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Welcome to another year’s annual Research Overview from the Genesis Breast Cancer Prevention Centre in Manchester. Once again we are proud to be supporting and sponsoring leading-edge research into breast cancer prediction, prevention, early detection and screening. Some of that research is lab-based basic science, and some is clinical research involving patients and volunteers.

This Overview explains what has been achieved during the last year, with particular emphasis on the work that has been published in scientific and medical journals. Those of you who are less scientific in background will be pleased to know that we also publish a summary of this work in a more lay-friendly format known as our Impact Report, and this can be accessed via our website www.genesisuk.org or through the Genesis office.

We hope you will find these overview documents interesting, and that you will pick up an exciting message contained within them; that breast cancer could potentially become a preventable disease for future generations if we work hard to make this goal possible.

Another message you will pick up from these pages is how collaborative our researchers are with other experts around the globe and how they work with a variety of fundraising bodies, trusts and charities.

The Genesis Prevention Centre itself is a collaborative venture with the NHS, and is part of a larger collaborative group of scientists and clinicians across Manchester known as the Manchester Breast Centre. This is not a building, but a ‘virtual centre’ of top experts in a variety of different fields who share our vision of making breast cancer a preventable disease. The Genesis Prevention Centre, however, is a real building – housing the UK’s largest Family History Clinic for women with a family history of breast cancer, and also housing one of the UK’s largest NHS Screening Units.

We enjoy the enormous good will of thousands of patients who come through our doors and who volunteer to take part in our research projects. We are also totally reliant on the dedicated Genesis supporters who through their fund-raising activities make all this work possible.

So thank you for picking up this Research Overview. We hope it inspires you to become long term supporters of what we are trying to achieve.

Lester Barr
Chairman of Genesis Breast Cancer Prevention
Overview of 2014/15
By Professor Tony Howell, Research Director

Genesis have always had breast cancer risk determination and prevention as the major focus of their research agenda since the inception of the charity in 1996. It is pleasing to see that other major research-based breast cancer charities are now also putting risk determination and prevention at the top of their list of research priorities.

Not only is this recognition of the prevention agenda important, but it should also lead to increased research funding in this area. One of those charities is Breast Cancer Now, who recently organised group meetings of senior investigators to decide the ‘gaps’ in our knowledge concerning breast cancer and breast biology.

‘Risk Determination and Prevention of Breast Cancer’ was identified as one of those gaps, and so a group of ten experts from across the UK (4 from Manchester) met to draw up a ‘Gap Analysis’ which would provide a blueprint for grant funding decisions. The analysis was published in the scientific journal, ‘Breast Cancer Research’, in September 2014.

As a measure of worldwide interest in this area, this paper has turned out to be one of the two most accessed in the journal Breast Cancer Research over the past year. The other most accessed paper was one on the ‘Angela Jolie effect’, which describes the increase in referrals to our clinic as a result of the publicity led by Gareth Evans. This paper also has the highest Altmetric score for any paper ever published in the journal.

The main gaps in our knowledge are shown in table 1, above. They fall into the four main areas of A) risk estimation B) prevention C) understanding the biology of breast cancer risk and prevention so that we can develop new prevention agents and D) applying our current prevention approaches (drugs and lifestyle change) to women at increased risk who

Table 1

A Gaps in risk estimation
1. The best standard model to estimate risk in the general population and in women at high risk.
2. What additional factors will give maximal improvement in a model?
3. Prediction of risk in the proportion of women with none of the current risk factors.

B Gaps in preventative therapy and lifestyle prevention.
1. Prediction of women who will benefit from current preventive therapy.
2. New agents for women who will not benefit from current preventive therapy.
3. Optimal measures for weight control and exercise; timings of this in the life course, who to target and type of interventions.

C Gaps in understanding the biology of breast cancer risk.
1. Mechanisms of the effects of pregnancy on breast cancer risk
2. Mechanisms of the lack of involution in some breasts with menopause?
3. Mechanism of energy restriction on reduction of risk.

D Gaps in implementing known preventive measures
1. Determination of the approximately 10% of women at high and moderate risk in populations.
2. How to make preventive therapy available to the subset of women who will benefit.
3. Optimal weight control and exercise programs for women at any age and in all countries and how we engage individuals in cancer prevention throughout the life course.
will benefit. Perhaps it is not surprising that this analysis summarises much of what we are doing in the Genesis Research Programme reviewed in this report!

Most of our clinical trial work is performed in a collaborative effort between Genesis and our colleagues in the Nightingale Breast Screening Centre. However there is usually a strong laboratory component to our studies. The laboratory work is performed in the Breast Cancer Now Unit and other laboratories at The Christie Hospital and with our collaborators on the main university campus, particularly in the Life Sciences, the Imaging Group at the Medical School, in Psychology and in Genetic Medicine at St Mary’s.

It is important for our research that Genesis, far-sightedly, funds laboratory and clinical work outside the Genesis Prevention Centre.

Our collaborative pathways are shown in figure 1, left, together with the individuals involved. Collectively we are all part of the Manchester Breast Centre (MBC) a group of 18 principle investigators, at least 11 of whom have a marked interest in risk and prevention.

Some years ago, two famous cancer biologists, Bob Weinberg and Doug Hanahan, published the widely regarded ten “Hallmarks of Cancer’ illustrating the main biological features of cancer.

**Figure 1.** The Genesis Prevention Centre forms the major clinical focus of risk estimation and prevention studies as part of the Manchester Breast Centre. Related laboratory and some clinical studies occur at the other sites indicated in the diagram. The named MBC investigators are those with an interest in prevention. The MBC provides an integrative focus for our research programmes.
We have suggested the eight ‘Hallmarks of Prevention’, see figure 2, above.

The upper four areas in the diagram are the ‘Hallmarks’ related to laboratory research (stem cells, sensor cells/hormones, stromal cells and the extracellular matrix) and the lower four areas (risk estimation, drug development, lifestyle prevention and imaging) are largely, but not completely, related to clinical research and the application of our work to appropriate populations of women. We show photos of the MBC investigators involved next to a particular ‘Hallmark’ but in practice each is usually associated with more than one.

In this report we outline the research progress we have made in relation to each ‘Hallmark’ and give an indication of our publications for each one.

Figure 2. The Genesis view of the key ‘Hallmarks’ of Breast Cancer Prevention. Work on the top four hallmarks is largely laboratory based and the lower four are largely clinically related although there is considerable overlap between them all. The principle investigators with interests in breast cancer prevention in Genesis/MBC are shown by a hallmark but, in practice, they are often interested in several.

1. Risk Estimation and Prevention of Breast Cancer
This and the next paper were the two most highly accessed papers published in 2014/15 in the journal ‘Breast Cancer Research’.

2. The Angelina Jolie effect: how high celebrity profile can have a major impact on provision of cancer related services.
Gareth Evans, Julian Barwell, Diana M Eccles, Amanda Collins, Louise Izatt, Chris Jacobs, Alan Donaldson, Angela F Brady, Andrew Cuthbert, Rachel Harrison, Sue Thomas, Anthony Howell, The FH02 Study Group, RGC teams, Zosia Miedzybrodzka, Alex Murray.
Referrals to our own and other Family History Clinics and Clinical Genetics Centres doubled as a result of the publicity surrounding the decision of Angelina Jolie to have risk reducing breast surgery after the discovery that she carries a mutation in BRCA1. Most of the referrals were judged to be appropriate indicating an unmet need for services for women at very high risk.

This is a brief overview if the management of women at high risk of breast cancer especially highlighting changes in management as a result of the new NICE guidelines ‘Familial Breast Cancer’ published in 2013.
Predicting risk of breast cancer

We now know a large number of risk factors for breast cancer but the major challenge surrounds how they might be optimally combined to give as precise an estimation of risk for women as possible so that they can make appropriate decisions concerning methods for prevention and the frequency of screening.

Work at Genesis surrounds the need for precise risk estimation in three groups of women, see figure 3, below.

1. Those in the general population who may or may not know their risk of breast cancer,
2. Women referred by their GPs to our Family History Clinic because of worries concerning breast cancer in the family, and;
3. Women who carry mutations in breast cancer genes such as BRCA1 and BRCA2.

The Genesis risk estimation programme covers all three groups in collaboration with our colleagues in the Nightingale Breast Screening Centre, and in Genetic Medicine at St Mary’s Hospital.

