of second malignancies have so far been inconclusive (Hosking et al, 2011), although women who carry rare deleterious ataxia-telangiectasia mutated (ATM) missense variants and who are treated with radiation may have an elevated risk of developing contralateral breast cancer (Bernstein et al, 2010). There is evidence that female gender increases the risk of second primary thyroid cancer after radiotherapy for a childhood cancer (Bhatti et al, 2010). A shorter latent time to develop a radiation-associated meningioma after treatment for childhood cancer has been associated with male gender, initial tumour type, radiotherapy volume and increased radiotherapy dose (Paulino et al, 2009).

In the field of pharmacogenomics, there is some evidence for women suffering greater toxicity than men following chemotherapy, with differences in pharmacokinetics and pharmacodynamics postulated as the underlying mechanism. More recent studies are increasingly likely to involve multi-modality therapy and, although recording of toxicity is increasing, sex-specific differences related solely to radiotherapy might be hard to elucidate.

3.1.2 Age

There is conflicting evidence for an effect of age on the prevalence and severity of radiation toxicity (Bentzen and Overgaard, 1991, 1994; Collette et al, 2008; Gagliardi et al, 2000; Kahan et al, 2007; Lilla et al, 2007; Peuckmann et al, 2009; Taylor et al, 1995; Turesson et al, 1996). Older age in adults is generally considered to be associated with an increased risk of toxicity (Table 3.2) due to decreased normal tissue tolerance. Progressive reduction of functional reserve due to depletion of tissue stem cells might enhance the damage of normal tissues and the risk of complications. Similarly, it has been hypothesised that reductions in blood flow, and age-related accumulation of mutations and a subsequent decline in DNA repair capacity (Lilla et al, 2007), may also contribute to reduced normal tissue tolerance with increased age. Patient age may not affect endpoints that do not directly depend on the physiological reserve capacity before irradiation (Bentzen, 2006). Older patients may be less likely to report breast pain, and this is in keeping with other reported studies (Barnett et al, 2011c; Peuckmann et al, 2009; Poleshuck et al, 2006).

3.1.3 Ethnicity

There are limited data on the effect of ethnicity on radiotherapy toxicity (Table 3.3). There is some evidence for an effect but it is not currently possible to draw any firm conclusions. Given genetic variation differs between ethnicities it is likely that some toxicity endpoints will be more severe in some groups due to specific mutations being present or more prevalent.

3.1.4 Body mass index and diet

Increased body mass index (BMI) correlates strongly with breast volume. In breast cancer patients increasing breast volume or BMI has been associated with increased acute and late toxicity (Table 3.4). However, a recent study of patients treated with chemoradiotherapy for locally advanced carcinoma of the cervix showed increased late toxicity in patients with low BMI. In addition, women with low BMI (below 22 kg m^{-2}) experienced greater acute toxicity during pre-operative chemoradiotherapy for rectal cancer ($p = 0.001$), although no significant effect of BMI was seen in male patients (Wolff et al, 2011). Dietary counselling or protein supplements may decrease acute toxicity (Table 3.4). An elemental diet is a chemically defined, nutritionally complete, liquid oral diet whose constituent nutrients have been broken down to their simplest form to enable passive absorption. Elemental diets have been proposed as a means of reducing damage to the small bowel during radiotherapy, although only limited compliance has been demonstrated (McGough et al, 2006).

3.1.5 Smoking and alcohol

There is clear evidence that smoking increases clinical radiosensitivity in pelvic, breast and head and neck cancer (Table 3.5). The evidence is less conclusive for lung cancers where several studies have shown a decreased prevalence of radiation pneumonitis in smokers. The largest study, which included 3489 cervical cancer patients treated with radiation, showed a dose-response relationship for increasing numbers of packs per day versus major small bowel complications (Eifel et al, 2002). Alcohol consumption has been considered to increase acute and late radiotherapy toxicity (Zimmermann et al, 1998). Smoking and alcohol use are risk factors for the development of osteo-radionecrosis after radiotherapy for head and neck cancer (O'Dell and Sinha, 2011) and have been correlated with the prevalence of oral candidiasis during radiotherapy (Epstein et al, 1993). However, *in vitro* studies have suggested that beer has a radioprotective effect (Monobe and Ando, 2002; Monobe et al, 2003). A study of 348 patients who underwent radiotherapy for breast cancer showed decreased acute skin toxicity in patients who reported regular wine consumption prior to starting treatment (Morganti et al, 2009).

3.1.6 Non-malignant systemic disease

Systemic diseases, such as diabetes mellitus, collagen vascular disease, hypertension and inflammatory bowel disease, are generally considered to be associated with a poor tolerance to radiotherapy. A review of the available literature was published in 2002 and reported that nine studies evaluated the effects of hypertension and diabetes on radiation tolerance, with all showing higher rates of late toxicities than in control groups (Chon and Loeffler, 2002). More recent literature is listed in Table 3.6.

There is good evidence that diabetes increases clinical radiosensitivity. Diabetics have increased blood viscosity and poor blood circulation and can have hypertension. Chronic diabetes is associated with microvascular occlusion, capillary hyalinisation, arteriolar obliteration, atheroschlerosis and tissue hypoxia (Chon and Loeffler, 2002). Chronic hypertension is associated with medial hypertrophy with luminal narrowing in the arterioles (Chon and Loeffler, 2002). Consistent with this suggestion, the use of anticoagulants/anti-aggregants was shown to protect against lower gastrointestinal toxicity in prostate patients (1124 cases, OR 0.65, $p = 0.04$) (Valdagni et al, 2008) and the use of anti-hypertensives reduced toxicity (718 cases, OR 0.31, $p = 0.05$, MVA) (Fellin et al, 2009). Two studies (Table 3.6) have shown a decreased prevalence of some toxicities in patients with hypertension. It is possible that these results may have arisen due to the effect of anti-hypertensive medication used by these patients. The use of anti-hypertensive medication has previously been associated with decreased acute diarrhoea (Vavassori et al, 2007), possibly mediated through a vascular mechanism (Liu et al, 2004). Statins may also have been commonly prescribed to patients in this group (see Section 3.1.7).

Collagen vascular disease (CVD) (eg rheumatoid arthritis, systemic lupus erythematosus, polymyositis and systemic sclerosis) is characterised by altered immunoregulation and inflammation. Chon and Loeffler (2002) identified 300 reported cases examining the potential risks associated with radiotherapy in patients with CVD. The largest study (Morris and Powell, 1997) reported no increased risk of acute or late toxicity in patients with rheumatoid arthritis. However, patients with other CVDs had an increased risk of late toxicity ($p = 0.0002$). A more recent study of 73 patients with CVD *versus* 222 matched controls (on sex, race, irradiated site, radiotherapy dose and age) showed that the CVD patients had an increased risk of any late toxicity (29.1% *vs* 14.0%, $p = 0.0010$) and a trend towards increased severe late toxicity

(9.3% *vs* 3.7%, p = 0.079) (Lin et al, 2008). A single-institution study showed the risk of toxicity was associated with connective tissue disorder severity (Gold et al, 2008).

There is evidence to suggest that patients with inflammatory bowel disease experience increased toxicity after radiotherapy to the abdomen or pelvis (Table 3.6).

3.1.7 Additional treatment (surgery, chemotherapy and endocrine therapy)

There is evidence that prior surgery increases radiotherapy toxicity (Table 3.7). Most of the studies in Table 3.7 compared toxicity in patients who underwent radiotherapy alone *versus* radiotherapy following surgery. In breast cancer, all patients undergo surgery followed by post-operative radiotherapy. The combination of surgery and radiotherapy results in increased late toxicity for several cancer sites.

The use of radiotherapy in combination with chemotherapy in lung, gynaecological, rectal and head and neck cancer is now commonplace. The use of such combination treatment results in improved overall survival, but at the expense of increased acute and late toxicity. Many randomised controlled trials of chemoradiotherapy *versus* radiotherapy alone have shown increased acute, but not late, toxicity with the addition of chemotherapy. However, there is concern that these phase III clinical trials have not revealed the true prevalence of late toxicity due to under-reporting, as subsequent non-randomised studies of chemoradiotherapy use in the clinic have shown enhanced late adverse effects (Bentzen et al, 2007).

A recent review of breast cancer patients showed increased acute toxicity when chemotherapy was given concurrently with radiotherapy (Bowden et al, 2006). Back et al (2004) found that chemotherapy, given either concurrently or sequentially, was associated with increased acute toxicity. Previous studies have shown a poorer cosmetic outcome and increased late toxicity when chemotherapy is given, and this appears to be more pronounced when chemotherapy is administered concurrently with radiotherapy (Bowden et al, 2006; Collette et al, 2008).

There is also evidence that the use of androgen-deprivation therapy prior to radical radiotherapy in prostate cancer patients decreases acute toxicity due to a reduction in the size of the prostate and seminal vesicles. Small studies have shown an increased prevalence of radiological pulmonary fibrosis in breast cancer patients receiving concurrent tamoxifen and post-mastectomy radiotherapy compared to those who underwent post-mastectomy radiation alone (Bentzen et al, 1996; Koc et al, 2002), although these findings were not confirmed in another study (Wennberg et al, 2002). Dorr et al (2005) found that the use of tamoxifen increased the prevalence of early pneumopathy detected on chest X-rays; on multivariate analysis only this pneumopathy at 15 weeks predicted the development of subsequent pulmonary fibrosis. A retrospective study of patients receiving radiotherapy to the conserved breast suggested an increase in breast fibrosis in patients receiving concurrent tamoxifen compared to those treated with sequential radiotherapy and tamoxifen (Wazer et al, 1992). However, two retrospective studies did not find a difference between the prevalence of late radiotherapy toxicity to the breast with either concurrent or sequential use of tamoxifen (Fowble et al, 1996; Harris et al, 2005). It has been reported that scheduling of hormonal therapy may affect the prevalence of toxicity.

There is emerging evidence that HMG-CoA reductase inhibitors (statins) used to reduce serum cholesterol protect against the development of radiotherapy toxicity (Fritz et al, 2011). Pravastatin exerts persistent

anti-inflammatory and anti-thrombotic effects on irradiated endothelial cells (Gaugler et al, 2005) and has a therapeutic effect on radiation-induced skin lesions in mice (Holler et al, 2009).

3.1.8 Infection

Post-operative infection was associated with an increased risk of telangiectasia in 1014 patients enrolled in the Cambridge Breast IMRT Trial (OR 3.39, 95% CI 1.94–5.91, $p < 0.0005$, MVA) (Barnett et al, 2011c). There are limited case reports suggesting an increased toxicity in HIV-positive patients (Smith et al, 1997).

3.1.9 Genetics

There is evidence that genetics influences clinical radiosensitivity. Syndromes associated with clinical radiosensitivity include ataxia telangiectasia (Taylor et al, 1975), LIG4 syndrome (Girard et al, 2004) and Nijmegen breakage syndrome (Little and Nove, 1990) involving *ATM*, *LIG4* and *NBN*, respectively (see Chapter 6). Other syndromes have been investigated but the results are equivocal. Such radiosensitivity syndromes illustrate that specific genes influence clinical radiosensitivity. These syndromes, characterised by Mendelian inheritance of germline mutations in genes involved in the detection of DNA damage or DNA repair (the DNA damage response, DDR), result in genomic instability and cancer predisposition. These syndromes are rare and probably of little relevance when assessing radiosensitivity in most cancer patients undergoing radiotherapy.

Heritability is defined as the proportion of phenotypic variance in a population attributable to additive genetic factors. Heritability of a disease is usually demonstrated by linkage studies involving family members. However, evidence of the heritability of radiotherapy toxicity is hard to obtain due to the difficulty in obtaining prospective toxicity data in cancer survivors and their offspring. There is therefore very limited literature on the heritability of radiation toxicity. Scott (2004) used a chromosomal damage assay to investigate the radiosensitivity of first-degree relatives of 16 sensitive and eight 'normal' breast cancer survivors. The author found that 62% of first-degree relatives of sensitive patients were also radiosensitive compared with 7% of first-degree relatives of 'normal' patients (Roberts et al, 1999). Unfortunately, this assay did not transfer well between laboratories, but recent studies of *in vitro* cellular radiosensitivity have suggested estimates of heritability of between 60 and 80% (Curwen et al, 2005, 2010; Finnon et al, 2008; Roberts et al, 1999; Schmitz et al, 2007; Wu et al, 2006).

3.1.10 Summary

No conclusions can be drawn on whether sex, ethnicity, body mass index, diet or alcohol consumption influence clinical radiosensitivity.

Studies in radiotherapy patients show that increasing age in adults, smoking, diabetes and collagen vascular disease tend to increase clinical radiosensitivity.

Genetic variation influences clinical radiosensitivity.

With increased standardisation and collection of toxicity data, future studies should be more informative.

Text continues on page 54

TABLE 3.1 Studies comparing radiotherapy toxicity in men and women

Source	Cancer	*n*	Toxicity	Toxicity system	Finding	Effect size (95% CI)	p
Ramaekers et al, 2011	HNC	396	Xerostomia	EuroQol-5D converted to RTOG score	↑ in men	Regression coefficient 0.052; SE 0.019	0.006 (MVA)
Siala et al, 2011	Nasopharynx	239	Hypothyroidism	Biochemical measurement	↑ in women		
Wolff et al, 2011	Rectal	196	Acute Haematological	CTCAEv3.0/LENT	↑ in women with low BMI ↑ in women	Not given	0.001 0.04
Dehing-Oberije et al, 2010	Lung	469	Acute dysphagia	CTCAEv3.0	↑ in women	OR 1.65 (1.12–2.43)	0.011 (MVA)
Roeder et al, 2010	Lung	242	Pneumonitis	Symptoms and radiography	No difference		
Palazzi et al, 2008	HNC	149	Pain	CTCAEv3.0	↑ in women	Not given	0.02
Bhandare et al, 2007	HNC	325	Ototoxicity	Otolaryngology and audiology records	No difference		0.80 (UVA) 0.90 (MVA)
Kong et al, 2006	Lung	109	Fibrosis	RTOG/SWOG/CTCAE	↑ in women	UVA HR 4.91 (1.8–13.7)	0.0024 (UVA) ns (MVA)
Metzger et al, 2006	Lymphoma	461	Hypothyroidism	Biochemical measurement	↑ in women	UVA HR 1.6 (1.2–2.1) MVA HR 1.4 (1.5–4.3)	0.002 (UVA) 0.03 (MVA)
Pieters et al, 2006	Various*	53	Neurological	Retrospective LENT	↑ in men	Not given	0.017 (MVA)
Tsujino et al, 2003	Lung	71	Pneumonitis	CTCv2	No difference		
Hernando et al, 2001	Lung	201	Pneumonitis	CTC	No difference		
Robnett et al, 2000	Lung	144	Pneumonitis	Modified RTOG	↑ in women	MVA OR 5.1	0.01 (MVA)

Source	Cancer	*n*	Toxicity	Toxicity system	Finding	Effect size (95% CI)	p
van der Voet et al, 1998	Glottis	383	Any late	Own scale	No difference		
Ho et al, 1999	Nasopharynx	294	Hearing loss	Pure tone audiogram	No difference		
Kwong et al, 1996	HNC	132	Hearing loss	Pure tone, impedance audiograms	↑ in men	Not given	0.013 (UVA) 0.018 (MVA)
Denham et al, 1995	Various	110	Erythema	Reflectance spectrophotometry	↑ in women		0.03 (UVA)
Mak et al, 1994	Rectum and rectosigmoid	224	Small bowel obstruction	Clinical diagnosis	No difference		

Abbreviations: CTCAE = common toxicity criteria for adverse events; HNC = head and neck cancer; HR = hazard ratio; LENT = late effects in normal tissues; MVA = multivariate analysis; ns = not significant; QoL = quality of life; RTOG = Radiation Therapy Oncology Group; SE = standard error; SWOG = South West Oncology Group; UVA = univariate analysis.

* Cauda equina irradiated.

TABLE 3.2 Effect of age on radiotherapy toxicity

Source	Cancer	*n*	Toxicity	Toxicity system	Finding	Effect size (95% CI)	p
Barnett et al, 2011c	Breast	1014	Telangiectasia Oedema Pain	Scale used in the START trial	↑ with age ↑ with age ↓ with age	OR 1.32 (0.97–1.79) OR 1.44 (1.18–1.79) OR 0.81 (0.70– 0.94)	0.076 <0.0005 (MVA) 0.007 (MVA)
Barnett et al, 2011a	Prostate	788	Proctitis	LENT/SOM	↑ with age	HR 1.06 (1.00–1.12)	0.04 (MVA)
Dehing-Oberije et al, 2010	Lung	469	Acute dysphagia	CTCAEv3.0	↓ with age	OR 0.97 (0.95–0.99)	0.003 (MVA)
Jereczek-Fossa et al, 2010	Prostate	973	Acute rectal Late rectal	RTOG	↓ with age No association	OR 0.66 (0.42–1.02)	0.02 (UVA) 0.06 (MVA)
Roeder et al, 2010	Lung	242	Pneumonitis	Symptoms and radiography	No association		
Dehing-Oberije et al, 2009	Lung	438	Dyspnea	CTCAEv3.0	↑ with age	OR 1.03 (1.002–1.005)	0.035 (MVA)
Peuckmann et al, 2009	Breast	2000	Chronic pain	Questionnaire	↓ with age	OR 0.48 (0.30–0.76)	0.0011 (MVA)
Huscher et al, 2009	Gynae	806	Late bowel	Need for hospitalisation or surgery	↑ with age	6% *vs* 3% > or ≤60 year RR per year 1.02	0.015 (UVA) 0.013 (MVA)
Taira et al, 2009	Prostate	226	Erectile dysfunction	IIEF-6	↑ with age	Potency preservation 74% (≤59 years), 48% (60–69), 33% (≥70)	0.001 (UVA)
Salama et al, 2008	HNC	95	Swallowing	Swallowing scale	Trend for ↑ with age	OR 1.04 (0.99–1.09)	0.08 (UVA)
Collette et al, 2008	Breast	5178	Fibrosis	None, minimal, moderate, severe	No association		
Palazzi et al, 2008	HNC	149	Acute dermatitis Acute weight loss	CTCAEv3.0	↓ with age ↑ with age	Not specified	0.03 (MVA)
Kahan et al, 2007	Breast	119	Pneumonitis Fibrosis	CTCAEv2.0	↑ with age ↑ with age	OR 1.05 (1.01–1.09) OR 1.06 (1.02–1.10)	0.015 (UVA) 0.008 (UVA)

Source	Cancer	*n*	Toxicity	Toxicity system	Finding	Effect size (95% CI)	p
Bhandare et al, 2007	HNC	325	Hearing loss	Otolaryngology and audiology records	↑ with age	Not specified	0.018 (UVA) 0.005 (MVA)
Lilla et al, 2007	Breast	416	Telangiectasia	RTOG/EORTC and LENT/SOMA	↑ with age	OR 2.11 (1.11–4.03) for age >70 *vs* ≤60 years	0.001 (MVA)
Mayahara et al, 2007	Prostate	287	Acute GI and GU	CTCAEv2.0	No association		
Merrick et al, 2007	Prostate	161	Late rectal function	R-FAS	No association		
Metzger et al, 2006	Lymphoma	461	Hypothyroidism	Biochemical measurement	No association	UVA HR 1.1 (0.8–1.5)	0.54 (UVA)
Feigenberg et al, 2005	Prostate	1204	Late toxicity	Modified LENT/SOMA	No association		
Dorr et al, 2005	Breast	451	Early pneumonapthy Late fibrosis	RTOG/EORTC and LENT/SOMA CT assessment	↑ with age No association	38% *vs* 22% for > or <58 years	0.0002 (UVA 0.013 (MVA)
Koper et al, 2004	Prostate	199	Rectal bleeding	Questionnaires	No association		
Tsujino et al, 2003	Lung	71	Pneumonitis	CTCv2	No association		
Jereczek-Fossa et al, 2003	Endometrium	317	Acute bowel	RTOG/EORTC	↑ with age	1.049 (1.006–1.094)	0.026 (UVA) 0.016 (MVA)
Cozzarini et al, 2003	Prostate	154	Rectal bleeding	Modified RTOG	No association		
Koc et al, 2002	Breast	111	Pulmonary fibrosis	Evidence of fibrosis on CT scan	↓ with age >50 years	MVA OR 0.64 (0.39–0.26)	0.007 (UVA) 0.01 (MVA)
Hernando et al, 2001	Lung	201	Pneumonitis	CTC	No association		

TABLE 3.2 *Continued*

Source	Cancer	*n*	Toxicity	Toxicity system	Finding	Effect size (95% CI)	p
Skwarchuk et al, 2000	Prostate	743	Late Gr 2/3 rectal bleeding	RTOG/EORTC	↑ with age	Regression coefficient 0.13 ±0.05*	0.02 (UVA)
Gagliardi et al, 2000	Breast	68	Pneumonitis	Retrospective, clinical and radiological assessment	↑ with age	Not specified	0.048 (UVA) 0.12 (MVA)
Ho et al, 1999	Nasopharynx	294	Hearing loss within 3 months	Pure tone audiogram	↑ with age	Not specified	<0.01 (UVA)
van der Voet et al, 1998	Glottis	383	Any late	Own scale	No association		
Kwong et al, 1996	HNC	132	Hearing loss	Pure tone and impedance audiograms	↑ with age	Not specified	0.0001 (UVA) 0.0001 (MVA)
Turesson et al, 1996	Breast	402	Erythema, acute reaction, telangiectasia	Peak reflectance erythema, photographic	No association		
Taylor et al, 1995	Breast	458	Poor cosmesis	Patient and physician questionnaires	↑ with age	Not specified	0.001 (UVA) 0.007 (MVA)
Fine et al, 1995	Cervix	189	Severe late toxicity	Own scale	No association		0.58 (MVA)
Mak et al, 1994	Rectum and rectosigmoid	224	Small bowel obstruction	Clinical diagnosis	No association		
Bentzen and Overgaard, 1993	Breast	163	Reduced shoulder movement	Movement relative to contralateral arm	No association		
Olsen et al, 1993	Breast	161	Brachial plexopathy	Neurological signs and symptoms	↓ with age	29% of patients age <47 years developed mild or disabling RBP, 11% age ≥47	0.04 (UVA)

Source	Cancer	*n*	Toxicity	Toxicity system	Finding	Effect size (95% CI)	p
Bentzen et al, 1989	Breast	163	Reduced shoulder movement	Movement relative to contralateral arm	↑ with age	Not specified	0.005 (MVA)
Koga et al, 1988	Lung	62	Pneumonitis	X-ray appearance	↑ with age	27/33 pneumonitis age <70 years, 27/29 age ≥70	ns

Abbreviations: CTCAE = common toxicity criteria for adverse events; GI = gastrointestinal; GU = genitourinary; HNC = head and neck cancer; HR = hazard ratio; Gynea = gynaecological cancer; IIEF-6 = International Index of Erectile Function-6; LENT = late effects in normal tissues; MVA = multivariate analysis; ns = not significant; RBP = radiation-induced brachial plexopathy; RR = risk ratio; RTOG = Radiation Therapy Oncology Group; UVA = univariate analysis.

TABLE 3.3 Effect of ethnicity on radiotherapy toxicity

Source	Cancer	*n*	Toxicity	Toxicity system	Finding	Effect size (95% CI)	p
Krasin et al, 2009	Sarcoma	76	Acute dermatitis	CTCv2	↑ in whites *vs* blacks	Not specified	<0.01
Ryan et al, 2007	Multiple sites	411	Mean skin problem score	Nationwide Symptom Inventory	No association		0.15
			Proportion reporting skin problems		↑ in blacks *vs* whites	56% black *vs* 23% white patients experienced severe reactions	0.001
Metzger et al, 2006	Lymphoma	461	Hypothyroidism	Biochemical measurement	↑ in whites *vs* blacks	UVA HR 2.8 (1.6–4.7) MVA HR 2.5 (1.5–4.3)	<0.001 (UVA) <0.001 (MVA)
Eifel et al, 2002	Cervix	3489	Bladder Rectal Small bowel Any	Major late complications	↑ in blacks ↑ in blacks ↓ in Hispanics ↓ in Hispanics	MVA HR 1.89 (1.24–2.89) MVA HR 1.73 (1.12–2.68) MVA HR 0.27 (0.12–0.57) MVA HR 0.74 (0.55–1.00)	0.003 (MVA) 0.01 (MVA) 0.001 (MVA) 0.05 (MVA)
Taylor et al, 1995	Breast	458	Poor cosmesis	Patient and physician questionnaires	↓in blacks	Not specified	0.0034 (UVA) 0.002 (MVA)

Abbreviations: CTCAE = common toxicity criteria for adverse events; HR = hazard ratio; MVA = multivariate analysis; UVA = univariate analysis.

TABLE 3.4 Effect of increased body mass index (BMI) and dietary intervention

Source	Cancer	*n*	Toxicity	Toxicity system	Finding	Effect size (95% CI)	p
BMI							
Kizer et al, 2011	Cervix	404	Gr 3/4 enteritis Gr 3/4 fistula GI obstruction Lymphoedema	CTCAEv4.0	↓ toxicity for >24.9 *vs* <18.5 *vs* >24.9 kg m^{-2}	13.6% *vs* 16.7 8.8% *vs* 11.1% 4.4% *vs* 33.3% 1.2% *vs* 5.6%	0.03 0.05 <0.001 0.02
Barnett et al, 2011c	Breast	1014	Acute and late	RTOG (acute) START trial scale (late) Photographic assessment of breast shrinkage	↑ toxicity with ↑ BMI and ↑ breast volume	MVA shrinkage OR per litre ↑ in volume =1.98 (1.41–2.78) Telangiectasia OR 3.94 (2.49–6.24) Oedema OR 3.65 (2.54–5.24) Pigmentation OR 1.75 (1.21–2.51)	MVA shrinkage, telangiectasia, oedema p < 0.0005 Pigmentation p = 0.003
Wedlake et al, 2010	Pelvis	193	Acute and 1 year	Modified Bowel Disease Questionnaire-Bowel subset	↓ acute toxicity	BMI <18.5 (*n* = 6) had worst toxicity during treatment	
Patil et al, 2009	Prostate	407	Acute toxicity	RTOG	No association		
Lilla et al, 2007	Breast	416	Telangiectasia/ fibrosis	RTOG/EORTC and LENT/SOMA	No association	OR 1.41 (0.76–2.64)	ns (MVA)
Werner et al, 1991	Breast	282	Arm oedema prevalence at 5 years	Difference of ≥ 2.5 cm in arm circumference between ipsilateral and contralateral arms	↑ toxicity	12.5% for BMI ≤27.2 27.4% for BMI >27.2	p = 0.002 (UVA) p < 0.0005 (MVA)
Diet							
Ravasco et al, 2005	Colorectal	111	Acute toxicity QoL	EORTC/RTOG and EORTC QoL Questionnaire v3.0	↓ toxicity ↑ QoL with diet counselling or protein supplements	Not stated	<0.05 (UVA) 0.02 (UVA)

Abbreviations: CTCAE = common toxicity criteria for adverse events; GI = gastrointestinal; LENT = late effects in normal tissues; MVA = multivariate analysis; ns = not significant; QoL = Quality of Life; UVA = univariate analysis.

TABLE 3.5 Effect of smoking (current/long-term) and alcohol consumption on radiotherapy toxicity

Source	Cancer	*n*	Toxicity	Toxicity system	Finding	Effect size (95% CI)	p
Smoking							
Defraene et al, 2012	Prostate	512	Rectal bleeding	Bleeding requiring laser treatment or transfusion	No association		0.16 (MVA)
Jenkins and Welsh, 2011	Lung	146	CT scan changes	SWOG	↓ toxicity	Not specified	0.02 (UVA) 0.15 (MVA)
Barnett et al, 2011b	Breast	1503	Overall toxicity (STAT)	START LENT/SOMA EORTC BR23	↑ toxicity	Regression coefficient 0.21* (0.12–0.30)	<0.0005 (MVA)
Chen et al, 2011	Oropharynx/ oral cavity	202	≥Gr 3 late toxicity	RTOG/EORTC	↑ toxicity	49% in active smokers *vs* 31% in former smokers	0.01
Barnett et al, 2011c	Breast	1014	Pigmentation	LENT/SOMA	↑ toxicity	MVA OR 2.06 (1.22–3.49)	0.007 (MVA)
Wedlake et al, 2010	Pelvis	193	Acute and at 1 year	Modified Bowel Disease Questionnaire – bowel subset	↑ toxicity	Current smokers had lowest presenting mean IBDQ-B score (63.7), suffered a fall during treatment (−12.0) and failed to recover at 1 year (4.3-point difference between baseline and 1 year)	Not specified
Roeder et al, 2010	Lung	242	Pneumonitis	Symptoms and radiographic finding	No association		
Dehing-Oberije et al, 2009	Lung	438	Dyspnea	CTCAEv3.0	↓ toxicity	OR 0.64 (0.39–1.04)	0.07 (MVA)
Purkey et al, 2009	HNC	52	Aspiration pneumonia	Clinical diagnosis	↑ toxicity	OR 1.04 per pack-year (1.01–1.07)	0.011 (MVA)
Zevallos et al, 2009	HNC	86	ORN	Hospitalisation	↑ toxicity	RR 1.46 (1.05–2.02) hospitalisation RR 1.32 (1.09–1.6) ORN	0.04 (UVA) 0.03 UVA)

Source	Cancer	*n*	Toxicity	Toxicity system	Finding	Effect size (95% CI)	p
Jin et al, 2009	Lung	576	≥Gr 3, 1 year pneumonitis	CTCAEv3.0	↓ toxicity	37% non-smokers *vs* 23% former smoker *vs* 14% smokers	0.001 (UVA)
Huscher et al, 2009	Gynae	806	Acute toxicity Late toxicity	Need for surgery	No association		
Lilla et al, 2007	Breast	416	Telangiectasia	RTOG/EORTC and LENT/SOMA	↑ toxicity	OR 2.3 (1.2–4.6)	0.004 (MVA)
Iraha et al, 2007	Gynae	1349	Enterocolitis	Enterocolitis requiring surgery	↑ toxicity	RR 4.05 (1.58–6.51)	<0.001 (UVA) 0.002 (MVA)
Merrick et al, 2007	Prostate	161	Late rectal function	R-FAS rectal function assessment score	↑ toxicity	Spearman's Rho = 0.18	0.02 (UVA)
Koper et al, 2004	Prostate	199	Rectal bleeding	Questionnaires	No association		
Tsujino et al, 2003	Lung	71	Pneumonitis	CTCAEv2.0	No association		
Hernando et al, 2001	Lung	201	Pneumonitis	CTC	↓ toxicity	OR 0.42	0.05 (UVA) 0.05 (MVA)
Eifel et al, 2002	Cervix	3489	Any late	Major late complications	↑ toxicity	HR 2.30 (1.84–2.87)	<0.0005
van der Voet et al, 1998	Glottis	383	Any late	Own scale	↑ toxicity	28% in smokers *vs* 15% in ex-smokers *vs* 16% in non- smokers	0.0014(UVA) 0.0038 (MVA)
Johansson et al, 1998	Breast/ oesophagus	606	Pneumonitis	X-ray changes combined with clinical symptoms	↓ toxicity	5/6 breast and 8/8 oesophageal patients with radiation pneumonitis were non-smokers	0.18 (UVA breast) 0.02 (UVA oesophagus)
Monson et al, 1998	Lung	83	Pneumonitis	Acute or sub-acute dyspnoea with no other aetiology	↑ toxicity	23% in smokers *vs* 0% in non-smokers	<0.01 (UVA)

TABLE 3.5 *Continued*

Source	Cancer	*n*	Toxicity	Toxicity system	Finding	Effect size (95% CI)	p
Kucera et al, 1987	Cervix	1304	Severe late	Not specified	↑ toxicity	Serious and irreversible effects: 28% in smokers *vs* 15.2% in non-smokers	<0.01 (UVA)
Alcohol							
Lilla et al, 2007	Breast	416	Telangiectasia/ fibrosis	RTOG/EORTC and LENT/SOMA	No association	OR 1.41 (0.76–2.64)	ns (MVA)
Morganti et al, 2009	Breast	348	Acute skin	RTOG	↓ in patients with regular wine intake	OR 0.49 (0.28–0.86) (MVA)	0.013 (MVA)

Abbreviations: CTCAE = common toxicity criteria for adverse events; HNC = head and neck cancer; HR = hazard ratio; LENT = late effects in normal tissues; MVA = multivariate analysis; ns = not significant; ORN = osteoradionecrosis; RR = risk ratio; STAT = standardised total average toxicity; UVA = univariate analysis.

TABLE 3.6 Non-malignant systemic disease

Source	Cancer	*n*	Toxicity	Toxicity system	Finding	Effect size (95% CI)	p
Diabetes							
Defraene et al, 2012	Prostate	512	Faecal incontinence	Incontinence of blood, mucus or stools (requiring use of pads >2 times per week)	↑ toxicity	D_{50} dose-modifying factor 0.61 (0.47–0.77) in LKB model	0.048 (MVA)
Barnett et al, 2011a	Prostate	788	Bladder and bowel	RTOG/LENT/SOMA/ RMH/UCLA-PCI	No association		
Barnett et al, 2011b	Breast	1503	STAT	START/LENT/SOMA/ EORTC BR23	↑ toxicity	Regression coefficient 0.17 (0.032–0.31)	0.016 (MVA)
Barnett et al, 2011c	Breast	1014	Breast shrinkage	Photographic assessment (START)	↑ toxicity	OR 2.08 (0.73–1.10)	0.0009 (UVA) 0.004 (MVA) 0.10 (MVA dichotomised endpoint)
Tucker et al, 2010	Prostate	1010	Gr ≥ 2 late rectal toxicity	RTOG	No association		0.91 (UVA)
Taira et al, 2009	Prostate	226	Erectile dysfunction	IIEF-6	↑ toxicity	HR 2.57 (UVA) HR 3.97 (MVA)	0.014 (UVA) 0.001 (MVA)
Valdagni et al, 2008	Prostate	1115	Acute lower GI toxicity	RTOG/EORTC and LENT/SOMA	↑ toxicity	OR 1.34	0.34 (MVA)
Lilla et al, 2007	Breast	416	Telangiectasia	RTOG/EORTC and LENT/SOMA	No association	OR 1.30 (0.61–2.76)	ns (MVA)
Mayahara et al, 2007	Prostate	287	Acute GI and GU	CTCAEv2.0	No association		
Iraha et al, 2007	Gynae	1349	Enterocolitis	Need for surgery	↑ toxicity	RR 9.02 (7.10–11.11)	<0.001 UVA <0.001 MVA

TABLE 3.6 *Continued*

Source	Cancer	*n*	Toxicity	Toxicity system	Finding	Effect size (95% CI)	p
Merrick et al, 2007	Prostate	161	Late rectal function	R-FAS	↓ toxicity	Spearman's Rho = −0.17	0.03 (UVA)
Feigenberg et al, 2005	Prostate	1204	Late toxicity	Modified LENT/SOMA	No association		
Koper et al, 2004	Prostate	199	Rectal bleeding	Questionnaires	No association		
Akimoto et al, 2004	Prostate	52	Rectal bleeding	RTOG/EORTC	↑ toxicity	MVA RR = 2.88 (1.23–9.83)	<0.001 (UVA) <0.05 (MVA)
Jereczek-Fossa et al, 2003	Endometrium	317	Acute toxicity	RTOG/EORTC	No association		
Cozzarini et al, 2003	Prostate	154	Rectal bleeding	modified RTOG	No association		
Skwarchuk et al, 2000	Prostate	743	Gr 2/3 bleeding	RTOG/EORTC	↑ toxicity	Regression coefficient 1.8	0.04 (MVA)
Mantz et al, 1999	Prostate	287	Erectile dysfunction	Physician reported	↑ toxicity	OR 2.01	0.002 (MVA)
Herold et al, 1999	Prostate	944	Acute Gr 2 late GI Gr 2 late GU	RTOG RTOG/modified LENT RTOG	No association ↑ toxicity ↑ toxicity	 28% *vs* 17% in non-diabetics 14% *vs* 6%	ns 0.007 (MVA) 0.0014 (MVA)
Debus et al, 1997	Skull base	367	Brainstem	Modified RTOG scale consistent with LENT/SOMA	↑ toxicity	RR 5.7	0.04 (UVA) 0.01 (MVA)
Mak et al, 1994	Rectum/ rectosigmoid	224	Small bowel obstruction	Clinical diagnosis	No association		
Kucera et al, 1987	Cervix	1304	Late	Not specified	No association		

Source	Cancer	*n*	Toxicity	Toxicity system	Finding	Effect size (95% CI)	p
Hypertension							
Barnett et al, 2011a	Prostate	788	↓ urine stream	LENT/SOMA	↓ toxicity	HR 0.25 (0.09–0.71)	0.007 (MVA)
Tucker et al, 2010	Prostate	1010	Gr ≥2 rectal	RTOG	No association		0.77 (UVA)
Taira et al, 2009	Prostate	226	Erectile dysfunction	IIEF-6	↑ toxicity	HR 1.72 HR 2.06	0.047 (UVA) 0.011 (MVA)
Merrick et al, 2007	Prostate	161	Late rectal function	R-FAS	No association		
Jereczek-Fossa et al, 2003	Endometrium	317	Acute toxicity	RTOG/EORTC	No association		
Cozzarini et al, 2003	Prostate	154	Rectal bleeding	Modified RTOG	No association		
Eifel et al, 2002	Cervix	3489	Bladder Small bowel	Major late complications	No association	HR 0.61 (0.33–1.10) HR 0.53 (0.28–0.99)	0.1 (MVA) 0.05 (MVA)
Mak et al, 1994	Rectum and rectosigmoid	224	Small bowel obstruction	Clinical diagnosis	No association		
Collagen vascular disease (CVD)							
Lin et al, 2008	Various	73 cases *vs* matched controls	Any late toxicity	RTOG/EORTC	↑ toxicity	29% *vs* 14%	0.0010
Gold et al, 2007	Various	20	Acute and late toxicity in scleroderma patients	CTCAEv3.0	High toxicity		N/A

TABLE 3.6 ***Continued***

Source	Cancer	*n*	Toxicity	Toxicity system	Finding	Effect size (95% CI)	p
Pinn et al, 2008	Various	21	Acute and late toxicity in SLE patients	CTCAEv3.0	Moderate risk of toxicity	≥Gr 1 acute 42% ≥Gr 3 acute 21% ≥Gr 1 late at 5 y 45% ≥Gr 1 late at 10 y 56% ≥Gr 3 late at 5 y 28% ≥Gr 3 late at 10 y 40%	N/A
Phan et al, 2003	Various	38 cases *vs* matched controls	Late	RTOG/EORTC	No association	Gr I 3% *vs* 7% Gr II 7% *vs* 3% Gr III 7% *vs* 7%	
Chen et al, 2001	Breast	36 scleroderma and 72 controls	Acute Late	RTOG/EORTC	No association ↑ late toxicity in scleroderma patients	14% *vs* 8% 17% *vs* 3%	
Morris and Powell, 1997	Various	209	Acute and late	RTOG/EORTC	No effect for RA ↑ toxicity in non-RA disease	21% *vs* 6% late toxicity for non-RA CVD *vs* RA	0.002
Cardiovascular							
Defraene et al, 2012	Prostate	512	Rectal bleeding	Bleeding requiring laser treatment or transfusion	↑ toxicity	D_{50} dose-modifying factor (dmf) 0.92 (0.87–0.95) in LKB model	0.015 (MVA LKB model)
Barnett et al, 2011c	Breast	1014	Acute and late	START LENT/SOMA EORTC BR23	No association		0.067 (UVA overall toxicity)
Koper et al, 2004	Prostate	199	Rectal bleeding	Questionnaires	No association		
Mantz et al, 1999	Prostate	287	Erectile function	Physician reported	↑ toxicity	OR 1.80	<0.001 (MVA)

Source	Cancer	*n*	Toxicity	Toxicity system	Finding	Effect size (95% CI)	p
Inflammatory bowel disease							
Barnett et al, 2011a	Prostate	788	Faecal urgency	UCLA-PCI	↑ faecal urgency	HR 3.59 (1.40–9.18)	0.008 (MVA)
Peters et al, 2006	Prostate	24	Late toxicity	CTCAE	Brachytherapy well tolerated	No Gr 3 or 4 rectal toxicity. 4 patients experienced Gr 2 late rectal toxicity	N/A
Song et al, 2001	Pelvic or abdominal tumours	24	Acute and late GI toxicity	RTOG/EORTC	Moderate prevalence of ≥Gr 3 toxicity	5 patients (21%) experienced ≥Gr 3 acute toxicity; 2 patients (8%) had ≥Gr 3 late toxicity	N/A
Willett et al, 2000	Pelvic or abdominal tumours	28	Severe acute GI Severe late GI	Failure to complete planned course of RT Need for hospital or surgery	Moderate prevalence of severe toxicity	21% 29%	N/A
Green et al, 1999	Rectal	15	Acute and late toxicity	RTOG/EORTC	Moderate prevalence of severe toxicity	3 patients (20%) had Gr ≥3 acute toxicity, including 2 cases of Gr 3 skin toxicity and 1 case of Gr GI toxicity No long-term toxicity	N/A
Grann and Wallner, 1998	Prostate	6	Acute and late GI toxicity	Not specified	Brachytherapy well tolerated	No unusual toxicity	N/A

Abbreviations: CTCAE = common toxicity criteria for adverse events; GI = gastrointestinal; GU = genitourinary; Gynae = gynaecological cancer; HR = hazard ratio; LENT = late effects in normal tissues; LKB = Lyman-Kutcher-Burman; MVA = multivariate analysis; N/A = not available; ns = not significant; RA = rheumatoid arthritis; R-FAS = Rectal Function Assessment Score; RMH = Royal Marsden Hospital; RR = risk ratio; RTOG = Radiation Therapy Oncology Group; SBO = small bowel obstruction; SLE = systemic lupus erythematosus; UCLA-PCI = University of California, Los Angeles, Prostate Cancer Index; UVA = univariate analysis.

