

STEROID HORMONE RECEPTORS IN THE  
PREDICTION OF ENDOCRINE RESPONSE IN  
CANCER OF THE BREAST

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M.D. Thesis

## ABSTRACT

This thesis describes a study of the predictive value of assays of oestrogen (RE) and progesterone (RP) receptors for endocrine response in mammary cancer.

The aim was assessment of the value of:-

1. Assays carried out routinely at the time of mastectomy
2. Classification by combinations of receptors
3. Assays of nuclear oestrogen receptors
4. Assays to show induction of progesterone receptor synthesis by tamoxifen

Nuclear RE and cytosol receptors were measured by multiple-point methods with Scatchard analysis. A new microassay for RP was also used and is described. Endocrine response was assessed by logrank analysis of the interval between the start of treatment and further progression of disease, and of survival both from presentation and 'first relapse'.

The results showed that, although considerable variation of receptor status occurred with time, assays of RE or RP on primary tumours, whether 'early' or 'advanced', indicated effectively the probability both of relapse & survival. Assays of RE immediately before treatment predicted neither relapse nor survival, while assays of RP then indicated only the prognosis for survival. Assay of both cytosol receptors offered no advantage over assay of RP alone, or RE when the concentration was considered. Nuclear RE assays did not

predict relapse, and gave only a weak indication of survival. Patients with increased concentrations of RP shortly after starting tamoxifen had a lower probability of relapse than those in whom RP declined, whose prognosis was similar to RP negative patients.

The conclusions were :-

1. Isoelectric focusing was a valid microassay.
2. Assays carried out on primary tumours were more reliable than assays done on metastases.
3. Assay of both receptors was unnecessary.
4. Assay of RP was preferable to RE.
5. Assays of nuclear RE were unreliable.
6. Assays of RP in biopsies taken before & shortly after starting tamoxifen indicated the prognosis more effectively than a single assessment.

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## CONTENTS

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|                                          |    |
|------------------------------------------|----|
| <u>TITLE PAGE</u>                        | 1  |
| <u>ABSTRACT</u>                          | 2  |
| <u>TABLE OF CONTENTS</u>                 | 4  |
| Text                                     | 5  |
| Tables                                   | 10 |
| Legends                                  | 11 |
| Appendices                               | 17 |
| <u>ACKNOWLEDGEMENTS</u>                  | 18 |
| <u>INTRODUCTION</u>                      | 20 |
| Endocrine response in mammary cancer     | 21 |
| Receptors and endocrine dependance       | 25 |
| Receptors and response in mammary cancer | 28 |
| Assessment of response                   | 32 |
| Assay of receptors                       | 35 |
| Variation of receptors                   | 39 |
| Induction of progesterone receptors      | 43 |
| Microassay of receptors                  | 44 |
| Summary                                  | 46 |
| <u>METHODS</u>                           | 47 |
| <u>Dextran-coated charcoal assay</u>     | 48 |
| Samples                                  | 48 |
| Reagents                                 | 48 |
| Preparation of cytosol                   | 48 |
| Preparation of non-radioactive solutions | 49 |
| Preparation of radioactive solutions     | 50 |
| Incubation of cytosol                    | 51 |
| Separation of free hormone               | 51 |
| Estimation of total activity             | 51 |

|                                                                          |    |
|--------------------------------------------------------------------------|----|
| Quality control                                                          | 52 |
| Charcoal blanks                                                          | 52 |
| Measurement of radioactivity                                             | 52 |
| Calculations                                                             | 52 |
| Measurement of cytosol protein                                           | 53 |
| Sensitivity of assay                                                     | 53 |
| Assay of nuclear oestrogen receptors                                     | 54 |
| <u>Isolelectric focusing assay</u>                                       | 54 |
| Reagents                                                                 | 54 |
| Solutions                                                                | 55 |
| Preparation of cytosol                                                   | 56 |
| Preparation of 'stock' radioactive solutions                             | 57 |
| Preparation of non-radioactive solutions                                 | 57 |
| Preparation of 'working' radioactive solutions                           | 57 |
| Preparation of gel                                                       | 58 |
| Isoelectric focusing                                                     | 59 |
| Measurement of radioactivity                                             | 61 |
| Calculations                                                             | 61 |
| Measurement of pH gradient                                               | 62 |
| Assay of cytosol protein                                                 | 62 |
| Precision of assay                                                       | 62 |
| Mobility of free <sup>3</sup> H-R5020                                    | 62 |
| Estimation of serum binding                                              | 62 |
| Use of acrylamide gels                                                   | 63 |
| Comparison of isoelectric focusing and the dextran-coated charcoal assay | 63 |

|                                                        |    |
|--------------------------------------------------------|----|
| Limits of detection                                    | 64 |
| <u>Variation of receptor status</u>                    | 65 |
| Samples                                                | 65 |
| Patients                                               | 66 |
| Analysis & statistical methods                         | 66 |
| <u>Receptors, response &amp; survival</u>              | 68 |
| Patients                                               | 68 |
| Previous treatments                                    | 68 |
| Sites of disease                                       | 69 |
| Treatments                                             | 69 |
| Clinical assessment                                    | 69 |
| Assessment of response                                 | 71 |
| Assessment of date of relapse                          | 74 |
| Statistical methods                                    | 74 |
| <u>Induction of progesterone receptor by tamoxifen</u> | 78 |
| Patients                                               | 78 |
| Treatment                                              | 78 |
| Timing of biopsies                                     | 79 |
| Techniques of biopsy                                   | 80 |
| Assay of receptors                                     | 80 |
| Expression of results                                  | 81 |
| Clinical assessment                                    | 81 |
| Statistical methods                                    | 82 |
| <u>Intra-tumour variation of progesterone receptor</u> | 83 |
| Samples & methods                                      | 83 |
| <u>RESULTS</u>                                         | 84 |

|                                                                            |     |
|----------------------------------------------------------------------------|-----|
| <u>Variation of receptors</u>                                              | 85  |
| Synchronous biopsies                                                       | 85  |
| Asynchronous biopsies                                                      | 85  |
| <u>Receptors, response &amp; survival</u>                                  | 93  |
| <u>Response</u>                                                            |     |
| General results                                                            | 93  |
| Overall effects of receptor status                                         | 98  |
| Assessment of receptor status at the time of treatment                     | 104 |
| Primary tumour receptor status                                             | 108 |
| Concentrations of receptor                                                 | 114 |
| Receptors in early vs advanced disease                                     | 119 |
| <u>Survival</u>                                                            | 122 |
| Overall effects of receptor status                                         | 122 |
| Primary tumour receptor status                                             | 129 |
| Assessments of receptor at the time of treatment                           | 139 |
| Concentrations of receptors                                                | 144 |
| <u>Assay of progesterone receptors by isoelectric focusing</u>             | 151 |
| Separation of receptor                                                     | 151 |
| Hormone binding by serum proteins                                          | 151 |
| Effects of molybdate ions                                                  | 152 |
| Gel medium                                                                 | 156 |
| Duration of electrofocusing                                                | 152 |
| Migration of free <sup>3</sup> H-R5020                                     | 152 |
| Precision of assay                                                         | 152 |
| Comparison of isoelectric focusing with the dextran-coated charcoal method | 153 |

|                                                            |     |
|------------------------------------------------------------|-----|
| <u>Assay of oestrogen receptor by isoelectric focusing</u> | 153 |
| Comparison with the dextran-coated charcoal method         | 154 |
| <u>Needle biopsies</u>                                     | 154 |
| Accuracy of sampling                                       | 154 |
| Weights & cytosol protein content                          | 154 |
| <u>Induction of progesterone receptor by tamoxifen</u>     | 164 |
| Changes in receptors                                       | 164 |
| Changes in receptors & response                            | 164 |
| Receptor status & probability of relapse                   | 167 |
| Induction of receptor & probability of relapse             | 167 |
| <u>Intra-tumour variation of progesterone receptor</u>     | 174 |
| <u>DISCUSSION</u>                                          | 176 |
| Variation of receptors                                     | 177 |
| <u>Receptors, response &amp; survival</u>                  | 182 |
| General results                                            | 182 |
| Cytosol receptors                                          | 182 |
| Nuclear oestrogen receptors                                | 184 |
| Timing of assays                                           | 185 |
| Extent of disease & response                               | 187 |
| Site of disease & response                                 | 187 |
| Conclusion                                                 | 188 |
| <u>Isoelectric focusing assay</u>                          | 189 |
| <u>Induction of progesterone receptor</u>                  | 194 |
| <u>REFERENCES</u>                                          | 198 |
| <u>APPENDICES</u>                                          | 221 |

## LIST OF TABLES

| <u>Introduction</u>                                                                               | Page |
|---------------------------------------------------------------------------------------------------|------|
| Table I: Oestrogen receptor status & endocrine response (Published results)                       | 29   |
| Table II: Variation of oestrogen receptor status                                                  | 42   |
| <u>Methods</u>                                                                                    |      |
| Table III: Interval between first biopsy & the start of treatment                                 | 79   |
| Table IV: Dose of tamoxifen & timing of second biopsy                                             | 79   |
| <u>Results</u>                                                                                    |      |
| Table VI(a,b): Variation of receptor status in synchronous & asynchronous biopsies                | 88   |
| Table VII: Initial receptor status & variation of receptor status in asynchronous biopsies        | 88   |
| Table VIII: Combined (RE & RP) receptor status in asynchronous biopsies                           | 89   |
| Table IX: Effect of endocrine treatment on variation of receptor status                           | 89   |
| Table X: Assessment of response (All patients)                                                    | 94   |
| Table XI: Oestrogen receptor status & endocrine response (All assays included)                    | 99   |
| Table XII: Progesterone receptor status & endocrine response (All assays included)                | 99   |
| Table XIII: Combined (RE & RP) receptor status & changes in progesterone receptor with tamoxifen. | 165  |
| Table XIV: Change in progesterone receptors with tamoxifen & response.                            | 165  |
| Table XV : Intra-tumour variation of progesterone receptor.                                       | 175  |

## LIST OF FIGURES

### Introduction

|                                                 |    |
|-------------------------------------------------|----|
| Figure 1: Action of oestrogen on target tissues | 27 |
| Figure 2: An example of Scatchard analysis      | 39 |

### Method

|                                                     |    |
|-----------------------------------------------------|----|
| Figure 3: Diagram of isoelectric focusing apparatus | 60 |
|-----------------------------------------------------|----|

### Results

|                                                                                                                                |     |
|--------------------------------------------------------------------------------------------------------------------------------|-----|
| Figure 4: Initial concentrations of RE or RP in<br>receptor-positive first biopsies, & receptor status of<br>subsequent biopsy | 90  |
| Figure 5: Variation of concentration of oestrogen<br>receptors in asynchronous biopsies                                        | 91  |
| Figure 6: Variation in concentration of progesterone<br>receptors in asynchronous biopsies                                     | 92  |
| Figure 7: Endocrine treatment & the probability of<br>relapse                                                                  | 95  |
| Figure 8: Extent of disease & the probability of<br>relapse.                                                                   | 96  |
| Figure 9: Site of distant metastasis & the probability<br>of relapse                                                           | 97  |
| Figure 10: Oestrogen receptor status & the probability<br>of relapse (All assays included)                                     | 100 |
| Figure 11: Progesterone receptor status & the<br>probability of relapse (All assays included)                                  | 101 |
| Figure 12: Nuclear oestrogen receptor status & the<br>probability of relapse (All assays included)                             | 102 |
| Figure 13 Combined (RE & RP) receptor status & the<br>probability of relapse (All assays included)                             | 103 |
| Figure 14: Oestrogen receptor status & the probability                                                                         |     |

|                                                                                                                                    |     |
|------------------------------------------------------------------------------------------------------------------------------------|-----|
| of relapse (Assays done at the time of treatment only)                                                                             | 105 |
| Figure 15: Progesterone receptor status & the probability of relapse (Assays done at the time of treatment)                        | 106 |
| Figure 16: Nuclear oestrogen receptor status & the probability of relapse (Assays done at the time of treatment)                   | 107 |
| Figure 17: Oestrogen receptor status & the probability of relapse (Receptors assessed in primary tumours)                          | 109 |
| Figure 18: Progesterone receptor status & the probability of relapse (Receptors assessed in primary tumours)                       | 110 |
| Figure 19: Nuclear oestrogen receptor status & the probability of relapse (Receptors assessed in primary tumours)                  | 111 |
| Figure 20: Combined (RE & RP) status & the probability of relapse (Receptors assessed in primary tumours)                          | 112 |
| Figure 21: Effect of progesterone receptor on the probability of relapse in RE +ve patients.                                       | 113 |
| Figure 22: Concentration of oestrogen receptors & the probability of relapse                                                       | 115 |
| Figure 23: Concentration of progesterone receptors & the probability of relapse                                                    | 116 |
| Figure 24: Effect of progesterone receptor status on the probability of relapse at different concentrations of oestrogen receptor  | 117 |
| Figure 25: Effects of oestrogen receptor status on the probability of relapse at different concentrations of progesterone receptor | 118 |



|                                                                                                                                                |     |
|------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| Figure 26: Early vs Late assessment of receptors in the prediction of relapse                                                                  | 120 |
| Figure 27: Primary tumour receptor status & the probability of relapse, allowing for results of assays done at the time of endocrine treatment | 121 |
| Figure 28: Oestrogen receptor status & survival from presentation (All assays included)                                                        | 123 |
| Figure 29: Oestrogen receptor status & survival after first relapse (All assays included)                                                      | 124 |
| Figure 30: Progesterone receptor status & survival from presentation (All assays included)                                                     | 125 |
| Figure 31 Progesterone receptor status & survival after first relapse (All assays included)                                                    | 126 |
| Figure 32: Nuclear oestrogen receptor status & survival from presentation (All assays included)                                                | 127 |
| Figure 33: Nuclear oestrogen receptor status & survival after first relapse (All assays included)                                              | 128 |
| Figure 34: Oestrogen receptor status & survival from presentation (Assays done on primary tumours)                                             | 130 |
| Figure 35: Oestrogen receptor status & survival after first relapse (Assays on primary tumours)                                                | 131 |
| Figure 36: Progesterone receptor status & survival from presentation (Assays on primary tumours)                                               | 132 |
| Figure 37: Progesterone receptor status & survival after first relapse (Assays on primary tumours)                                             | 133 |
| Figure 38: Combined (RE & RP) receptor status & survival from presentation                                                                     | 134 |
| Figure 39: Combined (RE & RP) receptor status & survival after first relapse                                                                   | 135 |

|                                                                                                                                               |     |
|-----------------------------------------------------------------------------------------------------------------------------------------------|-----|
| Figure 40: Effects of oestrogen receptor status on survival (a) & survival after first relapse (b) in progesterone receptor negative patients | 136 |
| Figure 41: Effects of progesterone receptor on survival (a) & survival after first relapse (b) in oestrogen receptor positive patients        | 137 |
| Figure 42: Nuclear oestrogen receptor status and survival after presentation (a) and after first relapse (b) (Assays on primary tumours)      | 138 |
| Figure 43: Progesterone receptor status & survival (Assays done at the time of treatment only)                                                | 140 |
| Figure 44: Progesterone receptor status & survival after first relapse (Assays done at the time of treatment)                                 | 141 |
| Figure 45: Oestrogen receptor status & survival after presentation (Assays done at the time of treatment)                                     | 142 |
| Figure 46: Oestrogen receptor status & survival after first relapse (Assays done at the time of treatment)                                    | 143 |
| Figure 47: Concentration of oestrogen receptors & survival after presentation                                                                 | 145 |
| Figure 48: Concentration of oestrogen receptors & survival after first relapse                                                                | 146 |
| Figure 49: Concentration of progesterone receptors & survival after presentation                                                              | 147 |
| Figure 50: Concentration of progesterone receptors & survival after first relapse                                                             | 148 |
| Figure 51: Concentration of oestrogen receptors & survival, allowing for concentration of progesterone receptor                               | 149 |

|                                                                                                                                                                       |     |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| Figure 52: Progesterone receptor status & survival,<br>allowing for the concentration of oestrogen receptor                                                           | 150 |
| Figure 53: Isoelectric focusing trace from a<br>progesterone receptor positive tumour                                                                                 | 155 |
| Figure 54: <sup>3</sup> H-R5020 binding by serum protein                                                                                                              | 156 |
| Figure 55: Binding of various steroids to serum<br>proteins                                                                                                           | 157 |
| Figure 56: Effect of molybdate on the proportion of<br>total receptor measured by IEF                                                                                 | 158 |
| Figure 57: Isoelectric focusing in agarose vs<br>acrylamide gels                                                                                                      | 159 |
| Figure 58: Migration of free <sup>3</sup> H-R5020                                                                                                                     | 160 |
| Figure 59: Comparison of results of assay of<br>progesterone receptors by IEF & DCC methods                                                                           | 161 |
| Figure 60: Total concentration of receptor & the<br>proportion measured by IEF                                                                                        | 162 |
| Figure 61: Comparison of results of assay of oestrogen<br>receptors by IEF & DCC methods                                                                              | 163 |
| Figure 62: Extent of change in concentration of<br>progesterone receptors with tamoxifen, and response                                                                | 166 |
| Figure 63: Initial oestrogen receptor status & the<br>probability of relapse (Induction study patients)                                                               | 168 |
| Figure 64: Initial progesterone receptor status & the<br>probability of relapse (Induction study patients)                                                            | 169 |
| Figure 65: Overall <sup>*</sup> progesterone receptor status & the<br><sup>*</sup> probability of relapse ( RP +ve if <u>either</u> 1st or 2nd<br>biopsy contains RP) | 170 |
| Figure 66: Change in progesterone receptors & the<br>probability of relapse (RP increased vs RP reduced or                                                            |     |

persistently absent) 171

Figure 67: Change in progesterone receptors & the  
probability of relapse in RP +ve patients (on initial  
assay). 172

Figure 68: Change in progesterone receptors & the  
probability of relapse in RP +ve patients (on either  
assay) 173

## LIST OF APPENDICES

|                                                                                    |     |
|------------------------------------------------------------------------------------|-----|
| APPENDIX 1: Calculation of volumes of tritiated<br>steroids for the DCC assay      | 222 |
| APPENDIX 2: Calculation of 'factors' for DCC assay                                 | 223 |
| APPENDIX 3: Exclusions from logrank analysis                                       | 224 |
| APPENDIX 4: Exclusions from logrank analysis                                       | 225 |
| APPENDIX 5: Proforma                                                               | 226 |
| APPENDIX 6: Variation of receptor status (synchronous)                             | 228 |
| APPENDIX 7: Variation of receptor status (asynchronous)                            | 230 |
| APPENDIX 8: Response; summary of data (Receptors unknown)                          | 235 |
| APPENDIX 9: Response; summary of data (Receptors known)                            | 238 |
| APPENDIX 10: Comparison of two methods of assay of<br>progesterone receptor        | 251 |
| APPENDIX 11: Binding of steroids by serum proteins                                 | 255 |
| APPENDIX 12: Effect of molybdate                                                   | 256 |
| APPENDIX 13: Effect of gel media                                                   | 257 |
| APPENDIX 14: Migration of free <sup>3</sup> H-R5020                                | 258 |
| APPENDIX 15: Precision of assay                                                    | 259 |
| APPENDIX 16: Comparison of two methods of assay of<br>oestrogen receptors          | 260 |
| APPENDIX 17: Needle biopsies; weights & cytosol<br>protein yield                   | 262 |
| APPENDIX 18: Induction of progesterone receptor;<br>clinical & biochemical details | 263 |
| APPENDIX 19: Intra-tumour variation of progesterone<br>receptor                    | 306 |

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\* \* \* \* \*

## INTRODUCTION

\* \* \* \* \*



At birth the breast consists of a number of rudimentary branching ducts. The male breast seldom develops further. In the female substantial changes occur that are manifestly associated with ovarian function. At puberty when the breast develops into adult form there is an increase in both epithelial and stromal elements. During reproductive life the epithelium undergoes changes in cellularity and differentiation in response to changes in concentrations of female sex hormones that occur with the menstrual cycle, pregnancy and lactation. After the menopause, when concentrations of oestrogens in the blood are reduced dramatically, the breast undergoes involution.

#### Endocrine response in cancer of the breast

A proportion of mammary cancers are affected by the presence or absence of oestrogens in a similar way to the normal breast. This phenomenon is of paramount importance for the treatment of disseminated cancer of the breast. Substantial changes in the concentration of circulating oestrogen such as occur when the ovaries are removed from premenopausal patients often result in gross reductions of the extent of disease with the healing of ulcers, calcification of lytic bone metastases and worthwhile symptomatic improvement.

The prevalence of these reductions, called regressions, is frequently used as a measure of the effectiveness of treatment. When this is done patients are classified into one of three states at a particular time after the start of treatment:

##### 1. Regression

## 2. Unchanged

## 3. Progression

It is usual to use the term 'response' to describe a beneficial effect of hormonal manipulation. Most workers regard only regression as a response and use the terms synonymously. Others regard stability of the disease as a response also.

Ovarian ablation, originally by oophorectomy and later by irradiation, was the first form of endocrine manipulation which was used (Beatson, 1896). Treatment with androgens, or with very high doses of oestrogens, were later shown to be effective (Ulrich, 1939. Haddow et al., 1944)

These treatments were only effective in a minority of patients, but there was no adequate alternative and such treatments were relatively free from untoward effects. There was little interest in the prediction of response to treatment.

The advent of major endocrine ablation, bilateral adrenalectomy or hypophysectomy, stimulated the search for an effective means of discrimination between patients who were likely to respond and those who were not. Many patients were subjected in the terminal stages of their illness to unpleasant treatment which had no effect. The need for a means of prediction of response was clear.

Clinical features which indicated the prognosis after oophorectomy (Boyd, 1900. Lett, 1905) were examined extensively. Some, such as the predominant site of involvement by the tumour, or the length of the interval

between mastectomy and the onset of secondary disease, defined small groups in whom the prognosis was appreciably different from the remainder (Fracchia, 1967. Taylor 1962. Nemoto & Dao, 1966). A trial of therapy was considered worthwhile in a majority. Other methods of selection of patients for endocrine treatment were sought.

The endocrine function of patients with disseminated breast cancer was studied extensively. The excretion of the classical oestrogens was an obvious factor which proved however to have no value in the selection of patients for operation (Bulbrook, 1974.).

Androgens, from the adrenal glands, may be converted to oestrogens within tumours (Miller & Forrest, 1974. Adams & Li, 1975. Abul-Hajj, 1975). Tests which were based on measurements of the excretion of androgen metabolites had some value in the prediction of response. An example of these was the 'discriminant function' of Bulbrook. Results with this discriminant showed that a clear regression occurred more often when the value was positive than when negative (Bulbrook, Greenwood & Hayward, 1960). Similar studies confirmed that these assays provided prognostic information before ablation (Sellwood et al., 1974. Juret, 1968. Perry, 1972) although the value of such hormone studies before other forms of hormone treatment was less clear (Ahlquist et al, 1967).

Indirect assessment of endocrine status by cytological examination of a vaginal smear also had some value as a prognostic test before ablation and progestogen treatment, although it did not prove valuable before

additive oestrogen therapy (Stoll, 1967. Fracchia et al., 1967).

The observation that oestrogens, when injected into animals, were concentrated in the mammary gland (Glascock & Hoekstra, 1959. Jensen & Jacobson, 1962) was followed by attempts to use this method to test for hormone dependence in cancer of the breast (Folca et al, 1961). Although this was not the first study to show selective uptake of an oestrogen by breast cancers (Lewison et al., 1951) it was the first study which sought to relate hormone response to the handling of oestrogens by tumours.

The ability of a cancer to take up oestrogens was estimated by the measurement of the radioactivity in a sample of the tumour after infusion of a radioactive preparation of a synthetic oestrogen, hexoestrol. The activity was compared with that in plasma and in striated muscle, a tissue which is not normally affected by oestrogens. With this method two groups with different prognoses were defined. In those patients in whom the radioactivity in the tumour was similar to that found in plasma and muscle there was a very low incidence of response. When uptake by the tumour was at least 50% greater than that in plasma, about half of the patients responded.

With further experience it was concluded that the reliability of the test may be affected by many factors including the stage in the course of the disease at which the test was undertaken (Braunsberg et al, 1973). This conclusion anticipated a major doubt concerning the value

of assays of receptors in the prediction of response.

### Receptors and endocrine dependance

The results of studies of uptake of oestrogen by tumours in-vitro indicated the presence of a receptor protein (Toft & Gorsky 1966, Jensen et al., 1968. Talwar et al., 1964).

Interaction of oestrogen with oestrogen receptor occurred in two steps. First oestrogen associated with the receptor spontaneously in the cytoplasm. Second the complex of receptor and oestrogen was passed to the nucleus (Jensen et al., 1968).

Studies with oestrogen were followed by reports of high-affinity binding of progesterone to a molecule which was similar in some respects to oestrogen receptor (Rao et al., 1973, Milgrom & Baulieu, 1970). This new protein was the progesterone receptor.

Early reports of progesterone binding by mammalian uteri indicated that prior treatment with oestrogens was essential for progesterone binding to be detected (Falk & Bardin, 1970. Milgrom & Baulieu, 1970). In experimental animals exposure to oestrogen was followed by a rise in the number of progesterone binding sites (Vu-Hai et al., 1977. Isomaa et al., 1979. Manni et al., 1981). When no more oestrogen was given the concentrations of progesterone receptor declined slowly, with a half life of 3-7 days. When oestradiol administration was continued the amount of progesterone receptor rose further, and after an initial reduction, oestrogen receptors rose to higher

concentrations than before treatment (Clark et al., 1977).. The secondary rise of oestrogen receptors was prevented by simultaneous treatment with progesterone.

Effects of oestradiol on protein synthesis had been observed before the discovery of oestrogen receptors. The actions of oestradiol on transcriptional events have been reviewed in detail (Baulieu et al, 1972. Chan & O'Malley, 1977).

These observations have led to an understanding of the action of oestrogen on target tissues (Figure 1).

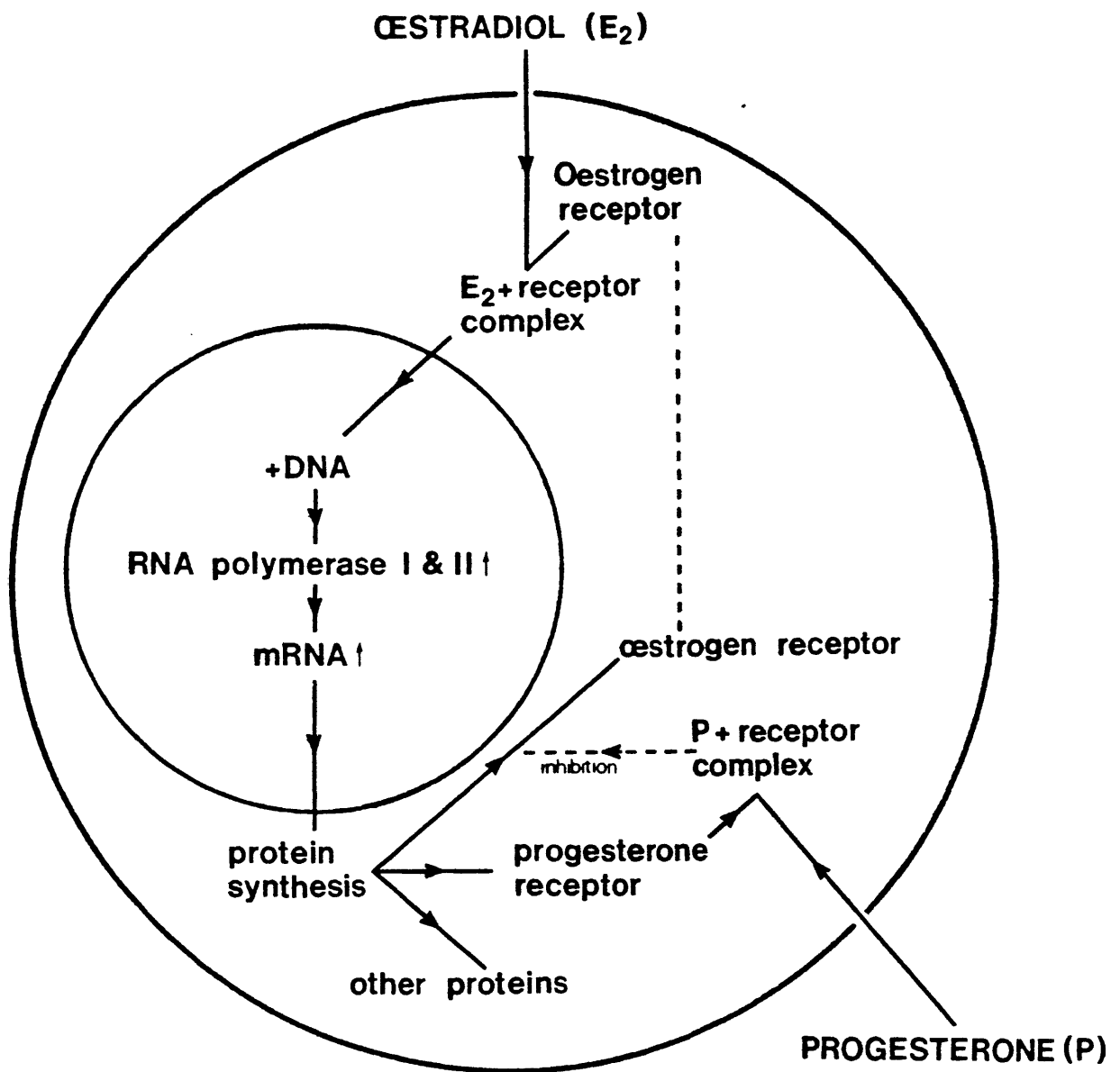


Figure 1: Mechanism of action of oestrogen on target tissues

### Receptors and response in mammary cancer

Jensen (1971) initially related the effects of adrenalectomy to the presence or absence of oestrogen receptor in 27 patients who had advanced cancer of the breast. Only one (5.5%) of 18 patients in whom oestrogen receptor was not detected responded as compared with 5 of 9 patients (55.5%) when assays had shown oestrogen receptor.

A substantial number of results are now available from different centres. These show that very few patients responded when no oestrogen receptor was detected in their tumour but about half of the patients responded when oestrogen receptor was present (Table I).

Many of these reports showed also that the results of assay of oestrogen receptors could be applied whatever form of endocrine treatment was given. This aspect of oestrogen receptor assay has been reviewed extensively (Hawkins, Roberts and Forrest, 1980).



TABLE I: Oestrogen receptor status and response

|                           | +ve             | -ve          |
|---------------------------|-----------------|--------------|
| Hahnel & Vivian, 1975     | 13/29 (45%)     | 2/52 (4%)    |
| Leung et al., 1975        | 27/45 (60%)     | 0/19         |
| Jensen et al., 1975       | 26/42 (62%)     | 2/56 (3%)    |
| Wittliff & Savlov, 1975   | 5/18 (28%)      | 0/15         |
| Heuson et al., 1977       | 8/25 (32%)      | 0/9          |
| Leclerq, 1977             | 2/6 (33%)       | 0/1          |
| Kiang et al., 1978        | 24/45 (53%)     | 1/38 (3%)    |
| Matsumoto et al., 1978    | 41/82 (50%)     | 5/52 (10%)   |
| Barnes et al., 1979       | 11/18 (61%)     | 5/33 (15%)   |
| Hawkins, 1979             | 22/53 (40%)     | 0/17         |
| King et al., 1979         | 19/50 (38%)     | 3/27 (11%)   |
| King, 1980                | 15/39 (38%)     | 2/18 (11%)   |
| Young et al., 1980        | 23/43 (53%)     | 3/11 (27%)   |
| Brooks et al., 1980       | 6/13 (46%)      | -            |
| Osborne et al., 1980      | 71/145 (49%)    | 5/53 (9%)    |
| McCarty et al., 1980      | 36/78 (46%)     | 3/20 (15%)   |
| Dao & Nemoto, 1980        | 64/119 (51%)    | 4/56 (7%)    |
| Holdaway et al, 1980      | 26/40 (65%)     | 3/32 (9%)    |
| Manni et al., 1980        | 70/114 (61%)    | 0/12         |
| Degenshein et al., 1980   | 29/47 (62%)     | 1/15 (7%)    |
| Nomura et al, 1980        | 29/45 (64%)     | 0/36         |
| Samaan, 1980              | 21/44 (47%)     | 1/18 (6%)    |
| Mosely et al., 1980       | 53/83 (64%)     | 6/35 (17%)   |
| Leclerq, 1980             | 14/38 (37%)     | 0/11         |
| Lippmann & Allegra, 1980  | 34/52 (67%)     | 3/33 (9%)    |
| Laurence et al., 1980     | 22/55 (40%)     | 3/7 (43%)    |
| Rubens et al., 1980       | 46/136 (34%)    | 5/55 (9%)    |
| MacFarlane et al., 1980   | 15/29 (52%)     | 1/11 (9%)    |
| Maass et al., 1980        | 64/93 (69%)     | 3/76 (4%)    |
| Paridaens et al., 1980    | 14/38 (37%)     | 0/11         |
| Sinhakowinta et al., 1980 | 20/30 (66%)     | 2/25 (8%)    |
| Rose et al., 1980         | 10/21 (48%)     | 2/19 (11%)   |
| Engelsman, 1980           | 23/31 (74%)     | 5/34 (15%)   |
| Loven & Rakosky, 1981     | 20/35 (57%)     | 2/13 (8%)    |
| Leake et al., 1981        | 36/62 (58%)     | 6/67 (9%)    |
| Cheix et al., 1981        | 26/38 (68%)     | 0/2          |
| Dittrich et al., 1981     | 25/43 (58%)     | 3/20 (15%)   |
| Campbell et al., 1981     | 17/55 (31%)     | 2/40 (5%)    |
| Stewart et al., 1982      | 42/103 (40%)    | 5/44 (11%)   |
| TOTAL                     | 1069/1982 (54%) | 88/1093 (8%) |

Although assay of oestrogen receptors was clearly of value as a means of prediction, the presence of oestrogen receptor did not indicate a very high probability of improvement with endocrine treatment. Horwitz and colleagues reasoned that the presence of progesterone receptor, which depended normally on the effective action of oestrogens, would give a clearer indication of endocrine dependence than the presence of oestrogen receptor (Horwitz & McGuire, 1975). In their initial report response to endocrine treatment was seen only in the 3 patients in whom progesterone receptors were detected. No response was seen in the seven remaining patients whatever their oestrogen receptor status.

Clearly these preliminary results were not conclusive. Although further experience has, in general, confirmed the high incidence of response when progesterone receptors are present within tumours there have been few reports in which this matter has been investigated extensively. Combination of results of five large studies suggest a prevalence of response of 70% (121/181) when progesterone receptors were present compared with 17% (21/125) when receptor was not found (Young et al., 1980. McCarty et al., 1980. Stewart et al., 1982. Degenshein et al., 1980). Although assay of progesterone receptors indicated the response (or lack of it) correctly in about 70% of patients the relatively high incidence of response when receptor was not found has discouraged most workers from recommending assay of progesterone receptors as the only test of endocrine dependence.

Often both oestrogen and progesterone receptors were measured. When both receptors were found between 50 and 90% (mean 78%) of patients responded (Young et al., 1980. Stewart et al., 1982. McCarty et al., 1980. McGuire, 1980. Degenshein et al., 1980. Dao & Nemoto, 1980). When neither receptor was present 0 - 10% of patients responded. About one third of patients whose tumours contained only oestrogen receptor responded. This group are generally regarded as one with an intermediate prognosis.

The presence of oestrogen receptor in the nucleus suggests that the processes of binding and translocation of receptor are intact. The few reports on the incidence of response in relation to the presence of nuclear oestrogen receptors suggests that the results of assays of both cytosol and nuclear oestrogen receptors may predict response more accurately than assay of cytosol oestrogen receptor alone (Leake et al., 1981, Barnes et al., 1979. MacFarlane et al., 1980).

Some studies have indicated that response was most likely when oestrogen receptors were present at high concentrations (Lippman & Allegra, 1980. Campbell et al., 1981. Paridaens et al., 1980. McGuire, 1980. Maass et al., 1980). It has been suggested that the measurement of other receptors may be unnecessary when the concentrations of oestrogen receptor are considered. In general, reports in which both oestrogen and progesterone receptors have been measured show a small advantage in favour of progesterone receptor assay over quantitative analysis of oestrogen receptor (McGuire, 1980. Young et al., 1980. Dao & Nemoto,

1980. McCarty et al., 1980).

### Assessment of response

An obvious regression is a reassuring sign that treatment is having a good effect but regression is sometimes difficult to recognise. The errors of clinical measurement are such that wide limits for changes in the extent of disease are set which must be reached before regression or progression is identified. Lesser changes are classified as stability of disease. The presence of this intermediate group reduces substantially the value of many reports of clinical trials. The assumption is usually made that only changes amounting to clear regression indicate benefit, although in some studies this group have had a similar prognosis to patients with obvious regression (Firusian et al., 1980. Ross et al., 1982. Glick et al., 1981. Pouillart et al., 1979). Arbitrary allocation of patients with stable disease to a response category, whether failure or success, introduces an error which has unpredictable effects on the assessment of the value of treatment.

Regression will not necessarily become obvious but progression inevitably will. Effective treatment will result, in general, in longer periods of freedom from progression than ineffective treatment. This period, between the start of treatment and progression of the disease may be analysed in the form of a 'life table' which gives an accurate estimate of the probability of remaining free from further progression of the disease at any time after the start of treatment. Such tables may be

compared by standard statistical methods (Mantel, 1966). The general justification and the application of logrank analysis to end-points other than death has been discussed in an authoritative article on the analysis of clinical trials (Peto et al, 1977.).

The accuracy of such tables and tests increases as the proportion of patients who have either suffered the event in question (in this case progression of the disease) or who have passed through the greatest period of risk, rises. For patients with advanced cancer of the breast progression is likely to occur in the early stages of treatment. This form of analysis is particularly suitable in this context, although it has been used only occasionally (Tormey et al, 1976. Glick et al., 1980). It is a standard method for the assessment of the effect of treatment in other malignant conditions, and when treatment is given as an adjuvant measure.

No system yet devised for the measurement of the effects of treatment conforms to the ideal, which would not exclude any patient from analysis and would give an unbiased and precise result from which a prognosis might be made without further qualification.

Apart from the imprecision due to the inaccuracy of clinical measurement the conventional system fails in a number of respects to conform to these ideals. Patients with manifestations of disease, such as serous effusions, for which no assessment can be made, are systematically excluded. Many of the recommendations for the assessment of patients are stringent (Hayward et al., 1977. British

Breast Group, 1974. Groupe Europeen de Chimiotherapie Anticancereuse, 1966) and a large proportion of patients whose assessment fails to conform must be discarded from analysis (Roberts et al., 1978). Selection in this way reduces efficiency and introduces a doubt about the validity of any conclusion.

In some patterns of disease the changes by which reponse is defined may be so extreme that a regression may easily go unrecognised. For example, sclerotic bone metastases can be shown to have improved only when the radiological appearances return to normal. This is a rare event and progression of disease is more likely to be recognised. Inclusion of such patients introduces a bias against the classification of regression. Oestrogen receptor positive tumours are said to metastasise to bone more frequently than oestrogen receptor negative tumours (Singhakowinta et al., 1976. Campbell et al., 1981. Stewart et al., 1981). In this way the assessment of response may be biased.

Different tests of whether a response has occurred are used for different patterns of disease. In lytic bone metastases some degree of sclerosis must occur for a response to be identified. It is not clear whether this change is a equivalent one to that which must occur when measureable disease is considered.

The course of the disease is split by the conventional system into two parts and two prognoses are given. The first is the probability of being in regression at the time of assessment. The second, given only for

patients in remission, is the probability of remaining free from progression thereafter. The latter prognosis is necessarily less accurate than the equivalent prognosis calculated for the whole group, irrespective of response. Comparisons made only between responders of one group and another are often inconclusive because of small numbers.

Although the use of logrank analysis depends on the same clinical measurements, progression is an event which is easier to recognise than regression, very few patients need be excluded and a clinically useful prognosis, the probability of relapse, is given directly and not by implication.

#### Assay of receptors

Several methods of measurement of receptors have been described. The biochemical methods are complicated but some elements of each assay are common to all.

As oestrogen and progesterone receptors are found in both the cytoplasm and the nuclei the first step in all biochemical techniques is the separation of the soluble cytoplasmic proteins from those held within the nuclei. To carry out this separation the tissue must be broken down to form a suspension of cell fragments and cytoplasm from which the nuclei and cellular debris are removed by centrifugation at high speeds. The supernatant, which contains the cytoplasm diluted in buffer, is called the cytosol. Below this is a pellet of cell fragments. This is a crude preparation of nuclei on which assays of nuclear receptor can be carried out, either directly or after

extraction procedures.

Most methods rely on the binding of a labelled oestrogen or progestin to its receptor. The usual 'label' is tritium, a beta-ray emitting isotope of hydrogen, which is incorporated into the hormone molecule. The natural hormone, oestradiol-17-beta, is used to measure oestrogen receptors. Curiously, progesterone is not the best hormone to use when measuring progesterone receptors, because it binds also to corticosteroid-binding globulin (a serum protein which may contaminate cytosol preparations) and does not have the highest affinity for the receptor of the compounds available (Raynaud et al., 1977. Duffy, 1979). Synthetic progestins which have a higher affinity for the receptor and which do not bind significantly to corticosteroid-binding globulin are used. Fortunately the names of these compounds are abbreviated. R5020 and ORG2058 are the progestins used commonly for this assay.

When incubated with the cytosol preparation the hormone binds strongly to the receptor. As the amount of hormone which is present is increased the amount attached to the receptor approaches a maximum beyond which no more will bind whatever the concentration of free hormone. The total receptor is given by the specific binding that would occur when an infinite amount of free hormone was present. The hormone also binds weakly to other proteins which may be present. This binding is unlimited and, unlike the receptor, cannot be saturated.

To distinguish the strong binding from the weak, other incubations are carried out in which both the



radioactively-labelled hormone and a much larger quantity of unlabelled hormone are present. The two hormones bind to the receptor according to their relative proportions. Because the weaker 'non-specific' binding is not affected by the excess of unlabelled hormone, the difference between the total amount of radioactivity bound when the unlabelled hormone is present and when it is not is a measure of the 'specific' binding to the receptor.

Before any estimate of the binding of labelled hormone to proteins can be made the hormone which has not been bound must be removed. Various methods have been used. Dextran-coated charcoal (DCC) which adsorbs the small molecules of free hormone, is both effective and convenient to use, and has become the standard preparation (King et al., 1979. Raam et al 1981). Other substances, such as hydroxylapatite which absorbs only the protein bound hormone, have been recommended but are not in widespread use (Rosner et al., 1980). When the free hormone has been removed the amount of radioactivity that is bound can be measured with a scintillation counter.

