International Agency for Research on Cancer
World Health Organization

Centre for Childhood Cancer Survivor Studies
UNIVERSITY OF BIRMINGHAM

Gene-Rad-Risk

Breast cancer after childhood cancer

Risk of breast cancer after childhood cancer in Britain,
particularly in relation to elements of treatment for childhood cancer

Study Protocol National Study
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Project overview

Title: Risk of breast cancer after childhood cancer in Britain, particularly in relation to elements of treatment for childhood cancer

Short title: Breast cancer after childhood cancer

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Prologue

The current study protocol relates to a national study within the UK which is embedded within an international collaboration led by the International Agency for Research in Cancer (IARC) in Lyon, France. Only those procedures specifically relating to the national study are described in this protocol. For a description of the international objectives and procedures see the protocol written by IARC (Appendix A). The IARC protocol has already been approved by the IARC Ethical Review Committee and a copy of this approval has been attached (Appendix B).

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Summary

Objective
The main objective of the current study is to quantify the risk of breast cancer, particularly in relation to the dose of radiation received by breast tissue and the dose of different types of chemotherapy received by survivors of childhood cancer in Britain.

Setting, participants, and design
The above objective will be investigated by means of a cohort and a nested case-control study. Eighty survivors of childhood cancer who have developed subsequent breast cancer within the underlying cohort will comprise the cases and 240 survivors without breast cancer selected from the cohort will act as controls.

General approach
The general practitioner (GP) of every survivor will be approached and his/her consent to contact the survivor will be sought. If the GP consents, a study package will be sent to the survivor including a request for them to telephone the Study Coordinating Centre (SCC) in order to arrange a mutually convenient time for a telephone interview. If the survivor agrees to participate then a research assistant will telephone her back at the scheduled time and will undertake the interview. After the interview, the survivor will also be asked whether she is willing to provide DNA in the form of saliva. With the permission of the survivor, a kit to collect and store DNA will then be sent to the survivor and she will be asked provide a suitable sample of saliva and return it to the SCC. The survivor will be asked to give written consent for storage and use of her DNA. In addition, the survivor will be asked (on the same consent form) to give permission to access her medical records. With consent of the survivor, a research assistant will contact the relevant hospitals and arrange for photocopies or photographs of the relevant medical records. Statistical analysis relating to the above objectives will take place at the SCC. Anonymised data will be sent to the International Agency for Research on Cancer (IARC) for statistical analysis relating to the objectives as described in the IARC protocol (Appendix A).

1 Study Overview

1.1 Background

There is increasing evidence that women exposed to radiation before the age of 40 are at an elevated risk of developing breast cancer.[1] In particular women treated with radiotherapy in the chest area are known to be at a greatly increased risk of subsequent breast cancer. Several studies[2-5] have found increased risks of breast cancer in survivors of Hodgkin's disease, however, few studies[6-8] have investigated breast cancer risk among survivors of all types of childhood cancer. The general consensus is that the risk is likely to be lower after childhood cancers other than Hodgkin's disease than after Hodgkin's disease, however little is known about the magnitude of the risk of breast cancer in the former group. Although it has been established that treatment with radiotherapy increases the risk of second primary breast cancer, most previous studies did not have detailed information on the radiation dose to which breast tissue was exposed, thereby excluding the possibility of a satisfactory investigation of dose-response. Also, little is known about the influence of potential modifying factors, such as chemotherapy and reproductive factors, for breast cancer after childhood cancer.

1.2 Objectives

We propose to conduct a cohort and nested case-control study; the latter allowing us to quantify the relative risk of radiation related breast cancer among survivors of childhood as reliably and in the most unbiased way that is practically possible within the UK. In addition, we will investigate to what extent other factors, such as reproductive and treatment factors other than radiotherapy, might play a role in the aetiology of breast cancer among survivors of childhood cancer.

1.3 Hypothesis

The hypothesis underlying our research objective is that treatment with radiotherapy for childhood cancer is associated with an elevated risk of second primary breast cancer, and that there is a dose-response relationship between the amount of radiation received by breast tissue in childhood and the risk of subsequent breast cancer.

1.4 Justification for study population

In order to investigate the above hypothesis it is important to include subjects who have been exposed to a range of doses, including high doses of radiation at a young age, since they comprise the group who are thought to be at the highest risk of breast cancer later in life. There are few opportunities to investigate such a hypothesis in other populations and survivors of childhood cancer provide the opportunity to investigate this hypothesis as a large proportion has received high doses of radiotherapy to the chest area at young ages,

and importantly the records are still available in relation to the vast majority of survivors to estimate the radiation dose to breast tissue reasonably accurately.

1.5 Potential implications of the results

The study outcomes will aid in identifying those groups of survivors of childhood cancer who are at an importantly increased risk of subsequent breast cancer. Such information is of great value to clinical practice in three principal ways. Firstly, the provision of such risk estimates is useful for counselling and otherwise informing survivors concerning their long-term risks of breast cancer. Secondly, it provides information useful in focusing clinical follow-up in late effects clinics on those survivors most at risk. Thirdly, it provides a rational basis for deciding upon future protocols taking into account both long-term prospects for cure and long-term risks of side effects of treatment.

2 Procedures

2.1 Location

The Study Centre will co-ordinate the study and is based at the University of Birmingham, Birmingham. Apart from accessing of medical records and obtaining tissue blocks, which will involve hospitals and pathology departments within Acute NHS Trusts, the research will take place outside the NHS.

2.2 Study population

In Britain, over 18 000 individuals were diagnosed with childhood cancer between 1940 and 1991, and survived for at least five years. These 18 000 long-term survivors of childhood cancer comprise the underlying cohort in this study. Cases are derived from the cohort and controls will be randomly selected from the same cohort taking into account specified matching criteria as defined below.

2.3 Study design

A cohort study
A case-control study nested within the underlying cohort

2.4 Case and control definition

- *Case definition*

Cases are those survivors in the cohort who have been diagnosed with a second primary breast cancer after surviving at least five years from diagnosis of childhood cancer.

- *Case inclusion criteria*

-Women diagnosed with a second primary breast cancer
-previously diagnosed with childhood cancer between 1940 and 1991 in Britain
-survived childhood cancer for at least five years

- *Case exclusion criteria*

None, but the GP acts as “gatekeeper” to the survivors and decides whether a survivor should be invited to participate in the case-control element of the study taking into account all relevant factors.

- *Control definition*

Controls will be randomly selected from among those survivors in the cohort who have not been diagnosed with second primary breast cancer.

- *Control inclusion criteria*

-Women never diagnosed with a primary breast cancer
-previously diagnosed with childhood cancer between 1940 and 1991 in Britain
-survived childhood cancer for at least five years

- *Control exclusion criteria*

None, but the GP acts as "gatekeeper" to the survivors and decides whether a survivor should be invited to participate in the case-control element of the study taking into account all relevant factors.

2.5 Matching criteria

Controls will be matched to cases on age of diagnosis (within 1 year), calendar year of diagnosis (within 3 years), and the matching control must have survived for at least the same period of time as the case without being diagnosed with a second primary breast cancer.

2.6 Patient identification, approach, recruitment, and consent

Details of those previously diagnosed with childhood cancer in Britain and satisfying cohort eligibility criteria were ascertained through the population-based National Registry of Childhood Tumours. All cohort survivors are flagged at the National Health Service Central Registries (NHSCR) by the Centre for Childhood Cancer Survivor Studies. The NHSCR notifies the Centre for Childhood Cancer Survivor Studies whenever a survivor has developed a second primary breast cancer, among other things. All those survivors who have been flagged and who have developed breast cancer will be eligible for inclusion in the study. Controls will be identified from within the underlying cohort and relating to each case three controls with similar 'age at diagnosis' and 'year of diagnosis', and 'period of follow-up' will be selected (see also matching criteria).
The GP of the survivor will be sent a letter (Appendix D) asking for their permission for the SCC to contact the survivor directly. This 'gatekeeper' approach will be used to guard against the inappropriate contacting of survivors. If the GP gives consent for the SCC to contact the survivor then a study package will be sent. However, approval from the Patient Information Advisory Group will have to be obtained before this approach can be used. The study package will contain a letter explaining the purpose of the study, a survivor information sheet, and the survivor questionnaire (Appendix C). The questionnaire will be included in order to give the survivor prior knowledge of the questions that we would wish to ask during a potential telephone interview. Such prior knowledge serves two useful purposes: firstly, it provides more information to a survivor so that they may decide on consenting in a more informed way; secondly, for questions which ask about issues for which the survivor's knowledge is incomplete, extra time is allowed for checking with relatives or others. If the survivor is willing to participate in the study, she is asked to telephone the SCC on the 'free' 0800 number to schedule a telephone interview.

2.7 Data collection

If the survivor telephones the SCC she will be asked if she is willing to make an appointment for a telephone interview. If the survivor agrees to participate then a research assistant will arrange a convenient time with the survivor for them to undertake the interview and subsequently telephone her back at the scheduled time to complete the interview. After the telephone interview the survivor will be asked whether she is willing to donate DNA in the form of saliva, and if she agrees, a saliva kit to obtain DNA will be sent to the survivor together with comprehensive instructions and reply paid packaging in which to return the specimen to the SCC. A consent form will be included as well and the survivor is asked to give consent for (A) storage and use of her DNA, and (B) for permission to access her medical records. If she agrees, a research assistant will contact the relevant hospitals and arrange photocopying or photography of the relevant medical records.

2.8 Study instruments

- *Questionnaire*

The survivor questionnaire as proposed in the IARC protocol will be used in this study (Appendix C). A research assistant will contact each participant and the questionnaire will be used as the basis of a telephone interview. Please refer to the IARC protocol for a more detailed description of the questionnaire (Appendix A).

- *Biological material*

A saliva kit (ORAGENE) will be used to collect DNA from the participants. The participant is asked to provide a specimen of her saliva using the kit and will be asked to send the kit back to the SCC. DNA purification and extraction will take place at the SCC. IARC will however develop a quality control system in order to ensure the quality of the DNA extraction. For deceased cases and controls, DNA from tumour tissue blocks will be obtained.

- *Medical records*

If the participant has given consent, detailed information will be obtained from her hospital medical records. This will include information on: radiotherapy, chemotherapy, diagnosis of breast cancer (for cases only), diagnostic x-rays, etc. For a more detailed overview please refer to the IARC protocol (Appendix A).

2.9 Data management

All data collection and storage will conform to the Data Protection Act. All personal data will be stored on a local computer network with no external connections. Only authorised personnel at the SCC will have access to the database and all computers will be password protected. Access to the SCC is by swipe card access and is restricted to SCC staff. Every six months, all relevant data (anonymised) will be sent to IARC in Lyon by uploading the data to a secured FTP-server at IARC. Data will be at this server for a maximum of 24 hours. Anonymised details on radiotherapy treatment will be sent to Institut Gustave

Roussy in France, where the radiation doses to the site of the breast will be estimated. Please refer to the IARC protocol for a more detailed description (Appendix A). Names, individual identity numbers like NHS numbers and contact details of the participant will be treated in the strictest confidence and will only be available to the authorised staff working at the SCC. Copies of medical records, together, with the (completed) questionnaires will be stored in locked filing cabinets at the SCC. All the filing cabinets and the computer network are located in the SCC to which only authorised personnel have access by swipe card. After working hours, this room is locked and a burglar alarm is set. No personally identifiable information will be disclosed from the SCC to any third party. All personnel at the SCC have to sign a confidentiality undertaking which if violated would lead to dismissal.

2.10 Data access right

Only authorised personnel at the SCC will have access to the data. The Principal Investigator will have control over the data and will act as a custodian for the data generated by the national study. Investigators who are involved in the overall international study will have only access to anonymised data which will be stored at IARC.

2.11 DNA storage and extraction

DNA will be extracted from the saliva samples upon receipt of the sample at the Study Centre. This DNA will be stored at a central repository at the University of Birmingham for as long as is required for the current project, or for any potential future studies for which a COREC application has been submitted before the end date of the current study. Otherwise the DNA will be transferred to a licensed research tissue bank. This approach is consistent with all conditions in the Human Tissue Act. A small proportion of the DNA will be sent to IARC, but this DNA will be destroyed after completion of the current study.

3 Statistical considerations

3.1 Estimated number of participants

Nested case-control study: 80 cases and 240 controls
Cohort study: approximately 8200 female survivors

3.2 Power calculations

All individuals diagnosed with childhood cancer in Britain between 1940 and 1991, who survived for at least five years, and who have developed subsequent breast cancer are eligible for inclusion (approximately 80 subjects in the UK). These survivors are identified through NHSCR and the British Childhood Cancer Survivor Study. The decision to include three controls per case in the case-control study is based on power calculations (see below). Power calculations for the international analysis, which will be conducted at IARC, can be found in the IARC protocol (Appendix A).

Case-control study
If the proportion of controls exposed to radiotherapy is assumed to be 0.5, then, it would be possible to detect any clinically important increase in the odds of developing breast cancer as a result of treatment with radiotherapy (OR>2.5; power ≥ 0.8).

Cohort study
If the UK population age and calender specific incidence rates of breast cancer would be applied to the cohort of survivors then the number of breast cancers in the cohort would be estimated at 27. With this expected number of breast cancers it would be possible to detect Standardised Incidence Ratios (O/E) in excess of 1.5 with a likelihood of at least 80%.

3.3 Statistical analysis

Case-control study
Odds ratios expressing the odds of breast cancer for those treated with high doses of radiotherapy relative to those treated without or with low doses of radiotherapy will be calculated in a matched analysis by means of multivariable conditional logistic regression. A dose-response analysis will be conducted in order to investigate whether there is a trend in the (log) odds of contracting breast cancer with increasing cumulative exposure of breast tissue to radiation. Other risk modifying and potential confounding factors will also be taken into account in the analysis. Analyses concerning gene-radiation dose interactions will be conducted at IARC (see IARC protocol). No statistical gene-analysis will take place at the Study Centre; however, DNA will be stored in order to conduct gene-analyses in the future.

Cohort study
Standardised Incidence Ratios (SIRs), defined as the ratio of the observed over the expected numbers of breast cancers among survivors, will be calculated by means of the STATA statistical software package. The expected number of breast cancers will be estimated by applying the age and calendar specific breast cancer rates in the UK general population to person-years at risk accumulated within the age and calendar specific strata in the cohort of survivors. Absolute Excess Risks (AER) of breast cancer attributed to surviving childhood cancer will be estimated by the number observed minus the expected number of breast cancers per 10,000 survivors per year. Both SIRs and AERs will also be calculated within levels of the following factors: *length of follow up*, *treatment decade*, *attained age, age at diagnosis,* and *initial treatment.* The cumulative incidence of breast cancer will be estimated by using both the Kaplan-Meier approach and also taking into account potential competing risks such as death.

Analysis of the national data will take place at the SCC in Birmingham, UK, by Mr R Reulen, Dr M M Hawkins and Prof M P Zeegers. The international analysis of the data will take place at the International Agency for Research on Cancer in Lyon, France. A team of several researcher, led by Dr Cardis, will be involved in this analysis.

4 Ethical considerations

4.1 Informed consent

Before contacting survivors, the GP of each participant will need to have provided written consent that they consider it appropriate to contact the survivor.
The survivor will be asked to contact the SCC in order to arrange a telephone interview. Agreement over the telephone with SCC staff will be regarded as consent for the undertaking of the telephone interview. The participant will however be asked to give written informed consent for the use of their DNA and access to their medical records.

4.2 Participant withdrawal

Participants can withdraw from the study at any time. If indicated by the participant, all relevant data and/or biological material will be destroyed at both the national SCC and at IARC.

5 Quality control/assurance

To assure the quality of the proposed research, IARC has developed a Quality Management Plan to which all collaborators should adhere. The main quality control will be review by the international collaborators within the overall project. In addition, IARC will carry out regular site visits to the national SCC in Birmingham to review progress and assist in resolution of problems arising from the implementation of the protocol. Also, IARC will perform internal consistency checks with regard to the data sent from the national SCC. For a more detailed description of the quality management plans please refer to Appendix E.

6 Dissemination, notification, reporting of results

6.1 Notifying participants of individual results

In the event that mutations in the genes BRCA1 and BRCA2 are found, we will provide feedback to the study participant (but only if indicated on the consent form) as there is convincing evidence from previous studies that mutations in these genes are associated with a high increase in breast cancer risk. We will contact the study participant's GP and inform the GP that something of significance *might* have been found and ask the GP to refer the individual concerned to a cancer genetics clinic. We will not provide feedback for the other genes on which we screen because the association with breast cancer incidence is less clear for these genes and at this stage of uncertain clinical significance. If individuals have indicated that they do not wish to receive feedback, then we would not do so.

6.2 Notifying participants of study findings

If indicated on the consent form that they like to receive one, study participants will be informed of the overall study findings by means of a Study Newsletter. A free telephone helpline will also be available to answer any queries relating to the study.