TABLE 3.7 Effect of other treatments

Source	Cancer	*n*	Toxicity	Toxicity system	Finding	Effect size (95% CI)	p
Surgery prior to radiotherapy							
Defraene et al, 2012	Prostate	512	Rectal bleeding Incontinence	Use of laser or transfusion Use of pads >2 times per week	↑ bleeding and incontinence	D_{50} dose-modifying factor (dmf) 0.91 (0.86–0.94) in LKB model for bleeding D_{50} dose-modifying factor (dmf) 0.52 (0.34–0.66) in LKB model for incontinence	0.012 (MVA LKB model) for bleeding <0.001(MVA LKB model) for incontinence
Ramaekers et al, 2011	HNC	396	Xerostomia	EuroQol-5D questionnaire converted to RTOG score	↑ xerostomia with previous surgery	Regression coefficient 0.062; SE 0.022	0.006 (MVA)
Barnett et al, 2011d	Breast	1145	Moderate/poor cosmesis	Photographic	Moderate/poor surgical cosmesis leads to moderate/poor overall cosmesis	OR 40.9 (25.4–65.8)	<0.0001 (UVA, MVA)
Huscher et al, 2009	Gynae	806	Acute toxicity Late toxicity	Need for surgery	No association with surgery for benign disease		
Fellin et al, 2009	Prostate	718	Rectal bleeding	Questionnaires based on LENT/SOMA	↑ bleeding	OR 2.94	0.018 (MVA)
Palazzi et al, 2008	HNC	149	Acute mucositis	CTCAEv3.0	↑ toxicity	Not specified	<0.05 (MVA)
Iraha et al, 2007	Gynae	1349	Enterocolitis	Enterocolitis requiring surgery	↑ toxicity	12.45 (6.4–18.5)	0.001 (UVA) 0.006 (MVA)
Jereczek-Fossa et al, 2003	Endometrium	317	Acute toxicity	RTOG/EORTC	No association		

Source	Cancer	*n*	Toxicity	Toxicity system	Finding	Effect size (95% CI)	p
Fine et al, 1995	Cervix	189	Any severe late toxicity	Complications requiring surgical repair or causing death	↑ with prior laparotomy ↑ with prior transperitoneal staging	Of 66 patients with serious toxicity, 60.6% had laparotomy *vs* 39.4% had not 13.6% of 66 patients had retroperitoneal *vs* 86.4% transperitoneal staging	0.001 (UVA) and 0.0003 (MVA) for laparotomy <0.0001 (UVA) and 0.0001 (MVA) for trans-peritoneal approach
Mak et al, 1994	Rectum and rectosigmoid	224	Small bowel obstruction	Clinical diagnosis	No association		
Chemotherapy							
Curran et al, 2011	Lung	610	Acute oesophagitis Late oesophagitis Acute lung toxicity Late lung toxicity	Not stated	↑ toxicity with concurrent *vs* sequential chemotherapy ↑ ≥Gr 3 acute lung toxicity with sequential chemotherapy No significant difference in late oesophagitis or lung toxicity	Acute oesophagitis ≥Gr 3 occurred in 4, 22 and 45% for sequential vin/cis, concurrent vin/cis, and concurrent etop/cis (arms 1, 2 and 3) Acute lung toxicity 14, 13 and 17% (arms 1, 2 and 3) Late oesophagitis 1–4% Late lung toxicity 13–17%	<0.001 (UVA acute oesophagitis)
Barnett et al, 2011b	Breast	1503	STAT	START trial EORTC BR23 LENT/SOMA	↑ toxicity in patients treated with sequential chemotherapy then radiotherapy	Regression coefficient 0.13 (0.046–0.21) MVA	0.0008 (UVA) 0.002 (MVA)
Dehing-Oberije et al, 2010	Lung	469	Acute dysphagia	CTCAEv3.0	↑ toxicity	OR 2.54 (1.64–3.91)	<0.001 (MVA)

TABLE 3.7 *Continued*

Source	Cancer	*n*	Toxicity	Toxicity system	Finding	Effect size (95% CI)	p
Braendengen et al, 2008	Rectal	207	≥Gr 3 acute Late toxicity	WHO WHO	↑ toxicity with CRT No association	29% *vs* 6% for radiotherapy alone	0.001 (UVA)
Vale, 2008	Cervix	>2000	Acute GI	5-point scale in all trials included in the meta-analysis	↑ toxicity for trials using platinum-based CRT	Available data suggest 1–3% experienced serious late toxicity	0.00002 (UVA)
Collette et al, 2008	Breast	5178	Fibrosis	None, minimal, moderate, severe	↑ with concurrent chemotherapy	HR 2.40 (99% CI 1.48–3.91) boost arm HR 2.52 (99% CI 1.38–4.62) no boost arm	<0.0001 (MVA)
Palazzi et al, 2008	HNC	149	Acute dysphagia Acute mucositis Acute weight loss Acute salivary changes	CTCAEv3.0	↑ with concurrent chemotherapy	Not specified	0.002 (MVA) 0.004 (MVA)
Kuoppala et al, 2008	Endometrium	156	Late GI	Need for surgery	↑ with concurrent chemotherapy	9.5% for CRT *vs* 2.7% for radiotherapy alone	Not specified
Ryan et al, 2007	Multiple sites	656	Patient-reported skin problems	Nationwide Symptom Inventory	No association		0.2 (UVA)
Bhandare et al, 2007	HNC	325	Ototoxicity Hearing loss	Review of records from otolaryngology and audiology departments	↑ acute otitis externa ↑ chronic otitis externa ↑ tympanic perforation ↑ labyrinthitis ↑ sensorineural hearing loss	41.8% of patients exhibited some ototoxicity: 33.2% had external ear complications, 28.6% had middle ear toxicity, 26.8% had inner ear toxicity	0.045 (MVA) 0.039 (MVA) <0.01 (MVA) <0.01 (MVA) 0.028 (UVA) 0.006 (MVA)
Metzger et al, 2006	Lymphoma	461	Hypothyroidism	Biochemical measurement	No association	HR 1.0 (0.7–1.6)	0.93 (UVA)
Gerard et al, 2006	Rectal	733	≥Gr 3 acute Late toxicity	WHO WHO	↑ toxicity No association	14.6% *vs* 2.7%	<0.0001 (UVA)

Source	Cancer	*n*	Toxicity	Toxicity system	Finding	Effect size (95% CI)	p
Bosset et al, 2006	Rectal	1011	≥Gr 2 acute Late toxicity	WHO	↑ toxicity No association	38% *vs* 17%	<0.001 (UVA)
Bernier et al, 2004	HNC	234	≥Gr 3 acute ≥Gr 3 late	CTCAEv2.0 RTOG/EORTC	↑ toxicity No association	41% *vs* 21%	0.001 (UVA)
Cooper et al, 2004	HNC	459	≥Gr 3 acute Late toxicity	CTCAEv2.0 RTOG/EORTC	↑ toxicity with CRT No association	77% *vs* 34% 21% *vs* 17%	<0.001 (UVA) 0.29 (UVA)
Denis et al, 2003; Calais et al, 1999	HNC	226	≥Gr 3 mucositis Late toxicity	RTOG, CTCAE RTOG, CTCAE and LENT/SOMA	↑ toxicity with CRT ↑ late toxicity with CRT	71% *vs* 39% 82% *vs* 47% ≥Gr 3	0.005 (UVA) 0.02 (UVA)
Green et al, 2001	Cervix	>2000	Acute leucopaenia Acute thrombo-cytopaenia Acute GU Acute GI	RTOG	↑ toxicity ↑ toxicity ↓ acute ↑ toxicity	OR 2.21 (1.72–2.93) OR 3.73 (1.53–9.10) 0.43 (0.20–0.92) 2.22 (1.58–3.11)	<0.0001 (UVA) 0.004 (UVA) 0.03 (UVA) <0.0001 (UVA)
Hernando et al, 2001	Lung	201	Pneumonitis	CTC	No association		
Whitney et al, 1999	Cervix	368	Gr 3/4 late	GOG	No association	16.2% *vs* 16.5%	
Tseng et al, 1997	Cervix	122	Any late	GOG	Non-significant increase	23% CRT *vs* 13% RT	0.13 (UVA)
Morris et al, 1999	Cervix	403	Gr 3/4 late	RTOG/EORTC	No association	12% *vs* 11% RT	
Hormones*							
Varga et al, 2011	Breast	328	Lung fibrosis	CT scan abnormalities	↑ toxicity	OR 2.44 (1.12–5.33)	0.03 (MVA)
Barnett et al, 2011c	Breast	1014	≥Gr 2 acute	RTOG/EORTC	↑ toxicity	OR1.21 (1.06–1.38)	0.0044 (UVA)

TABLE 3.7 *Continued*

Source	Cancer	*n*	Toxicity	Toxicity system	Finding	Effect size (95% CI)	p
Tucker et al, 2010	Prostate	1010	≥Gr 2 late rectal	RTOG/EORTC	No association		
Valdagni et al, 2008	Prostate	1132	Gr 2/3 acute rectal	RTOG/EORTC	↓ toxicity	OR 0.65	0.04 (MVA)
Mayahara et al, 2007	Prostate	287	Acute GI and GU	CTCAEv2.0	↑ Gr 2–3 acute bladder toxicity with ADT	2.2 (1.15–4.14)	0.05 (UVA) 0.017 (MVA)
Peeters et al, 2005	Prostate	336	≥Gr 2 acute rectal ≥Gr 2 acute bladder	RTOG/EORTC	↓ toxicity ↑ toxicity	27% *vs* 50% 72% *vs* 50%	<0.001 (MVA) <0.01 (UVA)
Dorr et al, 2005	Breast	451	Early pneumonopathy Late fibrosis	RTOG/EORTC and LENT/SOMA CT assessment	↑ toxicity No association	38.9% *vs* 20.9%	0.0065 (UVA) 0.001 (MVA)
Harris et al, 2005	Breast	278	Cosmesis and late toxicity	RTOG/EORTC	No association		0.31 (UVA)
Feigenberg et al, 2005	Prostate	1204	Late GU	Modified LENT/SOMA	↑ toxicity	8% no ADT, 8% short-term ADT, 14% long-term	0.02 (MVA)
			Late GI		↑ toxicity	17% no ADT, 18% short-term ADT, 26% long-term	0.02 (MVA)
Koper et al, 2004	Prostate	199	Rectal bleeding	Questionnaires	No association		
Cozzarini et al, 2003	Prostate	154	Rectal bleeding	Modified RTOG	No association		
Wennberg et al, 2002	Breast	121	Early pneumonopathy	Scale similar to CTCAE	No association		0.8 (UVA)
Koc et al, 2002	Breast	111	Lung fibrosis	CT scan	↑ toxicity	35.1% *vs* 13.5%	0.001 (UVA)

Source	Cancer	*n*	Toxicity	Toxicity system	Finding	Effect size (95% CI)	p
Zelefsky et al, 1999	Prostate	743	Erectile dysfunction	RTOG/EORTC	↑ toxicity	5-year actuarial likelihood of impotence, 69% with ADT and 56% without	0.002 (UVA) 0.01 (MVA)
Mantz et al, 1999	Prostate	287	Erectile dysfunction	Physician reported	↑ toxicity	OR 1.74	<0.001 (MVA)
Bentzen et al, 1996	Breast	196	Lung fibrosis	Lung density on radiographs	↑ toxicity	OR 2.9 (1.3–6.3)	0.007 (UVA)
Fowble et al, 1996	Breast	491	Cosmesis	Excellent, good, fair, poor	No association		

Abbreviations: ADT = androgen deprivation therapy; CRT = chemoradiotherapy; CTCAE = common toxicity criteria for adverse events; etop/cis = etoposide and cisplatin; GI = gastrointestinal; GU = genitourinary; Gynae = gynaecological cancer; HNC = head and neck cancer; LENT = late effects in normal tissues; LKB = Lyman-Kutcher-Burman; MVA = multivariate analysis; RTOG = Radiation Therapy Oncology Group; SE, standard error; UVA = univariate analysis; vin/cis = vinblastine and cisplatin.

*Tamoxifen for breast and androgen deprivation therapy for prostate.

3.2 Attempts at predicting a cancer patient's risk of developing toxicity following radiotherapy

3.2.1 Clonogenic, cytogenetic, DNA damage and apoptosis assays

Numerous publications report the results of studies investigating the relationship between laboratory measurements of radiosensitivity and a patient's likelihood of developing toxicity following radiotherapy. The earliest studies focused on individuals with very severe toxicity. Fibroblasts cultured from skin samples of such patients were shown to be unusually radiosensitive using clonogenic assays (Arlett and Priestley, 1983; Loeffler et al, 1990; Plowman et al, 1990; Smith et al, 1980; Woods et al, 1988). Generally, this clinical and cellular radiosensitivity is associated with undiagnosed genetic syndromes associated with DNA damage recognition and repair defects.

With the demonstration in the 1980s that there was variation in fibroblast radiosensitivity between cells cultured from individuals both with and without known genetic syndromes (Little et al, 1988; Malaise et al, 1987), studies were set up to investigate the relationship between cellular and clinical radiosensitivity with a goal of developing a test to predict a patient's probable reaction to radiotherapy. The first studies were retrospective and involved skin samples from patients who developed severe reactions to radiotherapy, which were compared with samples from patients with no/minimal toxicity. Although some studies showed a relationship between cellular and clinical radiosensitivity, overall the findings have been equivocal (Table 3.8).

As deriving fibroblast lines from skin samples takes two to three months, interest moved to investigating more rapid assays that would have greater clinical utility, with the main ones studied being chromosomal damage assays (Table 3.9), DNA damage assays (Table 3.10) and apoptosis (Table 3.11). Other assays have been explored, eg the ability of fibroblasts to undergo radiation-induced differentiation (Russell et al, 2000) and telomere length (Iwasaki et al, 2008). Combinations of assays have also been explored (Azria et al, 2008; Rzeszowska-Wolny et al, 2008). For example, a combination of low radiation-induced apoptosis and genetic testing was shown to predict the risk of severe late toxicity after radiotherapy (Azria et al, 2008). There is also interest in measuring the expression of cytokines in serum/plasma. For example, a combined two-centre analysis of 165 patients with non-small cell lung cancer showed that elevation of plasma transforming growth factor (TGF)-β1 during radiotherapy predicted for lung toxicity (Zhao et al, 2009).

Studies are also attempting to derive gene expression signatures (Badie et al, 2008; Henriquez Hernandez et al, 2009; Mayer et al, 2011). This work is challenging when attempting to measure radiosensitivity due to the need to choose whether to investigate baseline gene expression or radiation-induced gene expression (determining which dose and how long after irradiation is also difficult). Many in the field are now focusing on attempting to identify the genetic variants that predispose to radiotherapy toxicity because the assay is not subject to the same variability associated with other laboratory measurements of radiosensitivity (Section 3.2.2).

TABLE 3.8 Studies investigating whether cellular radiosensitivity predicts for clinical radiosensitivity: clonogenic or cell growth assays

Source	Type	Cancer	*n*	Cell type	Toxicity	Assay	p
Oppitz et al, 2001	Prospective	Breast	88	Fibroblast	Acute Late	Clono	<0.005 ns
West et al, 2001	Prospective	Cervix	83	Lymphocyte	Late	LDA	0.002
Peacock et al, 2000	Retrospective	Breast	104	Fibroblast	Late	Clono	0.19
Rudat et al, 1999	Prospective	HNC	25	Fibroblast	Late	Clono	0.50
Rudat et al, 1997	Prospective	HNC	25	Fibroblast	Acute	Clono	ns
Russell et al, 1998	Retrospective	Breast	79	Fibroblast	Fibrosis	Clono	0.13
Brock et al, 1995	Retrospective	Breast	22	Fibroblast	Acute Late	Clono	>0.21 >0.034
Ramsay and Birrell, 1995	Retrospective	Breast	56	LCL	Late	MTT	<0.02
Johansen et al, 1994, 1996	Retrospective	Breast	31	Fibroblast	Acute Fibrosis Telangiectasia	Clono	ns 0.009 ns
Begg et al, 1993	Retrospective	Breast	32	Fibroblast	Acute	Clono	>0.50
Geara et al, 1993	Prospective	HNC	21	Lymphocyte	Late	LDA	ns
Geara et al, 1993	Prospective	HNC	17	Fibroblast	Late	Clono	0.0013
Burnet et al, 1992, 1994	Retrospective	Breast	6	Fibroblast	Late	Clono	0.02

Abbreviations: Clono = clonogenic; HNC = head and neck cancer; LCL = lymphoblastoid cells; LDA = limiting dilution assay; MTT = 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay; ns = not significant.

TABLE 3.9 Studies investigating whether cellular radiosensitivity predicts for clinical radiosensitivity: chromosomal damage assays

Source	Cancer	*n*	Toxicity	Assay	Finding	p
Djuzenova et al, 2006	Breast	59	Acute	G2 MN	Association	<0.005
De Ruyck et al, 2005	Gynae	62	Late	G2	Association	0.002
Hoeller et al, 2003	Breast	86	Fibrosis	Aberrations	Trend	0.13
Barber et al, 2000a	Breast	116 123 47 47	Acute skin Acute skin Telangiectasia Fibrosis	MN G2 MN MN	No association Association Association No association	 0.004 0.042 0.055
Jones et al, 1995	Breast	16	Hypersensitive cases *vs* controls	Aberrations	↑ aberrations	0.009

Abbreviations: G2 = G2 assay; Gynae = gynaecological cancer; MN = micronucleus.

TABLE 3.10 Studies investigating whether cellular radiosensitivity predicts for clinical radiosensitivity: DNA damage assays

Source	Cancer	*n*	Toxicity	Assay	Finding	p
Bourton et al, 2011	Mixed	12	Atypical	γH2AX	↑ *vs* 10 with minimal toxicity	<0.0001
Werbrouck et al, 2011	HNC	31	Acute	γH2AX	No association	
Werbrouck et al, 2010	Gynae	29	Late	γH2AX	No association	0.59
Olive et al, 2008	Prostate	40	Late	γH2AX	No association	
Rzeszowska-Wolny et al, 2008	HNC	34	Acute	Comet	Weak association	0.039
Pinar et al, 2007	Breast	29	Late	PFGE	Association in parts receiving high dose	<0.031
Djuzenova et al, 2006	Breast	59	Late	Comet	No association	
Lopez et al, 2005	Breast	108 50	Acute Late	PFGE	No association	0.50 0.66
Twardella et al, 2003	Breast	113	Acute	Comet	No association	
Dickson et al, 2002	Breast	49	Fibrosis	PFGE	No association	0.48
Oppitz et al, 2002	Breast	30	Acute	Comet	Association	<0.00025
Dikomey et al, 2000	Breast	12	Fibrosis	CFGE	No association	
Alapetite et al, 1999	Breast	28	Acute and late	Comet	Association	<0.02
Kiltie et al, 1997	Breast	39	Fibrosis	PFGE	Association	<0.003

Abbreviations: CFGE = constant field gel electrophoresis; Gynae = gynaecological cancer; HNC = head and neck cancer; PFGE = pulsed field gel electrophoresis.

TABLE 3.11 Associations between low levels of radiation-induced apoptosis in pre-treatment peripheral blood lymphocytes and radiotherapy toxicity

Source	Cancer	*n*	Toxicity	Assay	Finding	p
Bordon et al, 2011	Cervix	94	Bowel Rectal Urinary	FCM	↑ toxicity	0.002 0.020 0.003
Henriquez-Hernandez et al, 2011	Breast	26	Late	High initial DSBs+FCM	↑ toxicity	0.008
Bordon et al, 2010	HNC	79	Xerostomia	FCM	↑ toxicity	0.035
Schnarr et al, 2009	Prostate	45	Late	FCM	↑ toxicity	0.010
Crompton et al, 2001	Mixed	60	Late	FCM-PI	↑ toxicity	N/A
Barber et al, 2000b	Breast	31	Late	FCM tunnel	No association	
Crompton et al, 1999	Mixed	12	Hypersensitive	FCM-PI	↑ toxicity	<0.0017

Abbreviations: DSBs = double strand breaks; FCM = flow cytometry (annexin assay); FCM-PI = FCM propidium iodide assay; HNC = head and neck cancer; N/A = not available.

There are several problems associated with research aimed at testing whether laboratory measurements of radiosensitivity predict clinical radiosensitivity.

a Assays are not standardised and there has been little or no attempt to ensure transferability across laboratories. The studies involve different radiation doses, dose rates, parameters and assay conditions. For example, DNA damage assays can be conducted in neutral or alkaline conditions, which affect the type of damage measured; initial damage, residual damage or the rate of repair can be assessed.

b Replication and validation studies are rarely carried out. Where they have been done, results have not been replicated for clonogenic assays (Burnet et al, 1992; Peacock et al, 2000) or DNA damage assays (Dickson et al, 2002; Kiltie et al, 1997).

c Patient cohorts are heterogeneous. Some studies involve severe atypical reactions; others investigate unselected series of patients. The factors determining radiosensitivity might differ between these two groups.

d Study designs vary considerably and few involve power calculations and multivariate analyses.

e Reproducibility is rarely reported but cell-based assays tend to have a large experimental variability compared with inter-individual variability in radiosensitivity.

Progress in the area requires standardised approaches for measuring radiosensitivity that have been shown to be transferable across laboratories and establishment of guidelines for carrying out studies.

3.2.2 Genetics as a predictor of radiotherapy toxicity

Common genetic variation is likely to be important in the differences seen between individuals. Single nucleotide polymorphisms (SNPs) are the most common form of variation in the human genome. Almost all SNP-association studies of radiotherapy toxicity, published to date, have used a candidate gene approach. Radiation-induced cell killing, for which DNA damage is a major mechanism, is thought to be a triggering event in the development of radiotherapy toxicity. Additionally, the release of cytokines is considered to initiate biological responses in multiple cell types, leading to the development of late toxicity. The focus of candidate gene studies has thus been on genes involved in DNA damage recognition and repair (eg *ATM*, *BRCA1*, *BRCA2* and *TP53*), free radical scavenging (eg *SOD2*) and anti-inflammatory response (eg *TGFB1*). Studies to date, reviewed recently (Alsner et al, 2008; Barnett et al, 2009; Popanda et al, 2009), have been underpowered, including fewer than 500 samples. The studies have tested many SNPs without adjusting for multiple comparisons and, although many reported positive associations, findings have proved difficult to replicate. Current thinking is that none will have a confirmed individual role at a clinically relevant effect size (Barnett et al, 2012). Research in this area is moving towards carrying out genome-wide association studies (GWAS) with no *a priori* assumptions of genes of interest. Results are starting to emerge (Hosking et al, 2011; Kerns et al, 2010; Michikawa et al, 2010) and the need for collaborative studies is being recognised (West et al, 2010).

In 2011, the findings of a GWAS were published investigating the development of second cancers in individuals who underwent radiotherapy for Hodgkin's lymphoma as children (Best et al, 2011). The study

involved 100 cases and 89 cancer-free controls and a replication cohort comprising 96 cases and 82 controls. The GWAS identified variants at 6q21 implicating *PRDM1* in radiotherapy-induced carcinogenesis. *PRDM1* (known also as *BLIMP1*) encodes a transcription factor that acts as a repressor of beta-interferon gene expression to coordinate response to viral infection. The gene regulates proliferation and differentiation. The finding implicates altered immune function in the risk of radiation-induced cancers. Another interesting finding from the GWAS was that the effect sizes associated with the SNPs identified were higher than expected from observations emerging for other traits. The highest odds ratio for the homozygote minor allele for the combined discovery and replication cohort was 11.4 (95% CI 3.23–40.25). The high effect size was attributed to genetic susceptibility being more pronounced in patients exposed to radiation at a young age in comparison with older patients for whom environmental factors might have a large influence. Genetic susceptibility to cancer induction is likely to decrease with age as environmental and lifestyle factors exert an increasing influence (Best et al, 2011).

3.2.3 Statistical issues relating to genetic association studies

Given the probable increase in the number of publications identifying potential genetic predictors of radiotherapy toxicity, it is useful to consider the statistical aspects of such studies which are crucial for correct interpretation. In designing a study to investigate the association between any exposure and any outcome, the most efficient and appropriate design will be informed by the nature of the underlying association. Thus, genetic association studies that seek to identify associations between germline genetic variation (exposure) and radiosensitivity (outcome) should be informed by the underlying genetic architecture of risk. The genetic architecture is an umbrella term to describe the range of risk alleles in terms of the allele frequency, the risks they confer and the genetic model for their effect (dominant, co-dominant or recessive). It should be noted that the phenotype of interest, radiosensitivity, as described earlier, is a complex construct encompassing a range of cellular and clinical phenotypes which are likely to be related, but which may differ in their underlying genetic architecture.

While there is good evidence for inter-individual variation in radiosensitivity and good evidence that a substantial proportion of that variation is caused by germline genetic variation, there are very few data to provide any information about the likely underlying genetic architecture for any given radiosensitivity phenotype. Nevertheless, some broad generalisations can be inferred from basic principles and from the genetic epidemiology of other complex human phenotypes. First, highly penetrant alleles are likely to be rare (0.1–1%) or very rare (under 0.1%). If this were not the case, the radiosensitive phenotype would be common in the population in which it is being studied. Such alleles would only account for a small proportion of the genetic component of the phenotypic variance. The remainder could be explained by a small number of common (over 5%) variants that confer modest risks to a very large number or very rare variants with small risks. Studies of other complex phenotypes have found few, if any, common variants that confer modest risks. For example, in breast cancer, common alleles that confer a relative risk of disease greater than 1.3 have not been found. Given that genetic association studies in breast cancer have virtually 100% power to detect such alleles, it seems reasonable to conclude that they do not exist. Common alleles conferring relative risks greater than two have not been identified for any complex

disease phenotype. Genome-wide association studies for common, complex diseases have been very successful and have identified large numbers of common alleles conferring weak effects, with each allele explaining less than 2% of the genetic component of disease. Rare and uncommon (1–5%) alleles conferring modest disease risks have been identified.

3.2.3.1 Searching for common alleles

Over the past decade there has been rapid progress in understanding the architecture of human genetic variation. In particular, projects such as the HapMap Project and the 1000 Genomes Project have provided a great deal of information about the extent and correlation structure of common variation across the genome in different populations. This, combined with major developments in genotyping technology, has made it possible to genotype tens of thousands of subjects for hundreds of thousands of SNPs that efficiently capture most of the common genetic variation across the genome.

What study design should be used?

The most appropriate study design depends on the radiosensitivity phenotype of interest. Some endpoints are quantitative and can be measured on a continuous scale; other quantitative endpoints may be measured on an ordinal scale. There are also endpoints that simply represent the presence or absence of the phenotype of interest. The presence/absence endpoints are perhaps the commonest and can be studied using a case–control design. In general, for a fixed sample size, such as when genotyping costs are a limiting factor, it is most efficient to have an equal number of cases and controls. However, sample size is often limited by the availability of subjects with the phenotype of interest and power can be increased by increasing the ratio of controls to cases if additional controls are available.

What statistical test should be used?

The simple answer to this question is 'the test that provides the greatest power to detect association'. However, the power of any given test for association depends upon the underlying genetic model. Consider a bi-allelic SNP, which has three possible genotypes, common homozygote, heterozygote and rare homozygote. In a case–control study this will generate the standard 2 x 3 contingency table and simple tests can be used to test for association. A general chi-squared test for heterogeneity (two degrees of freedom, df) can be used, but more powerful tests are available. Under a dominant genetic model the heterozygote and rare homozygote will confer the same risk and the greatest power would be achieved by grouping these two genotype categories and carrying out a one df chi-squared test on the resultant 2 x 2 contingency table. Similarly the common homozygote and heterozygote genotypes could be combined in order to test for a recessive allele. Under a co-dominant genetic model the heterozygote will be at intermediate risk between common homozygote and rare homozygote. Here a chi-squared test for trend (one df) will have the greatest power to detect association. It should be noted that the four tests – general, dominant, recessive and co-dominant – can detect association in all genetic models, but at reduced power.

As the genetic model is not usually known it is not possible to select in advance the test with the greatest power. Two possible approaches can then be used. The first is to apply all four tests and to choose the one with the smallest p-value. However, this p-value would need to be corrected for the fact that it was selected *post hoc*, usually using some sort of permutation procedure to allow for the fact that the tests

are not independent. The alternative is to use the test that has the greatest power across a range of genetic models. The majority of common, disease-susceptibility alleles detected to date seem to fit the co-dominant model best and so the chi-squared test for trend, which has reasonable power across a range of genetic models, is commonly used as a single test for association.

The simple chi-squared tests described above are generally used for univariate association tests. Where it is desirable to control for potentially confounding co-variates the equivalent tests can all be applied in a logistic regression framework. It should be noted that there are unlikely to be many, if any, true confounders of a true genetic association.

Dealing with population stratification

Confounding can occur in the context of cryptic population stratification. If the disease frequency in cases and controls is different in different populations and the allele of interest is not causal, but differs in frequency between the populations, then spurious association will be observed. Population stratification has not been found to be a major problem in carefully designed case–control studies restricted to populations of European origin. Furthermore, there are now well-established methods for dealing with the problem, such as principal components analysis.

However, perhaps the most important protection against false positives due to population stratification is through the replication of association signals in independent datasets. It is unlikely that population stratification causing a false positive for any given SNP in a study from one population will be the same in another study from a different population.

What should be considered 'statistically significant'?

The early literature reporting genetic association studies was littered with reports of statistically significant associations that subsequently failed to be replicated by independent studies. Several possible reasons were put forward to explain this, including population-specific differences in: (a) risk allele frequencies, (b) the correlation structure between marker polymorphisms and causal variants, (c) differences in the frequency of interacting alleles and lifestyle/environmental factors and (d) limited power of small, replication studies to detect alleles with weak effects (Pharoah et al, 2004). However, the major cause for failure of initial findings to be replicated has been because most of the initial findings were false positives caused by using an inappropriate threshold to declare statistical significance. The reason that the traditional $p < 0.05$ threshold is inappropriate for genetic association studies (and most other studies of observational epidemiology) is explained as follows.

The p-value, in itself, is not the probability of primary interest when interpreting data. The p-value is a conditional probability, namely the probability of observing data at least as extreme as those obtained if the null hypothesis is true. It is often incorrectly interpreted as the probability that the null hypothesis is true given the observed data. This latter probability can be considered to be the probability that an association that is declared as statistically significant is a false positive. The false positive probability depends on the prior probability of association (unknown), the power of the study to detect that association and the p-value. When the prior probability of association is small and the power to detect association is small, the false positive probability is high. The prior probability for a genetic association is unknown, but we know that there are around 10 million common variants in the human genome and

there can be, at most, 100 variants that each explain 1% of the genetic variance, the prior probability of association for a random variant is 1 : 100,000 at best. While some would argue that this prior probability can be improved by judicious selection of variants in candidate genes, even if it were improved by an order of magnitude the prior probability would still be small (1 : 10,000). It is worth noting that candidate gene studies in the pre-genome-wide association study (GWAS) era were notable for their lack of success and many risk alleles identified by GWAS have been in regions without any obvious candidate genes based on known gene function. Furthermore, some risk alleles have been identified in so-called gene deserts containing no gene coding sequences. Even at an unlikely prior probability of 1 : 1,000, a genetic association study with 80% power that declares a significant association at $p = 0.05$ would have a 98% chance of being a false positive. As a consequence, well-powered studies with stringent criteria for declaring statistical significance are required in order to provide robust evidence of association. Various p-value thresholds have been suggested as appropriate for genetic association studies, but $p < 5\ 10^{-8}$ is widely accepted as denoting 'genome-wide' significance.

What sample size is required?

The power of a given study to detect a specific risk allele depends on the frequency of the risk allele in the population, the magnitude of the effect of the risk allele, the sample size, the ratio of cases to controls and the type I error rate (p-value threshold). An added level of complexity arises from genome-wide association studies where there are likely to be multiple risk alleles and the goal is to identify one or more risk alleles.

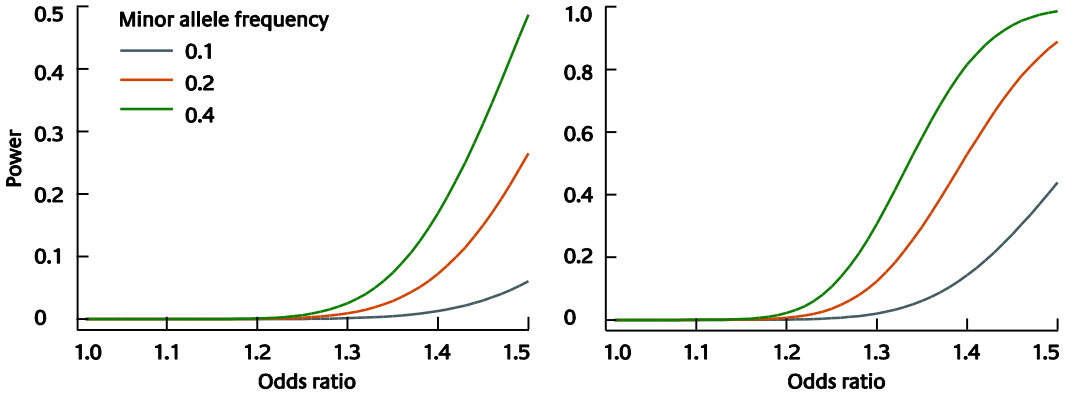
Figure 3.1(a) show the power to detect a single risk allele by allele frequency and risk at a type I error rate of $5\ 10^{-8}$ for a phenotype with a frequency of 10% (case–control ratio 1 : 9). As can be seen, the power to detect specific, modest-effect alleles at highly stringent levels of significance is low. However, if the presence of 10 risk alleles in a GWAS is assumed, the power to detect at least one is substantially greater (see Figure 3.1(b)).

Validation and replication

A fundamental requirement in evaluating genetic association is the need for replication of initial findings in independent datasets. What constitutes adequate replication is rarely defined and often misunderstood. There are two primary purposes of replication. First, where an initial association is of borderline statistical significance, replication can provide additional evidence of association such that when the data are combined the statistical evidence is strengthened. The strength of the statistical association as measured by the p-value in the replication study is less important than the requirement that the direction and magnitude of the effect in the replication study should be compatible with the initial association. Under these circumstances combining the data should result in a strengthening of the initial association. The second purpose of replication is to provide additional evidence that the initial association has not occurred as the result of bias or confounding. If a reported association is replicated in a second study with a different study design and carried out in a different population, it reduces the likelihood that the initial association was simply due to some unidentified bias or confounding. For example, if an association is observed in a study based on a European population it is possible that that association occurred as the result of population stratification. However, if the association is replicated in a study on an Asian population the probability that population stratification accounts for the association is

much less. Similarly technical biases, such as differences in DNA quality between cases and controls, is unlikely to be replicated in an independent study.

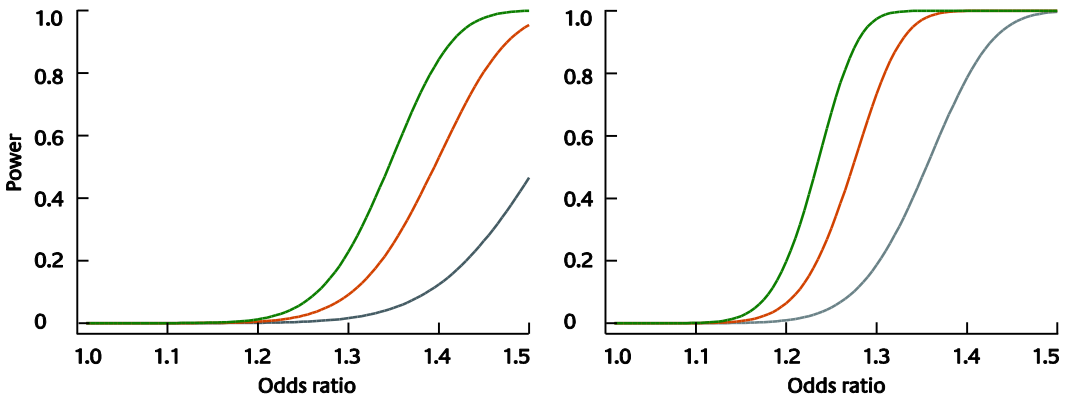
There can be no definitive rules to define replication. In practice, a pragmatic approach is needed. An association can be considered highly likely to be a true positive when the combined data from multiple studies carried out in different populations result in a highly significant association.



n = 400 cases/3600 controls

n = 800 cases/7200 controls

(a) Power to detect a single risk allele by allele frequency with a type I error rate of 5 10^{-8} for a phenotype with a frequency of 10% in populations of the stated size



n = 400 cases/3600 controls

n = 800 cases/7200 controls

(b) As above, but with the assumed presence of 10 risk alleles, indicating the statistical power to detect at least one significantly associating allele

FIGURE 3.1 Effects of allele frequency, magnitude of effect, sample size and type I error rate on the statistical power of genome-wide association studies

3.2.4 Searching for rare and uncommon risk alleles

The study of uncommon and rare variants is now becoming feasible. Rare variant genotyping arrays that capture the majority of the uncommon SNPs in the genome are now being developed and the costs of high throughput sequencing (next-generation sequencing) bring the costs of targeted and even whole-genome sequencing to levels where it will be possible to sequence thousands of samples. However, there are many issues with data management and analysis and the probability of success of studies aimed at identifying uncommon or rare risk variants is not known.

As with common variants, one of the major problems in the search for uncommon or rare alleles for complex phenotypes is that the statistical power to detect single alleles, even with large sample sizes, is modest. Recently published methods show that the power to detect rare risk variation can be greatly enhanced by combining information across variants in a target region such as a gene, when multiple variants influence phenotype.

The 'cohort allelic sums test' (CAST) (Morgenthaler and Thilly, 2007) and 'combined multivariate and collapsing (CMC) method' (Li and Leal, 2008) use this approach. CAST contrasts the number of individuals with one or more mutations between cases and controls. CMC, like CAST, pools all rare variants which are treated as a single count for analysis with common variants in a multivariate test. The CMC method permits a coherent test for common and rare variants (rare being defined arbitrarily, but usually at 1%).

Madsen and Browning (2009) introduced a non-parametric weighted sum test in which rare variants are grouped according to function (eg gene), and each individual is scored by a weighted sum of the mutation counts. The incorporation of weights improves the power of the test, and would be especially powerful when most of the rare variation is functionally relevant. While each of these rare variant tests differs in form, each seeks to assess the overall genetic burden due to rare variants, hence they are known as 'burden tests'. By design, they implicitly assume that all variation affecting phenotype acts in the same direction (increased risk). However, a gene harbouring phenotypically relevant variation could include a handful of rare Mendelian mutations that cause disease, some variants that moderately increase or decrease risk, along with numerous variants of no effect.

A well-established and powerful test for the presence of a mixture of effect and neutral alleles is the C-alpha score-test (Neyman and Scott, 1966; Zeleterman and Chen, 1988), which has recently been adapted for the analysis of sequence-level, case–control data (Neale et al, 2011). An alternative method proposed by Ionita-Laza and colleagues is based on assessing whether rare variants in a genetic region collectively occur at significantly higher frequencies in cases compared with controls (or vice versa) (Ionita-Laza et al, 2011). A main feature of the proposed methodology is that it is an overall test assessing a possibly large number of rare variants simultaneously, but the disease variants can be both protective and risk variants, with moderate decreases in statistical power when both types of variants are present. Simulations studies have shown that these approaches can be powerful under complex and general disease models, as well as in larger genetic regions where the proportion of disease susceptibility variants may be small. Comparisons with previously published tests on simulated data show that the proposed approaches can have better power than the existing methods.

It is likely that there will be further development of statistical methods for the analysis of sequence data over the next few years. For example, the admixture maximum likelihood (AML) test (Tyrer et al, 2006) was devised as a method for omnibus or 'burden' testing of multiple common genetic variants within a gene or pathway (Pharoah et al, 2007). A method has now been developed for the analysis of uncommon variants and the AML method has been used in the analysis of rare sequence variants identified through resequencing of 13 genes involved in the metabolism of cancer chemotherapy in 250 patients who had developed adverse, chemotherapy-related events after treatment (Pharoah, unpublished data). The AML method can also take account of variants which increase or decrease risk. Until the underlying architecture of uncommon and rare variants for complex disease susceptibility is elucidated, we cannot know for certain what the most powerful statistical method will be for data analysis.

3.2.5 Development of a test to predict radiotherapy toxicity

The ultimate aim of personalised medicine is to develop a predictive test that can discriminate between individuals who will develop the disease of interest and those who will not. There is interest in measuring a patient's radiosensitivity and in using genetic information to develop a test that predicts a patient's likelihood of suffering side-effects. This could be used to individualise radiation dose prescriptions in order to optimise tumour control while minimising normal tissue damage (Barnett et al, 2009). Development of a predictive assay or genetic risk profiles could, therefore, stratify patients into subgroups with different probabilities of developing toxicity; this would permit individualised dose prescription, to increase survival and decrease the morbidity associated with cancer. We are many years away from having a test of radiosensitivity suitable for routine clinical use. In terms of genetics, there is a need to identify genetic variants that are unequivocally associated with differences in radiation toxicity, even if their individual effect size is small.

Genome-wide association studies have proved more effective than candidate gene studies at identifying common genetic variants associated with traits and diseases. The loci identified to date from GWAS that are linked to the risk of breast, prostate, colorectal and lung cancer and melanoma individually confer only a modest risk of malignancy — increasing the relative risk of cancer by less than 50% of the baseline population risk (Easton and Eeles, 2008). In most cases the associated SNP is a tagging SNP rather than a causal variant and so the effect size may be underestimated. However, in many diseases the effects of different loci may be multiplicative (Pharoah et al, 2008).

Predictive clinical models/nomograms are now being developed to provide integrated, patient-tailored, user-friendly and clinically usable tools for predicting radiotherapy toxicity (Dehing-Oberije et al, 2010; Valdagni et al, 2009). These cancer type- or endpoint-specific models integrate currently available clinical factors (eg diabetes, age, smoking and hormonal therapy) and dosimetric factors (mean doses to critical normal tissues) to obtain an estimate of the probability of developing toxicity. These predictive nomograms are closer to clinical use than anything incorporating biological data.

The models could easily be extended to incorporate biomarker/genotyping data. For example, in the paper by Kerns et al (2010) a model using clinical factors only (eg age, stage, radiation dose, hormone use, diabetes and smoking) to predict erectile dysfunction following prostate radiotherapy yielded an

area under the curve of 0.75 but a genetic model based on four SNPs identified in their GWAS yielded an area under the curve of 0.98. This promising finding requires validation in replication studies and extending to other toxicity endpoints and cancer sites. Such work is being facilitated through the Radiogenomics Consortium (West et al, 2010).

The associations found between susceptibility markers and cancer predisposition have been too weak for risk prediction on an individual patient basis. For cancer incidence, the risks conferred by individual loci are small, but risk alleles appear to act multiplicatively (a log-additive model). The increase in risk for the susceptibility alleles at each locus identified by GWAS is generally 1.3-fold or less. Incorporating all known risk alleles and assuming a multiplicative model, the risk predictive power is still limited: the top 1% of the population has a risk that is approximately three-fold for prostate cancer and two-fold for breast cancer when compared to the mean population risk (Pharoah et al, 2008). In breast cancer, it has been suggested that, by targeting women who are at the greatest risk according to genotype, the efficiency of population-based prevention programmes, such as screening mammography, could be improved (Pharoah et al, 2008).

The five significant and replicated hits in the first GWAS of breast cancer incidence (Easton et al, 2007) and two additional susceptibility loci identified in a follow-up study (Ahmed et al, 2009), combined with previously known low penetrant genetic factors, together explain only approximately 6% of the excess familial risk of breast cancer (Moore et al, 2010). This contrasts with *BRCA1* and *BRCA2* mutations that together account for between 20 and 40% of familial breast cancer. However, the predictive power is likely to improve as more variants are found. In addition, risks associated with the presence of a causal variant in a known region of association are likely to be underestimated at present, because most of the causal variants have not been identified.

For radiotherapy toxicity, if 10 confirmed loci were identified, each with a risk allele frequency of 0.25 and a per-allele relative risk of 1.2, a log-normal risk distribution would be generated with a variance of 0.125. About 10% of the population would have a genetic relative risk over1.5. If the number of SNPs was doubled to 20, the top 10% would be at a relative risk of 1.67 or more and the top 5% at a relative risk of two or more (Pharoah 2011, personal communication). Identification of those individuals most at risk of toxicity would mean that radiotherapy could be avoided if possible in this subgroup, a dose reduction may be appropriate, or a hyperfractionated treatment regime followed to utilise the toxicity-sparing effect of smaller doses per fraction. The use of intensity modulated radiotherapy or image guided radiotherapy (IMRT or IGRT) would be appropriate to minimise the volume of normal tissue irradiated. In addition, the dose to the remaining population could be increased to maintain current levels of toxicity, perhaps with the addition of novel therapies, thereby increasing the chance of local control and cure (Burnet et al, 1996). This is illustrated in Figure 3.2.

The predictive value of a large number of multiple weak susceptibility variants may not easily improve (Janssens and van Duijn, 2008). This is because when multiple genes are considered, all individuals will carry at least one or more risk genotypes, even those individuals with lower than average risk of disease. In addition, individuals with the same number of risk genotypes may have differing risks of disease due to differences in effect sizes between loci. Furthermore, individuals will have a varying degree of protective genotypes which may outweigh the effect of the risk genotypes. Even if sufficient common risk variants could be identified, individual profiles will be based on different combinations of multiple variants and so each combination will be extremely rare, and therefore of limited use for the prediction of common disease.

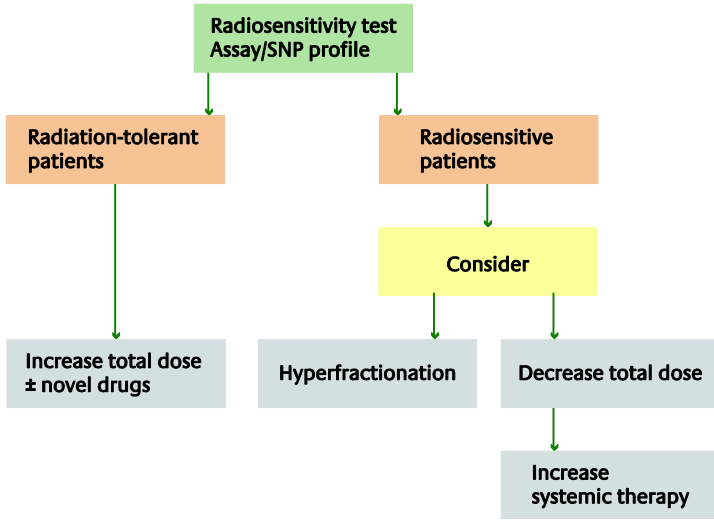


FIGURE 3.2 Potential utility of a test to predict radiotherapy toxicity

Genetic variants that predict for intermediate factors that cause disease will not remain significant when they are combined in multivariate analysis with these factors. For example, genetic variants that predict for dyslipidaemia or hypertension may predict for the development of cardiovascular disease, whilst genetic factors that predict for breast volume may predict for late radiotherapy toxicity on univariate but not multivariate analysis. Therefore genetic variants may improve disease prediction beyond traditional risk factors when they are involved in unknown pathways or in pathways with immeasurable intermediate factors. This is true for radiosensitivity, for which there are insufficient defined risk factors that predict a patient's likelihood of developing toxicity. It is possible that gene discoveries may identify novel aetiological pathways and novel intermediate biomarkers, which may be stronger predictors of disease than the genetic variants that led to its identification.

The mechanisms of radiation toxicity are poorly understood, but insight may be gained into its pathogenesis if new genetic loci predicting radiosensitivity can be identified. Measures to reduce the incidence of toxicity could follow, which could include the development of targeted drugs to prevent or abrogate radiation effects.

3.3 Summary

Studies of radiotherapy patients with severe normal tissue toxicity show there is heterogeneity in cellular radiosensitivity.

There is some evidence that laboratory measurements of radiosensitivity can predict for clinical radiosensitivity but the lack of assay standardisation, lack of replication and poor study design make it impossible to draw any firm conclusions.

Progress in the area requires standardisation of assays, reporting guidelines, larger collaborative multi-centre studies and identification of better predictive markers.

Results from genome-wide association studies are starting to emerge but currently there are no confirmed variants associated with radiotherapy toxicity.

3.4 References

Ahmed S, Thomas G, Ghoussaini M, Healey CS, Humphreys MK, Platte R, et al (2009). Newly discovered breast cancer susceptibility loci on 3p24 and 17q23.2. *Nat Genet,* **41**, 585–90.

Akimoto T, Muramatsu H, Takahashi M, Saito J, Kitamoto Y, Harashima K, et al (2004). Rectal bleeding after hypofractionated radiotherapy for prostate cancer: correlation between clinical and dosimetric parameters and the incidence of grade 2 or worse rectal bleeding. *Int J Radiat Oncol Biol Phys,* **60**, 1033–9.

Alapetite C, Thirion P, de la Rochefordiere A, Cosset JM and Moustacchi E (1999). Analysis by alkaline comet assay of cancer patients with severe reactions to radiotherapy: defective rejoining of radioinduced DNA strand breaks in lymphocytes of breast cancer patients. *Int J Cancer,* **83**, 83–90.

Alsner J, Andreassen CN and Overgaard J (2008). Genetic markers for prediction of normal tissue toxicity after radiotherapy. *Semin Radiat Oncol,* **18**, 126–35.

Arlett CF and Priestley A (1983). Defective recovery from potentially lethal damage in some human fibroblast cell strains. *Int J Radiat Biol,* **43**, 157–67.

Azria D, Ozsahin M, Kramar A, Peters S, Atencio DP, Crompton NE, et al (2008). Single nucleotide polymorphisms, apoptosis, and the development of severe late adverse effects after radiotherapy. *Clin Cancer Res,* **14**, 6284–8.

Back M, Guerrieri M, Wratten C and Steigler A (2004). Impact of radiation therapy on acute toxicity in breast conservation therapy for early breast cancer. *Clin Oncol (R Coll Radiol),* **16**, 12–16.

Badie C, Dziwura S, Raffy C, Tsigani T, Alsbeih G, Moody J, et al (2008). Aberrant CDKN1A transcriptional response associates with abnormal sensitivity to radiation treatment. *Br J Cancer,* **98**, 1845–51.

Barber JB, Burrill W, Spreadborough AR, Levine E, Warren C, Kiltie AE, et al (2000a). Relationship between *in vitro* chromosomal radiosensitivity of peripheral blood lymphocytes and the expression of normal tissue damage following radiotherapy for breast cancer. *Radiother Oncol,* **55**, 179–86.

Barber JB, West CM, Kiltie AE, Roberts SA and Scott D (2000b). Detection of individual differences in radiation-induced apoptosis of peripheral blood lymphocytes in normal individuals, ataxia telangiectasia homozygotes and heterozygotes, and breast cancer patients after radiotherapy. *Radiat Res,* **153**, 570–78.

Barnett GC, West CM, Dunning AM, Elliott RM, Coles CE, Pharoah PD and Burnet NG (2009). Normal tissue reactions to radiotherapy: towards tailoring treatment dose by genotype. *Nat Rev Cancer,* **9**, 134–42.

Barnett GC, De Meerleer G, Gulliford SL, Sydes MR, Elliott RM and Dearnaley DP (2011a). The impact of clinical factors on the development of late radiation toxicity: results from the Medical Research Council RT01 trial (ISRCTN47772397). *Clin Oncol (R Coll Radiol),* **23**, 613–24.

Barnett GC, West CM, Coles CE, Pharoah PD, Talbot CJ, Elliott RM, et al (2011b). Standardized total average toxicity score: a scale- and grade-independent measure of late radiotherapy toxicity to facilitate pooling of data from different studies. *Int J Radiat Oncol Biol Phys,* **82**(3), 1065–74.

Barnett GC, Wilkinson JS, Moody AM, Wilson CB, Twyman N, Wishart GC, et al (2011c). The Cambridge Breast Intensity-modulated Radiotherapy Trial: patient- and treatment-related factors that influence late toxicity. *Clin Oncol (R Coll Radiol),* **23,** 662–73.

Barnett GC, Wilkinson JS, Moody AM, Wilson CB, Twyman N, Wishart GC, et al (2011d). Randomized controlled trial of forward-planned intensity modulated radiotherapy for early breast cancer: interim results at 2 years. *Int J Radiat Oncol Biol Phys,* **82**, 715–23.

Barnett GC, Coles CE, Elliott RM, Baynes C, Luccarini C, Conroy D, et al (2012). Independent validation of genes and polymorphisms reported to be associated with radiation toxicity: a prospective analysis study. *Lancet Oncol,* **13**, 65–77.

