REQUITE Study Protocol

Validating predictive models and biomarkers of radiotherapy toxicity to reduce side-effects and improve quality-of-life in cancer survivors

Protocol Version 1.0, 02/12/2013

Principal Investigator (Print name)   Signature   Date

Prof. Dr. Jenny Chang-Claude
Observational Study Lead   Signature   Date

Prof. Catharine West
REQUITE Co-ordinator   Signature   Date

I confirm that I have read and understood REQUITE protocol v1.0, dated 02/12/2013. I agree to work in accordance with the procedures laid out in this document and to the research principles that have their basis in ICH GCP. I will ensure that all staff working on REQUITE at my centre are aware of their responsibilities and are compliant with the study protocol.

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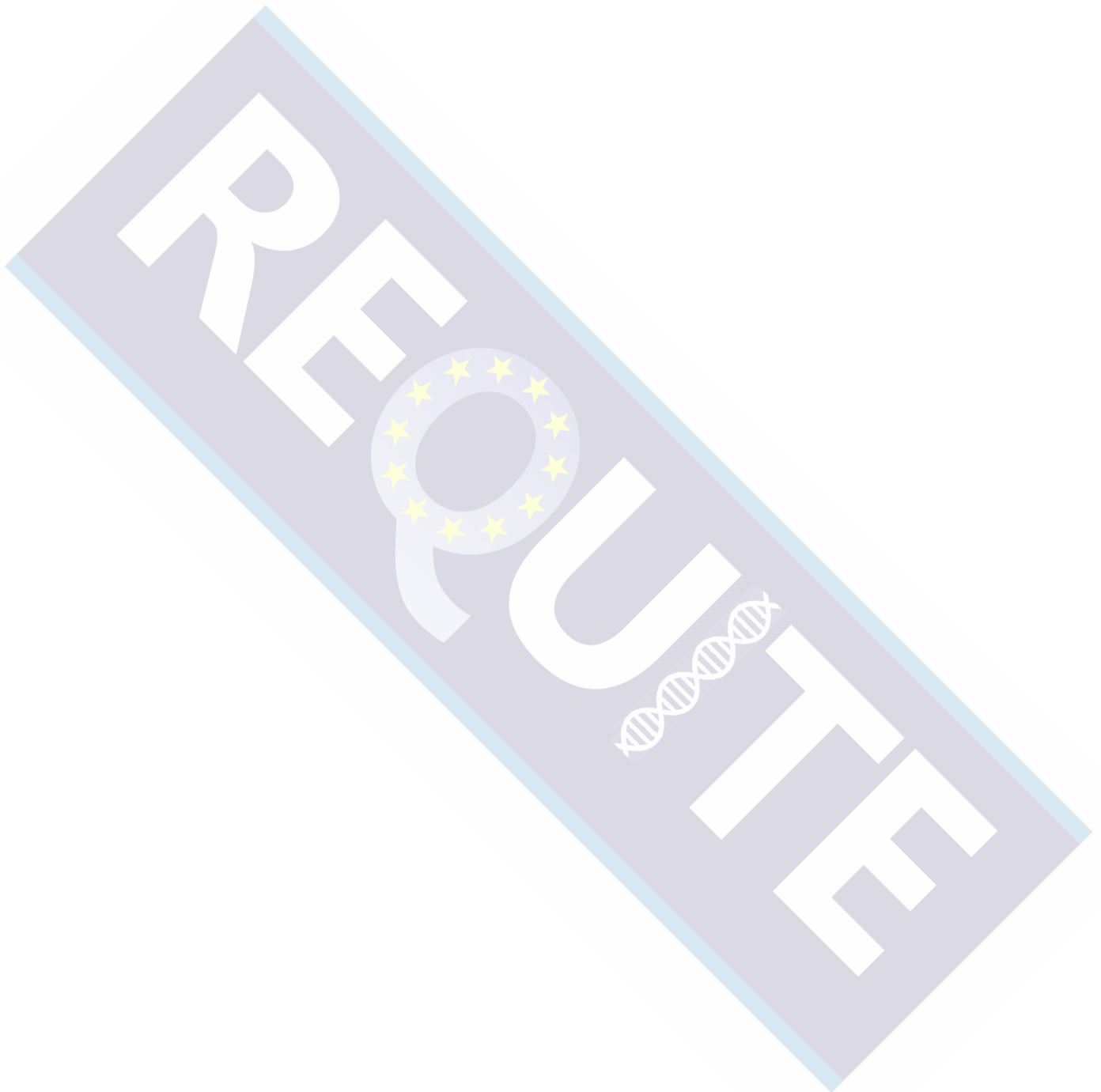Fax: +49 6221 422203
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ABBREVIATIONS

CIGMR	Centre for Integrated Genomic Medical Research
CNFT	The Christie NHS Foundation Trust, UK
CNV	Copy Number Variation
CRF	Case Record Form
CTCAE	Common Terminology Criteria for Adverse Events
DKFZ	German Cancer Research Center, Germany
FPGMX	Fundación Pública Galega de Medicina Xenómica, Spain
GCP	Good Clinical Practice
ICM	l'Institut régional du Cancer Montpellier, France
INT	Fondazione IRCCS Istituto Nazionale dei Tumori, Italy
KULEUVEN	Katholieke Universiteit Leuven, Belgium
LIMS	Laboratory Information Management System
MAASTRO	Maastricht Radiation Oncology, The Netherlands
MSSM	Icahn School of Medicine at Mount Sinai, USA
NTCP	Normal Tissue Complication Probability
PI	Principal Investigator
QoL	Quality of Life
SMG	Study Management Group
SNP	Single Nucleotide Polymorphism
STAT	Standardised Total Average Toxicity
UCAMB	University of Cambridge, UK
UGENT	Universiteit Gent, Belgium
ULEIC	University of Leicester, UK
UMM	Universitaetsmedizin Mannheim, part of University of Heidelberg, Germany
UMONT	University of Montpellier, France
UNIMAN	University of Manchester, UK