The most important recent advance is that we are beginning to understand the importance of combining standard risk factors (eg family history hormonal and lifestyle risk factors) with two other major risk factors, breast density and measurement of single nucleotide polymorphisms (SNPs) in the general population (the PROCAS Study), in the Family History Clinic (the FH-Risk Study) and in women with mutations in BRCA1 or BRCA2 (the Gene-Risk Study) (figure 3, right).

Predicting risk of breast cancer
The Predicting Cancer at Screening Study (PROCAS)

Over 56,000 women in the Nightingale Centre NHS Breast Screening Programme catchment area joined the PROCAS study between 2009 and 2015 (about 40% of total women who attended for screening).

During the five year follow up period over 700 women developed breast cancer. All had breast density estimated visually on their mammograms by our dedicated team of radiologists, radiographers and breast specialists. Ten thousand women also provided a saliva sample for estimation of ‘breast risk’ SNPs, figure 4, right.

To date we have estimated 18 SNPs in approximately 8,000 women. All women completed a questionnaire at entry to the study giving family history and hormonal risk factors and these ‘standard’ risk factors are entered into our risk estimation computer model (the Tyrer-Cuzick model [T-C]).

The effects of adding visually assessed mammographic density to the ten year risk distribution are shown in figure 5, right.

Importantly, the results indicate that when density is added to T-C more women fall into the NICE groupings of moderate (5-7.9% 10 year risk) and high (8%+ 10 year risk) risk categories which allows us to offer tamoxifen and raloxifene and an additional screen between the usual three yearly screening in the NHSBSP.

In the group of 8,000 women in PROCAS who had SNP18 measurements in addition to T-C and density the distribution of 10 year risk were ‘widened’ further, figure 6, table 2, page 9. Instead of 12% of women falling into the moderate and high risk groups, over 20% did so. Thus adding the three groups of risk factors allows more precise estimates of risk and we should consider using all three in the NHS Breast Screening Programme (NHSBSP).

We are now in a position to give risk feedback to women, a part of the original protocol. Risk feedback is based on T-C and adjusted density and, where available SNPs. On the trial entry questionnaires women were asked if they wished to be given their risk of breast cancer when available and, perhaps surprisingly, over 95% indicated they wished to do so. We asked women at...
The Predicting Cancer at Screening Study (PROCAS)

highest and lowest risk if they wished to have a face to face
or telephone consultation. To
date 70% of women invited at
high-risk attended counselling
(The results are summarised in
reference 7).

About 84% of women usually
re-attend for their next three
year mammogram but in this
high risk group it was 93%
indicating that giving feedback
risk information did not
inhibit, and actually increased,
subsequent screening uptake.
Women at low, average
and above average risk are
being informed by post
(20,000 to date with a further
33,000 to be contacted).
Risk feedback letters and
leaflets were developed by a
multidisciplinary team including
health psychologists, risk
communication experts, Family
History Clinic consultants,
researchers and our PPI panel,
who played a crucial role in this.

We are continuing to develop
and improve our risk feedback
resources through our PROCAS
Risk Communication study,
which is being run alongside
the PROCAS study. As part
of this we are conducting
qualitative interviews with
participants (performed by
Louise Gorman) of all risk
levels who have received a risk
feedback letter, so that we can
make improvements to our
letters and leaflets. We are also
investigating the psychological
effects of these letters via
a questionnaire sub-study.

Women at high risk based on
T-C and density or T-C, density
and SNPs continue to be offered
consultations.
The PROCAS study indicates that
it is possible to evaluate risk
and offer increased screening
and preventive measures to
women at moderate and high
risk in the context of a National
Breast Screening Programme.

In a new study, PROCAS-II,
we will automate the whole
process and evaluate giving risk
feedback soon after screening
attendance and we will include
psychological assessments of
this approach.

Figure 6, table 2

<p>| 10-year | Low | Ave | Above | Moderate | High | Total |</p>
<table>
<thead>
<tr>
<th>risks</th>
<th>&lt;1%</th>
<th>1-2%</th>
<th>2-3.5%</th>
<th>3.5-5%</th>
<th>5-8%</th>
<th>8%+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original T-C-no</td>
<td>15 1168 4416 1352 841 166</td>
<td>7958</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number reassigned to group</td>
<td>266 1328 798 1044 898 423</td>
<td>4570 (57.4%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast Cancer</td>
<td>4 32 31 50 45 28</td>
<td>190</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proportion with cancer</td>
<td>1.50% 2.40% 3.90% 4.80% 6.30% 6.60%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Reassignment of risk from original Tyrer-Cuzick 10-year risk category amongst 7958 women with SNP18 and density adjustment Reassignment of 57.4% of women appeared to be useful since those reassigned to lower risk groups had lower breast cancer incidence and those in higher risk groups higher.
The Family History Risk Study (FH-Risk)

The aim of the FH-Risk study is to determine whether we can improve risk estimation in the Family History Clinic (FHC) in women at moderate and high risk but who were shown not to carry BRCA1/2 mutations.

Women referred to the FHC are generally under the age of 50 with a family history of breast cancer. More than 10,000 women have been seen in the past 28 years and over 480 have developed breast cancer with 250 of these being tested negative for BRCA1/2 mutations.

Those without mutations were matched with 3 controls in a case control study. All women with breast cancer had a standard risk estimate and SNP 18 assessed (they have not had density assessed as yet). As with the PROCAS study, adding SNPs increased the number of women in high and low risk groups.

When the SNP scores are divided into quintiles women with the highest scores were 2.5 times more likely to develop breast cancer of women with low risk SNP18 (Figure 7, table 3, page 11).

These results may be improved further by the addition of density and will allow us to tailor our management advice in the Family History Clinic more precisely. 253 prospective breast cancers were age matched to controls at date of assessable mammogram. Mean lifetime risk by manual life tables approach were equivalent at around 30%. Women in the highest quintile of relative risk had a 2.5 fold increase in risk of prospective breast cancer compared with the lowest quintile. Exactly equivalent to the median predicted difference in relative risks. It is hoped using more SNPs (SNP100) will substantially improve risk discrimination further. (Reference: Michailidou K et al. Genome-wide association analysis of more than 120,000 individuals identifies 15 new susceptibility loci for breast cancer. Nat Genet 2015 Mar 9.)

Risk estimation of BRCA1 and BRCA2 carriers

The risks of breast cancer associated with BRCA1 and BRCA2 mutations vary considerably across studies but few have assessed prospective risks, which are likely to provide more reliable risk estimates for women undergoing testing before cancer development. Prospective breast cancer risks were assessed in 254
The Family History Risk Study (FH-Risk)

The probability of developing breast cancer was associated with stronger family histories and higher SNP aggregate scores in BRCA2. Prospective breast cancer risks in women in the UK are high especially for BRCA2 families ascertained on the basis of high risk. Women undergoing presymptomatic testing for BRCA2 should be quoted a wide range of possible breast cancer risks and should be steered within that range based on degree of family history, non-genetic risk factors and SNP testing.

**Risk reducing surgery**

Inherited mutations in BRCA1 or BRCA2 (BRCA1/2) confer very high risk of breast and ovarian cancers. Genetic testing and counselling can reduce risk and death from these cancers if appropriate preventive strategies are applied, including risk-reducing salpingo-oophorectomy (RRSO) and/or risk-reducing mastectomy (RRM).

However, some women who might benefit from these interventions do not take advantage of them. RRSO and RRM use was evaluated in a prospective cohort of 1,499 women with inherited BRCA1/2 mutations from 20 centres who enrolled in the study without prior cancer or RRSO or RRM and were followed forward for the occurrence of these events. Use of RRSO was 45% for BRCA1 and 34% for BRCA2 by age 40, and 86% for BRCA1 and 71% for BRCA2 by age 50. RRM usage was estimated to be 46% by age 70 in both BRCA1 and BRCA2 carriers. BRCA1 mutation carriers underwent RRSO more frequently than BRCA2 mutation carriers overall, but the uptake of RRSO in BRCA2 was similar after mutation testing and in women born since 1960. RRM uptake was similar for both BRCA1 and BRCA2.