Begg AC, Russell NS, Knaken H and Lebesque JV (1993). Lack of correlation of human fibroblast radiosensitivity *in vitro* with early skin reactions in patients undergoing radiotherapy. *Int J Radiat Biol,* **64**, 393–405.

Bentzen SM (2006). Preventing or reducing late side effects of radiation therapy: radiobiology meets molecular pathology. *Nat Rev,* **6**, 702–13.

Bentzen SM and Overgaard M (1991). Relationship between early and late normal-tissue injury after postmastectomy radiotherapy. *Radiother Oncol,* **20**, 159–65.

Bentzen SM and Overgaard M (1993). Early and late normal tissue injury after postmastectomy radiotherapy. *Recent Results Cancer Res (Fortschritte der Krebsforschung),* **130**, 59–78.

Bentzen SM and Overgaard J (1994). Patient-to-patient variability in the expression of radiation-induced normal tissue injury. *Semin Radiat Oncol,* **4**, 68–80.

Bentzen SM, Overgaard M and Thames HD (1989). Fractionation sensitivity of a functional endpoint: impaired shoulder movement after post-mastectomy radiotherapy. *Int J Radiat Oncol Biol Phys,* **17**, 531–7.

Bentzen SM, Skoczylas JZ, Overgaard M and Overgaard J (1996). Radiotherapy-related lung fibrosis enhanced by tamoxifen. *J Natl Cancer Inst,* **88**, 918–22.

Bentzen SM, Rosenthal DI, Weymuller EA and Trotti A (2007). Increasing toxicity in nonoperative head and neck cancer treatment: investigations and interventions. *Int J Radiat Oncol Biol Phys,* **69**, S79–82.

Bernier J, Domenge C, Ozsahin M, Matuszewska K, Lefebvre JL, Greiner RH, et al (2004). Postoperative irradiation with or without concomitant chemotherapy for locally advanced head and neck cancer. *N Engl J Med,* **350**, 1945–52.

Bernstein JL, Haile RW, Stovall M, Boice JD Jr, Shore RE, Langholz B, et al (2010). Radiation exposure, the *ATM* gene, and contralateral breast cancer in the women's environmental cancer and radiation epidemiology study. *J Natl Cancer Inst,* **102**, 475–83.

Berrington de Gonzalez A, Curtis RE, Kry SF, Gilbert E, Lamart S, Berg CD, et al (2011). Proportion of second cancers attributable to radiotherapy treatment in adults: a cohort study in the US SEER cancer registries. *Lancet Oncol,* **12**, 353–60.

Best T, Li D, Skol AD, Kirchhoff T, Jackson SA, Yasui Y, et al (2011). Variants at 6q21 implicate PRDM1 in the etiology of therapy-induced second malignancies after Hodgkin's lymphoma. *Nat Med,* **17**, 941–3.

Bhandare N, Antonelli PJ, Morris CG, Malayapa RS and Mendenhall WM (2007). Ototoxicity after radiotherapy for head and neck tumors. *Int J Radiat Oncol Biol Phys,* **67**, 469–79.

Bhatti P, Veiga LH, Ronckers CM, Sigurdson AJ, Stovall M, Smith SA, et al (2010). Risk of second primary thyroid cancer after radiotherapy for a childhood cancer in a large cohort study: an update from the childhood cancer survivor study. *Radiat Res,* **174**, 741–52.

Bordon E, Henriquez-Hernandez LA, Lara PC, Ruiz A, Pinar B, Rodriguez-Gallego C and Lloret M (2010). Prediction of clinical toxicity in locally advanced head and neck cancer patients by radio-induced apoptosis in peripheral blood lymphocytes (PBLs). *Radiat Oncol,* **5**, 4.

Bordon E, Henriquez-Hernandez LA, Lara PC, Pinar B, Rodriguez-Gallego C and Lloret M (2011). Role of CD4 and CD8 T-lymphocytes, B-lymphocytes and natural killer cells in the prediction of radiation-induced late toxicity in cervical cancer patients. *Int J Radiat Biol,* **87**, 424–31.

Borgmann K, Roper B, El-Awady R, Brackrock S, Bigalke M, Dork T, et al (2002). Indicators of late normal tissue response after radiotherapy for head and neck cancer: fibroblasts, lymphocytes, genetics, DNA repair, and chromosome aberrations. *Radiother Oncol,* **64**, 141–52.

Bosset JF, Collette L, Calais G, Mineur L, Maingon P, Radosevic-Jelic L, et al (2006). Chemotherapy with preoperative radiotherapy in rectal cancer. *N Engl J Med,* **355**, 1114–23.

Bourton EC, Plowman PN, Smith D, Arlett CF and Parris CN (2011). Prolonged expression of the gamma-H2AX DNA repair biomarker correlates with excess acute and chronic toxicity from radiotherapy treatment. *Int J Cancer,* **129**, 2928–34.

Bowden SJ, Fernando IN and Burton A (2006). Delaying radiotherapy for the delivery of adjuvant chemotherapy in the combined modality treatment of early breast cancer: is it disadvantageous and could combined treatment be the answer? *Clin Oncol (R Coll Radiol),* **18**, 247–56.

Braendengen M, Tveit KM, Berglund A, Birkemeyer E, Frykholm G, Pahlman L, et al (2008). Randomized phase III study comparing preoperative radiotherapy with chemoradiotherapy in nonresectable rectal cancer. *J Clin Oncol,* **26**, 3687–94.

Brock WA, Tucker SL, Geara FB, Turesson I, Wike J, Nyman J and Peters LJ (1995). Fibroblast radiosensitivity *versus* acute and late normal skin responses in patients treated for breast cancer. *Int J Radiat Oncol Biol Phys,* **32**, 1371–9.

Burnet NG, Nyman J, Turesson I, Wurm R, Yarnold JR and Peacock JH (1992). Prediction of normal-tissue tolerance to radiotherapy from in-vitro cellular radiation sensitivity. *Lancet,* **339**, 1570–71.

Burnet NG, Nyman J, Turesson I, Wurm R, Yarnold JR and Peacock JH (1994). The relationship between cellular radiation sensitivity and tissue response may provide the basis for individualising radiotherapy schedules. *Radiother Oncol,* **33**, 228–38.

Burnet NG, Wurm R, Nyman J and Peacock JH (1996). Normal tissue radiosensitivity – how important is it? *Clin Oncol (R Coll Radiol),* **8**, 25–34.

Calais G, Alfonsi M, Bardet E, Sire C, Germain T, Bergerot P, et al (1999). Randomized trial of radiation therapy *versus* concomitant chemotherapy and radiation therapy for advanced-stage oropharynx carcinoma. *J Natl Cancer Inst,* **91**, 2081–6.

Chen AM, Obedian E and Haffty BG (2001). Breast-conserving therapy in the setting of collagen vascular disease. *Cancer J,* **7**, 480–91.

Chen AM, Chen LM, Vaughan A, Farwell DG, Luu Q, Purdy JA and Vijayakumar S (2011). Head and neck cancer among lifelong never-smokers and ever-smokers: matched-pair analysis of outcomes after radiation therapy. *Am J Clin Oncol,* **34**, 270–75.

Chon BH and Loeffler JS (2002). The effect of nonmalignant systemic disease on tolerance to radiation therapy. *Oncologist,* **7**, 136–43.

Collette S, Collette L, Budiharto T, Horiot JC, Poortmans PM, Struikmans H, et al (2008). Predictors of the risk of fibrosis at 10 years after breast conserving therapy for early breast cancer: a study based on the EORTC Trial 22881-10882 'boost *versus* no boost'. *Eur J Cancer,* **44**, 2587–99.

Cooper JS, Pajak TF, Forastiere AA, Jacobs J, Campbell BH, Saxman SB, et al (2004). Postoperative concurrent radiotherapy and chemotherapy for high-risk squamous-cell carcinoma of the head and neck. *N Engl J Med,* **350**, 1937–44.

Cozzarini C, Fiorino C, Ceresoli GL, Cattaneo GM, Bolognesi A, Calandrino R and Villa E (2003). Significant correlation between rectal DVH and late bleeding in patients treated after radical prostatectomy with conformal or conventional radiotherapy (66.6–70.2 Gy). *Int J Radiat Oncol Biol Phys,* **55**, 688–94.

Crompton NE, Miralbell R, Rutz HP, Ersoy F, Sanal O, Wellmann D, et al (1999). Altered apoptotic profiles in irradiated patients with increased toxicity. *Int J Radiat Oncol Biol Phys,* **45**, 707–14.

Crompton NE, Shi YQ, Emery GC, Wisser L, Blattmann H, Maier A, et al (2001). Sources of variation in patient response to radiation treatment. *Int J Radiat Oncol Biol Phys,* **49**, 547–54.

Curran WJ Jr, Paulus R, Langer CJ, Komaki R, Lee JS, Hauser S, et al (2011). Sequential *vs* concurrent chemoradiation for stage III non-small cell lung cancer: randomized phase III trial RTOG 9410. *J Natl Cancer Inst,* **103**, 1452–60.

Curwen GB, Winther JF, Tawn EJ, Smart V, Whitehouse CA, Rees GS, et al (2005). G(2) chromosomal radiosensitivity in Danish survivors of childhood and adolescent cancer and their offspring. *Br J Cancer,* **93**, 1038–45.

Curwen GB, Cadwell KK, Winther JF, Tawn EJ, Rees GS, Olsen JH, et al (2010). The heritability of G2 chromosomal radiosensitivity and its association with cancer in Danish cancer survivors and their offspring. *Int J Radiat Biol,* **86**, 986–95.

Dasu A, Toma-Dasu I, Franzen L, Widmark A and Nilsson P (2011). Secondary malignancies from prostate cancer radiation treatment: a risk analysis of the influence of target margins and fractionation patterns. *Int J Radiat Oncol Biol Phys,* **79**, 738–46.

De Ruyck K, Van Eijkeren M, Claes K, Morthier R, De Paepe A, Vral A, et al (2005). Radiation-induced damage to normal tissues after radiotherapy in patients treated for gynecologic tumors: association with single nucleotide polymorphisms in *XRCC1, XRCC3,* and *OGG1* genes and *in vitro* chromosomal radiosensitivity in lymphocytes. *Int J Radiat Oncol Biol Phys,* **62**, 1140–49.

Debus J, Hug EB, Liebsch NJ, O'Farrel D, Finkelstein D, Efird J and Munzenrider JE (1997). Brainstem tolerance to conformal radiotherapy of skull base tumors. *Int J Radiat Oncol Biol Phys,* **39**, 967–75.

Defraene G, Van den Bergh L, Al-Mamgani A, Haustermans K, Heemsbergen W, Van den Heuvel F and Lebesque JV (2012). The benefits of including clinical factors in rectal normal tissue complication probability modeling after radiotherapy for prostate cancer. *Int J Radiat Oncol Biol Phys,* **82**(3), 1233–42.

Dehing-Oberije C, De Ruysscher D, van Baardwijk A, Yu S, Rao B and Lambin P (2009). The importance of patient characteristics for the prediction of radiation-induced lung toxicity. *Radiother Oncol,* **91**, 421–6.

Dehing-Oberije C, De Ruysscher D, Petit S, Van Meerbeeck J, Vandecasteele K, De Neve W, et al (2010). Development, external validation and clinical usefulness of a practical prediction model for radiation-induced dysphagia in lung cancer patients. *Radiother Oncol,* **97**, 455–61.

Denham JW, Hamilton CS, Simpson SA, Ostwald PM, O'Brien M, Kron T, et al (1995). Factors influencing the degree of erythematous skin reactions in humans. *Radiother Oncol,* **36**, 107–20.

Denis F, Garaud P, Bardet E, Alfonsi M, Sire C, Germain T, et al (2003). Late toxicity results of the GORTEC 94-01 randomized trial comparing radiotherapy with concomitant radiochemotherapy for advanced-stage oropharynx carcinoma: comparison of LENT/SOMA, RTOG/EORTC, and NCI-CTC scoring systems. *Int J Radiat Oncol Biol Phys,* **55**, 93–8.

Dickson J, Magee B, Stewart A and West CM (2002). Relationship between residual radiation-induced DNA double-strand breaks in cultured fibroblasts and late radiation reactions: a comparison of training and validation cohorts of breast cancer patients. *Radiother Oncol,* **62**, 321–6.

Dikomey E, Brammer I, Johansen J, Bentzen SM and Overgaard J (2000). Relationship between DNA double-strand breaks, cell killing, and fibrosis studied in confluent skin fibroblasts derived from breast cancer patients. *Int J Radiat Oncol Biol Phys,* **46**, 481–90.

Djuzenova CS, Muhl B, Fehn M, Oppitz U, Muller B and Flentje M (2006). Radiosensitivity in breast cancer assessed by the Comet and micronucleus assays. *Br J Cancer,* **94**, 1194–203.

Doi K, Mieno MN, Shimada Y, Yonehara H and Yoshinaga S (2011). Meta-analysis of second cancer risk after radiotherapy among childhood cancer survivors. *Radiat Prot Dosim,* **146**, 263–7.

Dorr W, Bertmann S and Herrmann T (2005). Radiation induced lung reactions in breast cancer therapy. Modulating factors and consequential effects. *Strahlenther Onkol,* **181**, 567–73.

Easton DF and Eeles RA (2008). Genome-wide association studies in cancer. *Hum Mol Genet,* **17**, R109–15.

Easton DF, Pooley KA, Dunning AM, Pharoah PD, Thompson D, Ballinger DG, et al (2007). Genome-wide association study identifies novel breast cancer susceptibility loci. *Nature,* **4474**(7148), 1087–93.

Eifel PJ, Jhingran A, Bodurka DC, Levenback C and Thames H (2002). Correlation of smoking history and other patient characteristics with major complications of pelvic radiation therapy for cervical cancer. *J Clin Oncol,* **20**, 3651–7.

Epstein JB, Freilich MM and Le ND (1993). Risk factors for oropharyngeal candidiasis in patients who receive radiation therapy for malignant conditions of the head and neck. *Oral Surg Oral Med Oral Pathol,* **76**, 169–74.

Feigenberg SJ, Hanlon AL, Horwitz EM, Uzzo RG, Eisenberg D and Pollack A (2005). Long-term androgen deprivation increases grade 2 and higher late morbidity in prostate cancer patients treated with three-dimensional conformal radiation therapy. *Int J Radiat Oncol Biol Phys,* **62**, 397–405.

Fellin G, Fiorino C, Rancati T, Vavassori V, Baccolini M, Bianchi C, et al (2009). Clinical and dosimetric predictors of late rectal toxicity after conformal radiation for localized prostate cancer: results of a large multicenter observational study. *Radiother Oncol,* **93**, 197–202.

Fine BA, Hempling RE, Piver MS, Baker TR, McAuley M and Driscoll D (1995). Severe radiation morbidity in carcinoma of the cervix: impact of pretherapy surgical staging and previous surgery. *Int J Radiat Oncol Biol Phys,* **31**, 717–23.

Finnon P, Robertson N, Dziwura S, Raffy C, Zhang W, Ainsbury L, et al (2008). Evidence for significant heritability of apoptotic and cell cycle responses to ionising radiation. *Hum Genet,* **123**, 485–93.

Fowble B, Fein DA, Hanlon AL, Eisenberg BL, Hoffman JP, Sigurdson ER, et al (1996). The impact of tamoxifen on breast recurrence, cosmesis, complications, and survival in estrogen receptor-positive early-stage breast cancer. *Int J Radiat Oncol Biol Phys,* **35**, 669–77.

Fritz G, Henninger C and Huelsenbeck J (2011). Potential use of HMG-CoA reductase inhibitors (statins) as radioprotective agents. *Br Med Bull,* **97**, 17–26.

Gagliardi G, Bjohle J, Lax I, Ottolenghi A, Eriksson F, Liedberg A, et al (2000). Radiation pneumonitis after breast cancer irradiation: analysis of the complication probability using the relative seriality model. *Int J Radiat Oncol Biol Phys,* **46**, 373–81.

Gaugler MH, Vereycken-Holler V, Squiban C, Vandamme M, Vozenin-Brotons MC and Benderitter M (2005). Pravastatin limits endothelial activation after irradiation and decreases the resulting inflammatory and thrombotic responses. *Radiat Res,* **163**, 479–87.

Geara FB, Peters LJ, Ang KK, Wike JL and Brock WA (1993). Prospective comparison of *in vitro* normal cell radiosensitivity and normal tissue reactions in radiotherapy patients. *Int J Radiat Oncol Biol Phys,* **27**, 1173–9.

Gerard JP, Conroy T, Bonnetain F, Bouche O, Chapet O, Closon-Dejardin MT, et al (2006). Preoperative radiotherapy with or without concurrent fluorouracil and leucovorin in T3-4 rectal cancers: results of FFCD 9203. *J Clin Oncol,* **24**, 4620–25.

Girard PM, Kysela B, Harer CJ, Doherty AJ and Jeggo PA (2004). Analysis of DNA ligase IV mutations found in LIG4 syndrome patients: the impact of two linked polymorphisms. *Hum Mol Genet,* **13**, 2369–76.

Gold DG, Miller RC, Petersen IA and Osborn TG (2007). Radiotherapy for malignancy in patients with scleroderma: the Mayo Clinic experience. *Int J Radiat Oncol Biol Phys,* **67**, 559–67.

Gold DG, Miller RC, Pinn ME, Osborn TG, Petersen IA and Brown PD (2008). Chronic toxicity risk after radiotherapy for patients with systemic sclerosis (systemic scleroderma) or systemic lupus erythematosus: association with connective tissue disorder severity. *Radiother Oncol,* **87**, 127–31.

Grann A and Wallner K (1998). Prostate brachytherapy in patients with inflammatory bowel disease. *Int J Radiat Oncol Biol Phys,* **40**, 135–8.

Green JA, Kirwan JM, Tierney JF, Symonds P, Fresco L, Collingwood M and Williams CJ (2001). Survival and recurrence after concomitant chemotherapy and radiotherapy for cancer of the uterine cervix: a systematic review and meta-analysis. *Lancet,* **358**, 781–6.

Green S, Stock RG and Greenstein AJ (1999). Rectal cancer and inflammatory bowel disease: natural history and implications for radiation therapy. *Int J Radiat Oncol Biol Phys,* **44**, 835–40.

Harris EE, Christensen VJ, Hwang WT, Fox K and Solin LJ (2005). Impact of concurrent *versus* sequential tamoxifen with radiation therapy in early-stage breast cancer patients undergoing breast conservation treatment. *J Clin Oncol,* **23**, 11–16.

Henriquez Hernandez LA, Lara PC, Pinar B, Bordon E, Rodriguez Gallego C, Bilbao C, et al (2009). Constitutive gene expression profile segregates toxicity in locally advanced breast cancer patients treated with high-dose hyperfractionated radical radiotherapy. *Radiat Oncol,* **4**, 17.

Henriquez-Hernandez LA, Carmona-Vigo R, Pinar B, Bordon E, Lloret M, Nunez MI, et al (2011). Combined low initial DNA damage and high radiation-induced apoptosis confers clinical resistance to long-term toxicity in breast cancer patients treated with high-dose radiotherapy. *Radiat Oncol,* **6**, 60.

Hernando ML, Marks LB, Bentel GC, Zhou SM, Hollis D, Das SK, et al (2001). Radiation-induced pulmonary toxicity: a dose-volume histogram analysis in 201 patients with lung cancer. *Int J Radiat Oncol Biol Phys,* **51**, 650–59.

Herold DM, Hanlon AL and Hanks GE (1999). Diabetes mellitus: a predictor for late radiation morbidity. *Int J Radiat Oncol Biol Phys,* **43**, 475–9.

Ho WK, Wei WI, Kwong DL, Sham JS, Tai PT, Yuen AP and Au DK (1999). Long-term sensorineural hearing deficit following radiotherapy in patients suffering from nasopharyngeal carcinoma: a prospective study. *Head Neck,* **21**, 547–53.

Hoeller U, Borgmann K, Bonacker M, Kuhlmey A, Bajrovic A, Jung H, et al (2003). Individual radiosensitivity measured with lymphocytes may be used to predict the risk of fibrosis after radiotherapy for breast cancer. *Radiother Oncol,* **69**, 137–44.

Holler V, Buard V, Gaugler MH, Guipaud O, Baudelin C, Sache A, et al (2009). Pravastatin limits radiation-induced vascular dysfunction in the skin. *J Invest Dermatol,* **129**, 1280–91.

Holthusen H (1936). Erfahrungen über die Verträglichkeitsgrenze für Röntgenstrahlen and deren Nutzanwendung zur Verhütung von Schäden. *Strahlentherapie,* **57**, 254–69.

Hosking FJ, Feldman D, Bruchim R, Olver B, Lloyd A, Vijayakrishnan J, et al (2011). Search for inherited susceptibility to radiation-associated meningioma by genomewide SNP linkage disequilibrium mapping. *Br J Cancer,* **104**, 1049–54.

Huscher A, Bignardi M, Magri E, Vitali E, Pasinetti N, Costa L, et al (2009). Determinants of small bowel toxicity in postoperative pelvic irradiation for gynaecological malignancies. *Anticancer Res,* **29**, 4821–6.

Ionita-Laza I, Buxbaum JD, Laird NM and Lange C (2011). A new testing strategy to identify rare variants with either risk or protective effect on disease. *PLoS Genet,* **7**, e1001289.

Iraha S, Ogawa K, Moromizato H, Shiraishi M, Nagai Y, Samura H, et al (2007). Radiation enterocolitis requiring surgery in patients with gynecological malignancies. *Int J Radiat Oncol Biol Phys,* **68**, 1088–93.

Iwasaki T, Robertson N, Tsigani T, Finnon P, Scott D, Levine E, et al (2008). Lymphocyte telomere length correlates with *in vitro* radiosensitivity in breast cancer cases but is not predictive of acute normal tissue reactions to radiotherapy. *Int J Radiat Biol,* **84**, 277–84.

Janssens AC and van Duijn CM (2008). Genome-based prediction of common diseases: advances and prospects. *Hum Mol Genet,* **17**, R166–73.

Jenkins P and Welsh A (2011). Computed tomography appearance of early radiation injury to the lung: correlation with clinical and dosimetric factors. *Int J Radiat Oncol Biol Phys,* **81**, 97–103.

Jereczek-Fossa BA, Badzio A and Jassem J (2003). Factors determining acute normal tissue reactions during post-operative radiotherapy in endometrial cancer: analysis of 317 consecutive cases. *Radiother Oncol,* **68**, 33–9.

Jereczek-Fossa BA, Zerini D, Fodor C, Santoro L, Serafini F, Cambria R, et al (2010). Correlation between acute and late toxicity in 973 prostate cancer patients treated with three-dimensional conformal external beam radiotherapy. *Int J Radiat Oncol Biol Phys,* **78**, 26–34.

Jin H, Tucker SL, Liu HH, Wei X, Yom SS, Wang S, et al (2009). Dose-volume thresholds and smoking status for the risk of treatment-related pneumonitis in inoperable non-small cell lung cancer treated with definitive radiotherapy. *Radiother Oncol,* **91**, 427–32.

Johansen J, Bentzen SM, Overgaard J and Overgaard M (1994). Evidence for a positive correlation between *in vitro* radiosensitivity of normal human skin fibroblasts and the occurrence of subcutaneous fibrosis after radiotherapy. *Int J Radiat Biol,* **66**, 407–12.

Johansen J, Bentzen SM, Overgaard J and Overgaard M (1996). Relationship between the *in vitro* radiosensitivity of skin fibroblasts and the expression of subcutaneous fibrosis, telangiectasia, and skin erythema after radiotherapy. *Radiother Oncol,* **40**, 101–9.

Johansson S, Bjermer L, Franzen L and Henriksson R (1998). Effects of ongoing smoking on the development of radiation-induced pneumonitis in breast cancer and oesophagus cancer patients. *Radiother Oncol,* **49**, 41–7.

Jones LA, Scott D, Cowan R and Roberts SA (1995). Abnormal radiosensitivity of lymphocytes from breast cancer patients with excessive normal tissue damage after radiotherapy: chromosome aberrations after low dose-rate irradiation. *Int J Radiat Biol,* **67**, 519–28.

Kahan Z, Csenki M, Varga Z, Szil E, Cserhati A, Balogh A, et al (2007). The risk of early and late lung sequelae after conformal radiotherapy in breast cancer patients. *Int J Radiat Oncol Biol Phys,* **68**, 673–81.

Kerns SL, Ostrer H, Stock R, Li W, Moore J, Pearlman A, et al (2010). Genome-wide association study to identify single nucleotide polymorphisms (SNPs) associated with the development of erectile dysfunction in African-American men after radiotherapy for prostate cancer. *Int J Radiat Oncol Biol Phys,* **78**, 1292–300.

Kiltie AE, Orton CJ, Ryan AJ, Roberts SA, Marples B, Davidson SE, et al (1997). A correlation between residual DNA double-strand breaks and clonogenic measurements of radiosensitivity in fibroblasts from preradiotherapy cervix cancer patients. *Int J Radiat Oncol Biol Phys,* **39**, 1137–44.

Kizer NT, Thaker PH, Gao F, Zighelboim I, Powell MA, Rader JS, et al (2011). The effects of body mass index on complications and survival outcomes in patients with cervical carcinoma undergoing curative chemoradiation therapy. *Cancer,* **117**, 948–56.

Koc M, Polat P and Suma S (2002). Effects of tamoxifen on pulmonary fibrosis after cobalt-60 radiotherapy in breast cancer patients. *Radiother Oncol,* **64**, 171–5.

Koga K, Kusumoto S, Watanabe K, Nishikawa K, Harada K and Ebihara H (1988). Age factor relevant to the development of radiation pneumonitis in radiotherapy of lung cancer. *Int J Radiat Oncol Biol Phys,* **14**, 367–71.

Kong FM, Hayman JA, Griffith KA, Kalemkerian GP, Arenberg D, Lyons S, et al (2006). Final toxicity results of a radiation-dose escalation study in patients with non-small-cell lung cancer (NSCLC): predictors for radiation pneumonitis and fibrosis. *Int J Radiat Oncol Biol Phys,* **65**, 1075–86.

Koper PC, Heemsbergen WD, Hoogeman MS, Jansen PP, Hart GA, Wijnmaalen AJ, et al (2004). Impact of volume and location of irradiated rectum wall on rectal blood loss after radiotherapy of prostate cancer. *Int J Radiat Oncol Biol Phys,* **58**, 1072–82.

Krasin MJ, Hoth KA, Hua C, Gray JM, Wu S and Xiong X (2009). Incidence and correlates of radiation dermatitis in children and adolescents receiving radiation therapy for the treatment of paediatric sarcomas. *Clin Oncol (R Coll Radiol),* **21**, 781–5.

Kucera H, Enzelsberger H, Eppel W, Weghaupt K (1987). The influence of nicotine abuse and diabetes mellitus on the results of primary irradiation in the treatment of carcinoma of the cervix. *Cancer* **60**, 1–4.

Kuoppala T, Maenpaa J, Tomas E, Puistola U, Salmi T, Grenman S, et al (2008). Surgically staged high-risk endometrial cancer: randomized study of adjuvant radiotherapy alone *vs* sequential chemo-radiotherapy. *Gynecol Oncol,* **110**, 190–95.

Kwong DL, Wei WI, Sham JS, Ho WK, Yuen PW, Chua DT, et al (1996). Sensorineural hearing loss in patients treated for nasopharyngeal carcinoma: a prospective study of the effect of radiation and cisplatin treatment. *Int J Radiat Oncol Biol Phys,* **36**, 281–9.

Li B and Leal SM (2008). Methods for detecting associations with rare variants for common diseases: application to analysis of sequence data. *Am J Hum Genet,* **83**, 311–21.

Lilla C, Ambrosone CB, Kropp S, Helmbold I, Schmezer P, von Fournier D, et al (2007). Predictive factors for late normal tissue complications following radiotherapy for breast cancer. *Breast Cancer Res Treat,* **106**, 143–50.

Lin A, Abu-Isa E, Griffith KA and Ben-Josef E (2008). Toxicity of radiotherapy in patients with collagen vascular disease. *Cancer,* **113**, 648–53.

Little JB and Nove J (1990). Sensitivity of human diploid fibroblast cell strains from various genetic disorders to acute and protracted radiation exposure. *Radiat Res,* **123**, 87–92.

Little JB, Nove J, Strong LC and Nichols WW (1988). Survival of human diploid skin fibroblasts from normal individuals after X-irradiation. *Int J Radiat Biol,* **54**, 899–910.

Liu M, Pickles T, Agranovich A, Berthelet E, Duncan G, Keyes M, et al (2004). Impact of neoadjuvant androgen ablation and other factors on late toxicity after external beam prostate radiotherapy. *Int J Radiat Oncol Biol Phys,* **58**, 59–67.

Loeffler JS, Harris JR, Dahlberg WK and Little JB (1990). *In vitro* radiosensitivity of human diploid fibroblasts derived from women with unusually sensitive clinical responses to definitive radiation therapy for breast cancer. *Radiat Res,* **121**, 227–31.

Lönn S, Gilbert ES, Ron E, Smith SA, Stovall M and Curtis RE (2010). Comparison of second cancer risks from brachytherapy and external beam therapy after uterine corpus cancer. *Cancer Epidemiol Biomarkers Prev,* **19**, 464–74.

Lopez E, Guerrero R, Nunez MI, del Moral R, Villalobos M, Martinez-Galan J, et al (2005). Early and late skin reactions to radiotherapy for breast cancer and their correlation with radiation-induced DNA damage in lymphocytes. *Breast Cancer Res,* **7**, R690–98.

McGough C, Baldwin C, Norman A, Frost G, Blake P, Tait D, et al (2006). Is supplementation with elemental diet feasible in patients undergoing pelvic radiotherapy? *Clin Nutr,* **25**, 109–16.

Madsen BE and Browning SR (2009). A groupwise association test for rare mutations using a weighted sum statistic. *PLoS Genet,* **5**, e1000384.

Mak AC, Rich TA, Schultheiss TE, Kavanagh B, Ota DM and Romsdahl MM (1994). Late complications of postoperative radiation therapy for cancer of the rectum and rectosigmoid. *Int J Radiat Oncol Biol Phys,* **28**, 597–603.

Malaise EP, Fertil B, Deschavanne PJ, Chavaudra N and Brock WA (1987). Initial slope of radiation survival curves is characteristic of the origin of primary and established cultures of human tumor cells and fibroblasts. *Radiat Res,* **111**, 319–33.

Mantz CA, Nautiyal J, Awan A, Kopnick M, Ray P, Kandel G, et al (1999). Potency preservation following conformal radiotherapy for localized prostate cancer: impact of neoadjuvant androgen blockade, treatment technique, and patient-related factors. *Cancer J Sci Am,* **5**, 230–36.

Mayahara H, Murakami M, Kagawa K, Kawaguchi A, Oda Y, Miyawaki D, et al (2007). Acute morbidity of proton therapy for prostate cancer: the Hyogo Ion Beam Medical Center experience. *Int J Radiat Oncol Biol Phys,* **69**, 434–43.

Mayer C, Popanda O, Greve B, Fritz E, Illig T, Eckardt-Schupp F, et al (2011). A radiation-induced gene expression signature as a tool to predict acute radiotherapy-induced adverse side effects. *Cancer Lett,* **302**, 20–28.

Merrick GS, Butler WM, Wallner KE, Galbreath RW, Allen ZA, Gutman S and Lief J (2007). Long-term rectal function after permanent prostate brachytherapy. *Cancer J,* **13**, 95–104.

Metzger ML, Hudson MM, Somes GW, Shorr RI, Li CS, Krasin MJ, et al (2006). White race as a risk factor for hypothyroidism after treatment for pediatric Hodgkin's lymphoma. *J Clin Oncol,* **24**, 1516–21.

Michikawa Y, Suga T, Ishikawa A, Hayashi H, Oka A, Inoko H, et al (2010). Genome wide screen identifies microsatellite markers associated with acute adverse effects following radiotherapy in cancer patients. *BMC Med Genet,* **11**, 123.

Monobe M and Ando K (2002). Drinking beer reduces radiation-induced chromosome aberrations in human lymphocytes. *J Radiat Res,* **43**, 237–45.

Monobe M, Koike S, Uzawa A and Ando K (2003). Effects of beer administration in mice on acute toxicities induced by X rays and carbon ions. *J Radiat Res,* **44**, 75–80.

Monson JM, Stark P, Reilly JJ, Sugarbaker DJ, Strauss GM, Swanson SJ, et al (1998). Clinical radiation pneumonitis and radiographic changes after thoracic radiation therapy for lung carcinoma. *Cancer,* **82**, 842–50.

Moore JH, Asselbergs FW and Williams SM (2010). Bioinformatics challenges for genome-wide association studies. *Bioinformatics,* **26**, 445–55.

Morganti AG, Digesu C, Panunzi S, De Gaetano A, Macchia G, Deodato F, et al (2009). Radioprotective effect of moderate wine consumption in patients with breast carcinoma. *Int J Radiat Oncol Biol Phys,* **74**, 1501–5.

Morgenthaler S and Thilly WG (2007). A strategy to discover genes that carry multi-allelic or mono-allelic risk for common diseases: a cohort allelic sums test (CAST). *Mutat Res,* **615**, 28–56.

Morris MM and Powell SN (1997). Irradiation in the setting of collagen vascular disease: acute and late complications. *J Clin Oncol,* **15**, 2728–35.

Morris M, Eifel PJ, Lu J, Grigsby PW, Levenback C, Stevens RE, et al (1999). Pelvic radiation with concurrent chemotherapy compared with pelvic and para-aortic radiation for high-risk cervical cancer. *N Engl J Med,* **340**, 1137–43.

Neale BM, Rivas MA, Voight BF, Altshuler D, Devlin B, Orho-Melander M et al (2011). Testing for an unusual distribution of rare variants. *PLoS Genet,* **7**, e1001322.

Neyman J and Scott E (1966). On the use of c(alpha) optimal tests of composite hypotheses. *Bull Int Stat Inst,* **41**, 477–97.

O'Dell K and Sinha U (2011). Osteoradionecrosis. *Oral Maxillofac Surg Clin North Am,* **23**, 455–64.

Olive PL, Banath JP and Keyes M (2008). Residual gammaH2AX after irradiation of human lymphocytes and monocytes *in vitro* and its relation to late effects after prostate brachytherapy. *Radiother Oncol,* **86**, 336–46.

Olsen NK, Pfeiffer P, Johannsen L, Schroder H and Rose C (1993). Radiation-induced brachial plexopathy: neurological follow-up in 161 recurrence-free breast cancer patients. *Int J Radiat Oncol Biol Phys,* **26**, 43–9.

Oppitz U, Baier K, Wulf J, Schakowski R and Flentje M (2001). The *in vitro* colony assay: a predictor of clinical outcome. *Int J Radiat Biol,* **77**, 105–10.

Oppitz U, Schulte S, Stopper H, Baier K, Muller M, Wulf J, et al (2002). *In vitro* radiosensitivity measured in lymphocytes and fibroblasts by colony formation and comet assay: comparison with clinical acute reactions to radiotherapy in breast cancer patients. *Int J Radiat Biol,* **78**, 611–16.

Palazzi M, Tomatis S, Orlandi E, Guzzo M, Sangalli C, Potepan P, et al (2008). Effects of treatment intensification on acute local toxicity during radiotherapy for head and neck cancer: prospective observational study validating CTCAE, version 3.0, scoring system. *Int J Radiat Oncol Biol Phys,* **70**, 330–37.

Patil N, Crook J, Saibishkumar EP, Aneja M, Borg J, Pond G and Ma C (2009). The effect of obesity on rectal dosimetry after permanent prostate brachytherapy. *Brachytherapy,* **8**, 218–22.

Paulino AC, Ahmed IM, Mai WY and Teh BS (2009). The influence of pretreatment characteristics and radiotherapy parameters on time interval to development of radiation-associated meningioma. *Int J Radiat Oncol Biol Phys,* **75**, 1408–14.

Peacock J, Ashton A, Bliss J, Bush C, Eady J, Jackson C, et al (2000). Cellular radiosensitivity and complication risk after curative radiotherapy. *Radiother Oncol,* **55**, 173–8.

Peeters ST, Hoogeman MS, Heemsbergen WD, Slot A, Tabak H, Koper PC and Lebesque JV (2005). Volume and hormonal effects for acute side effects of rectum and bladder during conformal radiotherapy for prostate cancer. *Int J Radiat Oncol Biol Phys,* **63**, 1142–52.

Peters CA, Cesaretti JA, Stone NN and Stock RG (2006). Low-dose rate prostate brachytherapy is well tolerated in patients with a history of inflammatory bowel disease. *Int J Radiat Oncol Biol Phys,* **66**, 424–9.

Peuckmann V, Ekholm O, Rasmussen NK, Groenvold M, Christiansen P, Moller S, et al (2009). Chronic pain and other sequelae in long-term breast cancer survivors: nationwide survey in Denmark. *Eur J Pain,* **13**, 478–85.

Phan C, Mindrum M, Silverman C, Paris K and Spanos W (2003). Matched-control retrospective study of the acute and late complications in patients with collagen vascular diseases treated with radiation therapy. *Cancer J,* **9**, 461–6.

Pharoah PD, Dunning AM, Ponder BA and Easton DF (2004). Association studies for finding cancer-susceptibility genetic variants. *Nat Rev,* **4**, 850–60.

Pharoah PD, Tyrer J, Dunning AM, Easton DF and Ponder BA (2007). Association between common variation in 120 candidate genes and breast cancer risk. *PLoS Genet,* **3**, e42.

Pharoah PD, Antoniou AC, Easton DF and Ponder BA (2008). Polygenes, risk prediction, and targeted prevention of breast cancer. *N Engl J Med,* **358**, 2796–803.

Pieters RS, Niemierko A, Fullerton BC and Munzenrider JE (2006). Cauda equina tolerance to high-dose fractionated irradiation. *Int J Radiat Oncol Biol Phys,* **64**, 251–7.

Pinar B, Lara PC, Lloret M, Bordon E, Nunez MI, Villalobos M, et al (2007). Radiation-induced DNA damage as a predictor of long-term toxicity in locally advanced breast cancer patients treated with high-dose hyperfractionated radical radiotherapy. *Radiat Res,* **168**, 415–22.

Pinn ME, Gold DG, Petersen IA, Osborn TG, Brown PD and Miller RC (2008). Systemic lupus erythematosus, radiotherapy, and the risk of acute and chronic toxicity: the Mayo Clinic Experience. *Int J Radiat Oncol Biol Phys,* **71**, 498–506.

Plowman PN, Bridges BA, Arlett CF, Hinney A and Kingston JE (1990). An instance of clinical radiation morbidity and cellular radiosensitivity, not associated with ataxia-telangiectasia. *Br J Radiol,* **63**, 624–8.

Poleshuck EL, Katz J, Andrus CH, Hogan LA, Jung BF, Kulick DI and Dworkin RH (2006). Risk factors for chronic pain following breast cancer surgery: a prospective study. *J Pain,* **7**, 626–34.

Popanda O, Marquardt JU, Chang-Claude J and Schmezer P (2009). Genetic variation in normal tissue toxicity induced by ionizing radiation. *Mutat Res,* **667**, 58–69.

Purkey MT, Levine MS, Prendes B, Norman MF and Mirza N (2009). Predictors of aspiration pneumonia following radiotherapy for head and neck cancer. *Ann Otol Rhinol Laryngol,* **118**, 811–16.

Ramaekers BL, Joore MA, Grutters JP, van den Ende P, Jong JD, Houben R, et al (2011). The impact of late treatment-toxicity on generic health-related quality of life in head and neck cancer patients after radiotherapy. *Oral Oncol,* **47**(8), 768–74.

Ramsay J and Birrell G (1995). Normal tissue radiosensitivity in breast cancer patients. *Int J Radiat Oncol Biol Phys,* **31**, 339–44.

Ravasco P, Monteiro-Grillo I, Vidal PM and Camilo ME (2005). Dietary counseling improves patient outcomes: a prospective, randomized, controlled trial in colorectal cancer patients undergoing radiotherapy. *J Clin Oncol,* **23**, 1431–8.

Roberts SA, Spreadborough AR, Bulman B, Barber JB, Evans DG and Scott D (1999). Heritability of cellular radiosensitivity: a marker of low-penetrance predisposition genes in breast cancer? *Am J Hum Genet,* **65**, 784–94.

Robnett TJ, Machtay M, Vines EF, McKenna MG, Algazy KM and McKenna WG (2000). Factors predicting severe radiation pneumonitis in patients receiving definitive chemoradiation for lung cancer. *Int J Radiat Oncol Biol Phys,* **48**, 89–94.

Roeder F, Friedrich J, Timke C, Kappes J, Huber P, Krempien R, et al (2010). Correlation of patient-related factors and dose-volume histogram parameters with the onset of radiation pneumonitis in patients with small cell lung cancer. *Strahlenther Onkol,* **186**, 149–56.

Rudat V, Dietz A, Conradt C, Weber KJ and Flentje M (1997). *In vitro* radiosensitivity of primary human fibroblasts. Lack of correlation with acute radiation toxicity in patients with head and neck cancer. *Radiother Oncol,* **43**, 181–8.

Rudat V, Dietz A, Nollert J, Conradt C, Weber KJ, Flentje M and Wannenmacher M (1999). Acute and late toxicity, tumour control and intrinsic radiosensitivity of primary fibroblasts *in vitro* of patients with advanced head and neck cancer after concomitant boost radiochemotherapy. *Radiother Oncol,* **53**, 233–45.

Russell NS, Grummels A, Hart AA, Smolders IJ, Borger J, Bartelink H and Begg AC (1998). Low predictive value of intrinsic fibroblast radiosensitivity for fibrosis development following radiotherapy for breast cancer. *Int J Radiat Biol,* **73**, 661–70.

Russell NS, Lara PC, Grummels A, Hart AA, Coco-Martin JM, Bartelink H and Begg AC (2000). *In vitro* differentiation characteristics of human skin fibroblasts: correlations with radiotherapy-induced breast fibrosis in patients. *Int J Radiat Biol,* **76**, 231–40.

Ryan JL, Bole C, Hickok JT, Figueroa-Moseley C, Colman L, Khanna RC, et al (2007). Post-treatment skin reactions reported by cancer patients differ by race, not by treatment or expectations. *Br J Cancer,* **97**, 14–21.

Rzeszowska-Wolny J, Palyvoda O, Polanska J, Wygoda A and Hancock R (2008). Relationships between acute reactions to radiotherapy in head and neck cancer patients and parameters of radiation-induced DNA damage and repair in their lymphocytes. *Int J Radiat Biol,* **84**, 635–42.

Safwat A, Bentzen SM, Turesson I and Hendry JH (2002). Deterministic rather than stochastic factors explain most of the variation in the expression of skin telangiectasia after radiotherapy. *Int J Radiat Oncol Biol Phys,* **52**(1), 198–204.

Salama JK, Stenson KM, List MA, Mell LK, Maccracken E, Cohen EE, et al (2008). Characteristics associated with swallowing changes after concurrent chemotherapy and radiotherapy in patients with head and neck cancer. *Arch Otolaryngol Head Neck Surg,* **134**, 1060–65.

Schmitz A, Bayer J, Dechamps N, Goldin L and Thomas G (2007). Heritability of susceptibility to ionizing radiation-induced apoptosis of human lymphocyte subpopulations. *Int J Radiat Oncol Biol Phys,* **68**, 1169–77.

Schnarr K, Boreham D, Sathya J, Julian J and Dayes IS (2009). Radiation-induced lymphocyte apoptosis to predict radiation therapy late toxicity in prostate cancer patients. *Int J Radiat Oncol Biol Phys,* **74**, 1424–30.

Scott D (2004). Chromosomal radiosensitivity and low penetrance predisposition to cancer. *Cytogenet Genome Res,* **104**, 365–70.

Siala W, Mnejja W, Abid M, Ghorbel A, Frikha M and Daoud J (2011). Thyroid toxicity after radiotherapy of nasopharyngeal carcinoma. *Ann Endocrinol (Paris),* **72**, 19–23.

Skwarchuk MW, Jackson A, Zelefsky MJ, Venkatraman ES, Cowen DM, Levegrun S, et al (2000). Late rectal toxicity after conformal radiotherapy of prostate cancer (I): multivariate analysis and dose-response. *Int J Radiat Oncol Biol Phys,* **47**, 103–13.

Smith KC, Hahn GM, Hoppe RT and Earle JD (1980). Radiosensitivity *in vitro* of human fibroblasts derived from patients with a severe skin reaction to radiation therapy. *Int J Radiat Oncol Biol Phys,* **6**, 1573–5.

Smith KJ, Skelton HG, Tuur S, Yeager J, Decker C and Wagner KF (1997). Increased cutaneous toxicity to ionizing radiation in HIV-positive patients. Military Medical Consortium for the Advancement of Retroviral Research (MMCARR). *Int J Dermatol,* **36**, 779–82.

Song DY, Lawrie WT, Abrams RA, Kafonek DR, Bayless TM, Welsh JS and DeWeese TL (2001). Acute and late radiotherapy toxicity in patients with inflammatory bowel disease. *Int J Radiat Oncol Biol Phys,* **51**, 455–9.

Taira AV, Merrick GS, Galbreath RW, Butler WM, Wallner KE, Kurko BS, et al (2009). Erectile function durability following permanent prostate brachytherapy. *Int J Radiat Oncol Biol Phys,* **75**, 639–48.

Taylor AJ, Little MP, Winter DL, Sugden E, Ellison DW, Stiller CA, et al (2010). Population-based risks of CNS tumors in survivors of childhood cancer: the British Childhood Cancer Survivor Study. *J Clin Oncol,* **28**, 5287–93.

Taylor AM, Harnden DG, Arlett CF, Harcourt SA, Lehmann AR, Stevens S and Bridges BA (1975). Ataxia telangiectasia: a human mutation with abnormal radiation sensitivity. *Nature,* **258**, 427–9.

Taylor ME, Perez CA, Halverson KJ, Kuske RR, Philpott GW, Garcia DM, et al (1995). Factors influencing cosmetic results after conservation therapy for breast cancer. *Int J Radiat Oncol Biol Phys,* **31**, 753–64.

Tseng CJ, Chang CT, Lai CH, Soong YK, Hong JH, Tang SG and Hsueh S (1997). A randomized trial of concurrent chemoradiotherapy *versus* radiotherapy in advanced carcinoma of the uterine cervix. *Gynecol Oncol,* **66**, 52–8.

Tsujino K, Hirota S, Endo M, Obayashi K, Kotani Y, Satouchi M, et al (2003). Predictive value of dose-volume histogram parameters for predicting radiation pneumonitis after concurrent chemoradiation for lung cancer. *Int J Radiat Oncol Biol Phys,* **55**, 110–15.

Tucker SL, Dong L, Bosch WR, Michalski J, Winter K, Mohan R, et al (2010). Late rectal toxicity on RTOG 94-06: analysis using a mixture Lyman model. *Int J Radiat Oncol Biol Phys,* **78**, 1253–60.

Tukenova M, Guibout C, Hawkins M, Quiniou E, Mousannif A, Pacquement H, et al (2011). Radiation therapy and late mortality from second sarcoma, carcinoma, and hematological malignancies after a solid cancer in childhood. *Int J Radiat Oncol Biol Phys,* **80**, 339–46.