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1.0 STUDY SUMMARY

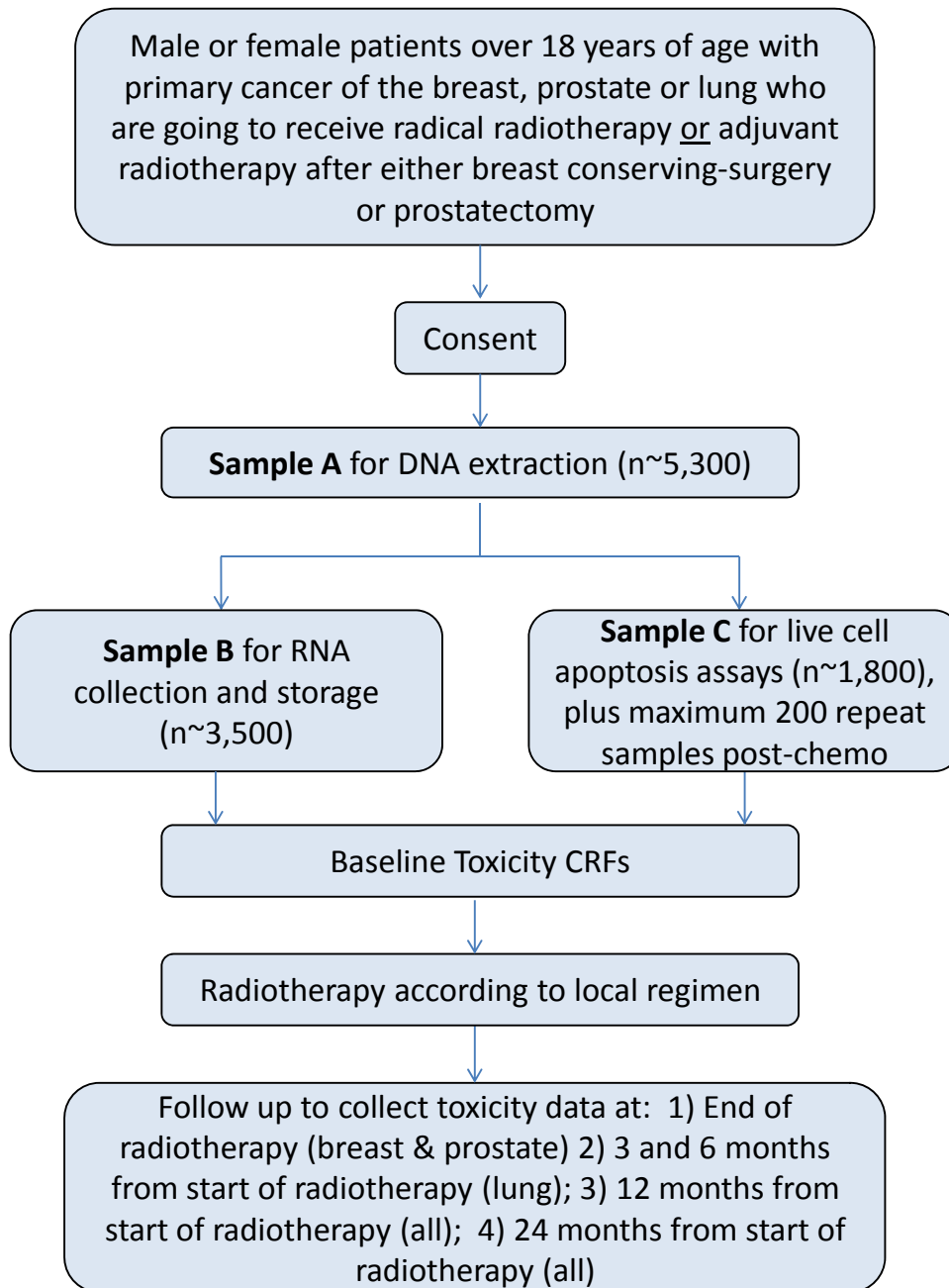|	
TITLE	REQUITE: Validating predictive models and biomarkers of radiotherapy toxicity to reduce side-effects and improve quality-of-life in cancer survivors
STUDY DESIGN	Observational/ cohort study
TARGET DISEASE	Cancer of the breast, prostate or lung
PRIMARY STUDY OBJECTIVE	To establish a prospective cohort of patients undergoing radiotherapy for breast, prostate or lung cancer according to local regimens and collecting standardised radiotherapy toxicity data, non-genetic risk factor data and samples for biomarker assays for the study of determinants of radiotherapy side-effects.
SECONDARY STUDY OBJECTIVES	To establish a comprehensive centralised database and sample collection as a resource for the prospective evaluation and validation of clinical models incorporating biomarker data to identify before treatment those cancer patients who are at risk of developing long term side-effects from radiotherapy.
PRIMARY ENDPOINTS	• Change in breast appearance at 24 months following start of radiotherapy (breast) • Rectal bleeding at 24 months following start of radiotherapy (prostate) • Dyspnea/ breathlessness at 12 months following start of radiotherapy (lung)
SECONDARY ENDPOINTS	• Other toxicity endpoints including but not limited to: fibrosis, induration and vascular changes (breast); rectal incontinence, urinary toxicity and erectile dysfunction (prostate); dysphagia and oesophagitis (lung) • Quality of life • Maximum grade of toxicity during follow-up period
STUDY POPULATION	Male or female patients over 18 years of age with primary cancer of the breast, prostate or lung who are going to receive planned radical radiotherapy or adjuvant radiotherapy after breast conserving surgery or prostatectomy.
RECRUITMENT TARGET	5,300 cancer patients over a 24 month accrual period

FOLLOW UP VISITS	Toxicity will be assessed and documented using REQUITE toxicity questionnaires based on the CTCAE v4.0 and EORTC Quality of Life at the following time points. Some site specific questionnaires will be used. • Baseline assessed prior to radiotherapy (all) • End of radiotherapy (breast and prostate); or first follow-up visit following implantation for prostate brachytherapy patients • 3 months from start of radiotherapy (lung) • 6 months from start of radiotherapy (lung) • 12 months from start of radiotherapy (all) • 24 months from start of radiotherapy (all) The follow-up period can be extended beyond 24 months. Further follow-up will be permissible and encouraged where possible as part of routine clinical care.
STUDY DURATION	• Minimum 24 months but actual follow-up period will depend on time of consent and duration of routine follow-up available at the recruiting centre (see follow-up visits) • 4 years overall (24 months recruitment period, 24 months minimum follow-up period)

SAMPLE COLLECTION	Pre-treatment blood samples will be collected for downstream analyses:
	
	• Sample A: One 10ml EDTA sample for DNA extraction to investigate genetic variation as a predictor of radiotherapy toxicity (n~5,300).
	
	• Depending on the recruiting site further samples can include:
	
	   ○ Sample B: A 2.5ml PAXgene sample for future RNA collection and storage (n~3,500)
	
	   ○ Sample C: A 10ml Lithium Heparin sample for live cell apoptosis assays (n~1,800). Repeat LiH samples will be collected from a sub-set of patients at University of Leicester only (n=200 max).

POWER CALCULATIONS	It is estimated that 2-year toxicity data (1-year for lung) will be available for 75% of enrolled patients. Based on effect sizes observed for genetic associations with radiation toxicity, a power calculation for the genetic assays shows that 1,575 patients for breast and prostate cancer has 80% power to detect a RR of >1.56 for at least grade 2 toxicity ($\alpha = 5 \times 10^{-5}$ for 1000 SNPs, allele freq = 0.25, toxicity rate = 20%), or with more stringent criteria a 90% power to detect a RR >1.66 ($\alpha = 1 \times 10^{-5}$, allele freq = 0.25, toxicity rate = 20%). For 825 patients with lung cancer the detectable RR would be 1.78 and 1.92.

1.1   Study Schema

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┌──────────────────────────────────────────────────┐
│  Male or female patients over 18 years of age with │
│  primary cancer of the breast, prostate or lung who│
│  are going to receive radical radiotherapy or      │
│  adjuvant radiotherapy after either breast         │
│  conserving-surgery or prostatectomy               │
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                  ┌───────────┐
                  │  Consent  │
                  └───────────┘
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        ┌──────────────────────────────────────┐
        │  Sample A for DNA extraction (n~5,300) │
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              ▼                          ▼
  ┌─────────────────────┐   ┌─────────────────────────────┐
  │  Sample B for RNA    │   │  Sample C for live cell       │
  │  collection and      │   │  apoptosis assays (n~1,800),  │
  │  storage             │   │  plus maximum 200 repeat      │
  │  (n~3,500)           │   │  samples post-chemo           │
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              ▼                          ▼
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        │          Baseline Toxicity CRFs         │
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        ┌──────────────────────────────────────┐
        │  Radiotherapy according to local regimen│
        └──────────────────────────────────────┘
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Follow up to collect toxicity data at: 1) End of radiotherapy (breast & prostate) 2) 3 and 6 months from start of radiotherapy (lung); 3) 12 months from start of radiotherapy (all); 4) 24 months from start of radiotherapy (all)

2.0   BACKGROUND

2.1   Cancer survivorship

In December 2011 the International Agency for Research on Cancer released its global cancer estimates for 2008[1]. The worldwide number of cancer survivors within five years of diagnosis was estimated to be ~28.7 million for 2008. Cancers of the breast (over 5 million survivors) and prostate (over 3 million survivors) had the highest prevalence in females (in 145 countries) and males (in 111 countries), respectively. Lung cancer has the highest overall prevalence globally and potentially curative treatment of lung cancer is increasing 2-year survival rates to ~75%[2].

With increasing life expectancies and improvements in diagnosis and treatment, the number of cancer patients and survivors is expected to continue to rise. As the illness increasingly becomes a chronic disease, cancer patients' quality-of-life needs to be addressed in a systematic manner in order to enhance their participation in society, including the workplace.

2.2   Long-term side-effects of radiotherapy impact on quality-of-life

Radiotherapy represents the most effective non-surgical modality in the curative treatment of cancer. Around a half of all cancer patients receive radiotherapy at some point in their treatment and nearly a half of all cancer survivors underwent radiotherapy as part of their care[3].