Childbearing influenced the use of RRSO and RRM in both BRCA1 and BRCA2. Uptake of RRSO is high, but some women are still diagnosed with ovarian cancer before undergoing RRSO. This suggests that research is needed to understand the optimal timing of RRSO to maximize risk reduction and limit potential adverse consequences of RRSO.

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**Table 3. Reassignment of risk from original Tyrer-Cuzick 10-year risk category amongst 7958 women with SNP18 and density adjustment.** Reassignment of 57.4% of women appeared to be useful since those reassigned to lower risk groups had lower breast cancer incidence and those in higher risk groups higher.

<table>
<thead>
<tr>
<th>Quintile</th>
<th>RR</th>
<th>Median RR</th>
<th>Number</th>
<th>Mean age entry</th>
<th>Mean lifetime risk</th>
<th>Breast cancers (n)</th>
<th>Mean age BC diagn.</th>
<th>Follow up (yrs)</th>
<th>Rate/1,000</th>
<th>Odds ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>0.40-0.80</td>
<td>0.68</td>
<td>236</td>
<td>41.5</td>
<td>29.8%</td>
<td>34</td>
<td>50.39</td>
<td>2196.8</td>
<td>15.48</td>
<td>ref</td>
</tr>
<tr>
<td>2nd</td>
<td>0.80-0.97</td>
<td><strong>0.89</strong></td>
<td>234</td>
<td>40.5</td>
<td>31.3%</td>
<td>40</td>
<td>50.73</td>
<td>2324.9</td>
<td>17.21</td>
<td>1.11</td>
</tr>
<tr>
<td>3rd</td>
<td>0.97-1.18</td>
<td>1.09</td>
<td>234</td>
<td>42.0</td>
<td>29.5%</td>
<td>47</td>
<td>51.41</td>
<td>2109.5</td>
<td>22.28</td>
<td>1.44</td>
</tr>
<tr>
<td>4th</td>
<td>1.18-1.44</td>
<td>1.30</td>
<td>233</td>
<td>42.1</td>
<td>29.5%</td>
<td>55</td>
<td>49.55</td>
<td>2175.5</td>
<td>25.28</td>
<td>1.63</td>
</tr>
<tr>
<td>5th</td>
<td>1.45-3.00</td>
<td>1.70</td>
<td>233</td>
<td>42.6</td>
<td>29.7%</td>
<td>77</td>
<td>50.97</td>
<td>1981.9</td>
<td>38.85</td>
<td>2.51</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>1169</td>
<td></td>
<td></td>
<td>253</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

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**Figure 7.** Prediction of breast cancer incidence in women under follow up in the Genesis Family History Clinic. The percentage of women who developed breast cancer is related to the time of follow up and SNP scores. The SNP scores were divided into quintiles. Women with the highest quintile of SNP scores (top curve) were 2.5 times more likely to develop breast cancer than those in the lowest quintile (bottom curve).
General population/PROCAS


In this study we estimated the number of young women eligible for annual screening and chemoprevention according to NICE guidelines and also according to risk given by the Tyrer-Cuzick model in women who entered the PROCAS study between the ages of 46 and 49 years in order to try and determine the proportion of young women a increased risk and the number referred to FHCs According to NICE guidelines (which only consider family history) 3.7% of women were at moderate or high risk whereas when the Tyrer-Cuzick risk model was used it was 8.6%. These women would meet criteria for additional mammography screening and consideration of chemoprevention with tamoxifen. The great majority of such women are likely to be unaware of their eligibility for these interventions as only 17.5% had been seen in our FHC or as part of the Clinical Genetics Service previously. The study also raises the question of the most appropriate tools for risk estimation.


This study of women in the control arm of the IBIS-1 study also shows that visually assessed density refines risk estimation of the T-C model and is helpful corroboration of the Manchester predictions. We have obtained similar results in PROCAS in a very much larger series using the same computational methods (see PROCAS result in figure 5, page 8).


This is a preliminary report of the results of PROCAS with respect to adding SNPs to Tyrer-Cuzick. In PROCAS, 18 of the known 100 SNPs associated with breast cancer risk were measured. The results indicated that SNP18 adds considerably to the Tyrer-Cuzick risk prediction model but we estimated that measuring more SNPs would add further refinement. However, importantly, it is estimated that SNP 18 gives 2/3 of the risk of SNP100.


This paper summarises the results of our risk feedback in women identified to be at high and low risk in the PROCAS study.

Family History Clinic


This study, in most of the women who have attended the Family History Clinic over the years, indicates that our own clinic risk estimation method based on published tables (Claus) modified by non-genetic risk factors is accurate. Comparisons with other risk models are underway.

BRCA1/2 carriers


10. Can multiple SNP testing in BRCA2 and BRCA1 female carriers be used to improve risk prediction models in conjunction with clinical assessment? Prosperi MC, Ingham SL, Howell A, Laloo F, Buchan JE, Evans DG.


This series of studies in women with BRCA1 or BRCA2 mutations show that both genes are becoming more penetrant\(^6,10\). For BRCA2 adding SNP data (but not other candidate genes\(^11\) or telomere length\(^12\)) improves estimation of the development of breast and ovarian cancer. In a collaborative study variants (n=3,248) located within or around 445 candidate genes were estimated. Disappointingly there was little evidence that any of the evaluated candidate variants act as modifiers of breast and/or ovarian cancer risk in BRCA1 or BRCA2 mutation carriers\(^11\).


Use of risk reducing salpingo-oophorectomy (RRSO) was 45% for BRCA1 and 34% for BRCA2 by age 40, and 86% for BRCA1 and 71% for BRCA2 by age 50. RRM usage was estimated to be 46% by age 70 in both BRCA1 and BRCA2 carriers. Many women not undergoing RRSO developed ovarian cancer. It will be important to encourage all women to consider this procedure.


This is a useful review of the indications for risk reducing contralateral mastectomy in women already diagnosed with primary breast cancer.
Drug Development

The introduction of new drugs to treat cancer, as is widely known, is a hugely expensive process. It would be even more prohibitively expensive to develop drugs specifically for prevention.

We believe that the way around this problem is to test drugs already in use for other conditions/illnesses in order to see if they have preventive activity in the clinic. This is called ‘drug re-purposing’. Tamoxifen, raloxifene, anastrozole and exemestane, were all developed to treat breast cancer and have been ‘re-purposed’ for breast cancer prevention.

All of these drugs act directly or indirectly act through the oestrogen receptor present in ‘sensor’ cells in tumours or the normal breast. A major aim of our laboratory programme is to find new drug targets for prevention and to develop ‘re-purposed’ drugs to inhibit these targets (see laboratory programme).

Preventive drugs in the clinic

In June 2013 NICE published new guidelines which indicated that tamoxifen and raloxifene should be ‘offered’ to women at high risk of breast cancer (>8% 10 year risk) and ‘considered’ for women at moderated risk (5-7.9% 10 year risk). This recommendation was on the basis of a review of the clinical trials reported which tested tamoxifen or raloxifene.

In Manchester we entered the first woman into the IBIS-I in 1992 and a further 500+ volunteers with the enormous help of our two trial nurses Rosemary Greenhalgh and Jenny Affen.

The ‘20 year’ results of the IBIS-I trial were presented at the San Antonio Breast Cancer Symposium in December 2014. The major, and perhaps quite surprising, result of this updated analysis indicates that the 40% preventive effect seen with tamoxifen at five years lasts as up to a further fifteen years and thus way beyond the five year period of active treatment (figure 8, above).

Another prevention landmark was the formal publication of the IBIS-II trial in The Lancet in March 2014.

In this trial over 4,000 women were randomised to be treated for five years with the oestrogen lowering aromatase inhibitor, anastrozole, or with placebo. The results indicate that over one half of breast cancers were
prevented (figure 9A, above) and, in common with the other tested aromatase inhibitor (AI), exemestane, suggest that that AIs are superior to tamoxifen and raloxifene and have a better side effect profile with the exception that anastrozole may reduce bone density. However, we published in Lancet Oncology in December 2014 that this negative effect can be virtually completely eliminated by the use of the bone sparing bisphosphonate risedronate (figure 9B, above.)
Uptake of tamoxifen

In the IBIS-I tamoxifen prevention trial just over 10% of women at increased risk approached in our FHC agreed to be randomised to placebo or tamoxifen.