In its simplest form the assay can be carried out in this way with a concentration of labelled hormone that is likely to fill almost all of the receptor sites. This is called a 'single point' or 'saturation point' assay.

Usually the incubations are carried out with a range of concentrations of the hormone. Although the data could be expressed as a plot of specifically bound hormone against the concentration of free hormone, they are more often expressed as a 'Scatchard plot' in which the ratio

of the specifically bound hormone to the free hormone ( $B/F$ ) is plotted against the concentration of specifically bound hormone  $[B]$  on the abscissa (Scatchard, 1949). When only one protein capable of binding hormone strongly is present a linear plot is obtained (Figure 2).

Extrapolation of the plot to the abscissa gives an estimate of the concentration of specifically bound hormone when the expression ( $B/F$ ) is equal to zero. This occurs when the concentration of free hormone ( $F$ ) is at infinity.

The high affinity of the receptor for the hormone is a fundamental characteristic. The dissociation constant, which is given by the reciprocal of the slope of the line of the Scatchard plot, is a measure of the affinity of the binding. Analysis in this way gives an estimate of this affinity for which confidence limits can be defined.

Other methods of assay rely solely on the suppression of binding by an excess of non-radioactive hormone as evidence of high-affinity binding. The receptor is identified by some other characteristic. Sucrose density gradient analysis is the most widely used of these other techniques. In this method receptor is separated on the basis of molecular size by spinning at very high speeds in tubes containing sucrose solutions of graded density. Much of the basic research has used this method, but its limited capacity make it unsuitable for routine use.

In the dextran-coated charcoal assay with Scatchard analysis 6 different concentrations of radioactive-hormone are used, with a parallel incubation containing the

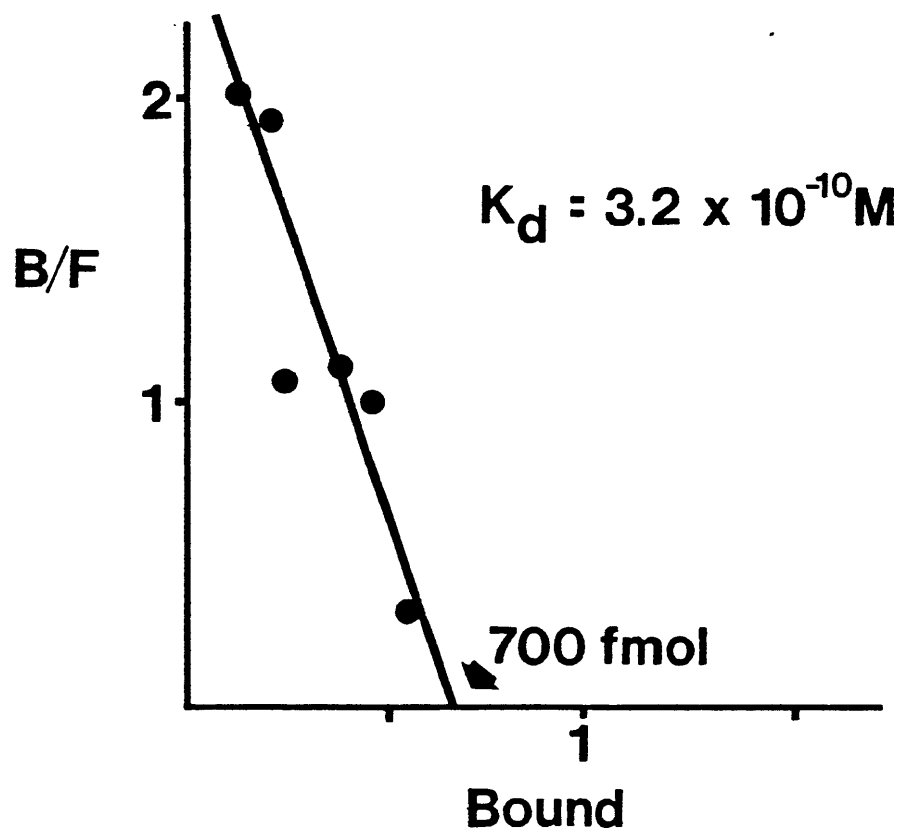


Figure 2: An example of a Scatchard plot

unlabelled competitor. 24 incubations are usually made to measure both oestrogen and progesterone receptors. A substantial volume of cytosol is needed.

When only a small amount of tumour is available, the concentration of the cytoplasmic proteins in the cytosol preparation is usually low. The assay becomes unreliable at low concentrations of cytosol (Powell et al., 1981). In practice a minimum of 200 mg. of tumour is necessary to measure both receptors. An open biopsy is usually needed to obtain this amount of tumour.

#### Variation of receptor

Secondary deposits are not often readily accessible for biopsy, or may be too small for these assays. Treatment is often decided on the basis of receptors assessed in the primary tumours before the need for systemic treatment has arisen. The value of assays carried out on the primary tumour may be reduced if receptor status changes with time, or with progression of the disease.

Two observations suggest that this may occur. First, the incidence of receptors is lower in metastases than in primary tumours (Hawkins et al., 1979. Maass et al., 1975. Jensen et al., 1975. Heuson et al., 1975). Such differences would also be explained if receptor negative patients were more likely to relapse. However the probability of a response declines as the number of involved sites increases even when oestrogen receptor positive patients only are considered (Lippman & Allegra, 1980).

The true extent of variation of receptors remains unclear. The numbers in reports which have suggested that oestrogen receptor status may vary have been too small to assess the significance of the changes observed. Assays of samples which had been taken at different times (asynchronous samples) show usually greater inconsistency than assays which had been carried out on samples removed at one operation (synchronous samples) (Table II).

The data on variation of progesterone receptor status are much less extensive. The few reports that are available suggest that progesterone receptors may vary to an extent similar to that of oestrogen receptor (Koenders, 1979. Matsumoto, 1978. Mobbs, 1982. Fernandez et al., 1982. King, 1979).

Previous reports have failed to consider the changes which may take place when both receptors are considered together.

Most reports have not stated unequivocally whether the sample of tumour for receptor assay was taken from the primary tumour at the time of mastectomy or from a secondary deposit shortly before endocrine treatment was started. Some studies of oestrogen receptor have shown little difference in the predictive value whether assays were carried out at the time of mastectomy or later (McGuire, 1980. DeSombre & Jensen, 1980. Singhakowinta et al., 1976. Stewart et al., 1982). On the other hand, the presence of both oestrogen and progesterone receptors may be less reliable as an indication of probable response when there is an interval between the assay and endocrine

treatment than when there is not (Stewart et al., 1982).

TABLE II: Published observations on variation of oestrogen receptor status (No. changed/ total)

|                             | Asynchronous | Synchronous |
|-----------------------------|--------------|-------------|
| Rosen et al, 1977           | 7/29 (24%)   | -           |
| Brennan et al, 1979         | -            | 10/29 (34%) |
| Peetz et al, 1982           | 17/58 (29%)  | 2/14 (14%)  |
| Walt et al, 1976            | 3/15 (20%)   | -           |
| Allegra et al, 1980         | 8/34 (24%)   | -           |
| Hoehn & Plotka, 1979        | -            | 7/37 (19%)  |
| Holdaway & Mountjoy<br>1978 | 0/1          | 1/6         |
| Webster et al, 1978         | 8/21 (38%)   | 8/25 (32%)  |
| Kiang & Kennedy,            | 3/6          | 0/9         |
| Fernandez et al, 1982       | -            | 6/26 (23%)  |
| Leake et al, 1981           | 14/50 (28%)  | -           |
| Hawkins et al, 1981         | -            | 1/23 (4%)   |
| Koenders, 1980              | 6/52 (12%)   | 1/19 (5%)   |
| Skinner et al, 1980         | 5/14 (36%)   | 2/10        |
| Mobbs, 1982                 | 3/25 (12%)   | 2/19 (10%)  |
| Manni et al, 1980           | 2/14 (14%)   | -           |
| Leung et al, 1975           | -            | 12/43 (27%) |
| Korsten et al, 1975         | 7/10         | -           |
| Nemoto & Dao, 1980          | -            | 2/18 (11%)  |
| Braunsberg et al, 1975      | -            | 0/2         |
| Nomura et al, 1977          | -            | 4/26 (15%)  |

### Induction of progesterone receptor

There are several possible reasons why receptor status is not completely reliable as a sign of endocrine dependance. First, the effects of oestrogens on progesterone receptor synthesis may occur seperately from the effects on the growth rate of tumours. This has been shown in some experimental tumours in which endocrine control progesterone receptor was normal, but which failed to respond to endocrine manipulations (Ip et al., 1981. Lee et al., 1981. Watson et al., 1980).

Second there may have been sampling and measurement errors.

Third, the assays may have failed to indicate response correctly because the cells had not had the necessary oestrogenic stimulus for the synthesis of progesterone receptors or because autonomous synthesis of progesterone receptor had occurred. The latter effect has been shown in one human breast cancer cell line (Horwitz, 1982) and there is a small proportion of progesterone receptor positive patients in whom oestrogen receptor is never found.

It is possible to test directly whether the synthesis of progesterone receptors depends on stimulation by oestrogens, by measuring progesterone receptors before and after a suitable stimulus. The use of oestrogens for this in patients with breast cancer may lead to harmful clinical effects. Tamoxifen is a anti-oestrogenic drug which is used widely for the treatment of mammary cancer. This drug also combines with the oestrogen receptor and in

experimental situations can produce some of the effects of oestrogens initially, including a rise in the concentration of progesterone receptors. These changes have been shown in the uteri of immature mammals, in DMBA-induced and the MTW-9B rat cancers, in the MCF-7 human breast cancer cell line and in patients with endometrial cancer (Davies et al., 1979. Pasqualini et al., 1980. Holinka et al., 1980. Isomaa et al., 1979. Horwitz & McGuire, 1980. Rochefort et al., 1981. Mortel et al., 1981).

Such increases in progesterone receptors have been described in patients with breast cancer, but the relationship of these changes to subsequent clinical response is not clear (Namer et al., 1980. Waseda et al., 1981). Increases in progesterone receptors which are induced by tamoxifen may reveal patients whose tumours retain a normal mechanism of endocrine control, and may be a specific test of endocrine dependance.

Such an approach requires repeated biopsies, and the difficulties of obtaining material at the time of dissemination have already been discussed. Conventional methods of assay are unsuitable for the extensive application of a 'dynamic' test of progesterone receptor synthesis. Methods of assay which can be carried out on very small samples would overcome many of the problems with this approach.

A number of methods have been proposed as suitable for the measurement of oestrogen and progesterone receptors in small samples (Wrange et al., 1976. Meyer et



al., 1978. Milano et al., 1983. Lloyd et al., 1982). All microassay techniques rely on a single 'saturation-point' incubation, and differ only in the means used to separate the free hormone from the protein bound activity.

The assay which has been developed in this study is based on the separation of receptor by isoelectric focusing (IEF). This is a form of electrophoresis carried out in a gel medium in which the pH drops progressively between the cathode and the anode. The charge of a protein molecule depends on the pH of its environment and each protein has a characteristic pH, its isoelectric point (pI), at which its net charge is zero. In such a gel medium migration of a protein molecule will cease when it reaches its isoelectric point and has no charge. This effect of concentration of a protein at its pI makes IEF a suitable technique for the resolution of small amounts of receptor. Variations of this technique have been described for the measurement of both oestrogen and progesterone receptors. Originally IEF was carried out in columns of gel, in which only one sample could be measured. It took a long time for focusing to occur in these columns, and they were never suitable for anything but occasional research use. The introduction of flat beds of gel allowed a number of samples to be assayed simultaneously and shortened the time taken for focusing to occur, so that the basic method became suitable for routine use (Vesterberg, 1973).

This method of IEF in flat beds of gel has been used for the measurement of oestrogen receptors (Lloyd et al., 1982. Coffer & King, 1976. Wrange et al., 1976). There has

been one report of its application to the assay of progesterone receptors, but the report was incomplete and failed to recognise some of the difficulties which may occur (Wrange et al., 1981).

### Summary

Despite a decade of experience of the measurement of receptors in mammary cancer several important aspects of the value of these assays remain unclear. For most patients assays can be done only at the time of mastectomy. The effects of time and dissemination on the value of these assays has not been established, particularly when both oestrogen and progesterone receptors are measured.

The effects of time on receptor status, and the relative merits of assay of oestrogen and progesterone receptors at different stages in the course of the disease is set out in this thesis which is presented to give a clearer understanding of how such assays may be used when treating patients.

It is already clear that assays of receptors fall short of the ideal test which would distinguish with certainty between patients who will and who will not respond to endocrine manipulations. Studies of the stimulation of progesterone receptor may be a sensitive means of showing normal endocrine response, and may improve discrimination. The use of this novel approach is investigated, and a new microassay which has been developed for these studies is described.

\* \* \* \* \*

## METHODS

\* \* \* \* \*

## METHOD OF ASSAY

### The dextran-coated charcoal (DCC) assay

#### Reagents

<sup>3</sup>  
[ <sup>3</sup>H]-oestradiol-17-beta & [ <sup>3</sup>H]-R5020 ((17-alpha methyl  
<sup>3</sup>  
H)promogestone), specific activity 87 Ci mmol, was  
obtained from New England Nuclear. Norethisterone (4  
Estren-17 alpha ethynyl beta-ol-3-one) was purchased from  
Koch Light. Diethylstilboestrol was supplied by the  
Christie Hospital Pharmacy. Dextran T70 was purchased from  
Pharmacia Ltd and gelatin from the Sterling Gelatin  
Company. Ready-Solv EP premixed scintillation fluid was  
supplied by Beckmann RIIC Ltd. Dithiothreitol and Norit-A  
charcoal were supplied by Sigma Ltd. All other reagents  
were Analar Grade. 3ml polycarbonate tubes were purchased  
from MSE Scientific Ltd and the Braun Microdismembrator  
was supplied by FT Scientific Instruments Ltd.

#### Samples

Samples of primary or metastatic lesions were taken  
at the Withington and Christie Hospitals in Manchester.  
Each sample was trimmed of excess fat and connective  
tissue, and then frozen rapidly in liquid nitrogen.  
Samples were transported and stored in liquid nitrogen  
until assayed.

#### Preparation of cytosol

The frozen sample was divided into small pieces when  
necessary. Each sample was put into a teflon vial which  
had been cooled in liquid nitrogen. A 10mm tungsten

carbide ball, also cooled in liquid nitrogen, was added and the vial sealed before further immersion in liquid nitrogen. When 'hard-frozen', each sample was disintegrated by rapid vibration in a Microdismembrator for 20 seconds. The powdered tissue was decanted from the vial into 10 ml beakers in an iced water-bath. The tissue powder was allowed to thaw and was suspended in about 2 mls of homogenisation buffer (see materials).

When mixed thoroughly the bulk of the cellular debris was removed by spinning at  $800 \times g$ , at  $4^{\circ}C$ , for 10 minutes. The supernatant was decanted into 3 ml polycarbonate tubes. The pellet was retained for the assay of nuclear oestrogen receptors.

The residue of the cell fragments were removed by spinning at  $100,000 \times g$  for 1 hour under a vacuum, again at  $4^{\circ}C$ . The resulting supernatant was retained as the cytosol preparation and was made up to 2.5 - 3 mls in volume with homogenisation buffer when an adequate nuclear pellet had been obtained. When the volume of nuclear and cellular debris was small the cytosol was not diluted, and the assay was carried out with fewer concentrations of radioactive hormone than usual.

#### Preparation of non-radioactive solutions.

##### i) Diethylstilboestrol

19.2 mg of diethylstilboestrol were dissolved in 50 mls of ethanol ( $1 \times 10^{-3}$  mol/l).  $2 \times 10^{-9}$  mol/l working solutions were made by dissolving 10 microlitres of stock solution in 50 ml of 0.1% gelatin buffer.

ii) Norethisterone

14.92 mg of norethisterone were dissolved in 50 ml of ethanol ( $1 \times 10^{-3}$  mol/l).  $8 \times 10^{-9}$  mol/l working solutions were made by dissolving 40 microlitres of the stock solution in 50 ml 0.1% gelatin buffer

iii) 1% Dextran-coated charcoal solution was made by dissolving 500 mg of dextran in 500 ml of 0.1% gelatin buffer. 5 g of charcoal was added and the solution was stirred vigorously for 1 hour.

Preparation of Radioactive solutions

i) Stock solutions

$^3$  [ H]-oestradiol and  $^3$  [ H]-R5020 were supplied dissolved in toluene (250 microCuries in 250 microlitres). The solvent was evaporated under a stream of nitrogen and the hormone redissolved in 5 ml ethanol (50 microCuries/ml).

ii) Working solutions

A volume (see Appendix 1) of stock  $^3$  [ H]-oestradiol was dried under a stream of nitrogen, and redissolved in buffer (see Materials). This working solution was diluted serially with buffer alone or with buffer containing 200nmol/l diethylstilboestrol (DES) to give 2 series of solutions. One series contained  $^3$  [ H]-oestradiol at 0.25, 0.375, 0.5, 0.75, 1.0 and 1.5 nmol/l in buffer, and the other contained the same concentrations of  $^3$  [ H]-oestradiol in buffer in the presence of a 60-fold excess of DES.

$^3$  [ H]-R5020 was prepared in the same way, with 0.014 ml of stock solution per ml final volume of working

solution. This was diluted further in buffer or buffer containing 800 nmol/l norethisterone to give concentrations of [<sup>3</sup>H]-R5020 of 0.66, 1.0, 1.33, 2.0, 2.66 and 4.0 nmol/l, with or without a 100-fold excess of norethisterone.

#### Incubation of cytosol

For every sample tested 0.1 ml of working solution at each concentration of radioactive hormone was pipetted into test-tubes resting in a slurry of ice and water.

0.1 ml of cytosol was added to each tube, and mixed. The incubation was continued overnight (16 - 20 hours) at 4 °C.

#### Separation of free hormone

A 1% solution of dextran-coated charcoal was mixed at 4 °C. 0.25 ml was added to each tube which contained cytosol. The tubes were shaken and allowed to stand for 15 minutes ([<sup>3</sup>H]-oestradiol) or 20 minutes ([<sup>3</sup>H]-R5020) in a slurry of ice and water. The dextran-coated charcoal was then removed by spinning at 10,000g for 15 minutes at 4 °C.

0.35 ml of the supernatant was carefully removed and put into scintillation vials containing 3 ml Beckmann EP scintillation fluid, for counting.

#### Total activity

The total radioactivity present at each concentration was measured by adding 0.1 ml of each concentration of hormone to test tubes at the time the incubations were carried out. 0.1 ml of buffer was added instead of

cytosol, and at the end of incubation 0.25 ml of buffer was added instead of dextran-coated charcoal. 0.35 ml was removed for counting, as before.

#### Quality Control

Cytosol was prepared from a tumour which contained oestrogen and progesterone receptors and was stored as 0.5 ml aliquots in liquid nitrogen. 0.1 ml of cytosol was incubated with the second highest concentration of each hormone, as described. When receptors were not detected in an assay and the quality control cytosol did not show the expected suppression of binding in the presence of competitor, the results were rejected.

#### Charcoal blanks

The four highest concentrations of hormone with competitor were incubated as described, with buffer instead of cytosol. Dextran-coated charcoal was added. When excessive radioactivity was found in the supernatant in all four tubes the efficiency of adsorption of the dextran-coated charcoal was suspect, and negative results interpreted accordingly.

#### Measurement of radioactivity

Radioactivity was measured by scintillation counting, using an Isocap-beta scintillation counter. Each sample was counted for 2 minutes. Results were given as counts per minute (cpm).

#### Calculations

The specific binding of tritiated hormone was given



by the difference in the amount bound in the presence or absence of the competitor. The free hormone present was given by the difference between the total radioactivity added and the amount bound when the competitor was not present.

These were calculated from the cpm with a factor which had been derived beforehand (Appendix 2), and were given by

$$[\text{Tritiated hormone}] = \text{cpm} \times \text{factor}.$$

A Scatchard plot was made of the ratio of specifically bound hormone to free hormone (B/F) plotted against the concentration of hormone bound specifically [B]. The total number of receptor sites was given by the intercept of this line with the abscissa. The dissociation constant was given by the reciprocal of the slope of the line.

Before 1980 these calculations and Scatchard plots were made 'by hand'. Since then they have been made with a computer.

The receptor content of cytosols was expressed as the amount of tritiated hormone bound (in fmol)/ mg cytosol protein.

#### Measurement of cytosol protein content

The protein concentration of the cytosol were determined by the method of Lowry (Lowry et al., 1951).

#### Sensitivity of assay

The minimum value of hormone binding which allowed a patient to be classified reliably as oestrogen receptor

positive was 5 fmol/mg cytosol protein. The minimum value for progesterone receptor (progesterone receptor positive) was 15 fmol/mg cytosol protein.

The usual range of dissociation constants ( $K_d$ ) was  $0.5 - 5.0 \times 10^{-10}$  M for the oestrogen receptor, and  $2.0 - 14.0 \times 10^{-10}$  M for the progesterone receptor.

#### Assay of nuclear oestrogen receptors

The pellet obtained after the first centrifugation was suspended and washed in buffered saline, spun down and washed again. The final pellet was suspended in buffered saline.

0.1 ml of the nuclear suspension was incubated with the four highest concentrations of [ $^3$ H]-oestradiol used in the DCC assay, with and without DES.

Free hormone was removed by washing in 0.9% saline, and filtration with a Millipore glass-fibre filter. After 3 washes the filter paper was put into scintillation vials containing 4 mls of Beckmann EP scintillation fluid. Radioactivity was measured as before, and results were calculated by Scatchard analysis. Results were usually expressed as fmol/microgram DNA, but in this study results are expressed only as 'positive' or 'negative'.

#### Isoelectric focusing (IEF)

##### Reagents

[ $^3$ H]-oestradiol-17-beta, [ $^3$ H]-dihydrotestosterone, and [ $^3$ H]-cortisol were obtained from Radiochemicals, Amersham. [ $^3$ H]-R5020, and [ $^3$ H]-R1881 were obtained from New England Nuclear. Norethisterone {4 Estren-17 alpha

ethynyl-17 beta-ol-3-one} was purchased from Koch Light. Dextran T70 and AgaroseIEF were purchased from Pharmacia Ltd, gelatin from the Sterling Gelatin Co. Ltd.. Ready-Solv NA and Ready-Solv EP premixed scintillation cocktails were supplied by Beckmann RIIC Ltd. Ampholine pH 3.5-9.5, Agarose-IEF and paper electrode wicks were purchased from LKB Ltd. Human haemoglobin Type IV, horse spleen ferritin, dithiothreitol, Norit-A Charcoal and molybdic acid (sodium salt) were supplied by Sigma Ltd.

Agarose gels were poured onto Gel-Fix sheets treated for agarose gels and supplied by Uniscience Ltd. All other reagents were Analar Grade.

1 ml cellulose nitrate and 5 ml polycarbonate centrifuge tubes were purchased from MSE Scientific Instruments Ltd.

### Solutions

Homogenisation buffer (for all samples): 10mmol/l Tris-HCl (pH 7.4) containing 1 mmol/l EDTA, 30 ml/100ml glycerol and 0.5 mmol/l dithiothreitol (added fresh for each assay).

Buffer for IEF steroid solutions: 10 mmol/l Tris-HCl (pH 7.4) containing 1 mmol/l EDTA.

Buffer for the steroid solutions used in the dextran-coated charcoal assay: 10 mmol/l Tris-HCl (pH 7.4) containing 1 mmol /l EDTA and 1 g/l gelatin.

A 300 mmol/l solution of sodium molybdate in distilled water was made up fresh for each assay.

### Preparation of cytosol

To compare IEF with the DCC assay a sample weighing about 200 mg was transferred to a Teflon vial containing 50 microlitres of homogenisation buffer and 200 microlitres of 300 mmol/l sodium molybdate in water, then refrozen in liquid nitrogen. A tungsten carbide ball pre-cooled in liquid nitrogen was added and the sample disintegrated by rapid vibration in a Microdismembrator (Braun) for 20 seconds. The frozen powder was then transferred to a 5 ml polycarbonate centrifuge tube and mixed by agitation with a further volume of homogenisation buffer. The temperature of the sample was kept at 0 - 4 degrees centigrade thereafter. Cellular debris was removed by spinning at 100,000 x g for 45 minutes. The supernatant was removed and the final volume adjusted with homogenisation buffer if necessary.

Cytosol used to investigate the effect of molybdate was prepared by lyophilising the frozen, powdered tissue before it was allowed to thaw. The mixed, lyophilised powder was divided and reconstituted with buffer, with or without molybdate.

When a needle biopsy specimen was prepared the sample was weighed and rapidly transferred to a Teflon vial containing 50 microlitres of homogenisation buffer and 1 microlitres per mg sample wt of sodium molybdate solution. The sample was disintegrated as before for 10 - 15 seconds. The frozen powder was transferred to 1 ml cellulose nitrate centrifuge tubes and mixed with a further 100 microlitres of buffer. The cellular debris was

separated by spinning down as before. The supernatant was drawn off with a graduated syringe and the final volume adjusted if necessary.

#### Preparation of non-radioactive solutions

##### A. Norethisterone

0.16 ml of stock norethisterone acetate solution ( $10^3$  mol/l in ethanol) was partially evaporated under nitrogen and redissolved in 5 ml of IEF buffer to give a  $32 \times 10^{-6}$  mol/l solution.

##### B. Diethylstilboestrol

0.06 ml of stock diethylstilboestrol solution ( $10^3$  mol/l in ethanol) was evaporated and redissolved in 5 ml of IEF buffer, as before, to give a  $12 \times 10^{-6}$  mol/l solution in buffer.

#### Preparation of radioactive solutions

The stock solutions which had been prepared for the DCC assay were used for the preparation of 'working' solutions.

Stock [ $^3$ H]-R5020 in ethanol was dried under a stream of nitrogen and then dissolved in buffer to a concentration of  $160 \times 10^{-9}$  mol/l. This solution was divided and further diluted 1:1 with either buffer alone (for the total binding) or norethisterone acetate  $32 \times 10^{-6}$  mol/l in buffer (for the non-specific binding) to give a working dilution of  $80 \times 10^{-9}$  mol/l [ $^3$ H]-R5020  $\pm 16 \times 10^{-6}$  mol/l norethisterone acetate (x200 excess).

[ $^3$ H]-oestradiol was prepared in a similar way, to give working solutions of  $30 \times 10^{-9}$  mol/l  $\pm 12 \times 10^{-6}$

diethylstilboestrol (x200 excess).

#### Preparation of gel

1 - 2 mm thick plates of agarose gel were prepared by dissolving sorbitol (10 g/100 ml if LKB Agarose-IEF was used or 12 g/100 ml if Pharmacia AgaroseIEF was used) in distilled water, followed by agarose (0.8 g/100 ml LKB Agarose-IEF or 1 g/100 ml Pharmacia AgaroseIEF) and heating. When hot (>75 degrees centigrade) 3 ml/100 ml of the carrier ampholyte (Ampholine pH 3.5 - 9.5) was added. Pre-coated plastic plates were mounted on a level bed and warmed with a hair dryer. The gels were poured when the agarose-sorbitol solution was just at boiling point, using 40 mls/gel. The gels were allowed to cool and then stored in an airtight container at 4 degrees centigrade for up to 48 hours before use.

Each acrylamide gel was made by dissolving 7.27g of acrylamide in 25 mls of water (29% w/v) and 0.225g of methyl-bis-acrylamide was dissolved in 25 mls of water (0.9% w/v). 10 mls of each solution were mixed together and the volume made up to 57 mls with 17% glycerol in water. The mixture was filtered and de-gassed under a vacuum for 15 minutes. When de-gassed 1.5 mls of ampholine mixture (0.9 mls of pH 3.5-10 and 0.6 mls of pH 5-8) and 1.5 mls of riboflavin (0.004% w/v) were added carefully. The solution was carefully decanted into a prepared mould and allowed to polymerise in direct sunlight. The total concentration of acrylamide (T) was 5% and there was 3% cross-linkage (C).

## IEF

A known volume of the sample, between 35 and 50 microlitres depending upon the volume prepared, was incubated with 25 microlitres of the label solution in small glass tubes. The same volume was used for incubation with the label and its competitor. A similar volume was used either for a duplicate assay or assay of oestrogen receptors and the residue, usually 10 - 20 microlitres, was used for protein estimation. Incubation was continued overnight at 0 - 4 degrees Centigrade. Before focusing the bulk of the free hormone was removed by adsorption onto dextran-coated charcoal at a final concentration of 1% charcoal, 0.1% dextran for 15 minutes and then spinning down at 800 x g for 10 minutes.

Isoelectric focusing was carried out with the LKB Multiphor system (Figure 3) in flat beds of agarose gel prepared in the way described. Thermal contact between the gel-plate and the cooling plate was made with water. The temperature of the gel was kept at 4 degrees centigrade by the circulation of water from an iced water-bath through the cooling plate. Paper strips were used as electrodes, wetted lightly with 1 mol/l NaOH for the cathode and 1 mol/l H<sub>3</sub>PO<sub>4</sub> for the anode. Electrofocusing was carried out with a constant power supply set at 1500 volts and 15 watts. It was not necessary to focus the gel before applying the sample.

It was possible to focus 6 samples on a gel, each consisting of a total and a non-specific binding. One

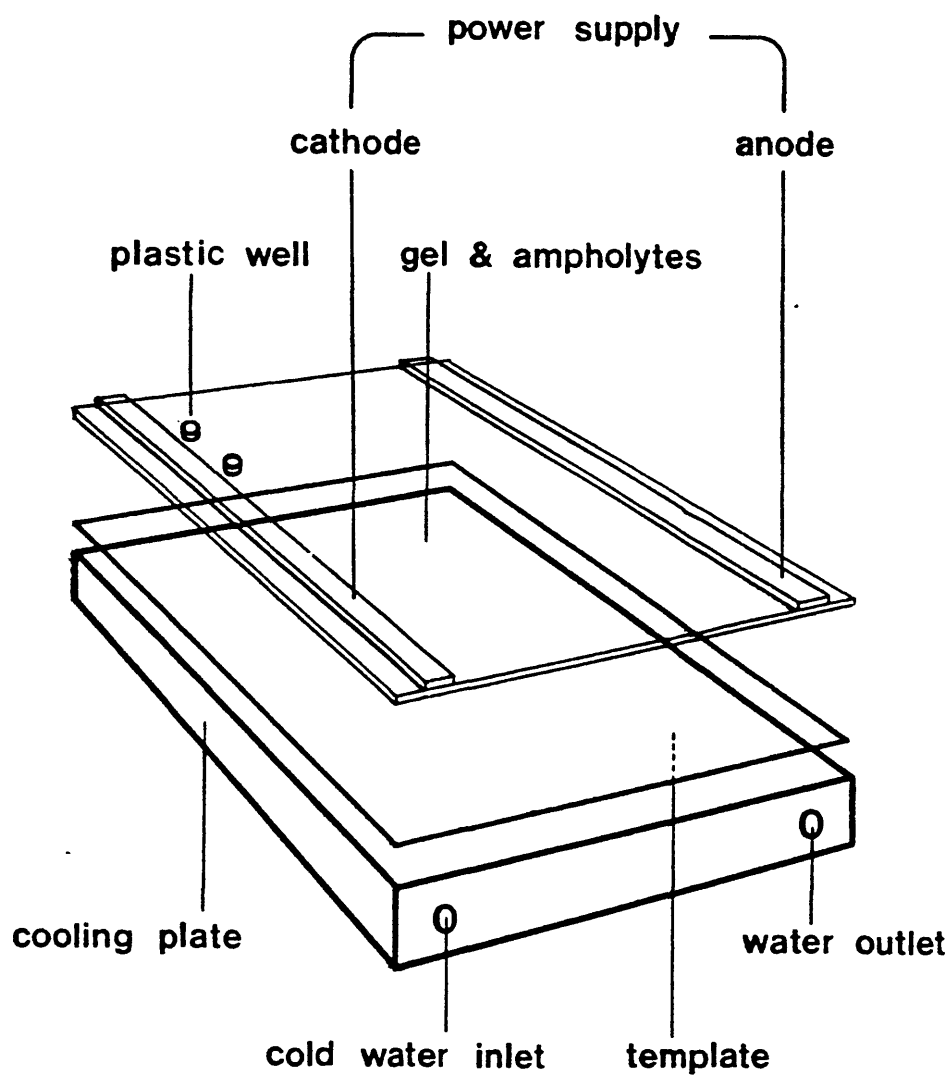


Figure 3: Diagram of isoelectric focusing apparatus.



sample was an internal quality control prepared from a tumour that was known to contain receptor. A 0.075 ml aliquot of the sample was applied by pipetting into a plastic well on the surface of the gel near to the cathode.

Coloured standards of haemoglobin (pI 7.6) and ferritin (pI ~5.0) were applied to each end of the gel and the duration of focusing was decided by reference to these, allowing 10% longer than the time taken for these to focus. This was usually 75 minutes. Initially the duration of focusing was measured in 'volt-hours' determined from the area under the curve of the voltage attained plotted against the time that had elapsed. Focusing of standards occurred at 1250 volt-hours.

Accumulation of water on the gel with excessive wetting of the electrodes was occasionally a problem, but this could be corrected by periodic drying of the electrodes with strips of filter paper during the procedure.

#### Measurement of radioactivity

After focusing the gel was cut to separate individual samples and then cut into 0.5 cm slices between the cathode and the anode. The radioactivity in each slice was measured by scintillation counting in plastic insert vials containing 2 mls of a premixed scintillation fluid for non-aqueous samples and using an Isocap 300 beta scintillation counter.

#### Calculation of binding sites

The specific binding was calculated from the sum of

the differences between the total and non-specific binding from fraction 7 or 8 to fraction 16 to 18. The equation used was:-

Specific binding (fmol/ml) =

$$\frac{(\text{Total dpm} - \text{non-specific dpm}) \times 1000 / \text{volume of cytosol used}}{\text{X Final volume of incubate/ volume applied to gel} \times 1 / (\text{specific activity} \times 2.2)}$$

Calculations were made with a computer.

#### Measurement of pH gradient

The pH across the gel was measured by eluting the ampholytes from the fractionated strips between each sample into distilled water and measurement with a pH meter, at 0 - 4 degrees centigrade.

#### Measurement of cytosol protein concentration

Cytosol protein content was estimated using the method of Lowry et al. The assay was 'scaled down' for small volumes. Bovine serum albumin was used as the standard.

#### Precision of assay

Results of reduplicated assays were used to assess precision

#### Mobility of free [<sup>3</sup>H]-R5020

The procedure was repeated in triplicate with the working solution of radioisotope.

#### Serum binding

On 19 occasions a 1:10 dilution of serum was

incubated with [3H]-R5020 +/- norethisterone acetate, as before. Isoelectric focusing was carried out as described. The pI of the peak of radioactivity was determined. The total radioactivity occurring in each peak in the presence or absence of an excess of norethisterone was calculated.

A duplicate incubation of diluted serum was made with the following tritiated steroids; cortisol, dihydrotestosterone, progesterone, R1881 and oestradiol. The pI's of the peaks of radioactivity were determined.

#### Acrylamide gels for isoelectric focusing

Cytosols were assayed by isoelectric focusing in acrylamide and agarose gels at the same time. The use of acrylamide gels differed from agarose in the following ways.

i) Gels were 'pre-focused' for 15 minutes before the sample was applied.

ii) Focusing was carried out at 1000 volts and 20 watts.

Results were expressed as the proportion of the number of binding sites found by a simultaneous DCC assay.

#### Comparison of IEF with the reference (DCC) method

DCC assays were carried out, as described, on the same cytosol preparations and at the same time as IEF, again with overnight incubations. Pooled, stored negative cytosol was used occasionally

For progesterone receptor, 90 IEF assays were carried out on cytosols prepared from 59 selected tumours for which a DCC assay was also performed. 4 cytosols in which

an excess of total over non-specific binding was seen at each concentration of [ $^3$ H]-R5020 used in the DCC assay did not give a satisfactory linear Scatchard plot. These were classed as receptor positive although no value could be used in regression analysis.

For oestrogen receptor 47 assays were carried out on cytosol from 36 selected tumours for which a DCC assay was also undertaken.

#### Estimation of the limit of detection

The value which gave best discrimination between receptor positive and negative cytosols (on DCC assay) was chosen.

## VARIATION OF RECEPTORS

### Samples

Specimens of primary or secondary cancer of the breast were obtained at the Withington and Christie Hospitals in Manchester. A portion of the tumour was trimmed of fat and connective tissue and frozen immediately in liquid nitrogen. Specimens were stored in liquid nitrogen until the assay was performed. The DCC assay was used in all but one case.

38 patients had more than one lesion excised at the same time for receptor assay (synchronous samples). In one case assay was carried out on the primary tumour and a bone metastasis. In two cases a bone metastasis and a regional recurrence were assayed. In 6 cases both assays were carried out on regional recurrences. Two patients who had two discrete, homolateral primary tumours were included among the synchronously biopsied patients. In the remainder the primary tumour and a lymph node were assayed.

91 second and third biopsies were taken after an interval ranging from 3 weeks to 58 months (asynchronous samples), from 83 patients. Both oestrogen receptor and progesterone receptor were known for each lesion on 84 occasions. In 4 patients oestrogen receptor but not progesterone receptor was known, and in 3 progesterone receptor only was known for both lesions.

In 8 of the asynchronous biopsies an advanced primary lesion was biopsied twice. In 62 the initial lesion was the primary tumour and the subsequent lesion a soft tissue

or lymph node metastasis. In the remainder all assays were carried out on soft tissue or nodal metastases.

No patient with bilateral primary tumours was included in either group.

#### Treatments & menopausal status

Patients were excluded if they had taken tamoxifen in the month preceding their biopsy. Apart from this, 20 patients had some form of hormone treatment in the interval between biopsies. 7 had tamoxifen, 6 ovarian ablation, 1 stilboestrol, 1 norethisterone, and 5 had a combination of some of these, in sequence. In one other, the natural menopause occurred between biopsies.

11 of the patients who had asynchronous biopsies were pre-menopausal, and 65 were post-menopausal at the time of both biopsies.

#### Histology

Three patients who had cutaneous lesions removed for receptor assay but not sent for histological confirmation of the clinical diagnosis of malignancy have been included. In all other patients malignancy was confirmed by histological examination.

#### Analysis & statistical methods

Comparisons were made between assay results. 4 patients had 3 biopsies taken at different times. Each permutation of their results was included to test the value of a single assay in the prediction of subsequent results.

The significance of differences in the incidence of receptors between groups was determined by contingency table analysis and the Chi-squared test. Means of receptor values were calculated on log-transformed data. Correlation between values was determined on raw data. Pearson's ( $r$ ) and Spearmans ( $\rho$ ) coefficients of correlation were used.

## RECEPTORS, RESPONSE AND SURVIVAL

### Patients

437 patients were treated for advanced breast cancer between the 1st of January 1976 and the 30th of November 1982 under the combined care of the Departments of Surgery and Medical Oncology at the Withington and Christie Hospitals in Manchester. 135 patients, 62 of whom had had receptors measured, did not receive any form of endocrine treatment. These patients were not included in this study. Of the remaining 302 patients with advanced disease who had had endocrine treatment, the group under study, receptor status was known in 215 patients.

56 patients were pre-menopausal at the time of their first endocrine treatment, 14 had last menstruated between 6 months and 3 years before their first hormone treatment and 232 were clearly post-menopausal.

### Presentation & primary treatment

98 patients had presented initially with Stage III or IV breast cancer. 8 had no surgery, 54 had a biopsy only and in 36 the tumour was excised.

153 patients had had excision of a Stage I or II breast cancer previously. Of these 31 had been given adjuvant cytotoxic treatment, 1 had received adjuvant tamoxifen, and in 5 patients the menopause was induced by irradiation, after mastectomy. 6 patients with 'early' disease were treated with tamoxifen. 2 were given radiotherapy.

In 9 patients who had had mastectomies the precise



stage of disease at presentation was not known.

### Treatments

19 patients (6%) received combinations of treatment which precluded an assessment (Appendix 3) and were excluded from all further analyses. 15 patients in whom treatment was started without objective evidence of progression were included only in analyses of survival (Appendix 4).

Of the remainder, 208 patients (69%) had tamoxifen alone. 11 patients had tamoxifen and a steroid (prednisolone or dexamethasone) and 1 had tamoxifen and aminoglutethimide. 25 (8%) patients had an oophorectomy. In an irradiation menopause was induced in 16 patients. 4 patients had stilboestrol and 3 had norethisterone acetate.

### Sites of disease

142 (53%) of those included had disease in the breast or mastectomy scar. 82 (31%) had disease in the axilla and 33 (12%) in one or other supraclavicular fossa. 11 (4%) had liver metastases, 58 (22%) had lung disease and 21 (8%) of patients had malignant pleural effusions. 120 (45%) had bone metastases.

### Clinical assessment

Patients were reviewed at 4 - 6 weekly intervals for six months from the start of treatment. Thereafter they were seen every 6 - 12 weeks.

A variety of medical staff were concerned with the treatment of patients over the years, although the bulk of

assessments were made by 4 practitioners only.

Certain manifestations of disease were considered unassessable. These were:-

1. Lymphoedema
2. Pleural or peritoneal effusion
3. Sclerotic bone metastases
4. Impalpable liver metastases
5. CNS metastases
6. Areas treated by local measures

Other manifestations of disease were assessed in the following ways.

#### A. Palpable disease

Areas of palpable disease were measured at each visit, with either calipers or metric ruler. When disease was widespread selected lesions were followed (usually the lesions which were easiest to measure).

The largest diameter and its largest perpendicular were noted.

When palpable disease was present which could not be measured subjective judgements of whether it was better or worse were made, often by reference to local anatomical landmarks.

#### B. Bone disease.

Plain x-rays of lumbar spine, pelvis, upper femora and the chest were the minimum requirement for assessment of the skeletal system before treatment. In general a full skeletal survey, and/or bone scan were performed.

Known areas of bone disease were reviewed by plain x-

ray between the fourth and eighth month after the start of treatment. Thereafter investigations were undertaken when indicated clinically.

#### C. Lung disease

Lung metastases were reviewed by serial chest x-rays. Measurements were made of nodular metastases. The extent of infiltrative disease (lymphangitis carcinomatosa) was compared.

#### D. Liver disease

Isotope or ultrasound scan evidence of secondary disease was essential before hepatomegaly was considered to be due to disease. The distance between the costal margin and the edge of the liver was measured in either the mid-clavicular or median line, at the peak of full inspiration.

