Breast cancer – diet to genes
Presented at the Department of Surgery, University of Cape Town, 20 July 2005

Consultant Surgeon, Level 2, Belfast City Hospital, Lisburn Road, Belfast, Northern Ireland

Summary
Frank Garfield Penman was a solicitor from England who died while on holiday in Cape Town in March 1963. Under a deed dated 9 November 1965, his widow Robina Douglas Penman established a Trust in his memory – the Penman Memorial Foundation. The object of the Foundation initially included scholarships to assist postgraduate medical students from South Africa, and in particular from Cape Town, to obtain teaching and further experience in the UK. Later, the Frank Penman Travelling Fellowship was established (the Visiting Professorship) to advance medical knowledge and practice in surgery by enabling a surgeon from the UK to give lectures and teach for a period of several weeks in South Africa. This paper is based on a lecture given on 20 July 2005 as part of the Penman Memorial Foundation Visiting Professorship to Cape Town.

Breast cancer
Breast cancer remains one of the most common cancers. In the UK the estimated annual incidence is 20 000 new cases per annum. Worldwide, the incidence of new cases approaches 1 million per annum.1 The incidence of this common tumour is rising throughout the world, although mortality, both in the UK and in the USA, is falling. This is due to many factors including early diagnosis, breast cancer screening programmes, the use of tamoxifen, the multidisciplinary team approach and, possibly more latterly, improved chemotherapy and targeted therapies such as Herceptin.2,4

The causation of breast cancer has been discussed in a multitude of publications and many factors appear to be involved, ranging from genetic to environmental. While 10% of breast cancer patients have a distinct genetic component to their disease, the other 90% or so have an environmental causation. The latter may range from hormonal factors, such as the contraceptive pill, HRT, early menarche and late menopause,23 to dietary factors, in which there has also been much interest.28,29 There is increasing evidence that high consumption of fruit and vegetables may lower the risk of certain cancers, including that of the breast.23,24 Increasing evidence from epidemiological and animal studies suggests that folate status may affect the risk of developing cancer in some tissues. This has been most clearly shown in cancer of the rectum and colon.25 Folate depletion appears to enhance carcinogenesis, whereas folate supplementation may give some protection. There has been some evidence28 that folate may play an important role in the prevention of breast cancer, especially in women who consume alcohol. It is unclear how folate can modulate cancer risk, but this vitamin plays a central role in DNA synthesis and methylation.27 Folate deficiency affects DNA stability through several pathways. 5,10-methyltetrahydrofolate donates a methyl group to uracil, converting it to thymine which is used for DNA synthesis and repair. If there is a deficiency of folate, the uracil becomes misincorporated into DNA. Subsequent double-strand breaks, chromosomal damage and cancer may occur.28 Folate also affects gene expression by regulating cellular S-adenosylmethionine (SAM) levels. 5-methyltetrahydrofolate is the methyl donor in the remethylation process of homocysteine to methionine, which is then converted to SAM.

SAM methylates specific cytosines in DNA and regulates gene transcription. As a consequence of folate deficiency, cellular SAM is depleted which, in turn, induces DNA hypomethylation and can induce proto-oncogene expression leading to cancer.28 Evidence linking folic acid to breast cancer has been limited and conflicting. However, several case control studies reported an inverse association between dietary folate and breast cancer risk.23-27 The overall trend in these studies showed a lowering of the risk of breast cancer with a higher intake of dietary folate.

A large population-based Shanghai breast cancer study between 1996 and 1998 showed an inverse relationship between breast cancer risk in those who had a higher dietary folate intake.32 On the other hand, there have been negative data. Other case control studies have failed to show any relationship and, in particular, any protective effect of folate against breast cancer.23-26 Evidence from large studies has shown that folate may be the factor that affects the association between alcohol intake and breast cancer.31 Strong evidence from meta-analysis studies currently supports a dose-response relationship between breast cancer risk and alcohol intake.34 However, there have been some other conflicting data.27

Dietary studies
Against this background, we studied folate status measured by red cell folate concentration in breast cancer and control patients, and related this to levels of DNA damage in both groups. In addition, we also assessed detailed dietary histories of a series of control patients compared with breast cancer patients. Ethical approval was obtained for the study from Queen’s University, Belfast, Research Ethics Committee. All patients gave written informed consent.