We wished to estimate whether this relatively low uptake was related to the fact that they were being asked to join a trial. Therefore we asked all women under follow up in our clinic between the ages of 33 and 46 whether they wished to take tamoxifen not in a trial scenario.

Of 1279 eligible women, 136 (10.6%) decided to take tamoxifen. Women >40 years (74 out of 553 (13.4%)) and those at higher non-BRCA-associated risk were more likely to accept tamoxifen (129 out of 1109 (11.6%). (Table 4, above.)

Interviews (performed by Louise Gorman) with women who did or did not choose to take tamoxifen highlighted four themes surrounding their decision of whether to take tamoxifen or not.

These four were, perceived impact of side effects, the impact of others’ experience on beliefs about tamoxifen, tamoxifen as a ‘cancer drug’, and daily reminder of cancer risk.

Interestingly, the women who did or did not take tamoxifen had similar concerns so that the final decision may be based on some other ‘gut’ feeling.

Thus, in our experience uptake of tamoxifen is about 10-15% irrespective of randomisation into a trial.

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**Table 4.** a BRCA (Breast Cancer 1 or 2, early onset gene mutation) negative refers to women who have known BRCA mutations in their family, but have personally tested negative for their familial mutation. BRCA untested represents women who have known BRCA mutations within the family but have not been tested themselves, thus are at a potential 51-85% risk. b TP53 mutation carrier.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Lifetime risk 17–25%</th>
<th>Lifetime risk 26–29%</th>
<th>Lifetime risk 40–50% not BRCA</th>
<th>BRCA negative</th>
<th>BRCA untested</th>
<th>Lifetime risk 51–85% (BRCA1/2 or TP53)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>33–35</td>
<td>2/40</td>
<td>8/63</td>
<td>0/26</td>
<td>0/4</td>
<td>0/17</td>
<td>0/11</td>
<td>11/161 (6.8%)</td>
</tr>
<tr>
<td>36–38</td>
<td>9/78</td>
<td>8/103</td>
<td>6/39</td>
<td>0/4</td>
<td>0/13</td>
<td>1/13</td>
<td>24/250 (9.6%)</td>
</tr>
<tr>
<td>39–40</td>
<td>6/52</td>
<td>10/95</td>
<td>4/31</td>
<td>0/9</td>
<td>0/9</td>
<td>0/5</td>
<td>20/201 (10%).</td>
</tr>
<tr>
<td>41–43</td>
<td>4/87</td>
<td>22/139</td>
<td>10/58</td>
<td>2/16</td>
<td>1/14</td>
<td>0/16</td>
<td>41/330 (12.4%)</td>
</tr>
<tr>
<td>&gt;43</td>
<td>11/101</td>
<td>21/151</td>
<td>8/46</td>
<td>3/22</td>
<td>0/9</td>
<td>0/8</td>
<td>42/337 (12.9%).</td>
</tr>
<tr>
<td>Total</td>
<td>32/358 (9.2%)</td>
<td>69/551</td>
<td>28/200 (14%)</td>
<td>5/55</td>
<td>1/62 (1.7%)</td>
<td>136/1279 (10.6%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 4. a BRCA (Breast Cancer 1 or 2, early onset gene mutation) negative refers to women who have known BRCA mutations in their family, but have personally tested negative for their familial mutation. BRCA untested represents women who have known BRCA mutations within the family but have not been tested themselves, thus are at a potential 51-85% risk. b TP53 mutation carrier.

The long-term results of this trial indicate that the reduction of risk of breast cancer continues for 10-15 years beyond the five years that tamoxifen is taken.


The reduction of risk of breast cancer was over 50% with little excess of side affects above the background seen in the controls apart from reduction in bone mineral density.


Anastrozole causes a reduction in bone mineral density. This study shows that this reduction can be prevented by the bisphosphonate, risedronate, taken once a week.


Of 1279 eligible women, 136 (10.6%) decided to take tamoxifen.


This study did not indicate, perhaps paradoxically, that the rate of metabolism of tamoxifen was relevant to its effectiveness
The Genesis Lifestyle Programme

There is considerable evidence that weight control/energy restriction (ER) and exercise can reduce breast cancer risk.

The Genesis Lifestyle programme involves women at moderate and high risk in the Family History Clinic and women in the NHS National Breast Screening Programme (PROCAS).

Our studies aim to determine:

1. How much weight control / ER and exercise influence breast cancer risk for these two groups of women,

2. What is the optimal method of ER and its mechanism of action on the breast tissue and fat compartments and;

3. How ER can be introduced for breast cancer prevention for women at high risk

Background

In 2006, we collaborated with investigators running the Iowa Women's Health Study in the United States to assess the effect of weight loss on breast cancer risk amongst 34,000 women. We demonstrated that women who lost over 5% of their body weight and maintained this reduction reduced their risk of breast cancer by approximately 25%-40% compared with women who continued to gain weight.

This finding was confirmed in a study of over 87,000 nurses in the Nurses Health Study, also in the USA. These reports stimulated us to introduce studies of weight control and ER into the Genesis Prevention Programme.

Weight loss and ER are well known to be difficult to achieve and maintain. In order to try and make it easier, Michelle Harvie developed an intermittent diet, the 2 Day Diet.

This involves 2 days of strict energy restriction and 5 days of normal eating each week. Randomised trials, conducted at the Genesis Centre between 2006 and 2012, demonstrated that the 2 Day Diet (Intermittent ER [IER]) was more effective when compared with standard continuous energy restriction (Continuous ER[CER i.e. dieting 7 days per week]).

Women were more able to maintain the intermittent diet and lost twice as much fat as daily dieters.

Importantly IER lead to greater reductions in insulin than daily restriction, suggesting additional metabolic advantages for IER. The BBC presenter, Michael Mosely, highlighted this intermittent approach on BBC Television in August 2012. Ongoing publicity about our research has made IER otherwise known as the 5:2 diet increasingly popular.

Genesis is proud that, despite the proliferation of 5:2 diet books, that the 2 Day Diet was developed and first tested here in the Prevention Centre. We currently have the only data indicating the superiority of IER compared with CER in randomised trials.

The intermittent approach to energy restriction seems to be effective for weight control in women at high risk of breast cancer.
breast cancer. However many questions arise concerning its effect on the breast (our major interest!) and on other key fat stores in the body (in the liver, pancreas and muscle) and whether it is effective in other clinical situations; for example whether it is more effective than CER in preventing the weight gain associated with chemotherapy and whether IER adds to chemotherapy in women with advanced breast cancer.

We have completed some studies to answer these questions as outlined above and several are in progress as shown in figure 10, above.

**Update of the BRRIDE-1 study of breast biopsy before and after one month’s intermittent energy restriction.**

We assessed changes in expression of a large number of genes before and after breast biopsies in women on the intermittent diet. The assays were performed in the laboratories of Robert Clarke at the Breakthrough Unit in Manchester and Dr Andrew Sims in Edinburgh.

We also measured changes in serum and urine metabolites in conjunction with Professor Roy Goodacre at the Manchester Institute of Biotechnology (http://www.isrctn.com/ISRCTN77916487). Not surprisingly many genes related to synthesis of fats were reduced in the breast cells. However we were surprised to see that, in the hundred top changing genes, an increase in the expression of genes which indicated differentiation of the breast which would potentially make the cells more stable and less likely to initiate cancer (Figure 11, see overleaf, page 20). These were genes for the milk proteins lactalbumin and casein and also mammaglobin and several mucins. Thus it appears that energy restriction reduces metabolism and increases differentiation of the breast which, may reduce cancers developing also, may be how it may prevent cancer recurring for women who have been diagnosed.

We also demonstrated that compared with CER, IER causes a change (some up and some down) of about 200 metabolites in serum and urine during the two day energy restriction period which return to baseline values over the subsequent five days of normal eating. The benefits or otherwise of these changes remain to be investigated.
Studies in progress

Here we briefly describe lifestyle studies in progress which are also illustrated in figure 11, right.

How much does weight control /ER and exercise influence cancer risk?

We are currently modelling the impact of current weight, adult weight gain, clothes size, exercise, and alcohol on risk of breast cancer amongst 10,000 high risk women in the family history clinic and 57,500 women in the National Breast Screening programme in collaboration with Prof Evans in the PROCAS and FH risk studies.

BRRIDE 2.

