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PROTOCOL  
Prospective Study of Outcomes in Sporadic versus Hereditary Breast Cancer  
(POSH Breast Cancer Study)

A prospective case controlled observational study of treatment choices and outcomes in young women with breast cancer

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## **Purpose**

The principal goal is to establish a first class biological resource linked to comprehensive clinical data that will allow a number of key research questions about hereditary breast cancer to be addressed. The proposal is to establish a collection comprising genomic DNA from 3,000 women newly diagnosed with breast cancers across participating centres in the UK. Data will be collected about diagnosis and treatment, conventional epidemiological risk factors and family history. A systematic pathology review of all cases will be carried out prospectively and tissue micro-arrays will be constructed. In addition, for centres where collection of fresh tumour tissue is routine, the existence of fresh tumour samples will be noted and linked to the central database creating in effect a “virtual” tissue bank.

Establishment of this biological resource will allow the principal research questions contained within this proposal to be answered but will also provide an invaluable resource for future valid and clinically important research questions.

The main clinical questions relate to prognosis of hereditary breast cancer compared to apparently sporadic cases and we have concentrated on the BRCA1 gene for the purposes of the current application although clearly other genes can be examined using the same proposed methods. The principal research questions are as follows:-

- Is there a measurable difference in relapse free and overall survival in BRCA1 carriers compared with contemporaneously treated age and stage matched sporadic breast cancers? (Is BRCA1 carrier status of independent prognostic significance?)
- What is the effect of adjuvant treatment on acute toxicity and on disease free survival in hereditary compared to sporadic cases and is there evidence of a chemopreventive effect from adjuvant treatments?
- If there is a difference, is this sufficient to influence clinical management where hereditary cancer is known or suspected?
- Can the observed pathobiological differences which appear to distinguish BRCA1 tumours from sporadic cases be replicated in this **prospective** and **age matched** cohort?
- Is there a pathological phenotype which can readily determine a subset of familial breast cancer cases not due to BRCA1 or BRCA2 which might facilitate identification of families for current studies aimed at discovering new breast cancer genes?

### Study End-points

Primary end points: 1) Breast cancer mortality and 2) distant metastasis free survival

Secondary endpoints: 1) ipsilateral breast tumour recurrence, 2) new primary breast cancer, 3) new primary cancer not in the breast.

Preliminary analysis of disease free survival will be possible at an early stage after completion of data collection (median follow up 5 years) but longer term prognosis will take long term follow up to establish (median follow up 15 years).

There are similar questions about other genes and other questions about cancer biology that could be answered using the proposed resource but it is anticipated that these will form the basis of future protocols and funding applications developed by this and other groups.

## **Background**

There are several areas of important clinical uncertainty regarding the management of hereditary

breast cancer. A striking area of uncertainty surrounds the prognosis for BRCA1 related breast cancers and current publications suggest the overall survival is better (Porter et al., 1994), the same (Eccles et al., 2001; Verhoog et al., 1998) or much worse (Stoppa-Lyonnet et al., 2000; Johannsson et al., 1998) than sporadic cancers. In addition, since BRCA1 and BRCA2 are involved in DNA repair, especially double stranded DNA breaks, the effect of adjuvant therapies in particular radiation therapy may differ in hereditary breast cancer compared to sporadic cases (Khanna and Jackson, 2001). This uncertainty is of critical importance and must be resolved since clinicians are already beginning to offer more radical surgical treatment to women at high genetic risk based on imperfect retrospective and biased studies. There is a need for good unbiased data to provide clear answers to the question of risk of death from the primary diagnosis versus risk of a second primary for each type of hereditary breast cancer in order that questions of treatment and prophylaxis can be given appropriate weight in the management decisions of patient and treating clinicians.

There is little useful information in the literature about any of these issues (Phillips and McKay, 2001; Phillips et al., 1999). There is a unique opportunity in the UK over the next 4-5 years for a trial of this type to collect unbiased information about treatment outcomes. In the UK at present most young women diagnosed with breast cancer have not had a genetic test. Technical challenges and cost limit the ability to perform rapid molecular genetic testing. Current data suggest that 5-10% of women diagnosed with breast cancer aged 40 years or younger will have a BRCA1 gene mutation – the published frequencies of mutation detection, in similar age groups, ranges from 2.6-15%. (Loman et al., 2001; Papelard et al., 2000; Malone et al., 2000; Turchetti et al., 2000; Eccles et al., 1998; Peto et al., 1999; Newman et al., 1998). Variations in prevalence of mutation carriers will depend on the sensitivity of analysis technique used and on the method of ascertainment of the population being studied. For example if BRCA1 *per se* confers a poor prognosis, studies where potential recruits have been omitted due to their early death will underestimate the prevalence of mutation carriers. In the UK there is no identified founder mutation, thus most newly diagnosed breast cancer cases will be treated in a conventional manner without knowledge of the patient's genetic status. The window of opportunity for this study is small. As technology moves on and genetic testing becomes more readily available, treatment decisions may be increasingly influenced by knowledge of the patient's genetic make-up, based on unreliable information from small, flawed retrospective studies.