Turesson I, Nyman J, Holmberg E and Oden A (1996). Prognostic factors for acute and late skin reactions in radiotherapy patients. *Int J Radiat Oncol Biol Phys,* **36**, 1065–75.

Twardella D, Popanda O, Helmbold I, Ebbeler R, Benner A, von Fournier D, et al (2003). Personal characteristics, therapy modalities and individual DNA repair capacity as predictive factors of acute skin toxicity in an unselected cohort of breast cancer patients receiving radiotherapy. *Radiother Oncol,* **69**, 145–53.

Tyrer J, Pharoah PD and Easton DF (2006). The admixture maximum likelihood test: a novel experiment-wise test of association between disease and multiple SNPs. *Genet Epidemiol,* **30**, 636–43.

Valdagni R, Rancati T, Fiorino C, Fellin G, Magli A, Baccolini M, et al (2008). Development of a set of nomograms to predict acute lower gastrointestinal toxicity for prostate cancer 3D-CRT. *Int J Radiat Oncol Biol Phys,* **71**, 1065–73.

Valdagni R, Rancati T and Fiorino C (2009). Predictive models of toxicity with external radiotherapy for prostate cancer: clinical issues. *Cancer,* **115**, 3141–9.

Vale C (2008). Reducing uncertainties about the effects of chemoradiotherapy for cervical cancer: individual patient data meta-analysis. *Cochrane Database of Systematic Reviews (Online)*, CD008285.

van der Voet JC, Keus RB, Hart AA, Hilgers FJ and Bartelink H (1998). The impact of treatment time and smoking on local control and complications in T1 glottic cancer. *Int J Radiat Oncol Biol Phys,* **42**, 247–55.

Varga Z, Cserhati A, Kelemen G, Boda K, Thurzo L and Kahan Z (2011). Role of systemic therapy in the development of lung sequelae after conformal radiotherapy in breast cancer patients. *Int J Radiat Oncol Biol Phys,* **80**, 1109–16.

Vavassori V, Fiorino C, Rancati T, Magli A, Fellin G, Baccolini M, et al (2007). Predictors for rectal and intestinal acute toxicities during prostate cancer high-dose 3D-CRT: results of a prospective multicenter study. *Int J Radiat Oncol Biol Phys,* **67**, 1401–10.

Wazer DE, DiPetrillo T, Schmidt-Ullrich R, Weld L, Smith TJ, Marchant DJ and Robert NJ (1992). Factors influencing cosmetic outcome and complication risk after conservative surgery and radiotherapy for early-stage breast carcinoma. *J Clin Oncol,* **10**, 356–63.

Wedlake LJ, Thomas K, Lalji A, Blake P, Khoo VS, Tait D and Andreyev HJ (2010). Predicting late effects of pelvic radiotherapy: is there a better approach? *Int J Radiat Oncol Biol Phys,* **78**, 1163–70.

Wennberg B, Gagliardi G, Sundbom L, Svane G and Lind P (2002). Early response of lung in breast cancer irradiation: radiologic density changes measured by CT and symptomatic radiation pneumonitis. *Int J Radiat Oncol Biol Phys,* **52**, 1196–206.

Werbrouck J, De Ruyck K, Beels L, Vral A, Van Eijkeren M, De Neve W and Thierens H (2010). Prediction of late normal tissue complications in RT treated gynaecological cancer patients: potential of the gamma-H2AX foci assay and association with chromosomal radiosensitivity. *Oncol Rep,* **23**, 571–8.

Werbrouck J, Duprez F, De Neve W and Thierens H (2011). Lack of a correlation between gammaH2AX foci kinetics in lymphocytes and the severity of acute normal tissue reactions during IMRT treatment for head and neck cancer. *Int J Radiat Biol,* **87**, 46–56.

Werner RS, McCormick B, Petrek J, Cox L, Cirrincione C, Gray JR and Yahalom J (1991). Arm edema in conservatively managed breast cancer: obesity is a major predictive factor. *Radiology,* **180**, 177–84.

West CM and Barnett GC (2011). Genetics and genomics of radiotherapy toxicity: towards prediction. *Genome Med,* **3**, 52.

West CM, Davidson SE, Elyan SA, Valentine H, Roberts SA, Swindell R and Hunter RD (2001). Lymphocyte radiosensitivity is a significant prognostic factor for morbidity in carcinoma of the cervix. *Int J Radiat Oncol Biol Phys,* **51**, 10–15.

West C, Rosenstein BS, Alsner J, Azria D, Barnett G, Begg A, et al (2010). Establishment of a radiogenomics consortium. *Int J Radiat Oncol Biol Phys,* **76**, 1295–6.

Whitney CW, Sause W, Bundy BN, Malfetano JH, Hannigan EV, Fowler WC Jr, et al (1999). Randomized comparison of fluorouracil plus cisplatin *versus* hydroxyurea as an adjunct to radiation therapy in stage IIB–IVA carcinoma of the cervix with negative para-aortic lymph nodes: a Gynecologic Oncology Group and Southwest Oncology Group study. *J Clin Oncol,* **17**, 1339–48.

Willett CG, Ooi CJ, Zietman AL, Menon V, Goldberg S, Sands BE and Podolsky DK (2000). Acute and late toxicity of patients with inflammatory bowel disease undergoing irradiation for abdominal and pelvic neoplasms. *Int J Radiat Oncol Biol Phys,* **46**, 995–8.

Wolff HA, Conradi LC, Schirmer M, Beissbarth T, Sprenger T, Rave-Frank M, et al (2011). Gender-specific acute organ toxicity during intensified preoperative radiochemotherapy for rectal cancer. *Oncologist,* **16,** 621–31.

Woods WG, Byrne TD and Kim TH (1988). Sensitivity of cultured cells to gamma radiation in a patient exhibiting marked *in vivo* radiation sensitivity. *Cancer,* **62,** 2341–5.

Wu X, Spitz MR, Amos CI, Lin J, Shao L, Gu J, et al (2006). Mutagen sensitivity has high heritability: evidence from a twin study. *Cancer Res,* **66,** 5993–6.

Zelefsky MJ, Cowen D, Fuks Z, Shike M, Burman C, Jackson A, et al (1999). Long term tolerance of high dose three-dimensional conformal radiotherapy in patients with localized prostate carcinoma. *Cancer,* **85**, 2460–68.

Zeleterman D and Chen C (1988). Homogeneity tests against central-mixture alternatives. *J Am Stat Assoc,* **83,** 179–82.

Zevallos JP, Mallen MJ, Lam CY, Karam-Hage M, Blalock J, Wetter DW, et al (2009). Complications of radiotherapy in laryngopharyngeal cancer: effects of a prospective smoking cessation program. *Cancer,* **115,** 4636–44.

Zhao L, Wang L, Ji W, Wang X, Zhu X, Hayman JA, et al (2009). Elevation of plasma TGF-beta1 during radiation therapy predicts radiation-induced lung toxicity in patients with non-small-cell lung cancer: a combined analysis from Beijing and Michigan. *Int J Radiat Oncol Biol Phys,* **74**, 1385–90.

Zimmermann JS, Kumpf L and Kimmig B (1998). Variability of individual normal tissue radiation sensitivity. An international empirical evaluation of endogenous and exogenous response modifiers. *Strahlenther Onkol,* **174**(Suppl 3), 16–19.

4 Radiosensitivity in Animals

In this chapter evidence from animal studies is reviewed for radiosensitivity at the level of the whole organism and for radiation-induced cancer.

4.1 Variation in acute whole organism radiosensitivity

There is genetic variation among strains of the same species. The mouse is the species where there is the greatest amount of evidence regarding individual variation in radiosensitivity, because of the large number of well-defined strains available. An isogenic strain (genetically identical) consists of multiple copies or clones of the same individual, and mice of particular strains can be produced in large numbers. The measurement of radiosensitivity using a specified endpoint *in vivo* requires a dose-response curve, with groups of isogenic mice exposed to different doses.

Several mouse strains were established in the early 1900s. A strain is regarded as inbred when it has been mated brother/sister for 20 or more consecutive generations, and can be traced to a single ancestral breeding pair in the 20th or a subsequent generation. At 20 generations, on average at least 98.6% of the loci in each mouse are homozygous. Many strains have been bred for more than 150 generations and are essentially homozygous at all loci. The first inbred mouse strain, DBA (which has the coat colour alleles, dilute, *d*, brown, *b*, and non-agouti, *a*), was started in 1909. Other inbred strains were generated over the next decade, including C57BL, C3H, CBA and BALB/c (Beck et al, 2000).

One widely used endpoint in the past was $LD_{50/30}$ (the radiation dose resulting in death or survival of 50% of animals within 30 days), and this endpoint for a single animal refers to haemopoietic failure/death or recovery/survival. Other endpoints have been used as well, but not in as many studies as with $LD_{50/30}$. These past studies have provided evidence of the amount of variation in radiosensitivity among strains of a single species. $LD_{50/30}$ can be subject to various confounding influences, which need to be considered when measurements of radiosensitivity are being compiled from different institutions. An important factor is their microbial status. Germ-free or specific-pathogen-free (spf) mice generally are more radioresistant than mice commonly called 'conventionally-housed' mice. The latter mice are nearer to man regarding microbial status than are spf mice, but their use declined in the 1990s in favour of the spf standard, and this corresponded with a decline in the use of the $LD_{50/30}$ assay which required large numbers of mice. Hence a review of values of $LD_{50/30}$ for different strains needs to look back to publications mainly from the 1950s to the 1980s. Also, various radiation sources were used, mainly 200–300 kVp X-rays, megavoltage X-rays or ^{60}Co gamma rays. These vary within about 10% in biological efficiency. In addition, because of the variability in response between the same strains in different

institutions, one common strain needs to be used as the baseline in each institution, and the response of other strains compared with reference to that strain. The determination of $LD_{50/30}$ was usually based on the response of 100–500 mice divided into various dose groups, and hence 95% confidence intervals on the values were small, within a few per cent.

Examples of the variation in strain radiosensitivity can be found in a review by Carsten (1984). He refers to studies by Kallman and Kohn (1956), where the ratios of $LD_{50/28}$, with reference to a baseline of 1.0 for C57BL mice, were 1.08 (C3H), 1.02 (A/HE) and 0.88 (BALB/c). Hence C3H mice were about 8% more resistant, and BALB/c mice about 12% more sensitive, than C57BL mice. Other values with reference to C57BL/6 mice were 0.94 (C3H/HE), 0.88 (A), 0.79 (BALB/c) and 1.08 (C57L/HE, a strain that shares a common origin with C57BL) (Grahn and Hamilton, 1957). Further reported values were 0.96 (A), 0.87 (BALB/c), 1.03 (CBA), 1.00 (C3HeB/Fe), 1.10 (C57BR/cd), 1.07 (RF), 1.16 (SJL) and 0.92 (SWR) (Yuhas and Storer, 1969). Hence the overall range in radiosensitivity among these various strains is about 30%, with C57BL being around the middle, using the $LD_{50/30}$ endpoint.

Later, 37 sets of primary data for mice in the early literature, where sample sizes and doses were available, were re-analysed by Hendry and Roberts (1990). The model used was Poisson-based, fitting ln(number of target-cell groups or tissue-rescuing units, TRU), and the D_0 for these TRU was deduced from the slope of the dose-response curve. (The model was based on the assumption that animal death results from the depletion of target cells below a critical number. If that number is a fraction, $1/K$, of the 'effective' original number at risk, then there are effectively K such groups of target cells in the unirradiated animal. The value of K at zero dose can be calculated by back extrapolation of the modelled transform of the dose-response curve, and the slope, D_0, for the TRU is assumed to parallel that for the target cells.) The conclusion was that the variations in TRU number between strains and datasets were greater than the variations in D_0. The average value of D_0 was 1.0 Gy, similar to many reported values for haemopoietic stem cells measured using colony assays. The TRU number is related to the relative tolerance of different strains to target cell depletion, and this is influenced also by different housing conditions in different institutions. For example, the bacterial status is an important variable, and germ-free mice of the Swiss-Webster strain were more resistant ($LD_{50/30}$) by 7% compared to 'conventionally-housed' mice of the same strain in the same institution (Wilson, 1963). Also, over a period of eight years in one institution, the dose required to reduce endogenous spleen colony counts to low levels in mice irradiated to deplete haemopoiesis in order to receive marrow grafts in transplantation studies, increased by 20% or more (Lord et al, 1984). The change occurred over the same period as an *increase* in the sensitivity of femoral CFU-S (Colony Forming Units in Spleen, now referred to as Multi-Potential Progenitor cells) to gamma rays by around 20%, but a *decrease* in sensitivity to X-rays (Hendry and Lord, 1983). A reduced differential in response to these two radiation types was known to apply to CFU-S that were rapidly cycling compared to their more-common quiescent state. Hence, changes in the basal cycling rates of CFU-S in mice over a period of time, potentially associated with variations in housing conditions, were considered a possible source of these changes. This reinforces the need to use radiosensitivity ratios within institutions, and well-controlled housing conditions, when comparing the responses of different strains.

The above variations in $LD_{50/30}$ are genetic in nature, because the radiosensitivity ratios were assessed in the same conditions within each institution. An example is that the strain DBA/2 has a rare allele within *Trp53.* This gene encodes transformation-related protein p53, which responds to diverse cellular stresses

to regulate target genes that induce cell cycle arrest, apoptosis, senescence, DNA repair or changes in metabolism. The strain BALB/c possesses two polymorphisms in the coding region of the *Prkdc* gene, which encodes the p450 DNA-dependent protein-kinase catalytic subunit, a component of the DNA-PK holoenzyme complex sensing DNA double strand breaks. It should be noted that the changes in radiosensitivity caused by these mutations are not restricted to the haemopoietic system, and most systems of the body are affected to various extents. An example is the *scid* (severe combined immune deficient) mouse of BALB/c origin, where there is an extra mutation in the *Prkdc* gene, which results in a truncated protein with drastically impaired kinase activity that virtually inactivates the function of the DNA-PK complex. This provides a large amount of sensitisation by around two-fold, which can be quantified accurately. The amount of radiosensitisation due to the *scid* mutation was positive in all tissues tested using colony techniques, and was greater in epithelial cells of the intestine and kidney than in haemopoietic and fibroblastoid cells in the bone marrow (Hendry and Jiang, 1994).

The importance of different environments and epigenetic influences can be seen in the $LD_{50/30}$ values for defined strains of mice housed in non-spf conditions, irradiated with orthovoltage X-rays at high dose rate in different institutions. Examples in the scientific literature around the year 1960 of $LD_{50/30}$ values (with uncertainties of a few per cent) for C57BL/6 mice can be found of 6.2, 6.3 and 8.0 Gy and, for more-radiosensitive Balb/c mice, of 4.5, 5.0 and 5.5 Gy (references can be found in Hendry and Roberts, 1990).

Variations in strain radiosensitivity are present in other species as well as mouse. Baverstock et al (1985) collected several primary datasets for $LD_{50/30}$ results using outbred swine, dog, monkey, sheep, goat and burro, using two dose rate ranges as criteria, in order to calculate LD_{95} and LD_{05} values for implications for human exposure. In general, $LD_{50/30}$ decreases with an increase in body size, indicating a lower tolerance to cell depletion in the larger animals (UNSCEAR, 1988). The datasets were re-analysed using the target cell number and radiosensitivity model described above (Hendry and Roberts, 1990). It was concluded that fitting with a common target cell number was compatible statistically with all the data for the different species, but a common D_0 was not acceptable. Differences in D_0 include both inter-strain and inter-species differences.

There is evidence that individual variation in susceptibility to radiation-induced normal-tissue injury is genetically controlled and heritable, from studies using inbred mouse strains. A genetic model for pulmonary fibrosis was developed from the fibrosis-prone C57BL/6J and the fibrosis-resistant C3HfIKam mouse strains. Inheritance of the fibrotic phenotype was characterised in Fl and F2 generations derived from the parental strains. Genetic mapping was used to determine whether the quantitative trait loci (QTL), which influence susceptibility to bleomycin-induced lung fibrosis in these progenitor strains, could be implicated in susceptibility to radiation-induced lung fibrosis (Haston and Travis, 1997). F2 inter-crossed mice, of fibrosis-prone and fibrosis-resistant phenotype, were genotyped with markers at the bleomycin loci on chromosomes 11 and 17 (the chromosome 17 marker is at the major histocompatibility complex). Genetic linkage was established for the marker on chromosome 17, which accounted for 6.6% of the F2 phenotypic variance, but not for the markers surrounding the QTL on chromosome 11. The inheritance data suggested that susceptibility to radiation-induced pulmonary fibrosis is a heritable trait controlled by two genetic loci, and through genomic mapping, a QTL on chromosome 17 was identified as one of the loci.

4.1.1 Summary

The overall range in radiosensitivity ($LD_{50/30}$) among various mouse strains is +/−15% about the mean, believed to be largely due to genetic factors.

The importance of non-genetic influences can also be seen in the $LD_{50/30}$ values for defined strains of mice housed in non-spf conditions in different institutions.

Mutations can cause many tissues to be variously more sensitive, eg mutations in a damage-sensing gene (*Prkdc*) sensitise epithelial cells more than haemopoietic or fibroblastoid cells.

Individual variation in susceptibility to radiation-induced lung injury is, at least in part, genetically controlled and heritable, shown in studies using cross-breeding and genetic linkage studies using susceptible and resistant inbred mouse strains.

Animal species larger than mouse also show inter-strain and inter-species variations in radiosensitivity, and hence individual human variation is expected.

4.2 Animal studies of sensitivity to radiation-induced cancer

The incidence of radiation-induced cancers in different mouse strains has been studied, which provides evidence of genetic influences in radiation carcinogenesis in a single animal species. Storer et al (1988) used four strains, and in unirradiated mice observed over a lifetime there was a preponderance of reticulum cell sarcomas in female BALB, female and male C57BL/6, and female RFM mice, and liver tumours in C3H males but not so much in females (see Table 4.1).

TABLE 4.1 Incidence of nine types of fatal neoplasms (% ± SE) in control mice and age ranges used in the correction procedures (from Storer et al, 1988, with permission)

	Strain, sex (F, female; M, male) and age range (days)					
Neoplasm	BALB (F) 101–950 d	C3H (F) 101–900 d	C3H (M) 101–900 d	C57BL/6 (F) 101–1050 d	C57BL/6 (M) 101–1100 d	RFM (F) 101–800 d
Lung carcinoma	8.0 ± 0.98	0.4 ± 0.28	2.0 ± 0.63	0.6 ± 0.35	0.6 ± 0.35	2.60 ± 0.59
Breast carcinoma	6.4 ± 0.87	8.9 ± 1.33	–	0.4 ± 0.29	–	0.4 ± 0.23
Myeloid leukaemia	0.7 ± 0.29	0	0	0	0.2 ± 0.20	0.7 ± 0.30
Thymic lymphoma	1.3 ± 0.40	0.2 ± 0.20	0	0.6 ± 0.35	0.2 ± 0.20	9.3 ± 1.12
Liver tumours	0.5 ± 0.24	10.7±1.46	73.5 ± 3.83	0.8 ± 0.41	1.0 ± 0.45	0.1 ± 0.13
Adrenal carcinoma	0.7 ± 0.29	0	0.6 ± 0.34	0	0	0.1 ± 0.13
Harderian carcinoma	0.2 ± 0.17	0.4 ± 0.28	0.2 ± 0.20	0	0	0.1 ± 0.13
Reticulum cell sarcoma	52.8 ± 2.52	5.6 ± 1.05	2.6 ± 1.05	43.4 ± 2.97	59.8 ± 3.45	42.1 ± 2.38
Ovarian tumours	1.9 ± 0.48	2.8 ± 0.74	–	0.6 ± 0.35	–	0.4 ± 0.23

The incidence of neoplasms after radiation doses of 0, 0.5, 1.0 and 2.0 Gy was assessed over most of the lifetime of these mouse strains. The investigators compared the use of EAR and RR models among strains, which is relevant to the discussions of transfer of risk (Sections 2.1 and 2.2). Storer et al (1988) concluded:

> "Intuitively, the absolute risk model seems an implausible method for extrapolating the risk of radiogenic cancers within a species. Its use requires the assumption that a population that is resistant (as estimated by the natural incidence) to a particular type of tumour would have a higher ratio of radiogenic cases to spontaneous cases than would a population that is naturally sensitive. In other words, the relative risk would be higher in the resistant population than in the sensitive population. The present study confirms this impression of implausibility, at least for mice. We examined the data for seven fatal types of mouse tumours for which there were major differences in the control incidence among the populations (Table 4.1). In all cases the absolute risk model could be convincingly rejected while the relative risk model gave an acceptable fit for five of the seven tumours. For two additional tumours, either model gave an acceptable fit, but this would be expected in view of the similarity of the incidence in the controls. We tentatively conclude, therefore, that relative risks, at least for some tumours, extrapolate directly across mouse strains."

Their conclusion was considered consistent with subsequent findings in defined cancer-prone rodent genotypes and human radiotherapy observations (ICRP, 1998: page 81).

The above data and other more recent data in mice were reviewed (together with data for other species including humans) by Suit et al (2007). Their conclusions with respect to inter-strain differences were:

> "Large and lifetime studies of several strains of inbred mice and F1 hybrid mice after whole-body irradiation have demonstrated quite substantial variations between strains with respect to natural cancer incidence, the increase in the incidence of cancer in the individual organs with dose to 2 Gy, and the impact of dose fractionation/dose rate on that relationship. In many assays, the data are consistent with a linear dose-response relationship to 2 Gy. However, for an important fraction of the studies, a linear relationship between dose and risk was not observed. The experimentally observed heterogeneity in risk between strains indicates a large genetic role in determination of risk in the individual."

The genetic role has been investigated extensively using mice in which single relevant genes have been silenced, eg repair and tumour suppressor genes, in order to assess their individual importance in radiation carcinogenesis (ICRP, 1998).

There is a considerable research effort being placed on the identification of disease susceptibility genes in mouse models (in addition to the lung-injury linkage analysis described above). The use of mice in mutagenesis programmes and systems genetics approaches provide routes to the identification of human susceptibility genes that do not require large genome-wide association studies in human populations (see Section 3.2). Large numbers of mouse candidate cancer susceptibility loci have been mapped in linkage and pedigree studies and a few genes have been identified (Dragani, 2003). The more recently available systems genetics approaches have also identified mouse cancer susceptibility genes (Quigley and Balmain, 2009). Some examples of the use of mouse models to map radiation-induced cancer

susceptibility loci are available. A number of candidate loci controlling susceptibility to radiation-induced acute myeloid leukaemia have been mapped in pedigree and association studies (Boulton et al, 2003; Darakhshan et al, 2006). Further candidate loci and genes have been identified in radiation-associated osteosarcoma (Rosemann et al, 2006) and there is evidence that the mouse *Rb1* gene makes a strong contribution to susceptibility to this tumour following irradiation (Gonzalez-Vasconcellos et al, 2011). The mouse radiation-associated thymic lymphoma system has also been exploited to identify susceptibility genes, some of which, such as *Anxa1* and *CD274*, act primarily in the tissue environment rather than in target cells themselves (Santos et al, 2009, 2010). The *Trp53* and *Hipk2* genes have been shown to interact to modulate susceptibility to thymic lymphomas in mice (Mao et al, 2012).

Some evidence is available for non-genetic factors, particularly diet, affecting cancer incidence in rodents. For example, high dietary fat intake was found to raise tumour frequency in the intestinal tract of predisposed Apc^{min} mice (Wasan et al, 1997). In contrast, there is now a considerable body of evidence that calorie restriction can serve to reduce cancer incidence (and incidence of other diseases) in experimental animals and humans (see, for example, Omodei and Fontana, 2011). In addition, calorie restriction can serve to reduce the frequency of radiation-induced leukaemias (Yoshida et al, 1997). It has also become clear that in mice the circadian clock can have a very profound effect on cancer yields following exposure to ultraviolet radiation at different times of day and this is due to circadian changes in gene expression (Gaddameedhi et al, 2011).

4.2.1 Summary

Large lifetime studies using several strains of inbred mice have shown substantial variations between strains with respect to natural cancer incidence, an increase in the incidence of cancer in individual organs after doses up to 2 Gy, and an impact of dose fractionation/dose rate on the dose-response relationship.

The observed heterogeneity in radiation risk between strains supports the proposition of a substantial genetic role in individual radiation risk.

A number of candidate susceptibility genes and loci controlling susceptibility to radiation-associated tumours have been identified.

Non-genetic factors can affect cancer risk in animals.

4.3 References

Baverstock KF, Papworth DG and Townsend KM (1985). Man's sensitivity to bone marrow failure following whole body exposure to low LET ionising radiation: inferences to be drawn from animal experiments. *Int J Radiat Biol Relat Stud Phys Chem Med,* **47**(4), 397–411.

Beck JA, Lloyd S, Hafezparast M, Lennon-Pierce M, Eppig JT, Festing MF and Fisher EM (2000). Genealogies of mouse inbred strains. *Nat Genet,* **24**(1), 23–5.

Boulton E, Cole C, Knight A, Cleary H, Snowden R and Plumb M (2003). Low-penetrance genetic susceptibility and resistance loci implicated in the relative risk for radiation-induced acute myeloid leukemia in mice. *Blood,* **101**, 2349–54.

Carsten AL (1984). Acute lethality – the hemopoietic syndrome in different species. In *Response of Different Species to Total Body Irradiation* (JJ Broerse and TJ MacVittie, Eds). Martinus Nijhoff Publishers, pp 59–86.

Darakhshan F, Badie C, Moody J, Coster M, Finnon R, Finnon P, et al (2006). Evidence for complex multigenic inheritance of radiation AML susceptibility in mice revealed using a surrogate phenotypic assay. *Carcinogenesis,* **27,** 311–18.

Dragani TA (2003). 10 years of mouse cancer modifier loci: human relevance. *Cancer Res,* **63,** 3011–18.

Gaddameedhi S, Selby CP, Kaufmann WK, Smart RC and Sancar A (2011). Control of skin cancer by the circadian rhythm. *Proc Natl Acad Sci USA,* **108,** 18790–95.

Gonzalez-Vasconcellos I, Domke T, Kuosaite V, Esposito I, Sanli-Bonazzi B, Nathrath M, et al (2011). Differential effects of genes of the Rb1 signalling pathway on osteosarcoma incidence and latency in alpha-particle irradiated mice. *Radiat Environ Biophys,* **50,** 135–41. Erratum: *Radiat Environ Biophys,* 2011, **50,** 143.

Grahn D and Hamilton KF (1957). Genetic variation in the acute lethal response of four inbred mouse strains to whole body X-irradiation. *Genetics,* **42**(3), 189–98.

Haston CK and Travis EL (1997). Murine susceptibility to radiation-induced pulmonary fibrosis is influenced by a genetic factor implicated in susceptibility to bleomycin-induced pulmonary fibrosis. *Cancer Res,* **57**(23), 5286–91.

Hendry JH and Jiang TN (1994). Differential radiosensitising effect of the scid mutation among tissues, studied using high and low dose rates: implications for prognostic indicators in radiotherapy. *Radiother Oncol,* **33**(3), 209–16.

Hendry JH and Lord BI (1983). The analysis of the early and late response to cytotoxic insults in the haemopoietic cell hierarchy. In *Cytotoxic Insult to Tissues: Effects on Cell Lineages* (CS Potten and JH Hendry, Eds). Edinburgh, Churchill Livingstone, pp 1–66.

Hendry JH and Roberts SA (1990). Analysis of dose-incidence relationships for marrow failure in different species, in terms of radiosensitivity of tissue-rescuing units. *Radiat Res,* **122**(2), 155–60.

ICRP (1998). Genetic Susceptibility to Cancer. ICRP Publication 79. *Ann ICRP,* **28**(1/2), 1–157.

Kallman RF and Kohn HI (1956). The influence of strain on acute X-ray lethality in the mouse. I. LD_{50} and death rate studies. *Radiat Res,* **5**(4), 309–17.

Lord BI, Hendry JH, Keene JP, Hodgson BW, Xu CX, Rezvani M and Jordan TJ (1984). A comparison of low and high dose-rate radiation for recipient mice in spleen-colony studies. *Cell Tissue Kinet,* **17**(4), 323–34.

Mao JH, Wu D, Kim IJ, Kang HC, Wei G, Climent J, et al (2012). Hipk2 cooperates with p53 to suppress γ-ray radiation-induced mouse thymic lymphoma. *Oncogene,* **31**(9), 1176–80.

Omodei D and Fontana L (2011). Calorie restriction and prevention of age-associated chronic disease. *FEBS Lett,* **585**(11), 1537–42.

Quigley D and Balmain A (2009). Systems genetics analysis of cancer susceptibility: from mouse models to humans. *Nat Rev Genet,* **10,** 651–7.

Rosemann M, Kuosaite V, Kremer M, Favor J, Quintanilla-Martinez L and Atkinson MJ (2006). Multilocus inheritance determines predisposition to alpha-radiation induced bone tumourigenesis in mice. *Int J Cancer,* **118,** 2132–8.

Santos J, González-Sánchez L, Matabuena-Deyzaguirre M, Villa-Morales M, Cozar P, López-Nieva P, et al (2009). A role for stroma-derived annexin A1 as mediator in the control of genetic susceptibility to T-cell lymphoblastic malignancies through prostaglandin E2 secretion. *Cancer Res,* **69,** 2577–87.

Santos J, González-Sánchez L, Villa-Morales M, Ors I, López-Nieva P, Vaquero C, et al (2010). The stromal gene encoding the CD274 antigen as a genetic modifier controlling survival of mice with γ-radiation-induced T-cell lymphoblastic lymphomas. *Oncogene,* **29,** 5265–73.

Storer JB, Mitchell TJ and Fry RJ (1988). Extrapolation of the relative risk of radiogenic neoplasms across mouse strains and to man. *Radiat Res,***114,** 331–53.

Suit H, Goldberg S, Niemierko A, Ancukiewicz M, Hall E, Goitein M, et al (2007). Secondary carcinogenesis in patients treated with radiation: a review of data on radiation-induced cancers in human, non-human primate, canine and rodent subjects. *Radiat Res,* **167,** 12–42.

UNSCEAR (United Nations Scientific Committee on the Effects of Atomic Radiation) (1988). *Sources, Effects and Risks of Ionizing Radiation*. Annex G: *Early Effects in Man of High Doses of Radiation*. Report to the General Assembly, with Annexes. New York, United Nations.

Wasan HS, Novelli M, Bee J and Bodmer WF (1997). Dietary fat influences on polyp phenotype in multiple intestinal neoplasia mice. *Proc Natl Acad Sci USA,* **94**(7), 3308–13.

Wilson BR (1963). Survival studies of whole-body X-irradiated germfree (axenic) mice. *Radiat Res,* **20,** 477–83.

Yoshida K, Inoue T, Nojima K, Hirabayashi Y and Sado T (1997). Calorie restriction reduces the incidence of myeloid leukemia induced by a single whole-body radiation in C3H/He mice. *Proc Natl Acad Sci USA,* **94**(6), 2615–19.

Yuhas JM and Storer JB (1969). On mouse strain differences in radiation resistance: hematopoietic death and the endogenous colony-forming unit. *Radiat Res,* **39**(3), 608–22.

5 Mechanisms contributing to Radiosensitivity

Exposure to ionising radiation induces a range of DNA lesions and activates several signalling responses. DNA double strand breaks (DSBs) are the major lethal DNA lesion induced by ionising radiation and a failure to repair DSBs causes dramatic radiosensitivity. However, aberrant signalling responses can also contribute to radiosensitivity. Most patients who show severe toxicity to radiotherapy probably have a DNA DSB repair defect, although for most patients who develop such severe reactions, the relationship to cellular endpoints has been equivocal. However, other processes must be at play as well as DNA repair. In addition to cellular radiosensitivity, other consequences need to be considered, including sensitivity to carcinogenesis and/or premature senescence, particularly following exposure to low radiation doses. Further, the mechanisms responding to high and low doses may be distinct. Here, the mechanisms of relevance for considering radiosensitivity after high and low dose exposures are briefly reviewed. The radiosensitive endpoint under consideration will be defined in each section. First, an overview will be provided of the DNA damage response (DDR) pathways (repair and signalling pathways) that impact upon the radiation response and then additional signalling responses, including stress, cytokine and inflammatory signalling responses, will be considered. Differences between the response to high and low doses will be highlighted.

5.1 Lesions arising from radiation exposure

Ionising radiation induces DNA base damage, single strand breaks (SSBs), DSBs and cross-links between DNA base pairs or between DNA and proteins. DNA base damage and SSBs are the most numerous lesions, but there are overlapping and highly efficient pathways for their repair. Thus, deficiency in these repair pathways normally causes only minor radiosensitivity. They will not be discussed here. DSBs are the most biologically significant lesions and the DDR to DSBs will be central to the ensuing discussion.

5.2 Overview of the DNA damage response

The DDR encompasses pathways of DNA repair and signal transduction pathways which are activated by one of two structures, a DSB or a region of single stranded DNA (ssDNA) (Figure 5.1). The major DNA repair pathways relevant to radiation damage are those repairing DSBs and the major signal transduction response is that activated by DSBs, which depends upon ataxia-telangiectasia mutated (ATM) protein. The signalling response initiated by ssDNA regions depends upon ataxia telangiectasia and Rad3 related protein (ATR). Base damage and SSBs (ie single stranded gaps but not single stranded regions of DNA) do not activate DDR signalling. Thus, although ionising radiation induces approximately 20-fold more

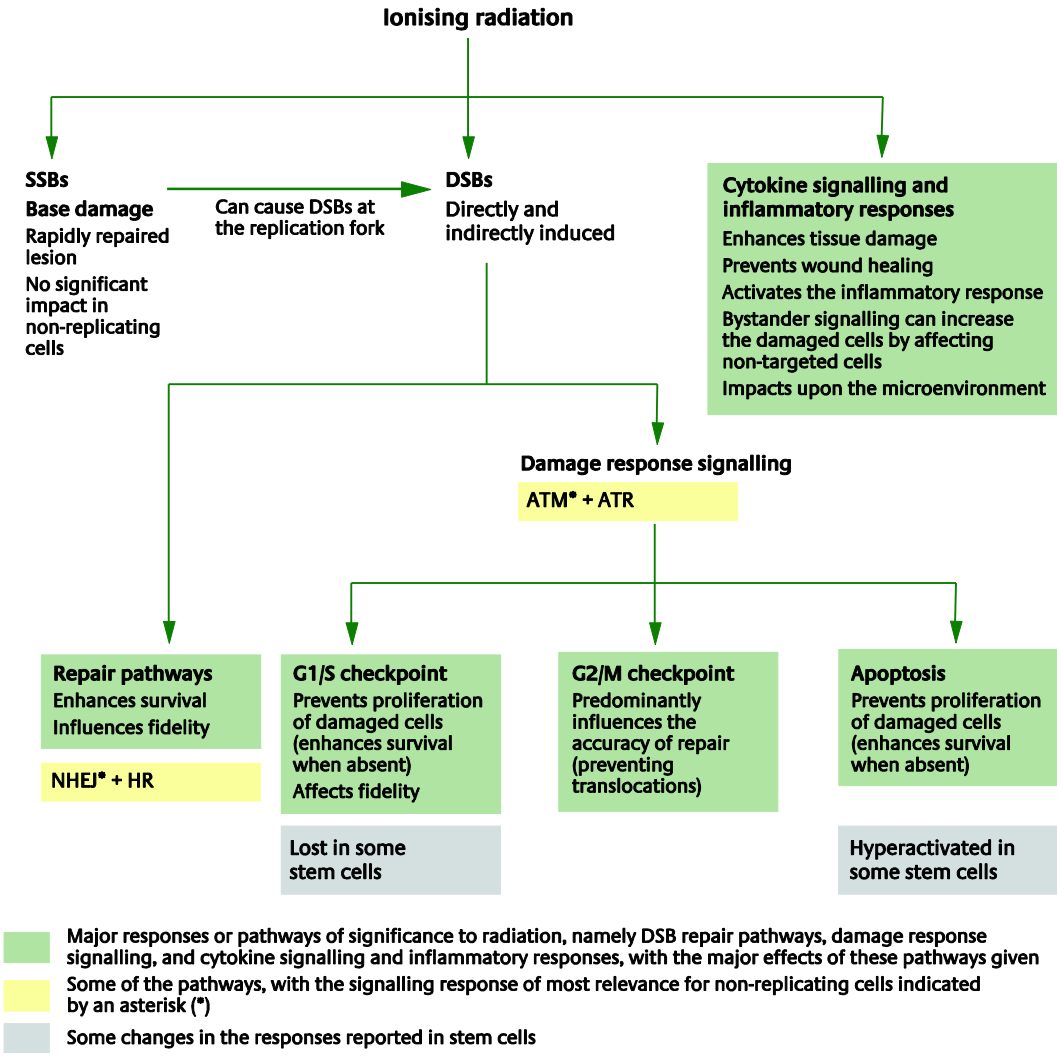


FIGURE 5.1 How the responses to DNA damage influence cellular outcome

Radiation can cause DNA damage (SSBs, base damage and DSBs) and also activate signalling pathways in both a DNA-dependent and DNA-independent manner.

Briefly, ionising radiation induces SSBs, base damage and DSBs. SSBs and base damage are rapidly repaired via multiple pathways. DSBs are slowly repaired and represent an important biological lesion induced by ionising radiation. In replicating cells, however, SSBs or base damage can cause DSB formation.

DSBs undergo repair and activate a signalling response that induces apoptosis and/or cell cycle checkpoint arrest. NHEJ is the major DSB repair pathway in non-replicating cells; homologous recombination (HR) can function in replicating cells. ATM is the major damage response signalling kinase, but ATR can function at the replication fork. Thus, ATR and HR can be important in replicating cells. Two pathways that can be altered in stem cells are highlighted. A detailed description of the signalling pathways activated in different cells has not been included.

base damage and SSBs than DSBs, the former do not activate a signalling response in non-dividing cells. However, ATR-dependent signalling can be activated following replication past any of these lesions. ATM and ATR are related protein kinases and share overlapping substrates. Current evidence demonstrates that DDR signalling causes marked changes to chromatin structure at the DSB site; pan-nuclear chromatin changes can also arise. ATM activation can also influence transcription via activation of transcription factors including p53 (Lavin, 2008). Relevant endpoints of the signal transduction pathway are cell cycle checkpoint arrest, apoptosis and an influence on DNA repair (see Section 5.5 for further discussion) (Figure 5.1).

5.3 Repair mechanisms

5.3.1 DSB repair

DNA non-homologous end-joining (NHEJ) represents the major DSB repair pathway in mammalian cells; homologous recombination also functions to repair DSBs in G2 phase. However, homologous recombination has its major role in S phase, enhancing recovery when lesions are encountered during replication.

In brief, NHEJ involves binding of the abundant Ku heterodimer (encompassing subunits Ku70 and Ku80) to double stranded DNA ends, which initiates NHEJ and protects DNA ends from nucleolytic digestion (Lieber, 2010). Once DNA bound, Ku recruits the DNA-dependent protein kinase catalytic subunit (DNA-PKcs) generating the DNA-PK holoenzyme (Ku + DNA-PKcs) and activating DNA-PKcs kinase activity. End-processing steps then ensue, potentially involving polynucleotide kinase (PNK), polymerases and nucleases. Artemis, a nuclease, appears to be an end-processing protein that requires DNA-PK for activation. Ligation is accomplished by recruitment of a second complex encompassing DNA ligase IV (LigIV), XLF/Cernunnos and XRCC4. Loss of any of the NHEJ proteins confers marked radiosensitivity. Additionally, NHEJ functions during V(D)J recombination, a critical step in immune development (Taccioli et al, 1993). Thus, loss of NHEJ proteins also confers immunodeficiency in animal models. Human disorders with mutations in NHEJ proteins have been identified. Such patients display radiosensitivity and immunodeficiency (Figure 5.2) (see also Chapter 6).

Homologous recombination has been reviewed previously (Holthausen et al, 2010). In brief, the process is initiated by resection, which involves the Mre11/Rad50/NBS1 (MRN) complex and CtIP, and generates 3′ss DNA tails. Further resection ensues by a cocktail of nucleases, generating long 3′ss DNA tails. The ssDNA binding protein, RPA, binds to these ssDNA regions. Subsequently, RPA is replaced by RAD51 forming D-loops, cross-over structures and branch migration. The process is finally completed by dissolution enzymes or Holliday junction resolvases. In addition to these steps, homologous recombination requires changes to chromatin around the site of damage, which is effected by the DDR. BRCA1 is required for efficient resection during homologous recombination.

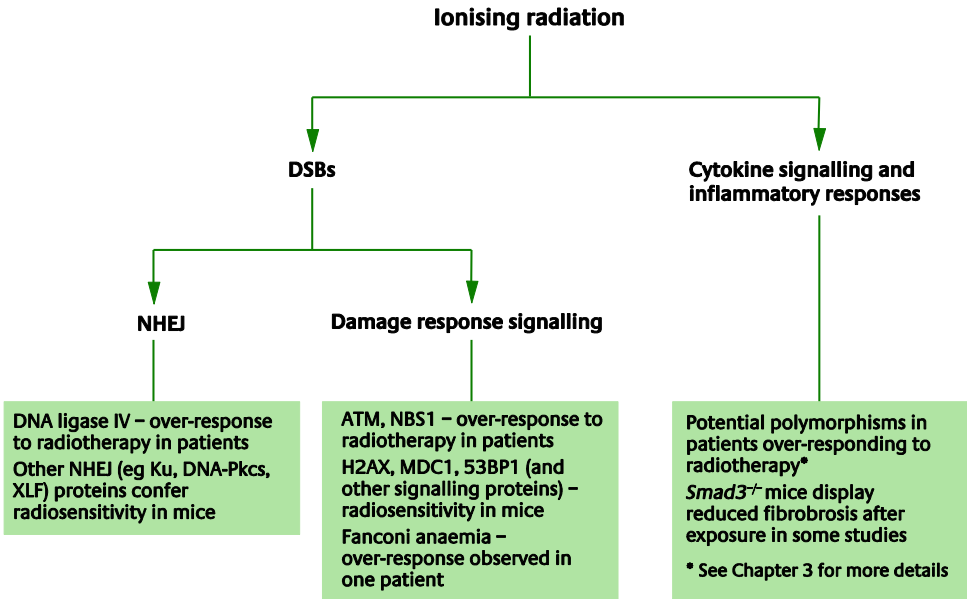


FIGURE 5.2 Responses to radiation exposure that are associated with sensitivity in patients or mice

5.3.2 DSB repair after low doses

DSB repair occurs in a relatively dose-independent manner. However, two studies have observed that unrepaired DSBs can persist for long periods after doses of less than 10 mGy (Grudzenski et al, 2010; Rothkamm and Lobrich, 2003). Further, in one study, it appeared that prior exposure to a dose of hydrogen peroxide overcame this phenomenon (Grudzenski et al, 2010). This finding is of importance for radiological protection but further work is required to substantiate it and to reveal the underlying mechanism.

5.4 DSB signal transduction response

An orchestrated assembly of DDR proteins occurs at the site of DSBs (for a review see Bekker-Jensen and Mailand, 2010). These changes are dependent upon ATM and involve the modification of histones around the DSB, including phosphorylation, ubiquitylation, methylation and sumoylation, and the recruitment of proteins, including 53BP1. One step that has been exploited as an assay to monitor DSB formation is phosphorylation of the histone variant, H2AX, generating γH2AX, which can extend megabase pair regions around the DSB and provides a marker for the presence of DSBs. The MRN complex is required to activate ATM at the DSB site and defects in this complex have been observed in radiosensitivity syndromes (see Chapter 6). RNF168, which is defective in RIDDLE syndrome, is another protein required for this signalling response. Defects in a failure to assemble DDR proteins and/or carry out the requisite histone modifications can lead to radiosensitivity, although the signalling response is not a prerequisite for DSB repair by NHEJ. There is, however, an influence on DSB repair as discussed below.

5.5 Endpoints of the DSB signalling response

5.5.1 Cell cycle checkpoint arrest

DNA damage checkpoints represent points where cell cycle progression can be delayed by the presence of DNA damage. Cells, therefore, possess mechanisms to assess genomic integrity and communicate the presence of damage to the machinery that regulates cell cycle progression (Lobrich and Jeggo, 2007). ATM and ATR regulate radiation-induced checkpoint arrest. Cell cycle checkpoints exist to prevent entry from G1 into S phase, from G2 into M phase and there is also an intra-S phase checkpoint. In addition, checkpoints exist to monitor steps during mitosis such as the integrity of the mitotic spindle.

5.5.2 G2/M checkpoint arrest

ATM phosphorylates and activates the transducer kinase, Chk2, whilst ATR activates Chk1. Chk1 and Chk2 phosphorylate and inactivate Cdc25 phosphatases. Mitotic entry is regulated by cyclin B1/Cdk1, the activity of which is inhibited by phosphorylation. Cdc25 phosphatases promote mitotic entry by dephosphorylating Cdk1. Thus, inhibition of Cdc25 activity prevents mitotic entry (Deckbar et al, 2011).

5.5.3 G1/S checkpoint arrest

S phase entry is regulated by phosphorylation of the retinoblastoma (Rb) protein, which regulates the transcription factor E2F, which in turn activates proteins required for S phase entry. Rb phosphorylation is regulated by Cyclin A-Ckd2. Two mechanisms regulating G1/S entry have been described: one process depends upon inactivation of the Cdc25 phosphatases via inhibitory phosphorylation by Chk1/Chk2, whilst the second process involves the activation of the Cdk inhibitor, p21. This inhibitor is transcriptionally regulated by p53, a target of ATM/ATR kinases. Thus, DNA damage activates ATM/ATR, which phosphorylates p53 and activates p21, a Cdk inhibitor (Deckbar et al, 2011).

5.5.4 Intra-S phase checkpoint arrest

DNA damage in S phase activates Cyclin A-Cdk2, which can impact upon the initiation of replication. This process leads to the inhibition of late-firing replicons and, therefore, progression through S phase.

5.5.5 Function and sensitivity of cell cycle checkpoint arrest and potential variation in the human population

Cell cycle checkpoint arrest has two major functions, as follows.

a It can permanently prevent progression of heavily damaged cells through the cell cycle providing an alternative to apoptosis to prevent the proliferation of heavily damaged cells. The G1/S checkpoint has a significant role in this function.

b Checkpoint arrest can allow additional time for DSB repair. This is important since both mitosis and replication in the presence of DSBs could create lesions that could lead to translocations or chromosome loss.

Thus, both functions of cell cycle checkpoint arrest are important in maintaining genomic stability and contribute to cancer avoidance (Lobrich and Jeggo, 2007). Checkpoint arrest can also influence survival as suggested by the phenomenon of low dose hypersensitivity (see below). However, checkpoint arrest exerts its greatest impact on the maintenance of genomic stability.

Recent studies have shown that cell cycle checkpoint arrest is not sensitive to a single DSB. Indeed, the G2/M checkpoint appears to have a defined threshold of 15–20 γH2AX foci, assumed to represent 15–20 unrepaired DSBs (Deckbar et al, 2007). This feature limits the ability of the G2/M checkpoint to prevent chromosome breakage and rearrangements in mitosis, which is of relevance for considering the exposure to low doses. In the context of checkpoint arrest, chromosomal instability can arise in several ways, as follows.

a Cells close to the mitotic entry point may escape arrest (defined as checkpoint escape).

b Low dose radiation damage may fail to activate checkpoint arrest.

c The release of checkpoint arrest occurs prior to the completion of DSB repair.

d The phenomenon of adaptation can allow the release of arrest after prolonged periods without the completion of DSB repair.

