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

Final Report Summary - REQUITE (Validating predictive models of radiotherapy toxicity to improve quality-of-life and reduce side-effects in cancer survivors)

Executive Summary:
REQUITE worked to address an ambitious and urgent need to reduce long-term side-effects and improve health-related quality-of-life in cancer survivors who received radiotherapy. The motivation for the project was the need to move away from a one-size-fits-all approach to treatment. Survivorship issues are particularly relevant for radiotherapy as it is an important potentially curative treatment in many cancers. Although many models and biomarkers have been developed that appear to identify patients with an
increased risk of long-term side-effects, validation is rare. Progress is hampered because data and samples are not generally collected in routine clinical practice and any datasets available are heterogeneous. Missing and harmonisation of data are problematic. The main achievement of REQUITE was completing an observational study, the largest of its kind, which is recognised as an exemplar of the type of multi-disciplinary, multi-national work that can and should be carried out in the radiotherapy-related research field.

As planned REQUITE produced standardised case report forms to collect epidemiology, treatment, side-effect and quality-of-life data from patients recruited in 12 main centres in Belgium, France, Germany, Italy, Spain, The Netherlands, UK and the USA. Questionnaires for collecting patient reported Common Toxicity Criteria for Adverse Events were translated into multiple languages and validated for use. Our forms are being shared worldwide to improve the standardisation of data collection. REQUITE succeeded in establishing a centralised database which is accessible on request by other researchers. 4,438 patients with breast, prostate or lung cancer were recruited prospectively. The quality controlled centralised database for electronic data capture and storage contains >100,000 completed case record forms; 11,563 breast photos; 17,107 DICOM and 12,684 DVH files. Raw genotype data are available for 4,777 and imputed data for 4,542 with European ancestry (1,948 breast, 1,728 prostate, 628 lung) patients. In summary, after quality control a total of 2.04 billion genotypes were directly typed, and 55.2 billion imputed genotypes were recorded.

REQUITE succeeded in establishing a centralised biorepository linked to the database, which stores DNA and PAXgene tubes that are easily accessible for validating future biomarkers. Genotyping for common genetic variants (single nucleotide polymorphisms) was successful with the data added to the centralised database. As planned, the radiation induced lymphocyte assay was carried out in three centres (France, Germany, UK) with data generated for a sub-set of 1,319 patients.

Validation of models and biomarkers will be completed after REQUITE ends, but trial designs and draft protocols were developed for future interventional studies using them to personalise radiotherapy and reduce risks of long-term side-effects. In addition to planned work, a process was established to provide access to the resource via submission of a concept form, and a public discovery platform to enable researchers to query the REQUITE resource.

The outputs of REQUITE are already serving as a resource for consortium partners and the wider radiotherapy research community. The short-term impact of the project will be a better understanding of side-effects in a multi-national setting. The results will inform stakeholders and increase knowledge of the health burden and of clinical risk factors. Use of the REQUITE resource to validate models and biomarkers and their use in future interventional trials will have a long-term impact on the health-related quality-of-life of cancer survivors.

Project Context and Objectives:
Overview
The central concept of REQUITE was to develop validated clinical models incorporating biomarker data to identify before treatment those cancer patients who are at risk of developing long-term side-effects from...
radiotherapy, to identify the best interventions for reducing the side-effects and to design interventional trials aimed at improving the quality-of-life of cancer survivors who underwent radiotherapy (West et al. 2014. PMID: 25267305).

Systematic reviews are required to identify approaches for ameliorating side-effects and research is required to design interventional clinical trials to reduce long-term side-effects and improve quality-of-life in cancer survivors.

Scientific Background
Approximately half of the ~28 million cancer survivors worldwide received radiotherapy as part of their cancer treatment. However, patients vary in how they react to radiation. Up to 5% of patients are sensitive and at risk of having long-term side-effects (radiation toxicity) that impact on their health-related quality-of-life. Predictive clinical models for radiotherapy side-effects are being developed to try to identify before the start of treatment those patients who are most sensitive. Some biomarkers show promise for incorporation into clinical models. However, the models and biomarkers are not yet ready to use in the clinic so radiation doses for all patients are currently limited by the risk of side-effects in the most sensitive patients.

The REQUITE project brought together clinicians, basic research scientists, patient advocates and clinical research teams from across Europe and the US. The consortium aims were to:
(1) develop validated clinical models incorporating biomarkers to identify before treatment cancer patients who are at risk of developing long-term side-effects from radiotherapy;
(2) design interventional trials aimed at improving the health-related quality-of-life of cancer survivors who underwent radiotherapy.

If successful, then in future it would be possible to identify the ‘radiosensitive’ patients before the start of treatment and move towards biologically personalised radiotherapy.

Main objectives of REQUITE
Objective 1: Perform a multi-centre, observational cohort study (WP2)
The first objective of REQUITE was to perform a multicentre observational study to collect standardised radiotherapy toxicity data, non-genetic risk factor data (e.g. dosimetry, chemotherapy use, age, diabetes, smoking history, gender) and samples for biomarker assays with centralised prospective data collection and biobanking. A key priority for the consortium was the collection of the same data in multiple centres. Also to minimise the amount of missing data that has traditionally hampered the research community’s ability to validate models that predict a cancer patient’s risk of side-effects following radiotherapy. Finally to provide an accessible centralised database for future radiotherapy related studies. The database was customised specifically to meet the needs of REQUITE, to allow for the collection of dose volume histograms, the centralised storage of digital photographs of breast cancer patients and for electronic data capture of patient reported outcomes. Although there are numerous cancers for which radiotherapy is an important part of potentially curative treatment, REQUITE focussed on the three most prevalent cancer sites: breast, prostate and lung.

Objective 2: Produce a centralised biobank (WP3)
The second objective of REQUITE was to produce a centralised biobank of DNA from the patients
enrolled in the observational study. This biobank would be linked to the clinical database. Biobanking is critically important for future developments in health research and there is a demand for high quality samples linked to accurate, reliable, and standardised clinical and laboratory data. Sample collection, processing, storage, tracking, and shipment of biospecimens would be optimised and ISO standards applied. REQUITE worked to produce a centralised, easily accessible, high quality resource linked to the clinical database (WP2) with centralised DNA extraction for use in biomarker studies not only those in REQUITE but also future studies (e.g. next generation sequencing).

Objective 3: Validate biomarkers of radiosensitivity (WP4)
The third objective was to validate published biomarkers of individual radiosensitivity by investigating common genetic determinants of radiosensitivity through exploring single nucleotide polymorphisms (SNPs). Ongoing collaborative work via the Radiogenomics Consortium will identify more SNPs that can be further replicated (i.e. in other cancers/for additional endpoints) in the samples collected in the REQUITE prospective observational study. An important aspect of this objective was SME involvement to ensure findings can be readily used in future interventional studies and clinically. WP4 also focussed on using functional assays of radiosensitivity. The most promising – an apoptosis assay – was tested within REQUITE. Standardised operating procedures (SOPs) were implemented across laboratories in three European countries thus developing the assay for use in a future interventional study and also clinically.