#### Assessment of response

A formal assesment of response was made retrospectively by two clinicians (myself, and Dr. A. Howell, Senior Lecturer in Medical Oncology at the Christie Hospital).

#### General requirements

The following general requirements were necessary for an assessment to be made

i) Assessable disease was present at the start of treatment.

ii) The bulk of disease was assessed before treatment and about six months after the start of treatment.

iii) Investigations for assessment of the changes in the disease were initiated in the 5th, 6th or 7th month after the start of treatment.

iv) A change of treatment without any documented evidence of an increase in the disease did not amount to progression of the disease.

#### Criteria for assessment of response

##### i) Palpable disease.

A regression was defined as a reduction in the sum of the product of the diameters of all lesions of at least 50%.

Progression was defined as either an increase of at least 25% in the sum of the products of the diameters of all lesions, a similar increase in the size of any one lesion, or the development of new areas of disease.

When palpable but unmeasurable disease was present the opinion of the clinician was accepted.

Both regression and progression were only accepted when the change was confirmed by a subsequent examination.

Neither the healing or development of ulceration was regarded as evidence of change.

##### ii) Bone disease

Regression was thought to have occurred when lytic disease became uniformly sclerotic or when sclerosis at the margins of a lesion reduced the bidimensional size of a lesion by at least 50%.

New areas of lytic disease or an increase of 25% in the bidimensional size of any existing lytic lesion was

evidence of progression. New areas of sclerosis with treatment were not necessarily evidence of progression.

In patients in whom bone metastases had been or were subsequently confirmed by plain x-ray, new areas of increased uptake that were not associated with benign lesions were regarded as progression. Other changes in isotope scans were not considered.

A pathological fracture was not accepted as evidence of progression.

iii) Lung

A reduction of 50% in the sum of the products of the two measurements of nodular lung metastases was considered to be a regression.

An increase of 25% in these measurements or the development of new lesions was considered progressive disease.

The extent and intensity of infiltrative lung disease was judged better, worse or little changed, with particular regard to the variation in penetration of serial x-rays. Sometimes it was felt that no accurate assessment was possible.

In no case was a response classification based on apparent change in hilar lymph nodes.

iv) Hepatomegaly

Regression was a reduction of 50% in the measurements of liver size. Progression was an increase of 25%.

v) The onset of confirmed but unassessable disease e.g. cytologically proven malignant effusion, was evidence of progression.

### Assessment of the date of relapse

The date of further progression was the first date on which objective evidence of progression was recorded. For x-rays and other special examinations this was the date of the examination rather than the date when it was arranged. For soft tissue disease this was the date at which the changes were first noted and not when they were subsequently confirmed.

### Statistical methods

Details of the patients' treatments, with dates, and response with date of relapse, were stored on a main computer data file. The proforma is shown in Appendix 5.

Data were read from the main file into one upon which analyses were performed. The program which selected patients for the secondary data file excluded patients who had not had any form of endocrine treatment, or who had an unacceptable combination of treatments.

The probability of relapse and of survival was estimated by life-table methods. These analyses were carried out with the following stratifications:-

#### A. Oestrogen receptor status

Patients were grouped according to the results of assays done at any time up to the start of the first endocrine treatment, those done only at the time of endocrine treatment, and assays carried out on the primary tumour.

Results assessed in primary tumours were subdivided into those of patients who presented with operable

disease (T1-3,N0-1,M0) on their original staging and those with advanced or inoperable cancer (which included 6 patients who presented with operable disease but who were suitable only for systemic treatment). Although 5 patients were re-staged in retrospect from 'early' to 'advanced' categories, they were retained in the 'early' group for analysis.

When contradictory results occurred in two or more assays done before treatment precedence was given first to primary tumour samples over secondary, and second to positive over negative. Patients in whom assays were carried out after the first endocrine treatment were not included.

#### B. Progesterone receptor status

Patients were grouped as before.

C. The combination of both oestrogen and progesterone receptors (Double receptor status).

This was assessed on primary tumours, and on all assays done before treatment.

D. Concentrations of oestrogen and progesterone receptor.

These were assessed only in primary tumours. Receptor positive patients were divided into those with more and those with less than 60 fmol/mg cytosol protein of either oestrogen or progesterone receptor.

#### E. Limited or extensive disease

'Limited disease' was defined as involvement of not

more than 2 areas of local soft tissue disease. The chest wall, axilla, each supraclavicular fossa and the opposite breast were each regarded as an area of local soft tissue disease. Involvement of more than 2 sites locally or of any distant site was regarded as widespread disease.

F. Involvement of bone or viscera (liver/lung/pleura) at the time of endocrine treatment, irrespective of any other sites of involvement. No further stratification was made with this variable.

#### Censoring variables

The interval between the start of treatment and the time when further progression of the disease was noted ('interval to progression'), the survival from initial treatment and the survival from 'first relapse' were used as censoring variables. When patients presented with advanced disease the date of presentation and of 'first relapse' were the same.

Deaths due to intercurrent disease were not distinguished from deaths due to cancer of the breast. When death occurred before progression was noted, the date of death was considered also to be the date of progression.

The influence of receptors on the interval to progression was analysed only for the first trial of hormone treatment. Surgical excision or radiotherapy at the same time as endocrine treatment did not exclude a patient from analysis of the probability of relapse or survival.



Logrank tests were used to test significance (Peto et al., 1977). Bi-variate analysis was done by logrank analysis within substrata, i.e. observed and expected frequencies for each explanatory variable were calculated within subgroups, then added to give a summary Chi-square statistic for the whole group (Mantel-Haenszel test).

## INDUCTION OF PROGESTERONE RECEPTOR BY TAMOXIFEN

### Patients

Forty patients aged 36 to 86 with advanced cancer of the breast were studied. One patient was studied twice, and each study is considered separately, as if on different patients.

All had soft tissue disease. In addition 10 had bone metastases, 4 had lung metastases, 2 had bone marrow infiltration and 1 had both bone lesions and malignant ascites.

Three patients had taken tamoxifen and 4 had had ovarian ablations previously (2 surgical, 2 by radiotherapy). 1 patient had had tamoxifen, stilboestrol and cytotoxic treatment in sequence. None of the remainder had received systemic treatment.

Two patients were premenopausal at the time of treatment.

### Initial treatment

Twenty-four patients were given a loading dose of tamoxifen. In 23 patients this was 160 mg. on the first day and in the other this was 120 mg over the first 3 days. Tamoxifen was continued at 20 mg once daily (23 patients) or twice daily (1 patient) thereafter. Seventeen patients started tamoxifen at 20 mg. daily.

### Maintenance treatment

Tamoxifen was continued until the time of second biopsy in all cases.

One patient underwent oophorectomy at the time of

the second biopsy and tamoxifen was discontinued then. One patient was treated with cyclical courses of tamoxifen and medroxyprogesterone acetate. In one patient prednisolone was added after the first month. All other patients continued tamoxifen.

### Timing of biopsies

A biopsy for assay of receptors was taken before the start of treatment. In 40 patients treatment was started within 21 days of the biopsy (Table III). In the other patient treatment was started after 45 days.

A further biopsy was taken within one month of starting treatment in 40 patients. In the other patient 35 days elapsed before the second biopsy. The intervals from the start of treatment to second biopsy were longer when conventional doses were given (Table IV).

TABLE III: Interval between the first biopsy & the start of treatment

|                 | Interval (days) |     |      |       |     |
|-----------------|-----------------|-----|------|-------|-----|
|                 | 0               | 1-7 | 8-14 | 15-21 | >21 |
| No. of patients | 21              | 6   | 11   | 2     | 1   |

TABLE IV: Dose of tamoxifen & timing of second biopsy

|               | Interval (days) |       |       |     |
|---------------|-----------------|-------|-------|-----|
|               | 7-13            | 14-20 | 21-28 | >28 |
| Standard dose | 4               | 4     | 9     | 0   |
| Loading dose  | 19              | 1     | 3     | 1   |

### Biopsy techniques

Open biopsies were taken from superficial lesions under local anaesthesia. Specimens were divided for assay of receptor and histological examination. Samples for assay were prepared, transported and stored as before.

Percutaneous biopsies were taken from soft tissue lesions with a Tru-cut biopsy needle (Travenol) under local anaesthesia. 2 cores were taken. Whenever possible each core was taken from a different part of the periphery of the tumour. When the lesion was small it was not possible to be so selective. A smear was made immediately from shed cells and air-dried before conventional cytological examination. Receptor samples were frozen, transported and stored as before.

In one patient a bone metastasis was biopsied, under general anaesthesia, by a percutaneous technique with a 3 mm Jam-Shidi trephine. The biopsies were carried out under x-ray guidance with image intensification. 2 cores were taken and divided for histological examination, assay of receptors and other studies. A small fragment from the receptor sample was also submitted for histological assessment.

### Assay of receptors

Both samples were assayed by the DCC method in 8 patients, by IEF in 26, and by both methods in 7 patients.

In all but one patient both oestrogen and progesterone receptors were assayed on the first biopsy. Progesterone receptors only were measured in the second sample in a majority. When possible assays were

duplicated.

### Expression of results

Results were expressed as the binding in fmol/mg cytosol protein. IEF gives an underestimate of the binding when compared with the DCC assay. 2 patients in whom the receptors were first measured by the DCC method had lower values of progesterone receptor in their second biopsies, which had been assayed by IEF. Their second values were converted, before correction for cytosol protein content, to give an estimate equivalent to the DCC technique, by a formula derived from a series of comparisons of the two assays, of:-

$$\text{Transformed value} = 55 + 3.13 \times (\text{IEF value})$$

When duplicate assays were performed the greater of the two values was used.

### Clinical assessment

Patients were followed at 4 to 6 weekly intervals for six months after the start of treatment, and at 6 to 8 weekly intervals thereafter. 2 patients were seen at other hospitals, at irregular intervals.

Clinical examination including measurement of palpable disease was carried out at each visit. Unless circumstances dictated earlier investigation plain radiographs of known areas of disease were repeated 3 months from the start of treatment. A full skeletal review was carried out 6 months after the start of treatment.

Response was assessed in the same way as before.

### Statistical methods

The effects of treatment were estimated from the prevalence of response and by analysis of the 'interval to progression', as before (except that an intercurrent death was treated as an uncensored event). The significance of differences of distributions between groups was assessed by contingency table analysis and the Chi-squared test. Means of receptor values were calculated on log corrected data, and the significance of differences between means was estimated by the t-test.

### INTRA-TUMOUR VARIATION OF PROGESTERONE RECEPTORS

Assay and sampling errors were estimated by measurement of progesterone receptors in multiple samples taken from tumours at the same time. 9 primary tumours in which malignancy had been confirmed by needle biopsy before operation were dissected from the breast immediately after mastectomy. a 2-4 mm thick slice was taken across the 'equator' of the tumour. The slice was trimmed of non-tumour tissue and frozen in liquid nitrogen. Routine assay of receptors and histology were carried out on the remaining tissue.

Each slice was scored and broken into smaller pieces when frozen. A cytosol was prepared from each piece as before. Up to 12 samples were taken from each tumour.

In 2 patients 3 peripheral and 1 central sample were taken from the tumour, but the whole slice was not processed. Receptors were measured by IEF in these patients.

Progesterone receptors were measured by both the DCC assay and IEF. Results were expressed as fmol/mg cytosol protein.

A total of 89 samples were taken from 9 patients. The coefficient of variation was calculated for each tumour. The effects of sampling on two sequential biopsies was assessed from the permutation of results in each tumour.

\* \* \* \* \*

## RESULTS

\* \* \* \* \*



## VARIATION OF RECEPTORS

### Synchronous biopsies

The assays disagreed on the presence or absence of oestrogen receptor and progesterone receptor and on the combinations of oestrogen receptor and progesterone receptor (Double receptor status) in 11%, 16% and 18% of biopsies respectively (Table V(a)).

### Asynchronous biopsies

Oestrogen receptor status varied in 23% of the paired assays. Although higher, this proportion was not significantly different from that found in synchronous biopsies (Table V(b)). The extent of variation was similar whether the initial lesion contained receptor or not (Table VII). Progesterone receptor status differed in 30% of the asynchronous samples. When the first lesion contained progesterone receptor 49% of patients did not have progesterone receptor in a later biopsy. When the first lesion did not contain progesterone receptor, it was found in 16% of the subsequent samples (Table VII, Chi-squared = 9.3,  $p = 0.0023$ ). When both receptors were considered together 43% of asynchronous biopsies did not maintain the same classification.

Five patients in whom there was progesterone receptor activity in one biopsy had evidence of progesterone receptor activity below the accepted limit of sensitivity in another. When these were accepted as containing progesterone receptor there was a small reduction in the extent of the variation, but the significance of

differences between synchronous and asynchronous samples was not affected (Table VI(b)).

When neither receptor was present initially one or the other was found in a subsequent biopsy in 28% of patients. When oestrogen receptor alone or both receptors were present in the first biopsy, 45 - 50% of patients did not maintain their receptor classification. 4 of 5 patients in whom progesterone receptor was the only receptor found in the first biopsy differed in a later receptor analysis. The differences in the extent of variation between the groups were not significant (Table VIII).

Exclusion of the patients who had had some form of hormone treatment in the interval between biopsies did not reduce the proportion of cases in which some variation occurred (Table IX).

Thirteen (50%) of the 26 patients in whom the interval between biopsies was less than one year had some change in their receptor status compared with 23 (40%) of the 58 patients in whom the interval was more than one year (Chi-squared = 1.45,  $p = 0.23$ ). When oestrogen receptor alone was considered 10 (38%) of the 26 patients who had a further biopsy within one year showed a change, compared with 10 (19%) of the remainder (Chi-squared = 4.0,  $p = 0.045$ ). The comparable figures for progesterone receptor were 6 (23%) in the first year, and 20 (33%) after a year had elapsed (Chi-squared = 0.23,  $p = 0.6$ ).

The initial levels of receptor were similar whether oestrogen receptor or progesterone receptor status changed

or not (Figure 4). The mean concentration of oestrogen receptor for all patients who were initially oestrogen receptor positive was 87 fmol/mg. 2 of 25 (8%) patients in whom the first oestrogen receptor lay above this mean became oestrogen receptor negative compared with 9 of 29 (31%) of patients who had values of oestrogen receptor below the mean (Chi-squared = 3.1,  $p = 0.08$ ).

The mean value of progesterone receptor in those who were initially progesterone receptor positive was 150 fmol/mg. 6 of the 18 patients (33%) who became progesterone receptor negative and 12 of the 19 patients (61%) who remained progesterone receptor positive had values above this mean in their first biopsies (Chi-square = 2.2,  $p = 0.14$ ). In those patients who remained oestrogen receptor or progesterone receptor positive there was only a weak correlation between the initial and subsequent values (Figures 5 & 6; for oestrogen receptor,  $\rho = 0.39$ ,  $p < 0.02$ ; for progesterone receptor  $\rho = 0.45$ ,  $0.05 < p < 0.1$ ).

The mean concentration of oestrogen receptor in those patients who were oestrogen receptor negative initially but in whom oestrogen receptor was found in a later biopsy was 22 fmol/mg (range 6 - 59 fmol/mg). When progesterone receptor was found only in the later biopsy the mean concentration was 41 fmol/mg (range 17 - 144 fmol/mg).

**TABLE VI:** Variation of receptor status in synchronous and asynchronous biopsies.(No. with receptor status changed/total).

(a) when the conventional limit of sensitivity for R<sub>Pc</sub> assay (15 fmol/ mg cytosol protein) is used.

|                                   | Synchronous | Asynchronous |           |
|-----------------------------------|-------------|--------------|-----------|
| R <sub>Ec</sub>                   | 4/38 (11%)  | 20/88 (23%)  | p = 0.176 |
| R <sub>Pc</sub>                   | 6/38 (16%)  | 26/87 (30%)  | p = 0.15  |
| R <sub>Ec</sub> & R <sub>Pc</sub> | 7/38 (18%)  | 36/84 (43%)  | p = 0.016 |

(b) when R<sub>Pc</sub> values between 0 and 15 fmol/mg are accepted.

|                                   | Synchronous | Asynchronous |            |
|-----------------------------------|-------------|--------------|------------|
| R <sub>Pc</sub>                   | 4/38 (8%)   | 23/87 (27%)  | p = 0.08   |
| R <sub>Ec</sub> & R <sub>Pc</sub> | 5/38 (11%)  | 33/84 (39%)  | p = 0.0075 |

**TABLE VII:** Variation in asynchronous samples and initial receptor status.

|                                         |     | Receptor status at second biopsy. |     |
|-----------------------------------------|-----|-----------------------------------|-----|
|                                         |     | +ve                               | -ve |
| R <sub>Ec</sub> status at first biopsy. | +ve | 43                                | 11  |
|                                         | -ve | 9                                 | 25  |
| R <sub>Pc</sub> status at first biopsy. | +ve | 19                                | 18  |
|                                         | -ve | 8                                 | 42  |

TABLE VIII: Double receptor status in asynchronous biopsies.

|                                       |         | Receptor status at second biopsy. |     |     |     |     |     |     |     |
|---------------------------------------|---------|-----------------------------------|-----|-----|-----|-----|-----|-----|-----|
|                                       |         | RE+                               | RP+ | RE+ | RP- | RE- | RP+ | RE- | RP- |
| Receptor<br>status at<br>first biopsy | RE+ RP+ | 16                                |     | 9   |     | 1   |     | 5   |     |
|                                       | RE+ RP- | 5                                 |     | 10  |     | 0   |     | 4   |     |
|                                       | RE- RP+ | 0                                 |     | 3   |     | 1   |     | 1   |     |
|                                       | RE- RP- | 0                                 |     | 6   |     | 2   |     | 21  |     |

TABLE IX: Effects of endocrine treatment between biopsies. (No. of patients with status changed/total).

|           | Treated    | Not treated |
|-----------|------------|-------------|
| REc       | 5/20 (25%) | 15/68 (22%) |
| RPc       | 6/20 (30%) | 20/67 (30%) |
| REc & RPc | 8/20 (40%) | 28/64 (44%) |

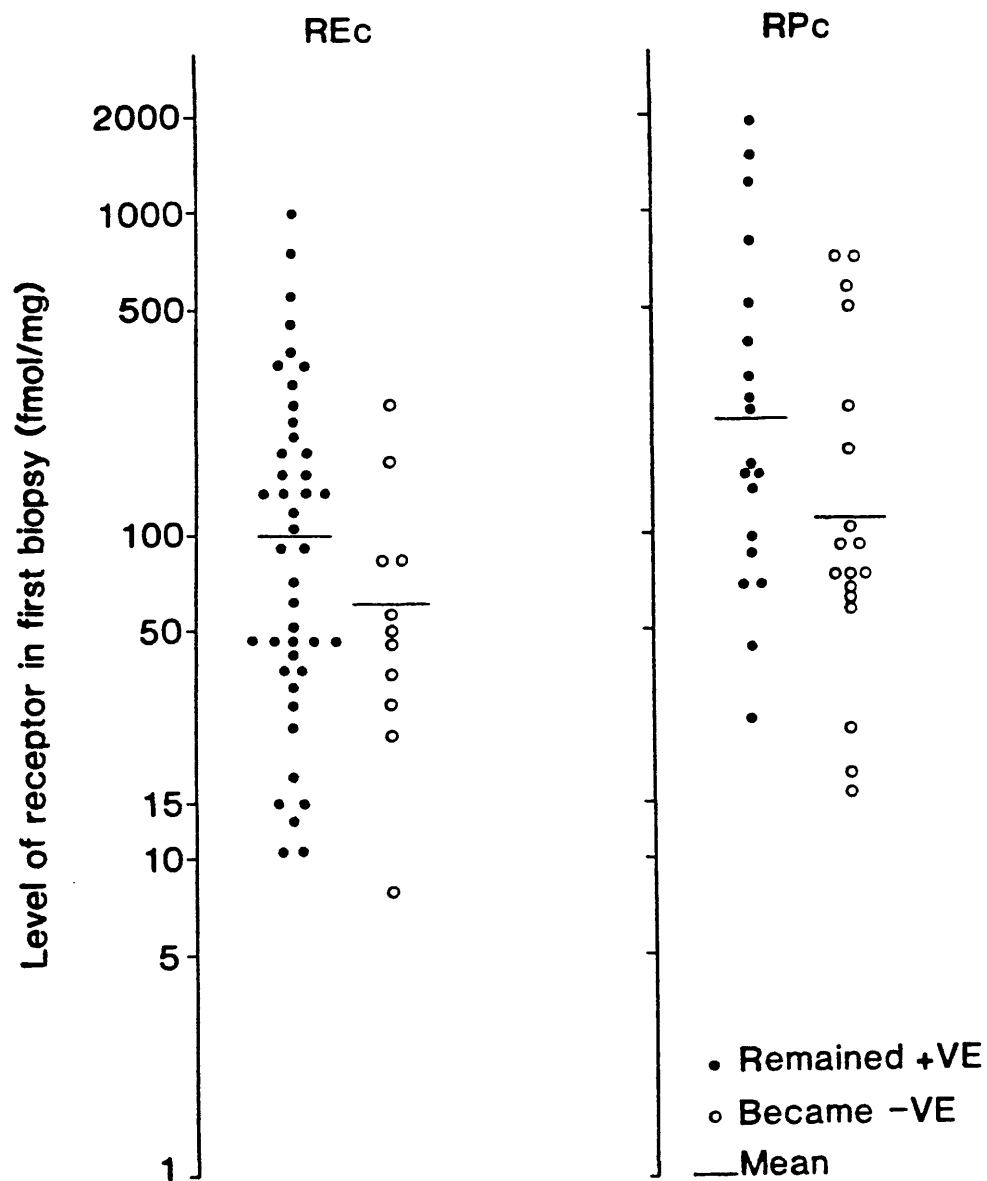


Figure 4: Initial concentration of RE or RP in receptor-positive first biopsies, & receptor status of subsequent biopsy.

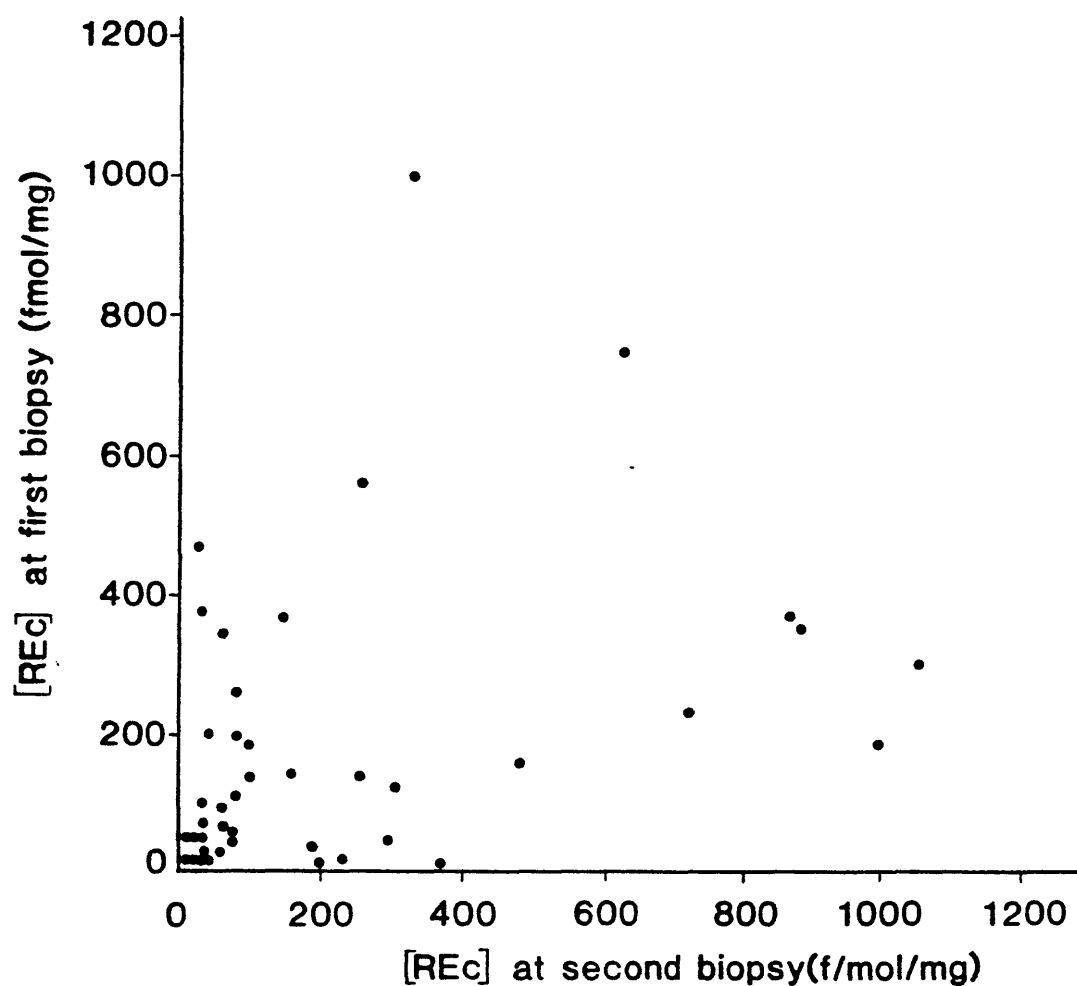


Figure 5: Variation in concentration of oestrogen receptors in asynchronous biopsies.

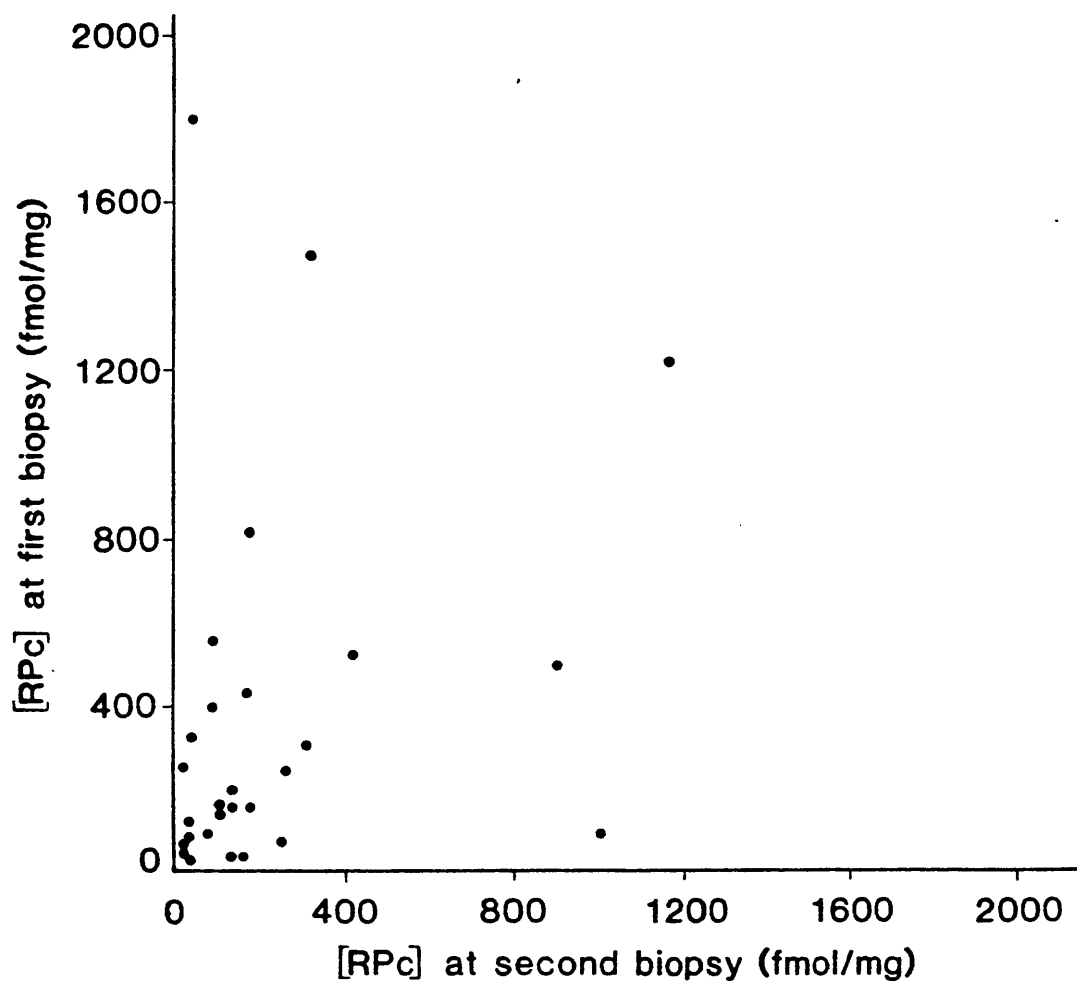


Figure 6: Variation in concentration of progesterone receptors in asynchronous biopsies.



## EFFECT OF TREATMENT

### General results

Life-table analysis of the interval from the start of treatment until further progression of the disease occurred ('interval to progression') showed that half of the patients had had further progression of the disease before six months had elapsed from the start of treatment (Figure 7).

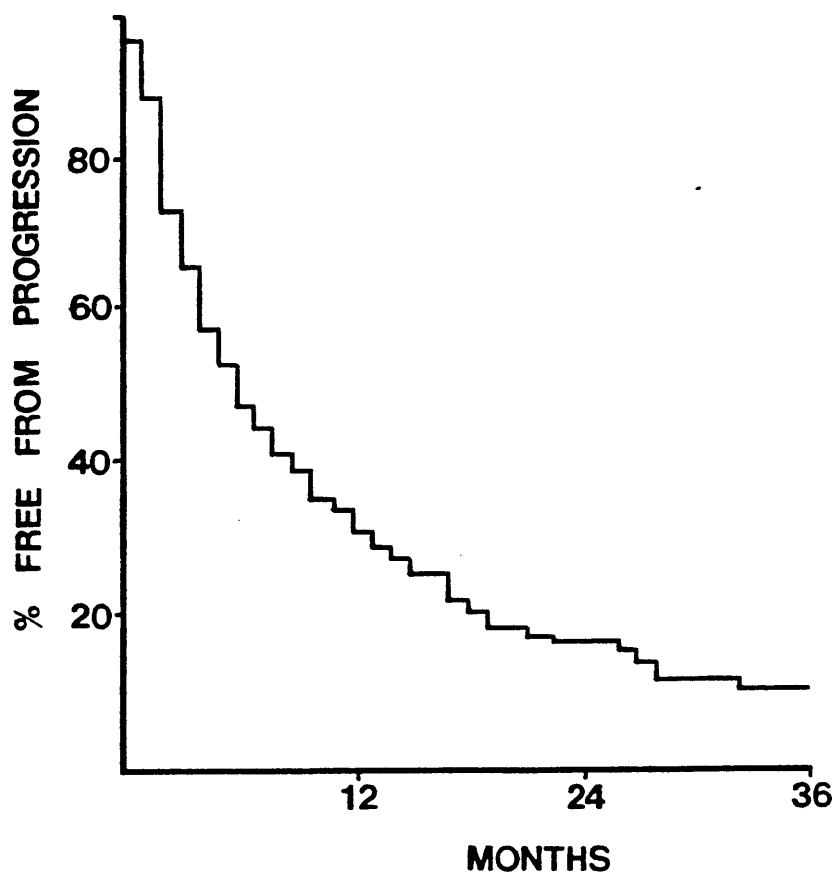
59 (34%) of 167 patients whose response had been assessed had had a regression (Table X)

Fewer patients with limited soft tissue disease (2 areas or less) had relapsed at any point after the start of treatment than patients with more extensive local disease, or with any distant metastases (Figure 8, Chi-squared = 14.9,  $p = 0.0001$ ).

The probability of remaining free from relapse was not significantly different whether distant metastases were present in bone, viscera or both (Figure 9, Chi-squared = 2.75,  $p = 0.097$ ).

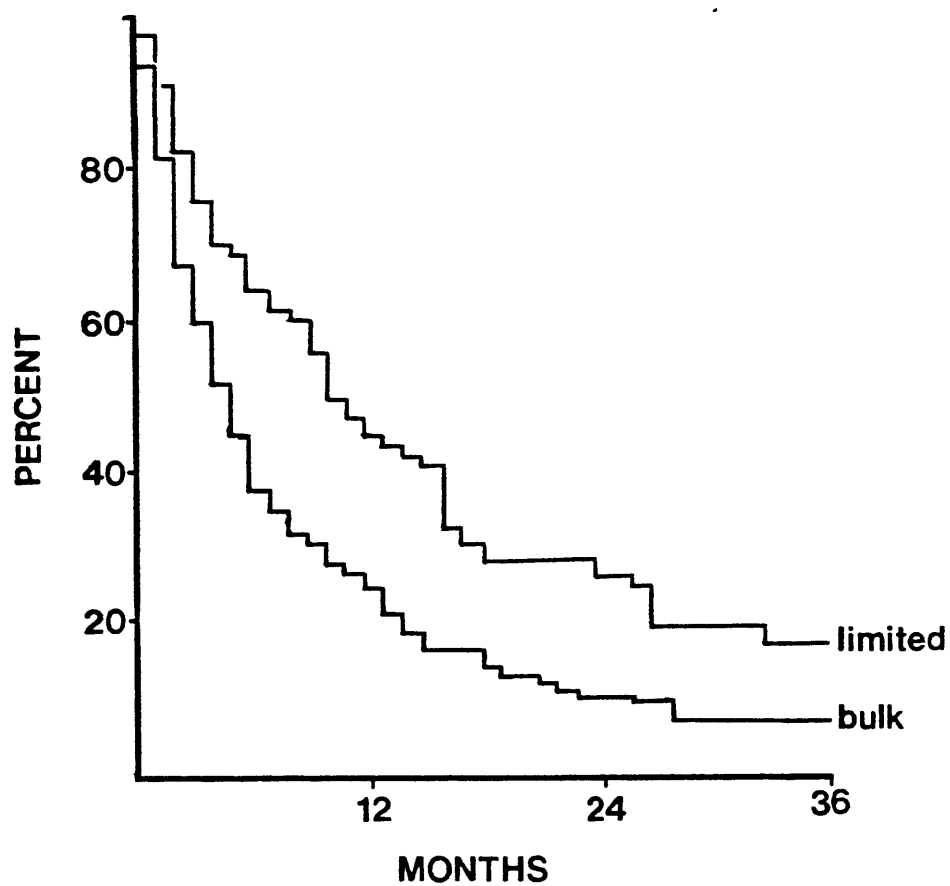
TABLE X: Assessments of response (all patients)

|                     |          |
|---------------------|----------|
| Progression         | 98 (57%) |
| Stable disease      | 16 (9%)  |
| Partial regression  | 42 (24%) |
| Complete regression | 17 (10%) |
| Early death         | 13       |
| Not assessable      | 41       |
| Not documented      | 16       |
| Recent treatment    | 26       |
| Exclusions          | 37       |



At risk: 265      120      68      42      29      18      10

Figure 7: Endocrine treatment & the probability of relapse.



|          |     |    |    |    |    |    |   |         |
|----------|-----|----|----|----|----|----|---|---------|
| At risk: | 94  | 57 | 35 | 24 | 18 | 11 | 7 | Limited |
|          | 174 | 64 | 33 | 18 | 11 | 6  | 2 | Bulk    |

Figure 8: Extent of disease & the probability of relapse.

(Limited = up to 2 areas of local disease: Bulk = more than 2 areas of local disease or any distant recurrence)

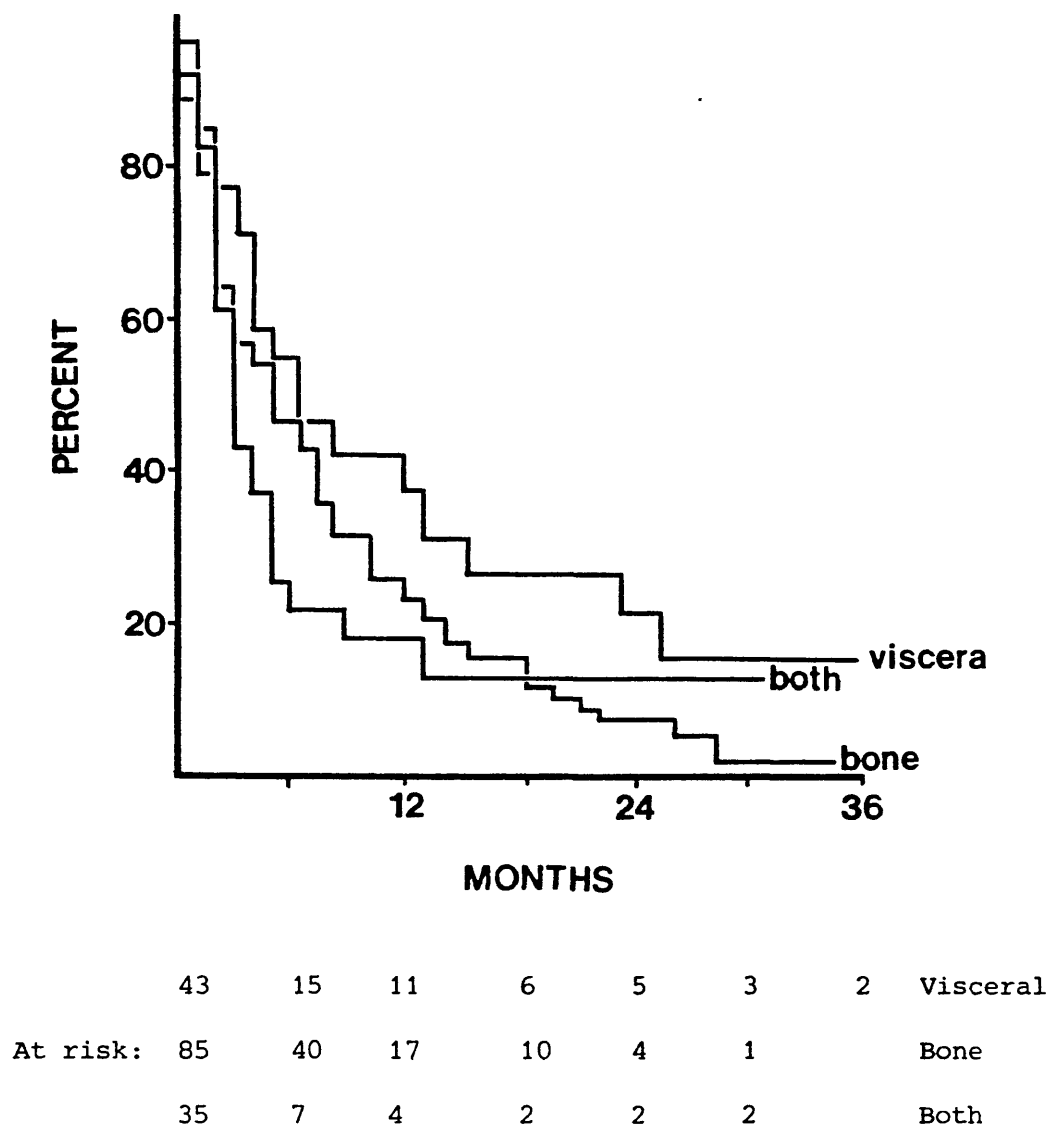


Figure 9: Site of disease & the probability of relapse.

### Receptor status - overall effects

There was a significant difference between the proportion of oestrogen receptor positive and negative patients who had relapsed when assays which had been carried out at any stage before the start of endocrine treatment were considered (Figure 10; Chi-squared = 12.06,  $p = 0.0005$ ). 39.3% of patients had a regression when oestrogen receptor was present, compared with 12.5% of patients in whom oestrogen receptor had not been detected (Table XI).

Similar differences were found when progesterone receptor was considered (Figure 11; Chi-squared = 13.21,  $p = 0.0003$ ). 42.3% of progesterone receptor positive and 19.6% of progesterone receptor negative patients had a regression (Table XII).

There was no difference in the proportion of patients who had relapsed when patients were grouped by nuclear oestrogen receptor status (Figure 12; Chi-squared = 1.26,  $p = 0.26$ ).

Proportionately fewer patients with both cytosol oestrogen and progesterone receptors had relapsed at any time than patients in whom progesterone receptor only was absent (Figure 13; Chi-squared = 4.03,  $p = 0.045$ ). There were 6 patients in whom progesterone but not oestrogen receptors were found.