The majority of chromosome breakage arises in cells released from checkpoint arrest prior to the completion of DSB repair. Although this represents only a low number of chromosome breaks per cell (one or two breaks per cell after 1 Gy gamma irradiation), it defines a significant mechanism for breakage since it occurs in the majority of cells. In contrast, a small fraction of cells may harbour multiple breaks at early periods post-irradiation due to escape from checkpoint arrest, but the contribution to chromosome breakage is minor since this represents only a small fraction of cells in the population. The phenomenon of low dose hypersensitivity appears to arise as a consequence of failed G2/M checkpoint activation after low doses of ionising radiation (Marples and Collis, 2008). An important aspect of genetic heterogeneity in the population could lie in the sensitivity of G2/M checkpoint arrest between individuals. Whilst this could impact upon sensitivity after exposure to low doses, there could be a significant impact also on genomic stability after ionising radiation exposure.

Although the G1/S checkpoint is activated by much lower doses, and possibly by a single DSB, there are also defined limitations to its efficacy (Deckbar et al, 2010). Importantly, the process involving Chk1/2 and Cdc25 phosphatases does not completely block S phase entry but rather slows the rate of entry (see Deckbar et al, 2011, for further discussion). Additionally, p21 transcriptional activatation takes four to six hours. Thus, a window exists when the G1/S checkpoint is not fully activated. Several polymorphisms in p53 have been described and whether they impact upon the efficacy of G1/S checkpoint arrest has not been carefully examined. Thus, this represents a further point of potential genetic variation.

Finally, the intra-S phase checkpoint is an insensitive checkpoint. Indeed, this process merely delays entry into G2 phase and does not appear to completely block S phase progression.

In summary, checkpoint arrest is important for maintaining genomic stability by preventing the progression of damaged cells through the cell cycle. Cells that completely lack these checkpoints show enhanced genomic instability. However, although generally efficient, the processes have defined limitations, leading to windows when damaged cells can progress through the cell cycle. Variations in the

efficacy of initiating and maintaining checkpoint arrest between individuals could be a source of genetic variation in the response to radiation exposure.

5.5.6 Activation of apoptosis

Phosphorylation of p53 by ATM/ATR can initiate activation of both mitochondrial and receptor mediated apoptosis, which are important mechanisms to remove damaged cells. The ability to activate apoptosis differs between tissues; some tissues sensitively activate apoptosis, whilst others do not activate apoptosis at all. In general, those tissues which sensitively activate apoptosis after ionising radiation exposure are those in which apoptosis functions during development. These also represent highly radiosensitive tissues. For example, in the haemopoietic system, cells harbouring unproductive recombination rearrangements are removed by apoptosis and haemopoietic stem cells are highly sensitive to radiation-induced apoptosis. There is also evidence that some stem cells may sensitively undergo apoptosis, eg the embryonic neuronal stem cells and stem cells in the intestinal crypt.

5.5.7 Impact of damage response signalling on DSB repair

Most DSB repair occurs by NHEJ independently of DDR signalling. However, recent studies have shown that ATM is required for the repair of around 15% of X- or gamma-ray-induced DSBs, that these represent DSBs located within more compacted regions of DNA (heterochromatin) and that the role of ATM is to promote heterochromatic relaxation (Goodarzi et al, 2010). This role of ATM additionally requires the DNA damage response signalling proteins (eg MRN, MDC1, RNF8, RNF168 and 53BP1) (Noon et al, 2010).

5.6 Fidelity of repair and the generation of mutations/translocations

The accuracy of DSB repair is important in considering the avoidance of carcinogenesis. The fidelity of repair can be influenced by the nature of the lesion, cell cycle stage, activation of checkpoint arrest and the fidelity of the repair process.

5.6.1 Nature of the lesion

The nature of the lesion generated is determined by the quality of the radiation. Radiation uniquely deposits its energy in tracks, allowing multiple damage to arise in close proximity generating clustered DNA damage. The linear energy transfer (LET) of radiation determines the extent of clustered damage. Clustered damage is difficult to repair accurately, particularly if there is loss of nucleotides since NHEJ cannot restore genetic information lost at the damage site. In contrast, homologous recombination, by using an undamaged template for repair, can more efficiently repair complex lesions. However, homologous recombination does not function in G0/G1 phase; thus, high LET radiation has the potential to result in loss of coding information at the repair junctions in G0/G1 phase.

5.6.2 Cell cycle phase

Since homologous recombination only functions in late S/G2 phase, this means that repair of complex lesions could be more accurate in G2 phase. Additionally, high LET radiation can induce complex SSBs with multiple base damage and SSBs in close proximity. There is evidence that such complex SSBs are slowly repaired. Thus, the rate of DNA replication could also influence the fidelity of repair. Such a consideration could influence the radiosensitivity of embryos where tissues can be rapidly replicating.

5.6.3 Activation of cell cycle checkpoint arrest

The ability of checkpoint arrest to enhance the accuracy of repair has been discussed above. Loss of the G1/S or G/M checkpoint can enhance the possibility of mis-repair by allowing cell cycle progression before repair is completed.

5.6.4 Repair process utilised

A critical issue is how translocations, an important type of mis-repair event, arise. There is evidence that the process generating chromosomal translocations involves CtIP-dependent resection (Zhang and Jasin, 2011). Unfortunately, precise details of mechanisms generating translocations remain unclear. Further, the impact of dose, dose rate and genetic factors is not well understood.

5.7 Genetic factors influencing radiosensitivity in cell lines and patients

Having considered the DDR pathways that impact upon repair of lesions induced by ionising radiation and the signalling response, the components that may be important in determining radiosensitivity, the impact of mutational changes identified in patients and the possibility that they could influence the variability in radiosensitivity between individuals will now be considered. For this discussion, radiosensitivity will be considered in terms of cell killing and, separately, with respect to sensitivity to other endpoints, such as carcinogenesis and ageing.

5.7.1 Factors conferring major radiosensitivity (consideration of survival)

NHEJ represents the major pathway determining sensitivity to ionising radiation; indeed, cell lines and mice lacking NHEJ proteins are dramatically radiosensitive. Importantly, mutations in several NHEJ proteins have also been identified in patients (see Chapter 6). In addition, mutations in DDR signalling proteins have been identified in patients and such patients also show marked radiosensitivity (Chapter 6).

5.7.2 Consideration of subtle changes in the DNA damage response genes

DDR-defective patients with highly impacting mutational changes, however, are extremely rare and normally have characteristic clinical features. The issue of relevance here is whether more subtle changes in the same genes confer radiosensitivity. Heterozygosity for such changes is predicted in around 1% of the population. Thus, it is important to understand how heterozygosity affects the response to radiation. Polymorphic variants in these genes have also been described. Although most may not affect protein function, a few examples have been described, including polymorphic variants in DNA ligase IV and Artemis (Girard et al, 2004; Woodbine et al, 2010). In these examples, the polymorphic change when combined with another mutational change, either in the same or the other allele, affected the radiation response.

5.7.3 Factors influencing the sensitivity to radiation-induced carcinogenesis rather than survival

The discussion above represents a description of patients who show marked sensitivity to high doses. Much less is known about sensitivity to low doses and low dose rates. ATM homozygous and heterozygous murine cell lines show DSB accumulation under chronic low dose rate exposure of non-replicating cells (Kato et al, 2006). These findings raise the possibility that, if the rate of DSB formation exceeds the DSB repair capacity, then DSBs will accumulate. In replicating cells, it is also possible that slower repair will allow more lesions to be encountered at the replication fork. In principle, the reduced activity of any component of the DSB DDR could confer a reduced rate of DSB repair and it is significant that these findings are observed in heterozygous cell lines.

Additionally, the fidelity of DSB repair is important when considering the impact of low doses and low dose rates. Thus, sensitivity to radiation-induced carcinogenesis, which is likely to be affected by the fidelity of DSB repair, may represent a more relevant form of sensitivity compared to cell killing, which might not be significant after low dose exposure. In contrast, a small increase in cells with chromosomal rearrangements or translocations could have a large impact on the rate of carcinogenesis. Unfortunately, currently little is known about factors conferring sensitivity to low dose rate irradiation, nor are the mechanisms regulating the fidelity of repair well understood.

5.7.4 Adaptive response to low dose exposure

The radio-adaptive response represents the ability of exposure to a low dose of radiation to enhance recovery from a subsequent exposure to radiation (Olivieri et al, 1984). Such a response has been reported in cell lines derived from individual donors, but is highly variable between donors (Bosi and Olivieri, 1989). The priming or adaptive dose is usually less than 100 mGy. The original experiments involved treatment of human lymphocytes with low doses of radioactive thymidine and revealed diminished chromosomal aberrations following a subsequent exposure to radiation compared to untreated cells (Olivieri et al, 1984; Wiencke et al, 1986). Subsequent studies revealed similar findings for additional endpoints (Shadley and Wolff, 1987; Shadley et al, 1987). There is some evidence that the

phenomenon is observed following clinical or occupational exposure (Barquinero et al, 1995; Monsieurs et al, 2000; Padovani et al, 1995). The underlying mechanism is unknown, but there is evidence that transcriptional changes could be involved (Wolff et al, 1989).

5.7.5 Impact of radiation exposure on senescence

A range of recent studies have demonstrating that exposure to radiation can enhance cellular senescence, a process that could impact upon cellular and patient ageing. Such premature senescence can arise from enhanced telomere erosion following radiation exposure, from the activation of a stress response or as a consequence of unrepaired DNA lesions. However, little is understood about the factors that regulate such responses.

5.8 Radiosensitivity of stem cells

The response of stem cells to radiation exposure could also be an important determinant of the overall tissue response if recovery necessitates stem cell replication and/or the de-differentiation of early progenitor cells. Additionally, the generation of mutations in stem cells could have a greater consequence than such a change in a differentiated cell. The response of cancer stem cells will not be considered here, since the focus is on the normal cell response. Thus, mechanisms that maintain genomic stability of stem cells could be particularly important, including cell cycle checkpoint responses and the induction of apoptosis. Further, the DDR mechanisms that ensure the maintained replicative capacity of a stem cell (eg ATR-signalling and homologous recombination) could be important in stem cells. It has also been suggested that it might be preferable for damaged stem cells to undergo cell death than to generate damaged daughter cells. A further consideration is that some stem cells exist in a hypoxic niche, which might reduce the magnitude of radiation-induced DNA damage. It is clear that at least some stem cells respond differently to radiation compared to differentiated cells. However, it is also clear that different stem cells respond differently. Here, some of the major differences that have been reported in stem cells are considered.

Embryonic stem cells fail to undergo G1/S checkpoint arrest after radiation exposure, potentially due to differences in cell cycle regulation, including an inactive pRB-E2F pathway, low expression of cyclin D1, loss of stress-induced p21 activation and changes in Chk2 localisation (Aladjem et al, 1998; Fluckiger et al, 2006; Hong et al, 2007). This could substantially influence the genomic instability of embryonic stem cells following radiation exposure. Embryonic neuronal stem cells, which like embryonic stem cells are rapidly replicating, have also been reported to lack a radiation-induced G1/S checkpoint and are highly sensitive to radiation-induced apoptosis (Gatz et al, 2011; Hoshino and Kameyama, 1988; Hoshino et al, 1991; Roque et al, 2012). The intestinal crypt stem cells, which also proliferate rapidly, also appear to lack a G1/S checkpoint and are hypersensitive to apoptosis (Potten, 2004). It is important to appreciate, however, that these three systems represent cells that proliferate rapidly and that such differences may not be observed in all stem cell compartments.

5.9 Oxidative stress, bystander and additional signalling responses

Reactive oxygen species (ROS) can arise directly from radiation exposure, creating directly induced (or targeted) DNA damage. Radiation can also lead to ROS production indirectly via signalling processes, release of ROS from the mitochondria or changes to the cell's microenvironment, including the activation of an inflammatory response. Further, ROS generation can arise without DNA damage – for example, following cytoplasmic irradiation (Hong et al, 2010). Frequently, the antioxidant capacity of a cell changes when ROS levels change, so that the steady-state level of ROS and/or ROS damage is minimised. If oxidative stress is defined as a situation where the generation of ROS can exceed the antioxidant capacity of a cell, then ionising radiation exposure can lead to oxidative stress.

5.9.1 Initial signal activation

The precise signal that initiates stress response signalling is unclear. There is evidence that extranuclear targets can mediate genotoxic effects of radiation. Additionally, the bystander response shows that signalling can be relayed from a damaged cell to an undamaged cell (Hei et al, 2011a). In addition to ROS, reactive nitrogen species (RNS) such as peroxynitrite anions ($ONOO^-$) can form through interaction of nitric oxide (NO) with superoxide anions. Further, oxidants, as well as other free radicals, can react with lipids, proteins and DNA. For example, lipid peroxyl radicals and their decomposition products, and intermediates of lipid peroxidation, can lead to the generation of reactive aldehydes including 4-hydroxynonenal (4-HNE), which has been implicated in oxidative stress conditions, including atherosclerosis. One model for signal generation is that changes in plasma membrane permeability occur following radiation exposure resulting in an influx of calcium into the cytosol, potentially affecting membrane potential, and ROS and NO (Lyng et al, 2011). It is also important to appreciate that radiation-induced DNA damage can activate stress response signalling either via ATM or ATR or pathways such as p38 signalling (see below). For example, ATM positively regulates AMP-activated protein kinase (AMPK), which stimulates the regulation of energy metabolism (Sanli et al, 2010).

5.9.2 Stress signalling responses activated by ionising radiation

Stress response signalling can be initiated in the cellular membrane by the rapid activation of cytokine and growth factor receptors. ROS and RNS, for example, activate acidic sphingomyelinase and ceramide, which directly promote membrane-associated receptor activation. These signalling cascades probably contribute to bystander signalling, where cytokines and other soluble factors can be transmitted to non-irradiated (bystander) cells. Additional intracellular transducers and signalling pathways activated in irradiated cells include the mitogen-activated protein kinase (MAPK) pathway, nuclear factor kappa B (NFκB), the phosphatidylinositol-3-kinase (PI3K) and associated protein kinase B (AKT), and mammalian target of rapamycin (mTOR) pathways. Of the cytokine signalling pathways, transforming growth factor β (TGFβ1) is particularly important following radiation exposure (Hei et al, 2011b). These signalling pathways regulate a range of cellular endpoints, including apoptosis, protein synthesis, cell growth and the inflammatory response. They can also impact on DDR signalling and/or DNA repair (Figure 5.2) (Dittmann et al, 2005; Kirshner et al, 2006).

5.9.3 Changes in antioxidants

Although ROS are required for normal physiological functions, excess ROS can have detrimental effects including the activation of apoptosis (Hunt et al, 1998). Consequently, a rapid response to an elevation in ROS levels is an increase in antioxidants or detoxification enzymes, such as superoxide dismutases (SOD), which catalyses the conversion of superoxide radicals into hydrogen peroxide and molecular oxygen (Christensen et al, 2000). Knockdown of Cytoglobin (CYGB), a recently identified vertebrate globin, which scavenges ROS and nitrosative species, sensitises glioma cells to oxidative stress and ionising radiation (Fang et al, 2011). Animal studies have also shown that expression of antioxidants such as SOD2, CAT or HO-1 can reduce the severity of fibrosis (Epperly et al, 2009; Hagiwara et al, 2000; Jin et al, 2011).

5.9.4 Cytokines, growth factor and chemokine signalling

In general, cytokines and growth factors cause a broad range of tissue-specific effects. They can impact on the microenvironment around the damaged cell/tissue by affecting the surrounding stroma, epithelial composition and growth stimulation. TFGβ, for example, acts via autocrine, paracrine and endocrine mechanisms, and, depending on the environment, can exert both tumour suppressor and tumour promoter impacts (Moses and Barcellos-Hoff, 2011). TFGβ signalling is efficiently activated after radiation exposure and orchestrates a complex network of cellular responses (Kruse et al, 2009; Rodemann and Blaese, 2007). TGFβ is a significant factor promoting radiation-induced fibrosis in a range of tissues, including the induction of pulmonary fibrosis (Leask and Abraham, 2004; Rabbani et al, 2003; Rube et al, 2000; Xavier et al, 2004). There has been some, but not reproducible, evidence in epidemiological studies that TFGβ1 single-nucleotide polymorphisms correlate with the severity of late tissue effects (see Chapter 3 for details).

The Erbβ1 family of receptors, eg epidermal growth factor receptor (EGFR), are particularly important for radiation responses. The signalling responses regulated by EGFR include cell cycle progression and proliferation, differentiation and cell death via apoptotic pathways. EGFR signalling has also been reported to regulate aspects of DNA repair (Dittmann et al, 2011).

5.9.5 MAPK-activated protein kinases

The mitogen-activated protein kinases (MAPKs) are serine/threonine kinases that function to relay extracellular signals to intracellular responses (see Cargnello and Roux, 2011, for a review). There is a large family of MAPKs, including ERK1/2, c-Jun kinases 1-3, p38 kinase and the ERK5 family of kinases. For the response to radiation and stress, p38 and JNK are the most significant. P38α plays a major role in the inflammatory response, both responding to inflammatory cytokines and increasing the activation of proinflammatory cytokines.

5.10 Activation of inflammatory responses

Inflammation is a significant endpoint of the activation of stress response signalling and ironically, since inflammation can itself lead to ROS generation, feedback occurs where ionising radiation activates ROS leading to endpoints that further activate ROS. This type of response may be particularly important clinically. Cytokines and particularly TGFβ, which is an important player in the inflammatory response, have been discussed in Section 5.9.4 and will not be discussed further in this short section.

Recent studies have revealed several links between inflammatory responses and radiation exposure. One study on the atomic-bomb survivors has suggested that radiation-induced T-cell immunosenescence may cause activation of inflammatory responses resulting in ageing-associated and inflammation-related disease, both of which are observed in the atomic-bomb survivors (Kusunoki et al, 2010). Further studies have also provided links between changes in pathways regulating innate immunity following radiation exposure (Schaue and McBride, 2010). These findings suggest that damaged 'self' molecules trigger an acute inflammatory response, which can enhance cell and tissue damage, and prevent wound healing. The mechanism resembles pathways in bacteria that recognise 'non-self'. Central to the process are pattern recognition receptors (PRRs), such as toll-like receptors (TLRs), which evolved to recognise non-self microbial products but appear able, in addition, to recognise damaged 'self' molecules. Whilst previous studies suggested that such pathways may function only after high dose radiation exposure, there is increasing evidence that changes in toll-like receptors, such as TLR4/MD2, can take place after doses of 50 mGy (Shan et al, 2007). Schaue and McBride (2010) provide an excellent review of this complex area.

An important issue is whether the inflammatory response determines the radiation response in humans, including the response to radiotherapy. One way to evaluate this is by determining whether polymorphic variants in any inflammatory response genes correlate with radiosensitivity. This approach is covered in Chapter 3. From the analysis of single nucleotide polymorphisms segregating with radiosensitivity, some in genes involved in inflammatory response signalling have been reported, including *TGF-B* and *COX-2*, although they are not reproducibly observed (Andreassen et al, 2005; Hildebrandt et al, 2010). Additionally, and of significance when considering low dose exposure and cancer risk, a recent study suggested that polymorphisms in oxidative stress and inflammatory pathway genes could potentially influence the breast cancer risk of US radiologic technologists (radiographers) (Schonfeld et al, 2010). However, the impact in this study was modest and further work is required to substantiate the findings.

5.11 Bystander signalling

A number of papers have reported effects on non-targeted cells, which are commonly described as a consequence of bystander signalling. An important question is whether this occurs *in vivo*. Importantly, delayed chromosomal aberrations, characteristic of chromosomal instability, have been shown in the bone marrow of irradiated mice (Watson et al, 2001). One significant study observed persisting genomic instability in macrophages obtained from the bone marrow of irradiated mice that activates a proinflammatory response, whereas this was not observed in mice that failed to show an anti-inflammatory phenotype, suggesting an involvement of proinflammatory cytokine signalling. Whilst this represents a form of bystander response, it should be appreciated that it occurs by activation of cytokine

signalling in macrophages and may not be a response of all cell types (Lorimore et al, 2008). Importantly, neither this response nor an inflammatory response was observed after exposure to doses below 1 Gy (Zyuzikov et al, 2011). However, it should be noted that mouse strains differ in their inflammatory response and in radiation-induced instability (Lorimore et al, 2011); genetic analysis suggests that multiple genes could regulate these differences. These findings provide evidence that factors other than DNA repair can regulate the response to radiation, that this could influence genomic stability and that the regulatory mechanisms could be complex. Nevertheless, they provide strong evidence that this signalling response to radiation exposure is genetically regulated.

5.12 Summary

The mechanisms responding to radiation after low dose or low dose rate exposure are similar to those after high doses but there are important distinctions.

A range of stress responses including the activation of an inflammatory response are also determinants of the response to radiation exposure.

Survival of damaged cells/tissues is important for the response to high radiation doses.

The induction of carcinogenesis and senescence are important endpoints after low dose exposure.

The response of stem cells to radiation is distinct to that of differentiated cells.

The regulation of cancer induction and the variation in individual sensitivity after low dose exposure is poorly understood and requires further work.

Knowledge of the response to radiation needs to be exploited to define biomarkers to identify radiosensitive individuals (to low and high dose exposures).

5.13 References

Aladjem MI, Spike BT, Rodewald LW, Hope TJ, Klemm M, Jaenisch R and Wahl GM (1998). ES cells do not activate p53-dependent stress responses and undergo p53-independent apoptosis in response to DNA damage. *Curr Biol,* **8,** 145–55.

Andreassen CN, Alsner J, Overgaard J, Herskind C, Haviland J, Owen R, et al (2005). TGFB1 polymorphisms are associated with risk of late normal tissue complications in the breast after radiotherapy for early breast cancer. *Radiother Oncol,* **75,** 18–21.

Barquinero JF, Barrios L, Caballin MR, Miro R, Ribas M, Subias A and Egozcue J (1995). Occupational exposure to radiation induces an adaptive response in human lymphocytes. *Int J Radiat Biol,* **67,** 187–91.

Bekker-Jensen S and Mailand N (2010). Assembly and function of DNA double-strand break repair foci in mammalian cells. *DNA Repair (Amst),* **9**(12), 1219–28.

Bosi A and Olivieri G (1989). Variability of the adaptive response to ionizing radiations in humans. *Mutat Res,* **211,** 13–17.

Cargnello M and Roux PP (2011). Activation and function of the MAPKs and their substrates, the MAPK-activated protein kinases. *Microbiol Mol Biol Rev,* **75,** 50–83.

Christensen PU, Bentley NJ, Martinho RG, Nielsen O and Carr AM (2000). Mik1 levels accumulate in S phase and may mediate an intrinsic link between S phase and mitosis. *Proc Natl Acad Sci USA,* **97,** 2579–84.

Deckbar D, Birraux J, Krempler A, Tchouandong L, Beucher A, Walker S, et al (2007). Chromosome breakage after G2 checkpoint release. *J Cell Biol,* **176,** 748–55.

Deckbar D, Stiff T, Koch B, Reis C, Lobrich M and Jeggo PA (2010). The limitations of the G1-S checkpoint. *Cancer Res,* **70,** 4412–21.

Deckbar D, Jeggo PA and Lobrich M (2011). Understanding the limitations of radiation-induced cell cycle checkpoints. *Crit Rev Biochem Mol Biol,* **46,** 271–83.

Dittmann K, Mayer C, Fehrenbacher B, Schaller M, Raju U, Milas L, et al (2005). Radiation-induced epidermal growth factor receptor nuclear import is linked to activation of DNA-dependent protein kinase. *J Biol Chem,* **280,** 31182–9.

Dittmann K, Mayer C, Fehrenbacher B, Schaller M, Kehlbach R and Rodemann HP (2011). Nuclear epidermal growth factor receptor modulates cellular radio-sensitivity by regulation of chromatin access. *Radiother Oncol,* **99**(3), 317–22.

Epperly MW, Melendez JA, Zhang X, Nie S, Pearce L, Peterson J, et al (2009). Mitochondrial targeting of a catalase transgene product by plasmid liposomes increases radioresistance *in vitro* and *in vivo*. *Radiat Res,* **171,** 588–95.

Fang J, Ma I and Allalunis-Turner J (2011). Knockdown of cytoglobin expression sensitizes human glioma cells to radiation and oxidative stress. *Radiat Res,* **176,** 198–207.

Fluckiger AC, Marcy G, Marchand M, Negre D, Cosset FL, Mitalipov S, et al (2006). Cell cycle features of primate embryonic stem cells. *Stem Cells,* **24,** 547–56.

Gatz SA, Ju L, Gruber R, Hoffmann E, Carr AM, Wang ZQ, et al (2011). Requirement for DNA ligase IV during embryonic neuronal development. *J Neurosci,* **31,** 10088–100.

Girard P-M, Kysela B, Harer CJ, Doherty AJ and Jeggo PA (2004). Analysis of DNA ligase IV mutations found in LIG4 syndrome patients: the impact of two linked polymorphisms. *Hum Mol Genet,* **13,** 2369–76.

Goodarzi AA, Jeggo P and Lobrich M (2010). The influence of heterochromatin on DNA double strand break repair: getting the strong, silent type to relax. *DNA Repair (Amst),* **9,** 1272–82.

Grudzenski S, Raths A, Conrad S, Rube CE and Lobrich M (2010). Inducible response required for repair of low-dose radiation damage in human fibroblasts. *Proc Natl Acad Sci USA,* **107,** 14205–10.

Hagiwara SI, Ishii Y and Kitamura S (2000). Aerosolized administration of N-acetylcysteine attenuates lung fibrosis induced by bleomycin in mice. *Am J Respir Crit Care Med,* **162,** 225–31.

Hei TK, Zhou H, Chai Y, Ponnaiya B and Ivanov VN (2011a). Radiation induced non-targeted response: mechanism and potential clinical implications. *Curr Mol Pharmacol,* **4,** 96–105.

Hei TK, Zhao Y, Zhou H and Ivanov V (2011b). Mechanism of radiation carcinogenesis: role of the TGFBI gene and the inflammatory signaling cascade. *Adv Exp Med Biol,* **720,** 163–70.

Hildebrandt MA, Komaki R, Liao Z, Gu J, Chang JY, Ye Y, et al (2010). Genetic variants in inflammation-related genes are associated with radiation-induced toxicity following treatment for non-small cell lung cancer. *PloS One,* **5,** e12402.

Holthausen JT, Wyman C and Kanaar R (2010). Regulation of DNA strand exchange in homologous recombination. *DNA Repair (Amst),* **9,** 1264–72.

Hong M, Xu A, Zhou H, Wu L, Randers-Pehrson G, Santella RM, et al (2010). Mechanism of genotoxicity induced by targeted cytoplasmic irradiation. *Br J Cancer* ,**103,** 1263–8.

Hong Y, Cervantes RB, Tichy E, Tischfield JA and Stambrook PJ (2007). Protecting genomic integrity in somatic cells and embryonic stem cells. *Mutat Res,* **614,** 48–55.

Hoshino K and Kameyama Y (1988). Developmental-stage-dependent radiosensitivity of neural cells in the ventricular zone of telencephalon in mouse and rat fetuses. *Teratology,* **37,** 257–62.

Hoshino K, Kameyama Y and Inouye M (1991). Split-dose effect of X-irradiation on the induction of cell death in the fetal mouse brain. *J Radiat Res,* **32,** 23–7.

Hunt CR, Sim JE, Sullivan SJ, Featherstone T, Golden W, Von Kapp-Herr C, et al (1998). Genomic instability and catalase gene amplification induced by chronic exposure to oxidative stress. *Cancer Res,* **58,** 3986–92.

Jin C, Zhou D and Lv F (2011). Beneficial effects of early (but not late) intervention of heme oxygenase-1 on bleomycin-induced pulmonary fibrosis in mice. *Respir Physiol Neurobiol*, **175,** 239–46.

Kato TA, Nagasawa H, Weil MM, Genik PC, Little JB and Bedford JS (2006). Gamma-H2AX foci after low-dose-rate irradiation reveal atm haploinsufficiency in mice. *Radiat Res,* **166,** 47–54.

Kirshner J, Jobling MF, Pajares MJ, Ravani SA, Glick AB, Lavin MJ, et al (2006). Inhibition of transforming growth factor-beta1 signaling attenuates ataxia telangiectasia mutated activity in response to genotoxic stress. *Cancer Res,* **66,** 10861–9.

Kruse JJ, Floot BG, te Poele JA, Russell NS and Stewart FA (2009). Radiation-induced activation of TGF-beta signaling pathways in relation to vascular damage in mouse kidneys. *Radiat Res,* **171,** 188–97.

Kusunoki Y, Yamaoka M, Kubo Y, Hayashi T, Kasagi F, Douple EB and Nakachi K (2010). T-cell immunosenescence and inflammatory response in atomic bomb survivors. *Radiat Res,* **174,** 870–76.

Lavin MF (2008). Ataxia-telangiectasia: from a rare disorder to a paradigm for cell signalling and cancer. *Nat Rev Mol Cell Biol,* **9,** 759–69.

Leask A and Abraham DJ (2004). TGF-beta signaling and the fibrotic response. *FASEB J,* **18,** 816–27.

Lieber MR (2010). The mechanism of double-strand DNA break repair by the nonhomologous DNA end-joining pathway. *Annu Rev Biochem* **79,** 181–211.

Lobrich M and Jeggo PA (2007). The impact of a negligent G2/M checkpoint on genomic instability and cancer induction. *Nat Rev Cancer,* **7,** 861–9.

Lorimore SA, Chrystal JA, Robinson JI, Coates PJ and Wright EG (2008). Chromosomal instability in unirradiated hemaopoietic cells induced by macrophages exposed *in vivo* to ionizing radiation. *Cancer Res,* **68,** 8122–6.

Lorimore SA, Mukherjee D, Robinson JI, Chrystal JA and Wright EG (2011). Long-lived inflammatory signaling in irradiated bone marrow is genome dependent. *Cancer Res,* **71**(20), 6485–91.

Lyng FM, Howe OL and McClean B (2011). Reactive oxygen species-induced release of signalling factors in irradiated cells triggers membrane signalling and calcium influx in bystander cells. *Int J Radiat Biol*, **87,** 683–95.

Marples B and Collis SJ (2008). Low-dose hyper-radiosensitivity: past, present, and future. *Int J Radiat Oncol Biol Phys,* **70,** 1310–18.

Monsieurs MA, Thierens HM, Vral AM, Van De Wiele C, De Ridder LI and Dierckx RA (2000). Adaptive response in patients treated with ^{131}I. *J Nucl Med,* **41,** 17–22.

Moses H and Barcellos-Hoff MH (2011). TGF-beta biology in mammary development and breast cancer. *Cold Spring Harb Perspect Biol,* **3,** a003277.

Noon AT, Shibata A, Rief N, Lobrich M, Stewart GS, Jeggo PA and Goodarzi AA (2010). 53BP1-dependent robust localized KAP-1 phosphorylation is essential for heterochromatic DNA double-strand break repair. *Nat Cell Biol,* **12,** 177–84.

Olivieri G, Bodycote J and Wolff S (1984). Adaptive response of human lymphocytes to low concentrations of radioactive thymidine. *Science,* **223,** 594–7.

Padovani L, Appolloni M, Anzidei P, Tedeschi B, Caporossi D, Vernole P and Mauro F (1995). Do human lymphocytes exposed to the fallout of the Chernobyl accident exhibit an adaptive response? 1. Challenge with ionizing radiation. *Mutat Res,* **332,** 33–8.

Potten CS (2004). Radiation, the ideal cytotoxic agent for studying the cell biology of tissues such as the small intestine. *Radiat Res,* **161,** 123–36.

Rabbani ZN, Anscher MS, Zhang X, Chen L, Samulski TV, Li CY and Vujaskovic Z (2003). Soluble TGFbeta type II receptor gene therapy ameliorates acute radiation-induced pulmonary injury in rats. *Int J Radiat Oncol Biol Phys,* **57,** 563–72.

Rodemann HP and Blaese MA (2007). Responses of normal cells to ionizing radiation. *Semin Radiat Oncol,* **17,** 81–8.

Roque T, Haton C, Etienne O, Chicheportiche A, Rousseau L, Martin L, et al (2012). Lack of a p21waf1/cip-dependent G1/S checkpoint in neural stem and progenitor cells after DNA damage *in vivo*. *Stem Cells,* **30,** 537–47.

Rothkamm K and Lobrich M (2003). Evidence for a lack of DNA double-strand break repair in human cells exposed to very low X-ray doses. *Proc Natl Acad Sci USA,* **100,** 5057–62.

Rube CE, Uthe D, Schmid KW, Richter KD, Wessel J, Schuck A, et al (2000). Dose-dependent induction of transforming growth factor beta (TGF-beta). in the lung tissue of fibrosis-prone mice after thoracic irradiation. *Int J Radiat Oncol Biol Phys,* **47,** 1033–42.

Sanli T, Rashid A, Liu C, Harding S, Bristow RG, Cutz JC, et al (2010). Ionizing radiation activates AMP-activated kinase (AMPK): a target for radiosensitization of human cancer cells. *Int J Radiat Oncol Biol Phys,* **78,** 221–9.

Schaue D and McBride WH (2010). Links between innate immunity and normal tissue radiobiology. *Radiat Res,* **173,** 406–17.

Schonfeld SJ, Bhatti P, Brown EE, Linet MS, Simon SL, Weinstock RM, et al (2010). Polymorphisms in oxidative stress and inflammation pathway genes, low-dose ionizing radiation, and the risk of breast cancer among US radiologic technologists. *Cancer Causes Control,* **21,** 1857–66.

Shadley J and Wolff S (1987). Very low doses of X-rays can cause human lymphocytes to become less susceptible to ionizing radiation. *Mutagenesis,* **2,** 95–6.

Shadley JD, Afzal V and Wolff S (1987). Characterization of the adaptive response to ionizing radiation induced by low doses of X rays to human lymphocytes. *Radiat Res,* **111,** 511–17.

Shan YX, Jin SZ, Liu XD, Liu Y and Liu SZ (2007). Ionizing radiation stimulates secretion of pro-inflammatory cytokines: dose-response relationship, mechanisms and implications. *Radiat Environ Biophys,* **46,** 21–9.

Taccioli GE, Rathbun G, Oltz E, Stamato T, Jeggo PA and Alt FW (1993). Impairment of V(D)J recombination in double-strand break repair mutants. *Science,* **260,** 207–10.

Watson GE, Pocock DA, Papworth D, Lorimore SA and Wright EG (2001). *In vivo* chromosomal instability and transmissible aberrations in the progeny of haemopoietic stem cells induced by high- and low-LET radiations. *Int J Radiat Biol,* **77,** 409–17.

Wiencke JK, Afzal V, Olivieri G and Wolff S (1986). Evidence that the [3H]thymidine-induced adaptive response of human lymphocytes to subsequent doses of X-rays involves the induction of a chromosomal repair mechanism. *Mutagenesis,* **1,** 375–80.

Wolff S, Weincke JK, Afzal V, Youngbloom J and Cortes F (1989). The adaptive response of human lymphocytes to very low doses of ionizing radiation: a case of induced chromosomal repair with the induction of specific proteins. In *Low Dose Radiation, Biological Basis of Risk Assessment* (KF Baverstock and JW Stather, Eds). London, Taylor and Francis, pp 446–54.

Woodbine L, Grigoriadou S, Goodarzi AA, Riballo E, Tape C, Oliver AW, et al (2010). An Artemis polymorphic variant reduces Artemis activity and confers cellular radiosensitivity. *DNA Repair (Amst),* **9,** 1003–10.

Xavier S, Piek E, Fujii M, Javelaud D, Mauviel A, Flanders KC, et al (2004). Amelioration of radiation-induced fibrosis: inhibition of transforming growth factor-beta signaling by halofuginone. *J Biol Chem,* **279,** 15167–76.

Zhang Y and Jasin M (2011). An essential role for CtIP in chromosomal translocation formation through an alternative end-joining pathway. *Nat Struct Mol Biol,* **18,** 80–84.

Zyuzikov NA, Coates PJ, Parry JM, Lorimore SA and Wright EG (2011). Lack of nontargeted effects in murine bone marrow after low-dose *in vivo* X irradiation. *Radiat Res,* **175,** 322–7.

6 Radiosensitivity Disorders and Familial Cancers

A number of rare recessive disorders are now known in which the affected individuals, with biallelic mutations of the particular gene, have a confirmed increase in radiosensitivity, both at the cellular level and in some cases also clinically. At the cellular level increased radiosensitivity has been commonly shown in several ways.

a Reduced colony forming ability following exposure of cells to ionising radiation.

b Elevated level of unrepaired chromosomal breaks.

c Reduction in the removal of phosphorylated H2AX foci over time.

d Failure of specific repair proteins to form foci at the sites of DNA damage.

e Inefficient activation of the intra-S phase and/or G2/M checkpoint following the induction of DNA damage.

While an increased sensitivity to ionising radiation is synonymous with a deficiency in the repair of DNA double strand breaks (DSBs), there are several mechanisms by which this can occur. The mechanisms that respond to damage induced by ionising radiation have been discussed in Chapter 5. In this chapter, the disorders resulting from gene defects in either of the two primary pathways used to repair DNA DSBs will be considered. Subsequently, other disorders will be described that are caused by gene defects in components of the signalling pathway involved in either recognition of the damage itself or coordinating the activation of the cell cycle checkpoint and/or repair of the damage.

The predominant human disorders linked with defects in the non-homologous end joining (NHEJ) repair pathway can result from mutations in DNA-PKcs, DNA ligase IV, Artemis and Cernunnos. Radiosensitivity disorders associated with defects in the homologous recombination repair (HRR) machinery can be caused by mutation in genes such as *FANCD1 (BRCA2), FANCD2, FANCI, FANCJ, FANCN, Rad51C* and possibly *BLM*.

In contrast to those disorders associated with a DSB repair deficiency caused by mutations in genes that encode for proteins that function as part of the core repair machinery, other human syndromes, again linked with an underlying DSB repair defect, have been documented in which the protein encoded by the mutated gene functions to signal the presence of the DNA break and/or to activate cell cycle checkpoints, such as ATM, the components of the Mre11/Rad50/Nbs1 (MRN) complex and RNF168.

Finally, there are examples of cellular radiosensitivity disorders arising as a result of mutation in proteins, termed cohesins, which function to hold chromosomes together during DNA replication and repair.

The precise nature of the 'cellular radiosensitivity phenotype' of a person with a radiation hypersensitivity disorder will depend on whether the mutation affects the repair machinery itself or the signalling molecules involved in transmitting the presence of the damage. These disorders are considered below.

Another endpoint of radiosensitivity, and perhaps the important endpoint when considering the risks of radiation exposure of the population, is cancer induction. The question is what evidence there is for predisposition to radiation-induced cancer, and this will be considered in Section 6.2.

6.1 Cellular radiosensitivity disorders

6.1.1 Disorders resulting from defects of non-homologous end joining (NHEJ)

6.1.1.1 SCID – ligase IV syndrome

Approximately 30% of severe combined immunodeficiency (T^-B^- NK^+ SCID) patients result from an underlying DSB repair defect caused by either ligase IV or Artemis mutations or, in some rare cases, DNA-PKcs.

DNA ligase IV was the first gene known to function during NHEJ-dependent repair shown to be mutated in a human DNA repair deficiency syndrome. Clinical features associated with DNA ligase IV mutations include immunodeficiency, microcephaly (a developmental and growth delay) and pancytopaenia. This disorder is characterised by a severe hypersensitivity to ionising radiation as shown by colony forming assay and chromosomal breakage, comparable to that observed for patients with classical ataxia telangiectasia (A-T) (O'Driscoll et al, 2001; Plowman et al, 1990; Riballo et al, 1999). Interestingly, LIG4 mutant cells exhibit normal activation of the intra-S phase and G2/M cell cycle checkpoints following exposure to ionising radiation, which differs to that of cells derived from A-T patients. *LIG4* mutation as a cause of T^-B^- NK^+ SCID without developmental defects was first described by van der Burg et al (2006).

6.1.1.2 SCID – Artemis

Approximately 15% of severe combined immunodeficiency T^-B^- SCID patients with increased radiosensitivity as measured by colony forming ability (Cavazzana-Calvo, 1993; Ege et al, 2005; Moshous et al, 2001; van der Burg et al, 2009) have been shown to have mutation of the *ARTEMIS* gene. However, the radiosensitivity appears not to be as severe as that seen in *LIG4* mutants. Interestingly, some of these patients have been described clinically as having Omenn syndrome (Ege et al, 2005), which exhibits clinical features not typically associated with Artemis deficiency, such as erythrodermia, hepatosplenomegaly, lymphadenopathy and alopecia. Hypomorphic mutations in *ARTEMIS* have also been reported (Moshous et al, 2003) in patients classified with common variable immunodeficiency in which the immune dysfunction is noticeably milder. These patients, however, still exhibit an increased sensitivity to ionising radiation as measured by colony forming assay.

6.1.1.3 SCID – DNA-PKcs

An SCID patient, under six months old, with virtually no B- and T-cells, with a defect in V(D)J recombination and requiring bone marrow transplant, was shown to have radiosensitivity by colony survival that was intermediate between normal and LIG4-deficient cells and similar to Artemis deficiency (van der Burg

et al, 2009). The proportion of γH2AX foci remaining 72 hours after ionising radiation exposure was greater than normal and similar to cells from an Artemis-deficient SCID patient. The radiosensitivity was shown to be due to missense mutation L3062P in DNA-PKcs, but did not affect DNA-PKcs kinase activity *in vitro* or prevent DNA-PKcs autophosphorylation. The consequence of the mutation was a reduction in activation of the Artemis nuclease.

6.1.1.4 Cernunnos

A group of patients with growth retardation, microcephaly and immunodeficiency characterised by a profound T- and B-cell lymphocytopaenia, but not SCID, were shown to have mutation of the *Cernunnos* gene. This resulted in impaired V(D)J recombination and DNA-end ligation process. Interestingly, cultured fibroblasts from four patients showed a variable level of radiosensitivity as determined by colony forming ability, with two cell lines being more sensitive than the A-T cell line used as a control. The γH2AX foci persisted in these patients' cells for 24 hours, indicating a DNA repair defect. The G1/S, intra-S phase and G2/M checkpoints were all normal following ionising radiation exposure (Buck et al, 2006).

6.1.2 Disorders and defects in homologous recombination repair (HRR)

6.1.2.1 Fanconi anaemia (FA) and FA-like clinical phenotypes

Fanconi anaemia occurs as a result of biallelic mutations in one of a growing number of genes. Most patients have a very similar clinical presentation, which includes small size, developmental defects, pancytopenia and a predisposition to the development of specific types of tumours, particularly myeloid leukaemia and some oral squamous cell carcinomas. Classical FA results from mutation of one of the 'core complex' genes (*FANCA, FANCB, FANCC, FANCE, FANCF, FANCG* and *FANCL*). Other patients have a more severe clinical presentation resulting from mutation of *FANCI, FANCD1 (BRCA2), FANCJ (BRIP1), FANCM, FANCN (PALB2)* and *FANCP (SLX4)*, which all act downstream of the 'core complex' to promote HRR. Cells from patients with core group gene mutations including *FANCA* and *FANCC* have been shown not to be radiosensitive by colony forming assay (Godthelp et al, 2006); in contrast, cells from patients mutant for the genes with functions downstream of the 'core complex' have been reported to have a mildly increased radiosensitivity – for example, *FANCD2* (Taniguchi et al, 2002) and *FANCD1/BRCA2* (Abbott et al, 1998). However, some reports suggest that patients with mutations in these two genes have no significantly increased radiosensitivity as measured by colony forming assay (Godthelp et al, 2006). It is likely that in the latter cases, the lack of a detectable increased sensitivity to ionising radiation, as measured by colony survival, is due to the presence of a mild hypomorphic mutation that retains sufficient function or that the identified alteration in the gene is not pathogenic. Despite this, an increased sensitivity to the radiomimetic chemotherapy agent bleomycin has been reported for both *FANCD1* and *FANCD2* cells (Godthelp et al, 2006). There is more agreement that the FA-D2 cells have a defect in radioresistant DNA synthesis following ionising radiation exposure (Godthelp et al, 2006; Taniguchi et al, 2002). These authors did not examine chromosomal damage in FA-D1 cells induced by ionising radiation (Godthelp et al, 2006).

6.1.2.2 RAD51 paralogues

RAD51 is the recombinase involved in homologous recombination repair. No individuals defective in *RAD51* have been described. There are, however paralogues of *RAD51* (*RAD51C, RAD51B, RAD51D, XRCC2* and *XRCC3*). A hypomorphic homozygous missense mutation of the gene *RAD51C* (with an important role in Rad51-mediated recombination) has been described in a single surviving patient aged 10 years with a Fanconi-anaemia-like disorder (Vaz et al, 2010). Rad51 focus formation in response to exposure to the cross-linking agent mitomycin C (MMC) was greatly reduced; however, there was only a modestly increased radiosensitivity and there was no report of sensitivity to ionising radiation of chromosomes from this patient. The Chinese hamster *Rad51C* mutant cell line CLV4B was only slightly sensitive by colony forming assay, to X-irradiation and bleomycin treatment (Godthelp et al, 2002), although another hamster cell mutant (irs3) showed approximately two-fold more sensitivity to ionising radiation (French et al, 2002). Analysis of spontaneous chromosomal aberrations revealed a high level of chromatid-type damage in irs3, although this was not examined after ionising radiation exposure. Both CLV4B and irs3 cells also showed a reduction in ionising-radiation-dependent Rad51 focus formation.

The loss of Rad51 focus forming ability is also shared by cells defective in *FA-D1/BRCA2* and *FA-N/PALB2*. Interestingly, there do not appear to be any published results on colony forming assays following ionising radiation exposure, using these cells.

6.1.2.3 Bloom's syndrome

While there is no evidence for increased sensitivity to ionising radiation of Bloom's syndrome cells by colony forming assays, these cells have been reported several times to have a large increased sensitivity to ionising-radiation-induced chromatid-type damage following G2 irradiation (Aurias et al,1985; Kuhn et al, 1980).

6.1.3 Disorders arising from mutation of genes involved in recognition and signalling the presence of radiation-induced damage

The response to DNA DSBs is initiated by an early stage of binding of the Mre11/Rad50/Nbs1 complex to the broken ends of DNA. This enables the recruitment of ATM kinase to the site of damage, which then promotes a signal cascade, first by phosphorylating the histone H2A variant H2AX (γH2AX) surrounding the break. The γH2AX then binds MDC1, which stabilises the Mre11/Rad50/Nbs1 complex at the site of the break. MDC1 also has an important role in binding the E3 ubiquitin ligase, RNF8, which modifies histones by the addition of ubiquitin molecules. The ubiquitylation of histone at the site of damage allows a second wave of repair protein accumulation, possibly as a result of a process of providing access to further repair proteins such as 53BP1 and subsequently BRCA1, which may be important in the step of removing 53BP1 in preparation for end resection and recombination repair. Indeed, RNF8 further recruits RNF168, another E3 ligase, which causes additional ubiquitylation of histones. When either RNF8 or RNF168 is absent there is failure of the second wave of recruitment and failure of appearance of 53BP1 and BRCA1 foci at the sites of DNA damage.