Objective 4: Validate models that predict risk of toxicity (WP5)
The fourth objective was to validate statistical models that use clinical and biomarker data to predict a patient’s risk of long-term side-effects following radiotherapy in patients with breast, prostate or lung cancer. REQUITE worked to collate available information and validate the models currently being developed using the data (WP2) and biological samples (WP3) from the multi-centre observational study. The aim was to select the best validated statistical models that predict risk of long-term side-effects for patients with cancers of the breast, prostate or lung for use in designing interventional trial protocols in WP6.

Objective 5: Design interventional trials (WP6)
The fifth objective was to design interventional trials aimed at reducing side-effects and improving the quality-of-life of long-term cancer survivors who underwent radiotherapy. Interventions to avoid, protect against, ameliorate or treat the toxicity of radiation were explored via systematic review. For the three cancers of REQUITE (breast, prostate & lung), the aim was to identify the best interventions to treat the endpoints of interest; the development of interventional trial designs and protocols making use of the validated models and biomarkers developed in earlier work packages.

Objective 6: Dissemination (WP7)
The sixth objective was to deliver a resource for dissemination and exploitation within the radiotherapy research community. The database allows for data sharing not only by project partners but also more widely within the radiotherapy research community. The resource has potential for wider application to explore the relationships between different radiotherapy side-effect endpoints and between the endpoints and quality-of-life items. The resource will increase knowledge of the health burden of long-term side-effects in the EU. The centralised biobank and patient samples linked to clinical data has potential for exploitation for further studies aimed at increasing understanding of the biological basis of radiosensitivity.
In particular, the resource will be exploitable for future studies using next generation sequencing.

Overall strategy of project plan
WP1 was responsible for overall management and scientific oversight of the project; co-ordination with patient advocates, the ethics group, the steering committee and the external advisory group; budgetary control and monitoring of project progress and milestones; co-ordination between work-packages and organisation of annual meetings. Management was based at The University of Manchester.

The central activity of the REQUITE project was the multi-centre observational study organised through WP2, led by DKFZ Heidelberg. Recruitment to these cohorts (breast, prostate and lung cancers) began after the protocols were finalised, the trial registered on a publically accessible database, ethics approvals gained, questionnaires for collecting patient reported toxicity translated and a primary data collection system (electronic physician reporting form and online database) installed. Enrolment was planned to proceed for two years in nine clinical centres. Patients would be followed for two years, by which time long-term side-effects of radiotherapy would be recordable. A prospective study was vital to ensure complete data and samples were obtained to validate existing models and biomarkers. The primary endpoints were selected because they show radiation dose response relationships, they have high enough prevalence to enable sufficient statistical power, i.e. >20%, for mild or moderate to severe side-effects and they have been used in building clinical models. However, many other endpoints were collected to enable validation/development of other clinical models for other toxicity endpoints.

Blood samples were collected from each patient before radiotherapy. Responsibility for the tracking and storage of the samples was handled in WP3. Some of the samples were for immediate use in assays as part of WP4, others were stored in the established biobanking facilities of CIGMR at The University of Manchester. This facility was also responsible for the preparation of DNA from the blood. The use of this facility ensured co-ordination between collection centres and uniformity of storage and extraction procedures.

Validation of biomarkers as predictive factors for long-term side-effects of radiotherapy in cancer survivors was carried out in WP4, led by the University of Leicester. Evidence suggested there was predictive value for a test of lymphocyte response to radiation, so this test of apoptosis was carried out on a sub-cohort of ~1,300 patients in three laboratories immediately following blood collection. Testing of genetic markers to select single nucleotide or copy number polymorphisms for predictive value was completed at the end of the study by the University of Cambridge.

Some clinical factors appear to have predictive value for radiotherapy side-effects, but there was no consensus in the field and little cross-replication of predictive models. WP5 planned to work with published models developed in existing cohorts already collected by the participants and validate them in the new REQUITE cohort collected as part of WP2. Biomarker data from WP4 are also being integrated to produce unified models with the highest predictive power achievable.

To move the REQUITE project towards patient impact WP6 designed clinical interventional trials. Draft protocols were produced ready to use the validated models from WP5 to personalise radiotherapy and reduce long-term side-effects in high-risk individuals without affecting tumour control.
The REQUITE project was keen to communicate its results effectively to a wide range of stakeholders including: radiobiology researchers, radiation oncologists, radiation physicists, professional organisations, biotechnology corporations, patients’ advocacy groups, cancer patients and policy-makers. Therefore WP7 was dedicated to devising and implementing a professional dissemination strategy, using a wide variety of media. This was led by Maastro Clinic which has excellent connections and a strong track record in dissemination.

The work packages were each contributing to a tightly integrated project with a clear long-term focus on improving quality-of-life of cancer survivors. The project built on existing evidence of clinical factors and biomarkers that were thought to affect risk of long-term side-effects following radiotherapy, worked to try and validate these in the new cohorts to create statistical models which can be applied in clinical practice for patient benefit. The leads of each WP have an existing track record of working together through the Radiogenomics Consortium, so were ideally placed to co-ordinate the different strands into an effective whole leading to a successful outcome. The principal investigators at each partner organisations are listed below.

- Prof. Catharine West; University of Manchester, UK
- Dr. Antony Payton; University of Manchester, UK
- Prof. Dr. Jenny Chang-Claude; Deutsches Krebsforschungszentrum, Heidelberg, Germany
- Dr. Liv Veldeman; Universiteit Gent, Belgium
- Dr. Chris Talbot; University of Leicester, UK
- Dr. Maarten Lambrecht; Katholieke Universiteit Leuven, Belgium
- Dr. Alison Dunning; University of Cambridge, UK
- Prof. Dr. David Azria; Université de Montpellier & Institut Regional du Cancer de Montpellier France
- Dr. Riccardo Valdagni; Istituto Nazionale dei Tumori, Milan, Italy
- Dr. Ana Vega; Fundación Pública Galega de Medicina Xenómica, Santiago, Spain
- Prof. Ananya Choudhury; Christie NHS Foundation Trust, Manchester, UK
- Dr. Tom Burr; Source Bioscience, Nottingham, UK
- Prof. Dr. Barry Rosenstein; Icahn School of Medicine at Mount Sinai, New York, USA
- Prof. Dr. Dirk De Ruyscher; Stichting Maastricht Radiation Oncology Maastro Clinic, Maastricht, The Netherlands
- Dr. Frederik Wenz; Universitätsklinikum Mannheim, Germany

Project Results:
The main results are summarised by work package and represent a brief overview of the work undertaken. Final analyses are ongoing and details of the research findings are currently being written up for publication with a number of papers ‘in press’.

WP2: Multi-centre observational study
REQUITE worked to establish an international observational study of patients undergoing radiotherapy for breast, prostate or lung cancer. The aim of the study was to try to predict which patients are more likely to have side-effects (toxicity) from radiotherapy.
The study was designed to recruit 5,300 patients across 26 hospitals in eight countries (Belgium, France, Germany, Italy, the Netherlands, Spain, UK, the United States). Hospitals in each country obtained the necessary local ethical and regulatory approvals to participate in REQUITE and followed a standard study protocol and documentation set throughout. Site initiation visits were organised to ensure all hospitals were following the same procedures, and monitoring visits were completed to check continued compliance. All patients gave written informed consent to participate in the study. The project started on 1st October 2013 and the first patient was recruited on target (six months later) on 10th April 2014.