An ideal cancer prevention diet would be easy to adhere to and preferentially reduce ‘bad’ fat stored in the liver and around organs in the abdomen and hence specifically reduce insulin resistance and levels of other circulating hormones and inflammatory blood marker which promote breast cancer.

At the same time, an ideal diet would preserve muscle mass and metabolic rate. In the BRRIDE 2 study, funded by Genesis, we are testing whether IER will lead to a greater reduction in fat within the liver and abdomen compared with a daily low calorie diet using magnetic resonance imaging in 26 obese women at increased risk of breast cancer.

In addition we will compare the effects of the two diets on insulin resistance, other breast cancer blood markers, muscle mass and metabolic rate. This study will define the effects of intermittent compared to standard dieting on metabolism and, indirectly, the potential effects on reducing breast cancer risk (figure 10, see page 19).
PROCAS – Lifestyle Breast Cancer Prevention Feasibility Study.

The NHS Breast Screening Programme includes approximately 70% of women aged 47 – 73 invited for screening every 3 years, but the programme does not currently offer any advice on risk estimation (see above) or programmes for prevention.

This Genesis funded study will determine how we can engage women in the NHSBSP to undertake a lifestyle breast cancer prevention programme. The study involves 120 women in the NHSBSP who have received their personalised risk of breast cancer as part of the PROCAS study. We are inviting women in all risk categories (http://public.ukcrn.org.uk/Search/StudyDetail.aspx?StudyID=17480).

The questions to be asked are: Does the level of risk of breast cancer influence uptake and adherence / Does advice/ information about risks of other conditions such as cardiovascular disease and diabetes with an NHS health check increase adherence to the programme?

Can women successfully lose weight and maintain weight loss with a supportive self monitoring 2 Day Diet website?

In another study (B-AHEAD2) we are assessing whether The 2 Day Diet is superior to CER to prevent weight gain and toxicity from chemotherapy and hence risk of breast cancer recurrence in a randomised trial amongst 170 women receiving adjuvant or neo-adjuvant chemotherapy.

We are also assessing the value of IER (B-AHEAD 3) when given with chemotherapy compared with chemotherapy alone in 134 women with advanced (metastatic) breast cancer with the aim of reducing toxicity and increasing duration of remission. (http://public.ukcrn.org.uk/Search/StudyDetail.aspx?StudyID=18070)

Selected presentations by Michelle Harvie


The workshop summarised changes that occurred in our society over the past sixty years including an increase in daily energy intake and, as a result increased, adult and adolescent obesity (figure 12, below.) The meeting emphasised the detrimental effects on health of shift-working and irregular meals on our circadian clocks and, in turn, our health. Also the potential beneficial effects of IER including improved cellular responses to stress, positive changes in metabolism, reduction of inflammation and improved cellular repair mechanisms 20.

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2. American Society for Clinical Oncology (Chicago May 2014) plenary seminar on “Special diets and Supplements” use in cancer. This was a review of evidence for lifestyle recommendations for cancer patients. There is evidence that weight control and exercise may reduce recurrence after a diagnosis of breast cancer. For supplements, unfortunately, perhaps with the exception of fish oil and vitamin D supplements, there is little evidence of benefit. Some antioxidant supplements could actually increase risk of cancer and cancer deaths. Michelle has also been asked to talk at this year’s ASCO conference on weight gain and cancer.


Publications


This review by leaders in the field summarises data of the effects on health of meal frequency and timing and intermittent versus continuous energy restriction.


This review indicated that many supplements have been shown to cause harm and, for a large number, there is little evidence for harm or benefit.


We have investigated the beliefs of high risk women concerning the effectiveness of lifestyle change in preventing breast cancer. This study involved in depth interviews with 20 women who had been in one of our Genesis weight loss programmes. Their beliefs around lifestyle and breast cancer depended mainly on women’s social networks, media reports and personal experience of breast cancer. We concluded that future counselling and health education material should be tailored to facilitate understanding of both genetic and modifiable lifestyle risk factors and that we should do more help individuals to visualise the weight and breast cancer link.


Resisting temptation has a greater relationship to weight loss than motivation!
Studies on breast imaging

Imaging of the breast by mammography is obviously vital for the NHS Breast Screening Programme and the use of mammography and magnetic resonance imaging (MRI) is also essential for following up women with mutations in BRCA1 and BRCA2.

However, here we summarise some newer aspects of imaging - either new uses of standard imaging measures or new measures of assessment of the breast in studies performed in the Nightingale Breast Screening Centre in association with the Genesis Prevention Centre and colleagues in the Centre for Imaging Sciences.

Mammography
Perhaps the current most important study concerning mammography is related to PROCAS. The radiologists, breast physicians and radiographers at the Nightingale Centre estimated the density of each of four views (two on each side) using a visual analogue scale (VAS) on over 50,000 women in the PROCAS study. The distribution of density is shown in figure 13, right.

By combining the percentage of the breast with dense tissue (VAS) and risk estimates derived from the Tyrer-Cuzick model the prediction of risk was improved by using the combination. Figure 5, page 8, shows that percentage density performs marginally better in predicting risk distribution compared with the Tyrer-Cuzick model alone but the optimal result is seen when the two methods are combined. The combination results in more women being found to be at high and low risk of breast cancer and is important when giving risk feedback in the clinic.

The Family History 2 (FH02) Study
An important question in the FHC is when should we begin to screen women found to be at increased breast cancer risk. We have previously reported, with others, that women in the FHC aged between 40 and 49 have a 20% reduction in breast cancer mortality as a result of annual mammographic screening (FH01 collaborative teams. Lancet Oncology 2010 11:1127-34).
Studies on breast imaging

Starting screening at 40 in women with a family history of breast cancer is indicated in NICE guidelines. However in the Manchester FHC we have instituted screening at the age of 35 since it’s inception in 1987. The FH02 trial is designed to answer the question of whether annual screening between the ages of 35 and 39 is useful.

This prospective study (FH02) in women with a lifetime breast cancer risk of ≥ 17 % is currently underway but will not report until 2018. Invasive tumour size, lymph node status and current vital status were all significantly better compared with two control groups of unscreened women. These data suggest that screening at this young age will worthwhile but we need to await the full results of FH02 in 2018.

Automated density estimation (Quantra and Volpara)

We are eternally grateful for the enormous effort of visually estimating density on so many mammograms. VAS had to be done in order to show proof of principle that density adds to the standard Tyrer-Cuzick model, and since area-based measures of percent density have provided much of the evidence for association between mammographic density and breast cancer risk. However, visual assessment is subjective, with different readers estimating the percentage differently. It is also time consuming, so if adding density is rolled out into the NHSBSP, automated measures of density would be preferable. Sue Astley, Reader in Imaging at the University, has been leading on the assessment of two automatic measures of the volume of density in the breast called Volpara and Quantra. Preliminary studies in over 38,430 women suggest that the automated measure Volpara, in particular, adds to
Studies on breast imaging

the Tyrer-Cuzick risk estimate and thus may be used to assess density in the National Screening Programme (figure 14, above). Automated methods also enable the assessment of volumes of fat and gland separately, rather than relative measurements produced by visual inspection of mammograms.

Another use of automated density measurement is for the assessment of response to tamoxifen for its preventive effect. In a previous study we reported that women who had a reduction in mammographic density during tamoxifen therapy (about one half of the women) were likely to have prevention of subsequent tumour development whereas if there was no change in density on the mammogram there was no preventive effect.

In a repeat of this study (called TAM/Prev) the same radiologist (Dr Ruth Warren from Cambridge) demonstrated that about half of the 106 women who completed one year of tamoxifen treatment ‘responded’ in this study also. However our own radiologists were unable to detect the decline in density in the same way indicating that it is unlikely that this method could be rolled out to other centres using visual methods.

In the same study we estimated change using the volumetric density measures Quantra and Volpara and found that volumetric density declined in response to tamoxifen with fair correlation with Dr Warren’s visual method (table 3, figure 15).

This is a very encouraging result but further work is necessary to evaluate the importance of the change in volumetric density and this is the subject of a new grant from Breast Cancer Now.
Another important issue regarding density is being addressed by Sue Astley and her colleagues using the Volpara volumetric technique. Although the breast density is a strong risk factor for breast cancer development most women who develop the disease do not have high density (see figure 13, page 23).