6.3 Disseminating results to public

Study findings will be published in peer reviewed clinical and scientific journals, internal reports, conference proceedings, and other related publications. The Principal Investigator for the national study (Dr Hawkins) is a full member of the United Kingdom Children's Cancer Study Group (UKCCSG). Most clinicians who treat children with cancer in the UK are members of the UKCCSG and attend its scientific meetings - this provides a very useful forum for communication between the national study and relevant clinicians throughout the UK.

7 Timeline

Month 0-6 Contacting GPs: asking consent for contacting survivors
Month 0-12 Administering of telephone interviews
Month 0-16 Collection of treatment data
Month 3-18 estimation of radiation dosimetry to the breasts (done externally)
Month 18-24 Statistical analyses and report results of national study
Month 24-36 Statistical analyses and report results of international study by IARC

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Appendix A

IARC Protocol

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER
WORLD HEALTH ORGANIZATION

GENE-RAD-RISK

Radiation exposures at an early age: impact of genotype on breast cancer risk

STUDY PROTOCOL

Last updated 28 April 2007

IARC, Lyon, 2006

IARC INTERNAL REPORT

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Objective

To test whether mutations or polymorphisms in specific DNA repair genes increase the risk of radiation induced cancer. The specific focus of this project is breast cancer.

In addition to studying the possible interactions between radiation exposure and genes, a secondary objective will be to study the possible modifying effects of reproductive factors and cancer therapies other than radiation on the risk of radiation induced breast cancer.

Background

Breast cancer is the most common cancer and one of the leading causes of death from cancer among women worldwide, with nearly 1.000.000 new cases per year (Globocan, 2000). Known risk factors for breast cancer include genetic susceptibility, reproductive factors, postmenopausal weight, history of benign breast disease and exposure to ionising radiation (UNSCEAR 2000, Stewart and Kleihues, 2003, Ronckers *et al*. 2005).

Epidemiological studies of atomic bomb survivors and medically irradiated populations show increased risks of female breast cancer (ranging from 1.1 to 2.7 at 1 Gy) following external irradiation before age 40 years (Boice *et al.* 1996). The relative risks of breast cancer for women exposed to external radiation in childhood and adolescence are substantially higher than for those exposed as adults (Bhatia *et al.* 1996, Hildreth *et al.* 1989, Pierce *et al.* 1996, Thompson *et al.* 1994, van Leeuwen *et al.* 2000, van Leeuwen *et al.* 2003.) and are among the highest known radiation related risks for any cancer type along with leukaemia and thyroid cancer following exposure in childhood (UNSCEAR, 2000). Results of a recent combined analysis of data from atomic bomb survivors and seven medically exposed cohorts (Preston *et al*. 2002) indicate clearly that, while radiation exposure at any age increases breast cancer risk, the relative and absolute excess risks tend to decrease with increasing age at exposure so that exposures after age 50 carry a much lower risk than exposures earlier in life. The increased risk of breast cancer starts to be observed 10 to 15 years after exposure, with relative risks decreasing as a function of attained age after reaching a peak, usually between ages 30 and 40. A study of children exposed repeatedly to low-doses of X-rays for monitoring of the curvature of the spine for scoliosis, has suggested that adolescence, when breast tissue is developing, is a vulnerable time for carcinogenic exposures (Doody *et al.* 2000). This is supported by evidence from studies of breast cancer following high-dose mantle radiotherapy for Hodgkin's disease (HD), in which little if any increased risk is seen for patients treated after age 30 (van Leeuwen *et al.* 2003, Wahner-Roedler and Petersen, 2004).

A multiplicative relationship between exposure to ionising radiation and other known risk factors of breast cancer, such as age at the time of a first full-term pregnancy, number of children and cumulative period of breast feeding has also been found in a case-control study nested in the Japanese Life Span Study cohort (Land *et al.* 1994), indicating that reproductive years are also important in radiation induced breast cancer.

A number of studies have clearly demonstrated that a fraction (of the order of 12%) of breast cancer risk is inherited (Goldgar *et al.* 1994; Lichtenstein *et al.* 2000; Pharoah *et al.* 2002; Amundadottir *et al.* 2004). Several of the genes that play important roles in the response to DNA damage produced by ionising radiation – that begins with signalling the presence of DNA double-strand breaks (DSBs), proceeds through activation of DSB repair (usually coupled to cell cycle arrest), and ends with repair of that damage – are now implicated as either high-risk or moderate-risk breast cancer susceptibility genes (see Figure 1). The first two major breast cancer susceptibility genes identified, *BRCA1* and *BRCA2*, were shown to play a role in DNA DSB repair (Venkitaraman, 2002; Powell *et al.* 2003). *BRCA1* has been

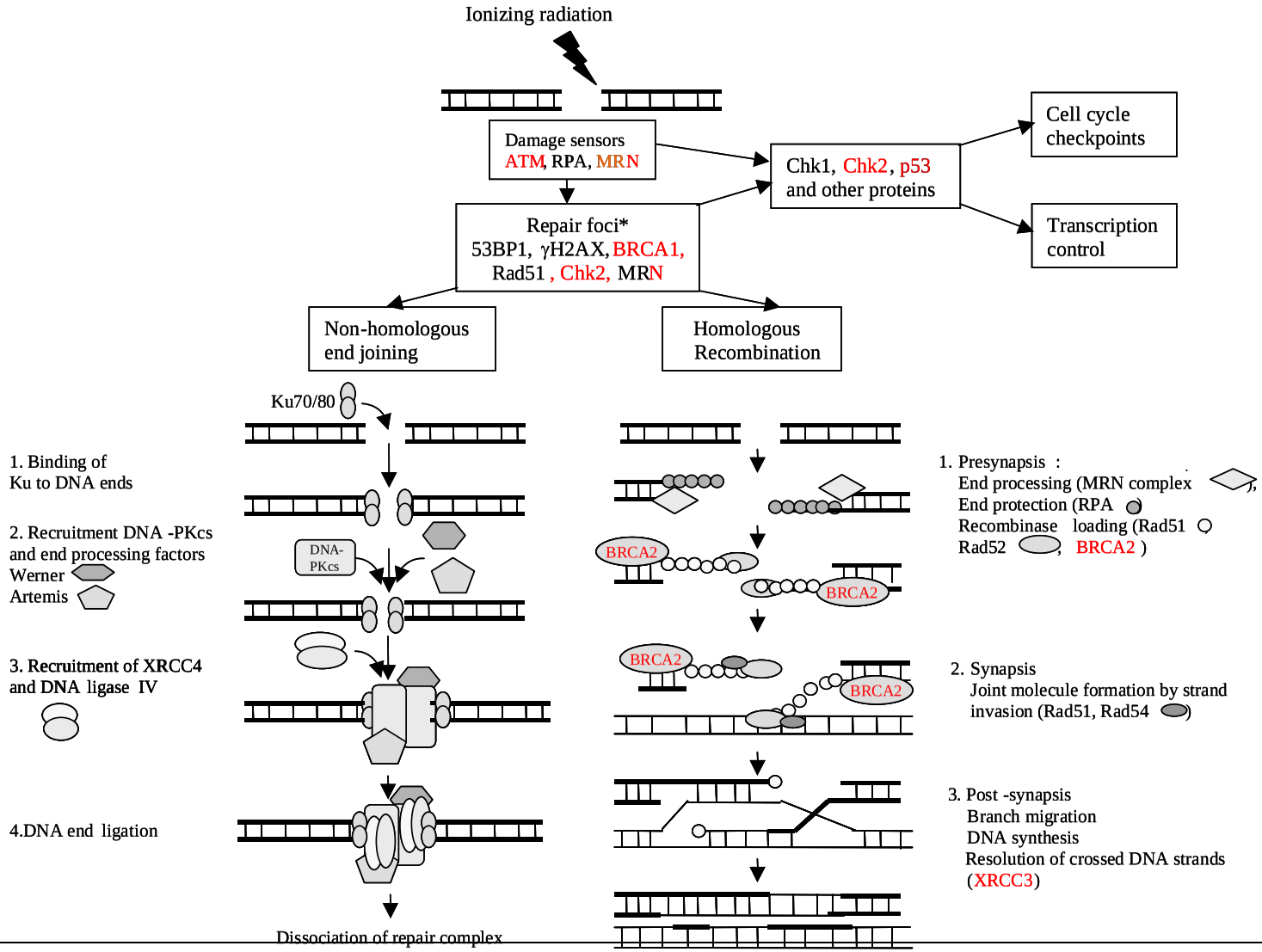
implicated both in the regulation of these repair processes, apparently facilitating the choice of the repair pathway: non-homologous end-joining (NHEJ) versus homologous recombination (HR) (see Figure 1). Both *BRCA1* and *BRCA2* play a role in HR.

The ATM protein, which is mutated in patients with ataxia-telangiactasia, plays a pivotal role in signalling the presence of DNA damage after exposure to ionising radiation (for review, see (Kurz and Lees Miller, 2004). It also appears to be important in breast cancer aetiology, as an excess breast cancer risk has consistently been observed among the relatives of *ATM* patients (Swift *et al.* 1987). *XRCC1* is another gene that has been associated with an altered cellular response to ionizing radiation and breast cancer risk. While the *XRCC1* protein *per se* is not directly involved in DSB repair, it is required for the efficient repair of DNA damage caused by ionising radiation, oxidative stress and DNA methylating agents. To date, the DNA damage response genes for which human genetic evidence of an association with breast cancer is quite solid include: *ATM* (Hall 2005; Cavacuiti *et al.* 2005), *BRCA1* (Miki *et al.* 1994; Castilla *et al.* 1994; Simard *et al.* 1994; Friedman *et al.* 1994), *BRCA2* (Wooster *et al.* 1995; Tavtigian *et al.* 1996), *CHEK2* (Meijers-Heijboer *et al.* 2002; *CHEK2, BCCC* 2004), *NBS1* (Gorski *et al.* 2003; Cybulski *et al.* 2004), *XRCC1* (Goode *et al.* 2002; Smith *et al.* 2003), and *XRCC3* (Kuschel *et al.* 2002; *Smith et al.* 2003). It has been suggested that *p53* mutations may influence the response of breast cancer patients to adjuvant therapy (Olivier *et al*, 2005).

There is increasing evidence that mild reductions in DNA repair capacity, assumed to be the consequence of common polymorphic variations in DNA repair genes, affect cancer predisposition (Mohrenweiser *et al.* 2003). Considerable inter-individual variation in the capacity to repair DNA damage is detected using cell-based assays and evidence for the importance of moderate reductions in DNA repair capacity in cancer risk has also come from experiments in mice. The complete loss of function for many DNA repair genes is incompatible with normal development and results in embryonic lethality. However studies on heterozygous mice have shown an altered cellular phenotype associated with reduced repair capacity. *ATM* haploinsufficiency results in increased sensitivity to sub-lethal doses of ionising radiation (Barlow *et al.*1999) and radiation exposure induces genomic instability and mammary ductal dysplasia, a precursor to mammary cancer (Weil *et al.* 2001). *Nbn* (the NBS1 mouse homologue) heterozygosity renders mice susceptible to tumour formation and IR-induced tumourigenesis (Dumon-Jones *et al.* 2003).

Therefore evaluating the association between sequence variation in DNA damage response genes and cancer occurrence in populations with and without prior radiation exposure may help to elucidate the mechanisms of cancer aetiology and to better estimate cancer risks. This need was recognized as early as 1998, when the International Commission for Radiological Protection (ICRP, 1998) stated that, despite the paucity of data, there seemed to be sufficient cause for concern that susceptible children and young adults exposed to radiotherapy may be at increased risk of subsequent breast cancer.

Figure 1 Role of genes of interest in cellular responses to DNA double-strand breaks. Adapted from information in special issue of DNA Repair, "Bridge over broken ends" 3 (2004) 779-1245*Note: MRN = MRE11/RAD50/NBS1 protein complex; genes that we plan to analyze are marked in red*



3

* Certain proteins (e.g. BRCA1 and the MRN complex) have been implicatedin the regulation of recombinational repair facilitating one or other of the processes (Zhang et al. , MCB 24(2004)708 -718)

Few studies have attempted to investigate whether variants in specific DNA damage response genes impact on breast cancer risks following radiation exposures. Three studies have investigated the frequency of some *ATM* variants in breast cancer cases and in controls from cohorts of Hodgkin Diseases patients treated with radiotherapy (Offit *et al.* 2002, Broeks *et al.* 2000, Nichols *et al.* 1999) and found no increased risk of radiation induced cancer. These studies were, however, limited both by their size and the techniques used for variant sequence detection.

A recent study by Mertens *et al.* (2004) investigated the association between the codon 399 variant in the *XRCC1* gene and risk of subsequent malignancy in survivor's of Hodgkins disease They found limited evidence of an increased frequency of the glutamine allele in women who developed breast cancer after treatment for HD indicating a possible role for this polymorphism in susceptibility (OR 1.4 95% CI 0.7-2.7).

The WECARE study, a multi-centre population-based case-control study of contralateral breast cancer (Bernstein *et al.* 2004) is currently underway to examine the joint roles of radiation exposure and genetic susceptibility in the aetiology of breast cancer, focusing on *ATM, BRCA1, BRCA2* and *CHEK2*. Results of this study are not yet available. While the study should allow such interactions to be addressed, it must be noted that the cases and controls are drawn from cohorts of women who were treated for a previous breast cancer, most of these after the age of 40, when susceptibility to radiation induced breast cancer is reduced.

A study of childhood cancer survivors in the US, the CCSS (Childhood Cancer Survivor Study), is also underway (Boice *et al.* 2003). Included in the study objectives is an evaluation of the relationship between therapeutic radiation and the risk of second cancers and the study of gene-radiation interactions. The study includes approximately 133 cases of breast cancer.

A study of interactions between environmental factors and mutations in *BRCA1* and *BRCA2* is also currently underway, based on 600 breast cancer cases within cohorts of mutation carriers in the Cancer Family Registries (http://www.cfr.epi.uci.edu/ic_registries/objectives.htm).

Several recurrent structural and numerical chromosomal abnormalities have been detected in primary breast cancer by cytogenetic analysis (Bieche and Lidereau, 1995). Using (classical) Comparative Genomic Hybridization (CGH), Wessels and coworkers (2002) showed that BRCA1 breast carcinomas exhibit specific somatic genetic aberrations which can be used to specifically identify such tumours with high efficiency (sensitivity 96%, specificity 76%). Based on the hypothesis that there is a specific radiation induced response-cascade, resulting in genetic aberrations different from those in sporadic and or familiar breast carcinomas, these results suggest that there must be a chromosomal pattern that would reveal the genes that are responsible for the development of breast cancer following radiation exposure.

The current challenge, as noted by Ronckers *et al.* (2005), is to study the combined role of genes and radiation in sufficiently large populations to ensure statistical power to detect such an interaction with reliable estimates of radiation dose, to the breast, genotyping on extracted DNA and an appropriate comparison group.

Study populations

Because of the low prevalence of mutation carriers and because of the generally low levels of ionising radiation exposure in the general population, population-based studies of breast cancer risk are unlikely to be informative for this purpose. The current project therefore focuses on the conduct of nested multinational case-control studies of breast cancer in two different but complementary populations chosen, on the basis of high prevalence of radiation exposure and/or high prevalence of known mutations in susceptibility genes, to maximize the

power to study the effects of radiation exposure on the risk of breast cancer and its interaction with relatively rare genetic mutations. The populations to be studied are as follows:

- Cohorts of patients who survived a first cancer diagnosed before the age of 35

  Persons treated for cancer can receive a wide range of radiation doses to the breast (Figure 2), from a few mGy to tens of Gy, and/or a wide variety of doses of anti-cancer drugs, such as alkylating agents and topoisomerase II inhibitors. A large number of studies have focused on the risk of second cancers in patients treated with radiotherapy or chemotherapy for a first cancer or on the risk of a first cancer following treatment for benign diseases (UNSCEAR 2000). Increased relative risks for cancer related to these therapies have been documented to be of the order of 2-100, depending on the organ and the level of exposure (de Vathaire *et al.* 1999) when received during childhood, adolescence and early reproductive years. Populations of cancer survivors may, moreover, be more likely than the general population to carry gene mutations predisposing them to treatment-induced cancer (Little *et al.* 1998). Retrospective individual dose estimation in these populations is generally possible from detailed radiotherapy records (Stovall *et al*. 2004; Diallo *et al.* 1996; Shamsaldin *et al.* 1998; Ligot *et al.* 1998).

  The objective of the study in these cohorts will be to assess the possible interactions between ionising radiation and sequence variation in DNA damage response genes.

- Cohorts of mutation carriers: cohorts of subjects with a known or suspected genetic predisposition to breast cancer – *BRCA1* and *2* mutation carriers.