TABLE XI: Oestrogen receptor status & response - overall effect

|                     | REc+       | REc-      |
|---------------------|------------|-----------|
| Progression         | 38 (45.2%) | 34 (85%)  |
| Stable disease      | 13 (15.5%) | 1 (2.5%)  |
| Partial regression  | 21 (25%)   | 5 (12.5%) |
| Complete regression | 12 (14.3%) | 0         |
| Early death         | 5          | 4         |
| Not assessable      | 19         | 9         |
| Not documented      | 5          | 2         |
| Recent treatment    | 15         | 6         |

TABLE XII: Progesterone receptor status and response - overall effects

|                     | RPc+       | RPc-       |
|---------------------|------------|------------|
| Progression         | 22 (37.3%) | 47 (77%)   |
| Stable disease      | 12 (20.4%) | 2 (3.3%)   |
| Partial regression  | 15 (25.3%) | 10 (16.4%) |
| Complete regression | 10 (17%)   | 2 (3.3%)   |
| Early death         | 5          | 3          |
| Not assessable      | 15         | 13         |
| Not documented      | 5          | 2          |
| Recent treatment    | 16         | 6          |

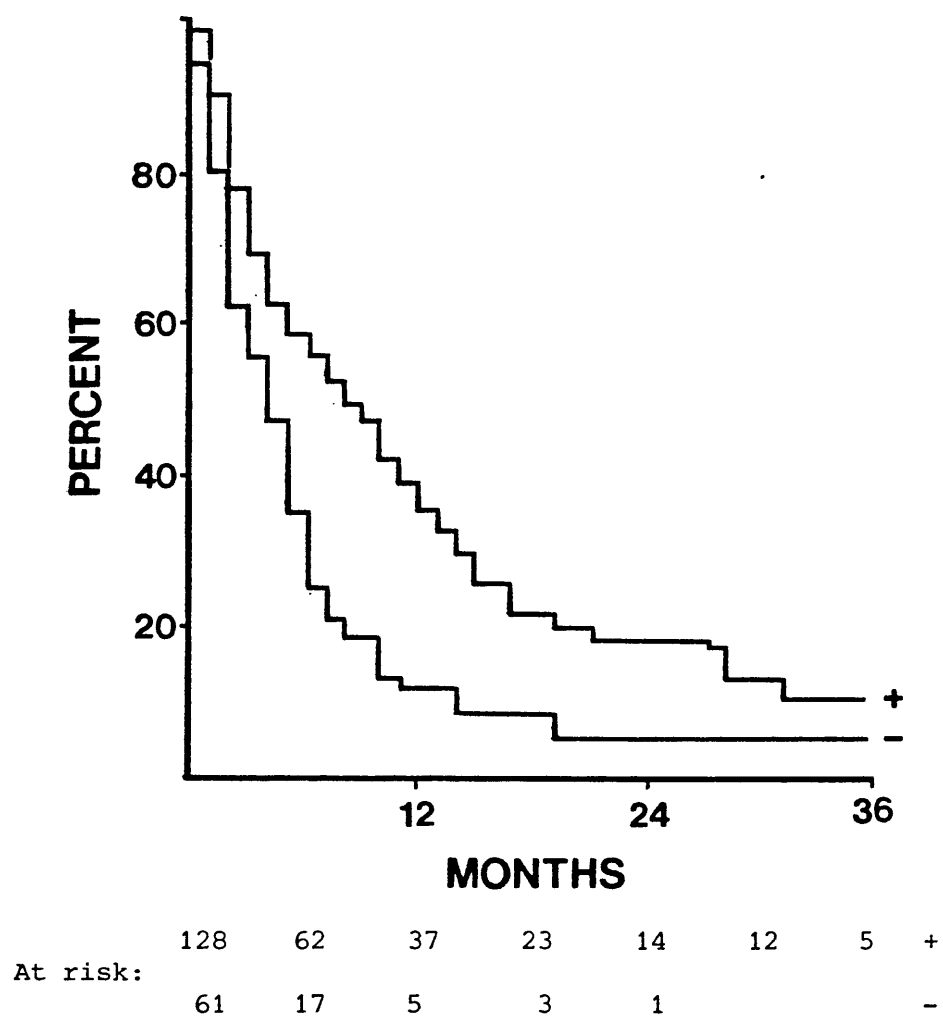
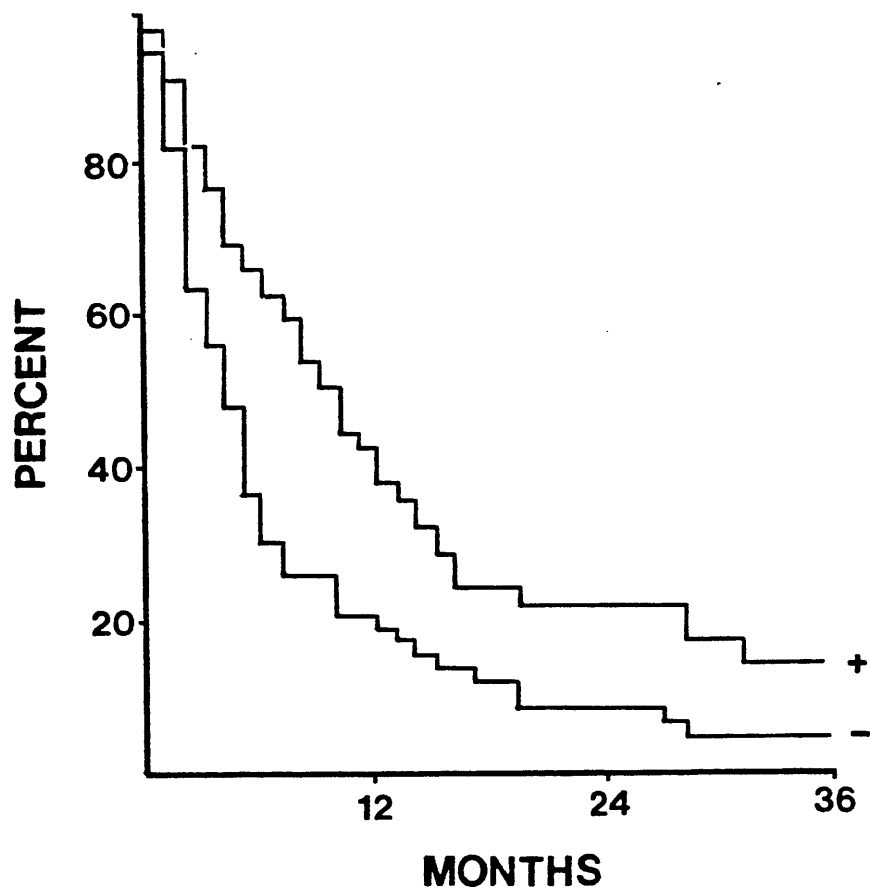


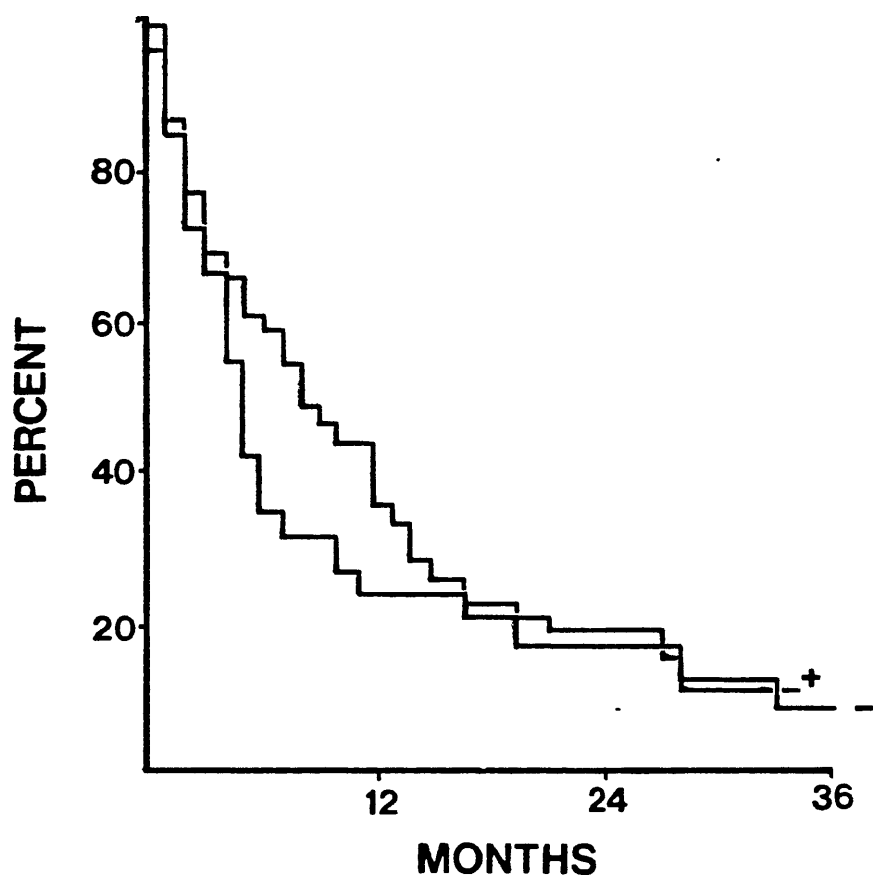
Figure 10: Oestrogen receptor status & the probability of relapse (All assays included)





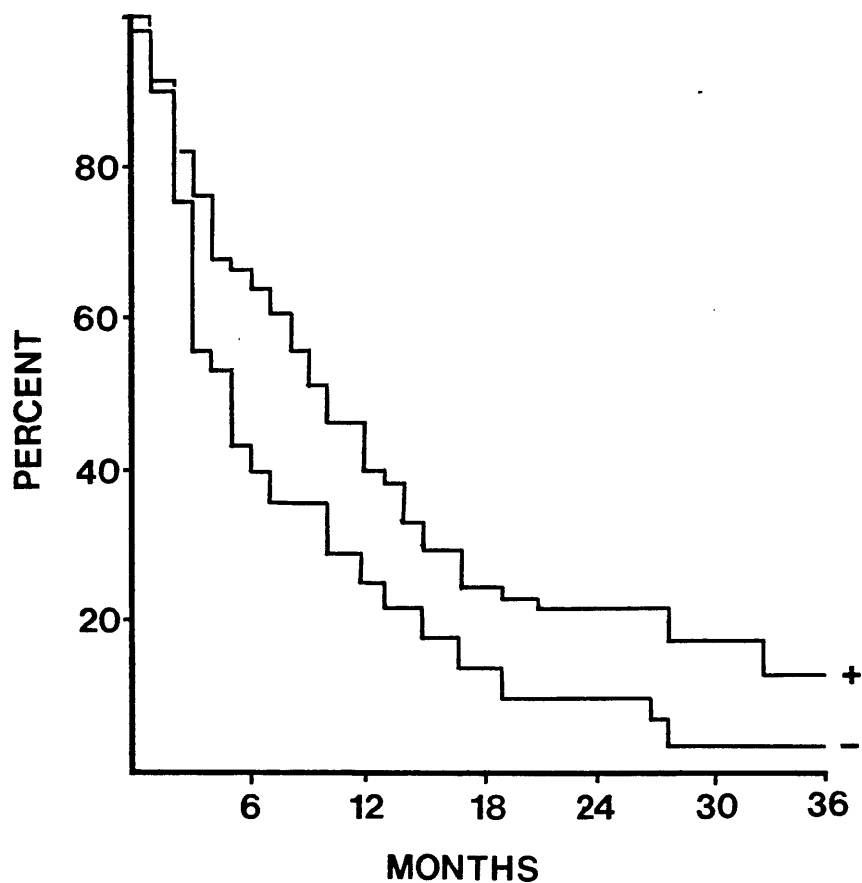
|          |     |    |    |    |    |   |   |   |
|----------|-----|----|----|----|----|---|---|---|
| At risk: | 100 | 51 | 29 | 14 | 11 | 5 | 2 | + |
|          | 85  | 27 | 13 | 7  | 4  | 2 | 2 | - |

Figure 11: Progesterone receptor status & the probability of relapse (All assays included)



|          |    |    |    |    |   |   |   |   |
|----------|----|----|----|----|---|---|---|---|
|          | 83 | 41 | 27 | 15 | 8 | 6 | 2 | + |
| At risk: | 50 | 17 | 8  | 7  | 4 | 4 | 2 | - |

Figure 12: Nuclear oestrogen receptor status & the probability of relapse (All assays included)



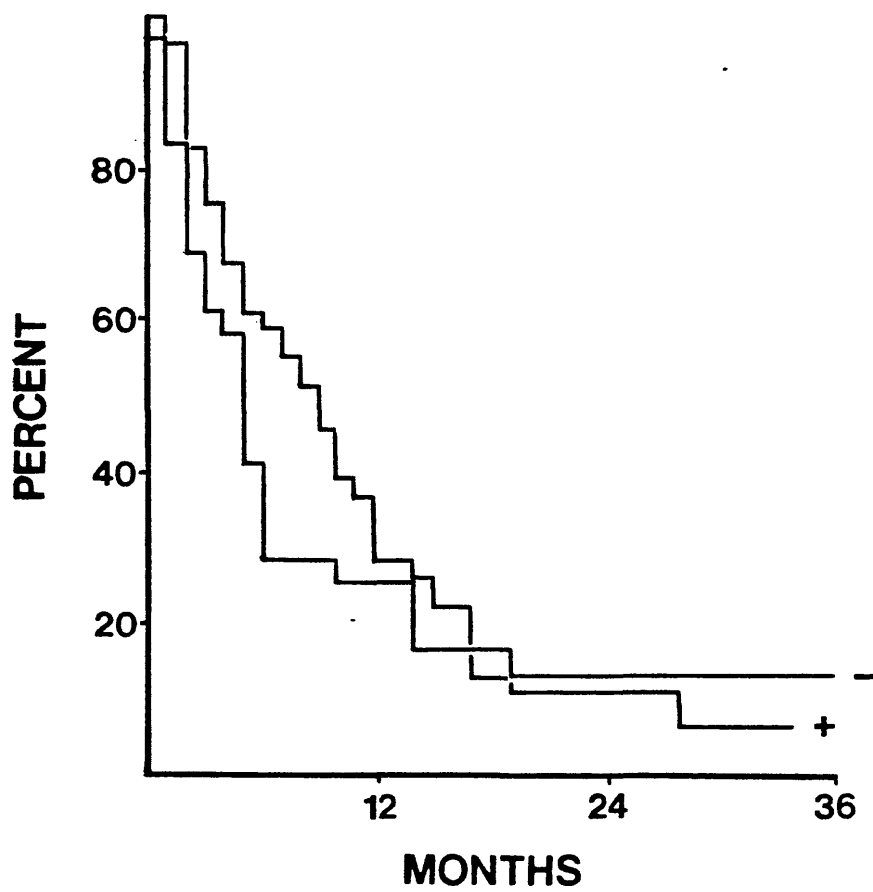
|          |    |    |    |    |    |   |   |   |
|----------|----|----|----|----|----|---|---|---|
|          | 91 | 47 | 28 | 13 | 10 | 7 | 1 | + |
| At risk; | 32 | 13 | 8  | 4  | 3  | 2 | 1 | - |

Figure 13: Effect of progesterone receptor status on the probability of relapse in oestrogen receptor positive patients (All assays included)

### Receptors assessed at the time of endocrine treatment

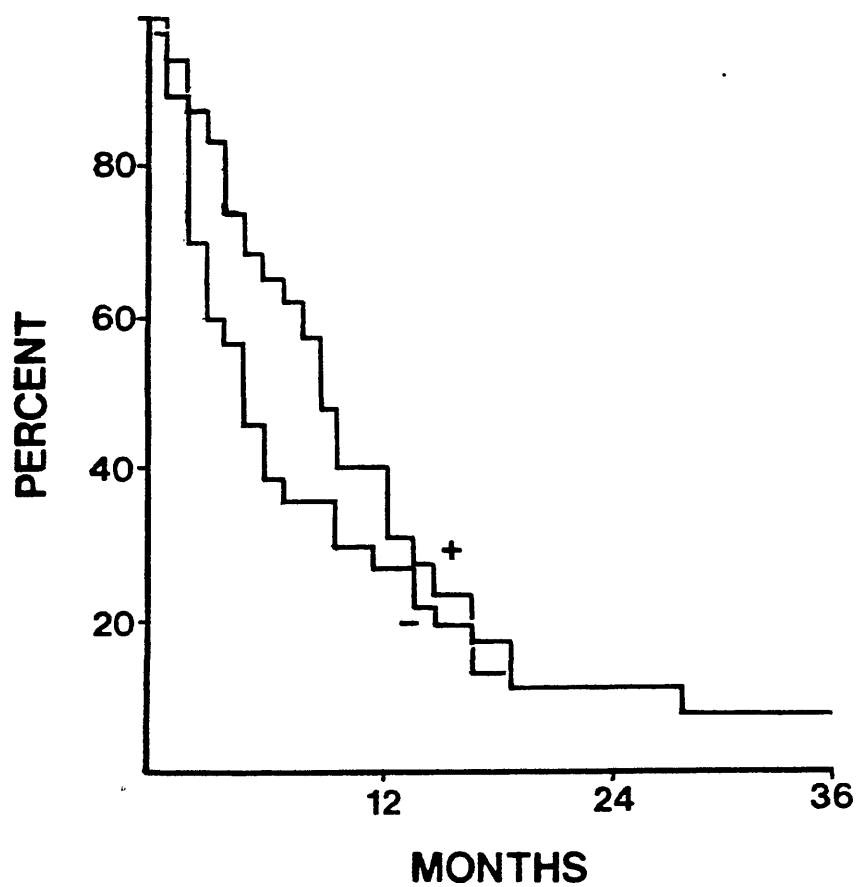
When receptors were assessed at the outset of endocrine treatment there was no significant difference in the probability of relapse whether receptor was detected or not (cytosol oestrogen receptor, Figure 14; Chi-squared = 0.6,  $p = 0.439$ . Progesterone receptor, Figure 15; Chi-squared = 1.18,  $p = 0.277$ . Nuclear oestrogen receptor, Figure 16; Chi-squared = 0,  $p = 0.996$ ).

Receptor status did not indicate the probability of relapse with treatment when allowance was made for the extent of disease at the time of treatment. (Oestrogen receptor, Chi-squared = 0.85,  $p = 0.36$ ).



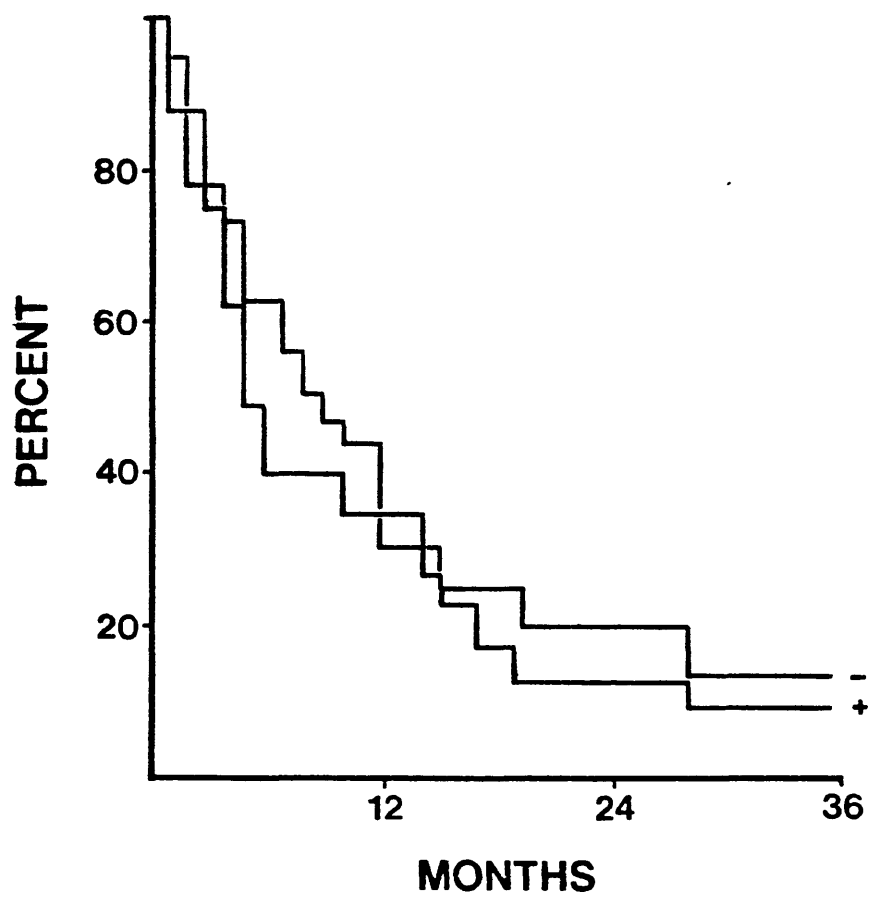
|          |    |    |    |   |   |   |   |   |
|----------|----|----|----|---|---|---|---|---|
|          | 73 | 36 | 17 | 6 | 4 | 2 |   | + |
| At risk: | 29 | 10 | 6  | 3 | 2 | 2 | 1 | - |

Figure 14: Oestrogen receptor status (assessed at the time of endocrine treatment) & the probability of relapse.



|          |    |    |    |   |   |   |   |
|----------|----|----|----|---|---|---|---|
|          | 54 | 27 | 12 | 4 | 3 | 2 | + |
| At risk: | 46 | 19 | 12 | 7 | 4 | 3 | - |

Figure 15: Progesterone receptor status (assessed at the time of treatment) & the probability of relapse.



|          |    |    |    |   |   |   |   |   |
|----------|----|----|----|---|---|---|---|---|
|          | 41 | 22 | 13 | 7 | 4 | 3 | 1 | + |
| At risk: | 24 | 11 | 7  | 5 | 3 | 3 | 2 | - |

Figure 16: Nuclear oestrogen receptor status (assessed at the time of endocrine treatment) & the probability of relapse.

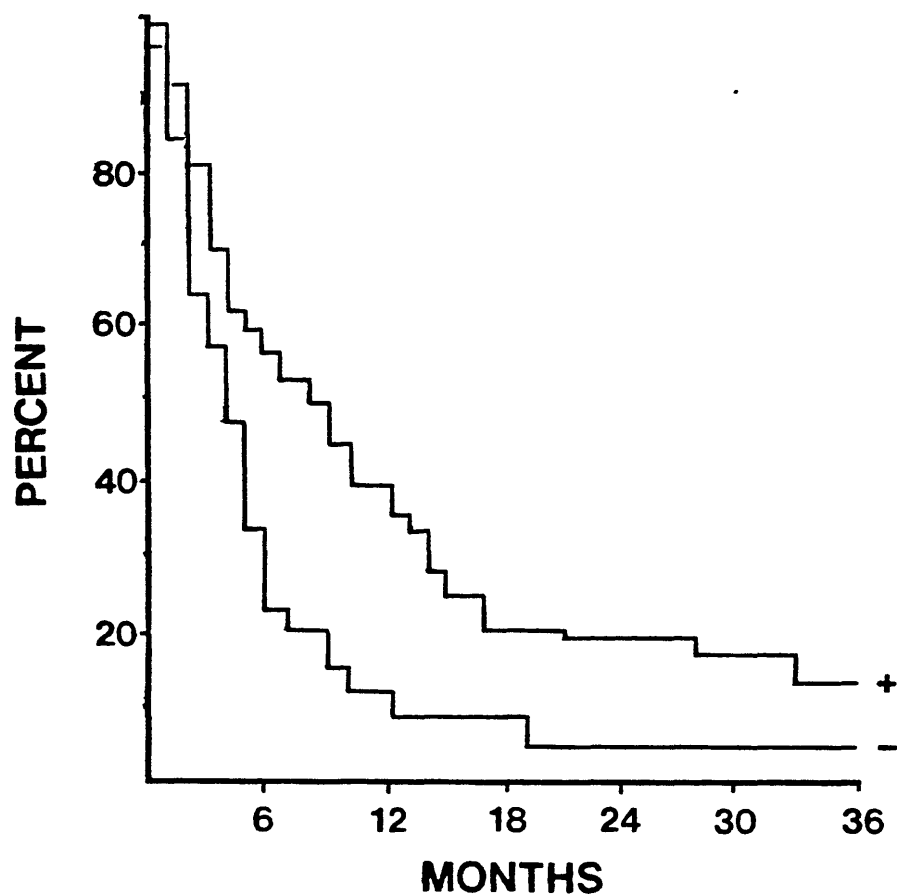
### Primary tumour receptor status

The differences in the proportion of patients who had relapsed were similar when the presence or absence of either cytosol oestrogen or progesterone receptor in samples of primary tumours was considered (Oestrogen receptor, Figure 17; Chi-squared = 10.54,  $p = 0.0012$ . Progesterone receptor; Figure 18; Chi-squared = 12.88,  $p = 0.0003$ ). Nuclear oestrogen receptor status of primary tumours gave only a weak prognosis (Figure 19; Chi-squared = 3.11,  $p = 0.078$ ).

When both cytosol oestrogen and progesterone receptors were considered together benefit was confined to the group of patients whose primary tumours contained progesterone receptor (Figure 20; Chi-squared = 13.17;  $p = 0.0003$ ). In the absence of progesterone receptor stratification by cytosol oestrogen receptor status did not yield groups with significantly different probabilities of progression at any given time after the start of treatment (Figure 21; Chi-squared = 0.18,  $p = 0.67$ ).

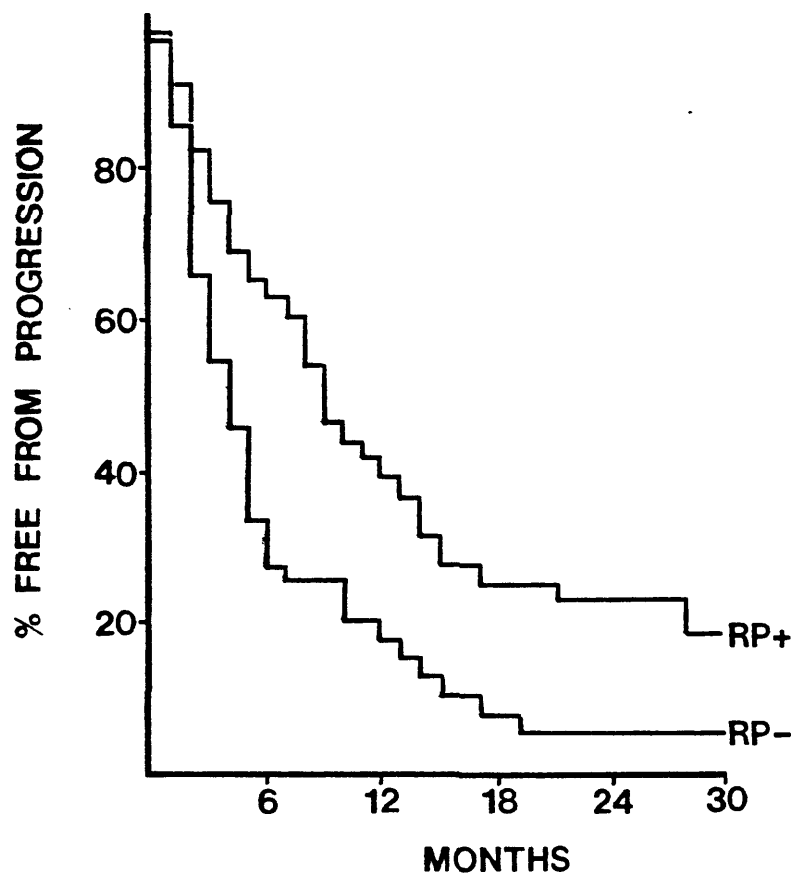
The predictive value of assays done on primary tumours was not affected by the stage of disease at presentation (Oestrogen receptor, allowing for stage; Chi-squared = 9.75,  $p = 0.0018$ . Progesterone receptor, allowing for stage; Chi-squared = 12.07,  $p = 0.0005$ ).





|          |     |    |    |    |    |   |   |   |
|----------|-----|----|----|----|----|---|---|---|
|          | 104 | 49 | 28 | 13 | 11 | 9 | 2 | + |
| At risk: | 47  | 12 | 4  | 2  | 1  | 1 | 1 | - |

Figure 17: Oestrogen receptor status (assessed on primary tumours) & the probability of relapse.



|          |    |    |    |    |    |   |   |
|----------|----|----|----|----|----|---|---|
|          | 65 | 18 | 9  | 3  | 2  |   | + |
| At risk: | 83 | 42 | 23 | 12 | 10 | 8 | + |

Figure 18: Progesterone receptor status (assessed on primary tumours) & the probability of relapse.

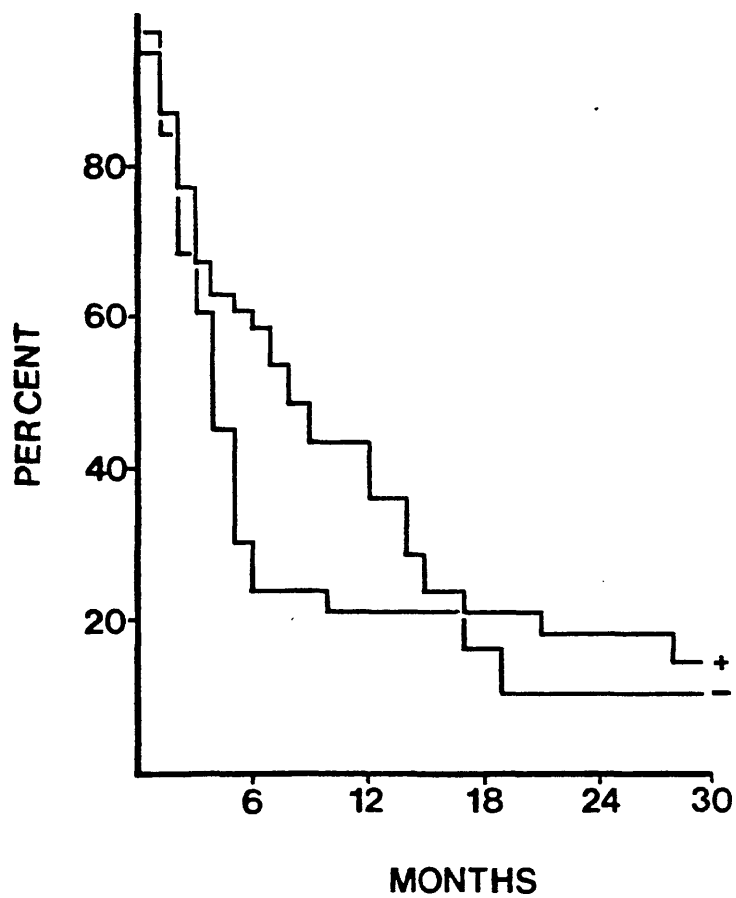
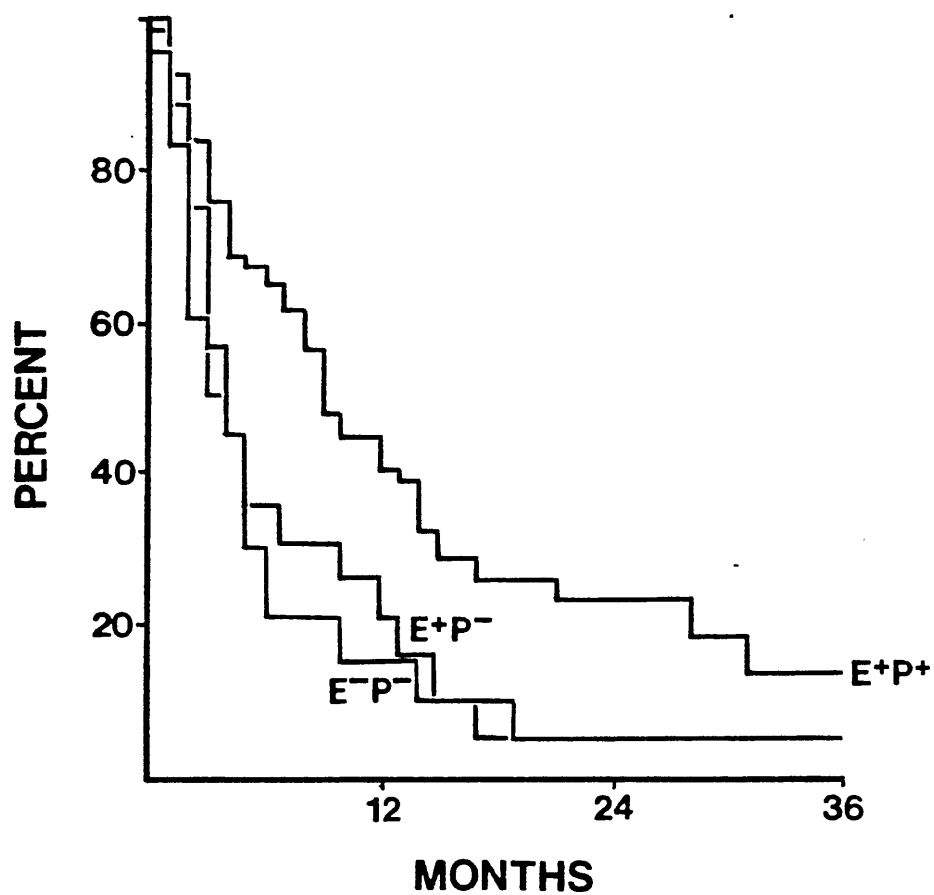
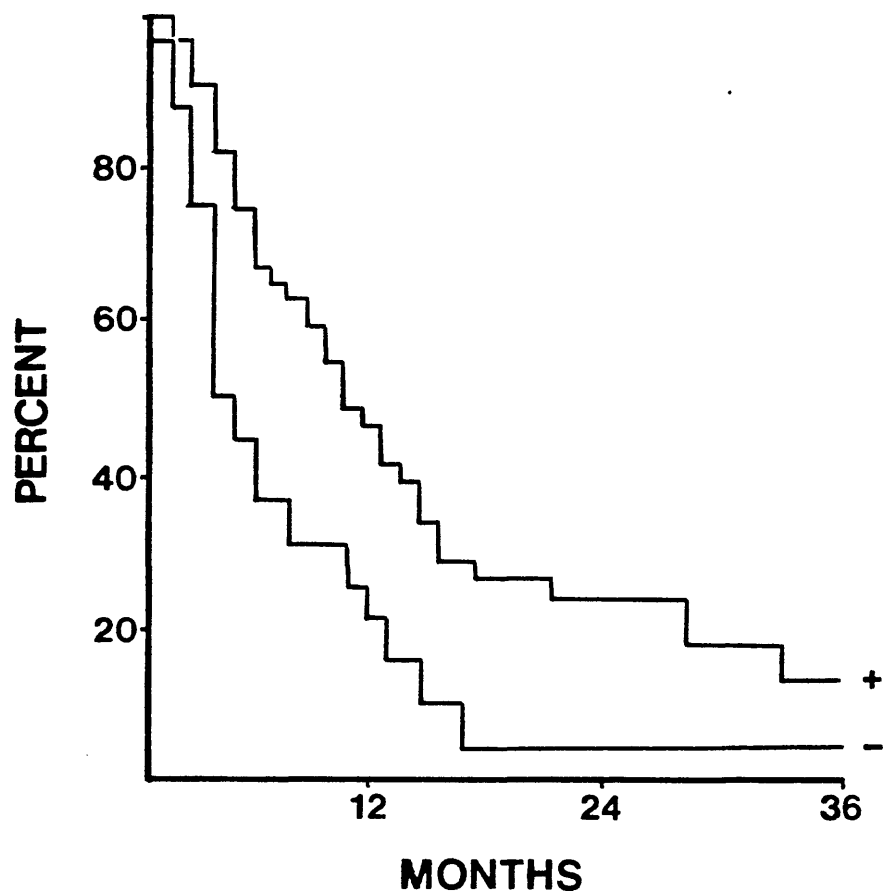


Figure 19: Nuclear oestrogen receptor status (assessed on primary tumours) & the probability of relapse.



|          |    |    |    |    |   |   |   |    |
|----------|----|----|----|----|---|---|---|----|
|          | 76 | 39 | 22 | 11 | 9 | 7 | 1 | ++ |
| At risk: | 24 | 8  | 5  | 1  |   |   |   | +- |
|          | 41 | 10 | 4  | 2  |   |   |   | -- |

Figure 20: Combined (RE & RP) receptor status & the probability of relapse.



|          |    |    |    |    |   |   |   |   |
|----------|----|----|----|----|---|---|---|---|
| At risk: | 76 | 39 | 22 | 11 | 9 | 7 | 1 | + |
|          | 24 | 8  | 5  | 1  |   |   |   | - |

Figure 21: Effect of progesterone receptor status on the probability of relapse in oestrogen receptor positive patients (Receptors assessed on primary tumours).

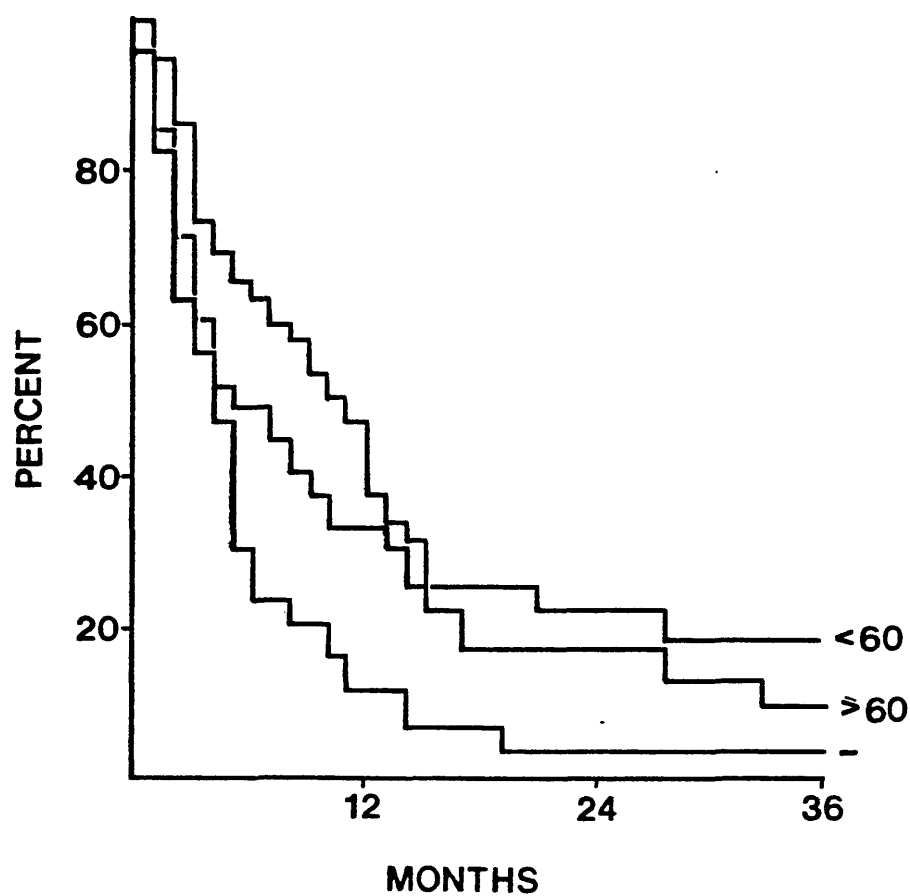
### Concentrations of receptor and response

A greater proportion of patients with oestrogen receptor concentrations below 60 fmol/ mg cytosol protein had relapsed at any time within the first 12 months after the start of endocrine treatment than when 60 fmol/ mg of protein or more of receptor was found (Figure 22; Chi-squared = 10.89,  $p = 0.001$ ). Beyond the 12th month after the start of treatment the prognosis (probability of relapse) of the two groups was similar. The proportion of patients with oestrogen receptor (whatever the concentration) who had relapsed was consistently lower than that of receptor negative patients.

A similar trend was seen when the concentration of progesterone receptor was considered (Figure 23; Chi-squared = 12.28,  $p = 0.0005$ ).

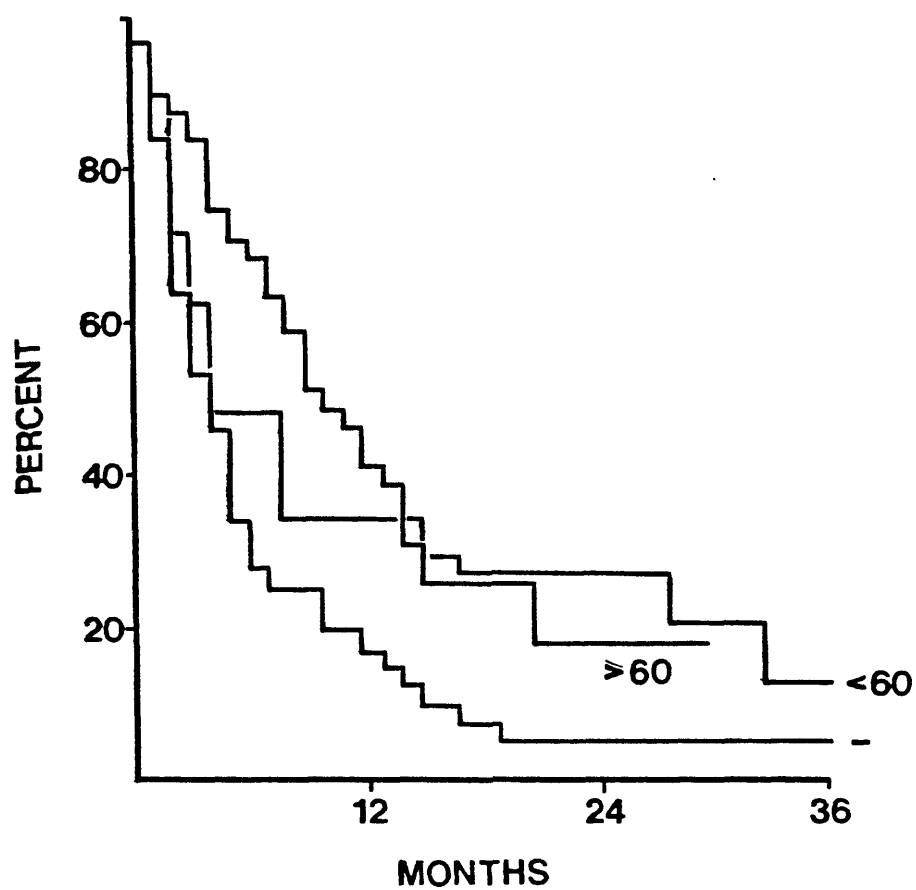
The concentration of receptor indicated the prognosis whether assays were carried out on 'early' or 'advanced' primary tumours.

When oestrogen receptor was present at concentrations above 60 fmol/mg protein, progesterone receptor status defined two groups with substantially different probabilities of relapse (Figure 24(a), Chi-squared = 11.95,  $p = 0.0005$ ). The adjusted figures, when other concentrations of oestrogen receptor were considered also, approached significance at the 5% level (Figure 24(a,b,c), Chi-squared = 3.69,  $p = 0.055$ ). Oestrogen receptor status had no predictive value when an allowance was made for the concentration of progesterone receptor (Figure 25; Chi-squared = 0.77,  $p = 0.38$ ).



|          |    |    |    |   |   |   |   |     |
|----------|----|----|----|---|---|---|---|-----|
|          | 57 | 27 | 16 | 8 | 7 | 6 | 3 | >60 |
| At risk: | 47 | 21 | 12 | 8 | 5 | 5 | 2 | <60 |
|          | 47 | 12 | 4  | 2 | 1 |   |   | -   |

Figure 22: Concentration of oestrogen receptors & the probability of relapse.



|         |    |    |    |    |   |   |   |      |
|---------|----|----|----|----|---|---|---|------|
|         | 61 | 32 | 19 | 11 | 9 | 8 | 2 | > 60 |
| At risk | 22 | 9  | 4  | 3  | 2 | 2 |   | < 60 |
|         | 65 | 18 | 9  | 4  | 2 | 2 | 2 | -    |

Figure 23: Concentration of progesterone receptors & the probability of relapse.