The radiotherapy schedule was prescribed by the local physician according to local standard-of-care. Adult breast and prostate cancer patients were recruited before receiving radiotherapy between April 2014 and September 2016 and were followed up by their local cancer care team for at least 24 months; similarly lung cancer patients were recruited before radiotherapy between April 2014 and March 2017 and were followed up for at least 12 months. The primary endpoints were change in breast appearance at two years (breast), rectal bleeding at two years (prostate) and breathlessness at 12 months (lung).

The study was registered with International Standard Randomised Controlled Trial Number Register (ref: ISRCTN98496463) http://www.controlled-trials.com/ISRCTN98496463

Data and sample collection
Management of the standardised collection of clinical, dosimetric and toxicity data with linked biosamples was handled centrally by the REQUITE observational study team at the German Cancer Research Center (DKFZ) in Heidelberg Germany. The standardised data collection forms developed by REQUITE included both physician reported and patient reported outcome questionnaires. These questionnaires were specific for each tumour type and were used to record information on side-effects. The patient reported outcome questionnaires were translated from English into multiple languages (German, Spanish, French, Italian & Dutch), and validated by testing the specific wording and overall acceptability in multiple, small patient cohorts. They are available on request via the website (www.requite.eu) to improve standardisation of data collection in other radiotherapy studies across the world. Anonymised patient data are held in a bespoke central database at The University of Leicester in the UK.

Data collection forms (comprising case record forms (CRFs), patient questionnaires and study documents) were created prior to the start of the observational study in April 2014, however, as the study progressed minor changes were implemented to improve clarity of the questions to better capture the correct information. A database manual was established to ensure standardised data input across centres.

The primary endpoint for breast is changes in breast appearance, to be assessed by comparing digital photographs taken before radiotherapy with photographs taken two years post-treatment. Each recruitment site followed the RQ12 SOP on Breast Photographic Assessment ensuring all photographs of breast patients excluded the head and were taken from three different viewpoints (anterior hands on hips, anterior hands above head, lateral hands above head). Immediately prior to the photographs being taken, key points of the breast are marked (using ink) on the skin, to ‘line-up’ the photographs and enable a direct comparison using BCCT.core software. Adherence to the guidelines helped to ensure comparability of the serial photos taken and reduce inter-site variation.
Radiotherapy imaging and dosimetry files (Digital Imaging and Communications in Medicine, (DICOM) and Dose-Volume-Histograms (DVH)) were collected for central storage. The incorporation of medical imaging and dosimetry files into the REQUITE database creates a substantial resource for future analyses in REQUITE and also for use by the wider research community. Many similar studies were unable to collect, store and analyse these large image files. All centres were able to simultaneously upload multiple anonymised DICOM and DVH files to the REQUITE servers, a huge time-saving for the personnel responsible for this work. DICOM files were validated on upload for i) modality and ii) inclusion of the correct REQUITE patient identifier, while DVH files are mapped to a standard organ naming scheme circumventing discrepancies raised by local language differences. The uploaded data were checked by colleagues working at INT, Milan Italy to ensure comparability of data across centres. There is also a second check of the uploaded DICOM files to remove potential personal data that may be present in the file headers.

Data validation & QC
The data manager at DKFZ performed regular quality control checks of the entered data. Furthermore, information was collected on the standard of care radiotherapy regimens at each recruiting centre and then compared against entered study data to check for data plausibility as part of the comprehensive data validation and QC process. The data manager worked closely with recruitment centres to ensure data completeness i.e. that any missing data and/or data queries were dealt with in a timely manner. Descriptive tables to examine completion of data input per time point were created on a monthly basis and shared throughout the consortium. This was a useful way to monitor and furthermore encourage data entry and upload of RT physics data and breast photos.

Finally University of Manchester conducted centralised transcribing error checks between December 2016 and July 2017. Checks were performed on a random sample of 5% of patients from each recruitment site, ensuring at least 5 patients in total, with ≥1 patient(s) of each tumour type. Scanned copies of the original paper CRFs for these patients were sent to The University of Manchester, and the entry for each data field was checked against the data available in the database. The number of discrepancies identified was very low from the outset and decreased as the study progressed; obviously any discrepancies were rectified in the database.

Cohort Recruitment & Follow-up
Patient recruitment ended on 31st March 2017 with accrual of 4,438 patients: 2,069 breast (99% of target), 1,808 prostate (86%) and 561 lung (51%). Furthermore ‘additional lung cohorts’ were identified at University of Gent, Belgium (n=82) and at University of Manchester, UK (n=301) such that samples from 383 additional lung cancer patients were genotyped alongside the REQUITE cohorts. The associated phenotype data are also available and will be included in the lung analyses (n= 944). Although the datasets associated with these additional cohorts are not as comprehensive as in REQUITE, 12 month follow up data were collected that included REQUITE’s primary endpoint for lung namely dyspnoea (or breathlessness). Detailed radiotherapy treatment information was available for 2,057 breast, 1,760 prostate and 530 lung cancer patients.

It was initially estimated that 24-month data would be available for 75% of the recruited patients, i.e. 1,575
of the original target of 2,100 breast or prostate cancer patients, respectively. This target was exceeded for both the breast and prostate cohorts. By 6th November 2018, data were received at 24 months from 1,707 breast cancer patients, which is 83% of the recruited 2,059 patients. For prostate cancer patients, 24 month data are available for 1,463 patients which is 81% of the recruited cohort. The lung cohort did not reach this target with data from 59% of recruited patients at the 12 months follow-up time point.

In total, 2,069 breast patients were recruited and more than 11,000 breast photos were uploaded to the database. Following guidance developed by the REQUITE consortium and detailed in the RQ17 SOP on Breast Photographic Analysis, these photographs were scored centrally at University of Manchester using the BCCT.core software v3.0 to assess changes in breast appearance and overall cosmetic result e.g. breast asymmetry, skin colour changes due to the radiotherapy, and surgical scar visibility. The assessment results are then grouped into excellent, good, fair and poor outcome. To date, 2,037 photographs were taken at baseline and photographs at 24 months of 1,582 patients have been scored. As a QC measure, University of Leicester has undertaken second scoring of 5% of these photographs to demonstrate accuracy and consistency of scoring. The concordance rate was 80.7% when comparing distinct values (excellent, good, fair and poor) and 93.2% when comparing dichotomised scores (excellent/good vs. fair/poor).

Overall, by the end of project in November 2018 the centralised database stored anonymised files of radiotherapy imaging (n=4,184), dosimetry (n=4,233). A data quality control check of 70,776 data entry points identified only minor and a low level of transcribing errors (<0.62%). Overall 330 breast, 186 prostate and 258 lung cancer patients with detailed radiotherapy data withdrew or dropped out. Reasons include death (17+39+133 patients), ‘do not wish to continue’ (176+77+8 patients) and ‘lost to follow-up’ (22+30+12 patients).

A manuscript “REQUITE: A prospective multicentre cohort study of patients undergoing radiotherapy for breast, lung or prostate cancer” that details the baseline characteristics of the patient cohort and the REQUITE study design was submitted to Radiotherapy & Oncology in November 2018.