  It has been estimated that the risk of breast cancer in *BRCA1/2* mutation carriers ranges from 50% by age 50 to ~80% lifetime risk (Ford *et al.* 1998). As indicated in section C (Preliminary studies), preliminary results of analyses carried out within the International *BRCA1/2* Carrier Cohort Study (IBCCS) show that women who carry a mutation in one of these two genes may be at higher risk of radiation induced breast cancer than the general population (Andrieu *et al*. 2006).

  The objective of the study in these cohorts will therefore be to assess the possible interactions between ionising radiation and mutations in BRCA1 and BRCA2.

It is proposed to carry out studies of breast cancer within two separate and complementary populations:

- cohorts of patients who survived a first cancer diagnosed before the age of 35 (in particular childhood cancer survivors and patients treated for Hodgkin's disease);
- cohorts of subjects with a known or suspected genetic predisposition to breast cancer – *BRCA1* and *2* mutation carriers.

Although the objective is to better understand genetic factors which may enhance the risk of cancer following low dose radiation exposure, the power to find modifying effects in epidemiological studies will be greater in populations with a high prevalence of high-dose radiation exposure (from cancer radiotherapy) and in populations who are carriers of a mutation or polymorphism known to be related to breast cancer risk.

Of particular interest for this project are radiation exposures that occurred in childhood, adolescence or early reproductive years and hence the age restrictions are applied in the definition of the cohorts above.

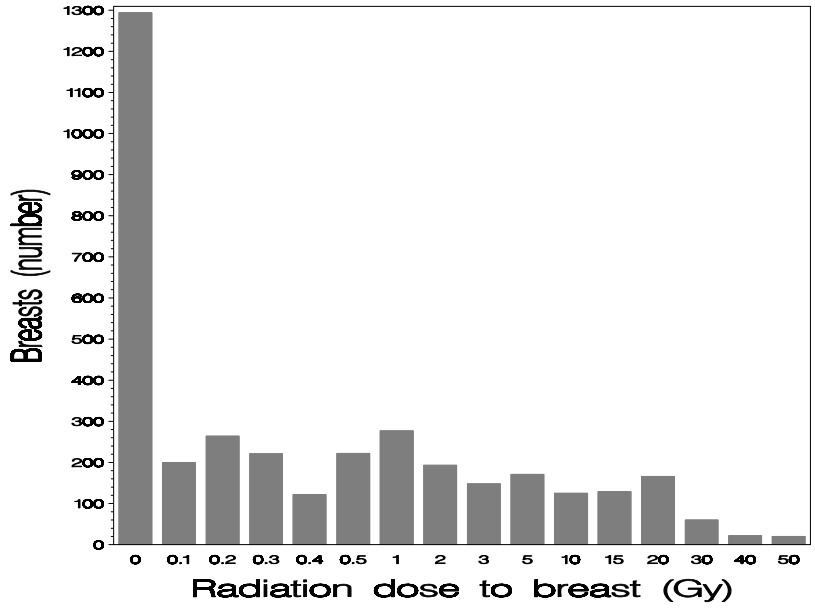


Figure 2. Distribution of radiation dose to the breasts among female childhood cancer survivors treated by external radiotherapy

Study design

Epidemiological studies will therefore be carried out in parallel in these two populations.

The study design will be as follows:

- **Cancer survivors:** *Nested case-control study* – this study, which will be nested within cohorts of cancer survivors, will allow the collection of detailed information on radiation exposure and genes.
- **Mutation carriers**: *Cohort study* – because of the high risk of developing breast cancer in mutation carriers, the proportion of affected carriers in the cohorts is extremely high and there will be no gain in cost in conducting a nested-case control study (it may in fact be impossible to find two unaffected controls for each case). The study will therefore include the entire cohort of mutation carriers.

Results will be compared (and combined if appropriate) and conclusions will be drawn about the possible modifying effect of mutations and polymorphisms in the genes considered.

Cases

Primary incident breast cancer cases occurring in the above mentioned cohorts before age 55. Ascertainment will be both retrospective (over the entire duration of follow-up of these cohorts) and prospective over a period of 2 years. Women are eligible as cases if they are diagnosed with a primary *in situ* or invasive breast cancer (behaviour is coded /2 or /3).

It is recognized that a substantial proportion of retrospective cases will have died before the beginning of the study. It is planned, however, to include in the study, wherever possible, both deceased and living cases in order to avoid a potential bias related to survival. Such a bias, if it exists, might occur if the genes of interest affected the prognosis of the disease.

Controls – cancer survivors only

Two controls will be selected for each case. Controls should be matched on: age at first primary cancer within 1 year, calendar year at first primary cancer within 3 years, and, when necessary for logistic reasons, on geographic region.

If there are less than two potential controls available then calendar period is relaxed one year at a time to a maximum of within 10 years. At each stage (within 4, 5, 6,..., 10 years) the pool available is examined to see if there are at least two potential controls. If the maximum limit of within 10 years of the calendar year of diagnosis of first cancer is reached and there are still less than two controls available the age at first cancer in potential controls should be relaxed by one year at a time to a maximum of within 3 years of age of the case at first cancer diagnosis.

For the Hodgkin's disease cohorts, should a situation arise when less than two controls fitting the limits of age at the time of diagnosis and calendar period are available, further relaxation of the matching criteria, keeping tighter limits on age than calendar period, is allowed to achieve the necessary two controls.

Careful record should be kept of the final extent of relaxation required for each matched set to achieve two controls.

It should be noted that:

- A control can be a control for more than one case
- A control from one matched set may later be found to be a case, at which point appropriate controls will be selected for the new set.

Ascertained breast cancer cases therefore should be included in the pool of potential controls as they are considered to be eligible if they have been free of breast cancer for at least as long as the interval between the first primary cancer and the breast cancer of the case to whom she is matched to within one year before diagnosis.

It should be noted that if controls are matched by hospital, there is a serious risk of overmatching on dose, since treatment may be more homogeneous in one hospital. Thus it would be advisable to match on a somewhat larger geographical area.

There will be no matching on primary type of cancer except for childhood cases with Hodgkin's disease where one control will be matched on disease of first primary cancer (Hodgkin's disease) and one not matched on disease of first primary cancer.

Deceased controls

If a selected control has died, she will be included in the study only if she is eligible, i.e. she must have survived (free of breast cancer) at least as long as the interval between the first primary cancer and the breast cancer of the case to whom she is matched.

Replacement in selection of controls

Deceased or untraced controls

It is acceptable to retain dead or untraced controls in the study. However, some participating centres may not be in a position to provide all elements of data in relation to such controls because of, for example, problems in obtaining ethical permission to approach relatives or to access medical records of such patients. In such circumstances it would be appropriate (as far as possible) to reselect until at least one fully informative control is obtained.

As reselection may introduce a selection bias (controls for whom all elements of data can be obtained might not be representative of the study population), analyses will be done with and without the reselected controls. It is therefore essential that as much information be obtained as possible even for the controls that are replaced. For example, if it is not possible to obtain interview data then every effort should be made to obtain medical record information (for radiation and chemotherapy exposure and reproductive factors) and the pathological blocks (for the second phase of genetic analyses after the specific sequence variants of interest are identified).

Controls who refused to participate

Again, these controls should be replaced and analyses are envisaged with and without the replaced controls as refusal may introduce a selection bias.

All available information on the controls should be noted (age, first cancer diagnosis) should be noted.

Reselection of controls missing at least one of: medical records of exposures, interview data or biological material

Every attempt should be made to secure at least one fully informative control matched to each fully informative case. Fully informative means that each of the three elements of information is available, that is: medical records, interview and biological material. In circumstances where it is not possible to obtain a fully informative subject the next best would include both medical record exposures and biological material - to enable gene-radiation interactions to be studied. Consequently, it would be acceptable to reselect controls to provide at least one fully informative control or, failing that, the next best would be a control for which both medical record exposures and biological materials are available.

A careful record of the reselection process should be kept so that later investigation for possible bias might be carried out. In particular, when a control is being reselected it is important to record all available information on this subject, in particular whether the control being replaced was exposed to radiotherapy or chemotherapy.

Collection of information

Information will be obtained from the following sources: questionnaire, medical records and biological samples.

Questionnaire

In-person or mailed self-administered questionnaire for live subjects (and proxies for deceased cases, wherever possible) containing:

- basic demographic information on vital status and education
- reproductive/gynaecological history
- history of exposure to medical radiation before age 40, including mammography, repeated routine fluoroscopy and x-rays, CT scans and radiotherapy for a previous cancer
- subject's and family's history of breast and ovarian cancer
- history of exposure to other putative risk factors for breast cancer, including physical activity, smoking, alcohol and anthropometry

Biological material for childhood cancer and Hodgkin's disease survivor cohorts

Two approaches will be used for the collection of material for genetic analyses. In some centres blood will be drawn where participants agree while in others centres where mailed questionnaires or telephone interviews are used, buccal cells will be obtained from saliva.

Blood samples

- 3 EDTA 5ml tubes should be drawn, labelled with ID code and a second identifier such as birth date, and sent to local coordinating centre within 48 hours (stored at room temperature in transit);
- on arrival the tubes should be frozen, at least at -20º C (-80º C would be optimal).
- 2 tubes should be sent to IARC on dry ice, 1 retained -20º C (-80º C would be optimal).in national centre as backup.

Of the 2 tubes sent to IARC, DNA will be extracted from one using Gentra equipment, while the other will serve as back-up at IARC stored in locked freezers. DNA will be stored at 4ºC until further processing – see section on Whole Genome Amplification below.

If DNA is extracted locally, then only 1 tube will be sent to IARC for quality assurance and backup.

Buccal cell collection

- Subjects will be provided with a labelled (ID code and a second identifier such as birth date) collection kit supplied by the company, Oragene, and clear instructions for collection of the biological sample;
- The filled collection kit will be returned within 48 hours at room temperature to the coordinating centre;
- The sample tube should be stored at room temperature until transferred to IARC or until the DNA is extracted locally.

The DNA will be extracted according to the Oragene manufacturer's instructions; the amount of DNA will be quantified and quality control procedures will be implemented.

DNA will be stored at 4ºC until further processing – see section on Whole Genome Amplification below.

Tissue blocks for dead subjects

DNA can be extracted from formalin fixed blocks, but the DNA from this source is fragmented resulting in extremely time consuming and expensive procedures to screen entire genes for mutations, particularly the very large genes such as *ATM, BRCA1 & 2*. However, the DNA can be used for genotyping specific mutations/SNPs of interest. Thus, if we obtain blocks from dead cases, DNA could be extracted to, further test any results obtained from the analyses of DNA from the live cases and controls. We will need formalin-fixed, paraffin-embedded blocks containing normal tissue (wherever possible blocks from lymph nodes, otherwise tumour blocks)

A pathologist will need to review the blocks to ensure that appropriate blocks are selected and that the slides are correctly prepared. The following material will be needed:

- One 3-micron thick section for haematoxylin staining (on a coated slide) and
- 10 x 5-micron sections on coated slides for DNA extraction;

The slides should be labelled with study ID and a second identifier such as birth date, and sent to IARC.

DNA can be extracted very simply from formalin fixed paraffin embedded material. The quality and quantity of DNA obtained will depend on the histology (how cellular, how necrotic the material is), how the material was handled prior to fixation and how it was fixed and embedded. The protocol to be used for DNA extraction is a simple proteinase K digestion that can be found in Armes et al (1999). Cut sections should be collected and stored dry at room temperature. DNA on these slides is very stable. DNA extracted from these slides can, however, suffer over time from degradation. DNA therefore will only be extracted from these sections **when** required for molecular work.

See Annex 1 and Annex 2 for protocol for extraction of DNA from paraffin blocks and slides.

Whole Genome Amplification of DNA from all sources

For DNA extracted from buccal cells and blood, Amersham Genomiphi amplification kits will be used. At the present time Sigma GenomePlex WGA amplification kits might be the best for DNA extracted from paraffin blocks, but evolving technology may indicate better techniques by the time we need to do this.

Quality control in relation to biological samples

IARC will work with those centres that extract their own DNA to develop a quality control system satisfactory to both partners.

Tracking biological samples

The progress in obtaining samples, specifications of type of material, dates of acquisition, storage information, dates of shipping and of each step taken to process the samples will be entered in the Field-work Follow-up database.

Shipping biological samples to IARC

The guidelines for shipment of biological samples to IARC are contained in Annex 3.

Biological samples of subject who have withdrawn from the study

When required, samples from subjects who withdraw from the study will be destroyed at IARC and the local study centre storage facility.

Information from medical records about the breast cancer (Form GRR-BCD):

The following information will be retrieved from medical records or through linkage with Cancer or/and Pathology registries and entered into the database:

- Date of diagnosis
- Exact diagnosis in words and topography and morphology codes
- Location of the breast cancer (from imaging and/or from the surgical report)
- Oestrogen and progesterone receptor status

When available, copies of the mammograms will be obtained and sent to the Institut Gustave Roussy for determining the precise location of the breast cancer.

Information from medical records for childhood cancer and Hodgkin's disease survivor cohorts

The following information will be retrieved from medical records and entered into the database:

- Diagnosis and radiotherapy information from medical records for first cancer – *this information should be obtained both for the primary treatment and for all recurrences.* (Forms GRR CS-D and GRR CS-D2):
    - Date or age at treatment
    - Diagnosis: morphology, topography, where available
    - Copies of the simulation films and radiotherapy records. If the copies of the simulation films and radiotherapy records are unavailable, the following information will be retried from the medical records:
    - Height and weight (and age /date at which this was measured)
    - Anatomical data (if available); trunk thickness, or width, etc.
    - Date of start for each course of radiotherapy
    - Type of radiotherapy
    - Clinical target volume
    - Dose specification point
    - Total dose delivered to the specification point (in Gy)
    - Total number of fractions
    - Total number of days
    - Name of hospital
- Information on chemotherapy from medical records for first cancer – *this information should be obtained both for the primary treatment and for all recurrences.* (Form GRR CS-ChT):
    - Date
    - Height and weight (and age /date at which this was measured)
    - Date of start and end of each cycle
    - Number of cycles
    - Name and code of cytotoxic drug used
    - Total dose (in mg/m^2)
    - Name of hospital
    - Weight dynamics in the course of treatment, if available
- Information about diagnostic procedures related to the diagnosis, treatment and follow-up of the first cancer (Form GRR CS-DX) – Year by year, number of:
    - Thoracic X-rays
    - Pelvic X-rays
    - Abdominal X-rays

- Arteriography
- Lymphography
- IV Urography
- CT, including bone scans

- Other risk factor information from medical records for both the breast cancer cases and the controls (Form GRR-RF)
  - Date of birth or age,
  - Height and weight (and age /date at which this was measured)
  - Age at menarche, when available
  - Parity and age at first pregnancy
  - Any reproductive / contraception / hormone information available

Other information available from cohort intake questionnaires (GRR-RF)

- Date of birth or age,
- Height and weight (and age /date at which this was measured)
- Age at menarche, when available
- Parity and age at first pregnancy
- Any reproductive / contraception information available

Dosimetry

Organ-specific doses for each study member of the medically-exposed cohorts will be calculated as well as the uncertainties in these estimates.

Radiation doses from radiotherapy received to the site of the breast cancer for cases (and to the same site for matched controls) will be individually estimated. Retrospective dose estimations will be performed using INSERM U605 software package for external radiotherapy (Diallo *et al* 1996, Shamsaldin *et al.* 1998) and ICTA (Ligot *et al* 1998, Shamsaldin *et al.* 2000) for brachytherapy. For this study, these two packages will be modified in order to include 16 additional sites of estimation for each breast (a total of 32). This will be done in order to have a representative dose estimate, whatever the breast cancer location.

In addition, various phantoms (accounting for different shapes and volumes of breast) will be included in the software, in order to account for patient morphology (derived from medical and radiotherapy records) in the estimation of dose to the 32 locations of interest.

Data input needed for reconstruction of the treatment and dose calculation will be extracted from technical and medical records collected. Centres will provide copies of the simulation films and radiation charts for those subjects who have undergone radiotherapy for a first cancer.

For case-controls studies for which breast doses have already been estimated by Marylin Stovall using MD Anderson software, a cross validation will be organized.

External radiotherapy

The software contains a typical treatment planning system, (TPS) extended to the whole body of the patient, that allows for the simultaneous positioning of the breast tumours and of the radiotherapy beams, as done in the MDAH system.

The approach does not, however, take into account the uncertainty in the breast tumour localisation. The TPS nevertheless improves the estimations of the peripheral doses, as the "global beam" model takes into account scattering and leakage. Phantoms used in the new approach allow simulating patient's gender, age, treatment position, body size, etc. For each patient a profile representing the variation of the dose according to the depth of the breast tumour can be created.

For each patient, individual retrospective doses will be computed separately for every beam and for every course of radiotherapy, and then cumulated to determine the radiation dose absorbed by each organ during the whole follow-up period defined for the study.