6.1.3.1 Ataxia telangiectasia

Ataxia telangiectasia (A-T) was the first human disorder to be described in which patients and their cells were shown to be unusually sensitive to ionising radiation. Recognition of the increased radiosensitivity in this disorder came about, initially, from observation of the acute effects of radiotherapy following treatment of tumours in these patients (Cunliffe et al, 1975; Gotoff et al, 1967; Morgan et al, 1968). Subsequently, this clinical radiosensitivity was confirmed at the cellular level as both a decreased colony forming ability and an increased level of chromosomal damage (at both G0 and G2) following exposure to ionising radiation (Taylor et al, 1975, 1976). Ataxia telangiectasia (A-T) is inherited in an autosomal recessive manner; all patients have biallelic mutations of the *ATM* gene. The birth frequency of A-T in the UK is approximately 1 in 300,000 (Woods et al, 1990) with approximately five to six new cases of A-T annually in the UK (Thompson et al, 2005). The most frequent causes of death in A-T are sinopulmonary infection and malignant disease (Reiman et al, 2011; Sedgwick and Boder, 1991). Median survival has been reported in one study as 19 and 25 years (Crawford et al, 2006), although there is a wide age range of survival as a consequence of the presence of some normal or mutant ATM protein, arising from either leaky splice site or missense mutations, which confer a milder form of the disorder, in terms of both age of onset and rate of progression. It is likely that some retained ATM kinase activity is the cause of this milder phenotype (Reiman et al, 2011). The consequence of these mutations, with some retained ATM kinase activity, is also seen in terms of radiosensitivity, where fewer induced chromosomal aberrations or a less severe decrease in cell survival is observed in cells from these individuals. Therefore, not all A-T patients are equally radiosensitive (Byrd et al, 2012).

6.1.3.2 Ataxia-telangiectasia-like disorder

Ataxia-telangiectasia-like disorder (ATLD) is very rare, with only around 20 cases published, four from the UK (Hernandez et al, 1993; Pitts et al, 2001; Stewart et al, 1999; Taylor et al, 2004), two from Italy (Delia et al, 2004), ten from Saudi Arabia (Fernet et al, 2005) and four from Japan (Matsumoto et al, 2011; Uchisaka et al, 2009). It is caused by biallelic mutation of the *MRE11* gene. The clinical features of ATLD are very similar to those of A-T, principally the progressive cerebellar ataxia, although onset is at a slightly later age of childhood and slower in progress; indeed, in two cases a diagnosis of benign hereditary chorea was initially made (Klein et al, 1996). There is the appearance, therefore, of a milder form of A-T. In contrast to A-T patients, ATLD patients do not show the presence of telangiectasia. ATLD patients also show normal levels of total IgM, IgA and IgG, although there may be reduced levels of specific functional antibodies. hMRE11 is a component of a complex containing the Nbs1 protein. It might be expected, therefore, that deficiency of hMRE11 would result in a clinical phenotype more like Nijmegen breakage syndrome where there is deficiency of Nbs1 protein (Carney et al, 1998; Taalman et al, 1983; Varon et al, 1998). Why this is not the case is not understood. The function of the MRE11 complex is required for full ATM activation and so loss of MRE11 might explain the A-T-like phenotype. Radiosensitivity of ATLD cells by survival is intermediate between A-T and normal and the same is true for G2 chromosomal radiosensitivity (Delia et al, 2004; Matsumoto et al, 2011; Stewart et al, 1999).

6.1.3.3 Nijmegen breakage syndrome (NBS)

In contrast to A-T and ATLD patients, NBS patients characteristically show microcephaly, some learning difficulties, immunodeficiency and a greater predisposition to lymphoid tumours; this is associated most often with the *NBN* c.657del5 founder mutation. There is no cerebellar degeneration. While A-T, ATLD and NBS can all be distinguished at the clinical level, they all show increased levels of chromosomal abnormalities involving chromosomes 7 and 14. At the cellular level, cells from NBS patients show a radiosensitivity by colony forming assay that is very similar to A-T (Taalman et al, 1983). Indeed, NBS lymphocytes also show an increased level of chromatid-type damage following ionising radiation exposure. All three disorders exhibit hypersensitivity to ionising radiation – with ATLD cells probably being the least radiosensitive.

Interestingly, two siblings compound heterozygous for two previously unreported *NBN* gene nonsense mutations did not show the developmental defects typical of NBS, but only the fertility defects. However, their lymphoblastoid cells showed increased radiosensitivity as measured by reduced survival (Warcoin et al, 2009).

6.1.3.4 NBS-like disorder

A single individual with microcephaly, growth retardation and slight, non-progressive ataxia was shown to have biallelic *RAD50* mutations. Her cells expressed a low level of Rad50 protein, because of the presence of a mutation in the *RAD50* stop codon, giving a slightly larger protein by 66 aa (Waltes et al, 2009). Her lymphocytes showed increased levels of spontaneous t(7;14) translocations (Barbi et al, 1991). Skin fibroblasts from the patient showed a level of colony forming ability following exposure to ionising radiation intermediate between normal and A-T, similar to *MRE11* patients' cells. The *RAD50*-deficient patient also showed a similar level of ionising-radiation-induced chromosomal abnormalities compared with A-T or NBS patients. There was indirect evidence of ionising-radiation-induced defects in the G1/S checkpoint and the intra-S phase checkpoint (as measured by radioresistant DNA synthesis and G2/M checkpoint defect), all defects also seen in A-T cells (Barbi et al, 1991; Waltes et al, 2009).

In contrast to *ATM*, the MRN complex genes (*hMRE11*, *NBS1* and *RAD50*) are all essential; total loss of *hMRE11*, like *NBS1* and *hRAD50*, is lethal. The mutations in *hMRE11* giving ATLD and those of *NBS* and *hRAD50* are, therefore, all hypomorphic mutations allowing expression of either truncated or full-length mutant protein, with some retained function. Despite the limited numbers of patients identified, it is becoming clear how prevalent clinical heterogeneity might be within these disorders. It is notable, however, that the ATLD patients of Fernet et al (2005) and Matsumoto et al (2011) had additional features, particularly microcephaly (more often associated with Nijmegen breakage syndrome). In contrast, NBS is genetically very uniform, the vast majority of patients having the *657del5 NBS1* mutation and, therefore, there is little scope for clinical heterogeneity. Radiosensitivity, therefore, is likely to be more uniform in NBS compared with ATLD, although some variations have been reported (Seemanová et al, 2006; Varon et al, 2006). Within this group it is A-T patients who are most likely to show the greatest heterogeneity of radiosensitivity.

6.1.3.5 RIDDLE syndrome

Two patients have so far been described with RIDDLE syndrome. In the first case an individual with an absence of IgG (and requiring IgG), mild dysmorphic features, learning difficulties, stunted growth and slight ataxic gait, was shown to have biallelic mutations of the *RNF168* gene. The patient's cultured skin fibroblasts were shown to be unusually radiosensitive by colony forming assay and an increased level of unrepaired chromatid-type damage following G2 irradiation of his blood lymphocytes was demonstrated (Stewart et al, 2007, 2009). The RNF168-deficient cells showed a defect in the G2M checkpoint and a mild intra-S phase checkpoint (RDS) defect following exposure to ionising radiation. Compared with A-T, the increased radiosensitivity is mild, as measured by both colony forming assay and G2 irradiation. The second patient described (Devgan et al, 2011) showed some clinical similarities to the first. A small increased radiosensitivity was demonstrated in lymphoblastoid cells in suspension from this patient.

6.1.4 Cohesinopathies and cellular radiosensitivity

These disorders are caused by mutations in proteins responsible for sister chromatid cohesion in chromosomes. A number of proteins are involved including cohesin subunits SMC1 and SMC3 as well as NIPBL and ESCO2.

6.1.4.1 Cornelia de Lange syndrome – NIPBL

Cornelia de Lange syndrome (CdLS) is a rare autosomal dominant disorder in which most affected individuals carry *de novo NIPBL* mutations but may also carry mutations of the cohesin subunits SMC1A or SMC3. CdLS cells have been found to be unusually sensitive to the DNA cross-linking agent mitomycin C (MMC), as measured both by reduced colony forming ability and chromosomally. In contrast, exposure of CdLS cells to ionising radiation did not lead to the same level of radiosensitivity as A-T cells, as measured by colony forming ability (Vrouwe et al, 2007). Irradiation of CdLS cells at the G1 phase of the cell cycle resulted in a similar level of chromosomal damage to normal cells, whereas a comparison of chromatid damage in CdLS and normal cells following G2 irradiation following 0.5 or 1.0 Gy ionising radiation revealed a much higher level of retained chromatid-type damage in the CdLS cells. The level of chromatid damage induced by MMC was also found to be approximately three-fold higher in the CdLS cells. A normal colony forming ability following ionising radiation exposure, together with a marked increased of chromatid-type damage following G2 irradiation of cells, is consistent with a defect principally in homologous recombination repair in CdLS syndrome with *NIPBL* mutations (Vrouwe et al, 2007).

6.1.4.2 Robert's syndrome – ESCO2

In contrast to Cornelia de Lange syndrome, Robert's syndrome is recessive and caused by biallelic mutation of ESCO2, an orthologue of an *S. cerevisiae* protein required for cohesion of sister chromatids during S phase. Robert's syndrome cells show 'premature chromatid separation'. There is some contradictory evidence regarding radiosensitivity (van Den Berg and Francke 1993; van der Lelij et al, 2009), with the most recent work agreeing that although Robert's cells are unusually sensitive to MMC they are not sensitive to ionising radiation, either by colony forming assay or by chromosomal analysis following G2 irradiation (van der Lelij et al, 2009).

6.1.4.3 Warsaw breakage syndrome – DDX11

A single patient has been described with severe microcephaly, pre- and post-natal growth retardation, and abnormal skin pigmentation with a biallelic mutation of the *DDX11* gene, an XPD helicase family member (van der Lelij et al, 2010). Chromosomally, separation of chromatids at the centromere ('railroads') were observed in metaphase spreads as well as total 'premature chromatid separation'. This is another cohesinopathy with cells showing a clear increased sensitivity to the DNA cross-linking agent MMC by colony forming assay. The authors reported sensitivity to ionising radiation in the normal range, although the results presented could be interpreted as showing a very slight increased radiosensitivity comparable to FA-D2. The authors did not examine chromosomal radiosensitivity following G2 irradiation, in contrast to Vrouwe et al (2007) with CdLS patients.

6.1.5 Other possible cellular radiosensitivity disorders

A single patient with a primary immunodeficiency disorder was identified with two compound heterozygous missense mutations of DNA ligase 1 (Barnes et al, 1992). The patient's skin fibroblasts were radiosensitive by colony forming assay. A group of primary immunodeficiency, characterised by a defect in class switch recombination of unknown cause, was described by Péron et al (2007); again the patient's skin fibroblasts were radiosensitive by colony forming assay. There also appear to be several NBS-like patients, but without NBS; an example of this is the patient described originally by Barbi et al (1991) who subsequently was shown to have *RAD50* mutations (Waltes et al, 2009). Another NBS-like patient (Berardinelli et al, 2007) showed increased chromosomal radiosensitivity following G2 irradiation. Finally, an NBS-like patient with mutation of neither *NBS1* nor *LIGIV* showed a chromosomal radiosensitivity intermediate between NBS and normal (Maraschio et al, 2003). A recent autosomal recessive disorder characterised by microcephaly, intractable seizures and developmental delay was described with patients showing biallelic mutation of the *PNKP* (polynucleotide kinase 3′-phosphatase) gene. A lymphoblastoid cell line from one patient, previously tested, was reported to show increased sensitivity to ionising radiation by a colony forming assay (Shen et al, 2010).

6.1.6 Cellular radiosensitivity results in clinical radiosensitivity

Several of the disorders described above have shown an unusual radiosensitivity at the clinical level including ataxia telangiectasia (Cunliffe et al, 1975; Gotoff et al, 1967; Morgan et al, 1968), LIGIV syndrome (Plowman et al, 1990) and Nijmegen breakage syndrome (Bakshi et al, 2003).

There have also been several reports of increased clinical radiosensitivity in Fanconi anaemia patients treated with radiotherapy, including a patient treated for tonsillar carcinoma. Cellular radiosensitivity assays, however, showed a normal response to ionising radiation (Djuzenova et al, 2004; Marcou et al, 2001). Establishing whether or not Fanconi anaemia patients are clinically sensitive to ionising radiation and conditioning regimens is important because of their requirement for bone marrow transplant.

6.1.7 Potential cellular radiosensitivity of heterozygotes

Increased cellular radiosensitivity, as measured by colony survival assay, has been reported previously in ataxia telangiectasia heterozygous normal individuals, although this has not been confirmed as a general finding. Indeed radiotherapy is part of the treatment for breast cancer occurring in *ATM* mutation carriers and there are no reports of an untoward clinical radiation response in these women. Interestingly, *ATM* mutation carriers may be at an increased risk of radiation-induced breast cancer arising in the radiation field (Bernstein et al, 2010) (see Section 6.2.2). Increased chromosomal radiosensitivity, measured by three-colour FISH chromosome painting, was reported in cells from both A-T and NBS heterozygotes and was intermediate between A-T/NBS homozygous and normal values (Neubauer et al, 2002). Conversely, a more normal level of chromosomal radiosensitivity in cells from NBS heterozygotes has also been reported (Tanzanella et al, 2003), The comet assay been used to show increased DNA damage in *NBS* carrier cells (Bürger et al, 2006).

6.1.8 Comparison of colony forming assay and S/G2-induced chromosomal damage following ionising radiation exposure, as measures of increased cellular radiosensitivity

An attempt should be made to interpret and compare the different measures of radiosensitivity that have been described above to characterise these different disorders.

For example, it is clear that the greatest radiosensitivity, as measured by colony forming ability, is associated with complete biallelic inactivation of the *ATM* gene and possibly also the *LIG4* and *Cernunnos*. With respect to the NHEJ genes, biallelic mutation of both *Artemis* and *DNA-PKcs* also give an unequivocal increased radiosensitivity, as measured by colony forming ability. Biallelic mutation of each of the genes of the MRE11/RAD50/NBS1 complex also give rise to a clear radiosensitivity as measured by colony forming assay, although *RAD50* and *MRE11* mutants give rise to a sensitivity intermediate between biallelic null A-T and normal.

In contrast, mutation of the genes involved in homologous recombination repair results in mildly increased radiosensitivity, at best, as measured by colony forming assay. An alternative view might be that mutation of HRR genes results in an essentially normal survival, ie no indication of increased radiosensitivity. Interestingly, however, an increased level of unrepaired chromatid-type chromosomal damage, following exposure of cells in S, G2 or early M phase in the cell cycle, is seen in cells from some HRR disorders. Indeed, this might be a general feature of cells deficient in HRR. These apparently contradictory results (normal colony forming ability and increased G2 chromosomal radiosensitivity) can be explained as the consequence of irradiating the cells at different stages of the cell cycle. In the case of chromosomal radiosensitivity, irradiation is in G2 phase. Normally, for a colony forming assay, most cells will be in G1 and only a minority in S phase or later. Therefore, if the defect is essentially in HRR, no increased radiosensitivity will be seen by the colony forming assay. Indeed, there are several examples of this apparent disparity between radiosensitivity measured chromosomally post-S phase and radiosensitivity measured by colony forming ability.

The corollary of this is to ask whether increased radiosensitivity as measured by colony forming ability is an indication that the repair defect is principally in NHEJ. Does the radiosensitivity phenotype of a NHEJ defect include unrepaired chromatid-type damage in cells irradiated post-S phase? What combinations of damage indicators suggest a defect in a particular form of repair? The following may be such combinations and indicators:

a Very radiosensitive by survival but no G2-induced chromatid-type damage – then principally NHEJ defect, eg DNA ligase IV.

b Not radiosensitive by survival but high level of G2-induced chromatid-type damage – then principally HRR defect, eg *RAD51C, BRCA2* and *PALB2* mutation.

c Very radiosensitive by survival and a high level of G2-induced chromatid-type damage – then both HRR and NHEJ defect, eg *ATM/NBS, MRE11* and *RNF168*, with the effect of loss of *ATM/NBS* greater than that of *MRE11*, which in turn is greater than that of *RNF168*.

There may also be intermediates such as Bloom's syndrome with no evidence of increased radiosensitivity by colony forming assay, but a small increased G2 chromosomal radiosensitivity.

Finally, it is clear that there are in the population some individuals with a severe cellular sensitivity to ionising radiation and with no readily recognisable clinical syndrome through which this radiosensitivity can be recognised (Byrd et al, 2012; Warcoin et al, 2009). While these individuals are likely to be very rare, we do not know the frequency with which they occur.

6.2 Predisposition to radiation-associated cancer

Here the evidence from studies of those individuals without inherited cellular radiosensitivity, but with predisposition to radiation-induced cancer, will be reviewed.

There exist in the human population a number of well-recognised cancer-prone disorders. The classical example is retinoblastoma (http://omim.org/entry/180200) which served to confirm the Knudson hypothesis (Knudson, 1986), which holds that loss of one copy of a cancer-predisposing gene in all somatic cells leads to a dominant cancer predisposition attributable to the high lifetime probability of loss or functional inactivation of the remaining wild-type allele. Other examples of this dominantly inherited cancer predisposition are provided by Wilm's tumour (http://omin.org/entry/194070) and the breast and ovarian cancer predisposition attributable to inheritance of the *BRCA1* or *BRCA2* genes (http://omim.org.entry/113705 and http://omim.org/entry/600185). Carriers of such dominant, strongly predisposing genes are rare, generally of the order of 1 in 25,000 live births. *BRCA1/BRCA2* carriers are somewhat more common, accounting for approximately 1 in 1,000 live births. As such, these familial predispositions do not contribute to population cancer risk very significantly.

Evidence of the increased cancer risk following ionising radiation exposure comes largely from studies of childhood and young adult cancer patients after radiotherapy. Overall, childhood cancer survivors carry a six-fold elevation in the risk of developing a second cancer; five-year survivors of cancer who received radiotherapy carry an eight-fold increased risk of a second cancer (Inskip and Curtis, 2007). Clearly, radiation exposure increases the risk and the fact that patients are young also contributes to the increased

risk. In addition, there are four well-documented cases where inherited genetic factors increase the risk of radiation-associated secondary tumours.

6.2.1 Radiation-induced cancer in inherited disorders

Retinoblastoma is a rare condition (1 in 10,000 live births) that occurs in a heritable and sporadic form, as originally described by Knudson (1971). Those with the heritable form of the disease inherit a germline loss or mutation of *RB1* and the disease is generally diagnosed at early age (one year or less) and retinal tumours can be observed in both eyes. Heritable retinoblastoma has frequently been treated by radiotherapy. Patients with heritable retinoblastoma are at an increased risk of tumours at several sites, including bone and soft tissue, brain and skin (melanoma). Radiotherapy has been found to increase the risk of secondary cancers further. Wong et al (1997) followed a cohort of heritable retinoblastoma survivors – bone and soft tissue sarcoma incidence was elevated in the radiation field of those treated by radiotherapy. The cumulative risk of second cancers in heritable retinoblastoma survivors has been found to be 36% at 50 years, compared to 5.7% in sporadic retinoblastoma survivors (Kleinerman et al, 2005). The cumulative risk of second cancers in heritable retinoblastoma survivors treated with radiotherapy was 38% compared to 21% in patients not receiving radiotherapy for the primary heritable retinoblastoma (Kleinerman et al, 2005). These findings strongly implicate *RB1* as a gene, variants of which can lead to elevated radiation-induced cancer risk.

Li-Fraumeni syndrome (LFS) is another rare disorder with dominant inheritance of cancer predisposition that is associated in most cases with germline mutations of *TP53* (Malkin et al, 1990, 1992). LFS patients are at a very high risk of cancer – the incidence is approximately 50% in 30 year olds and 90% in 60 year olds. These cancers can be at a wide range of sites, including breast, brain, adrenal glands and haemopoietic tissue (Li et al, 1988). The cumulative probability of second cancers in LFS patients surviving a primary cancer is very high, 57% at 30 years. An increased risk of sarcoma is observed after radiotherapy, with many of these tumours arising within the radiation field (Heyn et al, 1993; Hisada et al, 1998; Strong and Williams, 1987).

Patients with basal cell nevus syndrome (BCNS), also known as Gorlin syndrome, have a rare disorder characterised by multiple basal cell skin carcinomas that is attributable to inheritance of mutant forms of the *PTC* tumour suppressor gene (http://omim.org/entry/109400). Children with BCNS are at a high risk of developing multiple basal cell carcinomas in irradiated areas of skin from six months to three years after exposure (Evans et al, 1991; Strong, 1977). However, it appears that the high radiation risk does not apply to all patients (Southwide and Schwartz, 1979). BCNS is also associated with an increased medulloblastoma risk (see, for example, Evans et al, 1991). In a group of 88 childhood medulloblastoma patients receiving radiotherapy, four developed second cancers (a 39-fold elevated risk). Of these four second cancer cases, two had BCNS (Goldstein et al, 1997).

The final childhood cancer syndrome where an increased risk of radiation-induced cancer is observed is neurofibromatosis type 1 (NF1). NF1 is another rare syndrome (1 in 3,500 births) that is associated with neurofibroma and an increased risk of peripheral nerve sheath tumours, leukaemia and glioma (http://omim.org/entry/162200). NF1 patients receiving radiotherapy for primary optic pathway

gliomas were found to be at a three-fold greater relative risk of second cancers compared to non-irradiated NF1 glioma patients (Sharif et al, 2006). The second tumours in irradiated patients occurred within the radiation fields and with relatively short latency (mean 14 years). At least two other studies document an elevated risk of peripheral nerve sheath tumours in irradiated NF1 patients (Ducatman et al, 1986; Loree et al, 2000).

6.2.2 Radiation-induced cancer in carriers of mutations predisposing to breast cancer

Studies in general indicate that heterozygous carriers of *ATM* mutations are at an approximately two-fold elevated risk of breast cancer (Goldgar et al, 2011; Renwick et al, 2006). *ATM* mutations and radiation exposure were suggested by Broeks et al (2000) to interact to increase the risk of breast cancer. More recently, it has been found that rare missense variants of *ATM* and radiation exposure from breast cancer therapy together conferred a greater risk of contralateral second breast cancer than the sum of the individual effects (Bernstein et al, 2010). However, radiation has been found not to interact with *BRCA1* or *BRCA2* carrier status, with a *CHEK2* mutation, with gene variants that activate or are downstream targets of *ATM*, or with a variety of SNPs that associate with breast cancer (Bernstein et al, 2010; Brooks et al, 2012; Mellemkjaer et al, 2008; Teraoka et al, 2011). The frequency of carriers of risk increasing *ATM* variants in the population is judged to be sufficient to account for only a small proportion of second breast tumours in breast radiotherapy patients (Bernstein et al, 2010). The impact on radiogenic breast cancer in general may be greater.

In a case-only study, variants of *BRCA1*, *BRCA2*, *CHEK2* and *ATM* associated with an increased risk of secondary breast cancer in patients receiving radiotherapy for a primary breast cancer (Broeks et al, 2007). The gene variants conferred a relative risk of 2.18. More recently, variants of the breast cancer genes *BRCA1* and *BRCA2* have been found to increase substantially the risk of contralateral breast cancer – 4.5-fold for *BRCA1* and 3.4-fold for *BRCA2* (Malone et al, 2010), although the role of radiotherapy was not determined. There are numerous further reports of interactions between radiation and variants in genes associated with, for example, breast cancer risk, DNA repair and oestrogen metabolism that affect cancer risk; however, replication studies are required to confirm the findings (Bhatti et al, 2008a,b, 2010; Duell et al, 2011; Gronwald et al, 2008; Millikan et al, 2005; Rajaraman et al, 2008; Sigurdson et al, 2009a,b).

An elevated risk of breast cancer has also been reported in carriers of deleterious *BRCA1/BRCA2* mutations associated with multiple chest X-ray exposure (Andrieu et al, 2006). This study therefore can be taken as providing evidence for the *BRCA1/BRCA2* gene mutations conferring sensitivity to breast carcinogenesis at relatively low levels of exposure – perhaps a few tens of milligray; however, exposure to X-rays was determined by interview following disease diagnosis and is therefore subject to bias. In the context of medical screening there has been concern that the use of mammographic screening from young ages in *BRCA1/BRCA2* mutation carriers might carry a greater risk than benefit. Direct evidence for mammography screening for early detection of disease leading to an increased breast cancer risk in *BRCA1/BRCA2* mutation carriers is not, however, consistent and at best indicative that there may be a slight elevation in risk (Goldfrank et al, 2006; Narod et al, 2006). Indeed, it has been pointed out that the

size of the study population required to demonstrate an elevated breast cancer risk due to doses at the levels involved in mammographic examinations dictate that it will not be possible to obtain direct evidence (see, for example, Land, 1980). Risk modelling studies can estimate the breast cancer risk associated with mammography in *BRCA1/BRCA2* mutation carriers. On the basis of such calculations, regular mammography below the age of 34 years is not predicted to be beneficial; benefit would only be obtained from regular screening from the age of 35 years (Berrington de Gonzalez et al, 2009).

In addition to breast cancer, some studies have identified potential interactions between radiation and specific gene variants in childhood leukaemia (Chokkalingam et al, 2011; Infante-Rivard, 2003), lung cancer (Bonner et al, 2006; Hung et al, 2006) and thyroid cancer (Akulevich et al, 2009; Sigurdson et al, 2009b). None of these genetic influences on radiation-induced cancer risk has been verified in sufficiently large and robust studies to be very confident in the findings; the impacts on risk are also, in general, small. For leukaemia, it has further been suggested that those in the population who carry expanded clones of white blood cells carrying leukaemia-associated translocations acquired during fetal development are at an increased risk of radiation-induced disease (see Box 6.1).

6.3 Summary

Fifteen disorders showing increased cellular radiosensitivity are known. All except Cornelia de Lange syndrome are recessive disorders.

Cells may not show increased radiosensitivity using one assay but may do so using another. For example, cells may not show increased radiosensitivity by the standard colony forming assay, yet still show a large increased chromosomal radiosensitivity following S/G2 irradiation.

Assessment of cellular radiosensitivity requires an estimate of radiosensitivity both due to NHEJ and also due to HRR (colony forming assay *versus* chromosomal damage following ionising radiation exposure).

Increased cellular radiosensitivity is reflected in the increased clinical radiosensitivity reported for several of these disorders.

Some individuals exist in the population with a severe cellular sensitivity to ionising radiation and with no readily recognisable clinical syndrome, ie they are normally undetectable.

There is clear evidence that human cancer risk is influenced by inherited genetic factors in syndromes such as familial retinoblastoma.

Radiation-associated cancer risk is increased in several inherited disorders such as retinoblastoma, Li-Fraumeni syndrome and NF1.

Some evidence suggests that *ATM* variants increase the risk of second radiotherapy-related cancers in adults, studies implicating other gene variants require confirmation; an increased risk in gene-variant-carrying individuals after lower dose exposures may be present but is more difficult to detect.

BOX 6.1

Abnormal radiation response arising from early developmental events

This chapter considers heterogeneity in radiation response due to heritable genetic factors. Elsewhere in the report attention is focused upon the effect of environmental (eg lifestyle) factors. However, there is a third theoretical class of factors predisposing to an abnormal response to radiation that involves changes occurring early in life, usually before birth. These are genetic factors not inherited from parents but occurring after conception. Just as some lifestyle/environmental factors conferring radiosensitivity are specific for particular cancers (eg smoking and lung cancer), so these genetic factors may predispose to radiosensitivity for particular cancers. An example may be various leukaemias where there is evidence for an association with specific translocations in circulating lymphocytes that arise during gestation and are subject to clonal expansion. These genetic changes do not in themselves make the cells leukaemic without one or a small number of further mutations, but the cells can be regarded as effectively preleukaemic and individuals carrying clonally expanded numbers would be expected to be more at risk of developing leukaemia than the general population (Greaves, 2007; Mori et al, 2002).

If radiation-induced leukaemias arise because radiation induces second mutations in individuals carrying clonally expanded populations and not because of *de novo* induction of the translocations, then such individuals would constitute a radiosensitive population for leukaemia. This hypothesis has been stated and explored by Nakamura (2005). For paediatric ALL (acute lymphocytic leukaemia) associated with the t(12;21) translocation Nakamura estimates that the development of ALL after radiation exposure is almost exclusively attributable to the 1% of predisposed individuals in a population. The relative risk for these predisposed individuals is thus roughly 25 times higher than the conventional estimates of the risk to the population as a whole. Chronic myeloid leukaemia and (to some extent) acute myeloid leukaemia might behave similarly. Nakamura estimates that around 4% of the general population carry clonally expanded levels of cells with preleukaemic translocations which in situ hybridisation shows represents around 1 in 1000 lymphocytes. Individuals with these levels can be detected by RT-PCR of fusion transcripts (messenger RNAs). Almost all the risk associated with radiation exposure is estimated to be confined to these individuals, with the remainder of the population having a vanishingly small excess risk.

The fact that mice carrying a translocation-generated fusion gene (*IGH-BCL2*) are vulnerable to radiation-induced ALL if irradiated when young (Gibbons et al, 1999) could be regarded as proof of principle; mice carrying TEL/AML1 or AML1/ETO fusion cDNA (Tsuzuki et al, 2004; Yuan et al, 2001) could also be used. At present, such evidence as exists relates to paediatric ALL and the extent to which the hypothesis might apply to adult leukaemias is unclear.

Should this hypothesis be true it would have significant implications for certain areas of radiological protection. For example, following significant radiation exposure (whether accidental or therapeutic) children showing clonal expansion of cells carrying preleukaemic translocations would deserve particular specialised follow-up.

Individuals carrying clonally expanded populations of cells with preleukaemic translocations that have arisen during fetal life have been hypothesised to have a greatly increased sensitivity to leukaemia induction by subsequent exposure to radiation compared with the population as a whole.

6.4 References

Abbott DW, Freeman ML and Holt JT (1998). Double-strand break repair deficiency and radiation sensitivity in BRCA2 mutant cancer cells. *J Natl Cancer Inst,* **90**(13), 978–85.

Akulevich NM, Saenko VA, Rogounovitch TI, Drozd VM, Lushnikov EF, Ivanov VK, et al (2009). Polymorphisms of DNA damage response genes in radiation-related and sporadic papillary thyroid carcinoma. *Endocr Relat Cancer*, **16,** 491–503.

Andrieu N, Easton DF, Chang-Claude J, Rookus MA, Brohet R, Cardis E, et al (2006). Effect of chest X-rays on the risk of breast cancer among BRCA1/2 mutation carriers in the international BRCA1/2 carrier cohort study: a report from EMBRACE, GENEPSO, GEO-HEBON and IBCCS collaborators group. *J Clin Oncol,* **24,** 3361–6.

Aurias A, Antoine JL, Assathiany R, Odievre M and Dutrillaux B (1985). Radiation sensitivity of Bloom's syndrome lymphocytes during S and G2 phases. *Cancer Genet Cytogenet,* **16**(2), 131–6.

Bakhshi S, Cerosaletti KM, Concannon P, Bawle EV, Fontanesi J, Gatti RA and Bhambhani K (2003). Medulloblastoma with adverse reaction to radiation therapy in Nijmegen breakage syndrome. *J Pediatr Hematol Oncol,* **25**(3), 248–51.

Barbi G, Scheres JM, Schindler D, Taalman RD, Rodens K, Mehnert K, et al (1991). Chromosome instability and X-ray hypersensitivity in a microcephalic and growth-retarded child. *Am J Med Genet,* **40,** 44–5.

Barnes DE, Tomkinson AE, Lehmann AR, Webster AD and Lindahl T (1992). Mutations in the DNA ligase I gene of an individual with immunodeficiencies and cellular hypersensitivity to DNA-damaging agents. *Cell,* **69**(3), 495–503.

Berardinelli F, di Masi A, Salvatore M, Banerjee S, Myung K, De Villartay JP, et al (2007). A case report of a patient with microcephaly, facial dysmorphism, chromosomal radiosensitivity and telomere length alterations closely resembling 'Nijmegen breakage syndrome' phenotype. *Eur J Med Genet,* **50**(3), 176–87.

Bernstein JL, Haile RW, Stovall M, Boice JD Jr, Shore RE, Langholz B, et al (2010). Radiation exposure, the ATM gene, and contralateral breast cancer in the women's environmental cancer and radiation epidemiology study. *J Natl Cancer Inst,* **102,** 475–83.

Berrington de Gonzalez A, Berg CD, Visvanathan K and Robson M (2009). Estimated risk of radiation-induced breast cancer from mammographic screening for young BRCA mutation carriers. *J Natl Cancer Inst,* **101,** 205–9.

Bhatti P, Doody MM, Alexander BH, Yuenger J, Simon SL, Weinstock RM, et al (2008a). Breast cancer risk polymorphisms and interaction with ionizing radiation among US radiologic technologists. *Cancer Epidemiol Biomark Prev,* **17,** 2007–11.

Bhatti P, Struewing JP, Alexander BH, Hauptmann M, Bowen L, Mateus-Pereira LH, et al (2008b). Polymorphisms in DNA repair genes, ionizing radiation exposure and risk of breast cancer in US radiologic technologists. *Int J Cancer,* **122,** 177–82.

Bhatti P, Doody MM, Rajaraman P, Alexander BH, Yeager M, Hutchinson A, et al (2010). Novel breast cancer risk alleles and interaction with ionizing radiation among US radiologic technologists. *Radiat Res,* **173**(2), 214–24.

Bishop T and Hopper J (1997). AT-tributable risks? *Nat Genet,* **15,** 226.

Bonner MR, Bennett WP, Xiong W, Lan Q, Brownson RC, Harris CC, et al (2006). Radon, secondhand smoke, glutathione-S-transferase M1 and lung cancer among women. *Int J Cancer,* **119,** 1462–7.

Broeks A, Russell NS, Floore AN, Urbanus JH, Dahler EC, van T Veer MB, et al (2000). Increased risk of breast cancer following irradiation for Hodgkin's disease is not a result of ATM germline mutations. *Int J Radiat Biol,* **76,** 693–8.

Broeks A, Braaf CM, Huseinovic A, Nooijon A, Urbanus J, Hogervorst FBL, et al (2007). Identification of women with an increased risk of developing radiation-induced breast cancer: a case-only study. *Breast Cancer Res,* **9,** R26.

Brooks JD, Teraoka SN, Reiner AS, Satagopan JM, Bernstein L, Thomas DC, et al; Wecare Study Collaborative Group, Haile RW, Concannon P and Bernstein JL (2012). Variants in activators and downstream targets of ATM, radiation exposure, and contralateral breast cancer risk in the WECARE study. *Hum Mutat,* **33,** 158–64.

Buck D, Malivert L, de Chasseval R, Barraud A, Fondanèche MC, Sanal O, et al (2006). Cernunnos, a novel nonhomologous end-joining factor, is mutated in human immunodeficiency with microcephaly. *Cell,* **124**(2), 287–99.

Bürger S, Schindler D, Fehn M, Mühl B, Mahrhofer H, Flentje M, et al (2006). Radiation-induced DNA damage and repair in peripheral blood mononuclear cells from Nijmegen breakage syndrome patients and carriers assessed by the Comet assay. *Environ Mol Mutagen,* **47**(4), 260–70.

Byrd PJ, Srinivasan V, Last JI, Smith A, Biggs P, Carney EF, et al (2012). Severe reaction to radiotherapy for breast cancer as the presenting feature of ataxia telangiectasia. *Br J Cancer,* **106,** 262–8.

Carney JP, Maser RS, Olivares H, Davis EM, Le Beau M, Yates JR 3rd, et al (1998). The hMre11/hRad50 protein complex and Nijmegen breakage syndrome: linkage of double-strand break repair to the cellular DNA damage response. *Cell,* **93,** 477–86.

Cavazzana-Calvo M, Le Deist F, De Saint Basile G, Papadopoulo D, De Villartay JP and Fischer A (1993). Increased radiosensitivity of granulocyte macrophage colony-forming units and skin fibroblasts in human autosomal recessive severe combined immunodeficiency. *J Clin Invest,* **91**(3), 1214–18.

Chokkalingam AP, Bartley K, Wiemels JL, Metayer C, Barcellos LF, Hansen HM, et al (2011). Haplotypes of DNA repair and cell cycle control genes, X-ray exposure, and risk of childhood acute lymphoblastic leukemia. *Cancer Causes Control,* **22,** 1721–30.

Crawford TO, Skolasky RL, Fernandez R, Rosquist KJ and Lederman HM (2006). Survival probability in ataxia telangiectasia. *Arch Dis Child,* **91,** 610–11.

Cunliffe P, Mann J, Cameron A, Roberts K and Ward H (1975). Radiosensitivity in ataxia-telangiectasia. *Br J Radiol,* **48**(569), 374.

Delia D, Piane M, Buscemi G, Savio C, Palmeri S, Lulli P, et al (2004). MRE11 mutations and impaired ATM-dependent responses in an Italian family with ataxia-telangiectasia-like disorder. *Hum Mol Genet,* **13,** 2155–63.

Devgan SS, Sanal O, Doil C, Nakamura K, Nahas SA, Pettijohn K, et al (2011). Homozygous deficiency of ubiquitin-ligase ring-finger protein RNF168 mimics the radiosensitivity syndrome of ataxia-telangiectasia. *Cell Death Differ,* **18**(9), 1500–506.

Djuzenova C, Flentje M and Plowman PN (2004). Radiation response *in vitro* of fibroblasts from a Fanconi anaemia patient with marked clinical radiosensitivity. *Stahlenther Onkol,* **180,** 789–97.

Ducatman B, Scheithauer BW, Piepgras DG, Reiman HM and Ilstrup DM (1986). Malignant peripheral nerve sheath tumours: a clinicopathological study of 120 cases. *Cancer,* **57,** 2006–21.

Duell EJ, Millikan RC, Pittman GS, Winkel S, Lunn RM, Tse C-KJ, et al (2001) Polymorphisms in the DNA repair gene XRCC1 and breast cancer. *Cancer Epidemiol Biomark Prev,* **10,** 217–22.

Ege M, Ma Y, Manfras B, Kalwak K, Lu H, Lieber MR, Schwarz K and Pannicke U (2005). Omenn syndrome due to ARTEMIS mutations. *Blood,* **105**(11), 4179–86.

Evans DGR, Farndon PA, Burnell LD, Rao Gattamaneni H and Birch JM (1991). The incidence of Gorlin syndrome in 173 consecutive cases of medulloblastoma. *Br J Cancer,* **64,** 959–61.

Fernet M, Gribaa M, Salih MA, Seidahmed MZ, Hall J and Koenig M (2005). Identification and functional consequences of a novel MRE11 mutation affecting ten Saudi Arabian patients with the ataxia telangiectasia-like disorder (ATLD). *Hum Mol Genet,* **14**(2), 307–18.

French CA, Masson JY, Griffin CS, O'Regan P, West SC and Thacker J (2002). Role of mammalian RAD51L2 (RAD51C) in recombination and genetic stability. *J Biol Chem,* **277**(22), 19322–30.

Gibbons DL, MacDonald D, McCarthy KP, Cleary HJ, Plumb M, Wright EG and Greaves MF (1999). An E mu-BCL-2 transgene facilitates leukaemogenesis by ionizing radiation. *Oncogene,* **18**(26), 3870–77.

Godthelp BC, Wiegant WW, van Duijn-Goedhart A, Schärer OD, van Buul PP, Kanaar R and Zdzienicka MZ (2002). Mammalian Rad51C contributes to DNA cross-link resistance, sister chromatid cohesion and genomic stability. *Nucleic Acids Res,* **30**(10), 2172–82.

Godthelp BC, van Buul PP, Jaspers NG, Elghalbzouri-Maghrani E, van Duijn-Goedhart A, Arwert F, et al (2006). Cellular characterization of cells from the Fanconi anemia complementation group, FA-D1/BRCA2. *Mutat Res,* **601**(1–2), 191–201.

Goldfrank D, Chuai S, Bernstein JC, Ramon y Cajal T, Lee JB, et al (2006). Effect of mammography on breast cancer risk in women with mutations in BRCA1 or BRCA2. *Cancer Epidemiol Biomarkers Prev,* **15,** 2311–13.

Goldgar DE, Healey S, Dowty JG, DaSilva L, Chen X, Spurdle AB, et al (2011). Rare variants in the ATM gene and risk of breast cancer. *Breast Cancer Res,* **13,** R73.

Goldstein AM, Yuen J and Tucker MA (1997). Second cancers after medulloblastoma: population-based results from the United States and Sweden. *Cancer Causes Control,* **8,** 865–871.

Gotoff SP, Amirmokri E and Liebner EJ (1967). Ataxia telangiectasia: neoplasia, untoward response to X-irradiation, and tuberous sclerosis. *Am J Dis Child,* **114**(6), 617–25.

Greaves M (2007). Darwinian medicine: a case for cancer. *Nat Rev Cancer,* **6,** 193–203.

Gronwald J, Pijpe A, Byrski T, Huzarski T, Stawicka M, Cybulski C, et al (2008). Early radiation exposures and BRCA1-associated breast cancer in young women from Poland. *Breast Cancer Res Treat,* **112,** 581–4.

Hernandez D, McConville CM, Stacey M, Woods CG, Brown MM, Shutt P, et al (1993). A family showing no evidence of linkage between the ataxia telangiectasia gene and chromosome 11q22-23. *J Med Genet,* **30,** 135–40.

Heyn R, Haeberlen V, Newton WA, Ragab AH, Raney RB, Tefft M, et al (1993). Second malignant neoplasms in children treated for rhabdomyosarcoma. Intergroup Rhabdomyosarcoma Study Committee. *J Clin Oncol,* **11,** 262–70.

Hisada M, Garber JE, Fung CY, Fraumeni JF Jr and Li FP (1998). Multiple primary cancers in families with Li-Fraumeni syndrome. *J Natl Cancer Inst,* **90,** 606–11.

Hung RJ, Boffetta P, Canzian F, Moullan N, Szeszenia-Dabrowska N, Zaridze D, et al (2006). Sequence variants in cell cycle control pathway, X-ray exposure, and lung cancer risk: a multicenter case–control study in Central Europe. *Cancer Res,* **66,** 8280–86.

Infante-Rivard C (2003). Diagnostic X rays, DNA repair genes and childhood acute lymphoblastic leukemia. *Health Phys,* **85,** 60–64.

Inskip PD and Curtis RE (2007). New malignancies following childhood cancer in the United States 1973–2002. *Int J Cancer,* **121,** 2233–40.

Klein C, Wenning GK, Quinn NP and Marsden CD (1996). Ataxia without telangiectasia masquerading as benign hereditary chorea. *Mov Disord,* **11**(2), 217–20.

Kleinerman RA, Tucker MA, Tarone RE, Abramson DH, Seddon JM, Stovall M, et al (2005). Risk of new cancers after radiotherapy in long-term survivors of retinoblastoma: an extended follow-up. *J Clin Oncol,* **23,** 2272–9.

Knudson AG Jr (1971). Mutation and cancer: statistical study of retinoblastoma. *Proc Natl Acad Sci USA,* **68,** 820–23.

Knudson AG (1986). Genetics of human cancer. *Ann Rev Genet,* **20,** 231–51.

Kuhn EM (1980). Effects of X-irradiation in G1 and G2 on Bloom's syndrome and normal chromosomes. *Hum Genet,* **54**(3), 335–41.

Land CE (1980). Estimating cancer risks from low doses of ionizing radiation. *Science,* **209,** 1197–203.

Li FP, Fraumeni JF Jr, Mulvihill JJ, Blattner WA, Dreyfus MG, Tucker MA and Miller RW (1988). A cancer family syndrome in twenty four kindreds. *Cancer Res,* **48,** 5358–62.

Loree TR, North JH Jr, Werness BA, Nangia R, Mullins AP and Hicks WL Jr (2000). Malignant peripheral nerve sheath tumours of the head and neck: analysis of prognostic factors. *Otolaryngol Head Neck Surg,* **122,** 667–72.

Malkin D, Li FP, Strong LC, Fraumeni JF Jr, Nelson CE, Kim DH, et al (1990). Germline p53 mutations in a familial syndrome of breast cancer, sarcoma and other neoplasms. *Science,* **250,** 1233–8.

Malkin D, Jolly KW, Barber N, Look AT, Friend SH, Gebhardt MC, et al (1992). Germline mutations of the p53 tumour suppressor gene in children and young adults with second malignant neoplasms. *N Engl J Med,* **326,** 1309–15.

Malone KE, Begg CB, Haile RW, Borg P, Concannon P, Tehed L, et al (2010). Population based study of the risk of second primary contralateral breast cancer associated with carrying a mutation in BRCA1 or BRCA2. *J Clin Oncol,* **28,** 2404–10.

Maraschio P, Spadoni E, Tanzarella C, Antoccia A, Di Masi A, Maghnie M, et al (2003). Genetic heterogeneity for a Nijmegen breakage-like syndrome. *Clin Genet,* **63**(4), 283–90.

Marcou Y, D'Andrea A, Jeggo PA and Plowman PN (2001). Normal cellular radiosensitivity in an adult Fanconi anaemia patient with marked clinical radiosensitivity. *Radiother Oncol,* **60,** 75–9.

Matsumoto Y, Miyamoto T, Sakamoto H, Izumi H, Nakazawa Y, Ogi T, et al (2011). Two unrelated patients with MRE11A mutations and Nijmegen breakage syndrome-like severe microcephaly. *DNA Repair (Amst),* **10**(3), 314–21.

Mellemkjaer L, Dahl C, Olsen JH, Bertelsen L, Guldberg P, Christensen J, et al; WECARE Study Collaborative Group, Bernstein JL (2008). Risk for contralateral breast cancer among carriers of the CHEK2*1100delC mutation in the WECARE Study. *Br J Cancer,* **98,** 728–33.

Millikan RC, Player JS, deCotret AR, Tse C-K and Keku T (2005). Polymorphisms in DNA repair genes, medical exposure to ionizing radiation, and breast cancer risk. *Cancer Epidemiol Biomark Prev,* **14,** 2326–34.

Morgan JL, Holcomb TM and Morrissey RW (1968). Radiation reaction in ataxia telangiectasia. *Am J Dis Child,* **116**(5), 557–8.

Mori H, Colman SM, Xiao AM, Ford AM, Healy LE, Donaldson C, et al (2002). Chromosome translocations and covert leukaemic clones are generated during normal fetal development. *Proc Natl Acad Sci USA,* **99,** 8242–7.

Moshous D, Callebaut I, de Chasseval R, Corneo B, Cavazzana-Calvo M, Le Deist F, et al (2001). Artemis, a novel DNA double-strand break repair/V(D)J recombination protein, is mutated in human severe combined immune deficiency. *Cell,* **105**(2), 177–86.

Moshous D, Pannetier C, de Chasseval R, le Deist F, Cavazzana-Calvo M, Romana S, et al (2003). Partial T and B lymphocyte immunodeficiency and predisposition to lymphoma in patients with hypomorphic mutations in Artemis. *J Clin Invest,* **111**(3), 381–7.

Nakamura NA (2005). Hypothesis: radiation-related leukemia is mainly attributable to the small number of people who carry pre-existing clonally expanded preleukemic cells. *Radiat Res,* **163,** 258–65.