WP3: Biobanking
All patients donated at least two blood samples prior to the start of radiotherapy: an EDTA sample for SNP genotyping and a PAXgene and/or a Lithium Heparin (LiH) sample. DNA was extracted from the EDTA blood sample for genotyping, and PAXgene samples are stored in the centralised biobank for RNA extraction. These samples are held in the CIGMR Biobank at The University of Manchester, UK. A subset of patients from France, Germany and the UK gave LiH blood samples for prospective radiation induced lymphocyte apoptosis (RILA) assays using an established method developed by Prof David Azria at the Institute of Cancer in Montpellier France. All three recruitment centres followed the same SOP to ensure standardisation across the laboratories. Genotyping data were generated using the Illumina Infinium OncoArray-500K beadchip and imputed using the 1000 Genomes Project (version 3) as a reference panel. SOPs are available on request via www.requite.eu/.

SOPs and case report forms for blood collection and storage, sample tracking logs and sample kit request forms were developed specifically for use in REQUITE by all recruiting sites in the observational study.
Providing detailed SOPs and report forms ensured standardised blood collection and storage across sites leading to high quality DNA samples for downstream processing and genotyping. Modifications to the Thermo-Fisher Nautilus Laboratory Information Management System (LIMS) were tested and implemented such that it could fulfil the requirements of the REQUITE study. A barcode tracking system using bespoke labels carrying a 1D readable barcode in addition to a 2D spot was developed for internal sample tracking within LIMS.

CIGMR Biobank prepared, validated and shipped 59 kit requests, delivering 5,471 kits to recruitment sites across Europe & the US. In total, they receipted and logged 4,456 EDTA blood samples (including repeat bloods) and 3,039 PAXgene blood samples from REQUITE patients received in 42 shipments over the course of the project. In addition, a further 169 EDTA bloods and 132 DNA aliquots were received from The Christie NHS Foundation Trust, Manchester and were collected as ‘additional lung cohorts’. On receipt, samples were logged into LIMS and stored in -80°C temperature monitored freezers in accordance with in-house SOPs.

DNA extraction was performed using PerkinElmer Chemagic kits - a magnetic bead extraction process. The extractions were undertaken in accordance with the validated SOP. Blood volumes ranged from 1 ml to 9 ml with an average of ~8 ml. For six patients the samples provided were too small and there was insufficient DNA extracted for genotyping. The concentration of eluted DNA samples was measured using a NanoDrop spectrophotometer. This required 1 μl of sample per measurement. The NanoDrop absorbance ratios for 260 nm and 280 nm were used to evaluate purity. The concentration in ng/μl and the ratio data were uploaded directly into LIMS for future reference, and also uploaded to the central REQUITE database. The stock DNAs obtained had an average yield of 23 μg of DNA per ml of blood, with an average DNA concentration of 175 ng/μl and an average 260/280 purity ratio of 1.84. These data showed samples were fit for purpose, and there was sufficient DNA stored for use within REQUITE for genotyping as planned (input for the Illumina Oncoarray is 200 ng), but also for value-add studies carried out by members of the REQUITE consortium and for sample sharing with the wider research community.

To check that samples remain high quality and fit for purpose after long-term storage at 80°C, regular QC takes place to re-measure 20% of the DNA samples on a Nanodrop spectrophotometer. These are also re-runs on 1% agarose gels to demonstrate sample integrity.

WP4: Biomarker Assays
The REQUITE project collected blood samples for three purposes: radiation-induced lymphocyte apoptosis (RILA) assays, DNA extraction for genotyping and RNA for future gene expression experiments. All centres collected EDTA blood tubes for dispatch to WP1, for DNA extraction. Centres collected a second tube either for RNA stabilisation (a PAXgene tube) or for apoptosis assays (a lithium heparin tube). The lymphocyte apoptosis assay was carried out by three of the centres (University of Leicester, University of Heidelberg & Institute of Cancer Research, Montpellier); all other centres collected PAXgene tubes.

Radiation Induced Lymphocyte Assay
The RILA (radiation-induced lymphocyte apoptosis) test was demonstrated to have utility in predicting
adverse reactions to radiotherapy. Studies by David Azria and colleagues showed that there was substantial variation between patients in the amount of cell death (apoptosis) in their blood cells (lymphocytes) exposed to radiation. The patients with the lowest amount of cell death (low RILA score) had worse adverse reactions than those with the highest amount of cell death (high RILA score). That is there is an inverse correlation between RILA score and side-effects of the treatment. Several other research groups have confirmed this finding but it needed further verification in carefully controlled experiments before it was ready for widespread clinical use.

The RILA test required a small sample of fresh blood from the patients, which was then mixed with cell culture fluid and kept in a 37°C incubator for about 24 hours. Different flasks of the diluted blood were then either exposed to a high dose of X-ray radiation or left unexposed. Following the irradiation the samples were returned to the incubator for a further 48 hour period to give time for the blood cells to react to the radiation. At the end of this period the blood cells were separated from the fluid and stained with an antibody to identify the lymphocytes and a dye which quantifies the amount of DNA in the cell. Apoptotic cells (those that are in the process of being killed by the radiation) have less DNA and therefore it was possible to count the proportion of lymphocytes that were affected. The RILA score is the difference between the amount of cell death in the irradiated and unexposed samples for each patient.

From the start of the REQUITE project in October 2013 there was a six month period in which the test was set up in each of the three centres, the laboratory protocol was standardised and checks were carried out to compare the results between centres. The first patients were recruited in May 2014 and the test was then performed throughout the recruitment period. The end date for carrying out the tests was extended in line with the extension of patient recruitment to the end of September 2016.

Protocol optimisation
At the beginning of the project the RILA test was already being performed in Montpellier, and with a different protocol in Mannheim, but not in Leicester. All three centres adopted the Montpellier protocol which was adapted into a standard operating procedure (SOP).

Over the first six months a series of trials were carried out to investigate:
1. Whether the timings of the blood incubations are important for RILA results
2. The degree of standardisation needed of laboratory reagents and equipment
3. How similar the results were between the three centres

These experiments determined that the length of the incubations before and after irradiation of the samples have an important effect on the results. The timings needed to be carefully controlled and recorded – for this purpose a data collection form designated RQ9 was created. It was found that the source of one of the tissue culture fluids called FBS had an effect on results and so one manufacturer’s supply was specified in the SOP. Some compromises were necessary e.g. the three centres used different machines to measure cell death and this could not be standardised given the cost of purchasing new machines.

Two inter-laboratory comparisons were carried out, the first involved dispatching two blood samples from Leicester to the other centres, and the second involved sending six samples. These comparisons showed
that the changes to the SOP helped reduce differences between the centres but that these differences were not removed entirely.

Testing of patient samples
By the end of September 2016 the three centres had carried out the RILA test in duplicate on a total of 1,319 samples. These were 85% of all the patients recruited by the three centres: 67% at Leicester, 71% at Mannheim and 96% at Montpellier.

Leicester: 204 Breast; 25 Lung; 199 Prostate (428 Total)
Mannheim: 147 Breast; 0 Lung; 61 Prostate (208 Total)
Montpellier: 411 Breast; 27 Lung; 245 Prostate (683 Total)

All Centres: 762 Breast; 52 Lung; 505 Prostate (1,319 Total)

The test results were used to calculate a RILA score, which represents the increase in cell death as a result of radiation. The results of the RILA score for each centre were broadly similar in terms of average, range, risk cut-off points and distribution. Tertile cut-off points could be used to divide the patients into three equal groups of low, mid and high radiation-induced cell death. There was evidence for differences between cancer types, a novel finding not previously reported, with lung cancer patients having lower RILA scores than breast or prostate patients. This observation may be important when applying the test for use in clinical trials, with different cut-offs being used for each cancer type.