Brachytherapy

ICTA software (Ligot *et al* 1998, Shamsaldin *et al.* 2000) will be used for reconstruction of dose from brachytherapy. ICTA stands for individualized phantom based on CT slices of a real human body, using Auxological data. Individuals of various ages and the various brachytherapy applicators characteristics are included. Information about the position of the applicators on the patient's body is obtained from the patient's file (drawing, description, photographs). Doses delivered to 187 anatomical landmarks distributed throughout the body can be estimated with the ICTA dose calculation algorithm. The general structure of the ICTA software has previously been described, and the simulation of individuals has been detailed elsewhere (Ligot *et al.* 1998). Comparison of dose calculated by ICTA with Swedish results obtained at Radiumhemmet, Karolinska Hospital, have been published (Shamsaldin *et al.* 2000).

Diagnostic exposures

Estimation of radiation dose from diagnostic exposures will involve compilation of a table of average radiation dose (and typical range) for each diagnostic procedure of interest and type of equipment, by country and time period. Estimation of dose to individuals will then be based on the use of relevant information from the questionnaire coupled with the information from the table. Because of variations in radiation dose for the same diagnostic procedure between institutions in a given time period and country, the variability of the average doses will be estimated within the project. This will involve the following steps:

1) a literature review of published studies and institutional reports assessing radiation dose delivered to each main organ in the body from radiological examinations. The selected studies will be restricted to European studies performed on large patient samples, representative of patients and radiology services. A number of publications have been identified already.
2) estimation, if feasible, of the factors which play a role in the radiation dose received to breast during examination, using publication and all institutional reports available.
3) estimation of average radiation dose per country and of its variability, taking into account the factors identified in 2 as playing a role in dose delivered to the breast.

Gene analyses

The principal question under investigation in this study is whether there exists a significant interaction between exposure to ionizing radiation, genotype, and risk of breast cancer. Over the last 10 years, it has become clear that a significant fraction of the genetic attributable risk of breast cancer lies in reduced function and/ or loss of function alleles of genes that function in DNA DSB-repair pathways and/or cell cycle checkpoints that are activated by DNA damage. In humans, they are the most likely place to look for the gene-radiation interaction that we are investigating.

A vast number of association studies have identified functional variants in genes that are candidates for influencing breast cancer risk and very few have been replicated. A large multinational Breast Cancer Association Consortium (BCAC) is currently genotyping a selected set of the polymorphisms that had relatively strong evidence in previously published studies and conducting pooled analyses on about 15,000 cases and controls to distinguish between those that genuinely confer risk and those that do not. Of the first 16 selected SNPs five strong candidates have been already identified (BCAC, 2006), genotyping of these SNPs in additional BCAC studies is in progress and it is expected to inform a focused approach to selection of strong candidate or confirmed risk SNPs for the genotyping arm of this study.

Because of this, the approach proposed will be two-pronged:

- BRCA1, BRCA2, ATM and p53 will be mutation screened — because the risk-conferring alleles are individually extraordinary rare but very numerous (http://www-p53.iarc.fr/index.html).
- In parallel, for other genes involved in DNA repair, BCAC will provide a list of variants conferring modest risk that would be appropriate for genotyping while there would be little rationale for mutation screening the underlying genes. Genotyping of the specific variants involved in DNA repair will therefore be conducted.

Note: One complication in this study design is that BRCA1, BRCA2, and TP53 are all dominant high-risk susceptibility genes. It is becoming a normal part of clinical cancer genetics practice to report mutations found in these genes to patients. As such mutations are likely to be found over the course of this project, we must develop mechanisms (on a study centre by study centre basis) to inform healthcare providers and patients of potentially important findings so that the relevant patients can be referred to a licensed clinical testing service.

Power

As indicated in Table 1, it is expected that the study will include approximately 400 cases of breast cancer among cancer survivors. The power to estimate the effects of radiation by itself and the effects of age at exposure, attained age and time since exposures is very good in this study.

For the gene-radiation interactions in the cancer survivor cohorts, it is anticipated that records and biological samples will be available for only 90% of these on average, thus the expected numbers of cases with records and biological samples will be about 350.

Table 2 shows assumptions and results of calculations of the sample size needed to find interactions of different magnitudes between radiation and the pools of genotypes of interest. These calculations were made with the QUANTO software (University of Southern California); assumptions used for summed allele frequencies, exposure prevalence and associated relative risks are shown in the table. It is noted that calculations were made for a

binary dose variable. If possible, however, analyses will be based on a continuous dose variable or at least on a categorical variable with four or five levels.

For the interesting missense substitutions in *BRCA1/BRCA2* and *ATM*, the study will have 80% power to find interactions of the order of 3.5. For the *XRCC1* pool of mutations, for the frequent *ATM* SNP haplotypes (recessive), and for the undefined pool with summed allele frequencies of 0.1, the study will have 80% power to find an interaction of 2.5.

In the mutation carrier cohorts, as indicated above, the total number of cases is around 550. Given the relatively large effect seen in our preliminary analysis of chest X-ray data (Andrieu *et al*. 2006), the proposed study design should have 90% power to detect the effects of radiation exposure on these women at high risk and 80% power to detect an interaction of the order of 2.5. The study should also be able to provide important information about the most relevant periods of exposure.

Methodological issues

Assessment of interaction with polymorphic genes

When the candidate genes are highly polymorphic, the average number of subjects having each genotype will be modest. If analysed separately, the risks associated with most genotypes will be estimated imprecisely. A recently developed Bayesian partition model clusters genotypes according to risk, only allowing partitions that satisfy a particular assumption about the joint effect of the two alleles making up a genotype (Seaman *et al*. 2002). This assumption is genetically plausible, imposes structure on the set of genotype risks, and still leaves a highly flexible model. Such a partition model enables genotype risks to be estimated more accurately and the alleles to be ranked according to risk (Seaman *et al*. 2002). The method is readily extendable to determine interaction of polymorphisms with ionising radiation, taking account of measurement error.

Modelling dosimetric error

It is well recognised that measurement error can substantially alter the shape of the dose-response relationship and hence the derived radiation risk estimates (Thomas et al. 1993). The effect of measurement errors in medical radiation doses will be investigated within this project. A number of approaches have been recently developed, including a Bayesian approach (Richardson and Gilks 1993a, b) and a Monte-Carlo Maximum Likelihood approach (Stram and Kopecki, 2003) and will be applied to the analysis of the results of the study.

Participating centres

Because of the small number of cases that are expected from individual centres, and the need for large numbers of cases to allow sufficient statistical power to study interactions, a multi-centric collaborative study is proposed. The list of participating centres and the characteristics of the study populations are given in Table 1.

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Table 1. List of childhood and Hodgkin's disease cohorts

Name of study centre(s)	Definition of study population - Composition of the cohort • Date started • Inclusion criteria • Study region	Age at exposure / first cancer	Approximate size of study population	Period for retrospective case ascertainment	Expected number of cases of breast cancer
Hodgkin's disease cohorts					
Netherlands Cancer Institute	Females HL survivors 1966-1995, the Netherlands	<35	900	1966-1995	130
Institute of Cancer Research, UK	Women HD patients treated with Rx in 1971-2004 who survived 5+ years	<35	2500-5000	1976-	222
Institut Gustave Roussy					17
Istituto Nazionale di Tumori					25
Childhood cancer cohorts					
Institut Gustave Roussy	Childhood cancer survivors – 1942-1986 - France	<1-16	3744	1948-2007	31
Istituto G. Gaslini –ORT	Off-therapy Children treated at Centers of the Italian Association of Pediatric Hematology-Oncology (AIEOP)	0-15	4 000	1993-	10
Childhood Cancer Registry of Piedmont	Registry of children in Piedmont, diagnosed with cancer	0-14	3400	1967 - 2007	7
Istituto Nazionale di Tumori					16
Netherlands Cancer Institute	Childhood cancer survivors	0-15	2-3000	1980-2004	20
UK Childhood Cancer Survivor cohort	All UK, 1940-1991; 5 year surv.	<15	18 000	1945-1999	40

List of mutation carrier cohorts

Name of study centre(s)	Total expected number of cases
Centre René Huguenin	450
Netherlands Cancer Institute	590
Cambridge University	500

Table 2. Sample size calculations for the analysis of gene-radiation interactions – assuming a power of 80% to find an interaction with p=0.05 *(numbers shown are the number of cases needed, assuming two individually matched controls per case)*

Genes			Radiation		Size of the gene-radiation interaction							
Pools of mutations or variants of interest	Summed allele freq	RR_G	exposure prevalence	RR_E	10	5	4.5	4	3.5	3	2.5	2
BRCA1 + *BRCA2* + *ATM* interesting missense substitutions*	0.045	3	0.3	5	124	214	239	275	328	415	580	985
XRCC1 R194W, *XRCC1* R280H, *XRCC1* R399Gln	0.4	2.5	0.3	5	61	118	134	157	192	249	358	630
ATM SNP haplotypes	0.347	1.2[1]	0.3	5	47	91	103	121	147	190	271	472
Undefined pool with summed allele freq. of 0.1	0.1	2.5	0.3	5	70	125	140	162	195	249	351	601

Legend:

$RR_{G:}$ assumed relative risk of cancer among those that are positive for the pool of mutations/variants/SNPs

Exp prev.: assumed prevalence of the "higher" exposure group (based on a dichotomous categorisation of the exposure variable

RR_E : assumed relative risk among the higher exposure group compared to the lower

[1] RR assumed among heterozygotes

Annex 1
DNA preparation from formalin-fixed and paraffin-embedded blocks

1. Scrape off the region of interest from section on slide and put in an eppendorf tube containing 500 μL xylene, leave at RT for 15 minutes. If too many samples, do 5 by 5 vortex.
2. Centrifuge at 14000 room, 15 minutes.
3. Remove 2 x 180 μl supernatant.
4. Add ethanol 500 μl, vortex, centrifuge 10 minutes at 14000 rpm, discard supernatant (3 x 175 μl).
5. Repeat once more with 500 ethanol.
6. Remove supernatant (3 x 190 μl), add 50 μl acetone, do not vortex.
7. Spin 5 minutes at 14000 rpm, remove supernatant (all).
8. Dry at 53ºC for several minutes.
9. Add 30 to 50 μl proteinase K to the digestion buffer, mix well.
10. Incubate at 55ºC for 3 – 5 hours or O/N.
11. Check, and if needed, add proteinase K.
12. Inactivate at 99ºC for 10 minutes, put on ice, store at -20ºC.

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xylene; ethanol absolut, acetone

proteinase K: stock sol at 15.6 mg/μl, use 30 μl in 1 ml digestion buffer, final = 0.5 mg/ml

Digestion buffer: 1 mM EDTA; 50 mM tirs pH 8.5, (prepare fresh before use)

0.5M EDT pH 8.0	200 μl
1 MTris/HCl pH 8.5	5 ml
H_2O	94.8 ml
	100 ml

Annex 2
DNA extraction from slides

1. Remove the paraffin by incubating the slides a few minutes in the following solutions:
   - Xylene 5mn in 2 different baths
   - 100% EtOH 3mn
   - 95% EtOH 3mn
   - 70% EtOH 3mn
   - H_2O 3mn
2. Scrape the area of interest with a sterile scalpel blade.
3. Transfer the scraped area to a sterile mini-eppendorf tube containing usually 50 µl of the DNA Extraction Buffer.
4. Incubate 1 to 5 days at 56ºC.
   Add 2 µl of Proteinase K (20 mg/ml) twice a day (morning and evening).
5. At the end of the incubation, inactivate the proteinase K by heating the DNA solution 10 mn at 95ºC.
6. Centrifuge at about 12000 rpm to eliminate the debris and store the DNA solution at -20ºC.
7. Use about 5 µl for PCR reactions.

Very important: change gloves and scalpel blade after each sample extracted.

DNA Extraction Buffer:

TE pH9 with 0.1 µg/µl of Proteinase K (stock 20 mg/ml) and 0.25% of Nonidet P40.

TE: Tris 10mM, EDTA 1mM at pH9

For 10 ml of TE p9: 50 µl of PK (20 mg/ml) + 25 µl of NP40.

Annex 3
Guidelines for shipment of samples to IARC

The International regulations for the transport of infectious materials by any mode of transport are based upon the Recommendations of the United Nations Committee of Experts on the Transport of Dangerous Goods (UN). The Universal Postal Union (UPU) reflects these recommendations in its regulations, particularly for packaging. The International Civil Aviation Organization (ICAO) and the International Air Transport Association (IATA) have also incorporated the UN Recommendations in their respective regulations, as have other international transport organizations. The World Health Organization serves in an advisory capacity to these bodies.

Sample containers

All samples sent to IARC must be watertight, leak-proof sealed cryotubes with threads inside. It is recommended avoiding use of "Eppendorf" tubes. Before packing for shipment, sample tubes should be checked to ensure that they are tightly closed. If, before packing, the tubes have been contaminated by blood or other biological products, this must be removed with a dilute chlorine bleach solution.

Sample labels

It is recommended using printed and bar-coded labels to facilitate sample identification. Handwritten labels should not be used to avoid misidentification or loss of samples. No personal identifiers should be added to the specimens.

Sample packaging

Sample classification

Given the IATA regulations, there are two categories of infectious substances.

Category A is defined as an infectious substance which is transported in a form that, when exposure occurs, it is capable of causing permanent disability, life-threatening or fatal disease to humans or animals. Infectious substances in category A shall be assigned to UN2814 or UN2900. The proper shipping name for UN2814 is "Infectious Substance, affecting humans". The proper shipping name for UN2900 is "Infectious Substance, affecting animals only".

Category B is defined as an infectious substance which does not meet the criteria for inclusion in category A. Infectious substances in category B shall be assigned to UN3373. The proper shipping name of UN3373 is "Diagnostic Specimens" or "Clinical Specimens".

Biospecimens or derived products that have been specifically treated to neutralize infectious agents, or for which there is a minimal likelihood that pathogens are present, are not subject to these regulations. The proper shipping name for such substances is “Exempt Human (or Animal) Specimens

Most biological samples sent to IARC are classified in category B. Specimens from category A should not be sent to IARC except in very exceptional circumstances. Packing instructions for category A are stricter (Declaration for Dangerous Goods, in such case, please contact the IARC investigator and the IARC Biological Resources (caboux@iarc.fr). For category B, It is strongly recommended applying IATA Packing Instructions 650 which includes the triple packaging system.

Triple packaging system

For liquids, packages must be prepared as follows (IATA PI650):

1- Primary receptacle. The primary receptacle(s) must be leak-proof and must not contain more than 500mL.

2- Secondary receptacle. There must be absorbent material placed between the primary receptacle and the secondary packaging; if several fragile primary receptacles are placed in a single secondary packaging, they must be either individually wrapped or separated so as to prevent contact between them. The absorbent material, such as cotton wool, must be in sufficient quantity to absorb the entire contents of the primary receptacle(s) and the secondary packaging must be leak-proof.

3- Outer shipping package. The outer packaging must not contain more than 4L.

Specimen data forms, letters and other types of information that identify or describe the specimen should be taped to the outside of the secondary receptacle.

A list of sample ID numbers and a copy of the records should be enclosed in each batch shipped and a separate electronic list sent by e-mail. These documents must be placed in a plastic bag to ensure that the moisture in the box does not blur numbers. Another copy of the list and records should be kept at the consignee centre.

Each package and the "Nature and Quantity of Goods" item of the airway bill must show the text "Diagnostic specimen packed in compliance with IATA Packing Instruction 650".

Package label

The package (outer shipping package) must be clearly identified and described. An example of a label (used at IARC for shipment of specimens from category B, UN3373) could be:

Consignee:

International Agency for Research on Cancer
150, cours Albert Thomas
69372 LYON Cedex 08
FRANCE

Attention:

Dr…….
Tel: +33 (0)4 72 73…….
E-mail: ……@iarc.fr

DIAGNOSTIC SPECIMENS

PACKING INSTRUCTION 650

Substances shipped in refrigerated or frozen form

For frozen samples, a sufficient quantity of dry ice (solid CO_2) must be added. For example, for 3 kg of material travelling for 2 days, 20 kg of dry ice is needed. Ensure that all samples are frozen solid before they are placed in a plastic bag in the dry ice, as a sudden change in temperature may crack the tubes.

Dry ice or another refrigerant must be placed outside the secondary packaging. The outer packaging must permit the release of carbon-dioxide gas.

Indicate on the box: "**To be kept frozen at -20°**". For customs, specify also: "**NO COMMERCIAL VALUE: MATERIAL FOR SCIENTIFIC RESEARCH**". Make sure that accompanying customs documents are complete.

Shipment conditions

At least one week before the expected day of shipment, the responsible IARC scientist must be informed by e-mail or fax of all details of the impending shipment. **Do not ship samples without having received confirmation** from IARC that the message was received and that the proposed shipment details are acceptable.

All information regarding specimen shipment should be given by e-mail or fax: the name of the air company, flight number, date and time of arrival in Lyon, and AirWay Bill Number.