Yet, many patients receiving potentially curative radiotherapy will experience toxicity due to the unavoidable irradiation of surrounding healthy tissue. The toxicity varies in severity from minor to severe and in duration from weeks to a lifetime. Around 3-5% of people suffer with severe long-term side-effects but more experience moderate toxicity such as poor breast cosmetic outcome, incontinence, erectile dysfunction, chronic breathlessness, and chronic pain. Moderate toxicity, such as a poor cosmetic outcome following breast cancer treatment, can have a marked effect on subsequent psychological outcome[4]. The long-term side-effects of radiotherapy have been shown to impair quality-of-life in cancer survivors[5,6,7]. The ability to predict those patients likely to develop toxicity could potentially enable individual dose prescription to be made, which should improve survival and decrease morbidity.

2.3   Importance of genetics and biomarkers of radiosensitivity

Individual variation in toxicity was established from the early days of radiotherapy but it is only in the past 20 years that the role of genetics is widely recognised as being important. There is interest in measuring a patient's radiosensitivity to predict their likelihood of developing long-term

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[1] http://www.iarc.fr/en/media-centre/iarcnews/2011/globocan2008-prev.php
[2] Jeremić B, Miličić B, Milisavljević S. Radiotherapy Alone vs. Radiochemotherapy in Patients With Favorable Prognosis of Clinical Stage IIIA Non-Small-Cell Lung Cancer. Clin Lung Cancer 2013 epub Jan 4.
[3] Bentzen SM, Heeren G, Cottier B, et al. Towards evidence-based guidelines for radiotherapy infrastructure and staffing needs in Europe: the ESTRO QUARTS project. Radiother Oncol 2005;75:355-65
[4] Al-Ghazal SK, Fallowfield L, Blamey RW. Does cosmetic outcome from treatment of primary breast cancer influence psychosocial morbidity? Eur J Surg Oncol 1999;25:571-3.
[5] Barker CL, Routledge JA, Farnell DJ, Swindell R, Davidson SE. The impact of radiotherapy late effects on quality of life in gynaecological cancer patients.Br J Cancer 2009;100:1558-65.
[6] Sanda MG, Dunn RL, Michalski J et al. Quality of life and satisfaction with outcome among prostate-cancer survivors. N Engl J Med. 2008 Mar 20;358(12).
[7] Harden JK, Sanda MG, Wei JT et al Partners' long-term appraisal of their caregiving experience, marital satisfaction, sexual satisfaction, and quality of life 2 years after prostate cancer treatment. Cancer Nurs. 2013 Mar-Apr;36(2)

side-effects following radiotherapy. The first report that an individual with ataxia telangiectasia suffered severe toxicity following radiotherapy showed that fibroblasts cultured from a skin sample were approximately three times more radiosensitive than cells from normal individuals. A number of subsequent reports showed that individuals with severe life-threatening radiotherapy toxicity had radiosensitive fibroblasts. These severe reactors are extremely rare and up until the 1980s it was thought that radiosensitivity varied little between most individuals. However, during the 1980s there was increasing evidence for a spectrum of radiosensitivity within the population. This evidence led to the initiation of studies measuring the radiosensitivity of cells from cancer patients to investigate whether it was possible to predict their risk of developing side-effects.

There is interest in using rapid cell based functional assays for measuring radiosensitivity. Of particular relevance is a flow cytometry method for assessing radiation-induced apoptosis. High toxicity following radiotherapy has been linked with low levels of radiation-induced apoptosis in several independent studies. A number of assays were studied in the past and the apoptosis assay stands out because the findings have been replicated in several independent laboratories[8,9,10,11,12,13,14]. The apoptosis assay provides an assessment of the radiosensitivity of a patient at the time of radiotherapy, i.e. incorporating both genetic and epigenetic effects. This work will establish whether the apoptosis assay can be validated within a prospective multi-centre study using a standardised protocol. To do this, the assay will be standardised and quality assurance and control methods set up for cross laboratory analyses. The latter are required for clinical testing. If the assay can be neither standardised nor validated, then definitive evidence will be obtained that further studies using the assay are not warranted.

There is now good evidence for the heritability of radiosensitivity as a trait and growing interest in identifying the genetic variants associated with increased sensitivity to radiation[15]. Most studies in this area attempt to identify the SNPs (i.e. common genetic variation) associated with radiosensitivity and there are a large number of candidate genes that have been explored[53]. As for other traits and diseases, radiosensitivity is considered to be an inherited trait that is determined by common variation in a large number of genes each conferring small effects on the phenotype. Rare mutations in several DNA damage response genes (e.g. *ATM*) are known to confer large effects but these are associated with genetic syndromes and their rarity means they have limited use for the general population who undergo radiotherapy. Genetic association studies provided evidence linking SNPs in various candidate genes with radiotherapy side-effects,

[8]Crompton NE, Miralbell R, et al, Ozsahin M. Altered apoptotic profiles in irradiated patients with increased toxicity. Int J Radiat Oncol Biol Phys 1999;45:707-14.
[9]Barber JB, West CM, Kiltie AE, et al. Detection of individual differences in radiation-induced apoptosis of peripheral blood lymphocytes in normal individuals, ataxia telangiectasia homozygotes and heterozygotes, and breast cancer patients after radiotherapy. Radiat Res 2000;153:570-8.
[10]Crompton NE, Shi YQ, et al, Ozsahin M. Sources of variation in patient response to radiation treatment. Int J Radiat Oncol Biol Phys 2001;49:547-54.
[11]Ozsahin M, Crompton NE, et al, Azria D. CD4 and CD8 T-lymphocyte apoptosis can predict radiation-induced late toxicity: a prospective study in 399 patients. Clin Cancer Res 2005;11:7426-33.
[12]Schnarr K, Boreham D, Sathya J, Julian J, Dayes IS. Radiation-induced lymphocyte apoptosis to predict radiation therapy late toxicity in prostate cancer patients. Int J Radiat Oncol Biol Phys 2009;74:1424-30.
[13]Bordón E, Henríquez-Hernández LA, Lara PC, et al. Prediction of clinical toxicity in locally advanced head and neck cancer patients by radio-induced apoptosis in peripheral blood lymphocytes (PBLs). Radiat Oncol 2010;5:4
[14]Bordón E, Henríquez-Hernández LA, Lara PC, et al. Role of CD4 and CD8 T-lymphocytes, B-lymphocytes and Natural Killer cells in the prediction of radiation-induced late toxicity in cervical cancer patients. Int J Radiat Biol 2011;87:424-31.
[15]West CM, Barnett GC. Genetics and genomics of radiotherapy toxicity: towards prediction. Genome Med 2011;3:52.

but the associations have mainly not been replicated[16,17]. This situation is being resolved through meta-analyses organised through the Radiogenomics Consortium. These are excluding some genes such as *TGFB1* in breast cancer patients[18], and providing evidence for others e.g. *TNF*[19]. Several genome wide association studies (GWAS), underway or about to be published, are starting to provide a growing list of SNPs with evidence for predicting radiotherapy side-effects in different cancers[20,21].

Emerging evidence suggests that the SNPs will need targeting for specific endpoints. Several studies highlighted how information from SNP profiling can be incorporated into clinical models being explored to predict risk of radiotherapy toxicity[22,23]. With the increasing international co-operation, recognition for the need to collect harmonised data and acceptance of the importance of validation, the time is right for a project that validates the clinical models, alone and incorporating biomarker data.

REQUITE provides the international cooperation, data and samples to validate models in several cancers, prior to evaluation in interventional clinical trials. It is recognised that work in other fields highlights the challenges in linking genotype with phenotype, but the REQUITE focus on validation rather than discovery will enable definitive findings regarding the importance of individual genetic variants that are starting to emerge from GWAS meta-analyses.

2.4   Rationale for the study

In recent years predictive models have been developed that attempt to identify before the start of treatment patients at risk of long-term side-effects. These emerging models require systematic validation in a multi-centre collaborative setting. There are an increasing number of datasets available for validation but they are variable in terms of the data collected.

This multi-centre observational study will be the largest study of its kind collecting blood samples and standardised data longitudinally from 5,300 cancer patients. It will enable validation of models that predict a patient's risk of developing long-term side-effects following radiotherapy. It will be a unique (eventually widely accessible) resource for studying the relationships between side-effect endpoints and between side-effects and quality-of-life. It is known that genetics influence a patient's risk of developing side-effects and a number of assays/approaches have been explored to assess a patient's sensitivity to radiation. This prospective observational study will allow for the validation of the most promising biomarkers/approaches.