No significant difference was found between centres for breast or lung cancer patients, but the inter-centre difference was significant for prostate cancer patients. The explanation for this is probably differences in the pre-radiotherapy treatments the patients receive. For example a higher proportion of the Leicester prostate cancer patients received hormonal therapy than in Montpellier.

An analysis of patient-related factors that affect RILA testing showed that multiple variables had an independent effect:

• Patients with higher proportion of one type of lymphocytes had increased RILA scores. This may be important to the mechanism underlying the predictive value of the test.
• Smoking: Never & ex-smokers had an average RILA score 23.1%, compared with recent & current smokers who had an average of 18.0%. This may well be a confounding factor that will need to be corrected for when the RILA test is used as a predictive test.
• Depending upon the cohort, other treatments the patients have previously or are currently taking appear to affect RILA, including anti-depressants, alpha blockers & prior hip replacement.

Previous studies suggested that the RILA assay had no predictive value for acute radiotherapy toxicity, i.e. side-effects occurring during or in the weeks after treatment. An analysis of the REQUITE cohorts confirms this for prostate cancer acute reactions and for skin reddening (erythema) in breast cancer. In a novel finding the REQUITE data shows that RILA score does predict acute breast pain, with low RILA score associated with worse pain, the same inverse relationship seen with late toxicity.

Breast pain is an under-researched clinical end-point in radiotherapy research, despite patients regarding
it as the most important. In a paper published in March 2018 (Rattay et al. 2018. PMID: 29287972), ULEIC and Mount Sinai reported on REQUITE patients’ perspectives on a future radiogenomics test, finding that pain was the side-effect that would motivate patients to consider predictive testing.

A paper was submitted in October 2018 describing the RILA data, including the analysis of patient factors that affect RILA score and the analysis of acute toxicity data. As the data from the REQUITE project matures it will be possible to determine whether the test does predict late radiotherapy side-effects. That would allow future clinical trials that optimise a patient’s treatment based on their personal risk of serious side-effects.

SNP Genotyping

SNP genotyping was completed at University of Cambridge. Patient samples were genotyped using the Illumina Oncoarray, which contains 533,631 SNPs, including a set for genome wide association studies (GWAS) and 2000 SNPs specifically chosen for potential association with radiotoxicity.

In total, 4,442 REQUITE samples were genotyped, and passed through a validated multi-step quality control pipeline. This filters out samples with poor quality DNA and variants with low call rates or abnormal results on good quality DNA. After these quality control steps data were retained for 483,517 SNPs on 4,223 patients, of which there were:

- 1,948 breast cancer cases
- 1,728 prostate cancer cases
- 547 lung cancer cases

In addition, externally collected samples were genotyped from lung cancer patients recruited to the international CONVERT trial collected via University of Manchester and also cohorts from University of Gent. This increased the overall lung cohort to 866 patients with genotyping data available for analysis.

Following the quality control steps of the SNPs on the chip, imputation was performed of the genotypes of SNPs and copy number variants not directly typed by the Oncoarray. Imputation was carried out using IMPUTE2 software version 2.3.2 and 1000 Genomes project data as the reference panel. A total 21,465,139 polymorphisms were imputed of which 13,077,651 passed the imputation quality r2 metric >0.3 threshold. Considering common polymorphisms with a minor allele frequency (MAF) >0.05; a total of 7,431,964 were imputed of which 7,282,498 passed the quality threshold. In summary, after quality control, a total of 2.04 billion genotypes were directly typed, and 55.2 billion imputed genotypes were recorded. These data are now stored centrally and available for GWAS analyses, once final checks on the two-year toxicity data are completed.

RNA Extraction & RNAseq

In a pilot experiment REQUITE carried out RNA extraction on 50 PAXgene tubes from lung cancer patients with and without radiation toxicity (breathlessness and lung inflammation) and matched for sex and age. RNA sequencing was then carried out for the whole transcriptome using an Illumina HiSeq platform, which generated an average of 7477 Mb/sample. From the data obtained on 20 cases and 21 controls, 11 genes were identified as being differentially expressed, out of the 17811 genes in total. This experiment provided important preliminary data for grant applications such as for the RAD-PRECISE
To build the REQUITE resource further, RNA extraction from a further 1,032 PAXgene tubes was undertaken. RNA yield was >3,300 ng per sample with a generally high quality (RIN = 7.76 ± 0.43).

WP5: Validation of clinical predictors & models
WP5 deliverables to report the performance of models reported in literature were completed for breast, prostate and lung cancer cohorts over the course of the project. Furthermore, software was developed to integrate dose-volume histogram (DVH) data in prediction models with clinical and genetic (SNPs) variables. Work carried out in WP5 showed that overfitting and cohort heterogeneity are the two main causes of the failure to validate models predicting radiotherapy toxicity across cohorts (Mbah et al. 2016. PMID: 27479726). Cross-validation and similar techniques (e.g. bootstrapping) cope with overfitting, but the development of validated predictive models for radiation therapy toxicity requires strategies that deal with cohort heterogeneity. A linear method was proposed for building prediction models with high-dimensional data from multiple studies (Mbah et al. 2018. PMID: 30051767). WP5 also demonstrated that simultaneous development of prediction models for multiple toxicity endpoints improved the predictions of a given endpoint by borrowing information from other endpoints. Work showed that James-Stein Estimates can be used to identify variables associated with risk of multiple toxicity endpoints (Mbah et al. 2018. PMID: 29299946).

Breast models
In terms of the performance of prediction models in WP2 cohorts, the breast models available to try and validate were published by Lilla et al. (Lilla et al. 2007. PMID: 17221151) and Barnett et al. (Barnett et al. 2011. PMID: 21646002). Lilla et al. (2007) assessed late toxicities from radiotherapy and collected information on epidemiological factors in a cohort of breast cancer patients who received radiotherapy after breast-conserving surgery. Among 416 patients with complete follow-up data, a multivariate logistic regression model was used as the prediction model for the different toxicity endpoints. With a median follow-up time of two years, 131 (31.4%) patients presented with telangiectasia and 28 (6.7%) patients with fibrosis. Compared to the validation cohorts from WP2, the percentage for telangiectasia was significantly higher in Lilla et al. (2007). The AUC (area under the receiver characteristic curve) computed from cohorts from WP2 was 0.51. Barnett et al. (2011) included multiple endpoints. AUC values for REQUITE cohorts ranged from 0.55 (induration, shrinkage) to 0.70 (oedema).

The prevalence of the various toxicity endpoints were lower for the WP2 breast cohort compared to the cohorts in which the prediction models were constructed (Lilla et al. 2007. PMID: 17221151 and Barnett et al. 2011. PMID: 21646002). These low prevalences meant the models performed poorly on the REQUITE breast cohorts. Although oedema had an acceptable AUC of 70%, there was no concordance between predicted and observed probabilities of the toxicity. It is recommended that new prediction models should be constructed on the REQUITE breast cohorts taking into account the heterogeneity that exist between these cohorts. It is also recommended to use models that handle multiple endpoints simultaneously.