Narod SA, Lubinski J, Ghadirian P, Lynch HT, Moller P, Foulkes WD, et al, for the Hereditary Breast Cancer Clinical Study Group (2006). Screening mammography and risk of breast cancer in BRCA1 and BRCA2 mutation carriers: a case–control study. *Lancet Oncol,* **7,** 402–6.

Neubauer S, Arutyunyan R, Stumm M, Dörk T, Bendix R, Bremer M, et al (2002). Radiosensitivity of ataxia telangiectasia and Nijmegen breakage syndrome homozygotes and heterozygotes as determined by three-color FISH chromosome painting. *Radiat Res,* **157**(3), 312–21.

O'Driscoll M, Cerosaletti KM, Girard PM, Dai Y, Stumm M, Kysela B, et al (2001). DNA ligase IV mutations identified in patients exhibiting developmental delay and immunodeficiency. *Mol Cell,* **8**(6), 1175–85.

Péron S, Pan-Hammarström Q, Imai K, Du L, Taubenheim N, Sanal O, et al (2007). A primary immunodeficiency characterized by defective immunoglobulin class switch recombination and impaired DNA repair. *J Exp Med,* **204**(5), 1207–16.

Pitts S, Kullar H, Stankovic T, Stewart GS, Last JKK, Bedenham T, et al (2001). hMRE11: genomic structure and a null mutation identified in a transcript protected from nonsense-mediated mRNA decay. *Hum Mol Genet,* **10,** 1155–62.

Plowman PN, Bridges BA, Arlett CF, Hinney A and Kingston JE (1990). An instance of clinical radiation morbidity and cellular radiosensitivity not associated with ataxia telangiectasia. *Br J Radiol,* **63,** 624–8.

Rajaraman P, Bhatti P, Doody MM, Simon SL, Weinstock RM, Linet MS, et al (2008). Nucleotide excision repair polymorphisms may modify ionizing radiation-related breast cancer risk in US radiologic technologists. *Int J Cancer,* **123,** 2713–16.

Reiman A, Srinivasan V, Barone G, Last JI, Wootton LL, Davies EG, et al (2011). Lymphoid tumours and breast cancer in ataxia telangiectasia, substantial protective effect of residual ATM kinase activity against childhood tumours. *Br J Cancer,* **105,** 586–91.

Renwick A, Thompson D, Seal S, Kelly P, Chagtai T, Ahmed M, et al (2006). ATM mutations that cause ataxia-telangiectasia are breast cancer susceptibility alleles. *Nat Genet,* **38,** 873–5.

Revenkova E, Focarelli ML, Susani L, Paulis M, Bassi MT, Mannini L, et al (2009). Cornelia de Lange syndrome mutations in SMC1A or SMC3 affect binding to DNA. *Hum Mol Genet,* **18,** 418–27.

Riballo E, Critchlow SE, Teo SH, Doherty AJ, Priestley A and Broughton B (1999). Identification of a defect in DNA ligase IV in a radiosensitive leukaemia patient. *Curr Biol,* **9**(13), 699–702.

Sedgwick RP and Boder E (1991). Ataxia telangiectasia. In *Handbook of Clinical Neurology. Hereditary Neuropathies and Spinocerebellar Atrophies* (JBMV de Jong, Ed). Amsterdam, Elsevier Science Publishers, pp 347–42.

Seemanová E, Sperling K, Neitzel H, Varon R, Hadac J, Butova O, et al (2006). Nijmegen breakage syndrome (NBS) with neurological abnormalities and without chromosomal instability. *J Med Genet,* **43**(3), 218–24.

Sharif S, Ferner R, Birds JM, Gillespie JE, Gattamaneni HR, Baser ME and Evans DG (2006). Second primary tumours in neurofibromatosis patients treated for optic glioma: substantial risks after radiotherapy. *J Clin Oncol,* **24,** 2570–75.

Shen J, Gilmore EC, Marshall CA, Haddadin M, Reynolds JJ and Eyaid W (2010). Mutations in PNKP cause microcephaly, seizures and defects in DNA repair. *Nat Genet,* **42**(3), 245–9.

Sigurdson AJ, Bhatti P, Chang SC, Rajaraman P, Doody MM, Bowen L, et al (2009a). Polymorphisms in estrogen biosynthesis and metabolism-related genes, ionizing radiation exposure, and risk of breast cancer among US radiologic technologists. *Breast Cancer Res Treat,* **118,** 177–84.

Sigurdson AJ, Land CE, Bhatti P, Pineda M, Brenner A, Carr Z, et al (2009b). Thyroid nodules, polymorphic variants in DNA repair and RET-related genes, and interaction with ionizing radiation exposure from nuclear tests in Kazakhstan. *Radiat Res,* **171,** 77–88.

Southwide GJ and Schwartz RA (1979). The basal cell nevus syndrome: disasters occurring among a series of 36 patients. *Cancer,* **44,** 2294–305.

Stewart GS, Maser RS, Stankovic T, Bressan DA, Kaplan MI, Jaspers NG, et al (1999). The DNA double-strand break repair gene hMRE11 is mutated in individuals with an ataxia-telangiectasia-like disorder. *Cell,* **99,** 577–87.

Stewart GS, Stankovic T, Byrd PJ, Wechsler T, Miller ES, Huissoon A, et al (2007). RIDDLE immunodeficiency syndrome is linked to defects in 53BP1-mediated DNA damage signaling. *Proc Natl Acad Sci USA,* **104,** 16910–15.

Stewart GS, Panier S, Townsend K, Al-Hakim AK, Kolas NK, Miller ES, et al (2009). The RIDDLE syndrome protein mediates a ubiquitin-dependent signaling cascade at sites of DNA damage. *Cell,* **136**(3), 420–34.

Strong LC (1977). Genetic and environmental interactions. *Cancer,* **40,** 1861–6.

Strong LC and Williams WR (1987). The genetic implications of long term survival of childhood cancer. *Am J Podiatr Haematol Oncol,* **9,** 99–103.

Taalman RD, Jaspers NG, Scheres JM, de Wit J and Hustinx TW (1983). Hypersensitivity to ionizing radiation, *in vitro*, in a new chromosomal breakage disorder, the Nijmegen breakage syndrome. *Mutat Res,* **112,** 23–32.

Taniguchi T, Garcia-Higuera I, Xu B, Andreassen PR, Gregory RC, Kim ST, et al (2002). Convergence of the Fanconi anemia and ataxia telangiectasia signaling pathways. *Cell,* **109**(4), 459–72.

Tanzanella C, Antoccia A, Spadoni E, di Masi A, Pecile V, Demori E, et al (2003). Chromosome instability and nibrin protein variants in NBS heterozygotes. *Eur J Hum Genet,* **11**(4), 297–303.

Taylor AM, Harnden DG, Arlett CF, Harcourt SA, Lehmann AR, Stevens S and Bridges BA (1975). Ataxia telangiectasia: a human mutation with abnormal radiation sensitivity. *Nature,* **258,** 427–9.

Taylor AMR, Metcalfe JA, Oxford JM and Harnden DG (1976). Is chromatid type damage in ataxia telangiectasia after irradiation at G0 a consequence of defective repair? *Nature,* **260,** 441–3.

Taylor AM, Groom A and Byrd PJ (2004). Ataxia-telangiectasia-like disorder (ATLD) – its clinical presentation and molecular basis. *DNA Repair (Amst),* **3,** 1219–25.

Teraoka SN, Bernstein JL, Reiner AS, Haile RW, Bernstein L, Lynch CF, et al; WECARE Study Collaborative Group, Concannon P (2011). Single nucleotide polymorphisms associated with risk for contralateral breast cancer in the Women's Environment, Cancer, and Radiation Epidemiology (WECARE) Study. *Breast Cancer Res,* **13**(6), R114.

Thompson D, Duedal S, Kirner J, McGuffog L, Last J, Reiman A, et al (2005). Cancer risks and mortality in heterozygous ATM mutation carriers. *J Natl Cancer Inst,* **97,** 813–22.

Tsuzuki S, Seto M, Greaves M and Enver T (2004). Modeling first-hit functions of the t(12, 21) TEL-AML1 translocation in mice. *Proc Natl Acad Sci USA,* **101**(22), 8443–8.

Uchisaka N, Takahashi N, Sato M, Kikuchi A, Mochizuki S, Imai K, et al (2009). Two brothers with ataxia-telangiectasia-like disorder with lung adenocarcinoma. *J Pediatr,* **155**(3), 435–8.

van Den Berg DJ and Francke U (1993). Roberts syndrome: a review of 100 cases and a new rating system for severity. *Am J Med Genet,* **47**(7), 1104–23, Review.

van der Burg M, van Veelen LR, Verkaik NS, Wiegant WW, Hartwig NG, Barendregt BH, et al (2006). A new type of radiosensitive T-B-NK+ severe combined immunodeficiency caused by a LIG4 mutation. *J Clin Invest,* **116**(1), 137–45.

van der Burg M, Ijspeert H, Verkaik NS, Turul T, Wiegant WW, Morotomi-Yano K, et al (2009). A DNA-PKcs mutation in a radiosensitive T-B- SCID patient inhibits Artemis activation and nonhomologous end-joining. *J Clin Invest,* **119**(1), 91–8.

van der Lelij P, Godthelp BC, van Zon W, van Gosliga D, Oostra AB, Steltenpool J, et al (2009). The cellular phenotype of Roberts syndrome fibroblasts as revealed by ectopic expression of ESCO2. *PLoS One,* **4**(9), e6936.

van der Lelij P, Chrzanowska KH, Godthelp BC, Rooimans MA, Oostra AB, Stumm M, et al (2010). Warsaw breakage syndrome, a cohesinopathy associated with mutations in the XPD helicase family member DDX11/ChlR1. *Am J Hum Genet,* **86**(2), 262–6.

Varon R, Vissinga C, Platzer M, Cerosaletti KM, Chrzanowska KH, Saar K, et al (1998). Nibrin, a novel DNA double-strand break repair protein, is mutated in Nijmegen breakage syndrome. *Cell,* **93,** 467–76.

Varon R, Dutrannoy V, Weikert G, Tanzarella C, Antoccia A, Stöckl L, et al (2006). Mild Nijmegen breakage syndrome phenotype due to alternative splicing. *Hum Mol Genet,* **15**(5), 679–89.

Vaz F, Hanenberg H, Schuster B, Barker K, Wiek C, Erven V, et al (2010). Mutation of the RAD51C gene in a Fanconi anemia-like disorder. *Nat Genet,* **42**(5), 406–9.

Vrouwe MG, Elghalbzouri-Maghrani E, Meijers M, Schouten P, Godthelp BC, Bhuiyan ZA, et al (2007). Increased DNA damage sensitivity of Cornelia de Lange syndrome cells; evidence for impaired recombinational repair. *Hum Mol Genet,* **16,** 1478–87.

Waltes R, Kalb R, Gatei M, Kijas AW, Stumm M, Sobeck A, et al (2009). Human RAD50 deficiency in a Nijmegen breakage syndrome-like disorder. *Am J Hum Genet,* **84**(5), 605–16.

Warcoin M, Lespinasse J, Despouy G, Dubois d'Enghien C, Laugé A, et al (2009). Fertility defects revealing germline biallelic nonsense NBN mutations. *Hum Mutat*, **30**(3), 424–30.

Wong FL, Boice JD Jr, Abramson DH, Tarone RE, Kleinerman RA, Stovall M, et al (1997). Cancer incidence after retinoblastoma. Radiation dose and sarcoma risk. *JAMA,* **278,** 1262–7.

Woods CG, Bundey SE and Taylor AMR (1990). Unusual features in the inheritance of ataxia telangiectasia. *Hum Genet,* **84,** 555–62.

Yuan Y, Zhou L, Miyamoto T, Iwasaki H, Harakawa N, Hetherington CJ, et al (2001). AML1-ETO expression is directly involved in the development of acute myeloid leukemia in the presence of additional mutations. *Proc Natl Acad Sci USA,* **98,** 10398–403.

7 Cellular Radiosensitivity in Human Subpopulations

Here evidence for heterogeneity will be considered by comparing the radiosensitivity of cells from defined subpopulations with that of cells from ostensibly unselected members of the general population. It can of course be argued that none of the control samples is strictly unselected. They may come from students or laboratory staff whose lifestyle may not completely conform to that of the general population. Some will come from cell banks or laboratory collections of undefined origin. Many have arisen as healthy controls in studies of cancer patients; by definition these will be biased to some extent towards those less prone to cancer. Controls for breast cancer patients will generally be female and of a certain age. If the controls are non-cancer patients from the same hospital, these are by definition not the healthiest individuals. Nevertheless, despite these caveats, the available data may be expected to give some indication of whatever degree of heterogeneity may exist.

7.1 Breast cancer patients

There have been a number of studies showing that lymphocytes from breast cancer patients are more susceptible to radiation-induced chromosomal damage. It has been common for authors employing the G2 assay to use an arbitrary 90th percentile cut-off in the control population as a point determining increased sensitivity. In various studies the fraction of hypersensitives among breast cancer patients using the G2 chromosome assay was found to be 42% (Scott, 2000), 43% (Baeyens, 2005), 46% (Riches et al, 2001) and 40% (Barak et al, 1993). Using a 75% cut-off, breast cancer patients had a 2.83-fold increased odds ratio (Buchholz and Wu, 2001). Time-dependent repair in the G2 assay was reported to be suboptimal in 35% of normal controls compared to 93% of breast cancer patients (Patel et al, 1997).

Enhanced sensitivity to chromosomal damage in breast cancer patients has also been seen in the micronucleus assay. Out of 39 patients, 12 (31%) showed more than two standard deviations greater sensitivity than controls (two out of 42, 5%) (Scott et al, 1998).

The effect may be attributable to diminished repair of DNA damage. Shahidi et al (2007) found that the initial damage as assayed by both neutral and alkaline comet assay was similar for breast cancer patients and controls. When assayed after 24 hours, however, the cells from patients all showed more than 20% residual damage, in contrast to those from controls.

7.2 Cancer patients generally

Lymphoblastoid cell lines (lymphoid cells transformed by Epstein-Barr virus) have been used to examine radiosensitivity in the general population. By measuring the regrowth kinetics of irradiated and control cells the slopes of the exponential portions of the regrowth curves were determined and a grow-back ratio was calculated as a measure of radiosensitivity. Using chronic radiation exposure, 12% of 270 normal lines fell in the same range of sensitivity as 11 ataxia telangiectasia (A-T) lines. The other normal lines showed a reasonably wide range of sensitivity. In lines from 27 newly presenting cancer patients a higher proportion were more sensitive, but some were as resistant as the most resistant normals (Chadwick and Bridges, 1989; Gentner and Morrison, 1989; Gentner et al, 1988). Because of their transformed nature, there is a general reluctance to extrapolate from lymphoblastoid cell lines to primary human cells, nevertheless the results are not inconsistent with some results with primary cells.

In a pilot study Bondy and Ligon (1996) reported radiation-induced chromatid breaks in S and G2 phase cells in short-term lymphocyte cultures from 45 adult malignant glioma patients and 117 matched controls. They found an unadjusted odds ratio of 5.36 (95% CI 2.12–13.69) for radiosensitivity and brain tumour risk and an adjusted odds ratio of 5.79 (2.26–14.83) when they controlled for smoking, race, income and education. A subsequent study suggested that the radiosensitivity was modulated by polymorphisms in, and haplotypes of, the *RAD51L1* gene, which is involved in the DNA double strand break repair pathway (Liu et al, 2010).

Among juvenile cancer patients the proportion of hypersensitives (exceeding the normal 90th percentile) using the G2 assay on lymphocytes was 44% (Baria et al, 2002) and 52% (Curwen et al, 2005); among colorectal cases it was 30% (Baria et al, 2001); among head and neck cancer patients it was 26%, with the highest figure (38%) among oral cavity patients (De Ruyck et al, 2008). In a comparison of 57 patients with papillary thyroid cancer and 105 healthy matched controls, Xiong et al (2005) found an adjusted odds ratio of 4.54 (95% CI 2.7–9.95) and evidence of a dose response. Using the median of the controls, an adjusted odds ratio of 17.25 was found for patients with salivary gland tumours (Zheng et al, 2004). In principle, heterogeneity in the G2 assay could arise both from differences in rates of repair and differences in the operation of the G2/M checkpoint. However, recent evidence has failed to find any significant relationship between G2 checkpoint delay and G2 chromosomal radiosensitivity ($p = 0.284$) (Cadwell et al, 2011). Luipold et al (2006), using three-colour fluorescence in situ hybridisation for measuring chromosomal aberrations at metaphase, found that about one-third of cancer patients had an aberration count higher than the 99% confidence interval of the control's Gaussian distribution. About 5% were outside the 99% confidence interval for the cancer patients' normal distribution.

Scott (2000) has interpreted the results with the G2 assay as evidence that genes conferring radiosensitivity are present in the general population and that they are involved in low penetrance predisposition to breast and some other cancers (see above). In the Danish study of survivors of childhood and adolescent cancer, the proportion of offspring of survivors showing G2 chromosomal radiosensitivity (53%) was no different from the proportion of patients themselves (52%) (Curwen et al, 2005), consistent with inherited predisposition. However, genetic predisposition may not be the whole story, since 35% of the partners of the cancer survivors were also radiosensitive. The authors argue that this raises questions about the suitability of the partners of cancer survivors to act as an appropriate control

group. Put another way, it implies that lifestyle factors may influence radiosensitivity in the G2 assay. Indeed, it has been reported that the hormonal concomitants of pregnancy are associated with a change in lymphocyte radiosensitivity that is likely to be attributable to changes in gene expression (see discussion in Miles et al, 2011). The observation that lymphocytes from males are significantly more radiosensitive than those from females ($p = 0.03$) may also be relevant (Wang et al, 2000). Since lymphocytes are taken directly from the body and undergo very few divisions before being scored, it is also conceivable that their radiosensitivity might be modulated by systemic factors such as might be associated with inflammatory conditions. The observation that chromosomal radiosensitivity is significantly greater in lymphocytes from smokers is consistent with such a scenario (Wang et al, 2000).

If there is a fraction of the population that has radiosensitive lymphocytes and is cancer prone (whether for genetic or lifestyle reasons) the question arises as to whether such individuals are also predisposed to radiation-induced cancer. While this might seem a reasonable hypothesis, chromosomal hypersensitivity might equally well cause more cell death rather than more cancer induction. In principle, epidemiological studies on the risk of second cancers in therapeutically irradiated populations could shed light on this. If cancer patients are more susceptible to radiation-induced cancers, then the risks estimated from data on second cancers could be greater than those estimated from general population exposures such as the Japanese atomic-bomb survivors (Life Span Study cohort). This question was considered in detail in a previous AGIR report (AGIR, 2000), but no clear answer emerged largely because of the existence of confounding factors (such as toxicity associated with high therapeutic doses) in many of the available studies. The report concluded:

> "The finding from most of the studies considered here is that the ERR for the induction of second cancers is, in general, either compatible with or less than the ERR derived from the Japanese LSS data."

At present, therefore, second cancer data do not support the idea that chromosomal radiosensitivity in lymphocytes is associated with sensitivity to radiation-induced cancer.

There is good evidence that a substantial proportion of patients with several types of cancer (in addition to breast cancer) have lymphocytes showing chromosomal radiosensitivity.

We conclude that there is a fraction of the general population that shows chromosomal hypersensitivity to radiation and that is predisposed to a variety of cancers. While it might seem to be reasonable to suppose that these individuals would also be predisposed to radiation-induced cancer, the available evidence from second cancer epidemiology provides no support for this hypothesis.

7.3 Atherosclerosis patients

Fibroblasts from five patients with severe atherosclerosis were exposed to gamma radiation at a low dose rate (0.007 Gy min^{-1}) and exhibited a clonogenic radiosensitivity that was intermediate between that of healthy subjects and that of patients with the known radiosensitivity syndrome ataxia telangiectasia (A-T) (Hannan et al, 1994). There was a considerable inter-strain difference in the radiosensitivity of fibroblasts from patients with atherosclerosis, with their mean D_{10} values varying between 2.3 and 6.2 Gy, whereas

the mean D_{10} values for the cells from the A-T homozygote, A-T heterozygotes and healthy subjects were 2.0, 3.8 and 9.0 Gy, respectively. When exposed at a higher dose rate (8 Gy min^{-1}) cells from only one of the atherosclerosis patients could be differentiated from cells of the healthy subjects and that one showed cellular radiosensitivity similar to that of the A-T homozygote. Radioresistant DNA synthesis was also examined in four of the strains following exposure to radiation at 8 Gy min^{-1}. All four showed A-T heterozygote-like radioresistant DNA synthesis intermediate between that of A-T homozygotes and of healthy subjects, suggesting a partial deregulation of cell cycle in the patients with atherosclerosis.

In a subsequent study from the same laboratory with 19 atherosclerosis patients, the range of 37% survival doses was 0.6–3.1 Gy compared with 4.6–5.0 Gy based on three control strains. Most of the cell strains from atherosclerosis patients showed radioresistant DNA synthesis, with roughly 33% showing an A-T-like and the rest an A-T heterozygote-like response. All the strains with an AT-like response and one-quarter with a heterozygote-like response were found to be defective in the radiation-induced expression of both *p53* and *p21* (Nasrin et al, 1997).

Taken at face value, these results are surprising, given the relative commonness of atherosclerosis. Moreover, there are some issues regarding the use of concurrent controls and replication of experiments which lead to there being reservations about this work. Replication studies are required to confirm the findings.

Nevertheless, the work with cells from heart disease patients aged 32–69 years utilised low dose rate radiation procedures which would be expected to reveal partial deficiencies in radiation response that might not show up with the higher dose rates normally used (for example, in the work reported by Arlett et al, 2008). Even so, to explain these results it would be necessary to postulate that severe heart disease in middle-age is strongly associated with increased cellular radiosensitivity to exposures at low dose rate. It should be noted that although the study was carried out in Saudi Arabia, the actual ethnicity of the patients is not known. Work cited elsewhere should be noted in which lymphocytes from aged populations (including many with cardiovascular and cerebrovascular disease) were found to exhibit radiosensitivity (Harris et al, 1986).

It seems unlikely (though in principle possible) that an inherited mutation is responsible for enhanced radiosensitivity as well as predisposing to cardiovascular disease (see AGIR, 2010). But if the radiosensitivity is real and is a consequence of the cardiovascular disease (for example, due to an effect of the associated inflammatory condition), then there must be some persisting change (presumably epigenetic) in the fibroblasts that can be cultured from the patients.

7.4 Autoimmune disease patients

Harris et al (1985) had reported that peripheral blood lymphocytes cultured with ConA were more radiosensitive if they came from patients with conditions associated with autoimmunity, such as rheumatoid arthritis, systemic lupus erythematosus (SLE) and polymyositis. Subsequently, it was reported that repair of DNA single strand breaks was delayed in lymphocytes from children with such autoimmune conditions (McCurdy et al, 1997) and in lymphocytes from SLE patients (Bassi et al, 2008). Evidence has been presented that such patients have a reduced ability to deal with oxidative damage (Bashir et al, 1993). While causality remains to be established, a plausible explanation would be that the oxidative

species generated in inflammatory situations overloads the defence systems (scavenging and/or repair) resulting in enhanced sensitivity to radiation. SLE patients as a whole did not have a higher initial number of DNA double strand breaks, although SLE patients with anaemia, increased erythrocyte sedimentation rate and those with positive result for anti-La/SSB and anti-RNP antibodies showed significantly more DNA double strand breaks than those without them (Carrillo-Alascio et al, 2009).

7.5 Normal unselected population

Evidence for variation in radiosensitivity within the general population has also emerged from studies that did not use defined subpopulations.

7.5.1 Studies with peripheral blood lymphocytes

The ability to perform colony formation assays using peripheral blood lymphocytes irradiated in G0 became possible with the use of interleukin-2 (IL2) (James et al, 1983). Green et al (1991) examined the survival curves of lymphocytes from 34 normal donors and concluded that they were all similar and did not show significant heterogeneity. They were, however, using high dose rate radiation. Geara et al (1993), working with lymphocytes from 29 individuals, found that variation due to technical or sampling errors was significantly lower than variation between individuals ($p = 0.014$). This was true at both high and low dose rates. Elyan et al (1993), on the other hand, found that at an even lower dose rate, sparing of cell killing was seen and led to an increase in the spread of data between individuals such that inter-individual differences reached statistical significance for surviving fractions at 4 Gy ($p = 0.004$). They suggested that the dose rates used by Geara et al (and, by implication, Green et al) may not have been low enough to reveal heterogeneity in survival response.

The G2 cytogenetic assay is technically more demanding and at high dose rates variation between individuals and variation between repeat samples from an individual were found to be similar in one study (Vral et al, 2004), although in another study inter-individual variation between 19 healthy individuals was found to be highly statistically significant ($p = 0.001$) (Smart et al, 2003). The response of lymphocytes in the G2 assay is known to be altered by prior exposure to a very low dose of radiation (Olivieri et al, 1984). The yield of aberrations is reduced, a phenomenon that has been termed the adaptive response. This response is believed to contribute to the reduced effectiveness of radiation exposures given at low dose rate and presumably reflects the induction of more effective repair of DNA damage. Heterogeneity in the adaptive response has been reported in two studies. Sankaranarayanan et al (1989) found that lymphocytes from one out of five normal healthy volunteers (20%) showed no evidence of an adaptive response and Bosi and Olivieri (1989) obtained a similar lack in four out of 18 donors (22%). A study looking at chromatid aberrations in lymphocytes of individuals living in a high radiation area of Iran found that adaptation increased linearly with cumulative dose up to 1 Gy. Two out of seven individuals (28%), however, showed no evidence of adaptation (Montazavi et al, 2005).

There have been few studies on the effect of age on lymphocyte radiosensitivity. In the G2 assay young normal controls (3 months to 19 years old) were slightly more likely to have enhanced radiosensitivity than adult normals (20–60 years) (Baria et al, 2002), 15% versus 10%, but this was not statistically significant.

Harris et al (1986) had reported that greater radiosensitivity determined by colony formation was shown by lymphocytes from ageing donors. However, these donors were taken from patients classified as 'healthy' but being treated for chronic problems including cerebral and cardiovascular disease. In the light of subsequent work with fibroblasts from atherosclerosis patients (see Section 7.3) this may be significant. The observations made with the comet and G2 assays are not necessarily inconsistent since the standard comet assay measures apparent initial damage to the genome, whereas the G2 assay measures the outcome after repair and checkpoint processes have operated. Further work will be needed to clarify the effect of age in this system.

The initial damage most relevant to chromosomal aberrations is the DNA double strand break and this can now be considered by visualising the complex formed at double strand breaks by phosphorylated histone H2AX (the γH2AX assay). Quantitative assays have been developed by Roch-Lefevre et al (2010) and Bourton et al (2011) for peripheral blood lymphocytes. Hamasaki (2007) used a flow cytometry system in cultured T-lymphocytes to evaluate individual radiosensitivity *in vitro*. Irradiation of cells cultured for seven days showed significant inter-individual and reproducible differences in γH2AX content after 4 Gy of X-rays. However, these differences were not as marked in uncultured lymphocytes or in lymphocytes that were cultured for a prolonged period (around 13 days). There appear to be plans to combine this approach with an epidemiological study on the atomic-bomb survivors (Hamasaki et al, 2007).

Changes in gene expression following irradiation have been reported to vary from person to person, even *in vivo* (see, for example, Goldberg et al, 2006). Such changes may underlie some of the heterogeneity that has been documented in cellular assays, in post-Chernobyl thyroid carcinogenesis (Detours et al, 2008), as well as having a role in radiation-induced genomic instability (Aypar et al, 2011). Some gene expression changes are known to be able to persist through cell division.

The reports discussed above lead ineluctably to the conclusion that there are individuals in the population with lymphocytes that are more susceptible than the average to the chromosome-breaking effect of ionising radiation. They also show that among such individuals some are more than usually likely to develop cancer, implying that the heterogeneity manifest in lymphocytes is not confined to that cell type. The data do not, however, show whether such individuals are more than usually susceptible to radiation-induced cancer, although that would be a very reasonable hypothesis. Neither do they allow such individuals to be distinguished within the population; there may well be others above the 90th percentile cut-off who do not have any unusual propensity to develop cancer, and who are not hypersensitive for cancer induction by radiation. Similarly, the existence of heterogeneity means that there will be others whose lymphocytes are more resistant than the average; what such resistance might correlate with is speculative.

7.5.2 Studies with cultured skin fibroblasts

Primary fibroblast cell strains are untransformed and have undergone many more divisions in culture than lymphocytes before being scored. Their properties must therefore be ascribed either to their genetic make-up or to a stable change in gene expression profile. Fibroblasts were included in a monumental assemblage of radiosensitivity data from different cell types, which has shown clearly that experimental conditions have a large influence in the response of cells to radiation exposure (Deschavanne and Fertil,

1996). Comparisons therefore need to be confined to situations where such conditions are identical, ideally in the same laboratory during the same period of time. In working over many decades with strains from radiosensitive individuals, Arlett and colleagues have built up an impressive set of data for 'normal' controls. In 1991, they reported that there were significant differences among 34 donors in survival of fibroblasts exposed to acute high dose rate gamma radiation in contrast to lymphocytes where differences were not significant (Green et al, 1991). In a later study, of 53 'normal' strains, two were more than 1.96 standard deviations more sensitive than the mean, and one was more resistant (Arlett et al, 2008). A rather higher proportion of sensitives was reported by Nagasawa and Little (1988) who found three out of ten strains from the Coriell cell bank could be arbitrarily designated as sensitive in both the colony survival and G2 assays.

The use of low dose rate exposures has been found to widen the difference between 'normals' and 'radiosensitive' strains. Wilson et al (2008) used continuous exposure to low dose rate gamma radiation (0.5–8.4 cGy h^{-1}) and found that five out of 18 Coriell cell bank controls were significantly more sensitive than the remaining normal controls. Designation as 'sensitive' was arbitrary and based on the exposure rate that reduced relative survival to 1%; strains that needed less than 3.5 cGy h^{-1} were regarded as sensitive. The 1% survival dose rates for the 13 normals ranged from 3.9–5.6 cGy $h^{-1}$and for the five sensitives from 2.5–3.3 cGy h^{-1}.

In a later study, Wilson et al (2010) found that among five controls from the Coriell cell bank, two that were radiosensitive in the continuous exposure survival assay had significantly higher aberration frequencies than the other three 'normals' after 50 and 100 cGy. The authors speculate that these moderately radiosensitive individuals may harbour hypomorphic genetic or epigenetic changes that moderate genomic maintenance systems. Although the proportion of normal individuals showing apparent radiosensitivity was high in these two studies, the numbers were very small.

The use of assays for γH2AX or 53BP1 foci at sites of DNA double strand breaks has opened up a new and very sensitive approach to the study of human radiosensitivity. Initial results, however, are far from clear. Among 25 apparently normal fibroblast strains, seven were found to show slower focus formation after low radiation doses, similar to most repair-deficient mutant strains examined (Kato et al, 2006). After 24 hours post-irradiation, the normal strains having slower focus formation showed more efficient repair and the range of residual foci was at least as large as that observed for the repair-defective mutants. These workers also state that sensitivity in the G2 assay using low dose rates tends to track with sensitivity in the γH2AX assay, although the correlation is not absolute.

7.5.3 Studies with bone marrow stem cells

Kadhim et al (1992) reported a high frequency of non-clonal cytogenetic abnormalities in the clonal descendants of murine haemopoietic stem cells that survived low dose alpha particle irradiation. The data were compatible with the induction of stem cell lesions that transmit chromosomal instability to their progeny. Subsequent work showed that a similar delayed instability phenomenon could be demonstrated after exposure to alpha particles (but not X-rays) of bone marrow cells from two of four haematologically normal individuals (up to 25% abnormal metaphases) (Kadhim et al, 1994, 1995). Such inter-individual differences were confirmed in a limited number of other individuals (Wright, personal communication).

It is unclear what role delayed chromosomal instability has in radiation lethality or carcinogenesis, but the observed inter-individual differences, which should perhaps still be regarded as preliminary, suggest that responses to ionising radiation are not always the same from one person to another. Consistent with this is the finding that delayed instability varies quantitatively between inbred mouse strains (Kadhim et al, 1994).

7.5.4 Effect of dose and dose rate

Although the details are not fully understood, there is abundant mechanistic evidence that cells respond differently to radiation when exposed at different doses and at different dose rates. Indeed, most cell endpoints show greater resistance to radiation when exposed at low dose rates. There is therefore no reason to expect that any differences in radiosensitivity that might exist within the population will necessarily be the same at all doses and dose rates.

The endpoint of concern for differences in radiosensitivity depends upon the application. From a radiological protection perspective, very low doses and low dose rates are of interest when considering current exposure to the general population, whereas for radiation workers somewhat higher (though still 'low') doses are of interest. Cell death is not an issue of concern at very low doses; carcinogenesis is, together with circulatory disease at slightly higher 'low' doses. (The weight of evidence does not currently support a circulatory disease effect at very low doses.)

With very high doses and high dose rates such as are used in radiotherapy, cell killing is the primary concern, with carcinogenesis in exposed normal tissue a secondary consideration.

Radiation incidents and accidents potentially involve high, intermediate and low doses, but usually at a relatively high dose rate. When individuals are exposed to high doses in an incident or accident, knowledge of the dose to which they have been exposed would be the first concern, but knowledge of their radiosensitivity would also be important in the context of triage for emergency treatment.

There are relatively few studies in which differences in radiosensitivity have been studied at more than one dose rate, and even when low dose rates have been employed, the doses used have not been particularly low. Such evidence as exists is almost entirely concerned with cell killing and as such is likely to be more relevant to accidents or incidents involving relatively high dose exposures. Low dose rates have been reported to allow better discrimination of heterogeneity for killing of both lymphocytes (Elyan et al, 1993; West et al, 1995) and fibroblasts (Hannan et al, 1994; Wilson et al, 2010). However, radiotherapy involves high dose rates and heterogeneity only manifest at low dose rates may be of little relevance.

Heterogeneity of sensitivity to chromosomal damage might be viewed as a surrogate for carcinogenesis. Using dicentrics in lymphocytes as an endpoint, better discrimination among patients showing adverse reaction to radiotherapy was reported to occur at low compared with high dose rates (Jones et al, 1995). In the G2 assay, the adaptive response, which is believed to underlie the reduced effect of low dose rates, was reported to be absent in 20% (Sankaranarayanan et al, 1989), 22% (Bosi and Olivieri, 1989) or 28% (Montazavi et al, 2005) of individuals tested. This might lead to the expectation that the sparing effect of low dose rates for carcinogenesis might be reduced in about a quarter of individuals.

Finally, there is the special case of radon-induced lung cancer in smokers. At environmental exposures smokers are around 30 times more sensitive than non-smokers (AGIR, 2009). In the atomic-bomb survivors, exposed to high dose rate gamma and neutron radiation, the effect was less and was only seen in light to moderate smokers (Furukawa et al, 2010). However, from a radiation physics perspective no radon doses are received at low dose rate since all alpha particle tracks are high LET and deliver energy at a high dose rate to the affected cell.

It is important to realise that no direct extrapolation can be made from cellular radiosensitivity to carcinogenic risk following radiation exposure. This can be illustrated by considering an early paper on cell killing of fibroblasts from ataxia telangiectasia and basal cell nevus syndrome (BCNS) patients (Taylor et al, 1975). BCNS patients show multiple skin cancers in the radiation field (Walter et al, 1997) but show no overt cellular radiosensitivity under these conditions.

7.6 Evidence for heritability of cellular radiosensitivity in humans

Human cancer-prone disorders and radiosensitivity syndromes provide good evidence that inherited genetic factors can contribute to individual variation in risk. However, such studies do not allow a quantitative estimate of the contribution of genetics to the range of radiosensitivity in the general population. It is in principle possible to make such heritability estimates from human pedigree studies and twin studies. Heritability is defined as the amount of variation for a phenotypic trait that is accounted for by variation in inherited genetic factors. Heritability estimates are based on the correlation of offspring and parental phenotypes in pedigrees or, more commonly, comparison of correlation in monozygotic (MZ) and dizygotic (DZ) twin pairs. The mathematical models applied in twin study analysis are now highly sophisticated and methods to distinguish the relative contributions of inherited genetic factors, shared environmental factors and unique environmental factors are available. In twin studies, heritability is estimated as twice the difference in correlation between MZ and DZ twin pairs. The contribution of common, shared environmental factors is defined as the residual correlation after the heritability estimate is deducted. Estimating the role of unique (ie individual-specific) environmental factors requires studies of twins reared separately. Epidemiology suggests that the major environmental factors affecting radiation-induced carcinogenesis are, by and large, the same as those modulating spontaneous cancer incidence, of which the most notable are smoking and diet (see Chapter 2). Studies that do not allow for the operation of these factors in separately reared twins will not yield figures for genetic and environmental factors that are applicable to the general population. Furthermore, as most of the twin studies of radiosensitivity do not explicitly consider twins reared separately, it is possible that the role of these environmental/lifestyle factors is considerably underestimated.

There are of course no twin studies yielding radiation carcinogenesis data, but data for surrogate cellular endpoints are available. A number of heritability estimates are available for cellular radiosensitivity assessed in peripheral blood lymphocytes (see Table 7.1). Endpoints considered include G2 chromosomal radiosensitivity, apoptosis, micronucleus formation and cell cycle delay. The range of central estimates of heritability for these endpoints is 58–82% (Table 7.1). These estimates are based on moderate to high dose exposures (0.5–5 Gy) to low LET radiation at high dose rate. Therefore there is reasonably consistent evidence for a significant contribution of genetic factors to the range of cellular radiosensitivity observed

TABLE 7.1 Evidence for heritability of radiosensitivity as a human trait

Source	Study population	Assay*	Heritability estimate (95% CI)
Roberts et al, 1999	16 radiosensitive breast cancer survivors and 37 first-degree relative, 4 breast cancer survivors with normal radiosensitivity and 15 first-degree relatives	G2	82%
Curwen et al, 2005	23 cancer survivors, 29 partners, 38 offspring, 27 controls	G2	67%
Camplejohn et al, 2006	28 monozygotic and 26 dizygotic female twin pairs	Apoptosis	81% (67–89%)
Wu et al, 2006	148 monozygotic and 57 dizygotic twin pairs, 50 siblings	Apoptosis	63%
Schmitz et al, 2007	199 father, mother, offspring trios	Apoptosis	61%
Finnon et al, 2008	38 dizygotic and 16 monozygotic twin pairs	Apoptosis Cell cycle delay	68% (40–82%) 59% (22–79%)
Curwen et al, 2010	29 cancer survivors, 29 partners, 53 offspring	G2	58–78%
Surowy et al, 2011	39 monozygotic and 10 dizygotic twin pairs	MN	68%

* Peripheral blood lymphocytes used in all studies.
G2 assay involves scoring chromosomal damage in cells irradiated in G2 phase of the cell cycle.
MN (micronucleus) assay involves irradiating cells and preventing progression through mitosis. Radiation-damaged chromosomes form micronuclei which are counted.

in the general population. It is perhaps notable that the higher estimates tend to come from studies of cancer survivors and their families. This would be consistent with the observation of a higher excess absolute risk of second radiogenic cancers in some inherited cancer-prone disorders (see Chapter 6). However, the differences between cancer survivor families and normal individuals are not great or highly consistent.

7.7 Genetic predisposition for specific cancers

In addition to factors inherited from parents, there are genetic factors arising in offspring that can predispose to radiation-induced cancer. Just as some lifestyle/environmental factors conferring radiosensitivity are specific for particular cancers (eg smoking and lung cancer), so these genetic factors may predispose to radiosensitivity for particular cancers. An example may be various leukaemias where there is much evidence for an association with specific translocations in circulating lymphocytes. These translocations arise very early in gestation, probably during the first trimester, and are clonally expanded as the lymphocyte population expands during subsequent development. These genetic changes do not in themselves make the cells leukaemic without one or a small number of further mutations, but they can be regarded as effectively preleukaemic and individuals carrying clonally expanded numbers would be expected to be more at risk of developing leukaemia than the general population.

If radiation-induced leukaemias arise because radiation induces second mutations in individuals carrying clonally expanded populations and not because of *de novo* induction of the translocations, then such individuals would constitute a radiosensitive population for leukaemia. This hypothesis has been stated and explored by Nakamura (2005) who concludes that almost all the radiation risk is in the population with clonally expanded preleukaemic lymphocytes and the risk for the general population is vanishingly small. For paediatric ALL (acute lymphocytic leukaemia) associated with the t(12;21) translocation, Nakamura estimates that the development of ALL after radiation exposure is almost exclusively attributable to the 4% of predisposed individuals in a population. The relative risk for these predisposed individuals is thus roughly 25 times higher than the conventional estimates of the risk to the population as a whole.

7.8 Summary

There is good evidence that a substantial proportion of breast cancer patients have lymphocytes showing chromosomal radiosensitivity.

There is good evidence that a substantial proportion of patients with several types of cancer (in addition to breast cancer) have lymphocytes showing chromosomal radiosensitivity.

We conclude that there is a fraction of the general population that shows chromosomal hypersensitivity to radiation and that these individuals are predisposed to a variety of cancers. While it might seem to be reasonable to suppose that these individuals would also be predisposed to radiation-induced cancer, the available evidence from second cancer epidemiology provides no support for this hypothesis.

Fibroblasts from patients with severe atherosclerosis have been reported to be more sensitive to radiation, particularly at low dose rate.

Several pieces of evidence suggest that individuals with chronic inflammatory conditions are less well able to cope with radiation and may exhibit radiosensitivity.

Lymphocytes isolated and examined in culture demonstrate heterogeneity in response to radiation for colony formation, chromosomal damage and DNA strand break repair, although the proportions of 'sensitive' and 'resistant' individuals is unclear. Low dose rate exposure reveals more heterogeneity than acute dose exposure.

Three studies indicate that 20–30% of individuals have lymphocytes that do not show an adaptive response to radiation.

With fibroblasts, some heterogeneity of clonal radiosensitivity is seen with acute exposures.

Fibroblasts from normal individuals when exposed to radiation at low doses or dose rates show differences in response that are not obvious when high doses or dose rates are used. In particular, the distribution of responses is bimodal and cells from a high proportion (perhaps a third) of apparently normal individuals appear to show DNA repair capacities suggestive of increased radiosensitivity.

Significant differences between fibroblasts from different individuals have been reported with the H2AX assay for repair of DNA double strand breaks, but further work is needed.

Two out of four individuals demonstrated delayed chromosomal instability in bone marrow cells following exposure to alpha particles.

There is evidence from studies with cultured human cells that the spread of heterogeneity of radiosensitivity is greater at low than at high dose rates for cell killing and chromosomal damage.

Studies of human families and twins have provided estimates of the heritability of cellular radiosensitivity. These estimates vary between studies but are in general around 70%; however, it is difficult to completely exclude a contribution of common environmental factors in these estimates.

Individuals carrying clonally expanded populations of cells with preleukaemic translocations that have arisen during fetal life have been hypothesised to have a greatly increased sensitivity to leukaemia induction by subsequent exposure to radiation compared with the population as a whole.

7.9 References

AGIR (2000). Risks of Second Cancer in Therapeutically Irradiated Populations. Comparison with Cancer Risks in the Japanese Atomic Bomb Survivors and in Other Exposed Groups. Report of the Advisory Group on Ionising Radiation. *Doc NRPB,* **11**(1), 1–105.

AGIR (2009). Radon and Public Health. Report of the independent Advisory Group on Ionising Radiation. *Doc HPA,* RCE-11, 1–240. Available at www.hpa.org.uk

AGIR (2010). Circulatory Disease Risk. Report of the independent Advisory Group on Ionising Radiation. *Doc HPA,* RCE-16, 1–116. Available at www.hpa.org.uk

Arlett CF, Green MH, Rogers PB, Lehmann AR and Plowman PN (2008). Minimal ionizing radiation sensitivity in a large cohort of xeroderma pigmentosum fibroblasts. *Br J Radiol,* **81,** 51–8.

Aypar U, Morgan WF and Baulch JE (2011). Radiation-induced genomic instability: are epigenetic mechanisms the missing link? *Int J Radiat Biol*, **87**(2), 179–91.

Baeyens A, Van Den Broecke R, Makar A, Thierens H, De Ridder L and Vral A (2005). Chromosomal radiosensitivity in breast cancer patients: influence of age of onset of the disease. *Oncol Rep,* **13,** 347–53.

Barak Y, Juven T, Haffner R and Oren M (1993). *MDM2* Expression is induced by wild type p53 activity. *EMBO J,* **12**(2), 461–8.

Baria K, Warren C, Roberts SA, West CM and Scott D (2001). Chromosomal radiosensitivity as a marker of predisposition to common cancers? *Br J Cancer,* **84,** 892–6.

Baria K, Warren C, Eden OB, Roberts SA, West CM and Scott D (2002). Chromosomal radiosensitivity in young cancer patients: possible evidence of genetic predisposition. *Int J Radiat Biol,* **78,** 341–6.

Bashir S, Harris G, Denman MA, Blake DR and Winyard PG (1993). Oxidative DNA damage and cellular sensitivity to oxidative stress in human autoimmune diseases. *Ann Rheum Dis,* **52,** 659–66.

Bassi C, Xavier DJ, Palomino G, Nicolucci P, Soares C, Sakamoto-Hojo E and Donadi E (2008). Efficiency of the DNA repair and polymorphisms of the *XRCC1, XRCC3* and *XRCC4* DNA repair genes in systemic lupus erythematosus. *Lupus,* **17,** 988–95.

Bondy M and Ligon BL (1996). Epidemiology and aetiology of intracranial meningiomas: a review. *J Neurooncol,* **29,** 197–205.

Bondy ML, Kyritsis AP, Gu J, de Andrade M, Cunningham J, Levin VA, et al (1996). Mutagen sensitivity and risk of gliomas: a case–control analysis. *Cancer Res,* **1,** 1484–6.

Bosi A and Olivieri G (1989). Variability of the adaptive response to ionizing radiations in humans. *Mutat Res,* **211,** 13–17.

Bourton EC, Plowman PN, Smith DS, Arlett CF and Pariss CN (2011). Prolonged expression of the γ-H2AX DNA repair marker correlates with excess acute and chronic toxicity from radiotherapy treatment. *Int J Cancer,* **129**(12), 2928–34.

Buchholz TA and Wu X (2001). Radiation-induced chromatid breaks as a predictor of breast cancer risk. *Int J Radiat Oncol,* **49,** 533–7.

Cadwell KK, Curwen GB, Tawn EJ, Winther JF and Boice JD Jr (2011). G2 checkpoint control and G2 chromosomal radiosensitivity in cancer survivors and their families. *Mutagenesis,* **26**(2), 291–4.

Camplejohn RS, Hodgson S, Carter N, Kato BS and Spector TD (2006). Heritability of DNA damage-induced apoptosis and its relationship with age in lymphocytes from female twins. *Br J Cancer,* **95**(4), 520–24.

Carrillo-Alascio PL, Sabio JM, Núñez-Torres MI, López E, Muñoz-Gámez JA, Hidalgo-Tenorio C, et al; Grupo Lupus Virgen De Las Nieves (2009). In-vitro radiosensitivity in patients with systemic lupus erythematosus. *Lupus,* **18,** 645–9.

Chadwick KH and Bridges BA (1989). CEC/AECL workshop on methods for determining differential radiosensitivity in humans, 7–9 September 1988, Brighton, UK. *Int J Radiat Biol,* **55,** 483–9.