Shipment must be made without delay. If there is a transit time, it should be ensured that the parcel is stored in a cold room. Please send the parcel to Lyon at the beginning of a week, preferably on a Monday; do not allow the shipment to arrive at Lyon Airport on a Friday, weekend or holiday, as customs clearance cannot be processed then.

It is forbidden to carry specimens in person, either by a visitor to IARC or by an IARC staff member to an Institute. Carrying these types of specimens may cause considerable problems with customs.

IARC CONTACTS

- When in doubt of category of shipment or exceptional shipment of category A specimens, please contact the IARC investigator and Ms Elodie Caboux, the IARC Biological Resources Manager (caboux@iarc.fr).
- For information on importations, please contact Ms Fabienne Lelong, the Supply Assistant (lelong@iarc.fr).
- For information on exportations, please contact Ms Sophie Servat, the Administrative Assistant (servat@iarc.fr).

Appendix B

IARC Ethical Review

IARC ETHICAL REVIEW COMMITTEE

From: Dr R. Saracci, Chairman ERC

To: Dr Elisabeth Cardis, RAD

Subject: Project 04-28 submitted for ERC review

Date: October 4, 2004

The minutes of the Ethical Review Committee meeting held on 3rd September, 2004 have now been approved by the Committee members.

Please find reported here below the section relevant to the project you submitted:

04-28 Radiation exposures at an early age: impact of genotype on breast cancer risk (Dr E. Cardis)

The project is cleared after ethical review subject to the condition that clearance of the local/national ethics committee is provided. It is recommended that in the informed consent form: "genetic counselling" be replaced by "appropriate counselling will be provided"; the approximate number of subjects involved in the study should be mentioned.

cc. Dr D.M. Parkin, BEC Coordinator

Appendix C

Questionnaire for Telephone Interview

Gene-Radiation Interactions

GENE-RAD-RISK

Common Core Questionnaire:

Cancer survivor cohorts

12 April 2006

1. General information

1.1 Your full name:

last name: _____

first name: _____

maiden name (if changed): _____

1.2 Date of birth: day |__|__| month |__|__| year 19 |__|__|

1.3 Current address:

Street _____

City _____ postal code |__|__|__|__|__|__|__|

1.4 Telephone number: work |__|__|__|__|__|__|__|

home |__|__|__|__|__|__|__|

1.5 How many years of schooling (including professional training, technical or university studies) have you completed? |__|__|

1.6 To which ethnic group do you consider you belong? (Indicate more than one if applicable)

|__| White |__| Chinese |__| Jewish - Ashkenazi

|__| Black |__| South East Asian |__| Jewish Sephardi

|__| Maghrebian |__| South Asian

|__| Other (*specify*) _____

2. Reproductive History

It is important to know whether some forms of medical treatment during childhood or young adulthood have an effect on women's natural periods (natural menstrual bleeds).

2.1 At what age did you have your first period?

|__| I have never had a period (*go to question 2.7*)

|__| don't know (*go to question 2.7*)

|__|__| age at first period

2.2 How old were you when you started to have regular periods? Regular periods occur approximately once a month in a predictable way.

|__| I have never had regular cycles

|__| don't know

|__|__| age when you started to have regular periods

2.3 Did they later stop or become irregular (excluding pregnancies)?

|__| no *(go to question 2.4)*

|__| don't know *(go to question 2.4)*

|__| yes

If yes:

If your periods stopped for 3 months or more, when did this occur and for how long? If there was more than one such episode please enter the information for each episode on a separate line.

Age when periods stopped for at least 3 months	Total number of months without a period in this episode	OR	My periods did not restart
|__|__|	|__|__|	OR	|__|
|__|__|	|__|__|	OR	|__|
|__|__|	|__|__|	OR	|__|
|__|__|	|__|__|	OR	|__|

If the periods stopped completely, how did your periods stop?

|__| they stopped on their own at age *(in years)* |__|__|
|__| after surgery to remove the uterus at age *(in years)* → |__|__|
|__| after surgery to remove the ovaries at age *(in years)* → |__|__|
|__| after radiotherapy at age *(in years)* → |__|__|
|__| after chemotherapy at age *(in years)* → |__|__|

What was the reason for the surgery/radiotherapy/chemotherapy?

|__| cancer
|__| dysplasia
|__| polyps
|__| excessive bleeding
|__| other *(specify)* ____________________

2.4 Have you ever been pregnant (include all pregnancies regardless of outcome)?

|__| yes*, specify* how many times have you been pregnant? |__|__|
|__| no *(go to 2.6)*
|__| don't know *(go to 2.6)*

2.5 For each pregnancy (whether it resulted in a live birth or not), indicate in the following table:

Month/year of end of pregnancy	Outcome of pregnancy: 1=live birth 2=miscarriage before 5 months 3=induced abortion 4=stillbirth 5=ectopic pregnancy 9=don't know	How many babies were conceived in this pregnancy?	If the baby was born alive:	
			Did you breastfeed? 1=yes 2=no 9=don't know	If yes, for how many months did you breastfeed?
|__|__|/|__|__|	|__|	|__|	|__|	|__|__|
|__|__|/|__|__|	|__|	|__|	|__|	|__|__|
|__|__|/|__|__|	|__|	|__|	|__|	|__|__|
|__|__|/|__|__|	|__|	|__|	|__|	|__|__|
|__|__|/|__|__|	|__|	|__|	|__|	|__|__|
|__|__|/|__|__|	|__|	|__|	|__|	|__|__|
|__|__|/|__|__|	|__|	|__|	|__|	|__|__|
|__|__|/|__|__|	|__|	|__|	|__|	|__|__|
|__|__|/|__|__|	|__|	|__|	|__|	|__|__|
|__|__|/|__|__|	|__|	|__|	|__|	|__|__|

(for twins or other multiple conceptions please use a separate line for each)

2.6 Have you used the pill (oral contraceptives) or injections (of hormones) or implants or patches to avoid getting pregnant for at least 12 months?

|__| yes
|__| no *(go to question 2.7)*
|__| don't know *(go to question 2.7)*

For **each type of contraceptive used** please give age started and age stopped:

Brand name (if remembered) and type of contraceptive (pill, implant etc)	Age at start	Age last used	Total duration of use in months
_____	|__|__|	|__|__|	|__|__|
_____	|__|__|	|__|__|	|__|__|
_____	|__|__|	|__|__|	|__|__|
	|__|__|	|__|__|	|__|__|

2.7 Did you ever use hormones (hormone replacement therapy or estrogens) to induce menstrual bleeding
or alleviate menopausal symptoms or to prevent osteoporosis (fragile bones) or for some other reason?

|__| yes
|__| no *(go to section 3)*
|__| don't know *(go to section 3)*

If yes, specify:

Brand name (if remembered) and type (pill, gel, patch, etc.)	Age at start	Age at stop	Total duration of use in months	Reason for treatment
	|__|__|	|__|__|	|__|__|	
	|__|__|	|__|__|	|__|__|	
	|__|__|	|__|__|	|__|__|	
	|__|__|	|__|__|	|__|__|	

3. Medical History

3.1 Control: Have you ever had breast surgery or a breast biopsy for lumps before this past year?
Case: Have you ever had breast surgery or a breast biopsy at least a year before your breast cancer?

|__| yes
|__| no *(go to question 3.2)*
|__| don't know *(go to question 3.2)*

If yes, what was the reason for the surgery/biopsy?

|__| benign breast disease:
specify age at first surgery for benign breast disease |__|__|
|__| breast cancer *specify* age at first surgery for breast cancer |__|__|
If you had surgery for more than occurrence of breast cancer please specify age for each surgery
second breast cancer |__|__|
third breast cancer |__|__|
|__| other *(specify)* _____, at which age? |__|__|

Did any of the surgical procedures on your breast(s) involve a radical mastectomy?

|__| yes
|__| no
|__| don't know

3.2 Before age 40 did you ever have radiotherapy, that is, treatment involving radiation?

This treatment may be given for several reasons including such conditions as: benign diseases (of the breast, such as mastitis), ankylosing spondylitis, hormonal infertility, gynaecological disorders, tinea capitis (ringworm of the scalp), acne, haemangioma or for benign or malignant tumours?

|__| no *(go to question 3.3)*

|__| don't know *(go to question 3.3)*

|__| yes

If yes*:* Please list for each course of radiation treatment you have received:

Age	Part of body treated 1=head, 2=neck, 3=chest 4=abdomen, 5=pelvis, 6=arms-upper extremities, 7=legs-lower extremities	Reason for radiotherapy: 1=benign breast disease (specify) 2=ankylosing spondylitis 3=cancer (specify) 4=other (specify)	Name and address of treatment centre. If you do not have the precise address please note the city or town in which it was located	Name of the doctor who was treating you at the time
|__|__|	|__|	|__| _____	_____	_____
		_____	_____	
|__|__|	|__|	|__| _____	_____	_____
		_____	_____	
|__|__|	|__|	|__| _____	_____	_____
		_____	_____	
|__|__|	|__|	|__| _____	_____	_____
		_____	_____	
|__|__|	|__|	|__| _____	_____	_____

Age	Part of body treated 1=head, 2=neck, 3=chest 4=abdomen, 5=pelvis, 6=arms-upper extremities, 7=legs-lower extremities	Reason for radiotherapy: 1=benign breast disease (specify) 2=ankylosing spondylitis 3=cancer (specify) 4=other (specify)	Name and address of treatment centre. If you do not have the precise address please note the city or town in which it was located	Name of the doctor who was treating you at the time
|__|__|	|__|	|__| _____	_____	
			_____	_____	
|__|__|	|__|	|__| _____	_____	_____
			_____	_____	
|__|__|	|__|	|__| _____	_____	_____
			_____	_____	
|__|__|	|__|	|__| _____	_____	_____
			_____	_____	

At the age when you first received radiotherapy had you started to wear a bra regularly?

|__| no *(go to question 3.3)*
|__| don't know *(go to question 3.3)*
|__| yes
If yes, what size bra did you wear? *size* |__|__|__| *cup* |__|

Mammograms

3.3 Have you ever had a mammogram?

|__| no *(go to question 3.4)*
|__| don't know *(go to question 3.4)*
|__| yes

If yes*:* at what age did you have your first mammogram? |__|__|

What was the reason for this first mammogram?

|__| routine screening
|__| as a result of a lump found on a doctor's or self-examination of the breast
|__| preventive screening because of a family history of breast or ovarian cancer
|__| following symptoms or complaints
|__| other (specify) _____

In the grid below please mark the number of mammograms you had in each of three periods: up to age 19, between age 20 and 29, between age 30 and 39.

Note: If you had no mammograms at all in an age period, please fill in '0'.

	up to and including age 19	between age 20 and 29	between age 30 and 39
Number of mammograms	_____	_____	_____

How many mammograms in total have you undergone in your life? |__|__|

How old were you when you had your last mammogram? |__|__|

Fluoroscopy

From the 1930s and in particular after the Second World War, large groups of the population (nursing staff, educational staff and students) had fluoroscopies taken for tuberculosis.
During a fluoroscopy several images are taken during which the person moves slowly to the left and right.
Note: during a chest X-ray you are asked to stand still. Chest X-rays are not to be taken into account here.

3.4 Have you ever had a fluoroscopy?

|__| no *(go to question 3.5)*
|__| don't know *(go to question 3.5)*
|__| yes

if yes*:* at what age did you have your first fluoroscopy? |__|__|

In the grid below we have listed the most important reasons for having fluoroscopy. Please find the reason that applies to you and mark the number of times you had fluoroscopy in each of three age periods.

***Note**: If you had no fluoroscopy at all in an age period, please fill in '0'.*

Reason for fluoroscopy	Up to and including age 19		number	Between age 20 and 29		number	Between age 30 and 40		number
Example: routine chest examination	|_X|	no		|__|	no		|__|	no	
	|__|	yes	0	|_X|	yes	3-8	|_X|	yes	1
Routine chest examination (include tuberculosis screening)	|__|	no		|__|	no		|__|	no	
	|__|	yes	_____	|__|	yes	_____	|__|	yes	_____
Abdominal pain (including suspected peritonitis)	|__|	no		|__|	no		|__|	no	
	|__|	yes	_____	|__|	yes	_____	|__|	yes	_____
A spot found on a chest X-ray	|__|	no		|__|	no		|__|	no	
	|__|	yes	_____	|__|	yes	_____	|__|	yes	_____
Pneumothorax (collapsed lung)	|__|	no		|__|	no		|__|	no	
	|__|	yes	_____	|__|	yes	_____	|__|	yes	_____
Other, namely ……..	|__|	no		|__|	no		|__|	no	
	|__|	yes	_____	|__|	yes	_____	|__|	yes	_____
I do not recall the reason	|__|	no		|__|	no		|__|	no	
	|__|	yes	_____	|__|	yes	_____	|__|	yes	_____

*A*t what age did you have your last fluoroscopy? |__|__|

X-rays

3.5 Before age 40 did you ever **regularly** have x-rays to the upper part of your body (above the abdomen) as part of a routine screening program at work or school, or for continued monitoring of a chronic condition such as scoliosis or tuberculosis: **Exclude routine dental x-rays**

|__| yes
|__| no *(go to question 3.6)*
|__| don't know *(go to question 3.6)*

If yes*:* In the grid below we have listed the most important reasons for having more than one X-ray as a routine. Please find the reason that applies to you and mark the number of x-rays in each age period.

***Note**: If you had no x-rays at all in an age period, please fill in '0'.*

Reason for repeated X-rays	Up to and including age 19		number	Between age 20 and 29		number	Between age 30 and 39		number
Example: routine chest examination	|_X|	no		|__|	no		|__|	no	
	|__|	yes	|__|__|	|_X|	yes	3-8	|_X|	yes	1
Diagnosis of cancer before age 30	|__|	no		|__|	no		|__|	no	
	|__|	yes	|__|__|	|__|	yes	|__|__|	|__|	yes	|__|__|
Follow-up of cancer which occurred before age 30	|__|	no		|__|	no		|__|	no	
	|__|	yes	|__|__|	|__|	yes	|__|__|	|__|	yes	|__|__|
Routine chest examination (include tuberculosis screening)	|__|	no		|__|	no		|__|	no	
	|__|	yes	|__|__|	|__|	yes	|__|__|	|__|	yes	|__|__|
Scoliosis	|__|	no		|__|	no		|__|	no	
	|__|	yes	|__|__|	|__|	yes	|__|__|	|__|	yes	|__|__|
Pneumothorax (collapsed lung)?	|__|	no		|__|	no		|__|	no	
	|__|	yes	|__|__|	|__|	yes	|__|__|	|__|	yes	|__|__|
Other, namely ……..	|__|	no		|__|	no		|__|	no	
	|__|	yes	|__|__|	|__|	yes	|__|__|	|__|	yes	|__|__|
I do not recall the reason	|__|	no		|__|	no		|__|	no	
	|__|	yes	|__|__|	|__|	yes	|__|__|	|__|	yes	|__|__|

CT Scans

3.6 Before the age of 40 did you ever have a CT examination?
(In this procedure the patient lies down and a round part of the machine moves around the patient. It does not involve being in an enclosed space. In this technology transverse cuts of the body are being filmed and processed by a computer.)

|__| no *(go to question 3.7)*
|__| don't know*(go to question 3.7)*
|__| yes

if yes, please specify the CT scans you have undergone in the past (first to last).