Prostate models
Validation of a clinical/ dosimetric model of late rectal bleeding in prostate cancer patients not enrolled in
REQUITE considered three endpoints: Grade 1+, Grade 2+, Grade 3. Late rectal bleeding was defined as the maximum grade during follow-up with onset between 5 months and 3 years after the end of radiotherapy. The published models selected included the whole rectal dose-volume histogram (DVH), usually reduced to an Equivalent Uniform Dose (EUD) through fitting and use of a volume parameter.

Other factors selected for inclusion in the model were: patient-related dose-modifying factors, including both factors which were explicitly included in predictive models as dose modifying factors, and factors which emerged from studies not explicitly considering the dose. Published NTCP models were for: Grade 1+ (D’Avino et al. 2015. PMID: 25890376; Gulliford et al. 2012. PMID: 22119373); Grade 2+ (Rancati et al. 2011. PMID: 21741721; Michalski et al. 2010. PMID: 19577865; Gulliford et al. 2012. PMID: 22119373); and Grade 3 (Rancati et al. 2011. PMID: 21741721; Defraene et al. 2012. PMID: 21664059).

The combined meta-model included rectal EUD calculated with n=0.12 previous abdominal surgery, cardiovascular diseases, diabetes and use of neoadjuvant/concomitant hormone therapy. Evaluation of meta-model model performance was established in existing patient cohorts available to project partners involved: Airopros 0101, TROG 03.04 RADAR and DUE-01. The combined cohorts comprised ~1,700 patients. The best performance was seen for a Grade 3 model. Possible genetic risk factors (single nucleotide polymorphisms, SNPs) for late rectal bleeding to be added to the clinical/ dosimetric meta-model were based on those SNPs recently shown to be associated with late rectal bleeding in analyses undertaken by the Radiogenomics Consortium (papers by Kerns et al. 2016. PMID: 27515689 & Kerns et al. 2013. PMID: 23719583 and communication at the 2018 Radiogenomics Consortium meeting).

A preliminary analysis was carried out to test the performance of the meta-model and selected genetic risk factors in the REQUITE prostate cancer cohort. This analysis used the subpopulation of patients who received external beam radiotherapy with conventional fractionation and had completed two year follow-up by 1st September 2018 (933 patients). 15.8% with grade ≥1 and 3.1% with grade ≥2 late rectal bleeding events were scored in this selected population. Association of the selected features (from the meta-model and selected SNPs) with late rectal bleeding endpoints were investigated through logistic regression, and validation was evaluated through the correct direction of odd ratios (ORs) and their associated p-value. Rectal EUD, presence of abdominal surgery and cardiovascular diseases, together with two SNPs were confirmed for their association with an increased risk of late rectal bleeding.

Two final logistic models (one for grade ≥1 and one for grade ≥2 late rectal bleeding) including only validated predictors were fitted and two nomograms were developed. Confirmed SNPs were used to calculate a polygenic risk score which was included in the modelling as a single genetic parameter. Inclusion of a polygenic risk score improved calibration (i.e. the ability to predict the exact rate of toxicity as a function of risk factors), while the discriminative power of models was not improved. The complete model (including clinical/ dosimetric/ genetic risk factors) will be tested in the total REQUITE prostate cohort after the end of the project, and will also consider hypo-fractionated patients.

Lung models
This work focused on the testing of risk factors reported in the literature and building a multinomial logistic regression NTCP model. It has been written up and submitted for publication (‘Defraene et al. Development of a multinomial NTCP model for the severity of acute dyspnoea after radiotherapy for lung cancer’). Risk factors of symptomatic radiation pneumonitis derived by Appelt et al. (Appelt et al. 2014.
PMID: 23957623) from a literature meta-analysis were tested: mean lung dose (MLD) and six clinical factors (age at start of treatment, chemotherapy regimen, tumour location, current smoking, history of smoking and pre-existing pulmonary comorbidity). Additional published risk factors of pulmonary toxicity were added: baseline WHO performance status, planning target volume (PTV), relative total lung volume receiving more than 5 Gy (V5), the mean heart dose (MHD) and the maximal heart dose.

In a validation dataset from MAASTRO Clinic, 182 stage I-IV lung cancer patients treated with radical (chemo)radiotherapy were analysed. The lung toxicity endpoint was a patient-reported outcome measure of dyspnoea (shortness of breath) – the primary endpoint in REQUITE. Eleven risk factors were tested for associations with dyspnoea grade ≥2 and grade ≥3 at 6 months. Two factors were validated for grade ≥2 (WHO performance status and chemotherapy regimen), but none were significant for grade ≥3. Baseline dyspnoea grade was very significantly correlated to both dyspnoea endpoints at six months follow-up (AUC values of 0.76 and 0.67 respectively). A baseline symptom grade variable is thus likely to improve NTCP model accuracy and relevance. New multivariate logistic regression NTCP models incorporating baseline grades were generated and model discrimination assessed. The apparent AUCs were 0.79 and 0.74 for grade ≥2 and grade ≥3, respectively. An additional improvement was achieved optimizing a multinomial logistic regression model of dyspnoea grade. This model will be tested in the REQUITE cohort after the end of the project.

WP6: Interventional trial design
The first aim of WP6 was accomplished: a review of trial methodology in light of the design of trials based on predictive models (De Ruysscher et al. 2016. PMID: 27979370). The systematic review concluded that the interaction or risk profile-stratified design is considered to be the gold-standard. In this design, patients are allocated into risk categories, e.g. high vs. low or intermediate risk and afterwards randomised between standard treatment and an experimental therapy. The findings were summarised in a manuscript with patient representatives as co-authors: “De Ruysscher et al. Optimal design and patient selection for interventional trials using radiogenomic biomarkers: A REQUITE and Radiogenomics consortium statement”. With this knowledge it was then possible to move to the in silico modelling of the most suitable study endpoints for each cancer type and to write the clinical protocols.

BREAST CANCER
Most patients with breast cancer will have a favourable outcome with good to very good cosmesis after lumpectomy and postoperative radiotherapy. However, some patients have a radiosensitive phenotype that may lead to severe cosmetic side-effects. As it is expected that 5-10% of patients show a high radiosensitivity that may have clinical consequences, prior identification of these patients would ensure radiotherapy is avoided and patients could be offered an alternative therapy.

Based on results showing a good correlation between the in vitro radiosensitivity of lymphocytes (radiation induced lymphocyte apoptosis [RILA] score) and late radiation damage, a randomised phase II study is proposed. Patients would be tested for their radiosensitivity and according to their risk profile then be randomised between standard radiotherapy and partial breast irradiation.

The trial would test the hypothesis that partial breast irradiation will significantly reduce grade ≥2 breast
fibrosis in patients with a high risk of side-effects but low risk of breast cancer recurrence. The primary endpoint is breast fibrosis-free survival at three years. Secondary endpoints include local control.

Trial Design
This trial will investigate whether the RILA test can be used in a stratified medicine approach to reduce the level of grade ≥2 late side-effects in breast cancer patients undergoing radiotherapy. The proposed randomised phase II trial design comprises a standard arm delivering hypo-fractionated radiotherapy to the whole breast 42.5 Gy/16 fractions/3 weeks (Ontario trial) versus 40 Gy/15 fractions/3 weeks (START B trial).