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Patient recruitment
In all patients in the study the diagnosis of breast cancer or benign breast disease was histologically confirmed. The patients undergoing surgery for benign breast disease either had surgery at their own request, or because of repeated episodes of inflammation (duct ectasia).
Sixty-four pre- and postmenopausal breast cancer patients and 30 patients with benign breast disease (controls) were recruited. All patients completed a questionnaire, including details of past medical history, drug history, dietary history and lifestyle, including specific questions about smoking and alcohol consumption.
Patients who were taking any vitamin supplements, or those with gastrointestinal disease (particularly malabsorption or coeliac disease) were excluded from the study. Patients who had previously had gastric or intestinal surgery were also excluded. Patients who were taking medication known to interfere with folate metabolism, such as anti-epileptic drugs and the oral contraceptive pill, were likewise excluded.

Laboratory methods
These have been fully described in our paper. Folate was analysed using standard techniques. The relationship between DNA damage markers and folic status was examined in both the cases and the controls, with bioanalysis of blood samples for red cell folate using a microbiology assay. Plasma homocysteine levels were analysed using a standard immunoassay.
DNA damage biomarkers were measured in monocytes using alkaline comet assay and the modified comet assay, as described in detail in our paper.

Statistical analysis
The statistics were performed using the Statistics Package for the Social Services (SPSS) Version 10. For all tests, p-values less than 0.05 were considered significant. Plasma homocysteine, red cell folate and DNA damage (tail moment) data were skewed and were, therefore, log-transformed to normalise the data for comparison using the t-test, as previously described.

Results – dietary studies
The demographics of the patients and controls are shown in Table I.
The distribution of breast cancer control patients was homogeneous. None of the patients had a high-risk family history of breast cancer. Blood folate levels in the breast cancer patients and the controls are given in Table II.
The mean red cell folate value in the breast cancer patients was 369.0 (95% confidence interval (CI) 333.3 - 404.6) ng/ml, compared with 420.5 (CI 335.8 - 505.2) ng/ml for the controls. The mean plasma homocysteine value in the breast cancer patients was 13.5 (CI 10.6 - 16.4) µmol/ml in breast cancer patients, compared with 10.6 (CI 9.3 - 11.9) µmol/ml for the controls.
The data were log-transformed, as described previously. The results were presented as the geometric mean (95% CI) and compared using Student’s t-test, as outlined in Table II.
While the results were not statistically significant, breast cancer patients tended to have lower red cell folate and higher plasma homocysteine levels than the controls.

DNA damage analysis
The results of this part of the study are outlined in Table III. Following the basic alkaline comet assay, the mean tail moment for breast cancer patients was 5.0 (3.4) versus 1.1 (1.2) for control patients. The mean tail moment detected by the modified comet assay using Endonuclease III (which detects oxidised pyrimidines) for breast cancer patients was 7.5 (6.2) versus 3.1 (2.3) for control patients. The mean tail moment detected by the modified comet assay using formidopyrimidine glycosylase (FPG) (which detects oxidised purines) (as described in our paper) for breast cancer patients was 6.3 (3.6) versus 3.7 (2.7) for control patients (Table III).

<table>
<thead>
<tr>
<th>TABLE I. CHARACTERISTICS OF BREAST CANCER AND CONTROL PATIENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Breast cancer (SD)</strong></td>
</tr>
<tr>
<td>Mean age (yrs)</td>
</tr>
<tr>
<td>Weight (kg)</td>
</tr>
<tr>
<td>Height (m)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
</tr>
<tr>
<td>Menopausal status</td>
</tr>
<tr>
<td>Premenopausal</td>
</tr>
<tr>
<td>Postmenopausal</td>
</tr>
<tr>
<td>Alcohol consumption (g/d)</td>
</tr>
<tr>
<td>Smoking</td>
</tr>
<tr>
<td>Non-smokers</td>
</tr>
<tr>
<td>Smokers</td>
</tr>
</tbody>
</table>

* Chi-square test.
SD = standard deviation.
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As the tail moments were positively skewed, for normalisation purposes, these were log-transformed.