Curwen GB, Winther JF, Tawn EJ, Smart V, Whitehouse CA, Rees GS, et al (2005). G(2) chromosomal radiosensitivity in Danish survivors of childhood and adolescent cancer and their offspring. *Br J Cancer,* **93,** 1038–45.

Curwen GB, Cadwell KK, Winther JF, Tawn EJ, Rees GS, Olsen JH, et al (2010). The heritability of G2 chromosomal radiosensitivity and its association with cancer in Danish cancer survivors and their offspring. *Int J Radiat Biol,* **86**, 986–95.

De Ruyck K, de Gelder V, Van Eijkeren M, Boterberg T, De Neve W, Vral A and Thierens H (2008). Chromosomal radiosensitivity in head and neck cancer patients: evidence for genetic predisposition? *Br J Cancer,* **98,** 1723–8.

Deschavanne PJ and Fertil B (1996). A review of human cell radiosensitivity *in vitro*. *Int J Radiat Oncol Biol Phys,* **34,** 251–66.

Detours V, Versteyhe S, Dumont JE and Maenhaut C (2008). Gene expression profiles of post-Chernobyl thyroid cancers. In *Current Opinion in Endocrinology, Diabetes and Obesity* (PJ David, Ed). Volume 15.

Elyan SA, West CM, Roberts SA and Hunter RD (1993). Use of low-dose rate irradiation to measure the intrinsic radiosensitivity of human T-lymphocytes. *Int J Radiat Biol,* **64,** 375–83.

Finnon P, Robertson N, Dziwura S, Raffy C, Zhang W, Ainsbury L, et al (2008). Evidence for significant heritability of apoptotic and cell cycle responses to ionising radiation. *Hum Genet*, **123**, 485–93.

Furukawa K, Preston DL, Lönn S, Funamoto S, Yonehara S, Matsuo T, et al (2010). Radiation and smoking effects on lung cancer incidence among atomic bomb survivors, *Radiat Res,* **174,** 72–82.

Geara FB, Peters LJ, Ang KK, Wike JL and Brock WA (1993). Prospective comparison of *in vitro* normal cell radiosensitivity and normal tissue reactions in radiotherapy patients. *Int J Radiat Biol,* **27,** 1173–9.

Gentner NE and Morrison BP (1989). Determination of the proportion of persons in the population-at-large who exhibit abnormal sensitivity to ionising radiation. In *Low Dose Radiation: Biological Bases of Risk Assessment* (KF Baverstock and JW Stather, Eds). London, Taylor and Francis.

Gentner NE, Morrison DP and Myers DK (1988). Impact on radiogenic cancer risk of persons exhibiting abnormal sensitivity to ionizing radiation. *Health Phys,* **55,** 415–25.

Goldberg Z, Rocke DM, Schwietert C, Berglund SR, Santana A, Jones A, et al (2006). Human *in vivo* dose-response to controlled, low-dose low linear energy transfer ionizing radiation exposure. *Clin Cancer Res,* **12,** 3723–9.

Green MHL, Arlett CF, Cole J, Harcourt SA, Priestley A, Waugh A, et al (1991). Comparative human cellular radiosensitivity: III. Gamma-radiation survival of cultured skin fibroblasts and resting T-lymphocytes from the peripheral blood of the same individual. *Int J Radiat Biol,* **59,** 749–65.

Hamasaki K, Imai K, Nakachi K, Takahashi N, Kodama Y and Kusunoki Y (2007). Short-term culture and gamma H2AX flow cytometry determine differences in individual radiosensitivity in human peripheral T lymphocytes. *Environ Mol Mutagen,* **48,** 38–47.

Hannan MAF, Khougeer Z, Halees, Sanei AM and Khan BA (1994). Increased radiosensitivity and radioresistant DNA synthesis in cultured fibroblasts from patients with coronary atherosclerosis. *Arterioscler Thromb,* **14**(11), 1761–6.

Harris G, Cramp WA, Edwards JC, George AM, Sabovljev SA, Hart L, et al (1985). Radiosensitivity of peripheral blood lymphocytes in autoimmune disease. *Int J Radiat Biol,* **47,** 689–99.

Harris G, Holmes A, Sabovljev SA, Cramp WA, Hedges M, Hornsey S, et al (1986). Sensitivity to X-irradiation of peripheral blood lymphocytes from ageing donors. *Int J Radiat Biol,* **50,** 685–94.

James SE, Arlett CF, Green MHL and Bridges BA (1983). Radiosensitivity of human T-lymphocytes proliferating in long term culture. *Int J Radiat Biol,* **44,** 417–22.

Jones LA, Scott D, Cowan R and Roberts SA (1995). Abnormal radiosensitivity of lymphocytes from breast cancer patients with excessive normal tissue damage after radiotherapy: chromosome aberrations after low dose-rate irradiation. *Int J Radiat Biol,* **67,** 519–28.

Kadhim MA, MacDonald DA, Goodhead DT, Lorimore SA, Marsden SJ and Wright EG (1992). Transmission of chromosomal instability after plutonium alpha-particle irradiation. *Nature,* **355,** 738–40.

Kadhim MA, Lorimore SA, Hepburn MD, Goodhead DT, Buckle VJ and Wright EG (1994). α-particle-induced chromosomal instability in human bone marrow cells. *Lancet,* **344,** 987–88.

Kadhim MA, Lorimore SA, Townsend KM, Goodhead DT, Buckle VJ and Wright EG (1995). Radiation-induced genomic instability: delayed cytogenetic aberrations and apoptosis in primary human bone marrow cells. *Int J Radiat Biol,* **67**, 287–93.

Kato TA, Nagasawa H, Weil MM, Little JB and Bedford JS (2006). Levels of gamma-H2AX foci after low-dose-rate irradiation reveal a DNA DSB rejoining defect in cells from human ATM heterozygotes in two at families and in another apparently normal individual. *Radiat Res,* **166,** 443–53.

Liu Y, Shete S, Wang LE, El-Zein R, Etzel CJ, Liang FW, et al (2010 not 2011). Gamma-radiation sensitivity and polymorphisms in *RAD51L1* modulate glioma risk. *Carcinogenesis,* **31**(10), 1762–9.

Luipold VR, Neubauer S, Keller U, Sauer R and Grabenbauer GG (2006). Individual differences in chromosomal aberrations after *in vitro* irradiation of cells from healthy individuals, cancer and cancer susceptibility syndrome patients. *Radiother Oncol,* **81,** 257–63.

McCurdy D, Tai LQ, Frias S and Wang Z (1997). Delayed repair of DNA damage by ionizing radiation in cells from patients with juvenile systemic lupus erythematosus and rheumatoid arthritis. *Radiat Res,* **147,** 48–54.

Miles EF, Tatsukawa Y, Funamoto S, Kamada N, Nakashima E, Kodama Y, et al (2011). Biomarkers of radiosensitivity in A-bomb survivors pregnant at the time of bombings in Hiroshima and Nagasaki. *ISRN Obstet Gynecol,* 2011:264978. Epub 2010 Sep 29.

Montazavi SMJ, Shabestani-Monfared A, Ghiassi-Nejad M and Mozdarani H (2005). Radioadaptive responses induced in lymphocytes of the inhabitants in Ramsar, Iran. *International Congress Series,* **1276,** 201–3.

Nagasawa H and Little JB (1988). Radiosensitivities of ten apparently normal human diploid fibroblast strains to cell killing, G2-phase chromosomal aberrations, and cell cycle delay. *Cancer Res,* **48,** 4535–8.

Nakamura N (2005). A hypothesis: radiation-related leukemia is mainly attributable to the small number of people who carry pre-existing clonally expanded preleukemic cells. *Radiat Res,* **163**(3), 258–65.

Nasrin N, Mimish LA, Manogaran PS, Kunhi M, Al-Sedairy S and Hannan MA (1997). Cellular radiosensitivity, radioresistant DNA synthesis, and defect in radioinduction of p53 in fibroblasts from atherosclerosis patients. *Arterioscler Thromb Vasc Biol,* **17,** 947–53.

Olivieri G, Bodycote J and Wolff S (1984). Adaptive response of human lymphocytes to low concentrations of radoactive thymidine. *Science,* **223,** 594–7.

Patel RK, Trivedi AH, Arora DC, Bhatavdekar JM and Patel DD (1997). DNA repair proficiency in breast cancer patients and their first-degree relatives. *Int J Cancer,* **73,** 20–24.

Riches AC, Bryant PE, Steel CM, Gleig A, Robertson AJ, Preece PE and Thompson AM (2001). Chromosomal radiosensitivity in G2-phase lymphocytes identifies breast cancer patients with distinctive tumour characteristics. *Br J Cancer,* **85,** 1157–61.

Roberts SA, Spreadborough AR, Bulman B, Barber JB, Evans DG and Scott D (1999). Heritability of cellular radiosensitivity: a marker of low-penetrance predisposition genes in breast cancer? *Am J Hum Genet*, **65**, 784–94.

Roch-Lefevre S, Mandina T, Voisin P, Gaetan GB, Mesa JEG, Valente M, et al (2010). Quantification of γ-H2AX foci in human lymphocytes: a method for biological dosimetry after ionizing radiation exposure. *Radiat Res,* **174,** 185–94.

Sankaranarayanan K, von Duyn A, Loos MJ and Natarajan AT (1989). Adaptive response of human lymphocytes to low-level radiation from radioisotopes or X-rays. *Mutat Res,* **211,** 7–12.

Schmitz A, Bayer J, Dechamps N, Goldin L and Thomas G (2007). Heritability of susceptibility to ionizing radiation-induced apoptosis of human lymphocyte subpopulations. *Int J Radiat Oncol Biol Phys*, **68**, 1169–77.

Scott D (2000). Chromosomal radiosensitivity, cancer predisposition and response to radiotherapy. *Strahlenther Onkol,* **176,** 229–34.

Scott D, Barber JBP, Levine EL, Burrill W and Roberts SA (1998). Radiation-induced micronucleus induction in lymphocytes identifies a high frequency of radiosensitive cases among breast cancer patients: a test for predisposition? *Br J Cancer,* **77,** 614–20.

Shahidi M, Mozdarani H and Bryant PE (2007). Radiation sensitivity of leukocytes from healthy individuals and breast cancer patients as measured by the alkaline and neutral comet assay. *Cancer Lett,* **257,** 263–73.

Smart V, Curwen GB, Whitehouse CA, Edwards A and Tawn EJ (2003). Chromosomal radiosensitivity: a study of the chromosomal G(2) assay in human blood lymphocytes indicating significant inter-individual variability. *Mutat Res,* **528,** 105–10.

Surowy H, Rinckleb A, Luedeke M, Stuber M, Wecker A, Varga D, et al (2011). Heritability of baseline and induced micronucleus frequencies. *Mutagenesis*, **26**, 111–17.

Taylor AMR, Harnden DG, Arlett CF, Harcourt SA, Lehmann AR, Stevens S and Bridges BA (1975). Ataxia-telangiectasia, a human mutation with abnormal radiation sensitivity. *Nature,* **258,** 427–9.

Vral A, Thierens H, Beavens A and De Ridder L (2004). Chromosomal aberrations and *in vitro* radiosensitivity: intra-individual *versus* inter-individual variability. *Toxicol Lett,* **149,** 345–52.

Walter AW, Pivnick EK, Bale AE and Kun LE (1997). Complications of the nevoid basal cell carcinoma syndrome: a case report. *J Pediatr Hematol Oncol,* **19,** 258–62.

Wang LE, Bondy ML, de Andrade M, Strom SS, Wang X, Sigurdson A, et al (2000). Gender difference in smoking effect on chromosome sensitivity to gamma radiation in a healthy population. *Radiat Res,* **154,** 20–27.

West CML, Elyan SAG, Berry P, Cowan R and Scott D (1995). A comparison of the radiosensitivity of lymphocytes from normal donors, cancer patients, individuals with ataxia-telangiectasia (A-T) and A-T heterozygotes. *Int J Radiat Biol,* **68,** 197–203.

Wilson PF, Nagasawa H, Warner CL, Fitzek MM, Little JB and Bedford JS (2008). Radiation sensitivity of primary fibroblasts from hereditary retinoblastoma family members and some apparently normal controls: colony formation ability during continuous low-dose-rate gamma irradiation. *Radiat Res,* **169,** 483–94.

Wilson PF, Nagasawa H, Fitzek MM, Little JB and Bedford JS (2010). G2-phase chromosomal radiosensitivity of primary fibroblasts from hereditary retinoblastoma family members and some apparently normal controls. *Radiat Res,* **173,** 62–70.

Wu X, Spitz MR, Amos CI, Lin J, Shao L, Gu J, et al (2006). Mutagen sensitivity has high heritability: evidence from a twin study. *Cancer Res*, **66**, 5993–6.

Xiong P, Zheng R, Wang LE, Bondy ML, Shen H, Borer MM, et al (2005). A pilot case–control study of gamma-radiation sensitivity and risk of papillary thyroid cancer. *Thyroid,* **15,** 94–9.

Zheng R, Wang LE, Bondy ML, Wei Q and Sturgis EM (2004). Gamma radiation sensitivity and risk of malignant and benign salivary gland tumors: a pilot case–control analysis. *Cancer Lett,* **100,** 561–7.

8 Human Radiosensitivity and Ethics

This report lays out the known evidence relating to variations in human radiosensitivity. This chapter seeks to formulate and discuss a series of policy questions that arise in relation to the evidence of possible risks of harm. Ethics is central to many of them.

Is there anything we can do to reduce such risks?

How should we weigh such risks against other important considerations (eg financial cost)?

How should we respond to the evidence about interactions between the harm related to radiation exposure and other factors?

Whose job is it to protect individuals and the public from such risks?

What are the implications when we have evidence for differential impact due to variations in sensitivity to radiation?

8.1 Risks and harms

Once we have identified any potential risks, we still need to explore the idea and implications of risk in more detail. There are issues relating to the precise calculation of any risk of harm, and also how we factor such risks into our decision-making. Both tasks are more difficult than might be first thought.

Issues that emerge in the calculation of risks include considering both the magnitude of the possible harm and the likelihood of the possible harm emerging. Both magnitude and likelihood are scalar in nature (there is a gradient of each variable). However, they must be seen as two independent factors because magnitude and likelihood are not directly related. For example, a high degree of harm might be very likely or unlikely. A low risk of harm might also be very likely or unlikely, etc. In terms of policy discussions there is perhaps a tendency to focus on high impact harms, but, sometimes, low risk harms with a high degree of probability can collectively have more impact, and therefore might also be considered a high priority. We might also want to place a different degree of salience upon risks depending upon how certain we are about the evidence relating to a potential harm. Despite some views that hold that we ought to use some form of the precautionary principle, for policy makers the certainty of evidence relating to risks will be important to add into the calculation, along with the magnitude and likelihood of the risk. There is also a debate in the background about the nature of risks, in terms of whether probabilities are objective or subjective, and whether we can distinguish the reality of risk from perceptions of risk (Royal Society, 1992). For the purposes of this report, we assume that risks matter, whatever their metaphysical status.

We cannot simply identify a risk and then immediately act to reduce or remove it. Such a policy would be foolish and also impossible to implement. It would be impossible, as every action based on an attempt to reduce a risk would change things, resulting in the need for further risk assessment and further action, as risks were modified or further risks created. It would be foolish because it assumes that we ought to act to reduce or eliminate every risk. A more sensible policy would seek to consider weighing any risks against any potential benefits, and what other costs might be involved in risk avoidance or reduction. For example, we might reduce the risks involved in road traffic accidents by banning cars, but most people would feel the costs in terms of inconvenience and reduction in choice would be too high. So risks should not just be identified, but also evaluated.

Central to this is the idea that we are not really dealing with mere probabilities but the risk of a *harm* – that is, the likelihood or percentage chance of a harmful event occurring. This means that we need a clear idea about what counts as a harm, and this can be disputed. Harm can be defined in different ways, but the most common is in terms of something that results in damage to, or a negative setback to, our interests (Feinberg, 1987). In relation to radiation risks, we are mostly interested in physiological harms resulting in a negative impact on mortality and morbidity as a consequence of exposure to radiation. In at least some cases we can choose to prioritise certain kinds of harms as being of the utmost importance. Harms such as those resulting from radiation exposure are likely to be given high priority because of the likely negative impact upon human health. Once we know what the harm might be, we can then move on to think about which harms it is important to consider in relation to policy. This requires us to consider how to weigh different values and different considerations against each other. For example, preserving health is likely to be seen as a priority, as good health is very likely to be a precondition for the ability to make other choices.

One important consideration relating to risks is that it is often thought to be crucial how risks emerge or what control we have over them. For example, we might distinguish between exposure-related risks and sensitivity-related risks. Exposure-related risks cover the fact that everyone is exposed to ionising radiation (to some degree) during the course of daily life. However, such exposure is not equally distributed as it depends on where a person lives (eg local geology might increase exposure to radon, etc). It also depends on other factors such as exposure through employment, atomic material in the environment (through accidents or incidents) and medical treatment. Most of this exposure is to low doses over the course of a lifetime. Some will be higher level and involve more acute exposures. Some of these exposures can be reduced or minimised, but not eliminated. However, in addition to the unequal *exposure*, we must also notice that there is an inequality in relation to the *impact* of radiation. This is where the sensitivity-related issues come in. Two individuals might be at different levels of risk of harm, even if exposed to the same amount of ionising radiation, due to their genetic inheritance, other existing health conditions, or genetic interactions with environmental features, including certain lifestyle behaviours. Some elements of sensitivity-related risks might be under the control of individuals, such as some increased risk due to certain lifestyle factors (eg smoking), but many will not.

8.2 Whose responsibility?

How risks are created, and who can do something about them, is central to thinking about where responsibility lies for acting on those risks. For example, some will argue that we should just provide information to people about the various risks and let them decide what to do to avoid or mitigate them. Such a view might appeal to the idea of respecting an individual's decision-making autonomy or liberty, and argue that anything else, such as action by the state to reduce risks, would be inappropriate paternalism because it restricts choice and makes a decision on behalf of others about how to allocate resources that could be spent elsewhere. Such an approach sees risk reduction as being the responsibility of individuals, once they have been informed about any risks.

However, an alternative view might point out that, although some exposure-related risks (eg choice of employment) might be freely chosen and therefore the responsibility of individuals, this might also be doubted (eg where there is no real alternative employment available). In addition, many exposure-related risks are not easily open to 'choice' in the relevant sense. If the local environment contains radon-producing rocks, or a past accident at a distant nuclear facility increases the risk to an individual, is this something that they have chosen? Such doubts are likely to grow even stronger as we look to sensitivity-related risks, as we have not chosen our genes, or to be old, or to be ill with other health conditions. Some sensitivity-related factors might be chosen, such as continuing to smoke having had the relevant information related to such risks, but even this might not always be a simple matter of choice, given the nature of nicotine addiction and the norms in a particular society that influence how people behave. In addition, there are a number of possible problems to do with relying upon the provision of information to individuals as the answer. For example, we may not know what information people consider to be relevant to them. Information about risks from such things as radiation might be difficult to understand and cause anxiety, particularly if individuals can do little to counter any such risks.

Additionally, if it is thought that the risks of harm ought to be reduced or removed, it is important to see that often this cannot be achieved by individuals alone. Such risks can only be reduced by collective action and/or the action of the state on behalf of us all. For example, a government can pass laws to regulate risk-related activity, such as seeking to reduce the exposure of workers in nuclear facilities, or the state may use funds from general taxation to pay for remedial action to reduce the risk to families living in housing in a high radon area. Once it is accepted that governments might have responsibility to take action to reduce such risks because they are best able to do so, this thought can be supported by appeal to the idea that preventing or reducing risk of harm, particularly for those not able to take action themselves, is a matter of social justice. We should all be provided with the opportunity to lead a flourishing life. Such a conclusion is likely to impose strong obligations on the state to act to mitigate both exposure-related and sensitivity-related risks. The charge of paternalism is an easy one to make, but the matter is not so simple when considering policy that affects populations (Nys, 2008).

8.3 Exposure-related risks

As we saw above we can pick out different groups in society as a rough way to stratify risks relating to exposure. There are four groups with different characteristics. First, we have members of the general public who are exposed to environmental risks through general background radiation levels. This group is

large but generally at low risk. Second, a smaller, but still significant group is of those exposed to radiation in the course of medical treatment (eg radiotherapy) and preventive or diagnostic interventions (eg scans). In these cases, exposure may be to higher and/or repeated doses, but the decision is made that any possible risk is outweighed by some medical benefits. Third, there is also a small, but significant group of individuals exposed to higher and cumulative radiation through occupational exposure (eg nuclear powerplant workers and radiotherapists). Such risks can be minimised by adopting set regulations, but not eliminated. Any residual risks must be explained to such personnel, and presumably they choose to trade financial benefit against any residual risks. Fourth, each of the members of the above three groups might be vulnerable to a sudden, acute exposure through a miscalculation, accident or other incident. Such events are virtually certain to occur, even if general action is taken to minimise them. Since such events can be so catastrophic, we might choose not to seek certain benefits because of those risks. It should be apparent that only a small minority of the exposure-related risks are within the control of individuals to at least some degree (eg employment), many more are under the control of governments (eg legislation about safety regimes), but at least some are beyond all control (eg natural background radiation in food).

8.4 Sensitivity-related risks

How should we respond to differences in individual susceptibility to radiation? This report presents the existing evidence relating to the differential impact upon individuals after exposure. Although we cannot (yet) always individuate risks, we can see that certain general characteristics are associated with an increased risk of harm. Some of these are due to factors outside the control of individuals, but some can be influenced by individual behaviours.

For example, the earlier chapters of the report suggested that certain gene variants (eg *ATM* and possibly *BCRA1* and *BCRA2*) increase the bearer's radiosensitivity and consequent risk of developing certain cancers. Other characteristics, such as inflammation, might also increase radiosensitivity, and other physiological risk factors might be discovered. In addition, it seems that age and sex differences will tend to result in variations in the effects of radiation exposure. Children, the elderly and women all seem to be at greater risk. These discoveries all lead on to the question about what ought to be done in such cases? What obligations are there to protect those at higher risk from such potential harm? These questions can only be answered by looking at the different groups identified in the previous section. First, for example, those about to undergo radiotherapy could be tested for gene variants that increase long- or short-term toxicity following radiotherapy, where such a test exists. This might be a routine matter, particularly if the test is straightforward and cheap. Where it is complicated to use or interpret the result or extremely expensive, it might be used only if there is reason to suspect some problem (eg previous history of damage and family history). Second, we can explore the same issue for those with occupational risks. Once tests are available to detect more and more radiosensitive variants, should we offer to test this group? Should some individuals prove to be at high risk due to genetics, should they be excluded from employment in this area or merely warned about the risk? Such questions are complicated, but it is worth giving some thought to how to respond before such testing becomes widely available. Third, the population might be screened for such variants. However, this would be an expensive task, and is unlikely to be seen as a priority. The sudden, acute exposure case is also less relevant here. Unless this subgroup is

already part of the clinical or occupational high risk groups, we would just be talking about population screening once again. Here, even though the implications of a nuclear disaster would be catastrophic, the likelihood of it occurring is so low it is unlikely to justify such screening.

The second area of sensitivity-related risks worth thinking about relates to the evidence that we have that certain behaviours or elements of an individual's lifestyle (eg smoking, drinking alcohol and being overweight) might increase radiosensitivity. Of course, such risks may significantly increase in combination with other factors such as the gene variants described above and/or environmental factors such as the presence of radon. Although we know that certain lifestyle behaviours will increase risk, we have no evidence that if an individual changes their lifestyle such risks will be reduced, but it is a reasonable assumption given what we know about how risk levels return to normal levels in those who were smokers previously. What policy is justified in the case of such behaviours? One option would be to produce general information about such risks. This could be targeted at the general population or, perhaps more appropriately, at the high risk groups (clinical and occupational). Another option would be to explicitly warn those in these groups of the increased risk and urge them to change any relevant behaviour. A third option, for those at occupational risk, would be to ban those who refuse to change their behaviour from working in such environments as a way of protecting them from unnecessary harm. Such a policy would, no doubt, be labelled as paternalistic. However, one relevant consideration is that in a country with a health care system based on pooled risk and treatment in response to need, it can be argued that it is hard to simply accept that it is for individuals to put themselves at risk, when others share in the burden of paying for the consequences of such choices.

What this suggests is that a relevant consideration in the background to this discussion is the way that a health system is organised. Where health care costs are shared, differential impact in relation to radiosensitivity may be less important than where a health system assumes a more individualistic account of treatment and insurance payments. Any movement towards assigning responsibility for consequences as a result of failing to take provided information into account is problematic because of the difficulty in attributing responsibility fairly in relation to such risks. For example, suppose a 'voluntary' action such as the choice to smoke increases an individual's risk of lung cancer by 10% beyond that of those who live in the same local environment. Does this make that person more responsible for that outcome? Will they be expected to pay a supplement for their care? Once we move away from universal, shared health care, where there is no question of assigning responsibility before treatment, such questions about causation can become central to issues of responsibility attribution.

8.5 Priorities and risks

Given what we now know, and are likely to discover in the future, about differential degrees of radiosensitivity, how should we set 'safe' levels of exposure? Current limits are set for a fictional person averaged in terms of age and sex. Perhaps it is time to move to a more sophisticated analysis and set of policies related to subgroups for whom we can identify high risk or even individual risk assessment where we have the technology to determine this and it is important to protect individuals at higher risk (eg within the clinical and occupational context). The very idea of there being a 'safe level' of radiation makes little sense, given the evidence we have about gene variants, lifestyle behaviours, environmental

factors, etc, all possibly interacting and increasing risk. Such a move to protect those most at risk (once we can identify them) might appeal to ideas about fairness. Various accounts of justice might be used to justify unequal distributions of resources to ensure that those most at risk are not further disadvantaged. For example, some might argue in favour of giving priority to those worst off (in this case those most susceptible to the relevant potential risk). However, this is open to the objection that such an approach could result in a significant proportion of any budget being spent on just a few individuals, and this might not be thought to be just. In addition, once one group is 'compensated' for the risks they may run, others will at that point become a priority on the grounds of being 'worse off' and so on. Alternatively, others might suggest that we should prioritise trying to bring about a sufficient (or minimal) level of security from risk for all, but once this is achieved then justice has been served. This matter cannot be settled here but the question of priorities in relation to combating risks is something that requires urgent attention (Hansson, 2007, 2009).

8.6 Summary

Given what we know about differential individual radiosensitivity, and in the absence of perfect information about any individual's actual level of risk, it makes sense to seek to keep general levels of risk as low as possible across the population.

In the absence of routine individual testing it makes sense to focus on providing information to people about such risks and anything that can be done to reduce such risks. This is especially important when we have clear evidence (such as the significantly increased risks produced through a combination of radon exposure and smoking) or where individuals can change their behaviour to reduce risk (eg cease smoking in higher risk occupations).

As individual testing becomes more feasible, we must consider carefully the implications for the general population, high risk subgroups and individuals.

Policy ought to be formulated in response to what we know about different risks to different groups.

Even given present levels of knowledge, there is a strong argument for conducting routine testing of individuals to determine if they are particularly radiosensitive before they undergo clinical care such as radiotherapy.

All policies in this area will need to be flexible to respond to any changes in the evidence relating to risks of harm.

8.7 References

Feinberg J (1987). *Harm to Others.* Oxford, Oxford University Press.

Hansson SO (2007). Ethics and radiation protection. *J Radiol Prot,* **27,** 147–56.

Hansson SO (2009). Should we protect the most sensitive people? *J Radiol Prot,* **29,** 211–18.

Nys TRV (2008). Paternalism in public health care. *Public Health Ethics,* **1,** 64–72.

Royal Society (1992). *Risk: Analysis, Perception and Management.* London, The Royal Society.

9 Conclusions, Implications and Recommendations

9.1 Conclusions

Each chapter provided summaries of the conclusions specific to each topic. Some more general conclusions can be drawn and these are summarised here.

a The weight of evidence clearly shows that people differ in the way that they respond to ionising radiation exposure and that these differences can be seen to affect both the risk of radiation-induced cancer and (at higher doses) the extent of tissue damage. Evidence comes from human epidemiology and is backed up by cellular studies and animal studies. Both genetic and environmental (including lifestyle) factors are involved and currently the clearest example of the latter is the effect of cigarette smoking on radiation-induced lung cancer. The existence of heterogeneity in radiation-induced detriment has ethical and practical implications for both radiological protection and radiotherapy.

b There is evidence from epidemiological studies that the induction of cancer by ionising radiation is subject, at least to some extent, to the same risk factors as apply to cancer in the general population – for example, age. This implies that the response of individuals within the population (their radiosensitivity for cancer induction) will vary according to the extent to which these risk factors apply to them. This conclusion is supported by animal studies which indicate that the radiosensitivity for cancer induction in a variety of strains is often, but not always, related to the spontaneous incidence in each strain.

c There is ample evidence from cellular studies in humans that there is heterogeneity in radiosensitivity for a variety of endpoints, although none of these endpoints can be directly related to radiation carcinogenesis. The weight of evidence suggests that there is an unknown (but not trivial) fraction of the general population with lymphocytes that show chromosomal radiosensitivity: such individuals appear to have an enhanced risk of breast and some other cancers. It is conceivable that they might also be at a greater risk of radiation-induced cancers, but the available data on the epidemiology of second cancers in therapeutically irradiated populations does not support this. None of the cellular endpoints currently available is specific or reliable enough to identify particular individuals as being at greater risk, although it is likely that progress will be made towards this goal in the medium term.

d Cellular studies also indicate that the spectrum of radiosensitivity is broader at low dose rates. Currently it is not known whether a broader range of cancer risk exists in populations exposed at low dose rates by comparison with populations exposed at high dose rates. While, if this were

the case, it would be expected to be of particular relevance at the levels associated with normal population exposure, it is hard to justify taking account of this in prospective radiological protection limits, given the uncertainty that surrounds estimates of risk at low dose rates and given that such risk estimates are based on population averages.

e While the weight of evidence argues that lifestyle factors are important in influencing the cancer risk following irradiation, the only such factor that has been clearly established as increasing the risk of radiation-induced cancer is smoking in relation to lung cancer. We consider that this might well be taken into account in individual retrospective risk assessments involving doses over 100 mSv. This also raises some ethical questions for dose limits for radiation workers. There is a good case for smoking cessation advice to be provided when the health impact of radon is considered in both occupational and residential/public situations.

f Smoking is also a risk factor for normal cell toxicity associated with radiotherapy, along with other factors that are less well established. Despite much work there is no test currently available that can robustly and accurately predict the normal tissue toxicity in patients about to undergo radiotherapy, or measure it in individuals involved in a major radiation incident, although new molecular approaches may be anticipated to lead to advances in this area.

g Efforts to date to identify heritable gene variants predictive of individual risk or other predictive assays have not in general been successful in identifying tests of radiosensitivity to cancer. The exceptions might be emerging evidence that certain variants of genes, such as *ATM* and possibly *BRCA1 and BRCA2*, associate with a higher risk of breast cancer following radiation exposure – although much more evidence is required to validate any associations.

h There are more syndromes now known with a demonstrable increased cellular radiosensitivity and for which there is a better understanding of underlying genetics and mechanisms than in 1999 when the AGIR last addressed the issue of human radiosensitivity. Such increased radiosensitivity can result from different types of defect in the cell and therefore it is necessary to use the appropriate assay in order to demonstrate the increased radiosensitivity of any particular disorder.

i There is an increasing, but as yet incomplete, understanding of the mechanisms that determine radiosensitivity. These mechanisms are similar, but not identical, at high and low doses and dose rates. The G2/M cell cycle checkpoint, for example, has an apparent threshold for activation of around 15–20 unrepaired DNA double strand breaks per cell, ie around 0.5 Gy. In contrast, the G1/S checkpoint is probably activated by the presence of one DNA double strand break. Stress responses and inflammatory responses play a role in determining radiosensitivity as well as DNA damage response pathways.

j It has been proposed that radiation-induced leukaemias occur almost entirely in those members of the population who carry clonally expanded lymphocytes with preleukaemic translocations. On this hypothesis, the validity of which is yet to be confirmed, the excess absolute risk in these individuals would be some 25 times greater than the figure for the general population.

9.2 Implications for radiological protection

The preceding chapters have reviewed the evidence for variation in human radiosensitivity and the main conclusions are summarised above. It is clear that there is considerable evidence for such variation from a range of sources. Here we will consider the potential implications of this evidence for general radiological protection; this is followed by a detailed consideration of the implications for clinical practice. It must be emphasised, however, that almost none of the evidence available relates directly to the low dose (below 100 mSv) and low dose rate (below 5 mGy h^{-1}) exposure situations that are of concern because of the potential consequence of cancer induction; data relating to radon and smoking are, however, an exception. The potential implications for radiological protection can be divided into those relevant for the population in general, for radiation workers and for radiation accidents and incidents (public, occupational and emergency radiological protection, respectively).

9.2.1 Public radiological protection

There is good evidence that females and younger people are at a greater risk of radiation-induced cancer. For females much of the elevated risk is due to breast cancer risk. The influence of age is reflected in the ICRP recommended cancer risk estimates which are greater for the general population than for a working population. It is argued that for radiological protection purposes the use of age- and sex-averaged risk is appropriate as the effective dose limits that are derived are for use in prospective radiological protection planning (which aims to protect populations not individuals), not retrospective individual risk assessment. Age- and sex-specific risk information should, however, be used in retrospective assessment of the risks to health of exposed individuals.

In general, the cancer risk models recommended for use by the ICRP and other bodies depend to a large extent on excess relative risk as opposed to excess absolute risk. This suggests that the risk of radiation-associated cancer is to a great extent determined by the same factors that determine cancer risk in the general population. Therefore measures that reduce population cancer incidence and mortality should help reduce the incidence of radiation-associated cancer in populations.

There is good evidence of an interaction of radiation with smoking, from studies of occupational and residential radon exposure. These studies indicate, therefore, that it is prudent to avoid smoking to minimise the risk of cancer from both tobacco smoke and radiation exposure. From an ethical perspective it would seem sensible to make this information generally available. Given that the interaction between radon exposure and smoking is more than additive, there is justification to target this information to those living in high radon areas. Studies of clinical reactions to radiotherapy also provide some evidence that smoking can increase the severity of normal tissue reactions, furthermore there is some indication that alcohol consumption and weight (body mass index) can affect clinical radiosensitivity. The extent to which other common risk factors for cancer are also applicable to radiation carcinogenesis may be revealed by research in the future, but no firm evidence currently exists.

There is evidence for variation in radiosensitivity in the general population. In cellular assays this range can be several-fold and such assays also indicate that a considerable proportion of the variation may be

genetic in origin. In addition, twin studies indicate that genetic factors may account for approximately 70% of the variation but, as noted in Chapter 7, this estimate is likely to be very much an upper estimate. It is also unlikely that cellular assays can reflect completely the impact of lifestyle/environmental factors. In patients receiving radiotherapy, variation in radiation-induced cancer risk in those carrying known cancer susceptibility gene variants is approximately two-fold. The effect of the genetic component of disease risk modification on the small risks associated with low dose and low dose rate exposures is most probably small by comparison with known risk modifying factors, such as age and gender. Cellular studies suggest that differences in radiosensitivity are greater at low doses and dose rates. However, given the uncertainties on risk estimates below 100 mSv (the approximate limit of direct epidemiological evidence) it is hard to justify consideration of individual variation when setting limits for population exposure. It is also relevant to note that no reliable, simple assay of radiation-induced cancer risk is available. Prospects for the development of such assays remain distant, although some encouraging indicative data are now available (see, for example, Kabacik et al, 2011).

Those who present with certain cancers (eg retinoblastoma and breast) constitute a subpopulation whose lymphocytes may be more susceptible to radiation-induced chromosomal damage and who are thus potentially more susceptible to radiation-induced cancer. Some post-radiotherapy follow-up studies confirm the increased radiation-induced cancer risk in these situations. In the case of rare, but strongly predisposing gene variants, the impact at the population level will be small due to the scarcity of such individuals in populations. In other cases, and particularly where heterozygous carriers of risk variants are found to be at an increased risk (as may be the case for *ATM*), a larger impact on population risk can be expected. The potential impact on individual risk, however, should not be ignored.

9.2.2 Occupational radiological protection

Most of the points relating to public radiological protection made above apply also to occupational protection. Many of the concerns in occupational protection are linked to the feasibility (or otherwise) of predicting the sensitivity of individuals to radiation-associated diseases. It was noted above that there are no assays available that reliably predict individual radiation-induced cancer risk. There are, however, extensive efforts in research worldwide to identify disease risk biomarkers and it remains conceivable that biomarkers of individual sensitivity to radiation-induced disease may become available. The ethical implications associated with the application of such biomarkers will require careful consideration.

Evidence of elevated risk for some radiation-induced cancers, notably breast cancer in individuals carrying risk-enhancing variants of the *ATM* gene and some individuals with extreme cellular response to radiation, suggests that such individuals may benefit from greater protection from radiation exposure. Genetic tests for such risk variants are available and could, in principle, be applied in occupational protection. Genetic testing by employers as part of recruitment selection and discrimination on the basis of the results is a contentious issue. This practice is strongly discouraged by the International Labour Organisation and legislated against in several countries (see ILO, 2007, 2011) and so testing of this sort is unlikely to become common practice in the near future. However, such testing might be offered to prospective employees so that they may themselves take responsibility for accepting any greater than average risk. It is also possible that individuals may opt to have private genetic testing for such susceptibilities.

In the future it may be possible to use genetic tests to detect radiosensitivity as part of employment selection. However, we already have good evidence about increased radiosensitivity in relation to some lifestyle factors. This does have ethical implications; banning those who refuse to change their lifestyle, after receiving the relevant information about an increased risk of harm, from working with radiation would doubtless be labelled as paternalistic. An individual's responsibility for achieving and maintaining fitness for work is, however, recognised in some other professions (eg airline pilots, police and firefighters). A detailed consideration of the ethical implications of such a step is clearly required before it is considered in the context of radiological protection.

9.2.3 Emergency radiological protection

In radiation accidents and incidents the first action is to stop or minimise the exposure. When considering the medical management of casualties or, indeed, the larger number of 'worried well', individual susceptibility issues may be of relevance. Should reliable and simple assays of individual susceptibility to radiation-associated disease be available, the clinical management of casualties may be optimised and the follow-up screening of survivors could be tailored (for further information on high dose effects and accidents, see AGIR, 2009). Again, in the absence of good predictive assays, such approaches remain somewhat hypothetical. Nonetheless, as already noted, some promising assays of radiosensitivity may emerge in the next few years and their potential for use in casualty management and extended follow-up of exposed populations should be explored.

9.3 Clinical implications

9.3.1 Radiotherapy

With improved cure rates from cancer, survival issues have become increasingly important. Long-term toxicity associated with radiotherapy negatively affects the quality of life. The success of radiotherapy in eradicating a tumour depends principally on the total radiation dose given, but the tolerance of the normal tissues surrounding the tumour limits this dose. There is significant variation between patients in the severity of toxicity following a given dose of radiotherapy. As a result, the dose is suboptimal in the majority of individuals as current dose thresholds are set to limit toxicity in the most sensitive. Identification of patients who are more sensitive to radiation will allow safe dose escalation to be undertaken for the remaining population. In addition, strategies aimed at toxicity reduction are important.

The assessment of radiation toxicity is complicated. There are a variety of patient- and treatment-related factors that influence the development of radiation toxicity, and there is considerable variability in the scoring of normal tissue toxicity.

The determinants of clinical radiosensitivity should be interpreted with regard to their effect on individual patients receiving radiotherapy as part of their cancer treatment. In terms of advice to patients who are about to undergo a course of radiotherapy, potentially modifiable risk factors are most relevant. It is outside the remit of this document to provide specific clinical guidance.

9.3.2 Diagnostic exposure

Recent advances in medical imaging technology have led to a marked increase in diagnostic radiation exposure. For example, the marked increase in the use of computed tomography (CT) scanning and the increased use of nuclear medicine scans and interventional cardiology procedures have contributed to a doubling of the average radiation dose to which citizens of the USA are exposed in the last 30 years (Brenner and Hall, 2007). Patients who have been treated for cancer frequently have repeat CT scans to look for tumour recurrence. The evidence shows that the risk of radiation carcinogenesis from diagnostic exposure to an individual is small, but greatest in female and in younger patients (Wall et al, 2011). The relevant organ dose range for CT is 5–100 mSv. A recent study has demonstrated an increased cancer risk in those exposed to CT examinations (Pearce et al, 2012). The combined risk to the population from such diagnostic exposures becomes increasingly relevant as the use of radiological procedures increases (Brenner et al, 2003). As stated above, those who present with certain cancers constitute a subpopulation whose lymphocytes may be more susceptible to radiation-induced chromosomal damage and who are thus potentially more susceptible to radiation-induced cancer. Therefore, repeat diagnostic exposures in such patients may have a greater impact than in non-cancer patients.

When a diagnostic intervention is clinically warranted, the benefit of such a study will outweigh any possible individual risk, although the radiation exposure per scan should be minimised. It is essential to follow the principles proposed by the ICRP to ensure that each exposure is justifiable, the dose is optimised to be as low as reasonably achievable (ALARA) and relevant dose limits are set.

9.3.3 Patients with radiosensitivity syndromes

The major risk for patients with radiosensitivity syndromes, following radiotherapy, is death resulting from severe tissue reaction. Nowadays, however, most radiosensitivity syndrome patients will be known and, therefore, normal radiotherapeutic doses avoided. What the risk is for radiation carcinogenesis in long-term radiosensitivity syndrome survivors exposed either to moderated therapeutic doses or indeed diagnostic exposures is unknown, because of the rarity of these individuals and their shortened life span.

Exceptionally, patients with a radiosensitivity syndrome resulting in a significantly increased clinical and cellular radiosensitivity, because of the mildness of the presenting clinical features, are not detected before exposure to therapeutic doses of radiation. This has resulted in severe tissue damage, and the long-term consequences for these patients remain unknown. The proportion of such patients in the population is extremely low, being a proportion of a rare disorder.

9.4 Ethical implications

We are moving gradually to a situation where it will be possible to identify groups and sometimes individuals at greater than average risk from radiation exposure. Consideration as to how such groups and individuals might be protected raises new and important ethical questions, particularly in the occupational context. The more resources are devoted to protecting high risk groups and individuals, the more it may

be argued that spending a significant proportion of any budget on just a few groups and individuals lacks justice. This matter cannot be settled here, but the question of priorities in relation to radiological protection is something that needs to be addressed (see, for example, Hansson, 2007, 2009).

In the absence of routine individual testing it makes sense to focus on providing information to people about such risks and anything that can be done to reduce them. This is particularly important where there is clear evidence and where individuals can change their behaviour so as to reduce their individual risk.

9.5 Recommendations

a Work should continue in order to develop a better understanding of heterogeneity in cellular and molecular responses to radiation and their relevance to radiation-induced cancer. The implications of heterogeneity should be further examined in relation to clinical exposures and the societal response to radiation accidents and incidents. Improved toxicity recording will greatly facilitate future progress in determining genetic and non-genetic factors that influence radiotherapy toxicity.

b Associated efforts to identify and validate biomarkers/bioassays of sensitivity to radiogenic diseases based on cellular and molecular assays should continue.

c Efforts to identify gene variants that affect radiation-induced cancer risk should continue, and the costs and benefits of screening for such risk variants should be considered in the context of occupational protection and medical treatment or screening using ionising radiation.

d Existing epidemiological datasets should be examined:

- i regarding the possible interaction of radiation with diet, body mass index and alcohol consumption,
- ii regarding the possible interaction of smoking and radiation for smoking-related cancers other than lung.

e There is a need for further work to be carried out to examine the interaction of inflammatory responses and responses to irradiation.

f Work should be continued to enable a better understanding of apparent differences in radiation-induced cancer risk at low doses and dose rates.

g Further work is recommended to test the validity of the hypothesis that radiation-induced leukaemias arise almost exclusively among those individuals carrying preleukaemic translocations that have arisen during fetal life, together with a consideration of the possible implications for radiological protection.

h When undertaking retrospective assessments of the health risk in individuals exposed to radiation at doses of 100 mSv and above, the tobacco smoking history of the person should be taken into account when considering lung cancer risk, especially when alpha-particle exposures of the lung have occurred.

i Given that the interaction between radon and smoking is more than additive, it is recommended that the information about the consequences of this interaction should be targeted at those living in high radon areas/dwellings.

j While there are significant ethical issues relating to the influence of lifestyle factors in general on radiosensitivity, information on the interaction of smoking with radiation exposure should be made available to radiation workers at recruitment.

9.6 References

AGIR (1999). Genetic Heterogenecity in the Population and its Implications for Radiation Risk. Report of an independent Advisory Group on Ionising Radiation *Doc NRPB,* **10**(3), 1–47.

AGIR (2009). High Dose Radiation Effects and Tissue Injury. Report of the independent Advisory Group on Ionising Radiation. *Doc HPA,* RCE-10, 1–94. Available at www.hpa.org.uk

Brenner DJ and Hall EJ (2007). Computed tomography – an increasing source of radiation exposure. *N Engl J Med,* **357,** 2277–84.

Brenner DJ, Doll R, Goodhead DT, Hall EJ, Land CE, Little JB, et al (2003). Cancer risks attributable to low doses of ionizing radiation: assessing what we really know. *Proc Natl Acad Sci USA,* **100,** 13761–6.

Hansson SO (2007). Ethics and radiation protection. *J Radiol Prot,* **27,** 147–56.

Hansson SO (2009). Should we protect the most sensitive people? *J Radiol Prot,* **29,** 211–18.

ILO (International Labour Office) (2007). Equality at Work: Tackling the Challenges. Available at www.ilo.org/wcmsp5/groups/public/---dgreports/---dcomm/---webdev/documents/publication/wcms_082607.pdf

ILO (International Labour Office) (2011). Equality at Work: Continuing the Challenge. Available at www.ilo.org/wcmsp5/groups/public/---ed_norm/---relconf/documents/meetingdocument/wcms_154779.pdf

Kabacik S, Ortega-Molina A, Efeyan A, Finnon P, Bouffler S, Serrano M and Badie C (2011). A minimally invasive assay for individual assessment of the ATM/CHEK2/p53 pathway activity. *Cell Cycle,* **10,** 1152–61.

Pearce MS, Salotti JA, Little MP, McHugh K, Lee C, Kim KP, et al (2012). Radiation exposure from CT scans in childhood and subsequent risk of leukaemia and brain tumours: a retrospective cohort study. *Lancet,* **380**(9840), 499–505.

Wall BF, Haylock R, Jansen JTM, Hillier MC, Hart D and Shrimpton PC (2011). Radiation Risks from Medical X-ray Examinations as a Function of the Age and Sex of the Patient. Chilton, HPA-CRCE-028. Available at www.hpa.org.uk

Health Protection Agency
2nd Floor
151 Buckingham Palace Road
London
SW1W 9SZ
www.hpa.org.uk

Centre for Radiation, Chemical and Environmental Hazards
Chilton
Didcot
Oxfordshire
OX11 0RQ
T: +44(0)1235 831600
F: +44(0)1235 833891
E: ChiltonInformationOffice@hpa.org.uk

Documents of the Health Protection Agency
Radiation, Chemical and Environmental Hazards
RCE-21
March 2013
ISBN 978-0-85951-740-9
£50.00

This publication is also available in large print