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[16]Barnett GC, Coles CE, Elliott RM, et al, Bentzen SM, Dearnaley DP, Burnet NG, Pharoah PD, Dunning AM, West CM. Independent validation of genes and polymorphisms reported to be associated with radiation toxicity: a prospective analysis study. Lancet Oncol 2012;13:65-77.

[17] Fachal L, Gómez-Caamaño A, Sánchez-García M, Carballo A, Peleteiro P, Lobato-Busto R, Carracedo A, Vega A. TGFβ1 SNPs and radio-induced toxicity in prostate cancer patients. Radiother Oncol 2012;103:206-9.

[18]Barnett GC, Elliott RM, Alsner J, et al. Individual patient data meta-analysis shows no association between the SNP rs1800469 in TGFB and late radiotherapy toxicity. Radiother Oncol 2012;105:289-95.

[19]Talbot CJ, Tanteles GA, Barnett GC, et al. A replicated association between polymorphisms near TNFα and risk for adverse reactions to radiotherapy. Br J Cancer 2012;107:748-53.

[20]Kerns SL, Ostrer H, et al, Rosenstein BS. Genome-wide association study to identify single nucleotide polymorphisms (SNPs) associated with the development of erectile dysfunction in African-American men after radiotherapy for prostate cancer. Int J Radiat Oncol Biol Phys 2010;78:1292-300.

[21]Kerns SL, et al, Ostrer H, Rosenstein BS. A 2-stage genome-wide association study to identify single nucleotide polymorphisms associated with development of erectile dysfunction following radiation therapy for prostate cancer. Int J Radiat Oncol Biol Phys 2013;85:e21-8.

[22]De Ruyck K, Sabbe N, Oberije C, Vandecasteele K, Thas O, De Ruysscher D, Lambin P, Van Meerbeeck J, De Neve W, Thierens H. Development of a multicomponent prediction model for acute esophagitis in lung cancer patients receiving chemoradiotherapy. Int J Radiat Oncol Biol Phys 2011;81:537-44.

[23]Tucker SL, Li M, Xu T, et al. Incorporating Single-nucleotide Polymorphisms Into the Lyman Model to Improve Prediction of Radiation Pneumonitis. Int J Radiat Oncol Biol Phys 2013;85:251-7.

3.0   STUDY OBJECTIVES

3.1   Overall Design

This is an international observational cohort study. Eligible patients will have cancer of the breast (invasive or *in situ*), prostate or lung and be due to receive radical radiotherapy or adjuvant radiotherapy after breast conserving surgery or prostatectomy. Patients will be recruited from participating outpatient oncology clinics via cancer centres in multiple countries including Belgium, France, Germany, Italy, Spain, The Netherlands, UK and USA. Data on radiotherapy toxicity, non-genetic risk factors (e.g. dosimetry, chemotherapy use, age, diabetes, smoking history, co-morbidity) and quality of life will be collected at specified time points prospectively. Pre-treatment blood samples will be collected from all patients for downstream analyses. Patients will be required to have understood information about the study and given written informed consent.

3.2   Primary/ Secondary Objectives

Primary Objective

To establish a prospective cohort of patients undergoing radiotherapy for breast, prostate or lung cancers following local regimens and collecting standardised radiotherapy toxicity data, non genetic risk factor data and samples for biomarker assays for the study of determinants of radiotherapy side-effects.

Secondary Objective

To establish a comprehensive centralised database and sample collection as a resource for the prospective evaluation and validation of clinical models incorporating biomarker data to identify before treatment those cancer patients who are at risk of developing long term side-effects from radiotherapy.

3.3   Study Endpoints

Given the multiple endpoints of toxicity, REQUITE will focus on those that are considered most specific for radiotherapy, i.e. those that show a radiation dose response. In breast cancer, changes in breast appearance show a dose response relationship[24]. In prostate cancer survivors, dose response relationships have been shown for bleeding and faecal incontinence[25]. In lung cancer, dose and dose volume effects are strongly related to symptomatic late radiation toxicity[26]. For example, V20 (the percentage of lung receiving >20 Gy) is considered a good predictor of lung toxicity[27].

Toxicity can be progressive and continue to develop many years following radiotherapy. There is evidence that moderate toxicity at early time points predicts for severe toxicity later. It is agreed

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[24]Yarnold J, Ashton A, Bliss J, et al. Fractionation sensitivity and dose response of late adverse effects in the breast after radiotherapy for early breast cancer: long-term results of a randomised trial. Radiother Oncol 2005;75:9-17.
[25]Fellin G, Fiorino C, et al, Valdagni R. Clinical and dosimetric predictors of late rectal toxicity after conformal radiation for localized prostate cancer: results of a large multicenter observational study. Radiother Oncol 2009;93:197-202.
[26]Marks LB, Bentzen SM, Deasy JO, et al. Radiation dose-volume effects in the lung. Int J Radiat Oncol Biol Phys 2010;76:S70-6.
[27]Graham MV, Purdy JA, Emami B, et al. Clinical dose-volume histogram analysis for pneumonitis after 3D treatment for non-small cell lung cancer (NSCLC). Int J Radiat Oncol Biol Phys 1999;45:323-9.

that assessing toxicity at two years is ample time to express a significant proportion of late-effects[28]. Using a two-year time point minimises loss to follow-up but ensures sufficient time for late-effects to emerge. However, in lung cancer patients, late toxicity develops six months following the start of treatment and, as patient survival is lower than for cancers of the prostate or breast, one-year toxicity is a more appropriate time point.

Primary Endpoints

- Breast: Any change in breast appearance at 24 months following start of radiotherapy

- Prostate: Rectal bleeding at 24 months following start of radiotherapy

- Lung: Dyspnea/ breathlessness at 12 months following start of radiotherapy

Secondary Endpoints

- Other toxicity endpoints including but not limited to: fibrosis, induration and vascular changes (breast); rectal incontinence, urinary toxicity and erectile dysfunction (prostate); dysphagia, oesophagitis (lung)

- Quality of Life (QoL)

- Maximum grade of toxicity during follow-up period

3.4   Data Collection

REQUITE will have a centralised, online database for recording physician and patient-reported toxicity data, as well as treatment, physics, co-morbidity, epidemiologic, and quality of life data (see Section 6.3). Completion of online questionnaires by patients in the clinic (PC or tablet) is the preferred method of data capture. However, paper questionnaires will be available for patients for collecting information on patient-reported toxicity, personal characteristics and QoL as required. All data completed using paper questionnaires must be entered into the electronic database by local study centre personnel. Health care professionals will complete online case record forms (CRFs) entering data directly into the REQUITE database.

Treatment details and patient characteristics

To maximise the ability to validate clinical models predicting toxicity, information must be collected on a number of variables including but not limited to: radiation doses received by surrounding normal tissues and the volumes irradiated, age, weight, height, smoking history (past/current smoker, intensity, duration, time since quitting), co-morbidity (e.g. diabetes, collagen vascular disease, diverticulosis, haemorrhoids, lung diseases), menopausal status (women), other treatments received (chemotherapy, anti-hormonal therapy, trastuzumab etc.) and tumour type relevant characteristics (e.g. histological type, TNM, ER/PR, HER2, grade etc.).

Toxicity data

Based on the Common Terminology Criteria for Adverse Events (CTCAE v4.0), toxicity questionnaires have been developed for both healthcare professionals and patients to assist with

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[28]Trotti A, Bentzen SM. The need for adverse effects reporting standards in oncology clinical trials. J Clin Oncol 2004;22:19-22.

the collection, recording and managing of radiotherapy late effects[29]. Side-effects are often under reported, so the inclusion of patient reported outcome measures that are both sensitive and reliable will help to improve on data capture[30]. Information on acute toxicity will be collected according to standard clinical practice.

Quality of Life data

The EORTC QLQ-C30 questionnaire assesses the quality-of-life of cancer patients[31]. It has been translated and validated into 81 languages and is used in more than 3,000 studies worldwide. Other tumour specific questionnaires including but not limited to the breast EORTC BR23 module will be collected. Additional information on cancer-related fatigue will be collected using the Multidimensional Fatigue Inventory (MFI) [32] and, optionally, data on physical activity will be collected using the Global Physical Activity Questionnaire[33]. Both of these validated questionnaires are available in multiple languages.