Correlation analysis was performed between plasma homocysteine and red cell folic acid for all patients, and DNA damage (detected using the modified comet assay using FPG), revealed significant negative correlation between red cell folate values and DNA damage (Table IV). Further analysis looked at the impact of alcohol intake on DNA damage and folate status in breast cancer patients who consumed alcohol, and whose folate status was low (less than the median value of 363.98 ng/ml). A significant positive correlation between log alcohol intake and log tail moment was detected by modified comet assay (FPG) (Pearson correlation coefficient = 0.58, p = 0.04). In control patients with a low red cell folate value, there was no correlation between alcohol intake and DNA damage (Table V).

Discussion – dietary studies

This study showed that breast cancer patients tended to have reduced folate status, that is lower red cell folate and higher plasma homocysteine levels, compared with control patients. These data just failed to reach statistical significance. DNA damage in the monocytes of breast cancer patients was significantly higher than the control group, and red cell folate was negatively correlated with DNA damage detected by the modified comet assay (using FPG enzyme).

In contrast to these data, research from Washington County28 showed no evidence of an association between serum folate or homocysteine levels and the risk of breast cancer. There were, however, technical problems with this study, as outlined in our own paper.28 Other data have also shown DNA damage in breast cancer patients to be 2.5 times higher than controls.29 Our results confirm the data of others in that there is little correlation between low alcohol consumption and breast cancer (perhaps because so few women in our study consumed significant alcohol).43-45

Our study on folate and breast cancer showed that there was a trend towards lower red cell folate and higher plasma homocysteine concentrations in our breast cancer patients. DNA damage levels were found to be significantly higher in the monocytes of breast cancer patients compared with benign breast disease controls. This is the first study of its kind to investigate the level of DNA damage and folate status in breast cancer and benign breast disease patients. It has provided some evidence that reduced folate may be implicated in the development of breast cancer and raised the possibility that food supplementation with folic acid should be further studied as a tool in the armamentarium of breast cancer prevention.30

Genetic studies

In parallel with our dietary studies, serendipity played a role in our genetic studies. In 1997 Mulligan and his co-workers isolated a monoclonal antibody by inoculation of a G-CCM cell line derived from an anaplastic astrocytoma.44 This antibody recognises an epitope on an astrocytoma-associated glycoprotein named pMQ.

Further studies by Mulligan showed that this glycoprotein was a previously undescribed mammalian protein, sharing significant sequence homology with the gene product of a micro-organism (ORF6) implicated in cell adhesion.45 This protein was not expressed in normal tissue. It decreased with histological grade in astrocytomas. Mulligan suggested that this was an oncofetal protein with adverse potential. It was then recognised that pMQ was not confined to astrocytomas but was also observed in metastatic cells from a primary breast cancer. Clearly, one of the adverse sequences in breast cancer prevention.

**TABLE II. BLOOD FOLATE STATUS MEASUREMENTS IN BREAST CANCER AND CONTROL PATIENTS**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Breast cancer geometric mean (95% CI)</th>
<th>Controls geometric mean (95% CI)</th>
<th>Significance (Student’s t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red cell folate</td>
<td>339.07 (303.9 - 378.3)</td>
<td>379 (322.8 - 446.3)</td>
<td>p = 0.246</td>
</tr>
<tr>
<td>Plasma homocysteine</td>
<td>11.90 (10.61 - 13.25)</td>
<td>10.14 (9.01 - 11.43)</td>
<td>p = 0.073</td>
</tr>
</tbody>
</table>

*Significant (t-test).