## **Detailed research plan**

### ***Study design***

A large, well designed prospective cohort study is required in order to address the principal question of prognosis adequately. In designing this study a number of other possible options have been considered. A randomized controlled trial is not feasible because there is no accepted ideal treatment for hereditary breast cancer against which to examine an alternative. A case control study would suffer from ascertainment bias, the difficulties of appropriately matching controls for all potential variables and the need to collect data retrospectively.

#### *Inclusion criteria:*

All women diagnosed with invasive breast cancer at 40 years or younger in all participating centres.

Any woman diagnosed with invasive breast cancer with a known BRCA1 or BRCA2 gene mutation below age 50 years at diagnosis

#### *Exclusion criteria:*

Women who refuse their consent to retain diagnostic and follow up data [anonymised data on primary diagnostic and prognostic features to be retained wherever possible to clarify any biases of omission].

Previous invasive malignancy (with the exception of non-melanomatous skin cancer)

This cohort will comprise women with no known family history (50-70%), most of whom will not have mutations in analysed susceptibility genes and from which prognostically matched controls for identified “cases” will be drawn. Cases in the first instance will be women with identified BRCA1 gene mutations (by molecular analysis of the study cohort (see under statistics section for details).

#### Clinical Information and follow up

Initial patient questionnaires will collect information about epidemiological risk factors and family history of cancer. Study Clinical Trials Forms will be used to collect comprehensive data about diagnosis and all treatment modalities. Follow up Clinical Trials Forms will be sent annually for each patient to collect data on any new cancer related events including local and distant relapse and death.

#### Long term follow up

After 3-5 years most patients are usually discharged from routine follow up in the hospital. At this stage follow-up for patients recruited to the study will be most effectively facilitated by using the Medical Research Information Service (MRIS). MRIS will be able to provide mortality data – date of death and cause of death - for all recruits still resident in the UK at the time of death. This will be the most accurate record possible for the whole cohort and will not rely on the accuracy of local hospital records and the availability of local research personnel to search the hospital records. We will confirm mortality data reported in the first five years of follow up from recruiting centres and collect ongoing mortality data for the cohort. MRIS can also provide data that is not available from hospital records (for example if the patient did not die in hospital the date and cause of death may not be recorded). The collection of these data will provide a more accurate means of following the definitive endpoint for study patients. MRIS will inform the study about new cancer diagnoses but not currently about relapses from the original primary cancer. If a mechanism becomes available for flagging cancer relapses (not currently possible through MRIS but a high priority for Clinical Trials units and Cancer Registries), this will also facilitate more efficient follow up of patients. We intend to continue if funding allows, to collect data via hospitals about relapses, site of relapse, treatment and response but these data are not being returned comprehensively due to differing local priorities for research network staff in participating hospitals. The opinion of the National Information Governance Board (NIGB) regarding use of MRIS for long term follow up for this observational study will be sought via the MRIS application but it is not practical to re-consent all participants who consented to medical records review but not specifically to the use of MRIS to facilitate long term follow up. Patient representative views have been sought regarding this amendment to the POSH study protocol and briefly the view of the national Consumer Liaison Group was that “they would be unhappy to think that barriers were being raised on their behalf which meant that their contribution to research was being limited”.

#### ***Molecular analysis***

Since techniques for mutation scanning are developing rapidly, it is entirely possible that less expensive mutation screening technique may emerge in the future. For the present, the cost of molecular analyses has been estimated for BRCA1 using DHPLC (denaturing high performance liquid chromatography). The Wessex regional genetics laboratory has three DHPLC machines and considerable expertise in using this technique for large scale mutation screening. Grant funding to cover the cost of mutation analysis will be sought once the first 1000 samples are banked with a contemporary review of techniques balancing sensitivity and cost. Interim analysis

a cases using family history to estimate the likely proportion of gene carriers will allow re-assessment of the power of the study and will determine whether a larger collection might be preferable (this should be easier once the mechanism is well established in contributing centres and the cancer research networks well established). Collaboration with Dr Doug Easton in Cambridge has been agreed and newly diagnosed cases of breast cancer within the large UK cohort of BRCA1 and 2 gene carriers will enrich the ascertainment of contemporaneously treated gene carriers without compromising the study design.

One possibility for reducing the cost of mutation analysis for the purposes of the proposed study examining prognosis and treatment responses would be to select only high grade ER negative tumours which would be expected to include the majority of BRCA1 gene carriers. (Lakhani and et al, 1998; Turchetti et al., 2000). Individuals from the mutation negative and family history negative subgroup would provide ideal controls for identified gene carriers and the high grade of tumours would tend to be associated with a greater number of “events” for the early analyses.