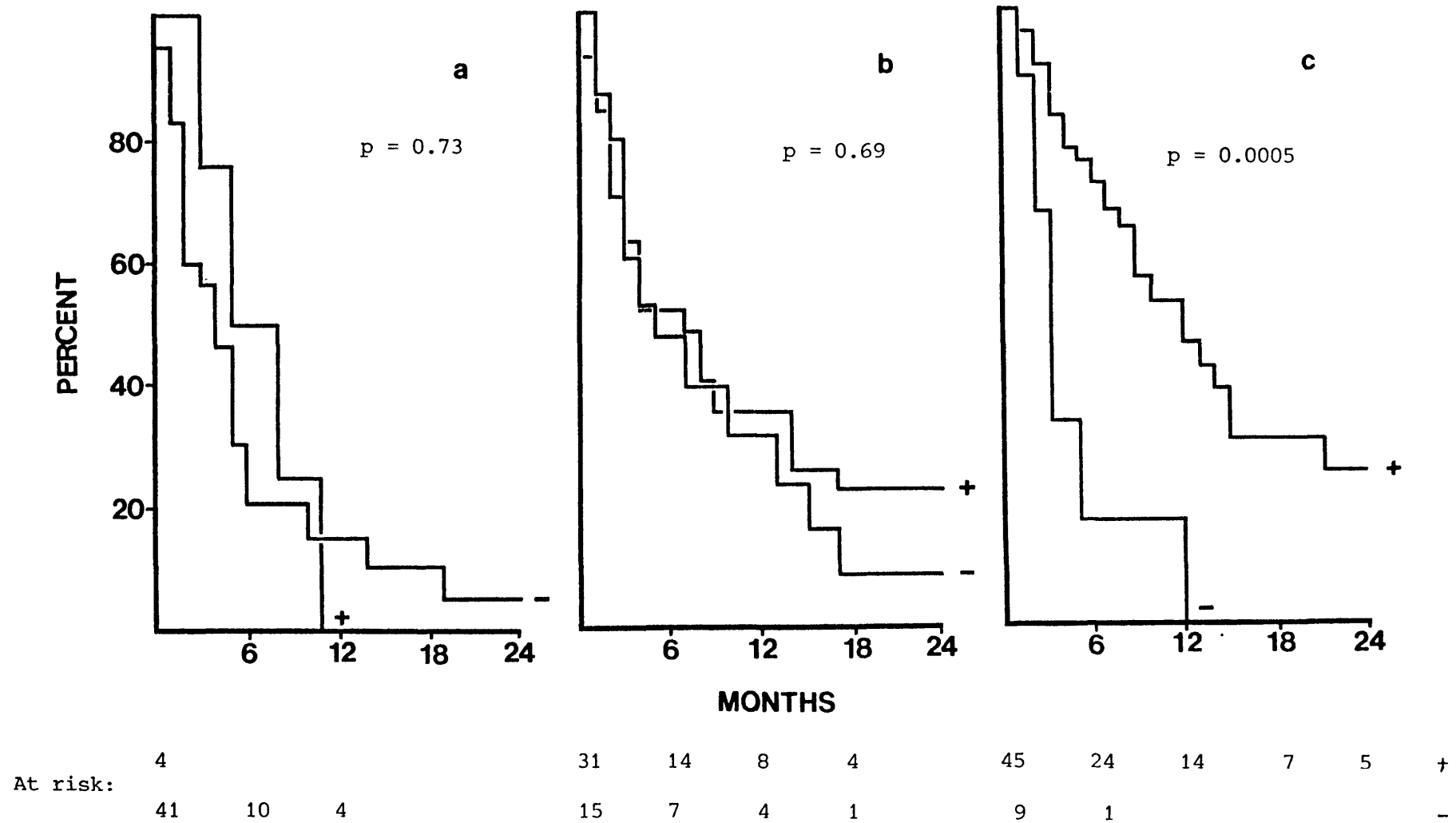


**Figure 24:** Effect of progesterone receptor status on the probability of relapse at different concentrations of oestrogen receptor ((a) RE negative, (b) RE less than 60 fmol/ mg protein, (c) RE greater than 60 fmol/mg protein)

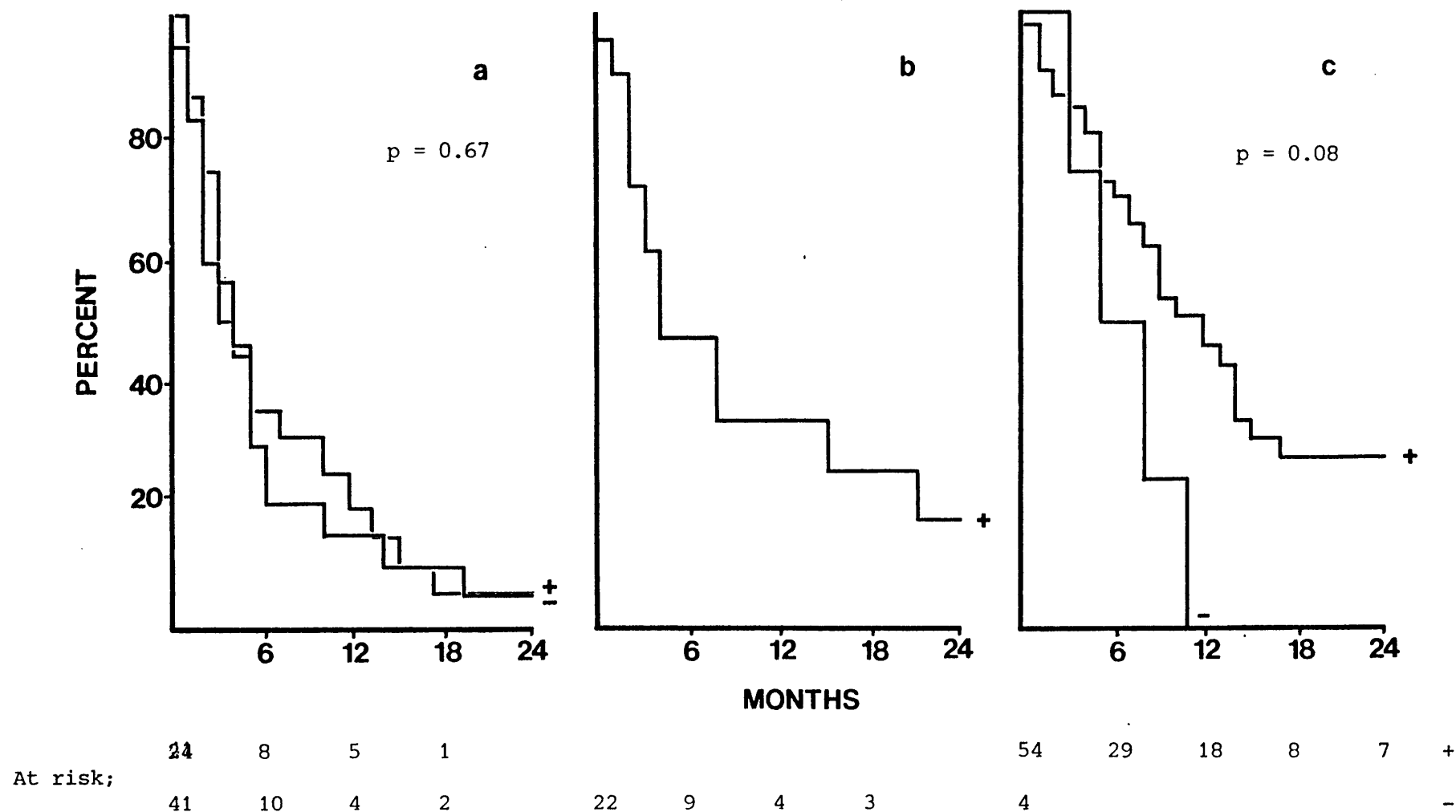


**Figure 25:** Effects of oestrogen receptor status on the probability of relapse at different concentrations of progesterone receptor. ((a) RP negative, (b) RP up to 60 fmol/mg protein, (c) RP greater than 60 fmol/mg protein).

### Comparison of receptors assessed in early and advanced disease

The difference between the proportion of receptor positive and negative patients who had relapsed in the first 6 months of endocrine treatment was similar whether receptors had been assessed before dissemination or shortly before endocrine treatment was given (Figure 26). Between the 6th and 12th month of treatment, patients assessed as receptor negative at the time of treatment fared better than those assessed as receptor negative on assays of primary tumours. Beyond the 12th month from the start of treatment the probability of relapse for patients classified on assays done at the time of treatment was similar whatever the result. When receptors had been assessed at the time of mastectomy proportionately more receptor negative than positive patients had relapsed at all times after the start of treatment (Figure 26).

Results of progesterone receptor assay on the primary tumour indicated the prognosis even when the results of progesterone receptor assay at the time of treatment did not agree (Figure 27 (a) & (c), Chi-squared = 6.8,  $p = 0.009$ ). Patients in whom oestrogen receptor was found in the primary tumour but not in a sample assayed at the time of treatment had a lower probability of relapse than patients assessed consistently as oestrogen receptor negative (Figure 27(d), Chi-square = 3.95,  $p = 0.047$ ). There was only one patient in whom oestrogen receptor was not found in the primary tumour but was detected at the time of treatment (Figure 27(b)).

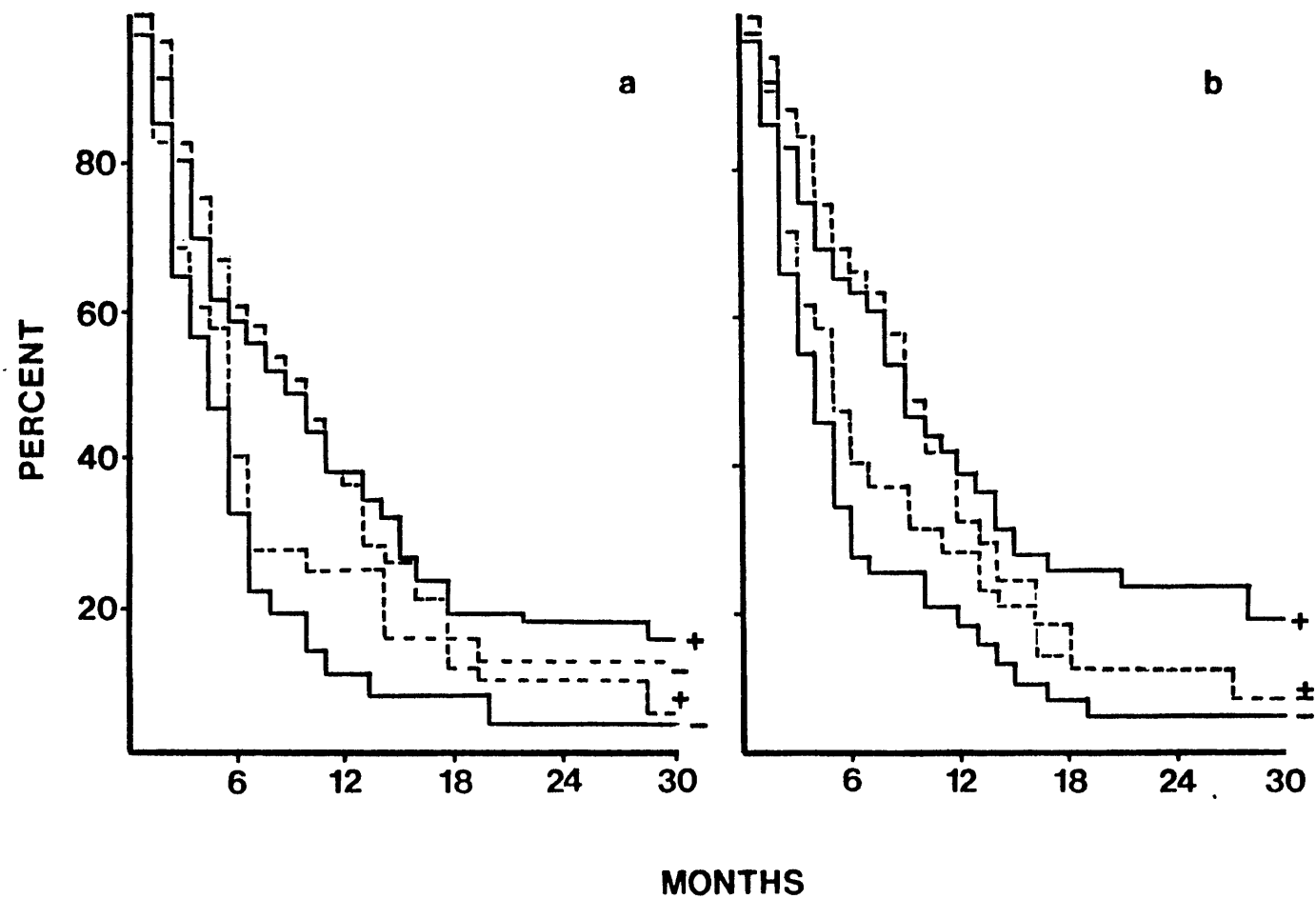


Figure 26: Early vs late assessment of receptors in the prediction of relapse ((a) RE; — = early, --- = late. (b) RP; — = early, --- = late).

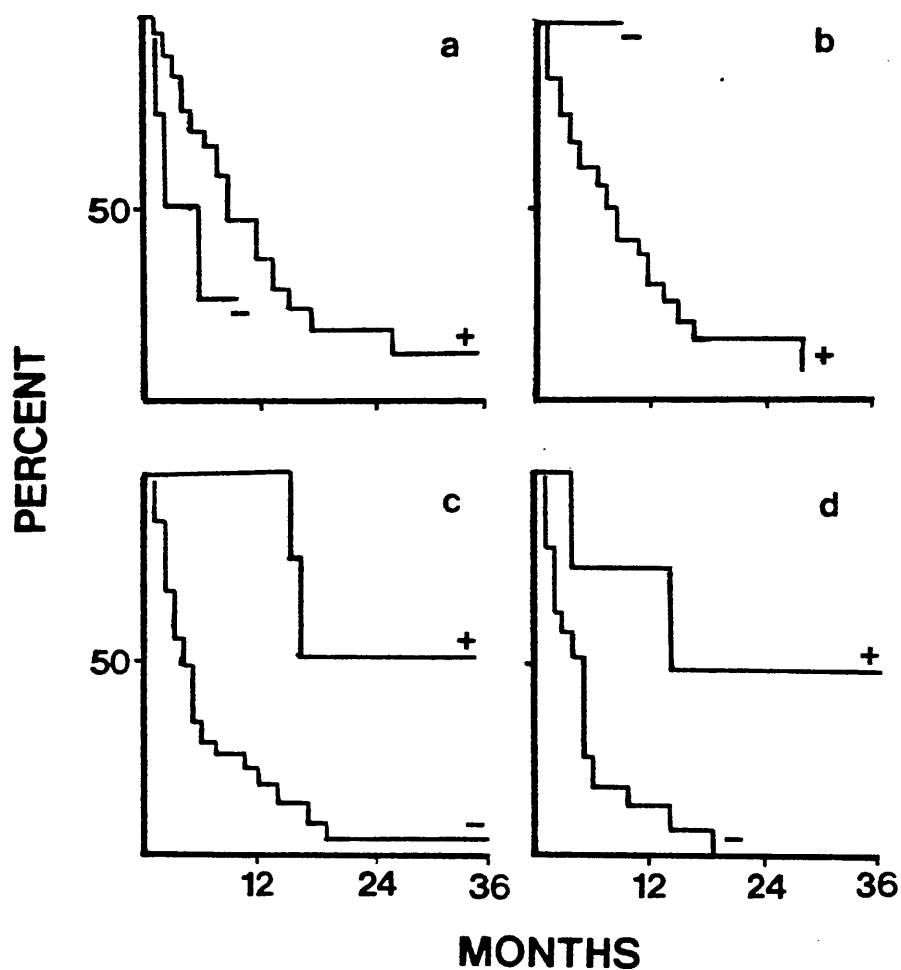


Figure 27: Primary tumour receptor status & the probability of relapse, allowing for results of assays done at the time of endocrine treatment ((a) RP positive at treatment, (b) RE positive at treatment, (c) RP negative at treatment, (d) RE negative at treatment.

Overall survival and survival from first relapse

Overall effect of receptors

A significantly greater proportion of patients whose tumours contained receptors were alive at any time after initial presentation, or after the first evidence of dissemination than of receptor negative patients (Oestrogen receptor, overall survival, Figure 28; Chi-squared = 9.76,  $p = 0.002$ . Survival from first relapse, Figure 29; Chi-squared = 12.5,  $p = 0.0004$ . Progesterone receptor, overall survival, Figure 30; Chi-squared = 19.12,  $p < 0.0001$ . Survival from first relapse, Figure 31; Chi-squared = 23,  $p < 0.0001$ ).

Nuclear oestrogen receptor status indicated groups with small differences in prospects of survival (Figures 32 & 33, Chi-squared = 2.51,  $p = 0.11$  for overall survival & Chi-squared = 5.44,  $p = 0.02$  for survival from first relapse).

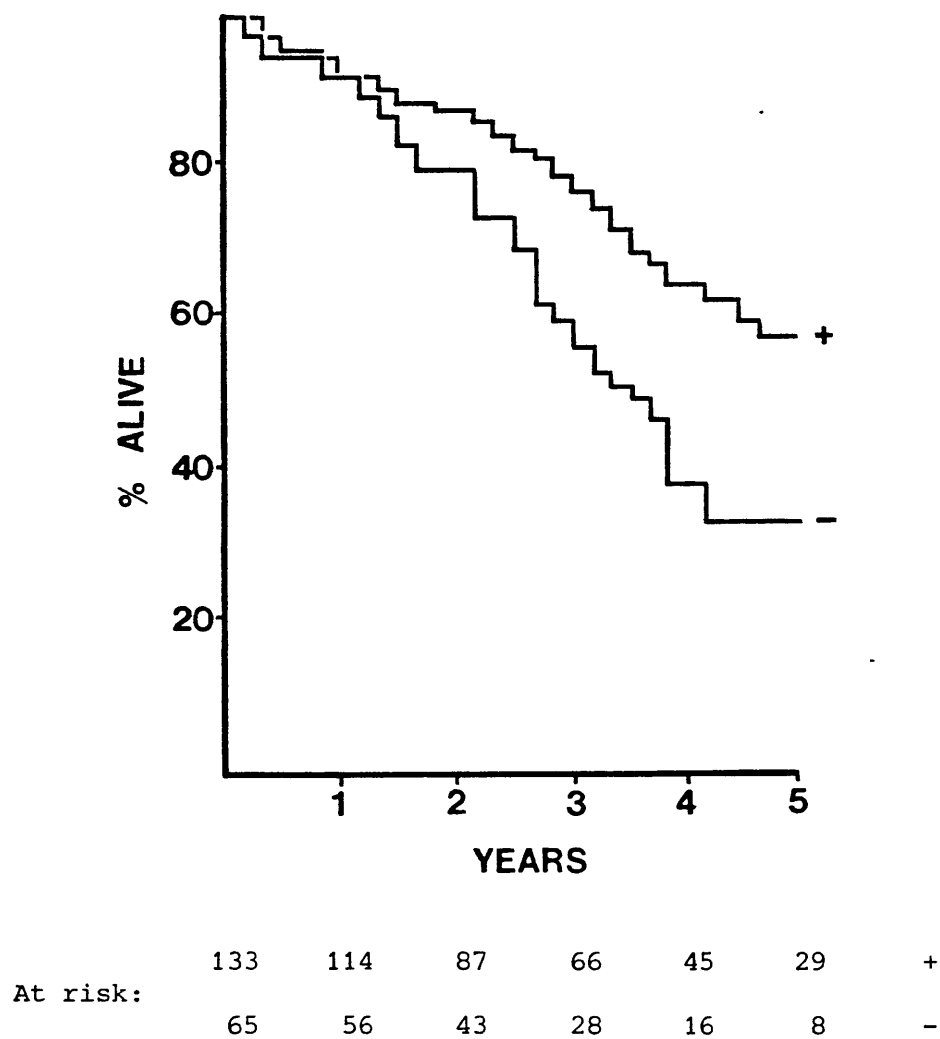
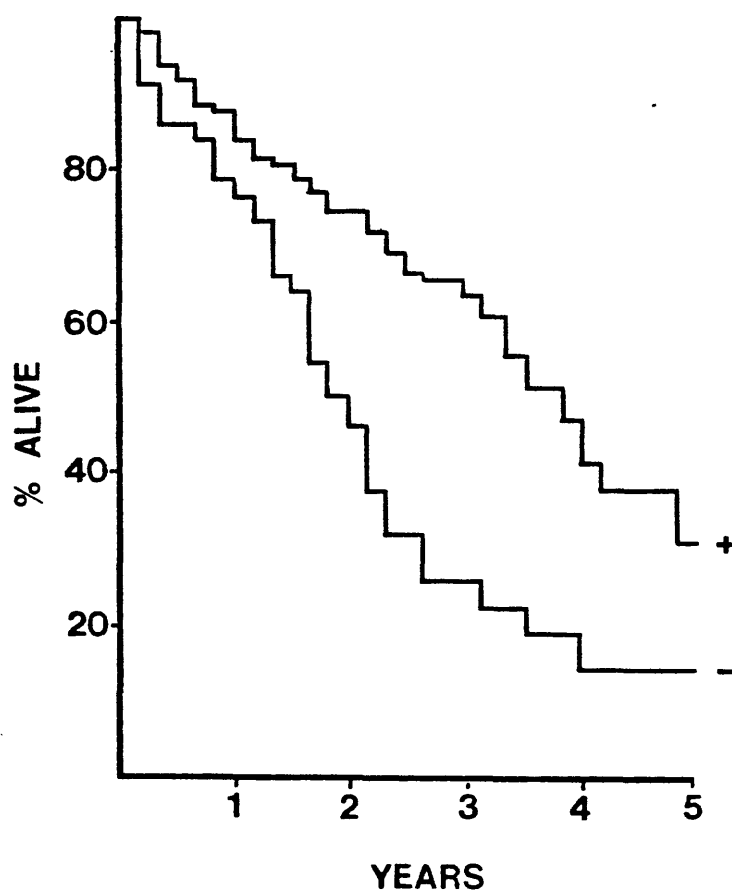


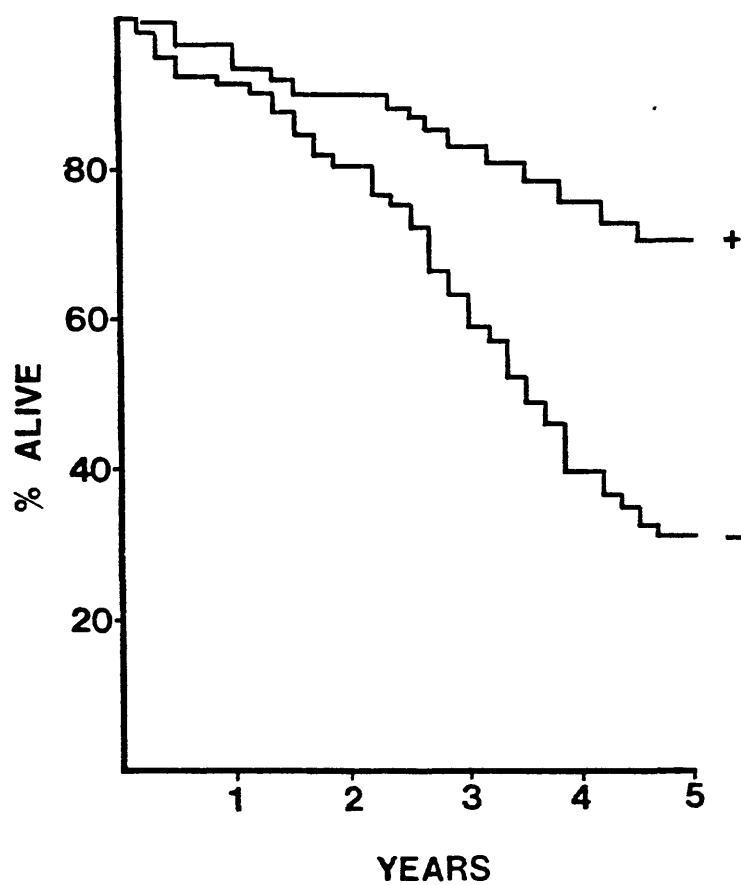
Figure 28: Oestrogen receptor status & survival from presentation  
(All assays included)



|          |     |    |    |    |    |   |   |
|----------|-----|----|----|----|----|---|---|
| At risk: | 133 | 89 | 53 | 31 | 13 | 3 | + |
|          | 65  | 41 | 18 | 8  | 3  | 2 | - |

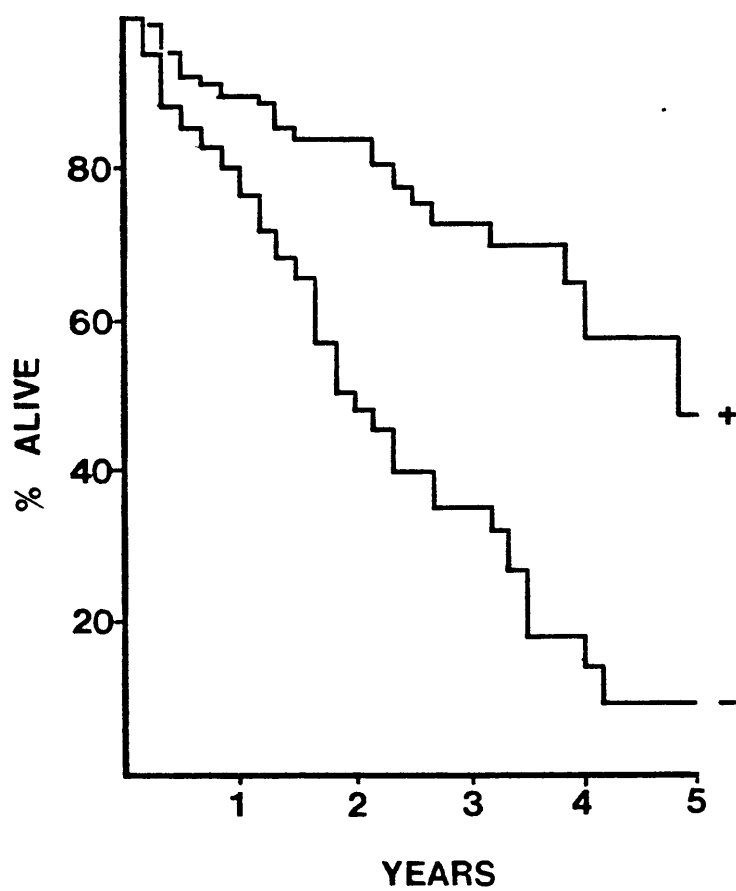
Figure 29: Oestrogen receptor status & survival after first relapse  
(All assays included)





|          |     |    |    |    |    |    |   |
|----------|-----|----|----|----|----|----|---|
|          | 102 | 87 | 66 | 52 | 39 | 26 | + |
| At risk: | 91  | 79 | 61 | 40 | 22 | 12 | - |

Figure 30: Progesterone receptor status & survival from presentation  
(All assays included)



|          |     |    |    |    |    |   |   |
|----------|-----|----|----|----|----|---|---|
|          | 102 | 71 | 41 | 24 | 14 | 4 | + |
| At risk: | 91  | 57 | 28 | 15 | 3  |   | - |

Figure 31: Progesterone receptor status & survival after first relapse. (All assays included)

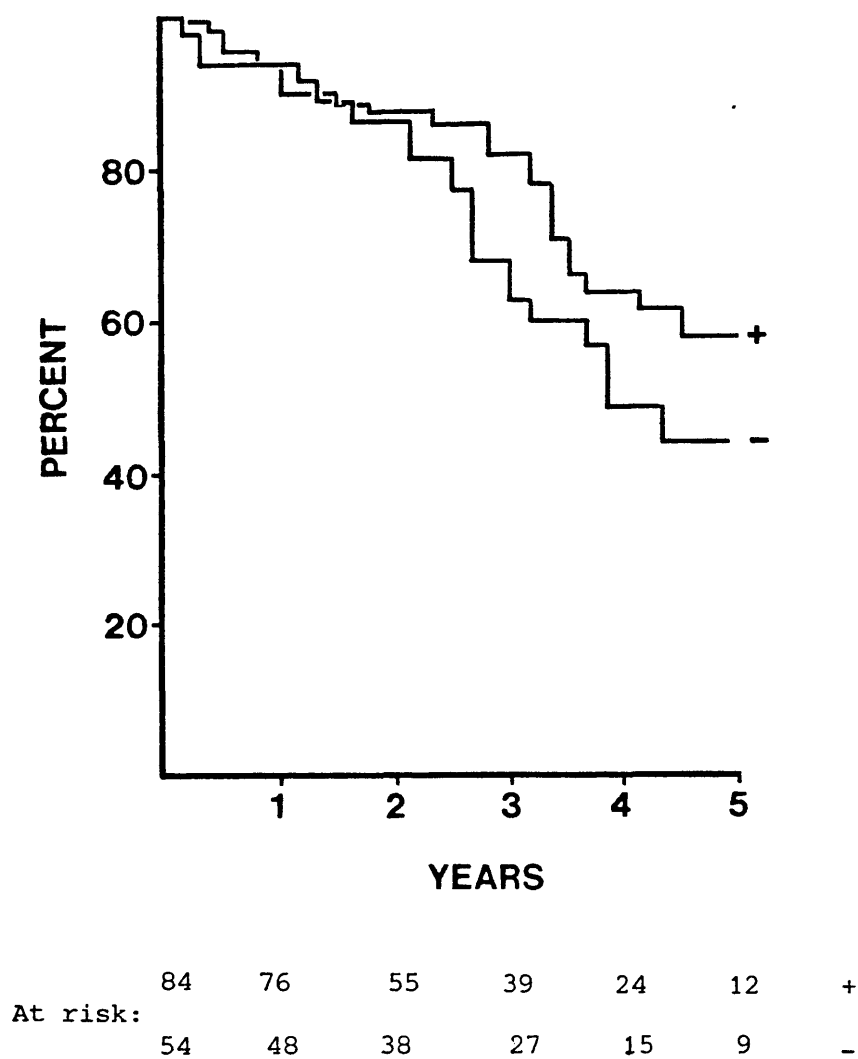
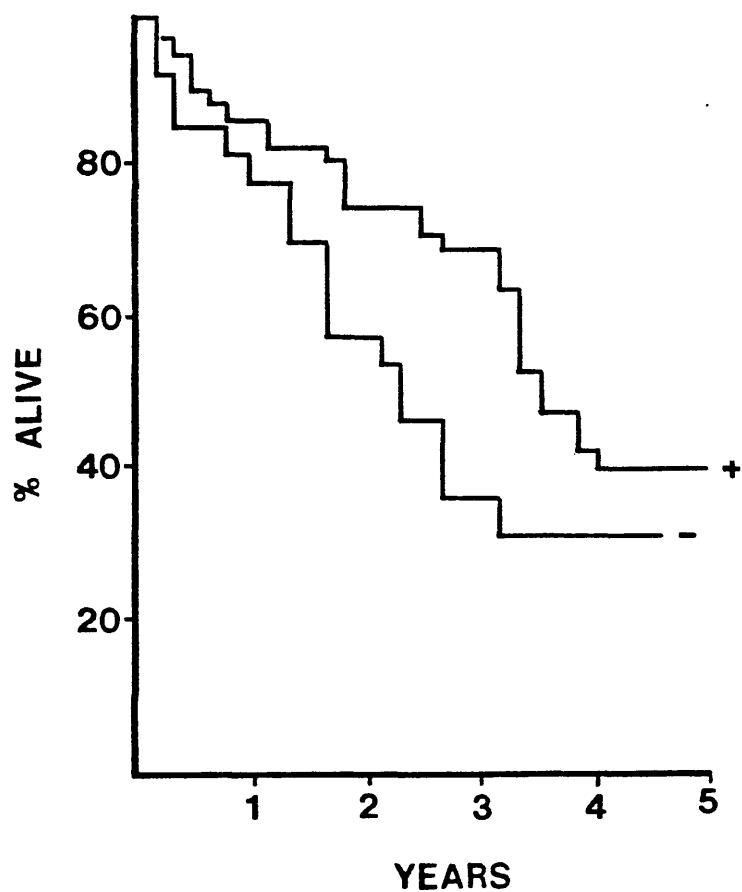


Figure 32: Nuclear oestrogen receptor status & survival from presentation (All assays included)



|          |    |    |    |    |   |   |   |
|----------|----|----|----|----|---|---|---|
|          | 84 | 57 | 31 | 16 | 7 | 3 | + |
| At risk: | 54 | 36 | 15 | 8  | 2 |   | - |

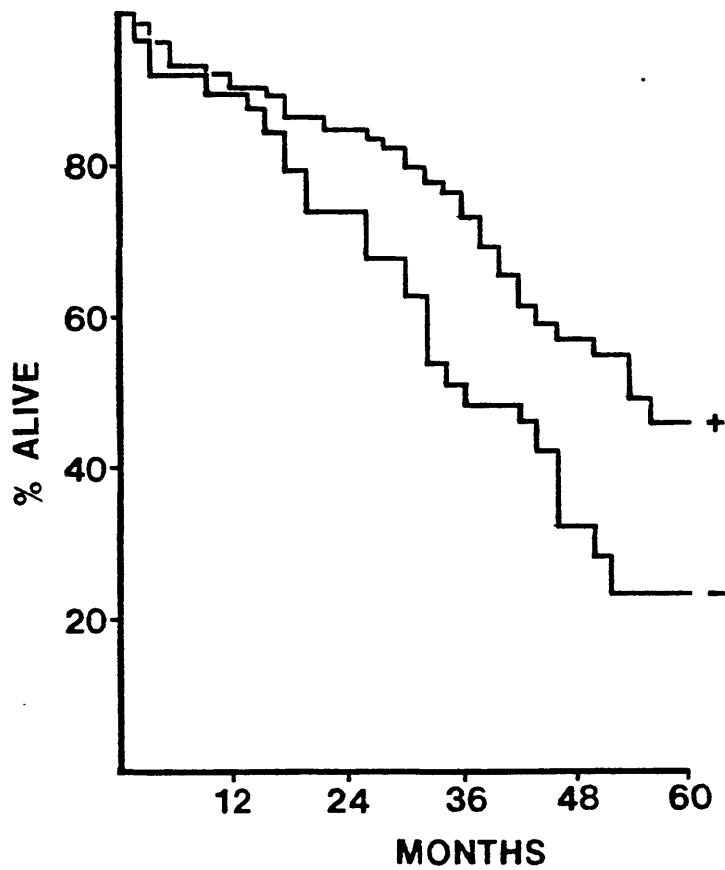
Figure 33: Nuclear oestrogen receptor status & survival after first relapse. (All assays included)

### Primary tumour receptors

Results of assays carried out on the primary tumours indicated groups with substantially different probabilities of survival at any time after mastectomy or first relapse (oestrogen receptor, Figures 34 & 35; overall survival, Chi-squared = 6.6,  $p = 0.01$ ; survival from first relapse, Chi-squared = 13.77,  $p = 0.0002$ . Progesterone receptor, Figures 36 & 37; overall survival, Chi-squared = 14.2,  $p = 0.0002$ ; survival from first relapse, Chi-squared = 19.36,  $p < 0.0001$ ).

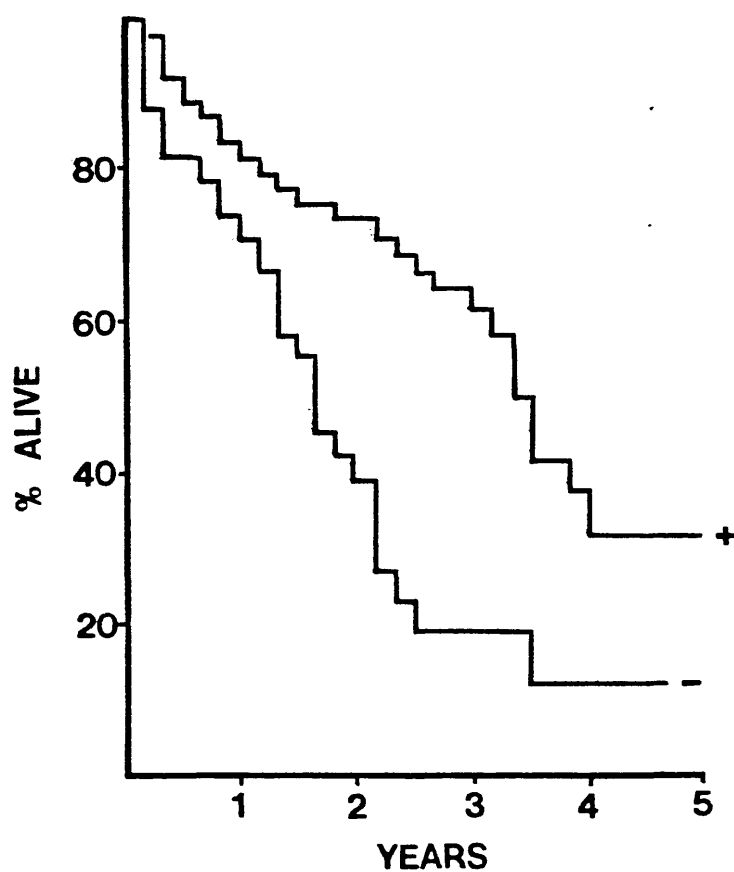
Survival after presentation and after the onset of secondary disease was more likely to be prolonged when both oestrogen and progesterone receptors were present than when oestrogen receptor only or neither receptor was present (Figure 38, Chi-squared = 20.84,  $p < 0.0001$ ). There was no significant difference in the proportion of progesterone receptor negative patients alive after initial presentation or the onset of secondary disease whatever their oestrogen receptor status (Figure 40; overall survival, Chi-squared = 0.02,  $p = 0.89$ ; survival from first relapse, Chi-squared = 0.66,  $p = 0.42$ ). Progesterone receptor status indicated groups with different prospects for survival, whatever the oestrogen receptor status (Chi-squared = 8.93,  $p = 0.003$ , Figure 41).

Nuclear oestrogen receptor status also defined groups with different probabilities of survival when assessed on the primary tumour (Figure 42; Chi-squared = 6.12,  $p = 0.013$ , for survival from first relapse).



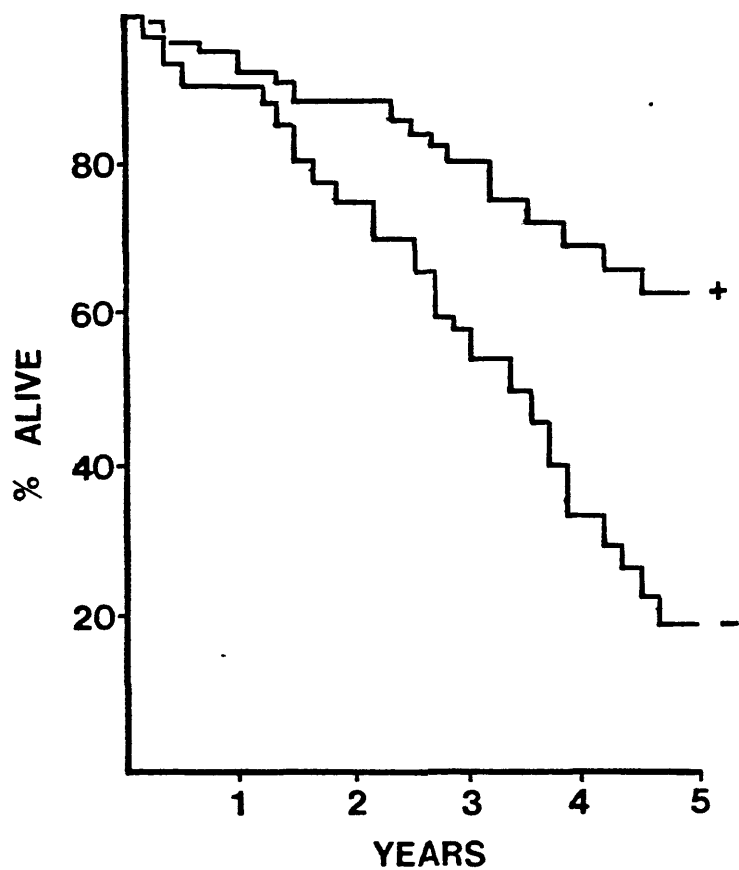
|          |     |    |    |    |    |    |   |
|----------|-----|----|----|----|----|----|---|
|          | 108 | 89 | 63 | 45 | 26 | 13 | + |
| At risk: | 50  | 42 | 31 | 18 | 10 | 3  | - |

Figure 34: Oestrogen receptor status (assessed on primary tumours) & survival from presentation.



|          |     |    |    |    |   |   |   |
|----------|-----|----|----|----|---|---|---|
|          | 108 | 69 | 41 | 21 | 6 | 2 | + |
| At risk: | 50  | 29 | 12 | 4  | 1 |   | - |

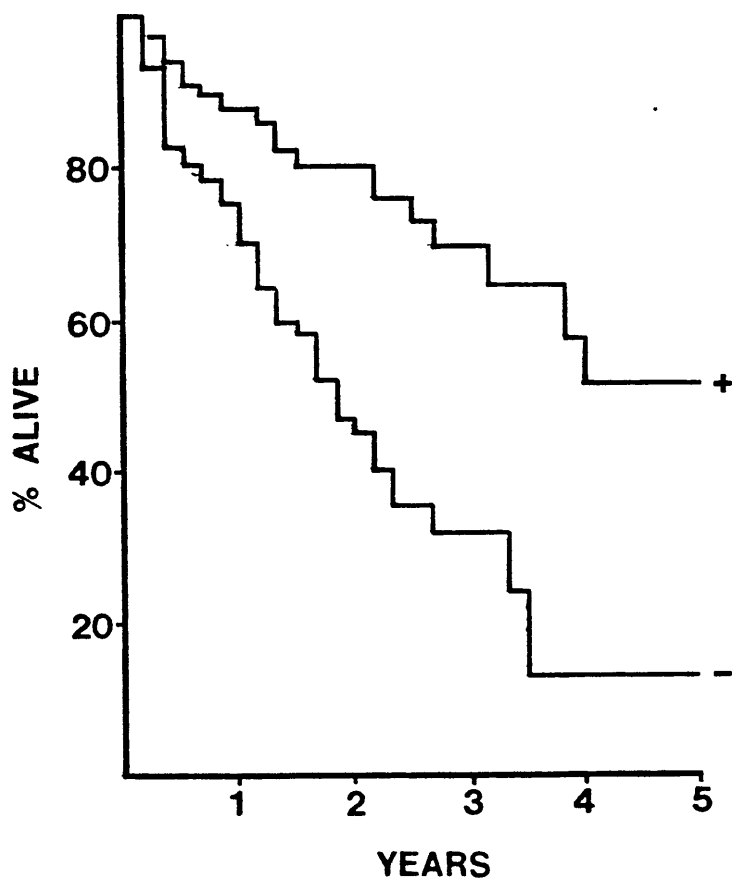
Figure 35: Oestrogen receptor status (assessed on primary tumours) & survival after first relapse.



|          |    |    |    |    |    |    |   |
|----------|----|----|----|----|----|----|---|
|          | 84 | 69 | 49 | 35 | 23 | 13 | + |
| At risk: | 70 | 59 | 43 | 27 | 13 | 4  | - |

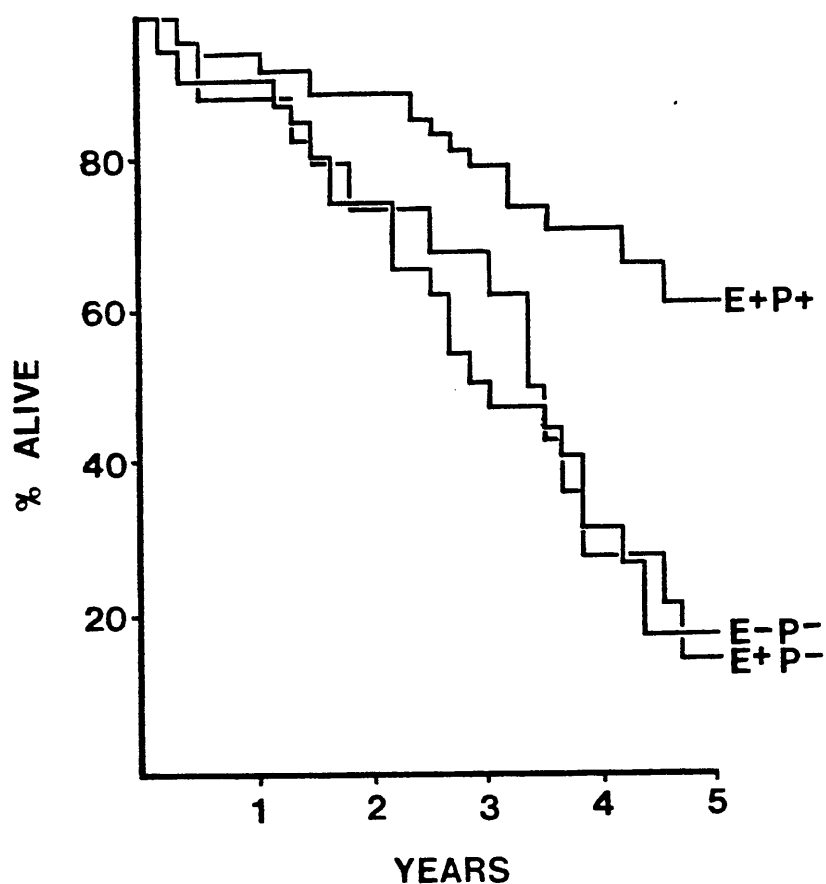
Figure 36: Progesterone receptor status (assessed on primary tumours) & survival from presentation.