**TABLE III. LEVELS OF DNA DAMAGE IN MONONUCLEAR CELLS OF BREAST CANCER AND CONTROL PATIENTS**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Breast cancer mean log TM (SD)(95% CI)</th>
<th>Control mean log TM (SD)(95% CI)</th>
<th>Significance (Student’s t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basic alkaline comet</td>
<td>1.46 (0.66) (1.26 - 1.66)</td>
<td>–0.177 (0.79) (–0.43 - 0.08)</td>
<td>p &lt; 0.0001*</td>
</tr>
<tr>
<td>Modified comet (Endo III)</td>
<td>1.77 (0.70) (1.56 - 1.98)</td>
<td>0.86 (0.81) (0.59 - 1.13)</td>
<td>p &lt; 0.0001*</td>
</tr>
<tr>
<td>Modified comet (FPG)</td>
<td>1.67 (0.62) (1.46 - 1.86)</td>
<td>0.99 (0.94) (0.72 - 1.26)</td>
<td>p &lt; 0.0001*</td>
</tr>
</tbody>
</table>

*Significant (t-test).

SD = standard deviation; 95% CI = 95% confidence interval; TM = tail moment (migrated DNA x tail length); Endo III = Endonuclease III enzyme (to detect oxidised pyrimidines); FPG = formamidopyrimidine glycosylase enzyme (to detect oxidised purines).
cancer disease progression is the development of widespread metastases. This piece of serendipity led us to study the glycoprotein, pMQ1, in breast cancer.

The methodology of our glycoprotein study has been published. In brief, 228 women diagnosed with breast cancer between 1984 and 1998 were studied; 25 control specimens were obtained and a considerable amount of clinical data was obtained on these patients, as outlined in Tables VI and VII. The methodology for detecting the glycoprotein pMQ1 is outlined in detail in our paper. The glycoprotein was detected using a monoclonal antibody raised against surface proteins expressed by the astrocytoma cell line G-CCM.

### TABLE IV. CORRELATION BETWEEN FOLATE STATUS AND LEVELS AND DNA DAMAGE IN MONONUCLEAR CELLS OF ALL PATIENTS

<table>
<thead>
<tr>
<th>Variable</th>
<th>Log mean tail moment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basic comet</td>
</tr>
<tr>
<td></td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>Red cell folate</td>
<td>0.82 (1.06)</td>
</tr>
<tr>
<td>Pearson correlation (r²)</td>
<td>−0.155 (p = 0.18)</td>
</tr>
<tr>
<td>Plasma homocysteine</td>
<td>0.08 (p = 0.48)</td>
</tr>
</tbody>
</table>

*Significant (Pearson correlation).

TM = tail moment (migrated DNA x tail length); Endo III = Endonuclease III enzyme; FPG = formamidopyrimidine glycosylase enzyme.

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### TABLE V. CORRELATION BETWEEN ALCOHOL INTAKE AND DNA DAMAGE IN MONONUCLEAR CELLS OF BREAST CANCER AND CONTROL PATIENTS WHO HAD LOW AND HIGH RED CELL FOLATE (RCF) VALUES

<table>
<thead>
<tr>
<th>Log alcohol intake g/day</th>
<th>Low red cell folate</th>
<th>High red cell folate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Breast cancer</td>
<td>Controls</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Breast cancer</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Controls</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>0.82 (1.06)</td>
<td>0.40 (p = 0.19)</td>
</tr>
<tr>
<td>Pearson correlation (r²)</td>
<td>0.25 (p = 0.42)</td>
<td>0.67 (p = 0.01)</td>
</tr>
<tr>
<td>(significance)</td>
<td></td>
<td>(p = 0.04)</td>
</tr>
<tr>
<td></td>
<td>−0.32 (p = 0.42)</td>
<td>−0.007 (p = 0.99)</td>
</tr>
<tr>
<td>(significance)</td>
<td></td>
<td>(p = 0.13)</td>
</tr>
<tr>
<td></td>
<td>−0.017 (p = 0.68)</td>
<td>−0.21 (p = 0.72)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(p = 0.01)</td>
</tr>
<tr>
<td></td>
<td>−0.57 (p = 0.13)</td>
<td>−0.75 (p = 0.13)</td>
</tr>
</tbody>
</table>

*Significant (Pearson correlation).

Low red cell folate (RCF) = less than median RCF value (363.98 ng/ml); high RCF = more than median RCF value (363.98 ng/ml).