3.5   Sample Collection

REQUITE will develop a centralised, accessible biobank linked to the observational study data. The Centre for Integrated Genomic Medical Research (CIGMR) will manage the biobank and co-ordinate with collection centres to organise shipping of frozen whole blood EDTA samples (Sample A) at regular intervals to Manchester, UK. This will ensure uniformity of storage prior to standardised DNA extraction. A second blood sample collected at the same time point will either be stored locally for RNA extraction in future gene expression studies (Sample B), or be used for immediate analysis in the live cell apoptosis assay (Sample C). See Section 6.2.

The CIGMR facility has multiple safeguards in place to maintain the integrity of frozen samples, including freezers monitored 24 hours a day, back-up power generation and ample spare freezer capacity for transferrals. All laboratory, data and management processes within the ISO9001:2008 certified biobank are fully ISO compliant and all processes carried out outside the biobank will seek to adhere to the ISO Quality Policy. REQUITE DNA samples will be held at two separate sites to mitigate against catastrophic loss.

3.6   Study Duration

Patients will remain on standard of care active treatment and will then be followed up for a minimum of 24 months. Follow-up beyond 24 months is encouraged where possible as part of routine clinical care. The study is expected to complete accrual within two years.

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[29]Farnell DJ, et al, West CM, Davidson SE. Development of a patient-reported questionnaire for collecting toxicity data following prostate brachytherapy. Radiother Oncol 2010;97:136-42.

[30]Ho KF, Farnell DJ, Routledge JA, Burns MP, Sykes AJ, Slevin NJ, Davidson SE. Comparison of patient-reported late treatment toxicity (LENT-SOMA) with quality of life (EORTC QLQ-C30 and QLQ-H&N35) assessment after head and neck radiotherapy. Radiother Oncol 2010;97:270-5.

[31]Aaronson NK, Ahmedzai S, Bergman B, Bullinger M, Cull A, Duez NJ, Filiberti A, Flechtner H, Fleishman SB, de Haes JC, et al. The European Organization for Research and Treatment of Cancer QLQ-C30: a quality-of-life instrument for use in international clinical trials in oncology. J Natl Cancer Inst. 1993 Mar 3;85(5):365-76.

[32]Smits et al. The multidimensional fatigue inventory (MFI) psychometric qualities of an instrument to assess fatigue. Journal of Psychosomatic Research 1995;39(5):315-25.

[33]Bull et al. Global physical activity questionnaire (GPAQ): nine country reliability and validity study. J Phys Act Health 2009;6(6):790-804.

4.0   SELECTION OF STUDY PATIENTS

4.1   Inclusion Criteria

- Patients suitable for adjuvant radiotherapy* for cancer of the breast (invasive or *in situ*) including breast patients receiving neo-adjuvant chemotherapy
- Patients suitable for radical radiotherapy or brachytherapy for prostate cancer; including post-prostatectomy patients
- Patients suitable for radical radiotherapy, sequential or concurrent chemoradiotherapy or stereotactic body radiation therapy for lung cancer
- No other malignancy prior to treatment for the specified tumour types except basal cell or squamous cell carcinoma of the skin
- No evidence of distant metastases
- Patients able to provide a venous blood sample
- Willingness and ability to comply with scheduled visits, treatment plans and available for follow up within country of origin
- Greater than 18 years of age; no upper age limit
- The capacity to understand the patient information sheet and the ability to provide written informed consent

*Breast patients receiving chemotherapy should have completed their course of chemotherapy (anthracyclines) at least one month prior to radiotherapy commencing.

4.2   Exclusion Criteria

- Patients with metastatic disease
- Prior irradiation at the same site
- Planned use of protons
- Breast patients receiving concomitant chemo-radiation
- Male breast cancer patients
- Mastectomy patients
- Bilateral breast cancer
- Small cell lung cancer
- Mental disability or patient otherwise unable to give informed consent and/or complete patient questionnaires
- Limited life expectancy due to co-morbidity
- Pregnant patients
- Partial breast irradiation
- Patients with breast implants if not removed during surgery

5.0   RECRUITMENT OF PATIENTS

5.1   Number of patients required

A total of 5,300 patients are to be recruited. Approximate breakdown: 2,100 breast patients; 2,100 prostate patients and 1,100 lung patients.

5.2   Identifying patients

Eligible patients will be identified in the respective multi-disciplinary meetings and out-patient clinics (women's clinics, urology and radio-oncology departments) at participating sites. A screening and recruitment log will be kept at each site detailing all patients who were considered for the study identifying which patients did not fulfill the eligibility criteria or declined to take part, and also those who consented to the study.

As REQUITE does not interfere with any standard or experimental, diagnostic or therapeutic procedures, patients may be included in REQUITE while participating in any other study/ trial.

The clinical leads representing the recruiting study centres have previously been involved in clinical trials and/or radiogenomics studies. Therefore, the logistics of patient recruitment already established at the participating clinics will be used for this study. Multiple clinics per recruitment centre will be involved, where necessary, to ensure adequate participant enrollment to reach the target sample size.

5.3   Consenting patients

Patients deemed eligible for entry into REQUITE will be provided with a verbal and written explanation of the study in accordance with local governance and regulatory departments. After adequate time has been given, all queries have been addressed and the clinical team is confident that the patient understands the study and the necessity for long-term follow-up, patients will be consented onto the study.

Consent will be taken by a member of the study team who is GCP trained, suitably qualified and experienced and who has been delegated by the Principal Investigator to undertake this activity (and this delegation is clearly documented on the delegation log).

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- Consent will be specifically sought for the following:
    - use of blood sample and/or DNA in molecular-genetic research
    - use of blood sample and/or DNA in future genomic studies
    - potential commercialisation

6.0   STUDY METHODOLOGY

6.1   Patient Registration

Issue of each patient's unique study identifier will be done by allocation of the next sequential REQUITE kit (provided by CIGMR). Each kit will contain a number of bar-coded labels displaying the unique study identifier for that patient, as well as a bar-coded BD Vacutainer EDTA blood

tube. The labels can be affixed to other blood tubes, consent form or patient notes as appropriate (see below).

Following signing of the consent form, the 'Consent' bar-code label from the next sequential REQUITE kit should be affixed to the consent form, and scanned into the appropriate table on the REQUITE centralised database. This will formally register the patient on the REQUITE study and represent their primary entry in the REQUITE database. The enrolment form confirming patient eligibility for the study should also be completed at this time.

6.2   Sample Collection and Tracking

Patients who are deemed eligible for the study following consent and all screening evaluations will be asked to gift pre-treatment blood samples that comprise 'Sample A' (10ml) and at least one of 'Sample B' (2.5ml) or 'Sample C' (10ml). A limited number of patients may be asked to give an additional blood sample to enable repeat analyses of the apoptosis assay for quality control purposes.

CIGMR will print and distribute all the bar-codes to be used in this study in individual REQUITE kits. Each bar-code label will display the following machine readable and human readable information:

- Human readable unique study identifier
- 1D barcode representing the unique study identifier
- Recruiting centre
- Sample type (EDTA blood, PAXgene or Lithium Heparin)

CIGMR will send out batches of individual REQUITE kits to each study centre. Each kit contains a bar-coded EDTA BD Vacutainer blood tube representing 'Sample A' in the REQUITE study. Depending on the recruiting site (see below) CIGMR will also provide additional bar-coded labels to be used on a PAXgene tube (Sample B) or a Lithium Heparin tube (Sample C). These labels should be affixed at site to the appropriate tube and blood collected at the appropriate time point (see below).

Sample A

- All patients in REQUITE (n ~5,300):

  One 10ml EDTA blood sample will be collected either:

    1) Prior to the start of radiotherapy for lung cancer patients;

    2) Prior to the start of radiotherapy for prostate cancer patients;

    3) Prior to the start of chemotherapy or radiotherapy for breast cancer patients*.

  * The timing for collection of the blood sample from breast cancer patients is dependent upon the recruiting site (see below).

  All EDTA bloods will be stored locally at -80°C as whole blood, no further processing is required. Following blood collection, study centres will scan the bar-code to complete a sample tracking form on the REQUITE centralised database, which can be accessed by

CIGMR. This will provide accurate and consistent tracking of samples at study centres and will be used to update the CIGMR Laboratory Information Management System (LIMS).