### **Statistics**

The proposed study group of 3,000 women will contain an estimated 200 (10%) BRCA1 gene carriers. The power estimates depend critically on the prevalence of mutation carriers and we have carried out power analyses for a variety of possible BRCA1 prevalence rates. If the prevalence rate is 10% the study has 97% power to detect a difference in 2 year event rate of 20% in gene carriers compared with 10% for sporadic cases. If the prevalence rate of carriers drops to 5%, the power drops to 78% to detect the same difference in event rates. Enrichment for newly diagnosed gene carriers diagnosed across the UK will be possible by the established collaboration with Dr Doug Easton’s EMBRACE study (a study to define genetic and epidemiological penetrance modifiers in BRCA gene carries).

The difficulty of “confounding by indication” whereby the prognosis partly determines the choice of treatment, is potentially a significant problem for non randomised trials such as this (Moses, 1995). This problem can be allowed for by careful documentation of the rationale for particular treatment choices, including prophylactic surgery where this is undertaken. This information will be requested at the 6 month follow up and will allow a more informative analysis to be made. Demographic data and features of the primary tumour will be compared using the two-sample t-test or the Mann-Whitney test, as dictated by the distribution of the response variable. Treatment modalities will be compared using the chi-squared test. Follow up data will be compared using Kaplan-Meier survival curves, with the log rank test being used to compare the distribution of recurrence-free times. Analysis comparing outcomes for patients treated differently will be adjusted for patient characteristics using logistic or Cox regression.

### **Ethical considerations**

This study received full approval from the South and West MREC committee in April 2001.

#### *Genetic test results*

Very careful thought was given to the feedback of genetic information, the design has been discussed with experts in ethics and the law and the MRC’s published guidelines (MRC working group, 2001a; MRC working group, 2001b) have been adhered to. In the context of this study indiscriminate disclosure of genetic test results is inappropriate. The information sheet for participants makes clear the appropriate clinical route by which participants can seek genetic advice regarding genetic testing. Results available from this research can be made available to clinical genetics departments on request and with written consent from the participant in order to help focus diagnostic mutation searching. The membership of the UK Cancer Genetics Group (now affiliated to the British Society of Human Genetics) has had a number of opportunities to debate and approve the proposed study at meetings of the group over the last few years.

#### *Storage of data*

Clinical data will be collected from medical records and epidemiological and family history data by patient questionnaire. Storage of data will be on a secure, password protected database within the clinical oncology system with access restricted to study personnel only (MRC working group, 2001a). Laboratory analyses for genetic status will use samples coded by the data co-ordinator so that test results can only be linked back to study participants within the protected study database.

#### *Storage of biological material*

All blood samples will be returned by post to Southampton. Initially lymphocytes will be frozen for future extraction of DNA. Samples will be stored at  $-70^{\circ}\text{C}$  in the Oncology Unit laboratories, bar coded to match the patient study code in the clinical database.

Frozen tumour tissue will be routinely collected in some centres (including for example Southampton and Portsmouth). Where this is routinely effected in participating centres and these centres wish to retain control of samples, the existence of a fresh tumour sample, its location and the responsible clinician will be logged on the database such that this “virtual” tissue collection can be accessed for specific subgroups in the future. Participating centres will be encouraged to send fresh tumour samples in a solution of RNA later (provided by the study centre) to allow successful transport of samples by post to the study centre. Particular efforts will be directed to ensure storage of tumour samples from patients with a significant family history of breast and/or ovarian cancer. Any use of this resource would be the subject of a future grant application and ethical review (MRC working group, 2001b).

#### ***The steering group and collaborators***

The steering group experience covers clinical cancer genetics, molecular genetics and genetic epidemiology, breast cancer treatment, trial design and statistical analysis and molecular pathology. Other collaborators will be clinicians treating breast cancer in breast units throughout the UK. We have used the estimated number of recruits available in each centre and our local experience of high acceptability of the study to patients to project our predicted recruitment levels. Many have expressed a willingness to “cast the net wider” via colleagues and Cancer Networks.

#### ***Study management***

The steering group and collaborators communicate electronically and the steering group have met twice during the study design phase. Once recruitment is running well, the steering group will aim to meet annually. Any change to the study design or any additional studies proposed will be reviewed by the steering group.

#### ***Pilot data***

Eligible patients in the Southampton breast unit were identified via the oncology clinic and via the pathology department records from June 2001. 20 patients currently undergoing treatment for breast cancer and eligible for the study have been approached. All have consented to take part and the patient questionnaires have been completed satisfactorily. Only one patient (with no family history) has been referred to the clinical genetics service at her request.

#### **Publication of results**

All active collaborators and steering group members will be named in publications as a result of this study. An appropriate writing committee will be convened from amongst participants according to the specific aspect of the study being reported.

### **Future studies utilising the resource**

Proposals for future studies will be invited once the resource is established and proposals will be reviewed by the steering committee, the members of which have widespread expertise in all aspects of breast cancer diagnosis and management. A Memorandum of Agreement, application form and draft Material Transfer Agreement must be completed to ensure compliance with the main study aims and the current ethical permission. If an application is thought not to be compliant with the current ethical agreement then appropriate separate permission must be sought by the applicant. No identifiable data or samples will be released at any time for research.

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