The experimental arm will deliver hypo-fractionated radiotherapy according to the level of breast fibrosis risk:
• If <10%: hypo-fractionated radiotherapy to the whole breast 42.5 Gy/16 fractions/3 weeks or 40 Gy/15 fractions/3 weeks (as per the standard arm).
• If ≥10%: hypo-fractionated radiotherapy to the tumour bed only (42.5 Gy/16 fractions/3 weeks or 40 Gy/15 fractions/3 weeks).

The cut-off of 10% comes from the fibrosis incidence in the low risk subgroup in the French multicentre RILA trial (Azria et al. 2015. PMID: 26844275). In both arms: no boost or nodal irradiation will be delivered. Brachytherapy or intra-operative radiotherapy (IORT) will not be allowed.

The primary endpoint is breast fibrosis-free survival at three years, where fibrosis is defined as the first observation of a grade ≥2 assessed using CTCAE v4.0.

The secondary endpoints linked with the secondary objectives:
- Incidence of acute and late side-effects
- Local recurrence
- Relapse-free survival
- Breast fibrosis-free survival
- Breast fibrosis-relapse-free survival
- Overall survival
- Quality of life

PROSTATE CANCER
Radiotherapy for prostate cancer may cause rectal injury, which may lead to bleeding and faecal urgency and incontinence. REQUITE together with other studies has identified risk factors for radiation-induced rectal injury such as rectal bleeding. These may be used to identify high-risk patients for rectal side-effects after radiotherapy. In these men, a rectal spacer, which is placed between the anterior rectal wall and the posterior part of the prostate will physically increase the distance from the prostate to the rectum. Hence, the dose to the rectum can be reduced.

However, the spacer and the procedure required to place it, can also have side-effects and requires general anaesthesia. It is therefore particularly important that only patients with the highest risk for rectal
complications are selected for this intervention.

To test the hypothesis that a rectal spacer would reduce rectal injury, we propose a non-randomised phase II study. We believe that randomisation would be difficult for the patient. The primary endpoint would be rectal complications.

Trial Design
This will be a non-randomised observational study. Patients will undergo stratification based on risk of gastrointestinal (GI) toxicity: radiation proctitis (bleeding, faecal incontinence, tenesmus, urgency, mucus discharge, urgency, spasms, and cramps). Patients at high risk will be selected for an implantation of a biodegradable implantable rectum-spacer (IRS) before the start of high-dose radiotherapy (EQD2 > 78 Gy). Patients in this proof-of-principle trial will be risk stratified based on normal tissue complication probability models for GI toxicity that include dosimetric parameters.

A simulation with a virtual spacer will confirm that using an implantable rectal spacer on high-risk patients (as selected by the probability model) will reduce the risk of toxicity. The dose distribution of the patient with and without virtual spacer will be compared. The dose distribution will be transformed in complication risk using validated multifactorial models with and without genetic information. A third module will calculate the cost-effectiveness of both treatments.

The main objective of this study is to decrease significantly the prevalence of two-year GI toxicity using rectal spacers in selected patients with a high risk of GI toxicities. Outcomes in stratified patients will be compared with a matched group of unstratified patients recruited between April 2014 and September 2016 into the REQUITE study. The REQUITE cohort comprises 1,808 patients with information on clinical, dosimetric and biological factors – sufficient to allow selection of a matched group.

The secondary objectives are to evaluate the impact of rectal spacers on all acute and late toxicities, toxicities, quality of life, and local recurrence (i.e. biochemical relapse-free survival).

LUNG CANCER
Many study designs are possible for an interventional lung trial that should follow the REQUITE project. However, the study should be, if possible, more than a prognostic assessment of patients for whom frequently no adequate alternative treatments are available. An example would be patients with an unresectable stage III non-small cell lung cancer, which is still the majority of locally advanced lung cancer patients.

In REQUITE, it was shown that the heterogeneity in the lung between patients, but even more interestingly within the same patient, could be identified and allows for selective sparing of the parts of the lungs that shows the highest susceptibility for damage (Defraene et al. 2015. PMID: 26255763; Botticella et al. 2016. PMID: 27732127).

In order to prepare the study protocol, a planning study was performed comparing a reference (standard) plan that assumes the lung is homogeneous with regard to radiation damage and a “lung damage avoidance” plan. This is the basis for our proposal for the study design for lung cancer: A randomised
phase II trial comparing standard radiotherapy to selective avoidance of susceptible parts of the lungs. The primary endpoint would be the incidence of grades 2-5 (CTCAE v4.0) radiation pneumonitis within 90 days after the end of radiotherapy.

**Trial Design**

With standard radiotherapy, the radiation-induced lung damage (RILD) rate is usually around 15% and with conformal avoidance radiotherapy it is aimed to reduce the RILD rate to 5%. For this reason, it was decided a non-comparative randomised phase II trial was the most appropriate design.

Patients, for which the conformal avoidance is possible, will be randomised using a 2:1 ratio to receive either conformal avoidance radiotherapy or standard radiotherapy. Patients randomised in the standard radiotherapy arm will be used to confirm the 15% RILD rate. Patients randomised in the conformal avoidance radiotherapy will be used to show that RILD rate is lower. To achieve this objective, a one-stage A’Hern design (alpha=5% and power=90%) will be used. Under the hypotheses H0: RILD rate=15% against H1: RILD rate=5%, 76 patients will be enrolled in the conformal avoidance arm. If fewer than six patients experience radiation induced lung damage, the functional conformal avoidance will be deemed successful. Therefore, 114 patients for which conformal avoidance is possible are required and will be randomised: 38 patients will receive standard radiotherapy and 76 patients will receive conformal avoidance.

The primary endpoint is the radiation-induced lung damage (RILD) rate defined as the incidence of grade 2-5 (CTCAE v4.0) radiation pneumonitis within 90 days after the end of radiotherapy.

The secondary endpoints will be exploratory and will include incidence of grades 2-4 cough (CTCAE v4.0) pulmonary function changes and:

- Overall survival
- Quality-of-life
- Proportion of patients for which conformal avoidance is possible
- Identification of CT characteristics that improve the prediction of RILD
- Identification of PET characteristics that improve the prediction of radiation-induced lung damage.

**Potential Impact:**

**WP7: Dissemination**

REQUITE database & biobank

The observational study recruited 2,069 breast (99% of target), 1,808 prostate (86%) and 561 lung (51%) cancer patients. 383 lung cancer patients from external cohorts were included for genotyping. The centralised, accessible database includes: physician- (47,025 forms) and patient- (54,901) reported outcomes; 11,563 breast photos; 17,107 DICOM and 12,684 DVH files. Raw genotype data are available for 4,777 patients and imputed data for 4,542 patients with European ancestry (1,948 breast, 1,728 prostate, 866 lung). Radiation induced lymphocyte apoptosis (RILA) assay data are available for 1,319 patients. DNA (n=4,409) and PAXgene blood/ RNA aliquots (n=3,039) are stored in the centralised biobank. Example prevalences of 2-year (1-year for lung) grade ≥3 toxicities are 13% atrophy (breast), 3% rectal bleeding (prostate) and 27% dyspnoea (lung).
These data demonstrate that the REQUITE consortium have worked to create a comprehensive centralised database and linked biobank that will serve as a valuable resource for the international radiotherapy community. It will provide data to validate models and biomarkers, and their use in future interventional trials will have a long-term impact on the health-related quality-of-life of cancer survivors.