Age	(or) Year	Part of the body scanned: 1=head, 2=neck, 3=chest, 4=abdomen, 5=pelvis, 6=arms-upper extremities, 7=legs-lower extremities	Reasons for CT scan	Name and address of treatment centre. If you do not have the precise address please note the city or town in which it was located
|__|__|	|__|__|__|__|	|__|	_____	_____
|__|__|	|__|__|__|__|	|__|	_____	_____
|__|__|	|__|__|__|__|	|__|	_____	_____
|__|__|	|__|__|__|__|	|__|	_____	_____
|__|__|	|__|__|__|__|	|__|	_____	_____
|__|__|	|__|__|__|__|	|__|	_____	_____
|__|__|	|__|__|__|__|	|__|	_____	_____
|__|__|	|__|__|__|__|	|__|	_____	_____

Radionuclide/radioisotope scans

3.7 Before the age of 40 did you ever have a radionuclide/radioisotope scan (for example, of the bone, thyroid, liver, heart, etc.)?

|__| no *(go to question 4.1)*

|__| don't know *(go to question 4.1)*

|__| yes

if yes, please list for all such scans:

Age	(or) Year	Reason for radionuclide/radioisotope scan	Name and address of treatment centre. If you do not have the precise address please note the city or town in which it was located
|__|__|	|__|__|__|__|		_____ _____
|__|__|	|__|__|__|__|		_____ _____
|__|__|	|__|__|__|__|		_____ _____
|__|__|	|__|__|__|__|		_____ _____
|__|__|	|__|__|__|__|		_____ _____
|__|__|	|__|__|__|__|		_____ _____
|__|__|	|__|__|__|__|		_____ _____
|__|__|	|__|__|__|__|		_____ _____
|__|__|	|__|__|__|__|		_____ _____
|__|__|	|__|__|__|__|		_____ _____

4. Family history

4.1 Do you have any sisters or half-sisters (exclude step sisters)?

|__| no
|__| don't know
|__| yes, how many? ⟶ |__|__|

4.2 Did your biological mother, sister(s) or either of your <u>biological</u> grandmothers ever have breast cancer?

|__| no *(go to question 4.3)*
|__| don't know *(go to question 43)*
|__| yes

If yes, please specify:

Relationship: 1= sister 2= half-sister 3= mother 4= maternal grandmother 5= paternal grandmother	Year of birth	At which age was the breast cancer diagnosed (if known)?	Was this before age 50? 1= yes 2= no 9= don't know
|__|	|__|__|__|__|	|__|__|	|__|
|__|	|__|__|__|__|	|__|__|	|__|
|__|	|__|__|__|__|	|__|__|	|__|
|__|	|__|__|__|__|	|__|__|	|__|
|__|	|__|__|__|__|	|__|__|	|__|

4.3 Did your biological mother, sister(s) or either of your biological grandmothers ever have ovarian cancer?

|__| no *(go to question 5.1)*
|__| don't know *(go to question 5.1)*
|__| yes

If yes, please specify:

Relationship: 1= sister 2= half-sister 3= mother 4= maternal grandmother 5= paternal grandmother	Year of birth	At which age was the ovarian cancer diagnosed (if known)?	Was this before age 50? 1= yes 2= no 9= don't know
|__|	|__|__|__|__|	|__|__|	|__|
|__|	|__|__|__|__|	|__|__|	|__|
|__|	|__|__|__|__|	|__|__|	|__|
|__|	|__|__|__|__|	|__|__|	|__|
|__|	|__|__|__|__|	|__|__|	|__|

5. Physical activity

5.1 Did you engage regularly in any of the following activities? Regularly means more than an hour a week for a period of one year or more?

If no, please check "never or rarely".
*If yes, H*ow many hours per week did you spend, on average, engaged in physical activity – when you were an adolescent, when you were a young adult, and in the more recent past?

		From 12-19 years of age	**From 20-39 years of age**	**Since age 40**
		On average	*On average*	*On average*
	Never or rarely	*Number of hours per week*	*Number of hours per week*	*Number of hours per week*
Walking (including to school, work, shopping or as a leisure activity)	|__|	|__|__|	|__|__|	|__|__|
Cycling (including to school, work, shopping or as a leisure activity)	|__|	|__|__|	|__|__|	|__|__|
Gardening	|__|	|__|__|	|__|__|	|__|__|
Housework (cooking, cleaning, childcare)	|__|	|__|__|	|__|__|	|__|__|
Non-competitive sport such as swimming, aerobics, tennis, skiing etc (specify) _____	|__|	|__|__|	|__|__|	|__|__|
Competitive sport or dance (specify) _____	|__|	|__|__|	|__|__|	|__|__|
Other (specify) _____	|__|	|__|__|	|__|__|	|__|__|

6. Tobacco

6.1 Have you ever regularly smoked cigarettes for at least a year? By regularly we mean:
at least 1 cigarette per day OR at least 5 cigarettes per week OR at least 1 pack per month

|__| yes
|__| no *(go to question 7.1)*
|__| don't know *(go to question 7.1)*

6.2 At what age did you start to smoke regularly *(in years)*? ⟶ |__|__|

6.3 Do you still smoke regularly?

|__| yes
|__| no*:* at what age did you stop? ⟶ |__|__|
|__| don't know

6.4 On average, how many cigarettes do/did you smoke per day? ⟶ |__|__|

6.5 What was the maximum number you ever smoked per day if different from above? |__|__|

6.6 For how long did you smoke this many? ⟶ |__|__| months *or* |__|__|years

7. Alcohol

7.1 We are aware that consumption of alcohol may vary over time. Try to remember average amounts consumed.

	Beer or cider	Wine	Fortified spirits (vodka, brandy etc)
Has there ever been a time when you drank beer, cider, wine, or spirits at least once a week?	|__| yes; |__| no; |__| don't know)	|__| yes; |__| no; |__| don't know)	|__| yes; |__| no; |__| don't know)
If yes: at what age did you start to drink at least once a week?	|__|__|	|__|__|	|__|__|
Do you still drink at least once a week?	|__| yes; |__| no; |__| don't know)	|__| yes; |__| no; |__| don't know)	|__| yes; |__| no; |__| don't know)
If No: at what age did you stop drinking at least once a week?	|__|__|	|__|__|	|__|__|

7.2 On average, how much did you drink per week at the following ages

Age	Beer or cider	Wine	Fortified spirits (vodka, whisky, brandy etc)
	Glass (300 ml)	*Glass (100 ml)*	*Shot*
20 years old	|__|__|	|__|__|	|__|__|
30 years old	|__|__|	|__|__|	|__|__|
40 years old	|__|__|	|__|__|	|__|__|

8. Anthropometry

8.1 At the time you were diagnosed with a malignant disease during childhood or adolescence was your weight:

|__| same as other girls/women your age
|__| less than other girls/women your age
|__| more than other girls/women your age

8.2 What is your current height in cm (in bare feet)?: |__|__|__|cm

8.3 What is your current waist size in cm: |__|__|__|cm

If unknown, what is your current dress size |__|__|

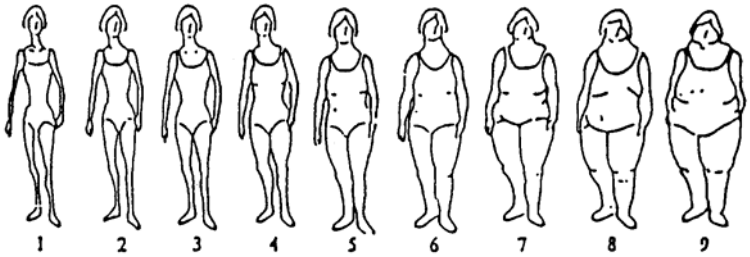
8.4 What is your current hip size in cm *(measure at widest part of hip)*: |__|__|__|cm

8.5 How much did you weigh at age 20? |__|__|__|kg

8.6 How much did you weigh at age 30? |__|__|__|kg

8.7 How much did you weigh at age 40? |__|__|__|kg

8.8 Body size in different periods of life (pictogram): please mark how you think you looked at different ages:



At around age 10 ○ ○ ○ ○ ○ ○ ○ ○ ○

At around age 20 ○ ○ ○ ○ ○ ○ ○ ○ ○

Now ○ ○ ○ ○ ○ ○ ○ ○ ○

9. Conclusion

This concludes the questionnaire. We would like to thank you very much for your time and effort in answering these questions. Is there any comment you would like to make or any information, which you think is relevant?

_____

_____

_____

_____

Time interview ended: hour |__|__| minutes |__|__|

10. Questions to the interviewer

Information to be completed by interviewer immediately after the interview:

10.1 Location of interview (*check one*)

1 polyclinic, hospital
2 at interviewee's home
3 other, specify _____

10.2 Was the subject responsive? (*check one*)

1 not at all (uninterested, reticent)
2 fairly co-operative and responsive
3 very co-operative, responsive and interested

10.3 In your opinion, how well did the respondent remember the information on reproductive history? *(check one)*

1 very well
2 well
3 fairly well
4 not well
5 not at all

10.4 In your opinion, how well did the respondent remember the information on medical history? *(check one)*

1 very well
2 well
3 fairly well
4 not well
5 not at all

10.5 In your opinion, how well did the respondent remember the information on family history? *(check one)*

1 very well
2 well
3 fairly well
4 not well
5 not at all

10.6 In your opinion, how well did the respondent remember the information on physical activity? *(check one)*

1 very well
2 well
3 fairly well
4 not well
5 not at all

10.7 In your opinion, how well did the respondent remember the information on tobacco use? *(check one)*

1 very well
2 well
3 fairly well
4 not well
5 not at all

10.8 In your opinion, how well did the respondent remember the information on alcohol consumption? *(check one)*

1 very well
2 well
3 fairly well
4 not well
5 not at all

10.9 Please add any additional comments on the interview which you think relevant:

_____

_____

Appendix D

Letters, Patient Information Sheets, and Consent Form

- GP letter case-version 1.0 – 21/08/2007
- GP letter control -version 1.0 – 21/07/2007
- GP consent form-version 1.0 – 21/07/2007

- Letter of invitation to participant case (1) - version 1.0 - 21/08/2007
- Letter of invitation to participant control (1) - version 1.0- 21/08/2007
- Participant information sheet case (1) - version 1.0 - 21/07/2007
- Participant information sheet control (1) - version 1.0 - 21/07/2007

- Letter of invitation to participant (2) - version 1.0- 21/08/2007
- Participant information sheet (2) -version 1.0- 21/08/2007
- Participant consent form-version 1.0-21/08/2007

(1) refers to first approach participant (2) refers to second approach participant

International Agency for Research on Cancer
World Health Organization

Centre for Childhood Cancer Survivor Studies
UNIVERSITY OF BIRMINGHAM

Free telephone helpline: 0800 328 9419

Dear Dr

Patient full name

Patient date of birth Patient NHS no

Diagnosed with

According to our records this patient was diagnosed with neoplastic disease in childhood and has subsequently developed breast cancer and is currently registered with your practice.

We are carrying out an international study, called GENE-RAD-RISK, which is investigating whether specific variations in DNA repair genes increase the risk of radiation induced breast cancer after treatment for childhood cancer. The study is being led by the International Agency for Research on Cancer which is part of the World Health Organization. The University of Birmingham is carrying out the British part of this study which concerns survivors of childhood cancer. The study will include survivors of childhood cancer who have developed subsequent breast cancer (cases) and survivors of childhood cancer who have not developed breast cancer (controls) and we shall compare differences in treatment and genotype between cases and controls. The identification of groups of survivors at particularly high risk, in terms of treatment history, genotype or both is likely to benefit both those treated in the past and children who are diagnosed in the future. Existing survivors should benefit through targeted surveillance and the potential for early diagnosis and treatment of subsequent breast cancer. Future survivors should benefit through the modification of future treatment protocols to avoid treatments (possibly in relation to specific genotypes) which are associated with a high risk of subsequent breast cancer.

At the Study Co-ordinating Centre in Birmingham we provide a free 0800 telephone helpline for patients who have difficulties or problems relating to the study.

We should like to invite the patient identified above to be interviewed over the telephone, to provide a sample of saliva, and to give permission for access to her medical records held at the relevant hospitals. To have confidence in the ultimate findings it is extremely important that we approach the maximum possible number of eligible survivors, thus avoiding the potential for bias. However, there may be compelling reasons why it is inappropriate to approach a small number of survivors and therefore we are initially approaching you as the patient is registered with you. We have considerable experience in approaching survivors of childhood cancer, in particular we have just completed the British Childhood Cancer Survivor Study which involved writing to 14 500 adult survivors and 10 500 returned a completed questionnaire.

We should greatly appreciate you indicating whether you consider it acceptable for us to contact your patient regarding this study, a consent form is enclosed for you to complete. With your permission, we shall then send the patient an invitation letter inviting them to participate in the study.

It is possible that some survivors may be sufficiently impaired and dependent upon others that it would be very difficult or impossible for them to complete the questionnaire. In such circumstances, or for cases where help is needed to understand the questionnaire, we are happy for a close relative or friend to help complete the questionnaire with as much input from the survivor as is practical.

If you would like to receive a newsletter concerning important new findings of the study then please indicate this on the consent form to be returned to us.

The study is overseen by a Steering Group including senior clinicians who are members of the United Kingdom Children's Cancer Study Group.

The study has been approved by the Main Research Ethics Committee. Any information provided in relation to this study will be treated in strict accordance with the Data Protection Act. Consent to contact General Practitioners and survivors has been obtained from the Patient Information Advisory Group. The study is funded by the European Union and Cancer Research UK.
Current general practitioners have been traced using the National Health Service Central Register and the National Strategic Tracing Service.

If you would like to receive further information about the study please indicate so on the consent form or visit our website at www.bccss.bham.ac.uk. It is also possible to contact Dr Hawkins using the telephone number above.

Thank you for your help with this study.

Yours sincerely,

M M Hawkins MSc DPhil
Centre Director

H C Jenkinson PhD FRCPCH
Consultant Paediatric Oncologist

Enc

International Agency for Research on Cancer
World Health Organization

Centre for Childhood Cancer Survivor Studies
UNIVERSITY OF BIRMINGHAM

Free telephone helpline: 0800 328 9419

Dear Dr

Patient full name

Patient date of birth Patient NHS no

Diagnosed with

According to our records this patient was diagnosed with neoplastic disease in childhood and is currently registered with your practice.

We are carrying out an international study, called GENE-RAD-RISK, which is investigating whether specific variations in DNA repair genes increase the risk of radiation induced breast cancer after treatment for childhood cancer. The study is being led by the International Agency for Research on Cancer which is part of the World Health Organization. The University of Birmingham is carrying out the British part of this study which concerns survivors of childhood cancer. The study will include survivors of childhood cancer who have developed subsequent breast cancer (cases) and survivors of childhood cancer who have not developed breast cancer (controls) and we shall compare differences in treatment and genotype between cases and controls. The identification of groups of survivors at particularly high risk, in terms of treatment history, genotype or both is likely to benefit both those treated in the past and children who are diagnosed in the future. Existing survivors should benefit through targeted surveillance and the potential for early diagnosis and treatment of subsequent breast cancer. Future survivors should benefit through the modification of future treatment protocols to avoid treatments (possibly in relation to specific genotypes) which are associated with a high risk of subsequent breast cancer.

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Thank you for your help with this study.

Yours sincerely,

M M Hawkins MSc DPhil
Centre Director

H C Jenkinson PhD FRCPCH
Consultant Paediatric Oncologist

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International Agency for Research on Cancer
World Health Organization

Centre for Childhood Cancer Survivor Studies
UNIVERSITY OF BIRMINGHAM

GENERAL PRACTITIONER'S CONSENT TO CONTACT PATIENT

Patient full name	Our ref:
Patient date of birth	
Diagnosed with	Patient NHS no

I give permission for my patient identified above to be contacted in relation to the GENE-RAD-RISK study.

GP signature ……

GP name (PLEASE PRINT) ……

The patient's address

……

……

……

……Postcode……

Patient's telephone number

……

IF YOU WOULD LIKE FURTHER INFORMATION PLEASE VISIT OUR WEBSITE www.bccss.bham.ac.uk OR TICK THE RELEVANT BOX(ES) TO RECEIVE A PAPER COPY:

Study Protocol ☐

Study Newsletter ☐

Study Questionnaire ☐

International Agency for Research on Cancer
World Health Organization

Centre for Childhood Cancer Survivor Studies
UNIVERSITY OF BIRMINGHAM

Free telephone helpline 0800 328 9419

Study of breast cancer in women who had cancer, tumour, leukaemia, or similar illnesses during childhood

Private and Confidential

Dear

We would like to invite you to take part in an international study on the risk of breast cancer in women who were treated in childhood for cancer, leukaemia, tumour or similar illness. We understand that you were treated during childhood for such an illness and that you were subsequently diagnosed with breast cancer.

Previous research suggests that the risk of breast cancer in women who were treated for a childhood illness such as cancer, tumour, leukaemia, or similar illnesses is in general low, but higher than in the general population. It is important to investigate why some people get breast cancer after such a childhood illness and others do not. If we could identify groups who are at an increased risk of breast cancer then this would help in three ways. Firstly, it would provide useful information for advising female survivors of childhood cancer, leukaemia, tumour or similar illness in relation to their risk of developing breast cancer. Secondly, it would provide evidence for focusing doctor's time on calling back those women most at risk and facilitate early diagnosis and treatment of subsequent breast cancer. Thirdly, it would provide a basis for planning future treatments of girls in a way to reduce their subsequent risk of developing breast cancer. It is only by comparing potential risk factors for breast cancer between women with and without the disease that we can identify those factors that might be related to the development of breast cancer.

We have contacted your family doctor (GP) and they gave us permission to approach you to ask if you might participate in the study. An information sheet explaining the study is enclosed. Please read this sheet carefully and if you have any additional questions then please telephone the Birmingham Study Centre on **0800 328 9419**. To have confidence in the results it is important that as many women as possible take part. However, you do not have to take part. If you do not take part in this study then this will in no way affect your future treatment by any doctor. This research is aimed at reducing the amount of breast cancer experienced by women surviving childhood cancer. We would greatly appreciate you telephoning the Study Centre's free telephone helpline on **0800 328 9419** to let us know whether you are willing to help us with this study by answering a questionnaire over the telephone.

We would prefer you to telephone the Study Centre, but if this would be difficult either because of some disability, impairment or handicap, or because you need some help to understand the questionnaire, then we are happy for a close relative or friend to help us complete the questionnaire over the telephone.