CIGMR will liaise with each of the study centres regarding the return of frozen EDTA blood samples by courier at regular intervals e.g. every six months or as required. CIGMR will log and receipt the frozen blood samples immediately raising any discrepancies with the originating centre. At an appropriate time point they will process the blood samples to extract DNA; perform both a quality assessment and DNA quantification using Nanodrop technology and dilute to a standard concentration of at least one aliquot and store the undiluted and diluted DNA at two different geographical sites to guard against catastrophic loss prior to transfer to Source Bioscience for genotyping (see Section 6.6.1)

CIGMR will ensure continuous maintenance and management of their storage facility to ensure all equipment is fit for purpose and safe to use. As a certified biobank, they will ensure all laboratory, data and management processes are fully compliant with ISO9001:2008.

Sample B

- Patients recruited at CNFT, FPGMX, UGENT, MSSM, INT, KULEUVEN, MAASTRO or third parties associated with these centres (n ~3,500).

One 10ml PAXgene tube will be labelled with the corresponding PAXgene bar-code clearly displaying the same study identifier as used previously for the EDTA sample (Sample A). This PAXgene tube is pre-loaded with 7.5ml of RNA stabiliser and 2.5ml of blood will be drawn from the patient. Bloods will be collected at the same time as 'Sample A' either:

1) Prior to the start of radiotherapy for breast cancer or lung cancer patients;

2) Prior to the start of radiotherapy for prostate cancer patients.

This PAXgene blood sample will be stored locally at -80°C as whole blood for future RNA collection and extraction. Note, the samples for RNA analysis will not be used further in REQUITE, but will be a resource for future exploitation in biomarker discovery projects or similar.

Sample C

- Patients recruited at UMM, ULEIC, ICM or third parties associated with these centres (n ~1,800)

One 10ml Lithium Heparin (LiH) blood tube will be labelled with the corresponding LiH bar-code clearly displaying the same study identifier as used previously for the EDTA sample (Sample A). The LiH blood sample should be collected at the same time as 'Sample A' either:

1) Prior to the start of chemotherapy and radiotherapy for lung patients;

2) Prior to the start of radiotherapy for prostate cancer patients;

3) Prior to the start of chemotherapy and radiotherapy for breast cancer patients.

This sample will be retained in the recruitment centre for immediate analysis in the live cell apoptosis assay work. Each centre will make local arrangements for the transport of samples from the clinical facilities to the analysis laboratory, and temporary storage at 4°C (see Section 6.6.2).

Repeat Sample C

- Patients recruited at ULEIC or third parties associated with this centre (n = 200 maximum)

An additional 10ml Lithium Heparin (LiH) blood tube will be labelled with a duplicate LiH barcode displaying the same study identifier as used previously. This LiH blood sample will be collected:

1) Prior to the start of radiotherapy from breast cancer patients who previously received chemotherapy.

This sample will be retained in the recruitment city for immediate analysis in the live cell apoptosis assay work. It will represent a repeat of 'Sample C' collected at an earlier time point and will be used for the live cell apoptosis assay work to allow researchers to compare differences between samples collected pre-chemotherapy and post-chemotherapy. Local arrangements will be made for the transport of samples from the clinical facility to the analysis laboratory, and temporary storage at 4°C (see Section 6.6.2).

Unused material
Unused material derived from samples A-C will be processed according to a common protocol followed by all participating sites. Subject to approval from the REQUITE management team this unused material specifically DNA, RNA, lymphocytes and plasma will be stored and made available for collaborators to use in externally funded and ethically approved projects (e.g. gene expression or epigenetic marker studies).

6.3   Data Collection

The primary method of data capture in REQUITE is the centralised online database. An online web browser will be available for patients to complete their questionnaires whilst in clinic, or alternatively at home if required. Where patients choose to use a paper based questionnaire then manual data entry must be performed by study centre personnel. Healthcare professionals will also have password protected access for completion of CRFs and file uploads (breast photos and physics data) as detailed below.

Case Record Forms (CRFs)

- Enrolment form (eligibility criteria)
- Blood collection form
- Patient factors form (epidemiology)
- Clinical and treatment data collection form
- Health professional toxicity data form (tumour specific; based on CTCAE v4.0)
- Patient reported outcomes questionnaire (tumour specific; based on CTCAE v4.0)
- EORTC Core 30 Quality of Life questionnaire (C30)
- Tumour specific questionnaires including but not limited to BR23
- Multidimensional Fatigue Inventory (MFI)
- Physical activity assessment (GPAQ)
- Withdrawal form

- Outcome data form

Time points for CRF collection

CRFs should be completed at routine clinic appointments specifically including:

- Baseline (prior to start of radiotherapy or within first five days of radiotherapy)
- End of radiotherapy (1-2 days prior to or the last day of treatment) for breast and prostate patients. For brachytherapy patients: at the first routine follow-up visit following implantation.
- 3 months from start of radiotherapy (+/- 4 weeks) for lung cancer patients
- 6 months from start of radiotherapy (+/- 4 weeks) for lung cancer patients
- 12 months from start of radiotherapy (+/- 4 weeks)
- 24 months from start of radiotherapy (+/- 4 weeks)

See schedule of assessments for timings of completion of each CRF for each tumour site (Section 12.0).

Physics File Uploads

Uploading of physics data and CT images to the REQUITE database can be completed at any point in the follow-up pathway prior to the observational study end. All personal data should be removed before files are uploaded. Only the unique study identifier should be used.

- DICOM-RT files
- CT images and contouring
- DVH data

Digital Photographs (breast cancer patients only)

Photographs should be of sufficient quality to show any skin changes (including telangiectasia), breast shrinkage or retraction. Photographs should include both breasts to compare any change between the irradiated and unirradiated breast. Photographs must exclude the head.

Two anterior views of the chest are required, one with hands on the hips and the other with hands raised as far as possible above the head. One lateral view of the chest with hands above the head is also required.

Study centres are advised to follow the guidelines detailed in the REQUITE 'Photographic Assessment Form' (Form B4)' for guidance on taking breast photographs for inclusion in this study. This will minimise inter-centre and intra-centre variation in photographic conditions.

Time points for digital photographs (breast only)

- Baseline (prior to start of radiotherapy or within first five days of radiotherapy)
- 24 months from start of radiotherapy (+/- 4 weeks)

6.4   Data Handling

All data entered into the REQUITE database by patients and healthcare professionals will be subject to an automatic comprehensive validation check program to identify missing, illogical and/ or inconsistent data before submission can be completed. In addition, the REQUITE data manager will assess data regularly and review any questionable data and correct any data entry errors with the help of the appropriate healthcare professionals.

The exact procedures for data entry and data clarification, including contact details for any related queries will be described in the study specific SOPs and instructions that will be sent to all REQUITE study centres as soon as they have completed the site initiation process. Reminders for any overdue data will also be sent out as necessary.

6.5   Radiotherapy

REQUITE is not a trial of radiotherapy, therefore the radiation dose and regimen is not prescribed. However, for breast patients receiving neo-adjuvant chemotherapy, it is important that radiotherapy should not commence until at least one month has elapsed following the end of chemotherapy.

All radiotherapy regimens will follow local standard of care decided by the treating clinician. Treatment details will be documented on the appropriate REQUITE CRF.

For lung cancer patients where possible, the heart should be outlined along with the pericardial sac. The pericardial sac surrounds the heart and extends superiorly to encompass the main pulmonary artery, the ascending aorta and the superior vena cava. Outlining should extend superiorly to the inferior limit of the aortic arch (the aortopulmonary window) and the superior limit of the trunk of the pulmonary artery if it can be identified on the radiotherapy planning CT scan.

For breast cancer patients, where possible, the following guidelines should be followed for heart delineation. Superiorly the heart starts just inferior to the left pulmonary artery. It includes the atria, ventricles, auricles, vessels and fat tissue within the pericardium. Since the cardiac vessels run in the fatty tissue within the pericardium, they should be included in the contours, even if there is no heart muscle visible in that area. Inferiorly, the heart blends with the diaphragm[32]. For breast delineation, a wire should be used on the CT scan around the palpable breast tissue to define the peripheral edges of the breast. The deep edge is the superficial side of the pectoral muscle/thoracic wall. The superficial edge is the skin. Any visible glandular breast tissue outside these margins should also be included.