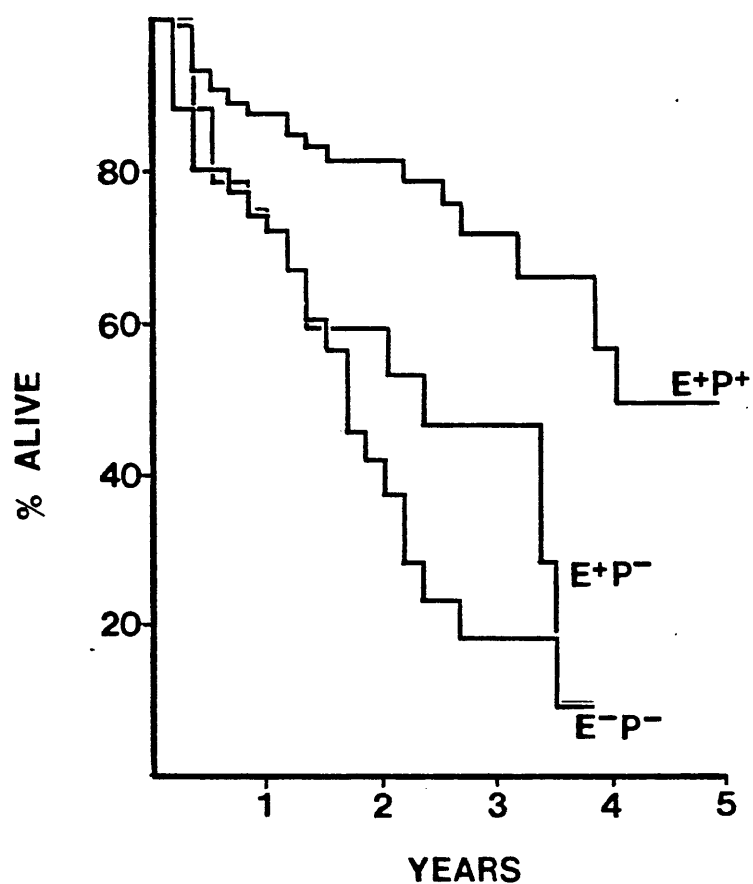
|          |    |    |    |    |   |   |   |
|----------|----|----|----|----|---|---|---|
|          | 84 | 56 | 31 | 15 | 8 | 2 | + |
| At risk: | 70 | 40 | 20 | 10 | 1 |   | - |

Figure 37: Progesterone receptor status (assessed on primary tumours) & survival after first relapse.



|          |    |    |    |    |    |    |    |
|----------|----|----|----|----|----|----|----|
|          | 77 | 63 | 44 | 31 | 20 | 10 | ++ |
| At risk: | 25 | 21 | 15 | 11 | 4  | 2  | +- |
|          | 4  |    |    |    |    |    | -+ |
|          | 44 | 37 | 27 | 15 | 8  | 1  | -- |

Figure 38: Combined (RE & RP) receptor status & survival from presentation (Survival of RE-RP+ patients has been omitted for clarity).



|          |    |    |    |    |   |   |    |
|----------|----|----|----|----|---|---|----|
|          | 77 | 51 | 28 | 13 | 6 | 2 | ++ |
| At risk: | 25 | 13 | 10 | 6  |   |   | +- |
|          | 4  |    |    |    |   |   | -+ |
|          | 44 | 26 | 10 | 3  |   |   | -- |

Figure 39: Combined (RE & RP) receptor status & survival after first relapse. (Survival of RE-RP+ patients has been omitted for clarity).

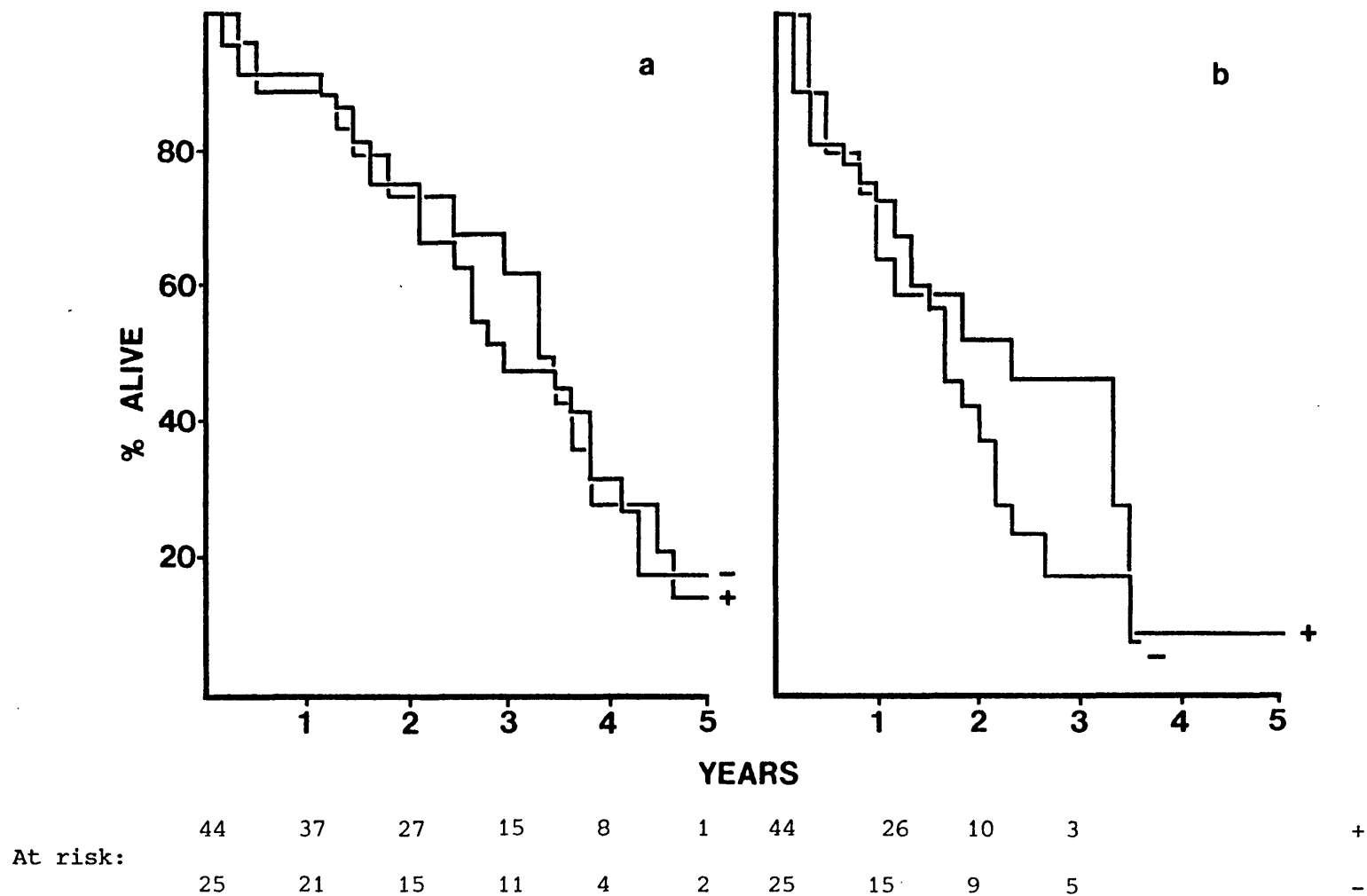
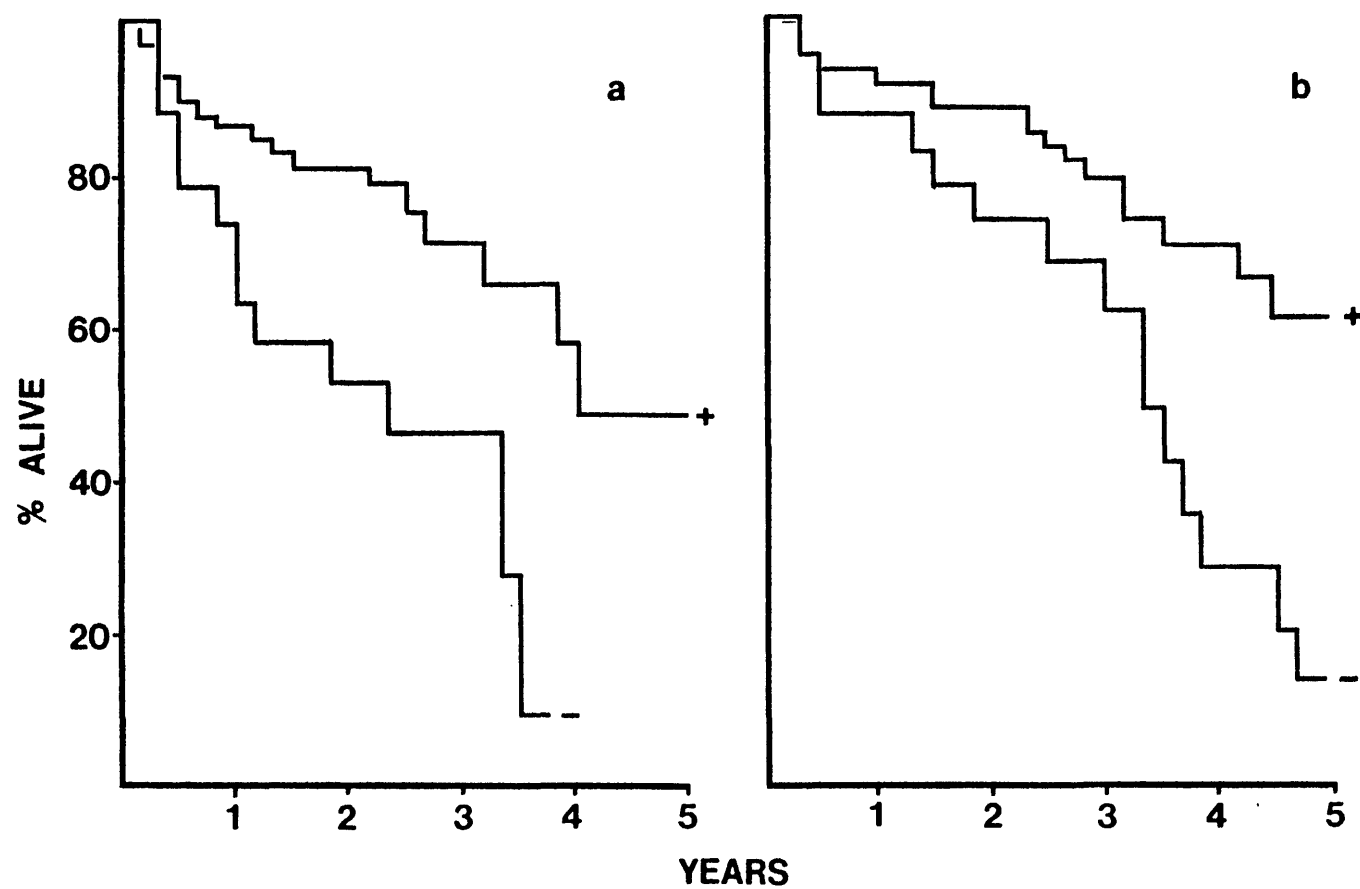
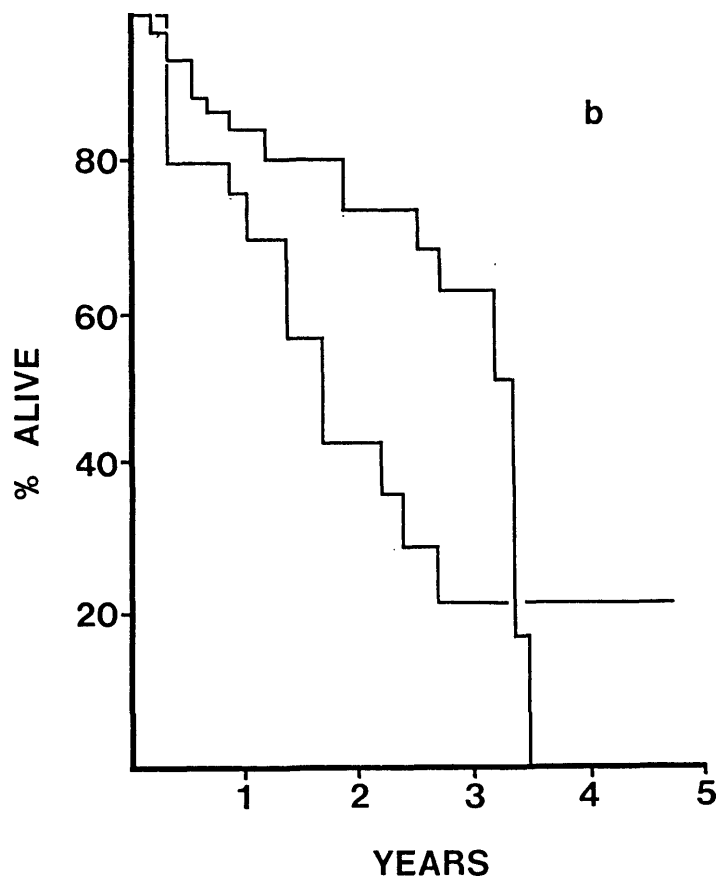


**Figure 40:** Effects of oestrogen receptor status on survival from presentation (a) & survival after first relapse (b) in progesterone receptor negative patients. (Assessed on primary tumours)



|          |    |    |    |    |   |  |    |    |    |    |    |    |   |
|----------|----|----|----|----|---|--|----|----|----|----|----|----|---|
|          | 77 | 51 | 28 | 13 | 6 |  | 77 | 63 | 44 | 31 | 20 | 10 | + |
| At risk: | 25 | 13 | 9  | 6  |   |  | 25 | 21 | 15 | 11 | 4  | 2  | - |

**Figure 41:** Effects of progesterone receptor status on survival after first relapse (a) & survival from presentation (b) in oestrogen receptor positive patients (Receptors assessed on primary tumours).

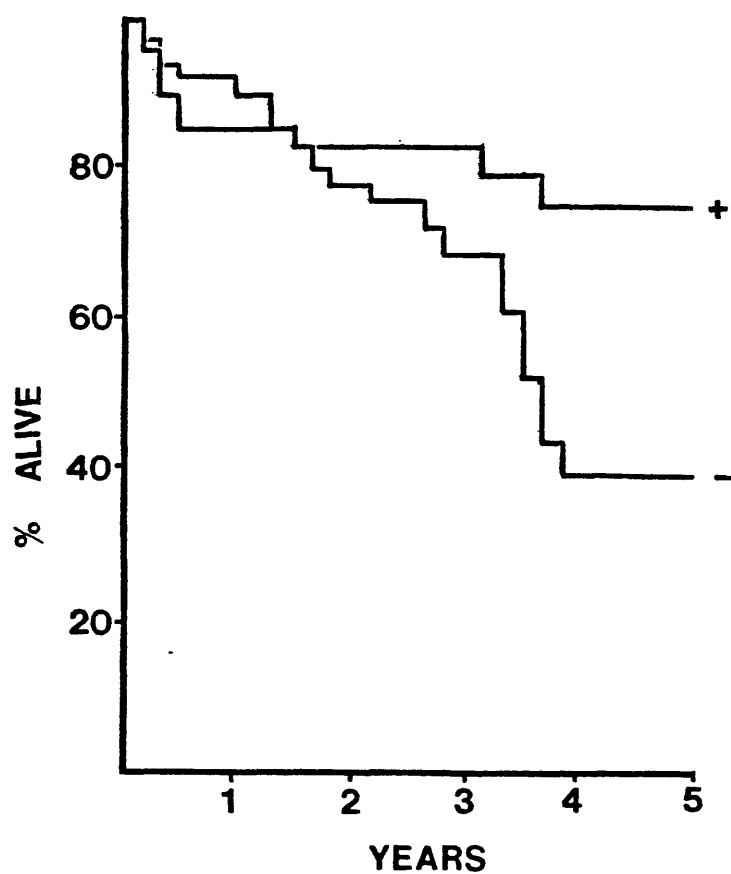


|          |    |    |    |   |   |
|----------|----|----|----|---|---|
|          | 64 | 43 | 21 | 7 | + |
| At risk: | 40 | 22 | 7  | 3 | - |

Figure 42: Nuclear oestrogen receptor status (assessed on primary tumours) & survival after first relapse.

### Assays at the time of treatment

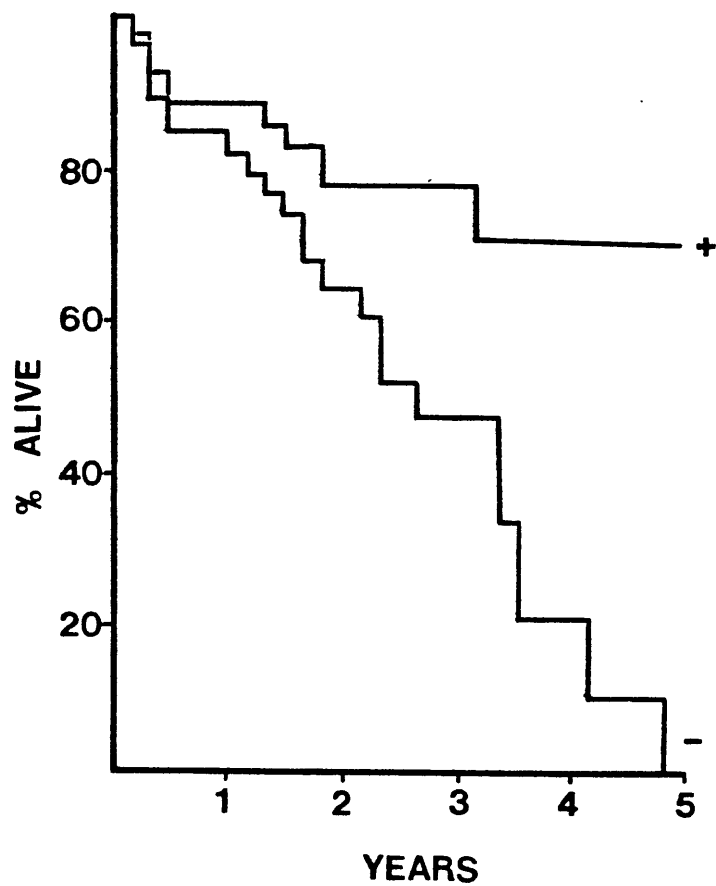
More patients in whom progesterone receptor had been detected at the time of treatment were alive at any given time after mastectomy or after the first relapse than when progesterone receptor was not present (Figures 43 & 44; overall survival, Chi-square = 8.77,  $p = 0.003$ ; survival from first relapse, Chi-square = 9.2,  $p = 0.002$ ). Assay of oestrogen receptors at the time of treatment failed to distinguish groups of clearly different survival (Figures 45 & 46; overall survival, Chi-squared = 2.58,  $p = 0.1$ ; survival from first relapse, Chi-squared = 1.65,  $p = 0.2$ ).



|          |    |    |    |    |    |   |
|----------|----|----|----|----|----|---|
|          | 56 | 43 | 25 | 22 | 18 | + |
| At risk: | 49 | 39 | 30 | 20 | 9  | - |

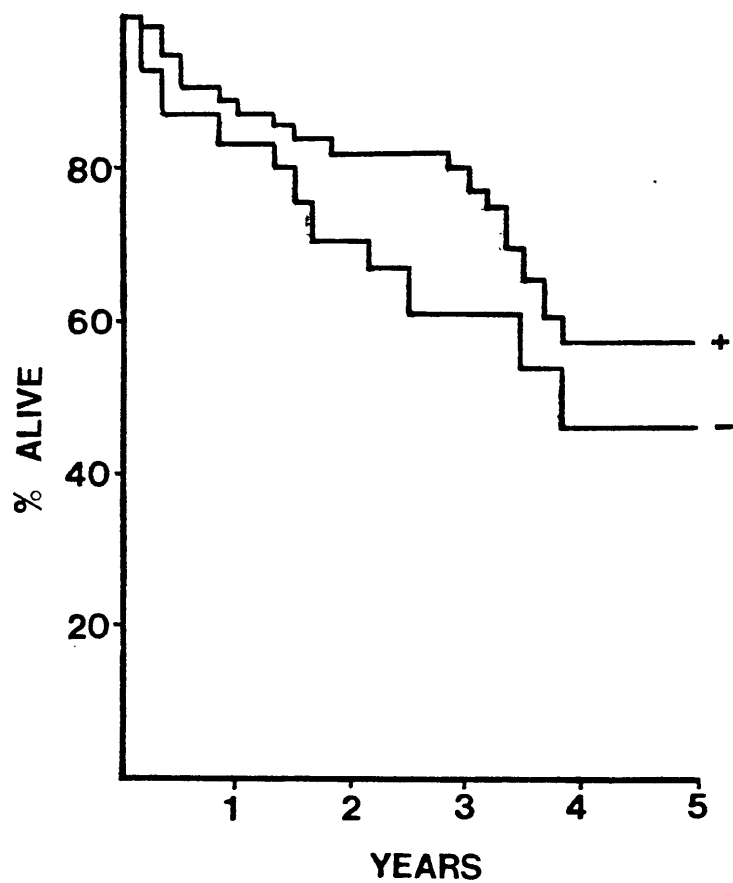
Figure 43: Progesterone receptor status (assessed at the time of endocrine treatment) & survival from presentation.





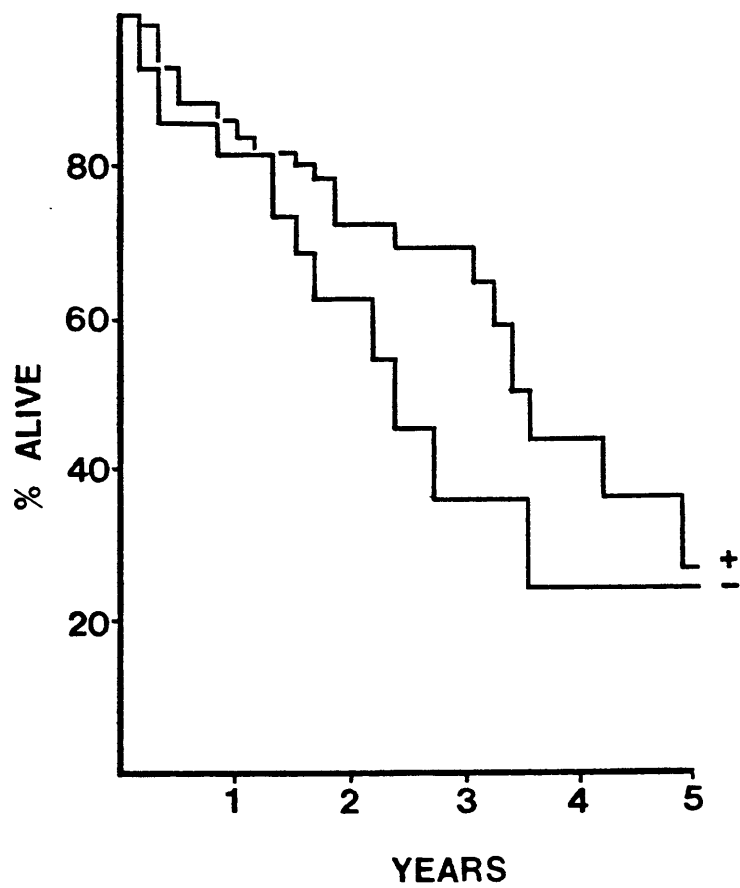
|          |    |    |    |    |   |   |   |
|----------|----|----|----|----|---|---|---|
|          | 56 | 35 | 14 | 11 | 6 | 4 | + |
| At risk: | 49 | 32 | 18 | 7  | 3 |   | - |

Figure 44: Progesterone receptor status (assessed at the time of endocrine treatment) & survival after first relapse.



|          |    |    |    |    |    |    |   |
|----------|----|----|----|----|----|----|---|
|          | 77 | 60 | 39 | 31 | 21 | 19 | + |
| At risk: | 30 | 23 | 16 | 11 | 7  | 4  | - |

Figure 45: Oestrogen receptor status (assessed at the time of endocrine treatment) & survival from presentation



|          |    |    |    |    |   |   |   |
|----------|----|----|----|----|---|---|---|
| At risk: | 77 | 49 | 23 | 16 | 6 | 3 | + |
|          | 30 | 19 | 9  | 3  | 1 |   | - |

Figure 46: Oestrogen receptor status (assessed at the time of endocrine treatment) & survival from first relapse.

### Concentrations of receptor in primary tumours & survival

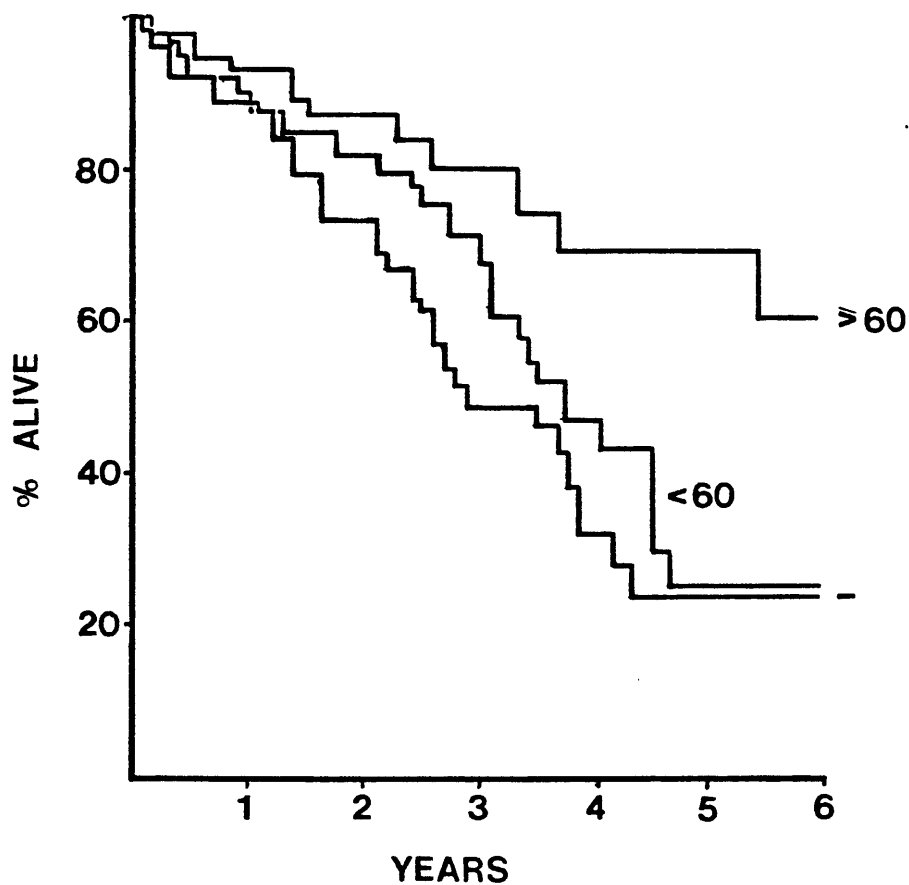
Patients with low concentrations of oestrogen receptor in their primary tumour had a similar probability of survival to oestrogen receptor negative patients.

Patients with more than 60 fmol/ mg cytosol protein of receptor were more likely to survive than those with low amounts (Figures 47 & 48; overall survival, Chi-squared = 9.65,  $p = 0.002$ ; survival from first relapse, chi-squared = 18.53,  $p < 0.0001$ ).

Similar effects were seen when patients were grouped according to the concentration of progesterone receptor status (Figures 49 & 50: Overall survival, Chi-squared = 14.54,  $p = 0.0001$ . Survival from first relapse, Chi-squared = 18.53,  $p < 0.0001$ ).

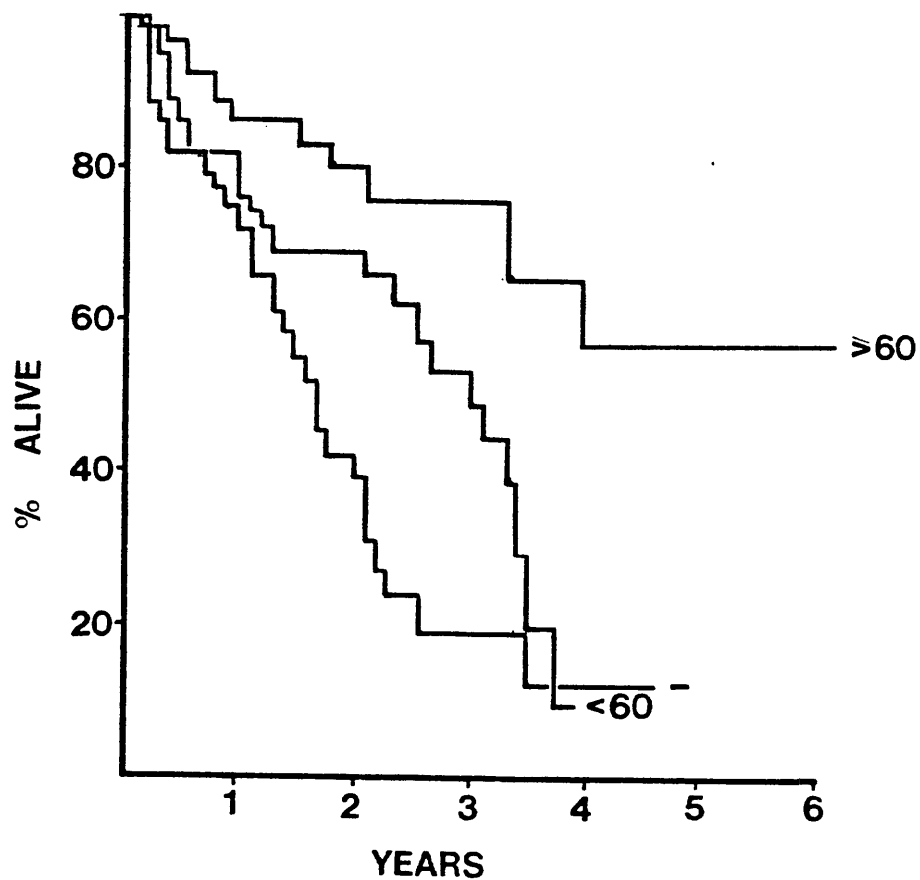
The concentration of oestrogen receptor did not indicate the probability of survival when an allowance was made for the concentration of progesterone receptor (Figure 51: Overall survival, Chi-squared = 2.1,  $p = 0.35$ ).

Conversely progesterone receptor status indicated groups with different survival even when allowing for the concentration of oestrogen receptor (Figure 52: Chi-squared = 5.11,  $p = 0.024$ )



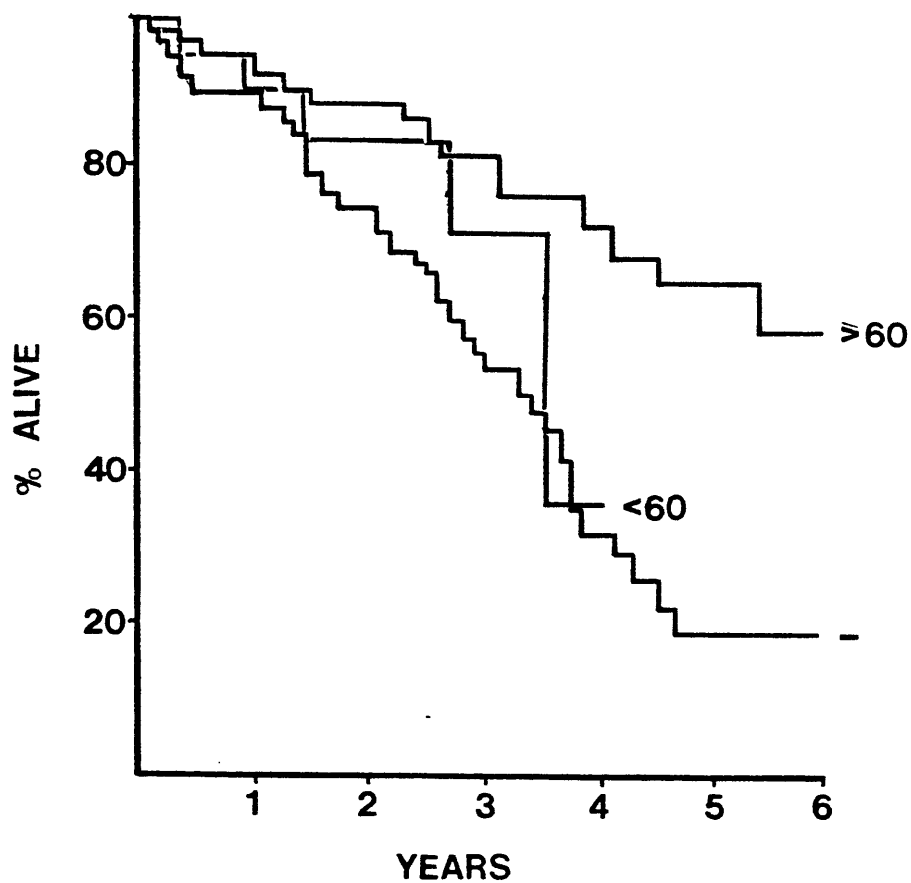
|          |    |    |    |    |    |   |   |     |
|----------|----|----|----|----|----|---|---|-----|
|          | 59 | 48 | 31 | 19 | 13 | 9 | 2 | >60 |
| At risk: | 49 | 41 | 32 | 26 | 13 | 4 |   | <60 |
|          | 50 | 42 | 31 | 18 | 10 | 3 | 1 | -   |

Figure 47: Concentration of oestrogen receptors & survival from presentation



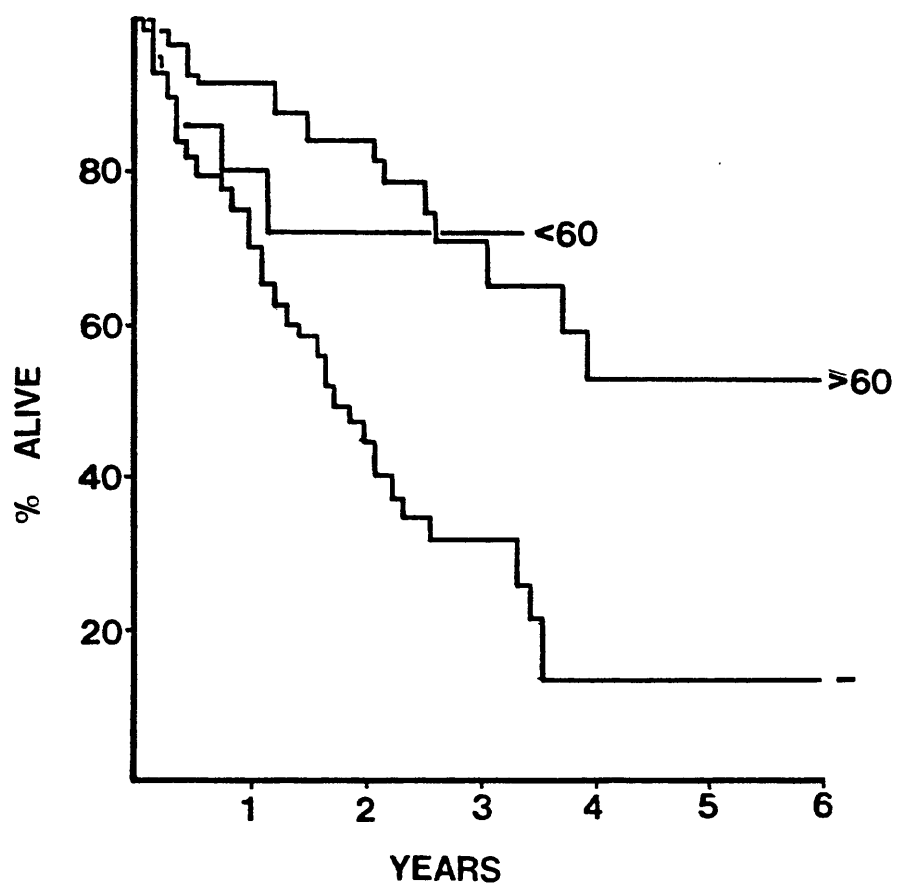
|          |    |    |    |    |   |   |   |     |
|----------|----|----|----|----|---|---|---|-----|
|          | 59 | 36 | 21 | 10 | 6 | 2 | 1 | >60 |
| At risk: | 49 | 33 | 20 | 11 |   |   |   | <60 |
|          | 50 | 29 | 12 | 4  | 1 |   |   | -   |

Figure 48: Concnetration of oestrogen receptors & survival after first relapse.



|          |    |    |    |    |    |    |   |     |
|----------|----|----|----|----|----|----|---|-----|
|          | 62 | 51 | 40 | 30 | 22 | 13 | 2 | >60 |
| At risk: | 22 | 18 | 9  | 5  | 1  |    |   | <60 |
|          | 70 | 59 | 43 | 27 | 13 | 4  | 2 | -   |

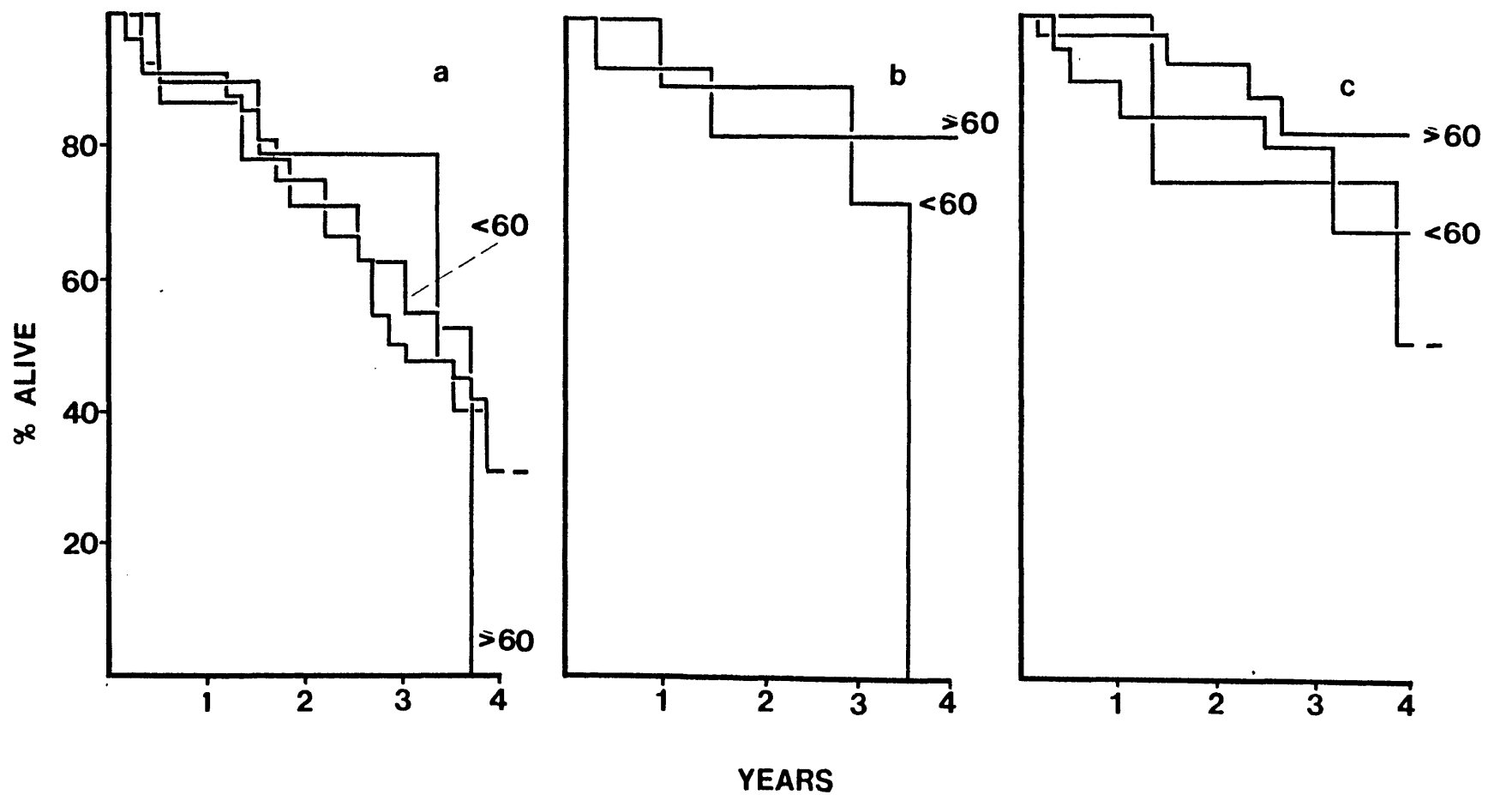
Figure 49: Concentration of progesterone receptors & survival from presentation.



|          |    |    |    |    |   |   |   |     |
|----------|----|----|----|----|---|---|---|-----|
|          | 62 | 45 | 27 | 13 | 8 | 2 | 1 | >60 |
| At risk: | 22 | 11 | 4  | 2  |   |   |   | <60 |
|          | 70 | 40 | 20 | 10 | 1 | 1 | 1 | -   |

Figure 50: Concentration of progesterone receptors & survival after first relapse





|          |    |    |    |    |   |    |    |   |  |    |    |    |    |    |     |
|----------|----|----|----|----|---|----|----|---|--|----|----|----|----|----|-----|
|          | 10 | 9  | 6  | 4  |   | 12 | 10 | 5 |  | 34 | 27 | 20 | 13 | 11 | >60 |
| At risk: | 15 | 12 | 9  | 8  | 4 | 10 | 8  | 5 |  | 21 | 18 | 15 | 13 | 8  | <60 |
|          | 44 | 37 | 27 | 15 | 9 |    |    |   |  | 4  |    |    |    |    | -   |

Figure 51: Concentration of oestrogen receptors & survival from presentation, allowing for different concentrations of progesterone receptors.