We shall prepare a newsletter containing important new findings of the study. If you would like to receive such a newsletter you will be given the opportunity to indicate this during the telephone interview.

All information obtained by this study is entirely confidential to the study and will not be passed on to anyone else. With your permission, the information will be kept to enable us to compare the health prospects of women included in this study with the health prospects of children who are treated in the future. In this way we will be able to assess the full impact of current and future treatments.

The study has been approved by the Main Research Ethics Committee. The study is funded by the European Union and Cancer Research UK. Your current GP has been traced using the National Health Service Central Register, which is a division of the Office for National Statistics in England and Wales and part of the General Register Office for Scotland.

With very many thanks for your help so far.

Yours Sincerely,

M M Hawkins MSc DPhil
Centre Director

H C Jenkinson PhD FRCPCH
Consultant Paediatric Oncologist

Enc

International Agency for Research on Cancer
World Health Organization

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UNIVERSITY OF BIRMINGHAM

Free telephone helpline 0800 328 9419

Study of breast cancer in women who had cancer, tumour, leukaemia, or similar illnesses during childhood

Private and Confidential

Dear

We would like to invite you to take part in an international study on the risk of breast cancer in women who were treated in childhood for cancer, leukaemia, tumour or similar illness. We understand that you were treated during childhood for such an illness and that you have **never** been diagnosed with breast cancer.

Previous research suggests that the risk of breast cancer in women who were treated for a childhood illness such as cancer, tumour, leukaemia, or similar illnesses is in general low, but higher than in the general population. It is important to investigate why some people get breast cancer after such a childhood illness and others do not. If we could identify groups who are at an increased risk of breast cancer then this would help in three ways. Firstly, it would provide useful information for advising female survivors of childhood cancer, leukaemia, tumour or similar illness in relation to their risk of developing breast cancer. Secondly, it would provide evidence for focusing doctor's time on calling back those women most at risk and facilitate early diagnosis and treatment of subsequent breast cancer. Thirdly, it would provide a basis for planning future treatments of girls in a way to reduce their subsequent risk of developing breast cancer. It is only by comparing potential risk factors for breast cancer between women with and without the disease that we can identify those factors that might be related to the development of breast cancer.

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Study of breast cancer in women who had cancer, tumour, leukaemia, or similar illnesses during childhood

Free Telephone Helpline 0800 328 9419

Participant Information Sheet

You are being invited to take part in a research study. Before you decide whether you wish to take part in this research study it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Talk to others about the study if you wish. Feel free to phone the Study Centre on the free telephone helpline if there is anything that is not clear or if you would like more information.

- Part 1 tells you the purpose of this study and what will happen to you if you take part.
- Part 2 gives you more detailed information about the conduct of the study.

Part 1

1. What is the purpose of the study?

Some women who have been diagnosed with cancer, tumour, leukaemia or similar illness during childhood will develop breast cancer several years later. The number of women who develop breast cancer after such a childhood illness is, in general, low, but greater than expected from the general population. We want to investigate potential reasons why some women get breast cancer after such a childhood illness and others do not. To this end, an international study is being carried out to investigate the possible links between breast cancer and an individual's medical history, treatment history, and genetic factors following diagnosis in childhood of an illness such as cancer, tumour, leukaemia, or a similar illness.

2. Why have I been chosen?

You are being asked to participate in this study because, according to our records, you were diagnosed with cancer, tumour, leukaemia, or a similar illness during childhood and breast cancer after your childhood illness. Information on your childhood diagnosis and breast cancer were obtained from national registries. Your contact details were provided by your General Practitioner. It is only by comparing people with and without breast cancer that we will be able to detect the factors related to the development of breast cancer after a childhood illness such as cancer, tumour, leukaemia, or a similar illness. In total, approximately 1200 women from all over Europe who have all had a similar illness to you will be included in the study, of whom 320 are British.

3. Do I have to take part?
You do not have to take part. However, to have confidence in the results it is important that everyone we approach takes part in the study, therefore we hope you will agree to participate in this important international study.

4. What to do if I would like to take part?
If you wish to take part, we would ask you to phone the Study Centre's free telephone helpline on 0800 328 9419. A research assistant will answer your call and will arrange a time that is convenient for you to answer a telephone interview. The questions asked during the telephone interview will be about your reproductive history (periods, pregnancies, pill usage etc.), medical history (any breast surgery, mammograms etc.), treatment (any radiotherapy, regular x-rays etc.), family history of breast cancer, and lifestyle factors (physical activity, smoking, alcohol). A copy of the questionnaire is enclosed. The duration of the telephone interview will be at least 15 minutes.

5. What to do if I do not wish to take part?
If you decide not to take part, then this will not affect your current or future treatment by any doctor. If you do not wish to take part then please call the Study Centre's free telephone helpline on 0800 328 9419 to let us know. We will then update our records so that you will not receive any further communications about the study. Should you choose to take part you would be free to change your mind at any time without giving a reason.

6. What are the possible disadvantages and risks of taking part?
There are no foreseeable disadvantages or risks in taking part.

7. What are the possible benefits of taking part?
This study is likely to benefit both those treated in the past for childhood cancer, leukaemia, tumour or similar illnesses and children diagnosed in the future. Existing survivors should benefit through targeted surveillance and the potential for early diagnosis and treatment of subsequent breast cancer. Future survivors should benefit through the modification of future treatment protocols to avoid treatments (particular in the presence of specific genes) which are associated with a high risk of subsequent breast cancer.

Part 2

8. What if there is a problem?
If you have a concern about any aspect of this study, you should phone the Study Centre on the free telephone helpline (0800 328 9419) and staff will do their best to answer your question.

9. Will my taking part in this study be kept confidential?
All data obtained in the course of the study from you will be kept in the strictest confidence. All collected data will be stored securely and only authorised personnel at the Study Centre will have access to your data. Anonymised (name, address and other identification information removed) data will be transferred for the purpose of analysis to associated researchers at the International Agency for Research on Cancer in France. No personal information about you will ever be released to anyone outside the Study Centre. All procedures for handling, processing, storage and destruction of data comply with the Data Protection Act 1998.
Data will be stored as long as possible in order to carry out future studies. However, all future studies would need to obtain ethical approval from appropriate Research Ethics Committees before the data is used.

10. Will I be notified of the study results?
If you wish to be informed of the study findings you will be given the opportunity to indicate this during the telephone interview. A study newsletter containing the study findings will then be sent to you as soon as the study findings are available. However you should be aware that it may take some time before the results are available (may be up to a few years).

11. What will happen to the results of the research study?
Findings from the research will be published in clinical and scientific journals. Only statistical summaries of the information obtained from participants will be published. Your name will never appear in any report.

12. Who is organising and funding the research?
The study is being led by researchers at the International Agency for Research on Cancer in Lyon, France, which is part of the World Health Organization (WHO), and involves researchers from more than ten different countries. Researchers at the University of Birmingham coordinate the study within the UK. The research is funded by the European Union and Cancer Research UK.

13. Who has reviewed the study?
This study was given a favourable ethical opinion for conduct by the Main Research Ethics Committee. Scientific experts have given a favourable opinion regarding the scientific merit of the study on behalf on the European Union and Cancer Research UK.

14. What if I have any other questions?
If you have any questions relating to the study please phone the Study Centre's free telephone helpline at 0800 328 9419.

International Agency for Research on Cancer
World Health Organization

Centre for Childhood Cancer Survivor Studies
UNIVERSITY OF BIRMINGHAM

Study of breast cancer in women who had cancer, tumour, leukaemia, or similar illnesses during childhood

Free Telephone Helpline 0800 328 9419

Participant Information Sheet

You are being invited to take part in a research study. Before you decide whether you wish to take part in this research study it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Talk to others about the study if you wish. Feel free to phone the Study Centre on the free telephone helpline if there is anything that is not clear or if you would like more information.

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2. Why have I been chosen?

You are being asked to participate in this study because, according to our records, you were diagnosed with cancer, tumour, leukaemia, or a similar illness during childhood. Information on your childhood diagnosis was obtained from a national registry. Your contact details were provided by your General Practitioner. It is important that we collect information from people who have not developed breast cancer following their childhood as it is only by comparing people with and without breast cancer that we will be able to detect the factors related to the development of breast cancer after a childhood illness such as cancer, tumour, leukaemia, or a similar illness. In total, approximately 1200 people from all over Europe who have all had a similar illness to you will be included in the study, of whom 320 are British.

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Part 2

8. What if there is a problem?
If you have a concern about any aspect of this study, you should phone the Study Centre on the free telephone helpline (0800 328 9419) and staff will do their best to answer your question.

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International Agency for Research on Cancer
World Health Organization

Centre for Childhood Cancer Survivor Studies
UNIVERSITY OF BIRMINGHAM

Free telephone helpline 0800 328 9419

Study of breast cancer in women who had cancer, tumour, leukaemia, or similar illnesses during childhood

Private and Confidential

Dear

A few days ago you completed a telephone interview with one of the research assistants at the Centre for Childhood Cancer Survivor Studies at the University of Birmingham. You indicated that you would be happy to provide us a sample of your saliva for the genetic part of this study. We have enclosed a Consent Form for you to complete if you are still willing to do this and we suggest that you carefully read the Information Sheet also enclosed. Feel free to telephone the Study Centre if you would like more information or if you have any questions.

Together with this letter the saliva collection kit should have been delivered to your home. Provided you are happy to provide a sample of saliva, please read the instructions included with the kit carefully and follow them to produce a sample to send to us.

In addition, we would like to ask whether we may access your medical records. We would like to do this because we would like to know the types of medical treatments and diagnostic procedures which you have experienced (including diagnostic x-rays, drugs, radiotherapy, and chemotherapy) because we wish to investigate whether such exposures increase the risk of breast cancer in some survivors of cancer, tumour, leukaemia or similar illnesses during childhood.

Please read, complete and sign the Consent Form and post it back in the reply paid envelope. Also, please enclose the saliva kit containing your sample, if you are content to provide it.

Thank you for your help with this study.

Yours sincerely,

M M Hawkins MSc DPhil
Director, Centre for Childhood Cancer Survivor Studies

H C Jenkinson PhD FRCPCH
Consultant Paediatric Oncologist

Enc

International Agency for Research on Cancer
World Health Organization

Centre for Childhood Cancer Survivor Studies
UNIVERSITY OF BIRMINGHAM

Study of breast cancer in women who had cancer, tumour, leukaemia, or similar illnesses during childhood

Free telephone helpline 0800 328 9419

Participant Information Sheet

Why would we like access to your medical records?

We would like to investigate whether specific medical treatments or diagnostic procedures are related to an increase in breast cancer risk. Only by collecting this data from medical records will we be able to investigate precisely whether specific medical treatments or diagnostic procedures are related to an increased risk of breast cancer after a childhood illness such as cancer, tumour, leukaemia or similar illnesses. Your medical records are probably based at the hospital where you were treated for your childhood illness. If you give consent for us to access your medical records, then we will contact the relevant hospital(s) and arrange for photocopies to be made of the relevant medical records. We will then extract relevant information from these records.

Will my medical records in this study be kept confidential?

All data collection and storage will conform to the Data Protection Act. This means that only authorised personnel at the Study Centre will have access to your medical records, and that the data abstracted from your medical records will be treated in the strictest confidence, and no identifiable information will ever be released from the Study Centre.

What is the purpose of the genetic study?

This part of the study will investigate whether there are people with particular genes which render them more susceptible to breast cancer following exposure to radiation from radiotherapy or diagnostic radiology.

What is required from me?

We would like you to provide a sample of your saliva using the saliva collection kit provided. The saliva kit should then be posted back to the Study Centre in the reply paid package.

What will happen to the saliva sample?

DNA will be extracted from the saliva sample and will be stored in a repository at the University of Birmingham and a small proportion will be sent to the International Agency for Research on Cancer in Lyon, France in a completely anonymised form. Special arrangements have been made to transfer DNA samples to the International Agency for Research on Cancer in France. Specific genetic analyses will then be carried out by researchers at the International Agency for Research on Cancer. In addition, we would like to use the DNA sample donated as part of this study for future research. By giving your consent to donate your DNA you will be offering your DNA sample as a gift for our research.

Will I be notified of the results of genetic tests which could be important to me?

Some of the genes for which we plan to test are clearly related to an increased risk of breast cancer. However, we expect that very few people in this study are likely to carry such genes. In the unlikely event that we find evidence for such a gene relating to you then we would tell you, provided that you indicated that you would like us to do this on your Consent Form. Before we request your consent you will be given detailed information on risks by study staff over the telephone. If you would like to discuss the risks further with a specialist in genetics independent from the study before deciding whether to take part then this would be possible.

In the unlikely event that we find evidence that you might carry a clear cancer gene then with your consent we could arrange for an appointment to be made for you to attend your regional cancer genetics centre where you would be retested. Such clear cancer genes, if confirmed, could have implications for medical insurance and the risks of cancer among your relatives. We shall only feedback the results of tests which are concerned with clear cancer genes because these are the ones which may be given a meaningful interpretation for individuals.

How long will the samples be stored?

The DNA stored at the International Agency for Research on Cancer will be destroyed after study completion. The DNA stored at the University of Birmingham might be used for future analyses related to the current study and will be kept for as long as necessary to conduct these analyses. Feedback on individual results will not be available, except as described

above. However, any important general findings which emerge will be reported back through the newsletter. Later genetic studies might take place, but such studies will first have to get ethical approval from the Research Ethics Committee before using your DNA. If no such studies are developed then your DNA sample will be destroyed.

International Agency for Research on Cancer
World Health Organization

Centre for Childhood Cancer Survivor Studies
UNIVERSITY OF BIRMINGHAM

Private and Confidential

CONSENT FORM

Please tick box

Permission to obtain confidential information

☐ I **give** my consent for a representative of the Study Centre to contact the relevant hospital(s), General Practitioner(s), or Cancer Registries to obtain further details relating to my previous illness(es) and treatment(s). I understand that my medical records will be accessed and information abstracted by authorised personnel only. I give permission for such individuals to have such access to my records. I understand that strict confidentiality will be maintained.

Permission to keep confidential information

☐ I **give** my consent for information collected on me to be kept for as long as necessary, under conditions of strict confidentiality, to carry out current and future research projects which have received appropriate scientific and ethical approval.

Permission to store and use of DNA

☐ I **give** consent for my DNA to be stored and used for current and future research projects which have received appropriate scientific and ethical approval.

Feedback clinically significant results

In the unlikely event that we find that you might have an increased risk of breast cancer, because of the presence of a mutation in a specific gene, would you like us to inform you? In such unlikely circumstances and with your permission we could liaise with your GP for an appointment to be made for you to be retested for the relevant gene mutation in your regional cancer genetics centre.

☐ I **do** wish to be informed of the results of genetic tests which could be important to me

FULL NAME……

SIGNATURE……DATE …../……/…..

We shall prepare a newsletter to inform those who take part in the study about the main study findings. Would you like to receive a copy of such a newsletter?

☐ Yes

Appendix E

Quality Management Plan

Quality Management Plan for the GENE-RAD-RISK project

The GENE-RAD-RISK project is comprised of two separate multi-national and multidisciplinary epidemiological studies.

All partners in the project are experienced institutions that abide by good epidemiological and laboratory practices.

The main approach to quality control within the project is review by the peers within the project (the Project Board (PB), the General Assembly (GA) and the Consortium Group (CG) as a whole). The consortium is composed of a large number of well-qualified scientists with considerable experience in the design and conduct of epidemiological studies, in dose-reconstruction, gene analysis, statistical analysis and methods development. The ultimate check of the quality of the studies will be the peer review by the scientific journals to which the publications arising from this project will be submitted.

To ensure the highest quality and completeness of information collected and developed in this project, however, the following approaches to Quality Arrangements will be implemented. Compliance will be obligatory for all partners.

Collection of data and biological samples

Study documents

All multinational epidemiological studies at IARC and in particular in the Radiation group are based on the use of a common core protocol, questionnaire and data abstraction forms in all centres and the development of centre specific procedures detailing the way in which the study will be implemented in each centre.

The study protocol

The study protocol outlines the scientific rationale for the study and provides the essential outlines of the design of the study. The protocol specifies the definition of the study population, of the cases and the controls, the data to be collected, the eligibility criteria for cases and controls, the methods used to assess exposure, and the analytic strategy.

The first draft of the protocol will be prepared at IARC. It will be circulated to the Epidemiological Methods Expert Group (EG) for detailed comments and suggestions, revised and then circulated to the PB and consortium for further comments. It will be discussed in detail at the first meeting of the consortium and finalised by month 6.

The procedures document

As indicated above, the main approach to quality control within the project is review by the peers within the project. During the first 6 months of the project, IARC, in collaboration with the Partners, will prepare a draft of the detailed procedures document.