REQUITE Study Documentation Set & Patient Reported Outcome (PRO) Questionnaires
An important dissemination goal of the REQUITE consortium was to improve and facilitate data collection of radiotherapy side-effects. Dissemination activities such as peer reviewed publications, presentation at conference, newsletters & the health professional REQUITE video all provide excellent platforms to highlight the REQUITE study documentation set developed in WP2. This includes the series of validated patient reported outcome (PRO) questionnaires which are available in multiple languages to researchers wishing to make use of them in the clinic or for research purposes. The validated PRO questionnaires are available in English, French, German, Dutch, Spanish and Italian and can be requested via the REQUITE website.

Many analyses are hindered by the problems encountered when trying to work with heterogeneous datasets. Therefore the standardisation of radiotherapy toxicity data collection by use of a standard dataset and validated PRO questionnaires is of huge benefit to the wider research community and will facilitate use of shared datasets in future meta-analyses. There is also an obvious benefit to both patients and their treating clinicians who are introducing routine use of these questionnaires into the clinic to better record radiation side-effects. As such, the work of the REQUITE consortium is proving to be successful in harmonising radiotherapy data collection across Europe and internationally. During the course of the project, REQUITE shared PRO questionnaires with 11 groups for use in the clinical area and/ or clinical trials from as far afield as Australia, India, and the USA. All PRO questionnaires acknowledge EU REQUITE funding and quote our grant agreement number.

Audio Visual Outputs
Two video shorts were produced in collaboration with a media company: one was aimed at current/recently diagnosed patients and the second was for health professionals/researchers.
https://www.requite.eu/node/189
https://www.requite.eu/node/190

The talking head style videos summarise i) the core aims of the project, explaining the importance and motivations of the REQUITE consortium, the success of the observational study and what impact it will have on future treatments and research and ii) highlighting the REQUITE resource to increase visibility and promote data discoverability.

The REQUITE patient video highlights the goals of this multi-centre international study and the progress so far. The audio of the patient video is in English but is subtitled in Spanish, French, German, Italian, Dutch and also English.

• Erik Briers, member REQUITE patient advisory group: "For the REQUITE project, the optimal outcome would be that it could find a marker that could be analysed in future patients".
• Chris Talbot, deputy lead of REQUITE: "A big thank you has to go to every patient, because their involvement will help patients in the future who are undergoing cancer treatment."
Working with a Dutch scientific animation company, REQUITE collaborated to produce an animation describing the efforts of the consortium to reduce long-term side-effects and improve health-related quality-of-life in cancer survivors. Using lay terminology throughout and with both audio & text translated into each of the languages of the patients who participated in REQUITE, the animation addresses the concerns and questions of patients who have recently been diagnosed with cancer and expect to receive radiotherapy as part of their care. It highlights the work of REQUITE, progress to date and describes the breadth of the resource for future research that aims to help more patients survive and improve their quality of life.

The English version of the animation premiered at the REQUITE Symposium Dissemination Day on 18th July 2018 and was very well received by all present. It is on YouTube (currently on two channels – REQUITE & REQUITE EU) with >150 views to date. More recently the translated animations were completed and posted to YouTube and these are currently being disseminated by colleagues working in each country. 
https://www.requite.eu/node/193

Data Discovery
The data discovery platform acts as a ‘shop window’ to the REQUITE database allowing researchers from across the world to find out what data were collected in REQUITE and could be made available to them (subject to approval of a concept form and a data transfer agreement). The search facility accessed via the ‘discoverData’ link on the REQUITE homepage (discover.requite.eu) allows researchers to identify how many patients/ samples are available for specific descriptors of a cohort of interest e.g. how many patients “do not have diabetes AND have toxicity data at 12 months available AND have genotyping data”. This obviously facilitates future data sharing while allowing the REQUITE consortium to retain control over the data in line with current legislation. Note that only patients who agreed for their data (and/or samples) to be shared beyond the REQUITE consortium partners and also for research that could extend beyond cancer studies are included on the data discovery platform.

Value-add projects
In total over the full duration of REQUITE 42 concept forms were received and to date, 34 were approved. These concept forms provide details of new analyses or ‘value add projects’ that are beyond the original scope of the project. Proposed by members of the REQUITE consortium and more widely from those in the radiotherapy research community they describe analyses that make use of REQUITE data and samples to address new research questions. Hypothesis generating research is not permitted and concept forms must clearly describe the specific analyses planned providing justification for the data and/or samples requested.

One of the value-added projects led by the University of Leicester recently published a finding that time of treatment affects radiotherapy response in some breast cancer patients according to genotype in circadian rhythm genes (Johnson et al. 2018. PMID: 30389261).

Interventional clinical trial
One of the interventional trials developed by members of the REQUITE consortium (SAHARA-01) will be
SAHARA-01 is a randomised phase III trial comparing the risk of breast fibrosis between the standard arm (hypofractionated) breast adjuvant radiotherapy (RT) and a personalised arm based on a predictive nomogram (NovaGray Breast®). Personalisation involves a new minimally invasive blood test developed by NovaGray. It is the only test that was validated prospectively as a measure of radiosensitivity and predictor of radiotherapy side-effect risk. The main objective of SAHARA-01 is to decrease significantly the incidence of severe breast fibrosis using the late toxicities predictor nomogram (including a biological assay and clinical independent factors) and by reducing the volume of irradiated breast in the group who are at low-risk of local recurrence. The secondary objectives are to evaluate the impact of personalised radiotherapy on acute toxicities, late toxicities (other than breast fibrosis) and the quality of life. This trial will investigate whether the NovaGray Breast® test can be used in a stratified medicine approach to reduce the level of ≥grade 2 late side-effects in breast cancer patients undergoing radiotherapy.

Training the next generation of clinicians & scientists
The REQUITE consortium have supported four PhD students throughout the course of the project. Each successfully defended their thesis that made use of REQUITE data, samples and resources. Two students Gilles Defraene & Chamberlain Mbah were REQUITE funded PhD students.
- Gilles Defraene defended his PhD and had the thesis accepted (January 2018): “Image based quantification of radiation induced lung damage”.
- Tim Rattay defended his PhD and had the thesis accepted (February 2018): “Predicting acute radiation toxicity in breast cancer”. This thesis is not currently available due to an embargo requested by the REQUITE consortium that core publications are prioritised above other research/ analyses making use of REQUITE resources.
- Kerstie Johnson defended her MD and the thesis accepted (July 2018): “Predicting radiotherapy toxicity in patients treated with radical radiotherapy using predictive assays and circadian rhythm”.
- Chamberlain Mbah defended his PhD and had the thesis accepted (September 2018): “Challenges in predicting normal tissue toxicity in radiotherapy”.

In addition, the following students made use of REQUITE data for their undergraduate/ postgraduate dissertations (not available online): two intercalated medical students; seven MSc students; four BSc students.

List of Websites:
www.requite.eu

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