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and a grading system was devised as outlined in the paper.59 Detailed analysis to determine observer variation was also carried out. The tumours were all reviewed to re-assess tumour size, histological grade, subtype, lymphovascular invasion, lymph node status, oestrogen receptor positivity and p53 and Ki-67 positivity.59

**Statistical analysis**

The statistical analysis was performed using the Package for Social Sciences Version 9.59 The association between pMQ expression and currently known prognostic indicators was evaluated using chi-square and bivariate Spearman’s correlation analysis. Kappa analysis was performed to measure the level of agreement between the two observers and to ensure that inter-observer variation was within acceptable limits.

The results of pMQ in each tumour were expressed as either positivity or negativity. Thereafter, the analysis was carried out to determine the correlation between pMQ status and the patient’s clinical outcome, as defined by either local or distant recurrence, disease-free survival, event-free survival and overall survival.

The difference in outcome between pMQ-positive and negative cancer patients was analysed by the Kaplan-Meier method, using the cumulative hazard option. Separation survival curves were tested for significance using log rank analysis.

**Results – pMQ studies**

In normal tissue, the staining intensity for pMQ was either absent or weak and distributed basally in contact with the basement membrane. A similar pattern was found in benign breast disease, such as fibroadenomas and benign fibrocystic disease of the breast. In essence, while all benign breast tissue and normal breast tissue was negative for pMQ expression, 60% (137/228) of the archival breast cancer specimens were pMQ positive. The staining pattern for pMQ was distributed at the cell surface membrane and intracytoplasmic regions. The pMQ staining was identified as being either focal or diffuse. In most tumours (94) pMQ was diffusely expressed throughout the entire cancer. As described previously,57 detailed subset analysis indicated that the tumours with a worse grade (grade III) were mostly pMQ positive (74.4%, 58/78). Of the 225 tumours 44 (19%) were graded histologically as grade I, 103 (46%) were grade II and 78 (35%) were grade III. There was a positive correlation between pMQ expression and increasing histological grade (Spearman’s correlation coefficient \( r = 0.271, p < 0.01 \)).

Sixty-six of 169 patients (39%) demonstrated lymphovascular invasion, and this was positively correlated with histological grade. pMQ was expressed in a significantly higher number of tumours with lymphovascular invasion (74% (49/66), \( r = 0.182, p = 0.018 \)).

Other analyses showed that there was an inverse correlation between pMQ and age; that oestrogen receptor (ER)-positive breast cancer was less likely to express pMQ (Table VII); and that cancers that were pMQ positive were more common among patients who had a higher Nottingham Prognostic Index. Detailed statistical analysis is reported in our paper.55 Of interest, there was no significant correlation between pMQ and tumour size, lymph node status and p53 status.

### Table VI. Correlation between pMQ expression and primary prognostic indicators in human breast cancer

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>( N )</th>
<th>pMQ-positive cases</th>
<th>Correlation coefficient</th>
<th>( p )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt; 40</td>
<td>56</td>
<td>70%</td>
<td>( r = -0.135</td>
<td>0.042</td>
</tr>
<tr>
<td></td>
<td>41 - 55</td>
<td>55</td>
<td>62%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>56 - 70</td>
<td>86</td>
<td>55%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt; 70</td>
<td>31</td>
<td>55%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumour size</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>T1 = &lt; 2 cm</td>
<td>73</td>
<td>60.3%</td>
<td></td>
<td>0.227 NS</td>
</tr>
<tr>
<td></td>
<td>T2 = 2 - 5 cm</td>
<td>139</td>
<td>69.8%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>T3 = &gt; 5 cm</td>
<td>15</td>
<td>69.2%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histological grade</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Low (grade I)</td>
<td>44</td>
<td>36.4%</td>
<td>( r = 0.271</td>
<td>&lt; 0.0005</td>
</tr>
<tr>
<td></td>
<td>Moderate (grade II)</td>
<td>103</td>
<td>58.3%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>High (grade III)</td>
<td>78</td>
<td>74.4%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histological subtype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Invasive ductal carcinoma</td>
<td>166</td>
<td>64%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Invasive lobular carcinoma</td>
<td>42</td>
<td>43%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphovascular invasion (LVI)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>66</td>
<td>74.2%</td>
<td>( r = 0.182</td>
<td>0.018</td>
</tr>
<tr>
<td></td>
<td>Absent</td>
<td>103</td>
<td>56.3%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymph node status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>I (no nodes involved)</td>
<td>93</td>
<td>65.6%</td>
<td></td>
<td>0.523 NS</td>
</tr>
<tr>
<td></td>
<td>II (1 - 3 nodes involved)</td>
<td>51</td>
<td>67.7%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>30</td>
<td>66.7%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( p < 0.05 \) significant.