It is recognised that, because regional and national conditions and epidemiological resources vary and because of regional and national legal, ethical and logistic constraints, the implementation of the protocol will vary from centre to centre. The proposed centre/region-specific procedures for the implementation of the protocol will therefore be discussed between IARC and the national/regional investigators and included in the draft procedures document.

They will then be reviewed by the Epidemiological Methods Expert Group, and then by the Project Board and consortium, to ensure that these groups are satisfied that the country/region-specific procedures will be adequate for the purpose of the project. If not, alternative procedures will be discussed, tested if necessary, and implemented.

The questionnaire

The study questionnaire, which will be administered to the study subjects (or a suitable proxy respondent where necessary), will be designed to elicit information on the most important known and suspected risk factors for breast cancer.

The first draft of the questionnaire will be prepared at IARC, and will draw on other questionnaires successfully used for similar studies at IARC, in the partner institutes and elsewhere.

The first draft of the protocol will be prepared at IARC. It will be circulated to the Epidemiological Methods Expert Group (EG) for detailed comments and suggestions, revised and then circulated to the PB and consortium for further comments. It will be discussed in detail at the first meeting of the consortium and finalised by month 6.

Other data abstraction forms

Data abstraction forms will be prepared for extraction of information on the study subjects from other sources (radiotherapy records, other treatment records, current and past medical records). The purpose of these forms is to ensure a coherent approach amongst all partners in retrieving information on disease status of the subjects, both current and historic and on the risk factors of interest, in particular detailed information on radiotherapy (for radiation dose reconstruction) and chemotherapy.

The first draft of these abstraction forms will be prepared by partners 1, 5 and 7 (based on experience and expertise). They will be circulated to the Epidemiological Methods Expert Group (EG) for detailed comments and suggestions, revised and then circulated to the PB and consortium for further comments. They will be discussed in detail at the first meeting of the consortium and finalised by month 6.

Instructions for completing the questionnaire and data abstraction forms

Instructions will be prepared to assist local coordinators, interviewers and research assistants in the collection of data in a coherent and consistent manner.

The first draft will be prepared at IARC. It will be circulated to the Epidemiological Methods Expert Group (EG) for detailed comments and suggestions, revised and then circulated to the PB and consortium for further comments. It will be discussed in detail at the first meeting of the consortium and finalised by month 6.

Instructions for collecting biological samples

Instructions will be prepared to assist local coordinators, nurses and research assistants in the proper procedures for collecting, processing, storing and shipping the biological samples needed in the study.

The first draft will be prepared at IARC. It will be circulated to the Epidemiological Methods Expert Group (EG) for detailed comments and suggestions, revised and then circulated to the PB and consortium for further comments. It will be discussed in detail at the first meeting of the consortium and finalised by month 6.

Database

A common database will be created for use by the partners for these studies. The choice of the database management programme will be made after consultation with all Partners, based on availability, reliability and ease of use.

The database will be composed of four related sub-databases:

- A main sub-database in which to record basic information on all eligible study subjects, and in which the fieldwork follow-up is recorded, such as obtaining physician authorisation to contact subject; outcome of attempt to interview and obtain informed consent; retrieval of information related to first disease outcome; retrieval of information

related to breast cancer; retrieval of radiation treatment or diagnostic information for dosimetry; progress in collection of biological material.

- A sub-database containing the information needed for dosimetry
- A sub-database containing diagnostic information
- A sub-database for the questionnaire information

A set of data entry screens will be provided with the database to facilitate the entry of all collected data (allowing double entry and incorporating consistency checks at entry). A set of additional validation tools, which will allow checking the consistency and coherence of the data entered in different fields will also be provided, as well as tools for the exploitation of the data (summary statistics, exposure gradients, etc.).

Detailed documentation of the database and instructions for using the entry screens, data validation and data exploitation tools will be provided in a User Manual.

The importance of daily back-ups of the data will be stressed in the User Manual.

The database, entry screens, data validation and exploitation tools and user manuals will be developed at IARC by a small group of scientists and technical assistants. A Database Expert Group may be appointed by the PB to discuss the database structure and tools.

Arrangements for testing will be made with individual members of the consortium.

It is noted that, in those centres with pre-existing studies, databases are already in use. The compatibility of the local databases with the central GENE-RAD-RISK database will be ensured.

Training of local coordinators, research assistants and interviewers

In order to ensure the coherence and consistency of data collection, a series of training workshops will be conducted (either at IARC or locally for groups of countries). Within these workshops, a thorough review of the study instruments will be made and test interviews conducted.

The issue of regular (weekly or monthly, as appropriate on the basis of the number of subjects included) review of data collected and feedback to interviewers and research assistants will be particularly stressed.

Review of information collected

Site visits

Site visits will be carried out by staff from the coordinating centre to review progress and assist in resolution of problems arising from the implementation of the protocol. A review of information collected by questionnaire and data abstraction forms will be conducted at that time and discussion and feedback provided.

QA on the biological samples

Samples received at IARC will be processed to check their quality.

Periodic sending of de-identified data to IARC for checking

Every 6 months partners should send a copy of their database to IARC where the data will be checked for internal consistency. Feedback will be provided to the centres.

Adaptation of methods and programmes for gene-rad interaction analyses and for adjustment for errors in doses

The software used in the first instance for fitting the models will be WinBUGS (Spiegelhalter *et al.* 2003). Additional software in a high level language such as FORTRAN or C may also need to be written for the adaptation of the methods.

Testing procedures

Any special software written will use standard structured programming techniques, as a series of independent modules. Each module will be debugged as necessary, using line-by-line validation of calculations using Excel (Microsoft 1999). In addition, wherever possible the results of fitting such software, and any code written using WinBUGS (Spiegelhalter *et al.* 2003) will be validated against the results of statistical packages such as S-Plus (2001).

Documentation

Any code written, using WinBUGS (Spiegelhalter *et al.* 2003) or in a high level language such as FORTRAN or C, will have in-line comments, which will be the main means of documenting what is done. The in-line comments within each self-contained code will include a brief description of the function of each module, where relevant, including the data input to the module (which variables should in general remain unchanged) and the data output (variables changed), the input data required (the general sort of data contained, the format of data files, and their location), and the format of output files (the general sort of data contained, and the format of data files). The codes and all data will be archived on CD-ROM, and these together with hard copies of the codes will be kept by the coordinator and leaders of each relevant WP.Dose reconstruction

Dose reconstruction model

Software

1) Body phantom construction

The software package Dos_EG, was used to simulate patients anatomy as closely as possible, according to available information at the time of treatment, i.e; sex and height or age. This phantom was constructed using auxological tables, and adapted to each patient at the time of each treatment, using recorded anatomical information about the patient when available , i.e.; lateral diameter of different sections of the body, ant-post thickness, and organ heights. The anatomy so generated is a considerable improvement on a previous model, in that: (1) the individual phantom is articulated allowing for trunk inclination and back extension of the head as for mantle treatments, (2) the parameters used to fit the generated phantom to the patient, increase to 12, allowing for better adaptation, (3) it localises 151 anatomical points using a Cartesian co-ordinate system against 64 in the previous method, (4) the outline shape of transverse slices permit a better adaptation to the morphology of the actual patient.

2) Dose calculation

Once the individual anatomy was constructed the treatment conditions were simulated using the recorded technical data, i.e; total dose delivered to the target volume or to the point of maximum build-up, the type of treatment machine, radiation quality (gamma from Cobalt-60, X-rays from orthovoltage tubes, high energy X-rays from accelerators or electrons) and energy, source-skin distance, field size and shape, beam direction and wedges if any, and weighted dose from each beam. The number of fractions and the time from first to last fraction were also introduced into the software. This information is useful for epidemiological studies, i.e; the study of fractionated dose dependence effects. Dos_EG allows the input of the complex form of each field when shielding blocks are present.

This algorithm takes into account a wide range of photon beam qualities (50 kV to 31 MV) and electron beams from 4 to 32 MeV produced by different treatment machines (28

machines used in 8 treatment centres), but also lung heterogeneity, shielding block and wedge modifications, and all possible field shapes and sizes. The distance at which the dose could be estimated was extended from 50 cm to 180 cm from the central axis, permitting the calculation of doses to all sites of all patients from all treatments. In addition, it uses the actual spectrum emitted by the treatment machine in the estimation of the energy flux, for all beam energies from conventional orthovoltage tubes and linear accelerators. Absorbed doses were computed for each patient and for each course of radiotherapy. When more than one course of radiotherapy was performed for the same patient, the doses received at each anatomical point during the different treatments were cumulated.

The dose calculation algorithm, considers all principle sources of radiation doses, i.e; scattering from the patient, beam limitation devices, walls, and other obstacles, leakage from the head of the machine, bremsstrahlung scattering for high energy electron beams, etc... With the exception of the lungs, the whole phantom is considered water equivalent. Therefore the lung heterogeneity was considered, but no correction for bone density was introduced.

Documentation of the dose reconstruction software

A guide for user of the software has been written in French (9 pages). For physicists and computer scientist, a guide of the source (9 pages), of the menus (2 pages) of functions (4 pages) and of the general architecture (7 pages) of the software have been written in French. These documents are in possession of U605 INSERM of Gustave Roussy Institute and me be consulted by any person.

The following publications present the methods and results obtained with Dos_EG software.

François P, Beurtheret C, Dutreix A, de Vathaire F. A mathematical child phantom for the calculation of dose to the organs at risk. Medical physics, 1988; 15:328-333.

Diallo I, Lamon A, Shamsaldin A, Grimaud E, de Vathaire F, Chavaudra J. Estimation of the radiation dose delivered at any point in the body for individual patient in external beam radiotherapy. Radiotherapy and Oncology, 1996; 38:269-271.

Shamsaldim A, Grimaud E, Hardiman C, Diallo I, de Vathaire F, Chavaudra J. Dose distribution throughout the body for radiotherapy for Hodgkin's disease in childhood. Radiotherapy and Oncology, 1998 ; 335 :85-90.

Diallo I, Lamon A, Shamsaldin A, Grimaud E, de Vathaire F, Chavaudra J. Estimation of the radiation dose delivered at any point in the body for individual patient in external beam radiotherapy. Radiotherapy and Oncology, 1996; 38:269-271.

Shamsaldin A, Grimaud E, Ligot L, François P, de Vathaire F, Chavaudra J. Theoretical models for retropsective dosimetry. IRPA 100, Japon, 2000. Proceedings 2000, p-3a-208:1-6.

Shamsaldin A, Grimaud E, François P, de Vathaire F, Chavaudra J. Dosimetric evaluations applied to second cancer studies: experience and last developments at the Institut Gustave-Roussy, Chicago 2000 World Congress on Medical Physics and Biomedical Engineering. Abstract in proceedings, CD-Rom WE-E325-06, Chicago, July 23-28 2000.

Modification of existing programmes to include additional points in breast

- Model description

Using the present position of the breast in the mathematical phantom of body used in DOS_EG, 16 additional sites of dose estimation will be used. These new sites will be localised on star of 8 arms centred on the yet existing site. Two new sites per arm, at 2 and 4 cm of the yet existing site will be added.

- Validation of modification

The validation of the dose to the 16 additional site in breast will be performed by an expert group including 2 hospital physicists of Gustave Roussy Institute and 2 hospital physicists from other radiotherapy centres.

Validation of method

Comparisons with the software used by Marylin Stovall's group in MD Anderson Hospital of Houston has been performed in 1989, including 20 patients treated by various machines (including High Energy photon, 200 KV photons and Cobalt) and 8 organs (including the breast). For this purpose a physicist of Gustave Roussy Hospital has spent 2 months in the MD Anderson Hospital. A good agreement has been evidenced.

Comparison with dose estimates derived by Marilyn Stovall's group

New comparisons with dose estimated to breast for Dutch subject by software used by Marylin Stovall's group in MD Anderson Hospital of Houston will be performed, including 15 patients treated by various machines, including Electrons, High Energy photon, 200 KV photons and Cobalt, and various age at radiotherapy: 1, 5 and 15 years. Doses from MD Anderson are already available for this purpose. A meeting will be organised to discuss the differences in doses.

Quality control for dose reconstruction within project

For 30 patients included in the study and treated by various machines including Electrons, High Energy photon, 200 KV photons and Cobalt, and various age at radiotherapy: 1, 5 and 15 years, the dose estimation will be performed by 2 different experts : the hospital physicist of Gustave Roussy Institute and another external expert.

Data analysis

Analyses of the data will be carried out in parallel at the regional/national and European levels. Comparisons of results will be made to ensure that no errors have been made.

Sensitivity analyses will be performed, in addition, to evaluate the robustness of results to various changes in analytical strategies.

Publications

The project board will review all proposals for publication arising from the joint European analyses. Drafts will be prepared and circulated to all partners for comments and suggestions prior to submission to peer reviewed journal.

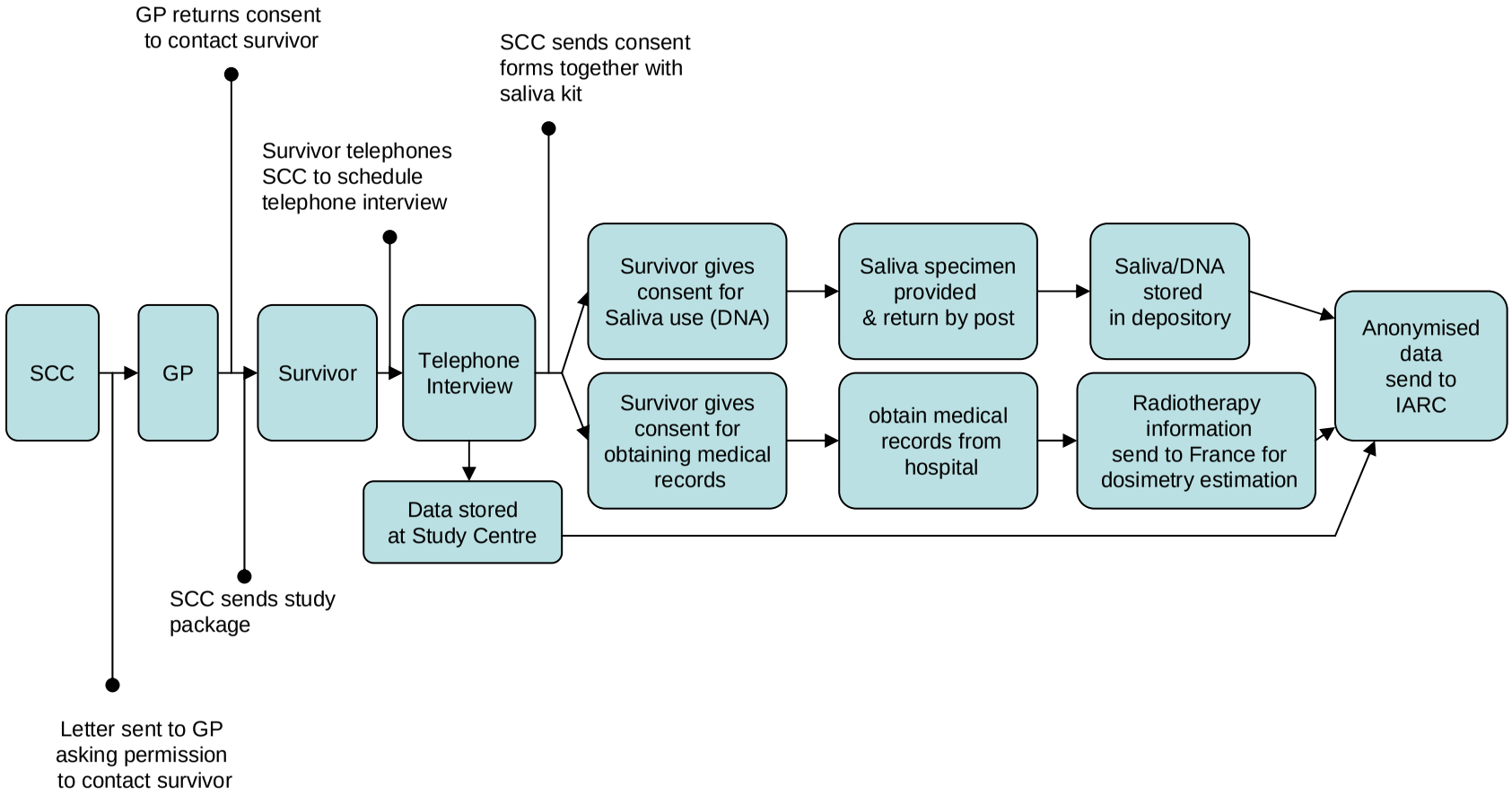
The ultimate evaluation of the quality of the work under this project will be the peer review by the scientific journals to which the manuscripts will be submitted.

Management quality control

The PB will review the technical reports sent by all partners, periodic progress reports and the cost statements for consistency with the schedule of implementation of the work and of resource utilisation. Any major discrepancies will be discussed with the concerned partners and solutions devised where appropriate.

Appendix F

Study Procedures Flow Chart



SCC: Study Coordinating Centre

GP: General Practitioner