For prostate cancer patients, details of the rectum and bladder delineation should be specified on form P3, stating whether the entire rectum is delineated from anus to sigmoid; or alternatively, 2 cm cranial and caudal of the PTV; either as solid or hollow organ. For bladder delineation, it should be specified whether the entire bladder is delineated or the posterior wall only. Additionally, the filling level of the bladder should be stated.

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[32] Feng, M, Moran, JM, Koelling, T, et al. Development and Validation of a Heart Atlas To Study Cardiac Exposure to Radiation Following Treatment for Breast Cancer. Int. J. Radiat. Oncol. Biol. Phys. 2011;79.

6.6   Biomarker Assays

6.6.1 Genotyping

Genotyping will be carried out by commercial partner Source Bioscience in their facilities in Eire, UK or Germany.

The selection of genetic markers to be typed will be made by University of Cambridge in association with the University of Leicester and other members of REQUITE. The published literature will be evaluated for high quality genetic studies showing strong evidence of association with one of the clinical radiation toxicity endpoints. These markers will be collated into a single list such that patients for each cancer are typed for all markers. This maximises the chance of validating the associations whether they affect a single or multiple clinical endpoints.
Genotyping will be performed with the best available technology at the time, currently Fluidigm or GoldenGate custom arrays. CNVs would be typed either with a SNP genotyping platform or a qPCR based approach. A genome-wide approach may also be feasible, either by arrays or sequencing. If whole genome data are collected the information from the chosen markers will be extracted for the predictive markers, with the whole data being included in a separate association analysis. Control samples will be repeat typed in each laboratory to ensure reproducibility between sites.

Genotyping Quality Assurance

Quality assurance measures are already in place to produce negative controls on each experimental plate, and 5% of the samples from each plate will be duplicated on another plate as a reproducibility check. The genotype assay will be optimised on a panel of 80 unique DNA samples plus 12 duplicates and 4 negative controls. Duplicated samples must give greater than 99% concordance before the assay is accepted. These procedures are currently part of laboratory procedure.

6.6.2 Live cell apoptosis assays

The samples will be analysed at three centres: University of Leicester, UK; University of Heidelberg, Germany and University of Montpellier, France. Each centre will follow the current REQUITE standard protocol for 'Radiation Induced Apoptosis Assays' to reduce variability between centres. As a quality assurance measure for reproducibility both inter-lab and intra-lab variation will be assessed. Completion of the apoptosis assays will occur in Year 3 when patient recruitment closes.

7.0   STATISTICAL PLANS AND DATA ANALYSIS

7.1   Power calculations

Live Cell Assays

For the apoptosis assays with 800 breast, 600 prostate and 400 lung patient samples there is 80% power to detect relative risks (RR) of >1.31, 1.36 and 1.44 respectively, (α = 0.05, low apoptosis freq = 0.39, toxicity rate = 20%), or a 95% power to detect RR of >1.40, 1.47 and 1.57.

Genotyping

It is estimated that 2-year toxicity data (1-year for lung) will be available for 75% of enrolled patients. Based on effect sizes observed for genetic associations with radiation toxicity, a power calculation for the genetic assays shows that 1,575 patients for breast and prostate cancer each has 80% power to detect a RR of >1.56 for at least grade 2 toxicity ($\alpha = 5 \times 10^{-5}$ for 1000 SNPs, allele freq = 0.25, toxicity rate = 20%), or with more stringent criteria a 90% power to detect a RR >1.66 ($\alpha = 1 \times 10^{-5}$, allele freq = 0.25, toxicity rate = 20%). For 825 patients with lung cancer the detectable RR would be 1.78 and 1.92.

7.2 Statistical Data Analysis

In a first stage the data from the REQUITE multi-centre observational study will be used to validate published statistical models that use clinical and biomarker data to predict a patient's risk of long-term side-effects following radiotherapy in patients with prostate, breast or lung cancer. Variables considered will be dose-volume data of organs and tissues at risk, dose fractions and overall treatment time, patient-related cofactors (e.g. age, weight, diabetes, and smoking), use of concurrent treatment (e.g. chemotherapy) and genotyping data. If necessary, individual clinical toxicity endpoints will be combined to derive tissue, organ or overall radiosensitivity measures (e.g. by STAT score or principal component analysis). Validation of risk factors will involve univariate analysis with Bonferroni correction and multivariate analysis. The strength of association of predictors will be assessed by calculation of the odds ratios.

Nomograms, logistic regression-, ordinal logistic regression-, and NTCP (normal tissue complication probability) models published in the literature will be considered for the prediction model validation. Validation of the prediction models in the REQUITE cohort will be evaluated by: (a) Calibration: assessing the agreement between observed and predicted probabilities using the Hosmer-Lemeshow "goodness-of-fit" test. (b) Discrimination: assessing the ability of the models to discriminate between those with and those without toxicity. For binary endpoints the area under the receiver operating curve (ROC) will be used with derivation of sensitivity and specificity for a chosen probability threshold. For polychotomous endpoints c-statistic will be used as rank order statistic. (c) Clinical usefulness: the "net benefit" (NB) will be calculated (NB=(TP−wFP)/N, where TP is the number of true-positive classifications, FP the number of false-positive classifications). w is a weight equal to the "ratio of harm to benefit" (w=cutoff/(1−cutoff), where cutoff is the probability threshold which is chosen to define the treatment rule (e.g.: the level of non-acceptable toxicity)). A clinically useful model should have an NB>cut-off.

In a second stage existing models will be improved and extended using the biomarker data (SNPs, apoptosis data) in large cohorts recruited via the REQUITE study. Derivation of these models will involve separating cohorts into discovery and validation cohorts. The following performance measures will be considered for improvement and extension of existing models: (a) Likelihood-ratio test: comparing the fits of two models, the null model versus the alternative model. (b) Reclassification table (i.e. percent of individuals who change risk category when applying an advanced model). (c) Net Reclassification Improvement (NRI; i.e. for patients with toxicity any upward shift in risk classes implies prediction improvement and any downward shift indicates reduced reclassification. The reverse holds for patients without toxicity.

In both stages appropriate statistical methods are used as described in the statistical operating procedure (SOP) for statistical analysis.

8.0 COMPLIANCE, DATA PROTECTION AND DATA SHARING

8.1 Compliance

REQUITE will be conducted according to the protocol, relevant Standard Operating Procedures (SOPs), ICH-GCP and relevant national regulatory requirements.

By participating in the REQUITE study, the Principal Investigators at each study centre are confirming agreement to ensure that:

- Sufficient data are recorded for all participating patients to enable accurate linkage between hospital records and CRFs;
- Source data and all study related documentation are accurate, complete, maintained and accessible for monitoring and audit visits;
- Study-related monitoring, audits, and regulatory inspection(s) are permitted and direct access to source data/documents is provided as required.

8.2 Data Protection

Patients will be assigned a unique study identifier by allocation of the next sequential bar-coded 'Sample A' blood tube (provided by CIGMR), which will be used throughout their participation in the study. Any personal data recorded will be regarded as confidential, and any information which would allow individual patients to be identified will not be released into the public domain.

Each investigator should keep the screening and recruitment log and all other study documents (including participant's written consent forms) which are to be held at the recruiting study centre, in strictest confidence. The investigator must ensure the patients' confidentiality is maintained.

All investigators and researchers involved with the study must comply with the national requirements for data protection with regard to the collection, storage, processing and disclosure of personal information and agree to uphold the appropriate core principles.

Patient notes and study files at site must be kept in a secure storage area with limited access. Access to the REQUITE database will be strictly limited via usernames and passwords. Published results will not contain any personal data that could allow identification of individual patients.

8.3 Data Sharing

The REQUITE steering committee will eventually seek to combine samples and data collected in this observational study with those collected in other radiogenomics studies. This collaboration via the Radiogenomics Consortium with other researchers worldwide should enable a significant sample size to be reached via meta-analyses for detailed study into the genetic differences which lead to variation in radiosensitivity between individuals. Consent will be sought from participants to allow their donated samples and data to be shared with other research groups. Before any data are sent to an external research group, a written proposal detailing the proposed work, including the study hypothesis, inclusion and exclusion criteria, type of statistical analysis, and the variables to be considered, must be submitted to and approved by the REQUITE steering committee. Any samples and data transferred to third parties would not contain any personal information about the patients and therefore confidentiality would be maintained.