It is possible that rather than all of the breast being dense there are localised areas of high density where tumours arise. Dr Astley’s team have demonstrated that this may be the case by comparing, in the PROCAS study, localised areas of density. She has demonstrated that before cancers arise there are localised areas of high density compared with the mirror image area in the opposite breast (figure 16, above). Development of these observations may lead to new methods for screening depending on differences between breasts.

**Digital breast tomosynthesis (DBT)**

A major problem in women with very dense breasts is that tumours may be missed (this is called masking). Tony Maxwell (Senior Lecturer in Radiology) at the Nightingale with Sue Astley are assessing the method of mammography called Digital Breast Tomosynthesis. (DBT). DBT is a relatively new technique which produces three-dimensional images of the breast in a series of thin (usually 1mm) slices much like a CT scan. A number of small studies have demonstrated that DBT has a higher sensitivity and specificity for cancer detection than conventional 2D digital mammography.

As participants in the UK’s major DBT screening trial (the Tommy Trial) we now have considerable experience with this imaging method.

The Tommy trial showed that DBT used with mammography is better at detecting cancers in dense breasts and for detecting small invasive cancers. The major benefit was that specificity was improved, so fewer women would be recalled if this was used for screening.

Since DBT may be of particular benefit in screening younger women (40 to 49 years of age) who tend to have denser breasts than older women we have a trial underway in women in our FHC to assess whether DBT can increase the specificity of screening these women, many of whom have dense breasts.

**The use of Magnetic Resonance Imaging**

Women with a genetic predisposition to breast cancer tend to develop the disease at a younger age with denser breasts making mammography screening less effective as noted above.

The introduction of magnetic resonance imaging (MRI) for familial breast cancer screening programmes in recent years was intended to improve outcomes in these women.
We aimed to assess whether introduction of MRI surveillance improves 5- and 10-year survival of high-risk women and determine the accuracy of MRI breast cancer detection compared with mammography-only or no enhanced surveillance and compare size and pathology of cancers detected in women screened with MRI + mammography and mammography only.

We used data from two prospective studies where asymptomatic women with a very high breast cancer risk were screened by either mammography alone or with MRI also compared with BRCA1/2 carriers with no intensive surveillance (28). 63 cancers were detected in women receiving MRI + mammography and 76 in women receiving mammography only.

Sensitivity of MRI + mammography was 93% with 63% specificity. Fewer cancers detected on MRI were lymph node positive compared to mammography/no additional screening. There were no differences in 10-year survival between the MRI + mammography and mammography-only groups, but survival was significantly higher in the MRI-screened group (95.3%) compared to no intensive screening (73.7%; p = 0.002).

There were no deaths among the 21 BRCA2 carriers receiving MRI. There appears to be benefit from screening with MRI, particularly in BRCA2 carriers. Extended follow-up of larger numbers of high-risk women is required to assess long-term survival.

We are also exploring the use of MRI in two new studies supported by Genesis and Breast Cancer Now. One is exploring the effect of the anti-progestin, Ullipristal, in women at high risk of breast cancer (PI Dr S Howell) and the other is assessing the value of MRI in detecting the response of the breast to raloxifene and tamoxifen in postmenopausal women (PI Prof G Evans).

Although MRI is expensive and cannot be used on a population basis we hope that MRI will give us insights into the mechanism of the effects of these anti-hormones on the breast. Tony Maxwell is leading the MRI aspects of these studies. Sue Astley is leading research into computer-based analysis of breast images.

There are three main areas of focus. Firstly, with colleagues in Imaging Science, she is developing software to reliably estimate the volume of the compressed breast. This in turn will result in more accurate measurement of breast density.
Secondly, methods for analysis of breast texture are being investigated.

These mimic radiologists’ assessment of mammographic patterns and may be useful in association with volumetric breast density to better characterize breast cancer risk.

Finally, analysis of DBT projection images, which are taken at different angles, is being used to gain an insight into the relationship between area-based breast density methods and volumetric methods.

**Publications**


This paper reports a preliminary analysis which suggests that screening in high risk women age 35-39 will be beneficial. However we now await confirmation in the ongoing FH02 trial.


The Tommy trial showed that DBT used with mammography is better at detecting cancers in dense breasts and for detecting small invasive cancers. The major benefit was that specificity was improved, so fewer women would be recalled if this was used for screening.


27. Local mammographic density as a predictor of breast cancer Mayu Otsuka, Elaine F Harkness, Xin Chen, Emmanouil Moschidis, Megan Bydder Soujanya Gadde, Yit Y Lim, Anthony J Maxwell, D Gareth Evans, Anthony Howell, Paula Stavrinos, Mary Wilson, Susan M Astley SPIE Medical Imaging in press.

This study shows that areas in the breast where cancers will arise have increased density compared with similar sites in the contralateral breast.


MRI added to mammography is associated with some improvement in survival in women with BRCA1/2 mutations.
The four ‘Hallmarks’ of prevention related to the Genesis/MBC laboratory programme comprise work on the ‘compartments’ of the breast including stem cells, sensor cells and cellular and non-cellular stroma. All of these are thought to be related to the development of breast cancer but most of the mechanisms involved are unclear.

Alterations in stem cells in the ducts and acini of the breast are probably key to breast cancer. However we know that the activity of the stem cell can be influenced factors released by other cells, such as adjacent hormone sensing cells and fibroblasts, and also the non-cellular part of the breast, the extracellular matrix, can specifically interact with stem cells (figure 17, above).

Thus, not only the stem cell, but also the other cells and the matrix may be targets for preventive therapy.

Figure 18, right, shows the basic structure of the breast. Terminal ducts lead to lobules and the whole is called the terminal duct lobular unit (TDLU). Each lobule has several finger-like ductules which are seen in cross section. An electron micrograph shows the epithelial cells of the ductile surrounded by stromal cells. The fatty and collagenous stroma are seen in the main picture.

In this section of the Annual Report we give some examples of the studies attempting to unravel the complexity of the breast related to our four laboratory ‘Hallmarks’.
The stem cell as a prevention target

There is considerable worldwide effort to target the stem cell for cancer treatment and prevention. Our interest is in the prevention arena is to develop re-purposed drugs which may be used for prevention by targeting epithelial stem cells 29-31.

Recent studies indicate that stem cells may differ from differentiated cells by having greater numbers of mitochondria. We know that many common antibiotics target the mitochondrion.

The effects of antibiotics and other inhibitors of mitochondria against stem tumour stem cells (Mammosphere forming cells) have been tested in the Breakthrough Unit (Gillian Farnie, Michael Lisanti & Federica Sotgia).

In figure 19, above, we show the results of one experiment. It it indicates that the antibiotic doxycycline has little inhibitory effect on tumour cells overall, some effect when the cells are cultured with fibroblasts but a major effect when added in a tumour stem cell assay (mammosphere formation)31.

There is evidence that doxycycline can inhibit tumours in animals and man and so future work will be on how such antibiotics affect stem cells in patients and whether it is likely to be clinically useful.

Figure 19. The effect of the antibiotic, doxycycline, on MCF-7 cells in culture.

A When MCF-7 cells are grown in monolayer that are resistant to doxycycline (light blue). However when co-cultured with fibroblasts they become somewhat more sensitive (red). However maximal sensitivity to doxycycline is seen when cells are grown as mammospheres suggesting that the mammosphere forming stem cell is the most sensitive cell and suggests that doxycycline could be used in the clinic for cancer treatment and

Other stem cell inhibitors we have assessed include lapatinib32, graphene oxide33, and sulphoradex34 all look promising but require further investigation.

Sulphoradex is a long acting form of sulphoraphane, an anticancer agent found in broccoli and other cruciferous vegetables. Sulphoradex inhibits breast stem cells in-vitro but also primary and secondary breast cancers in immune deprived mice in patient derived xenograft models (PDX)35 by over 50%. It has completed phase I trials in man and now can be tested in the clinic.

This is the first study to show that the stem cells (these form mammospheres in culture) have marked differences in protein expression to the bulk of tumour cells.


Proteins involved in regulating the synthesis of other proteins were particularly upregulated in stem cells. Protein synthesis inhibitors reduced tumour cell growth. Interestingly reduction of the amino acid methionine, known to inhibit protein synthesis also reduced drug. Methionine restriction has been suggested as a treatment for cancer equivalent to calorie restriction.