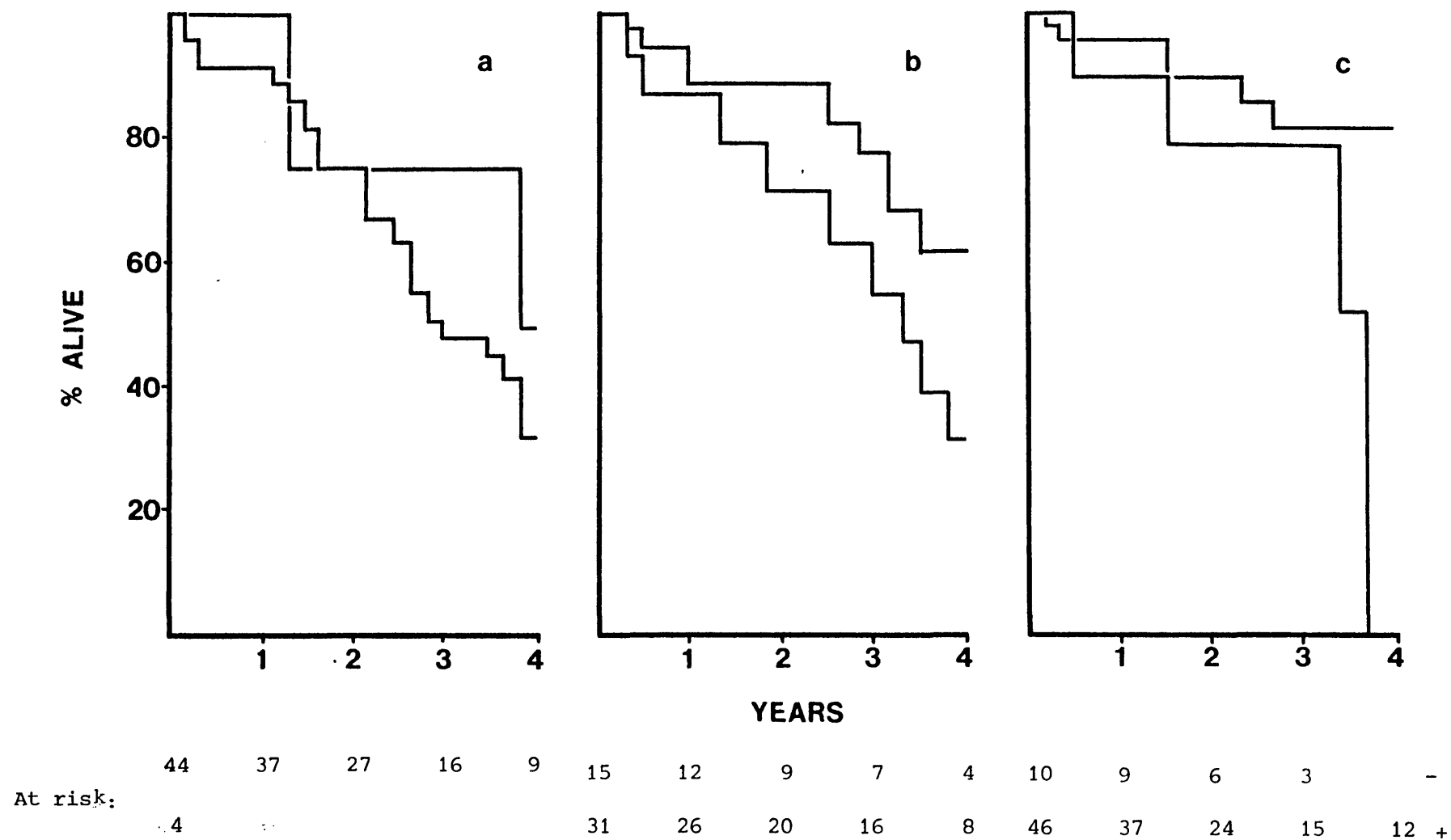


**Figure 52:** Progesterone receptor status & survival from presentation, allowing for the concentration of oestrogen receptors. ((a) ER negative, (b) ER <60 fmol/mg protein, (c) ER ≥60 fmol/mg protein).

## MEASUREMENT OF PROGESTERONE RECEPTOR BY IEF

### Separation of progesterone receptor & pI's

When a cytosol contained receptor 1 major peak of activity which could be suppressed by excess progestogen was seen (Figure 53). This occurred between pH 5.0 and 5.8 (mean = 5.46, s.e.mean = 0.05). Lesser peaks at pI ~6.4 were sometimes seen.

At the application point the specific and non-specific binding were well matched except at high levels of binding when an excess of total over non-specific binding was found in all the fractions.

### Limit of sensitivity

The lower limit of sensitivity was 30 fmol/ml cytosol. This was established in cytosols which ranged in concentration from 0.7 to 7.0 g protein/l.

### Factors affecting the assay

#### Binding of steroids by serum proteins

The main component of progestin binding in serum focused in the range pH 4.6 - 5.4 (mean = 4.95, s.e.mean = 0.06). Serum binding could not be suppressed by an excess of norethisterone (Figure 54.). Binding in the presence of norethisterone was 106% of that found when it was not present (s.e.mean = 7.7%, n = 19).

Oestradiol and androgens (dihydrotestosterone and R-1881) bound to a protein which focused at pI 6.2, while all steroids except dihydrotestosterone bound to one which focused at pI ~4.8. (Figure 55).

### Effect of molybdate

64.3% (s.e.mean = 15.1%) of the receptor found by DCC assay was focused at the pI when molybdate was present. When molybdate was absent only 6% (s.e.mean = 2.05%) of the receptor was focused (Figure 56,  $t = 3.77$ ,  $p < 0.01$ ). No consistent effect was seen on the amount of receptor found by the DCC assay.

### Gel medium

Similar amounts of receptor were found by IEF whether agarose or acrylamide gel was used. However acrylamide gels did not usually show signs of focusing of the receptor (Figure 57) and were more difficult to interpret.

The pI of progesterone receptor in acrylamide gel was 6.39 (s.e. mean = 0.097). The same cytosols gave a pI of 5.23 (s.e. mean = 0.08) in agarose gel ( $t = 6.4$ ,  $p < 0.001$ ).

### Duration of electrofocusing

The mean concentration of receptor estimated after focusing had continued for 2000 volt-hours was 356 fmol/ml cytosol (range of  $\pm 1$  s.d. = 116 - 1465) compared with 413 fmol (range of  $\pm 1$  s.d. = 100 - 1263) found at 1250 volt-hours.

### Migration of free <sup>3</sup>H-R5020

Free <sup>3</sup>H-R5020 migrated towards the cathode. Activity between fractions 6 and 16 was 1% or less of that found in the maximum peak, rising to ~10% in fraction 18 (Figure 58).

### Precision

27 samples were assayed at least in duplicate. The mean of each set of results was taken as 100% and the scatter of the results about their means expressed as a percentage deviation. The standard deviation of this distribution was 28%.

### Comparison of IEF with the DCC assay

53 (91%) of 58 assays agreed on the presence of progesterone receptor and 27 (84%) of 32 assays agreed on the absence of receptor.

The relationship between IEF and the DCC assay was linear (Figure 59), and was described by the regression equation :-

$$Y = 33.8 + (0.21 \times X)$$

where Y = [progesterone receptor] by IEF and X = [progesterone receptor] by DCC assay.

Pearson's coefficient of correlation was 0.81 (t = 12.64, p < 0.001). Spearman's rank correlation coefficient was 0.87 (t = 16.14, p < 0.001).

IEF usually underestimated the binding, finding on average 39.9% (s.e.mean = 3.73%) of that found by Scatchard analysis. The proportion of binding which focused decreased as the number of sites increased (Figure 60). IEF found 58.9% of the activity found by the DCC assay at levels (on the DCC assay) up to 400 fmol/ml.

### Assay of oestrogen receptors by IEF

#### Isoelectric points & comparison of IEF with the DCC assay

Two peaks of activity were seen at pI 6.8 (s.e. mean

= 0.09) and 5.5 (s.e. mean = 0.08) in variable proportions. The relationship of IEF with the DCC assay was linear (Figure 61), and was described by the regression equation:

$$Y = -25 + (0.816 \times X)$$

where Y = [oestrogen receptor] by IEF, and X = [oestrogen receptor] by the DCC assay.

Pearson's correlation coefficient was 0.95 (t = 21.05 p < 0.001) and Spearman's rank correlation coefficient was 0.90 (t = 14.45, p < 0.001).

IEF gave an underestimate when compared to the DCC assay, finding on average 56% (s.e.mean = 6%) of that found by the DCC assay. The DCC assay gave a positive result in 5 of 24 cytosols which gave negative results by IEF. There was no 'false positive' result by IEF.

### Precision

Multiple assays were carried out on 4 cytosol preparations. The standard deviation of the scatter of results about their means was 36% (20 results).

### Needle biopsies

Cytological smears were made from 60 needle biopsy samples. 56 (93%) confirmed malignancy.

46 samples (mean wt. 30 mg) were prepared in the way described. The protein concentration for these samples ranged from 0.7 to 30 g/l. The median concentration was 7 g/l. 95% of samples contained more than 1 g protein/l.

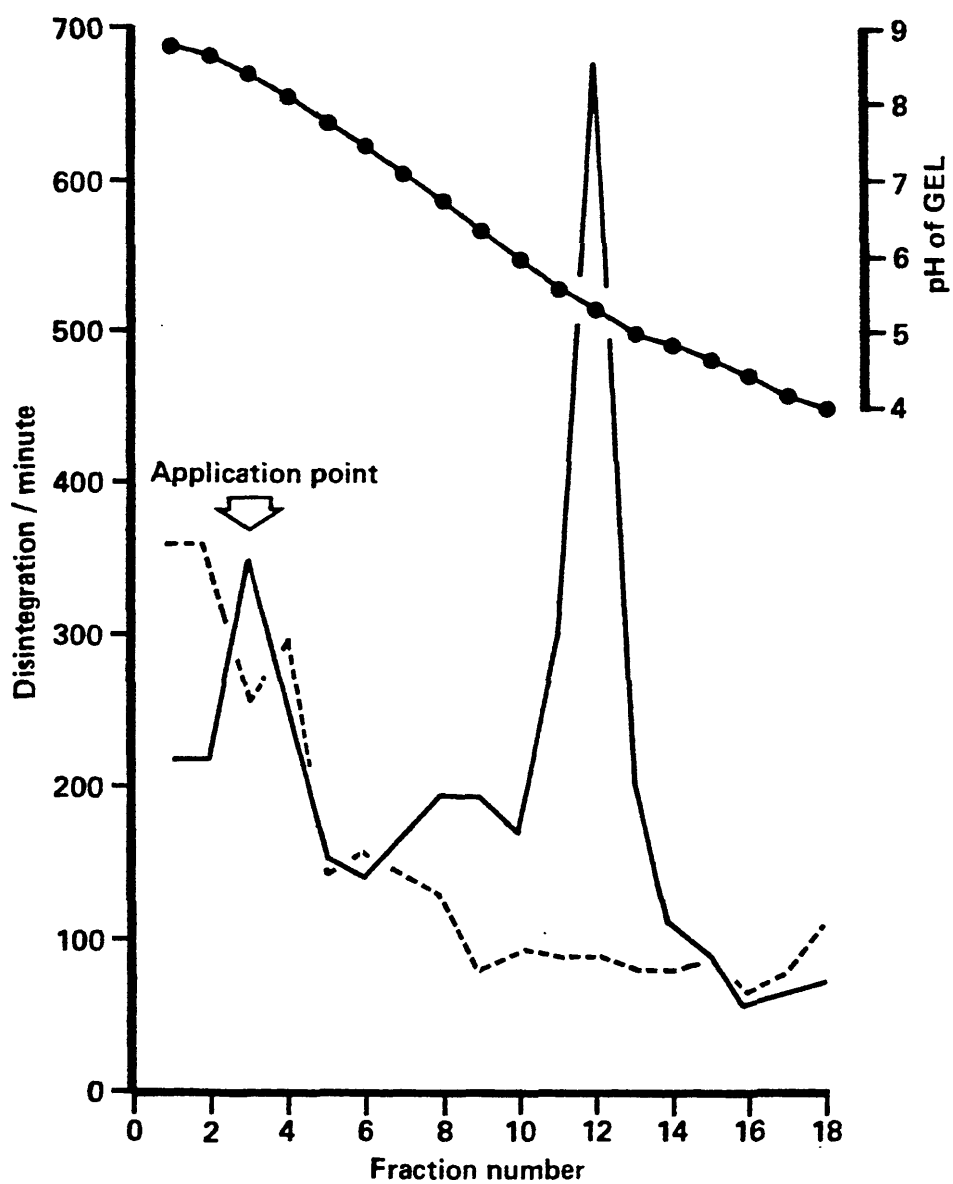


Figure 53: Isoelectric focusing trace from a progesterone receptor positive tumour ( ●● = pH: — = Sample +  $^3\text{H}$ -R5020: --- = Sample +  $^3\text{H}$ -R5020 + Norethisterone)

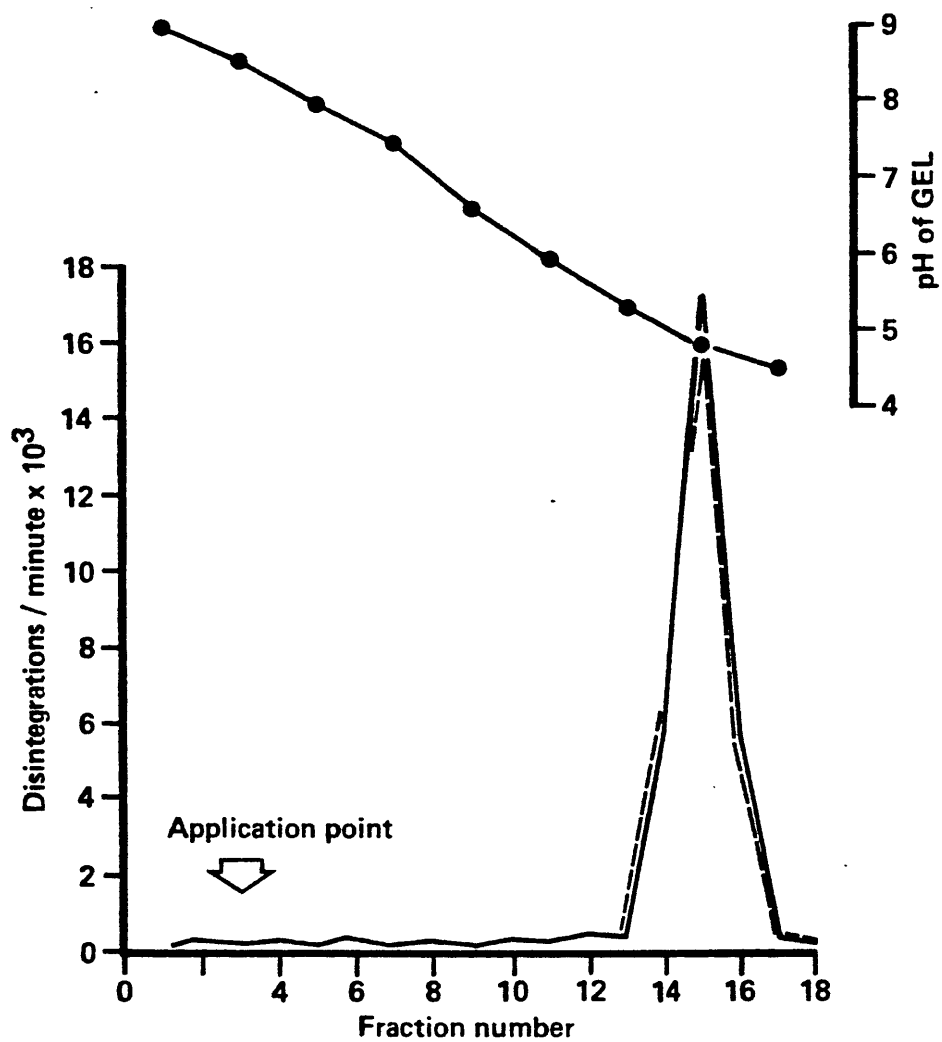


Figure 54:  $^3\text{H}$ -R5020 binding by serum protein ( ●—● = pH:  
 — = Serum +  $^3\text{H}$ -R5020: --- = Serum +  $^3\text{H}$ -R5020 + norethisterone)



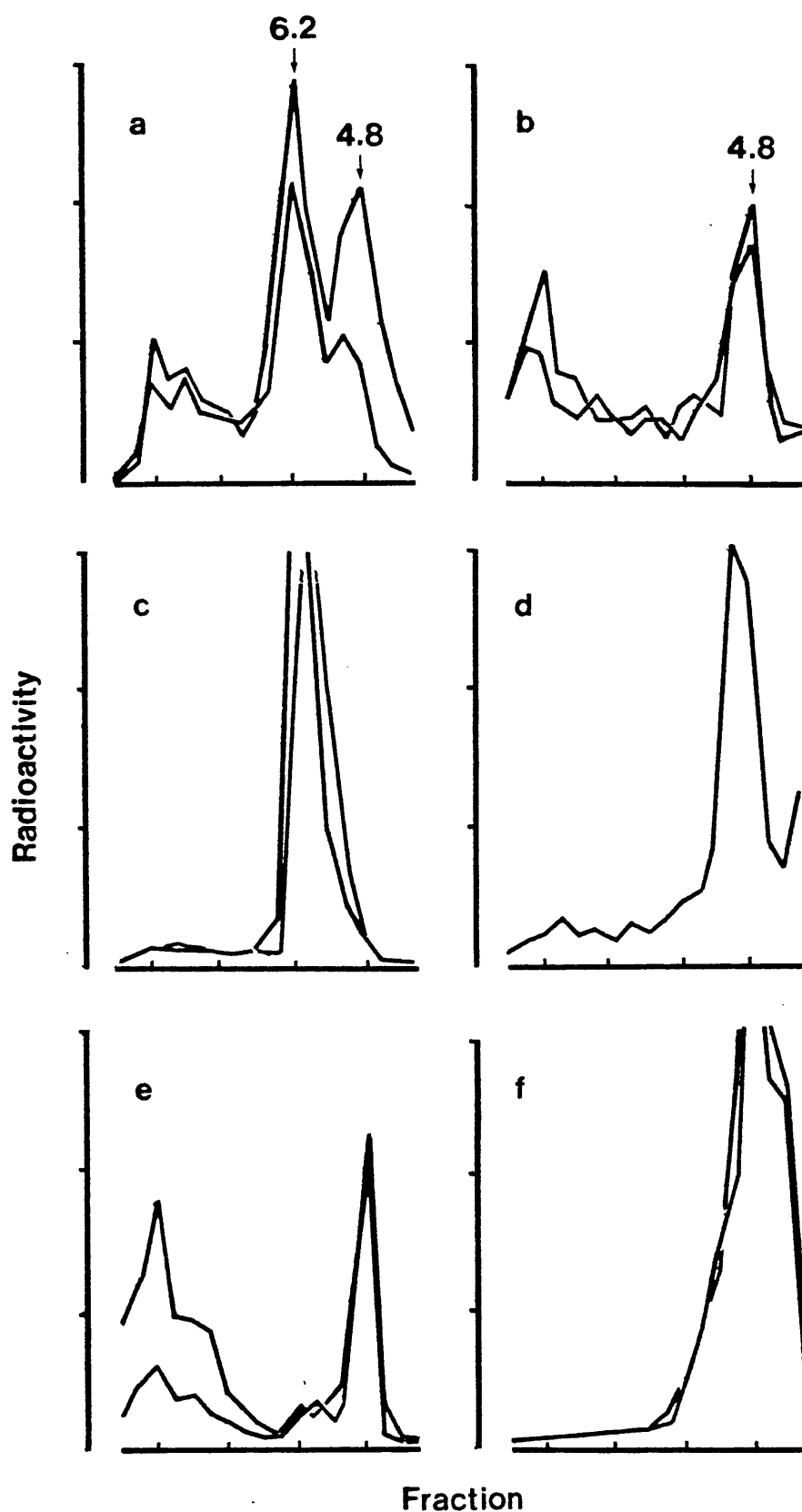


Figure 55: Binding of various steroids to serum proteins

((a)  $^3\text{H}$ -oestradiol X2, (b)  $^3\text{H}$ -R5020 X2, (c)  $^3\text{H}$ -dihydrotestosterone X2, (d)  $^3\text{H}$ -progesterone X1, (e)  $^3\text{H}$ -R1881 X2, (f)  $^3\text{H}$ -cortisol X2).

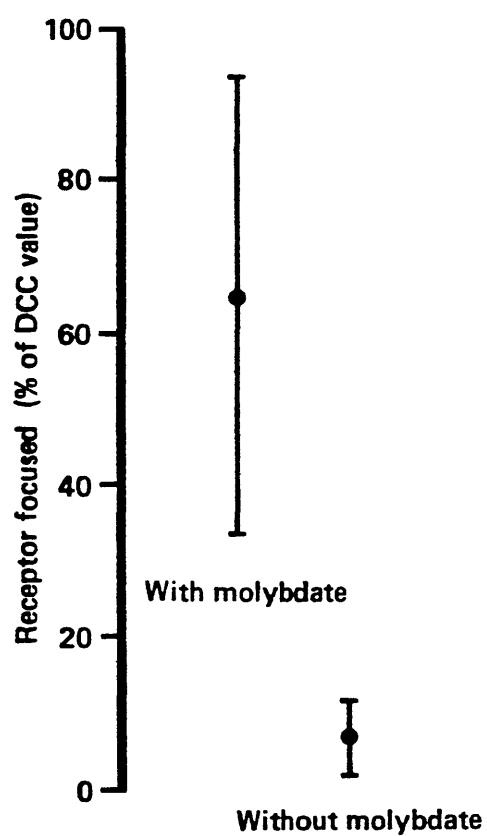


Figure 56: Effect of molybdate on the proportion of total receptor measured by IEF (Mean  $\pm$  2 X standard error)

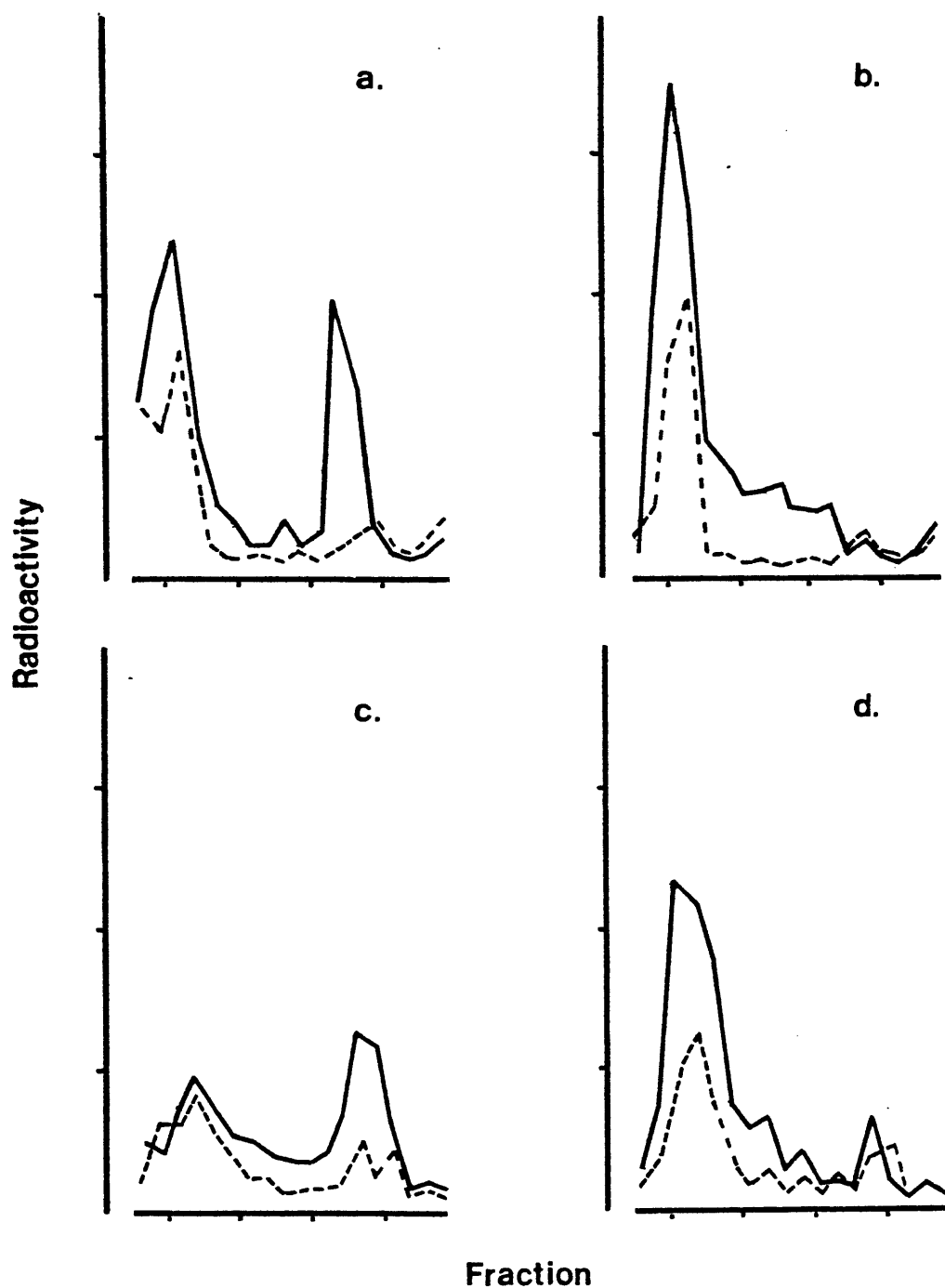


Figure 57: Isoelectric focusing in agarose vs acrylamide gels  
 ((a) = agarose, (b) = same sample in acrylamide. (c) = agarose,  
 (d) = same sample in acrylamide).

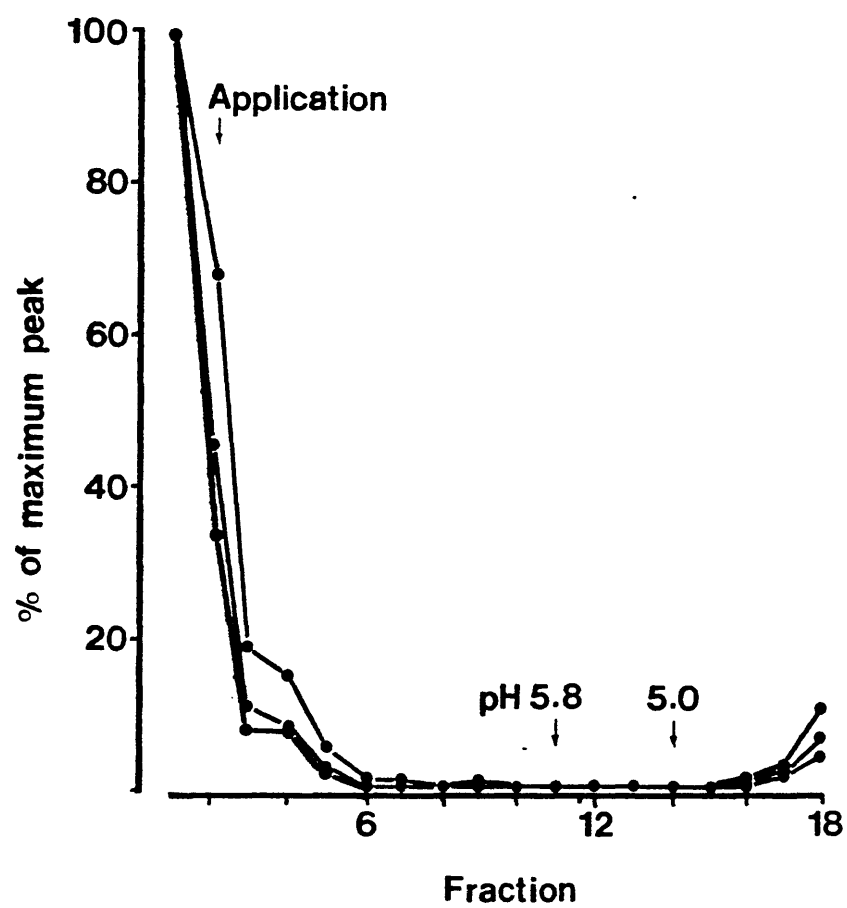


Figure 58 : Migration of free  $^3\text{H}$ -R5020 during IEF.

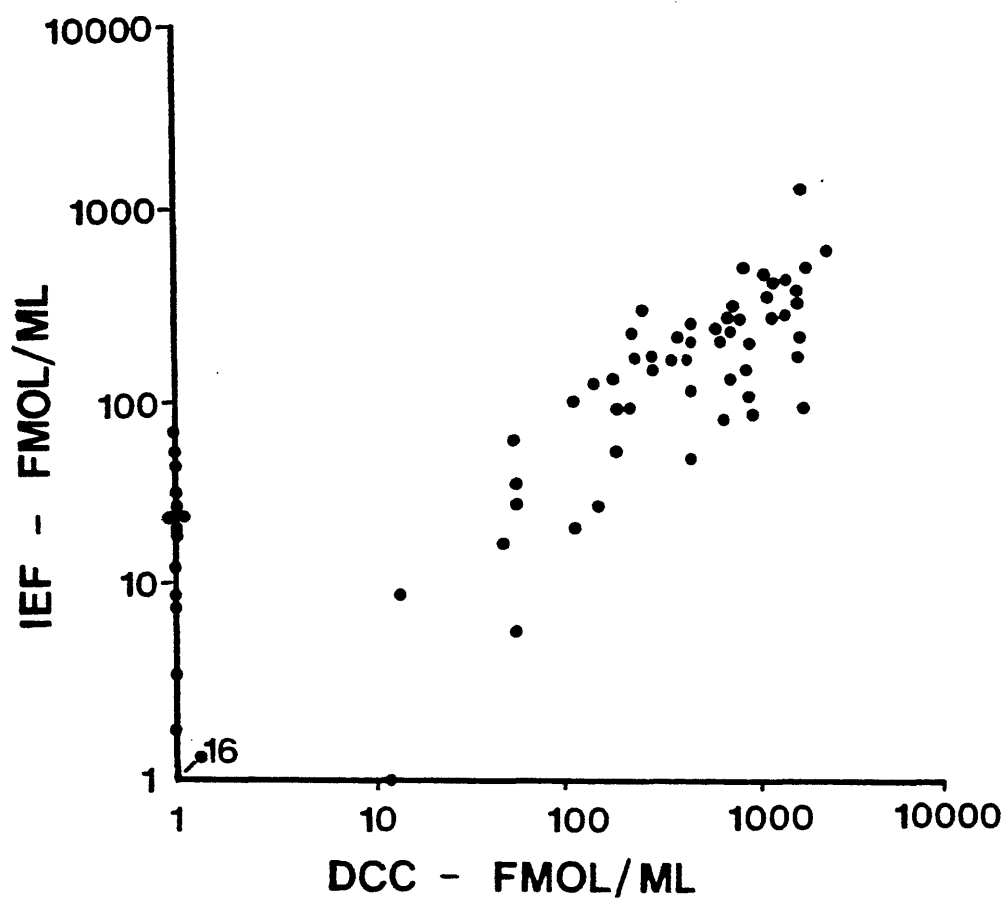


Figure 59: Comparison of results of assay of progesterone receptors by IEF and DCC methods.

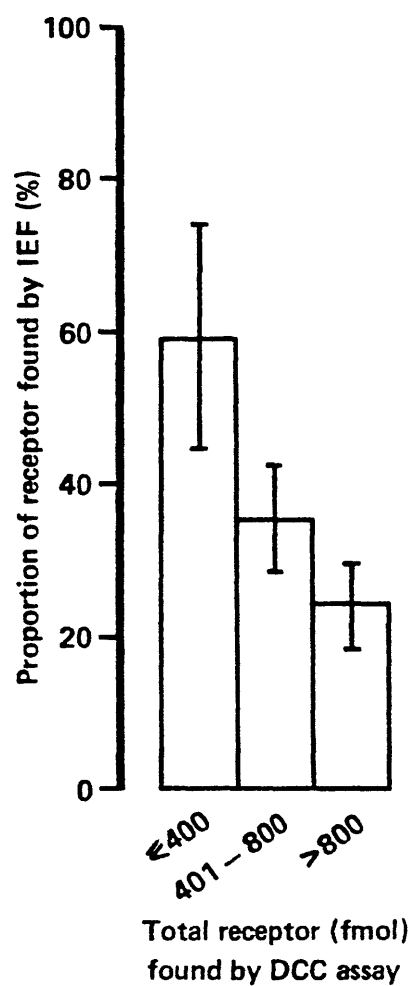


Figure 60: Total concentration of receptor & the proportion measured by IEF (Total receptor in fmol/ml) ( Mean  $\pm$  2 X standard error)

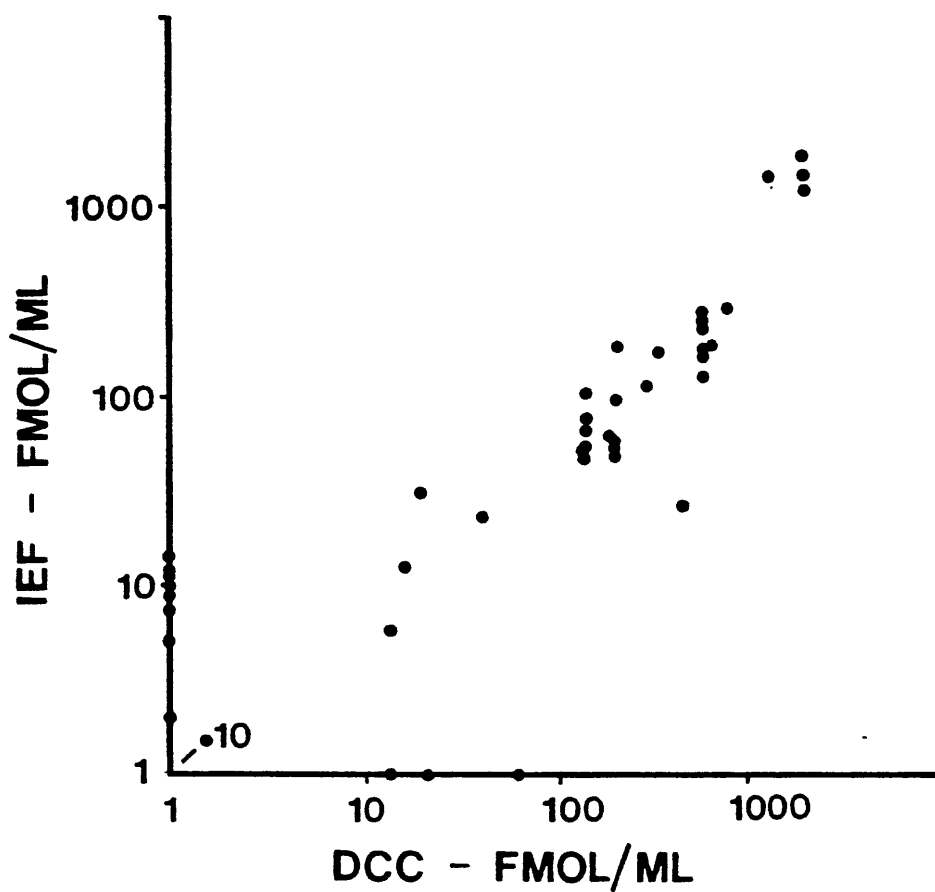


Figure 61: Comparison of results of assay of oestrogen receptors by IEF and DCC methods.

## INDUCTION OF PROGESTERONE RECEPTOR

### 'Changes' in progesterone receptor

Twenty seven patients were progesterone receptor positive before tamoxifen was given. 17 (63%) of those in whom progesterone receptor was found initially had higher values of progesterone receptor in the second biopsy and in 10 patients concentrations of receptor were reduced. In 4 patients progesterone receptor was found only in the later biopsy, and in 10 patients PR was not found in either sample (Table XIII).

Increased concentrations of progesterone receptor occurred in 59% of oestrogen receptor positive patients compared with 30% of oestrogen receptor negative patients.

### Changes in progesterone receptor & response

16 (76%) of 21 patients in whom progesterone receptor had risen, or appeared de novo, responded favourably to therapy. The prevalence of response when concentrations of progesterone receptor were lower with tamoxifen was significantly less than that of patients in whom receptor content rose ( $p = 0.018$ ), and was similar to that of progesterone receptor negative patients (Table XIV). 16 (84%) of 19 patients, in whom there was an increase of at least 1.4-fold over initial concentrations of receptor, responded, compared with 4 (19%) of the remaining 21 patients (Figure 62:  $p = 0.0001$ ).



TABLE XIII: Combined oestrogen (RE) and progesterone (RP) receptor status & change in progesterone receptor (No. of patients).

|              | RP reduced<br>or absent | RP increased |
|--------------|-------------------------|--------------|
| RE+ RP+      | 8                       | 14           |
| RE+ RP-      | 3                       | 2            |
| RE- RP+      | 2                       | 2            |
| RE- RP-<br>* | 7                       | 2            |
| RE? RP+      | 0                       | 1            |

\*Assay of RE not done

TABLE XIV: Change in progesterone receptor & response  
(No. of patients

|                | RP- | RP+        |              |
|----------------|-----|------------|--------------|
|                |     | RP reduced | RP increased |
| Regression     | 0   | 2          | 7            |
| Stable disease | 2   | 0          | 9            |
| Progression    | 8   | 7          | 5            |

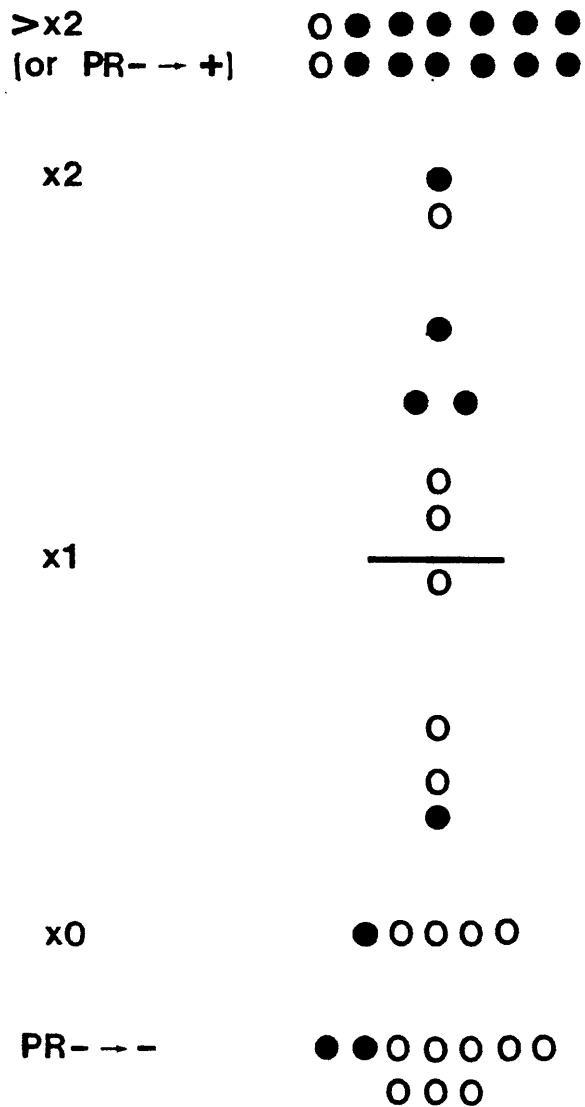


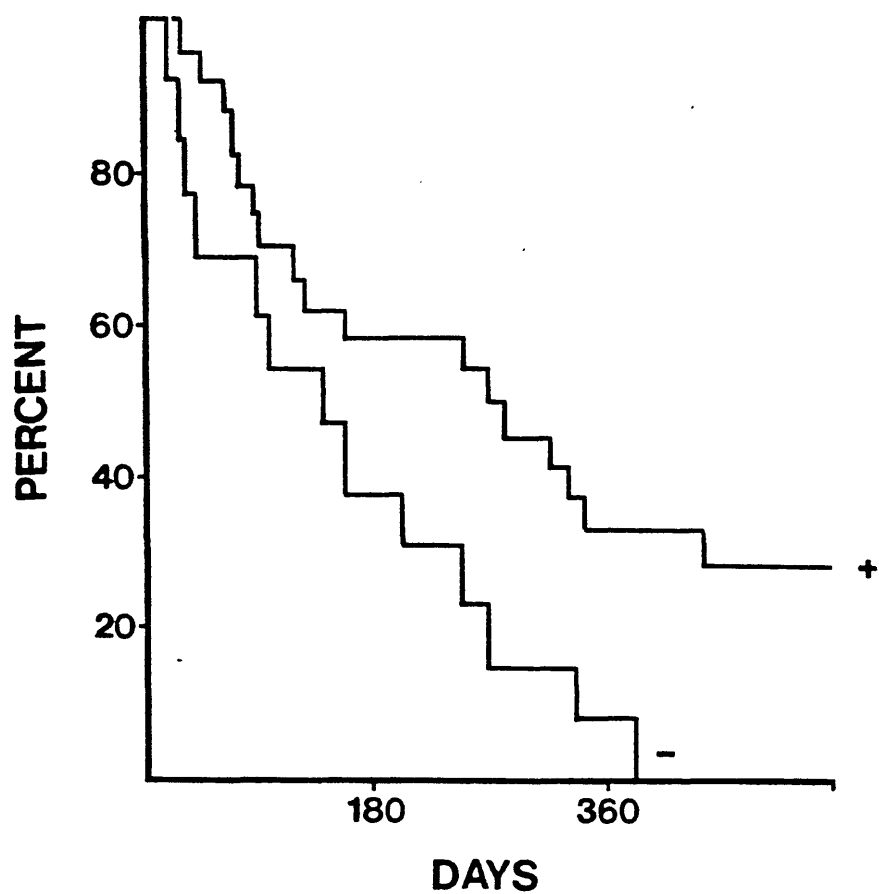
Figure 62: Extent of change in concentration of progesterone receptors with tamoxifen, and response (●= Response: 0= No Response).