9.0 ETHICAL AND REGULATORY REQUIREMENTS

Research Ethics approval for this study has been sought from xxxxx and a favourable opinion granted (ref. xxxxxxx). Individual participating centres must apply to their local research governance departments for management permission prior to opening.

The study will be conducted in accordance with the current approved protocol, the Declaration of Helsinki, ICH Guidelines for Good Clinical Practice (ICH GCP), relevant regulations and standard operating procedures.

The local Principal Investigator (PI) must ensure that the study protocol, patient information sheet, consent form, family doctor letter and submitted supporting documents have been approved by the appropriate regulatory body(ies) and research ethics committee(s) prior to any patient recruitment.

Any agreed substantial amendments must also be submitted and receive ethical and regulatory approval prior to implementation. It is the responsibility of the PI at each site to ensure that the study has all the necessary approvals in place. A site initiation meeting must be completed prior to each study centre opening to recruitment.

9.1   Indemnity

Each participating site will be responsible for arranging their own indemnity or insurance for their participation in the study, as well as for compliance with local law applicable to their participation in the study.

9.2   Informed Consent

A patient information sheet will be presented to potential participants detailing the nature of the study, the implications of participating and any potential risks or inconveniences involved in taking part. It will be clearly stated that the participant is free to withdraw from the study at any time, for any reason, without prejudice to future care and with no obligation to give the reason for withdrawal. The participant will be allowed sufficient time to consider the information. Written informed consent will then be obtained by means of patient dated signature and dated signature of the person who presented and obtained the informed consent.

Molecular and genetic information collected in this research will be in a linked anonymised format such that individual patient information will not be available to anyone in the research team, holding or analysing the data. However, unique study identifiers will be assigned that allow the supplier of the data, such as the person who obtained informed consent, to be able to identify people from it.

9.3   Patient Withdrawal

Lung cancer patients will be withdrawn from the study if they experience a recurrence or second malignancy within the thorax (including breast cancer).

Breast cancer patients will be withdrawn from the study if they have a secondary mastectomy due to relapse.

Patients wishing to withdraw from the study will not be replaced. If a participant decides to withdraw, their coded blood samples and information will be retained for use within REQUITE and for future medical research unless the patient makes a specific request otherwise. If specifically

requested, the blood samples will be destroyed and the medical information removed from the REQUITE database.

10.0   STUDY MANAGEMENT AND OVERSIGHT ARRANGEMENTS

10.1   Study Management Group

A Study Management Group (SMG) will be established and will include those individuals responsible for the day-to-day management of the study including the Chief Investigator, co-investigators and identified collaborators, Principal Investigators, the study statistician and the study manager(s). The SMG will monitor overall progress of the study to ensure the protocol is adhered to and will take appropriate action to safeguard the patients and the quality of the study where appropriate.

The SMG will hold teleconferences at least quarterly once the study is actively recruiting. Minutes will be taken at SMG meetings and copies of the minutes will be filed in the Study Master File. The study manager and Chief Investigator will ensure that all relevant issues and actions discussed during the meeting are followed up and resolved. Details of significant issues will be made available to participating sites and other relevant parties as appropriate.

10.2   Study Data

Submitted data will be checked for errors, inconsistencies and omissions. If missing or questionable data are identified, the REQUITE data manager will request that the data be clarified.

10.3   Study Monitoring

There will be a set-up/ site initiation meeting at each main study centre (personnel from third parties who are recruiting patients should also attend). This is very important to ensure that the correct procedures and guidelines are fully understood and that all members of staff working at each study centre have an opportunity for dedicated training on the protocol, data collection, electronic data input, sample collection and storage procedures.

There will also be a subsequent monitoring visit per study centre. The purpose of these visits is:

- To verify that the rights and well-being of patients/participants are protected.
- To verify accuracy, completion and validity of reported study data from the source documents.
- To evaluate the conduct of the study within the institution with regard to compliance with the currently approved protocol, GCP and with the applicable regulatory requirements

11.0 PUBLICATION

For the main publication(s) of this study, it is anticipated that all contributors will be authors with the proviso that clinicians contributing patients must have contributed 20 patients or more. A formal publications policy will be generated, which all participants will be asked to sign up to.

The main results of the REQUITE study will be published in a peer-reviewed journal, on behalf of all collaborators. The manuscript will be prepared by a writing group, appointed from amongst the REQUITE Steering Committee and high accruing clinicians. All recruiting study centres and clinicians will be acknowledged in this publication. All presentations and publications relating to the study must be authorised by the Steering Committee. No investigator may present or attempt to publish data relating to REQUITE without prior permission from the REQUITE Steering Committee.

12.0 SCHEDULE OF ASSESSMENTS

Additional annual follow-up beyond 24 months is encouraged for all patients recruited to REQUITE, and particularly those recruited in the first year of the study.

12.1 Breast Patients

ACTIVITY	FORM LABEL	TIME POINT			
		Baseline (prior to radiotherapy unless otherwise indicated)^	End of radiotherapy*	12 months from start of radiotherapy+	24 months from start of radiotherapy+ (1° endpoint)
Enrolment Form	B1	X			
Consent	RQ1	X			
Blood Collection^	RQ2	X^			
Patient Factors Form	B2	B		F	F
Clinical and Treatment Data Collection Form	B3		X		
Photographic Assessment Form	B4	X			X
Health Professional Toxicity Data Form	B5	X	X	X	X
EORTC C30	B6a	B	F	F	F
EORTC BR23	B6b	B	F	F	F
MFI	B6c	B	F	F	F
GPAQ	B6d	B	F	F	F
Withdrawal Form	B7				X
Outcome Data Form	B8				X

^ Consent and blood collection can either be prior to chemotherapy or radiotherapy (see Section 6.2)
* 1-2 days prior to end of radiotherapy or last day
+ Plus/ minus four weeks
X: Same version for every time point
B: Baseline version
F: Follow-up version

12.2 Prostate Patients

ACTIVITY	FORM LABEL	TIME POINT			
		Baseline (prior to radiotherapy)^	End of radiotherapy*&	12 months from start of radiotherapy+	24 months from start of radiotherapy+ *(1° endpoint)*
Enrolment Form	P1	X			
Consent	RQ1	X			
Blood Collection	RQ2	X			
Patient Factors Form	P2	B		F	F
Clinical and Treatment Data Collection Form	P3		X		
Health Professional Toxicity Data Form	P4	X	X	X	X
Patient Reported Outcomes Questionnaire	P5a	B	F	F	F
EORTC C30	P5b	B	F	F	F
MFI	P5c	B	F	F	F
GPAQ	P5d	B	F	F	F
Withdrawal Form	P6				X
Outcome Data Form	P7				X

^ Prior to radiotherapy

* 1-2 days prior to end of radiotherapy or last day

& For brachytherapy patients, the end of treatment time point should take place at the first routine follow-up visit following implantation

+ Plus/ minus four weeks

X: Same version for every time point

B: Baseline version

F: Follow-up version

12.3 Lung Patients

ACTIVITY	FORM LABEL	TIME POINT				
		Baseline (prior to radiotherapy)	3 months from start of radiotherapy[+]	6 months from the start of radiotherapy[+]	12 months from start of radiotherapy[+] *(1° endpoint)*	24 months from start of radiotherapy[+]
Enrolment Form	L1	X				
Consent	RQ1	X				
Blood Collection	RQ2	X				
Patient Factors Form	L2	B			F	F
Clinical and Treatment Data Collection Form	L3		X			
Health Professional Toxicity Data Form	L4	X	X	X	X	X
Patient Reported Outcomes Questionnaire	L5a	B	F	F	F	F
EORTC C30	L5b	B	F	F	F	F
MFI	L5c	B	F	F	F	F
GPAQ	L5d	B	F	F	F	F
Withdrawal Form	L6					X
Outcome Data Form	L7					X

[+] Plus/ minus four weeks
X: Same version for every time point
B: Baseline version
F: Follow-up version.