Mitochondria, the power houses of the cell, were also up-regulated in stem cells. Proliferation of stem cells could be inhibited by relatively low concentrations of antibiotics. These are worth testing in the clinic.


34. Sulforadex targets breast cancer stem-like cells in patient-derived cells and xenograft tumoursBruno Simões, Denis Alferz, Rachel Eyre, Kath Spence, Angelica Santiago-Gomez, Iris Tanaka, Bertram Kohler, David Howat, Sacha Howell, Robert Clarke American Association for Cancer Research 2015 in press

The long acting form of sulforaphane, Sulforadex, inhibits breast tumour stem cell growth by over 50% in vitro and against human tumours growing in patient derived tumour xenografts.


Recently, there has been an increasing interest in the development and characterisation of patient-derived tumour xenograft (PDX) models for cancer research. PDX models mostly retain the principal histologic and genetic characteristics of their donor tumour and remain stable across passages. These models have been shown to be predictive of clinical outcomes and are being used for preclinical drug evaluation, biomarker identification, biologic studies, and personalised medicine strategies. This article summarises the current state of the art in this field, including methodological issues, available collections, practical applications, challenges and shortcomings, and future directions, and introduces a European consortium of PDX models. PDX models are increasingly used in translational cancer research. These models are useful for drug screening, biomarker development, and the preclinical evaluation of personalised medicine strategies. This review provides a timely overview of the key characteristics of PDX models and a detailed discussion of future directions in the field.
The ‘sensor’ cell as a target for prevention

Many years ago we demonstrated that the activity of the stem cell is controlled by so called ‘sensor’ cells which, because they express oestrogen and progesterone receptors, allow the breast to respond to systemic hormones. Stem cells divide in response to signals from sensor cells which include notch, growth hormone and RANK ligand. Inhibition of the effects of oestrogen on the sensor cell with drugs such as tamoxifen, raloxifene and anastrozole (indirectly) is the basis of all current preventive therapies.

Recently with our collaborator, Professor Rama Khokha in Toronto we demonstrated that blocking the sensor cell with antiprogestins inhibited the proliferation of normal breast stem cells during the menstrual cycle. This forms the basis of our studies in the laboratory on developing new antiprogestins and testing them on women at high risk of breast cancer. These studies will begin later this year (2015).


The breast stroma as a target

The non-epithelial part of the breast is called the stroma. It comprises stromal cells (including fibroblasts, adipocytes immune cells and vascular cells) and also the non-cellular extracellular matrix including growth factors and cytokines, proteoglycans and collagens (ECM, figure 16, page 26). The importance of all of these factors in breast development was summarised by Beatrice Howard at the Breakthrough Centre in London and by Paul Lu in Life Sciences at the University and formerly at the Breakthrough Unit in Manchester.


This review focusses on the dynamic interactions that the stroma engages with the epithelium during mammary specification, cell differentiation, and branching morphogenesis of both the embryonic and postnatal development of the mammary gland. Similar stromal-epithelial interactions underlie the aetiology of breast cancer, making targeting the cancer stroma an increasingly important and promising therapeutic strategy to pursue for breast cancer treatment.


“Breast density” gene signature consisting of>1250 transcripts that were significantly increased in fibroblasts taken from women with dense breasts (HD) were compared with those from non-dense breast (LD). Relative to LD fibroblasts. HD fibroblasts

Stromal cells

One of the most important stromal cell is the fibroblast which is involved in synthesis of ECM molecules such as collagen, proteoglycans, cytokines and growth factors. Recently we demonstrated that fibroblasts taken from women with high mammographic density are highly active and synthesise more growth factors than fibroblasts from low density areas. This has some similarities with the ‘activated’ cancer associated fibroblasts seen in breast tumours (figure 20, overleaf page 33). We are investigating a number of new drugs that can reverse these processes and which could be potentially used for prevention of breast cancer. We have already shown that one of the mechanisms of action of tamoxifen is to reduce breast density presumably by reduction of collagen production by fibroblasts (See ‘Imaging’).
show the up-regulation and/or hyper-activation of several key cellular processes and several key signaling pathways including JNK1, iNOS, Rho GTPase(s), FGF-R, EGF-R, and PDGF-R-mediated signal transduction, thereby creating a pro-inflammatory, pro-proliferative, cytokine, and chemokine-rich microenvironment. JNK1 stress signaling is the single most significant biological process that was upregulated. Similarities between the HD fibroblast gene signature, wound healing, and the cancer-associated fibroblast phenotype were reported. Thus, this unbiased informatics analysis of high breast density provides a novel framework for additional experimental exploration and new hypothesis-driven breast cancer prevention research. The papers below emphasise the importance of interactions between cancer fibroblasts and tumour cells: a mechanism which may be happening in women with high density breasts.


Soluble stromal factors acting via classical cell surface receptors on epithelial cells

Fibroblasts and other cells in the stroma may produce soluble growth factors and cytokines which may dramatically affect epithelial cell function via classical receptors on the epithelial cell surface.

42. FGF ligands of the postnatal mammary stroma regulate distinct aspects of epithelial morphogenesis. Zhang X, Martinez D, Koledova Z, Qiao G, Streuli CH, Lu P. Development. 2014 Sep;141(17):3352-62. doi: 10.1242/dev.106732. This study demonstrated that fibroblast derived fibroblast growth factors (FGFs) stimulates the early development of the epithelial branching of the mouse mammary gland. FGF10 regulates branch initiation, which depends on directional epithelial migration. By contrast, FGF2 controls ductal elongation, requiring cell proliferation and epithelial expansion both via the epithelial cell surface receptor FGF2. Thus different FGF ligands regulate distinct aspects of epithelial behaviour of the breast.

43. Modulation of fibroblast growth factor signaling is essential for mammary epithelial morphogenesis. Zhang X, Qiao G, Lu P. PLoS One. 2014 Apr 9;9(4):e92735. This study demonstrated that fibroblast growth factor (FGF) signaling is reduced by an enzyme called sprouty2 in the epithelium which may act as a brake and protect the epithelium against tumorigenesis.

44. A differential role for CXCR4 in the regulation of normal versus malignant breast stem cell activity. Ablett MP, O'Brien CS, Sims AH, Farnie G, Clarke RB. Oncotarget. 2014 Feb 15;5(3):599-612. The epithelial cell cytokine receptor, C-X-C chemokine receptor type 4 (CXCR4) on epithelial stem cells is stimulated stromal derived factor 1 in tumours but not normal stem cells. Thus cancer formation may be related to a change in the activity of the CXCR-4 receptor. Inhibitors of CXCR-4 are in clinical development.

Stromal components which act directly via integrins

Integrins are cell surface receptors for extracellular matrix components, such as collagens and laminins, which sense the microenvironment of the cells.

Charles Streuli working with the surgeons (Ashu Ghandi, Cliona Kirwan) has shown that biopsies from dense areas of the breast show remodelled collagen architecture, which in turn increases stiffness of the breast. Several studies indicate that stiffness of the breast may lead to epithelial dysfunction and which, in turn, may stimulate epithelial dysfunction and tumour development.

Other component of the ECM include long sugar molecules associated with proteins which are called called proteoglycans and have many functions including regulating growth factor activity and interacting with integrins. The problem is that until recently it has been extremely difficult to determine the importance of proteoglycans in the breast. Using a new in-vitro assay comprising short protein octamers (hydrogels) Gillian Farnie has demonstrated that heparin sulphate proteoglycan and laminin can stimulate the appearance of malignancy in mammary cells. This assay holds great promise in being able to work out how the ECM affects epithelial cells.

Gillian has also recently demonstrated that a kinase related to signalling from the surface of the cell via integrins (Focal Adhesion Kinase FAK) is over-expressed in pre-invasive cancer which is leading to testing of FAK inhibitors in the clinic.

Integrin mediated changes

45. Signalling pathways linking integrins with cell cycle progression. Moreno-Layseca P, Streuli CH. Matrix Biol. 2014 Feb;34:144-53. Integrins are adhesion receptors that allow cells to sense and respond to microenvironmental signals encoded by the extracellular matrix. They are crucial for the adhesion, survival, proliferation, differentiation and migration of most cell types. This paper is a review of recent studies to elucidate mechanisms of integrin-dependent cell growth.