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Follow-up

There was complete follow-up in 211 patients; 17 were either lost to follow-up or data were deemed to be inaccurate. Overall, 13% of patients (28/211) developed local recurrence at the site of the primary tumour; 25% (53/211) died from distant recurrence.

Kaplan-Meier analysis, using the cumulative hazard option, revealed that local recurrence was significantly more common in pMQ1-positive tumours (22/124) than pMQ1-negative tumours (6/87; p = 0.036) (Fig. 1). However, the incidence of distant tumour recurrence was similar in both groups (p = 0.677). There was no difference between pMQ1-positive and pMQ1-negative patients with regard to disease-free survival, event-free survival and overall survival. Multivariate analysis demonstrated that pMQ1 expression was an independent variable in survival analysis. pMQ1 correlated positively with higher histological grade and the presence of lymphovascular invasion.

Discussion – pMQ1 studies

Our study suggests that pMQ1 is a new glycoprotein which may be a cell adhesion molecule or receptor. It seems to be a tumour-associated protein. In this context, other tumour-associated markers have found clinical application in diagnosis, detection and monitoring of cancer progression.

In future, if this study can be confirmed in a larger patient setting, pMQ1 expression may have potential as an additional tool for the histological diagnosis of difficult pathological conditions, such as atypical ductal hyperplasia and ductal carcinoma in situ. It is also of interest that there was a significant inverse correlation between pMQ1 expression and ER status (p = 0.001). Aggressive tumours are more likely to be pMQ1 positive and ER negative.

It may be that positivity of pMQ1 glycoprotein is associated with increased basal membrane invasiveness, and the ability of the cancer cells to proliferate and migrate. Overall, women with pMQ1-positive tumours seem to have a higher risk of local recurrence. Recurrence normally develops in the first 2 to 3 years after surgery, and is usually associated with increased risk of distant metastases. In future, it is possible that pMQ1 could be used to select women who have a risk of developing local recurrence for, perhaps, more radical therapies, possibly including chest wall radiotherapy after mastectomy, even in the absence of other indications.

**TABLE VII. CORRELATION BETWEEN pMQ1 AND SECONDARY PROGNOSTIC VARIABLES IN HUMAN BREAST CANCER**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>N</th>
<th>pMQ1 expression</th>
<th>Correlation coefficient</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oestrogen receptor (ER)</td>
<td>ER negative</td>
<td>58</td>
<td>79%</td>
<td>r = -0.278</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>ER weakly positive</td>
<td>21</td>
<td>71%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ER strongly positive</td>
<td>63</td>
<td>51%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ki-67</td>
<td>Positive</td>
<td>68</td>
<td>63%</td>
<td>0.387 NS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>61</td>
<td>70%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p53</td>
<td>Positive</td>
<td>75</td>
<td>75%</td>
<td>0.878 NS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>50</td>
<td>74%</td>
<td></td>
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p < 0.05 significant.

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![Fig. 1. Kaplan-Meier cumulative hazard analysis comparing the risk of local recurrence by pMQ1 expression (p = 0.36; log rank test). The risk of developing a local recurrence was significantly higher in women with pMQ1-positive cancers. (Fon et al., copyright British Journal of Surgery Society Ltd., reproduced with permission from John Wiley & Sons Ltd on behalf of BJS Ltd.)](image)
should be professional and public debate on folic acid supple-
mentation in foodstuffs. These studies indicate the value of this two-pronged
approach of epidemiology and pure science (not forgetting serendipity) in advancing our knowledge of breast cancer
with possible preventative, diagnostic and therapeutic impli-
cations.

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