In this review Ucar and Streuli highlight data which indicate that progesterone secretion during pregnancy activates beta3 integrin and increases stem cell numbers during pregnancy.


Another example of integrin stimulation stem cell proliferation in the breast


Density is associated with altered periductal collagen and stiffness.

49. Merry CLR, Meade K, Lowe E, Saiani A, Miller A. Use of hydrogels for stem cell culture. PCT Submission WO 2013124620 A1, filed in the USA and Europe October 2014

The patent submission for the hydrogels


This important paper indicates that the signaling system between the ECM and the cell is upregulated in DCIS. A member of this pathway is focal adhesion kinase (FAK). Inhibitors of FAK stop DCIS growth and are now being considered for the clinic.


Our highlights of 2014

1. Publication of “Hallmarks of risk and prevention”
2. Collaboration with MBC
3. Over 50 publications
4. PROCAS recruitment completed
5. Density and genes improve standard risk estimation
6. Tomo-trial started
7. Anastrozole reduces risk by over 50%
8. Lifestyle alters gene expression in breast
9. Tamoxifen effective for up to 20 years
10. Altered metabolism found in dense breast tissue
11. New stem cell inhibitor
12. New inhibitor of DCIS
Lead investigators
in the Genesis Breast Cancer Prevention Centre

**Professor Tony Howell**
*Professor of Medical Oncology, University of Manchester*

Research Director of the Genesis Breast Cancer Prevention Centre.
Co-Chairman of the IBIS- II trial.
Vice Chairman of the Steering Committees of the FH01 and FH02 screening trials.
Co-Chairman of Manchester and Cheshire Cancer Prevention Network.
Former Director of the Breakthrough Breast Cancer Research Unit.
Past Chairman of the Manchester Breast Centre and of the ATAC Trial.
Member of the Board of the International Society for Cancer Prevention.
Member of the Editorial Board of Cancer Prevention Research.

**Professor Nigel Bundred**
*Professor of Surgery, University of Manchester*

Principle Investigator on National Institute for Heath Research Programme Grant entitled ‘Individualising breast cancer treatment to improve survival and minimise complications’.
Member of Editorial Board of Endocrine Related Cancer and The Breast journals.
NIHR Senior Investigator.
North West Surgical Trials Centre Director.

**Dr Sue Astley**
*Reader in Imaging Sciences, University of Manchester*

PhD (Faculty of Medicine, Manchester) 1988
MSc (Computer Science, UCL) 1984
BSc (Maths and Astronomy, Sheffield) 1978
Honorary Lectureship in Computer Science, University of Manchester, 1994-date
Research Posts in Medical Biophysics, University of Manchester 1987-94
Honorary Member of the British Society of Breast Radiologists
NHS Breast Screening Digital Steering Group 2004-2006
NHS Breast Screening Working Party on Digital Breast Tomosynthesis 2009-10
OPTIMAM Steering Group 2010-date

**Dr Michelle Harvie**
*Research Dietician*

Member of World Cancer Research Fund (WCRF) grant reviewing committee.
Expert advisor for the breast cancer charities Breast Cancer Care and Breast Cancer Now, guiding their policy and advice sheets for breast cancer prevention and patients after diagnosis.
Member of WCRF/AICR protocol development group for cancer survivors as part of WCRF/AICR Continuous Update Project.
Member of National Cancer Research Institute Lifestyle and behaviour change sub-group

**Professor Gareth Evans**
*Professor of Cancer Genetics and Epidemiology, University of Manchester*

Lead Clinician and ex Chairman of the NICE Committee on services for women at increased risk of breast cancer.
Principle investigator NIHR Programme Grants.
NIHR Senior Investigator.
Chairman Scientific Advisory Board of Breast Cancer Now.
Ex officio Chairman of the Cancer Genetics Group.
Director Manchester Breast Centre
Member of the NIHR breast CSG committee.
Chairman of the ICG on familial breast and ovarian cancer.
Dr Anthony J Maxwell
Consultant Academic Breast Radiologist, Nightingale Centre and Genesis Prevention Centre.

Honorary Senior Lecturer in Imaging, Institute of Population Health, University of Manchester.
North West Regional Director of Breast Screening Quality Assurance, Public Health England.
Clinical Vice-President of the UK Radiological Congress (UKRC).
Member of the National Cancer Intelligence Network Site Specific Clinical Reference Group for Breast Cancer.
Member of the Sloane Project Steering Group (a national audit of breast atypias and non-invasive breast cancer).
Former Honorary Secretary of the British Society of Breast Radiology (previously the Royal College of Radiologists Breast Group).

Cliona Kirwan
Clinician Scientist and Consultant Surgeon

National Institute for Health Research (NIHR) Clinician Scientist Award for research into breast cancer.
Principal Investigator of the CHAMPION, T-Poetic and TUFClot Studies.
Member of the IMPORT and Fast Forward Trial management groups.
Member of the NIHR Early Breast Cancer CSG Committee.
Consultant Oncoplastic Breast Surgeon at University Hospital of South Manchester.

The Genesis team and collaborators

Programme to predict risk in the NHSBSP and in the Family History Clinic population (PROCAS)
Paula Stavrinos, Sarah Sampson, Jill Fox, Lynne Fox, Donna Watterson, Jake Southworth, Bill Newman, Helen Byers, Elaine Harkness, Iain Buchan, Wendy Watson, Fiona Harrison, Katherine Payne, Michelle Harvie, Mary Wilson, Sue Astley, Alan Hufton, Tony Howell, Gareth Evans, David French.

Mammographic Density and Screening group programme
Mary Wilson, Ursula Beetles, Sue Astley, Alan Hufton, Ruth Warren, Jamie Sergeant, Yit Lim, Anil Jain, Nicky Barr, Sally Bundred, Emma Hurley, Megan Bydder, Soujanya Godde, Anthony Maxwell, Val Reece, Claire Mercer, Alix Hartley.

Preventive therapy and biomarker programme
Louise Gorman, Rosemary Greenhalgh, Julia Wiseman, Nicola Fisher, Emma Buckley.

Breast Research Nurses and team
Sue Grassby, Tracey Platt, Anam Asif, Susan Mbale, Faiza Idries, Deidre Leonard.

Radiographers and Radiographer Assistants

Family History Clinic service

Risk reducing surgery service
Susan Wisely, Stuart Wilson, Lester Barr, Ged Lambe, Richard Johnson, Ashu Ghandi.

Asian Breast Cancer Support Group
Anil Jain, Saima Rashid.
National Hereditary Breast Cancer Helpline
Wendy Watson at www.breastcancergenetics.co.uk

Genesis Scientific Advisory Board
Gareth Evans (Chair), John Winstanley, Andrew Renehan, Tom Warnes, Alan Stewart

Genesis staff team and volunteers
Nikki Barraclough, Louise Parker, Judi Hibbert, Angela Wrobel, Saima Rashid, Suzanna Dhawan, Jane McLaughlin, Tom Allen, Gill Kay, Sheila Jagota, Caroline Bennison, Michelle Cohen; at www.genesisuk.org

Medical Advisory Board
Gareth Evans, Ashu Gandhi, Richard Johnson, Cliona Kirwan, James Harvey, John Murphy, Nicola Barnes, Soujanya Gadde, Tony Maxwell.

International Advisors
Rowan Chhlebowski (Los Angeles), Anne McTiernan (Seattle), Peter Boyle (Lyon), Jack Cuzick (London), John Forbes (Newcastle Australia).

National and International Collaborators

Diet and lifestyle research team

Dietetic team
Michelle Harvie, Mary Pegington, Nina Brogden, Grace Cooper, Claire Lindsay, Sarah Abdula, Sarah McDiarmid.

Physiotherapy and exercise specialists
Debbie McMullen, Claire Edwards, Karen Livingstone.

Data management
Kath Sellers, Helen Ruane, Suzanne Slack.

Genesis Volunteers
Susan Rowe, Jane Eaton, Ali Parr, Jo O’Neill.

Radiographers
Pam Coates, Sandhya Solanki.

Psychology Research
Louise Gorman, David French, Helen Ruane.
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Nightingale Centre & Genesis Prevention Centre
University Hospital of South Manchester NHS Foundation Trust
Manchester M23 9LT.

For further information, please contact us on 0161 291 4400 or via our website
www.genesisuk.org

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