### Receptor status and the probability of relapse

The presence or absence of oestrogen receptor indicated groups with different probabilities of relapse during treatment (Figure 63, Chi-squared = 5.88,  $p = 0.015$ ). Assay of progesterone receptor on samples of tumour taken before treatment failed to indicate groups of patients in which the proportion of patients on treatment who remained free from further progression of disease differed significantly (Figure 64. Chi-squared = 0.7,  $p = 0.4$ ). When the presence of progesterone receptor in either of the biopsies was considered progesterone receptor status indicated groups with significantly different probabilities of relapse (Figure 65, Chi-squared = 12.7,  $p = 0.0004$ ).

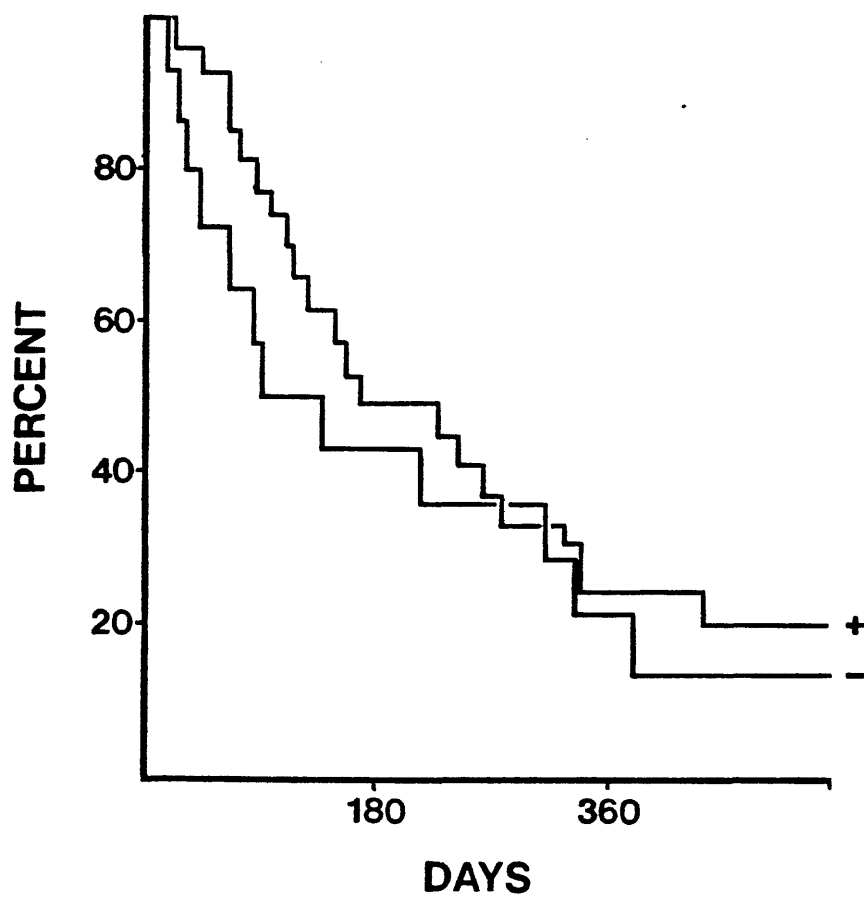
### Induction of receptor & the probability of relapse

When the time from the start of treatment until further progression of disease was analysed there was a highly significant difference in the probability of relapse between patients in whom concentrations of progesterone receptor had risen and the remainder (Figure 66. Chi-squared = 18.5,  $p < 0.0001$ ). This was true when only patients in whom progesterone receptor was present before treatment were considered (Figure 67. Chi-squared = 10,  $p = 0.0015$ ), and when analysis was confined to those who could be classified as progesterone receptor positive on the result of either of their biopsies (Figure 68: Chi-squared = 10.6,  $p = 0.001$ ).



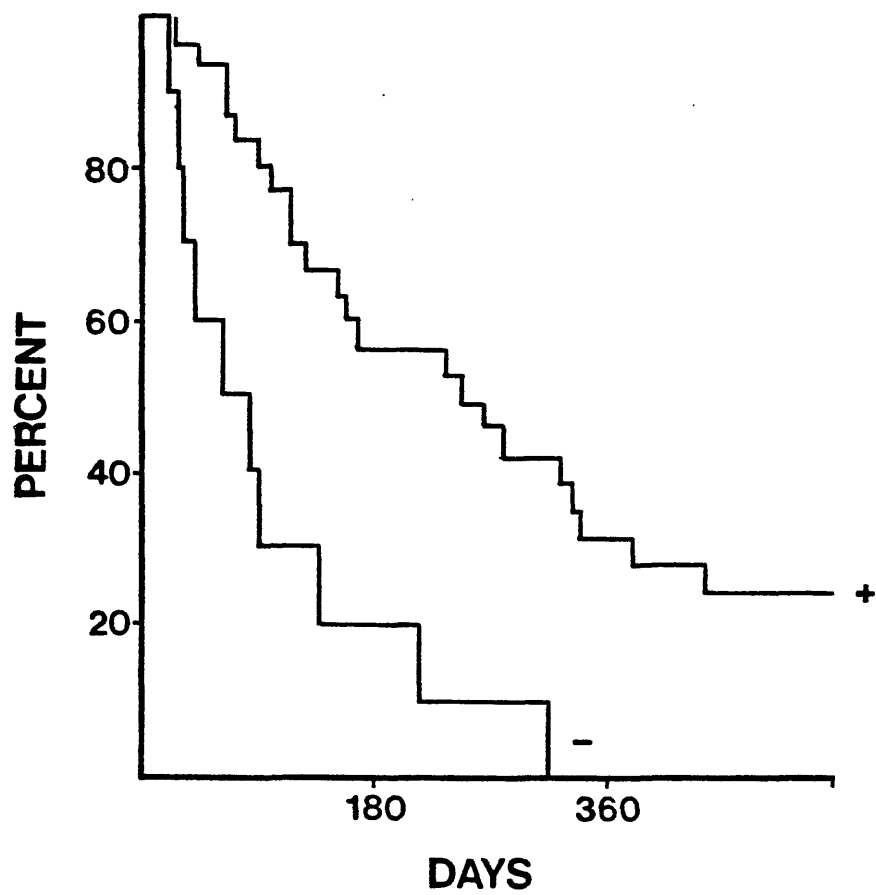
|          |    |    |   |   |   |
|----------|----|----|---|---|---|
|          | 27 | 17 | 8 | 5 | + |
| At risk: | 13 | 7  | 1 |   | - |

Figure 63: Initial oestrogen receptor status & the probability of relapse (Induction study patients)



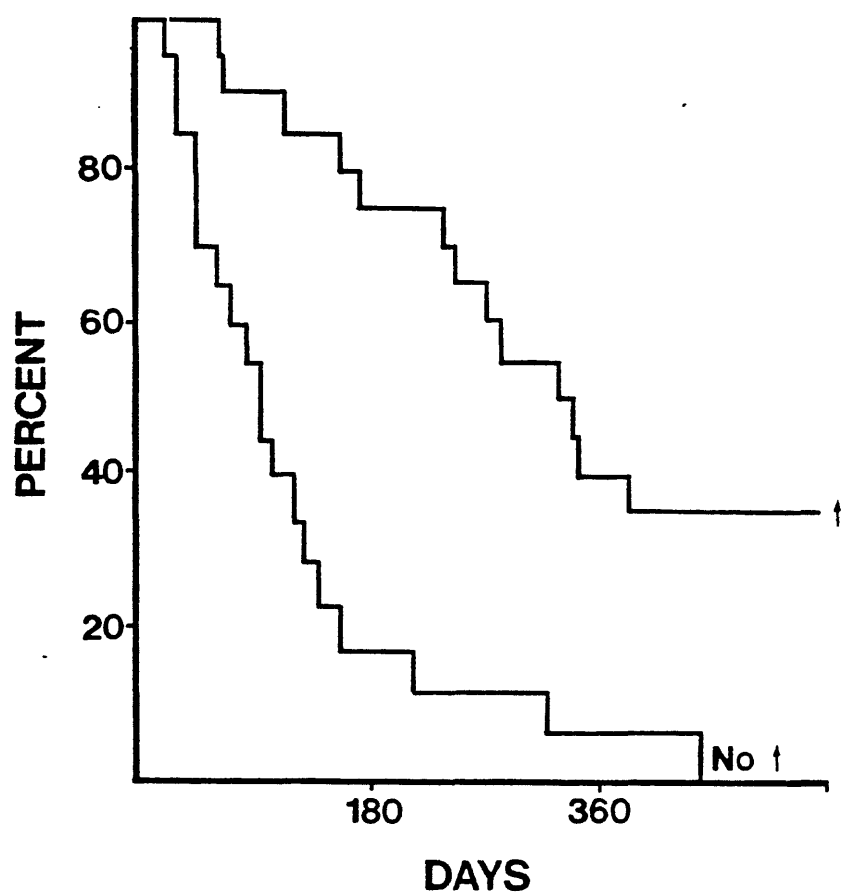
|          |    |    |   |   |   |
|----------|----|----|---|---|---|
|          | 27 | 13 | 7 | 3 | + |
| At risk: | 14 | 6  | 3 | 2 | - |

Figure 64: Initial progesterone receptor status & the probability of relapse (Induction study patients)



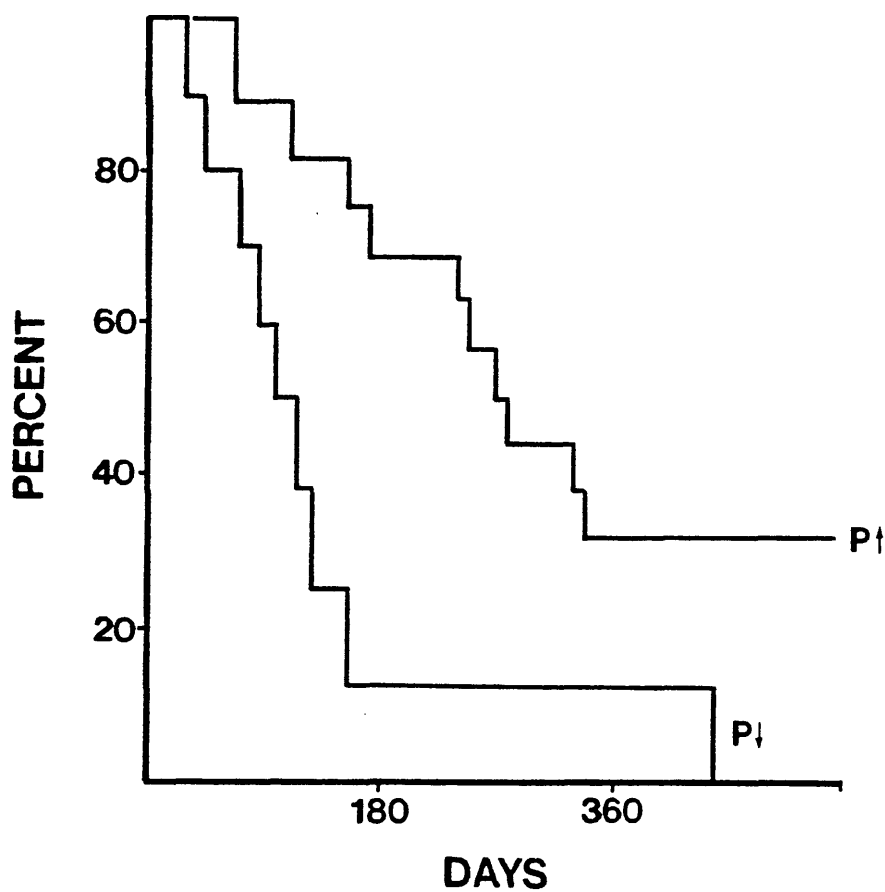
At risk: 31 16 9 5 +  
10 2 -

Figure 65: Overall progesterone receptor status & the probability of relapse (+ = RP positive if either 1st or 2nd biopsy contained progesterone receptor, - = neither biopsy contained progesterone receptor).



|          |    |    |   |   |
|----------|----|----|---|---|
|          | 21 | 15 | 8 | 5 |
| At risk: | 20 | 3  | 1 |   |

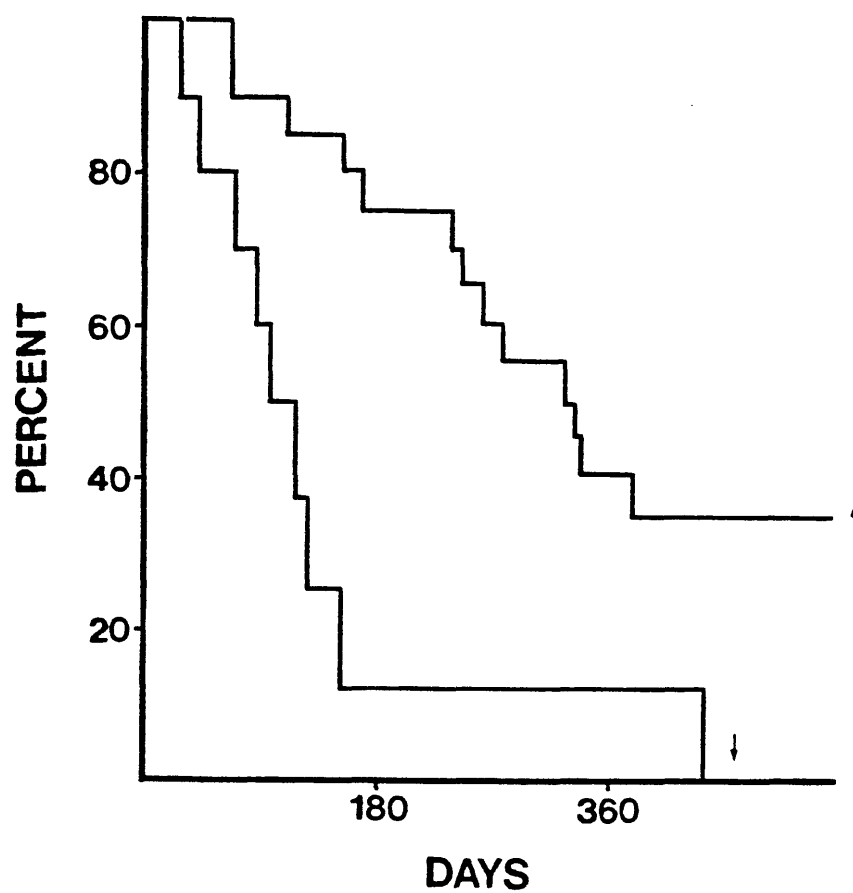
Figure 66: Change in concentration of progesterone receptors & the probability of relapse. (↑ = [RP] increased in second biopsy, No ↑ = [RP] reduced or persistently negative).



|          |    |    |   |   |   |
|----------|----|----|---|---|---|
|          | 17 | 11 | 5 | 3 | P |
| At risk: | 10 | 1  | 1 |   | P |

Figure 67: Change in concentration of progesterone receptors & the probability of relapse in RP positive patients (on initial assay). (P↑ = concentration rose between biopsies, P↓ = concentration of RP fell).





|          |    |    |   |   |
|----------|----|----|---|---|
|          | 21 | 15 | 8 | 5 |
| At risk: | 10 | 1  | 1 |   |

Figure 68: Change in concentration of progesterone receptors & the probability of relapse in RP positive patients (on either assay). ( ↑ = concentration rose, ↓ = concentration fell).

### Intra-tumour variation of progesterone receptor

In 1 patient the tumour was uniformly progesterone receptor negative on DCC assay, 4 patients had uniformly positive tumours and in 2 patients both receptor positive and negative areas were present (Table XIV.). The Coefficients of variation ranged from 0.45 to 2.42 (DCC assay, mean = 0.93). In the uniformly positive tumours the coefficients of variation ranged from 0.45 to 0.73 (DCC assay, mean = 0.6, s.d. = 0.14).

7 patients had tumours which showed both positive and negative areas when assayed by IEF. One patient had a uniformly negative and one patient had a uniformly positive tumour. The mean coefficient of variation (excluding the uniformly negative tumour) was 1.48 (s.e. mean = 0.33).

Permutation of results from tumours showing positive areas confirmed that about 40% of pairs of samples would have shown 'increases' if sampled sequentially whether IEF or DCC assay was used (Table XV (a,b)).

Table XV . Intratumour variation of progesterone receptor

a) DCC assay

| Sample> | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12 |
|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|----|
| DC      | 61  | 27  | 33  | 142 | 130 | 42  | 85  | 118 | 63  | 94  | 101 | 84 |
| NA      | 267 | 135 | 67  | 323 | 163 | 127 | 162 | 153 | 137 | 173 | 413 | 25 |
| JM      | 60  | 115 | 73  | 39  | 83  | 94  | 114 | 9   | 11  | 23  | 30  | 9  |
| HB      | 146 | 34  | -   | -   | -   | -   | -   | -   | -   | 46  |     |    |
| MG      | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -  |
| LLo     | 292 | 266 | 297 | 142 | 160 | 221 | 109 | 221 | 53  | 369 | 391 | 11 |
| LLa     | 8   | 31  | 16  | 11  | -   | 23  | -   | 24  | -   | -   | -   |    |

b) IEF assay

|     |    |     |     |     |    |    |    |    |    |    |     |    |
|-----|----|-----|-----|-----|----|----|----|----|----|----|-----|----|
| DC  | 9  | 15  | 5   | 76  | 65 | 9  | 9  | 65 | 25 | 53 | 76  | 44 |
| NA  | 72 | 36  | 16  | 133 | 33 | 27 | 46 | 70 | 60 | 65 | 200 | 16 |
| JM  | 35 | 51  | 33  | 7   | 35 | 8  | 43 | -  | 2  | 4  | 13  | -  |
| HB  | 23 | -   | -   | -   | -  | 6  | -  | -  | -  | 12 |     |    |
| MG  | 12 | -   | -   | -   | -  | -  | -  | -  | -  | -  | -   | -  |
| LLo | 35 | 179 | 215 | 108 | 17 | 94 | 65 | 34 | 18 | 75 | 148 | 3  |
| LLa | 35 | 23  | 53  | 11  | -  | 19 | 30 | 38 | -  | -  | 21  |    |
| EC  | -  | -   | -   | 32  |    |    |    |    |    |    |     |    |
| VC  | -  | -   | -   | -   |    |    |    |    |    |    |     |    |

\* \* \* \* \*

## DISCUSSION

\* \* \* \* \*

### Variation of receptor

The proportion of patients in whom assays on two or more lesions agreed on the presence of oestrogen receptor was similar to that quoted in other reports which give a range of 66 to 85% agreement between biopsies (Brennan et al., 1979. Webster et al., 1978. Rosen et al., 1977. Allegra et al., 1980. Kiang & Kennedy, 1977. Koenders, 1980. Paridaens et al., 1980). Less data are available on the variation of progesterone receptor between sites and with time, but other workers have noted a similar inconsistency (Koenders et al, 1980. Matsumoto et al, 1978. Fernandez et al., 1982. Mobbs, 1982). In this study loss of progesterone receptor was particularly common. In only one patient could this have been due to the menopause occurring in the interval between the biopsies.

Previous reports have not considered the changes which may take place in the combined receptor status. The finding of a change in 43% of asynchronous biopsies casts serious doubt on the value of classification according to the presence of both oestrogen and progesterone receptor.

Although the overall changes in receptor were not related to the interval between biopsies, when oestrogen receptor alone was considered there was a higher probability of change when the interval was short. This is not consistent with the findings of Webster et al (1978) who found that variation in receptor status occurred in patients with longer intervals between

biopsies. In this study the difference was not highly significant, and may well have been fortuitous.

Variation was neither confined to the patients who were initially oestrogen receptor positive as has been suggested (Leake et al, 1981), nor to those with only low concentrations of receptor (Campbell et al., 1981).

Some authors have suggested that a response is more likely with high concentrations of oestrogen receptor than with low ones (Lippman & Allegra, 1980. Osborne et al., 1980. Campbell et al., 1981. Paridaens et al., 1980). Concentrations of oestrogen receptor showed a similar inconsistency and it cannot be assumed that initial high concentrations will be maintained later in the course of the disease.

The concentrations of receptor that occurred when receptor was found only in a later biopsy were usually low. The changes from oestrogen receptor negative to oestrogen receptor positive that occurred may not indicate a substantially higher probability of response.

There are several possible reasons for this variation. Other workers have found that the variation was greatest in patients who had received endocrine treatment between biopsies (Allegra et al., 1980). However in their patients only 10 -14 days had elapsed between stopping treatment with oestrogens or anti-oestrogens and their second biopsy. Oestrogen receptor may remain undetectable for several weeks after stopping tamoxifen (Koenders et al., 1980), and their data may be explained by this. There were only 20 patients who had

had endocrine treatment between biopsies in this study. Clearly no extensive differences existed between treated patients and the remainder, although the numbers were too small to show any subtle effect of treatment upon the concentration of receptors. These findings support those of others (Taylor et al., 1982) that endocrine treatment does not lead to the selective growth of receptor negative cells. The generally high incidence of endocrine response after previously successful endocrine treatment also suggests that this is unlikely.

In some cases anoxia of the tumour during mastectomy may have caused low concentrations of receptor to denature and led to false negative status for some primary tumours. Again this has been cited as a cause of variation in receptors (Hasson et al., 1981).

Both oestrogen and progesterone receptor are easily destroyed by proteolytic enzymes. Autolysis may lead to loss of receptor activity rapidly after the sample has been excised. In this unit a flask of liquid nitrogen was routinely available at the times when biopsies were carried out.

Several precautions were taken within the assay to ensure that the protein was not denatured. Tissues were stored in liquid nitrogen. All manoeuvres were carried out at 4 °C. Dithiothreitol which protects the disulphide bridges against disruption, was included at the time of homogenisation. All buffers contained EDTA, which chelates the calcium ions needed as cofactors by many enzymes. Systematic errors seem equally probable in

synchronous and asynchronous biopsies.

Considered alone, the difference in variation between synchronous and asynchronous biopsies suggests a true discrepancy of receptor status with time, due either to a widespread change in hormone dependence, or increased heterogeneity of the tumour. The former possibility is plausible when loss of receptor is considered, but seems very unlikely when receptor is apparently gained. If true, then the value of assays would be less when carried out at an early stage of the disease. Subsequent results of this study indicate that this is not so. If heterogeneity of tumours increased with time, which also seems plausible, the effects on the value of receptors would be unpredictable.

A substantial number of 'second biopsies' in the asynchronous group were superficial recurrences, which may have contained fewer tumour cells than the synchronous 'controls', which were mainly primary tumours and axillary nodes sampled at the time of mastectomy. In some cases, despite evidence of malignancy and satisfactory cytosol protein concentrations much of the material analysed may not have been tumour. Differences in cellularity have been cited as an explanation for variation of receptor between samples (Hawkins et al, 1982. Singhakowinta et al., 1976. Hoehn et al., 1979). Unfortunately no information on the cellularity of these samples was available.

The chest wall or the regional nodes have not been



shown to be preferential sites of recurrence for either oestrogen receptor positive or oestrogen receptor negative primary tumours. Bone and viscera may be preferred sites of metastasis for oestrogen receptor positive and oestrogen receptor negative primary tumours respectively (Campbell et al., 1981. Stewart et al., 1982. Singhakowinta et al., 1976). The receptor status of secondary deposits at these sites may be easier to predict and information on receptor status at these sites in relation to previous biopsies would be of interest.

## Receptors and response

### General results

The results in general are similar to those of others. The overall prevalence of regression was as expected. The prevalence of regression among the receptor positive patients was similar to that reported by others in the U.K. (Hawkins et al., 1979. Rubens Hayward, 1980. Campbell et al., 1981. Leake et al., 1981), but less than that in reports from elsewhere (Table I). The response of receptor negative patients was slightly higher than usual, at 12%.

The conclusions which have been reached were not based on the conventional assessment of response. The two methods seem to give a different insight into the data and it is difficult to translate the results, as expressed in this study, into a form which allows easy comparison with other series. The proportion of patients who had not relapsed at 6-7 months after the start of treatment was equivalent to the proportion of patients who would have been assessed as having regressed or remained stable.

### Cytosol oestrogen and progesterone receptor

Groups with grossly different prognoses were defined by assay of either cytosol oestrogen or progesterone receptors. The differences in the probability of relapse with time, overall survival and survival from the first relapse between receptor positive and negative groups were consistently greater with progesterone receptor than with oestrogen

receptor.

These findings are compatible with the reported experience of receptor analyses. A few centres have reported that the assay of progesterone receptors offered no benefit over the assay of oestrogen receptor alone (Holdaway et al., 1980. Manni et al., 1980) but a majority of reports indicate that response was most prevalent when progesterone receptors were present. In this study 70% of patients in whom progesterone receptors were present were free from further progression of the disease six months after the start of treatment.

When progesterone receptor was not present oestrogen receptor status did not define groups with prognoses that differed significantly. Other workers have reported a 'response rate' of 25-30% when only oestrogen receptor was present (Stewart et al., 1982. Osborne et al., 1980) and this group is regarded usually as one with an intermediate prognosis. Few authors have suggested that such patients should be excluded from treatment on the basis of receptor status alone. Although the proportion of patients that responded in this study (27.5%) was similar to that in other reports the analysis has shown that there was a difference in the probability of relapse only between the 6th and 12th month of follow-up. The probability of remaining free from further progression thereafter was identical for both groups, and the value of oestrogen receptor status as an indication of response when

progesterone receptors are not present can only be regarded as trivial.

Patients with both oestrogen and progesterone receptors had a significantly greater probability of remaining free from relapse long after the start of treatment than patients in whom progesterone receptor was not found whether oestrogen receptor was present or not. There were very few patients in whom progesterone receptor only was present, and while they appeared to form an unresponsive group there were too few to make any accurate observations. Their effect on the analysis was so small it can be safely ignored.

#### Nuclear oestrogen receptors

In this study assays of nuclear oestrogen receptor by a simplified technique failed to predict response adequately. The technique differed slightly from that used by other workers who have reported that the measurement of nuclear oestrogen receptors indicated response more accurately than measurement of cytosol receptor alone (Leake et al., 1981. MacFarlane et al., 1980). In the assay used for this study only four concentrations of [<sup>3</sup>H]-oestradiol were used, compared with six points in other series. False classifications due to random errors are more likely when fewer points are used, and this may explain the poor prediction by nuclear receptors in this study.

Other minor differences between the assays, for example in the homogenisation of the tissue, were

unlikely to have contributed to the negative results.

At 4 °C little exchange occurs over the course of the assay between free oestrogen and that already bound by receptor (Leake et al., 1979). Assay in this way measures only unoccupied receptor sites. As a large proportion of receptor in the nucleus is occupied by oestrogen (Edwards et al., 1979), assays using exchange or extraction procedures may give more accurate prognostic information.

#### Primary tumour receptors & the timing of assays

The results of assay of receptors on samples of primary tumours gave excellent discrimination whether this was carried out at or before the time of treatment. Previous reports on the value of receptors assessed in primary tumours have been contradictory. Most data are available for oestrogen receptor, and these reports found no difference in the prevalence of response whether oestrogen receptor assay was carried out at an early or late stage in the course of the disease (McGuire, 1980. Hawkins et al., 1980. Desombre & Jensen, 1980).

One report has concluded that assays done at an early stage of the disease were less reliable than assays carried out at the time of treatment (Stewart et al., 1982). Variation of receptor status with time was cited as a possible cause of the discrepancy. In this study, on the rare occasions when the results of assays at the time of endocrine treatment disagreed with those done on primary tumours there was a strong trend in favour of the results of

primary tumour assay, whether this was positive or negative. In only one case was the assay of oestrogen receptor negative on the primary and positive at the time of treatment, and that patient continued without relapse 10 months after the start of treatment. In this respect the numbers of patients were too small for anything but a guarded opinion, that secondary assays are more likely to give unreliable indications of response than primary ones.

The failure of assays carried out at the time of endocrine treatment to discriminate adequately between patients with good or bad prognoses apparently contradicts the experience of all other reports in which it was clear that assays carried out at the time of treatment gave excellent discrimination. Close examination shows that the lack of prediction was confined to some receptor-negative patients who had a better long-term prognosis than the assays indicated. At six months from the start of treatment there was a greater difference in the proportions who had remained free from progression between receptor positive and negative groups when secondary assays were considered than with receptors assessed in operable primary tumours. This is a similar finding to those of other workers (Stewart et al., 1982), and again shows the value of considering the relative risks for all groups throughout the period of risk rather than limiting the assessment to the time when the maximum difference is likely to occur, and the most optimistic result obtained.

### Extent of disease

Patients with limited disease had a better prognosis for both relapse and survival than patients with widespread disease. This divided prognosis applied only to receptor positive patients. This finding is compatible with that of others who found that the prevalence of response was lower when more sites were involved (Lippman & Allegra, 1981). In this study the extent of disease did not affect the prognosis when patients were grouped according to the results of assays performed at the time of treatment. Such a finding is hard to explain.

### Sites of metastasis

The prognosis was not affected by the distribution of metastases. About half of the patients had no distant spread when the first endocrine treatment was given. Whatever sites oestrogen receptor positive or negative tumours metastasise to most frequently such metastases often become apparent after the first endocrine manipulation. The poor indication of response given by the site of disease may hide some effect in a small group of, for example, patients with liver metastases. The incidence of liver secondaries was lower in the patients who received endocrine treatment than in the others, and some patients may have been excluded from endocrine treatment because of serious visceral disease. As the numbers in this category are small it is unlikely that a different conclusion would

have been reached if they had received treatment.

### Conclusion

These data indicate that,

1. Classification by the presence or absence of both oestrogen and progesterone receptors is subject to great variation between sites and with time.

2. Measurement of receptors is an effective means of assessment of the prognosis of patients treated by endocrine methods.

3. Assays carried out on metastases are less reliable in general than those done on primary tumours.

4. Assay of both oestrogen and progesterone receptors offers no significant advantage over the measurement of progesterone receptor alone, or of oestrogen receptor when concentrations of receptor are considered. Measurement of progesterone receptor is preferable.



### Isoelectric focusing assay

IEF has been found by others to be a sensitive method of measuring oestrogen receptors (Lloyd et al., 1982, Wrangé et al., 1976. Coffey & King, 1976). The modified technique which has been described has also proved suitable. Although there was close correlation with the DCC assay there was a small incidence of false negative results at the limit of sensitivity chosen. IEF was proposed as a single point assay and the effect of duplicate assays on the reliability of the result was not tested. In general, IEF should not be used when there is sufficient tissue available for DCC assay but it is of considerable value when only small amounts of tissue are available. Tru-cut needle biopsies are a suitable source of material for assay of oestrogen receptors by IEF (Lloyd et al., 1982). IEF therefore extends the range of circumstances in which receptor data may be obtained.

The limit of sensitivity of IEF was expressed in an unorthodox way. Usually such limits are referred to the protein or DNA content of the cytosol. The measurement of DNA in very small specimens proved impossible, and so the comparisons between IEF and the DCC method in larger samples were not related to DNA content. The limit of sensitivity of IEF depends on chance variation between total and non-specific traces which occur in the absence of receptor, even in the absence of protein. It would need a considerable effort to clarify the relationship between the scatter of such variations and the protein content. The approach which was used was to define a

limit which gave the best discrimination between positive and negative cytosols over a range of concentrations of protein that was comparable to the concentrations of cytosol that had occurred in the previous experience of the laboratory. In fact the protein concentration of cytosol made from needle biopsies was generally higher than that found in the routine DCC assay, and it was assumed that the limit of sensitivity was valid at higher concentrations.

The peak of progestin binding activity which has been described has the specificity of progesterone receptor (Raynaud., 1977. Duffy, 1979), and does not correspond to any progestin-binding protein of serum which may have contaminated the cytosol preparation. Correlation between the results of IEF and those of the DCC assay was reasonable, and the two methods agreed closely on the presence or absence of receptor.

The use of IEF for the assay of progesterone receptors has been described previously (Wrange et al., 1981) but extensive comparisons were not made with established methods. The initial effort to reproduce the method in this study was not particularly successful. It was clear that 'focusing' (the phenomenon of a distinct peak of radioactivity clearly separate from the application point) occurred rarely. Usually there was either a small peak or a 'shoulder' at pH 6.2-6.4, as has been illustrated. This was in continuity with a large application peak, and was hard to identify as a separate entity. With agarose gel more distinct peaks were seen

allowing greater confidence in the identification of the receptor, although the absolute activity found over the same fraction range was the same with either medium.

The estimate of the pI of progesterone receptor given in this study differs from that of the other report of flat-bed IEF in which the pI of mammary carcinoma progesterone receptor was given as 6.2 (Wrange et al., 1981). The same group found a pI for endometrial progesterone receptor of 5.0 when focusing was carried out in sucrose gradients (Gustafsson et al., 1977). In this study when polyacrylamide was used, the pI was ~6.4, and occasionally small peaks were seen in agarose at this pI. It is not clear whether the difference between media was due to chemical interaction of the gel with the protein or ampholyte buffers, or whether there was another difference. Mobility of molecules is limited in polyacrylamide gels by the 'pore-size' which results from the cross-linkage which occurs with polymerisation, which in turn depends on the total concentration of acrylamide and the proportion of bis-acrylamide which is used. The gel used for this and other studies is regarded as a large-pore gel, suitable for molecules of up to MW 400,000. However, the presence of a large peak of specific activity which persisted at the application point suggested that the receptor was present in different forms, perhaps as aggregates, and that only one of these focused in polyacrylamide gel.

In another report the pI of endometrial receptor (in glycerol) was given as 4.8 (Pollow et al., 1977). In this

report no competitor was included, and this binding would be hard to distinguish from that of albumin (pI 4.8) (Documenta Geigy). Although the pI of progestin-binding proteins of serum was different from that of receptor some overlap could occur, and a parallel incubation for non-specific binding was always included, so that binding due to contamination by serum did not enter the calculation. Free [<sup>3</sup>H]-R5020 did not migrate into the area of the gel in which receptor focused, and treatment of the cytosol with DCC may have been unnecessary, although it proved useful when plotting the results to avoid dominant application peaks.

In the comparison of DCC and IEF assays the absolute activity in the fractions selected was used. It was not essential for radioactivity to be 'focused' at the correct pI before accepting a cytosol as receptor positive, although the test seemed more reliable when some focusing had occurred in the right pH range. Again, formal assessment of this would require considerable, carefully-controlled experience of assays carried out when only low concentrations of receptor were present.

The inclusion of molybdate at the time of disintegration of the tissue is an important feature of this technique. Molybdate is said to prevent degradation of the receptor (Anderson et al., 1980. Lukola & Punnonen, 1983. Tilzer et al., 1981). When IEF was used molybdate acted to increase the proportion of receptor which focused. The mechanism of its action was not investigated in this study.

IEF usually underestimated the concentration of receptor. The estimates were lower for progesterone receptor than oestrogen receptor. It is not clear how such an underestimate could have arisen, although it may be explained to some extent by incomplete saturation of the receptor. This is not likely to explain the major part of the discrepancy. Electroendoosmosis, the movement of water towards the cathode, may have been responsible, but the mechanism is not clear.

### Induction of progesterone receptor

None of the data presented in this study has confirmed that tamoxifen increases progesterone receptors in patients with cancer of the breast. Mean concentrations of receptor before and after the start of treatment did not differ significantly even among the patients who responded, and the prevalence of 'induction' was not substantially higher than could have occurred by chance. Much larger numbers of patients would be needed to establish this point. Other studies of induction of progesterone receptor in human mammary cancer were also not conclusive (Namer et al., 1980. Waseda et al., 1981). Induction of receptor by tamoxifen has been demonstrated experimentally in animals and in cell culture work (Davies et al., 1979. Holinka et al., 1980. Isomaa et al., 1979. Horwitz & McGuire, 1980. Pasqualini et al., 1980. Rochefort et al., 1981). It has also been found in patients with endometrial cancer (Mortel et al., 1981).

Increases of progesterone receptor by tamoxifen are said to be associated strongly with oestrogen receptor positivity (Waseda et al., 1981. Baulieu et al., 1980). In this study rises in progesterone receptor were more common among receptor positive patients. Oestrogen receptor had been found in other biopsies in 2 of the 3 oestrogen receptor negative patients who showed increases in progesterone receptor, and in the third there was some evidence of the presence of oestrogen receptor at concentrations below the cut-off value for the assay. This suggests that the induction of progesterone receptor

may be more closely associated with the presence of oestrogen receptor than these results indicate.

The dissociation of response from increases of receptor within the progesterone receptor positive group may be, in part, an effect of the inherent variation between samples. When progesterone receptors are not affected by tamoxifen a proportion of patients would have higher values in a second biopsy by chance. In our patients 5 of the 12 progesterone receptor positive patients who failed to respond had had increases between biopsies, but the rise was more than 1.4-fold in only 3.

The improved discrimination which occurred when only those patients who had had substantial increases were considered indicated that the size of any differences which are observed is an important factor. In this group a 1.4-fold limit gave excellent discrimination between patients with endocrine-responsive and unresponsive tumours. Although discrimination was not absolute, these findings show that 80% of patients whose progesterone receptor was 'induced' by tamoxifen remained free from further progression of their disease for at least 6 months after starting treatment, when only 17% of those with lesser changes were free from progression in that time.

In some experimental situations tamoxifen may affect the synthesis of progesterone receptors but not cell growth (Ip et al., 1981. Lee et al., 1981), or vice versa (Allegra et al., 1981). It has been suggested that the oestrogen-receptor complex may act at several sites on

the chromatin, and that the tamoxifen-receptor complex may act selectively on occasions. These results do not exclude this effect, but suggest that it does not explain entirely the dissociation between receptor status and response.

These results, however, are influenced greatly by the behaviour of 4 patients in whom progesterone receptor was found only in the second biopsy. In 2 neither oestrogen nor progesterone receptor was found initially; in one the lack of receptors agreed in simultaneous biopsies from soft tissue and bone, assayed by both methods; in the other the IEF trace suggested the presence of oestrogen receptor at concentrations below the limit of sensitivity. In the other patients oestrogen, but not progesterone receptor had been found. There was a gross difference in the probability of relapse between patients in whom progesterone receptor was never found and those whose status changed from negative to positive, and this contributed substantially to the significance of induction when allowance was made for the initial progesterone receptor status. Larger numbers would clarify this point.

Earlier results of this study have shown that sampling at the time of endocrine treatment is unreliable. It could be argued that improved discrimination when 'induction' occurred stemmed from improved detection of receptor. Certainly the presence of progesterone receptor in either of the biopsies indicated prognosis to a similar extent as 'induction' of receptor.



However 'induction' continued to distinguish groups of different prognosis in patients in whom progesterone receptor had been found at any time. This suggests that the effects were not due solely to improved sampling.

Induction indicated prognosis to a similar extent whatever the oestrogen receptor status of the patient (42% of the expected relapses occurred).

Heterogeneity of the tumour with regard to the concentrations of progesterone receptor, may have contributed to the imperfection of discrimination according to changes in receptor. Gross heterogeneity was shown, and would explain some of the failures of the test, even at a 1.4 fold confidence limit for increases.

This approach requires repeated biopsies and special methods of assay. The information gained does not seem to offer substantial advantages over reliable measurement of progesterone receptor status, and in its present form, cannot be recommended as a routine test of endocrine sensitivity.

Oestradiol may have more immediate effects on the concentration of progesterone receptors than tamoxifen, and might improve the value of 'induction' as a sign of response. Oestrogens stimulate the synthesis of many cell proteins, and proteins which are more closely associated with cell growth, or which can be measured more reliably, would be better suited to the 'dynamic' assessment of endocrine sensitivity.

\* \* \* \* \*

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\* \* \* \* \*

## APPENDICES

\* \* \* \* \*

APPENDIX 1: Calculation of volumes of tritiated steroids used in the DCC assay

$$\begin{aligned}\text{Let Specific Activity of isotope} &= A \text{ Ci/mmol} \\ &= A \times 10^{-9} \text{ Ci/pmol}\end{aligned}$$

$$\text{Let desired concentration} = B \text{ pmol/ml}$$

$$\text{Given that stock solution contains } 50 \times 10^{-6} \text{ Ci/ml}$$

$$\text{Then, volume required} = \frac{(A \times B) \times 10^{-9}}{50 \times 10^{-6}}$$

$$= (A.B./50) \times 10^{-3} \text{ ml/ml final volume.}$$

e.g <sup>3</sup>[ H]-oestradiol (specific activity 104 ci/mmol) @ 3 pmol/ml

$$\begin{aligned}\text{volume required} &= (3 \times 104/50) \times 10^{-3} \text{ ml/ml} \\ &= 6.2 \times 10^{-3} \text{ ml/ml}\end{aligned}$$

## APPENDIX 2: Calculation of factors for DCC assay

Given  $2.2 \times 10^{12}$  dpm/Ci

Let specific activity of isotope = A Ci/mmol,  
 $= A \times 10^{-9}$  Ci/pmol

Then each pmol gives  $(2.2 \times 10^{12}) \times (A \times 10^{-9})$  dpm  
 $= (2.2 \times A \times 10^3)$

Given average efficiency of counting = 35%

Then dpm =  $\frac{\text{cpm} \times 100}{35}$

Then each pmol gives  $(2.2 \times A \times 10^3) \times 0.35$  cpm  
 $= (2.2 \times A \times 10 \times 35)$

But 0.35 ml is counted, out of 0.45 ml

Therefore pmol/sample = cpm/pmol  $\times 0.45/0.35$

Each sample contains 0.2 ml of incubate (label + cytosol)

Therefore pmol/incubate = pmol/sample  $\times 5$

$= (2.2 \times A \times 10 \times 35) \times 5 \times 0.45/0.35$

e.g  $^3$  [H]-estradiol (specific activity = 104 ci/mmol)

Factor =  $2.2 \times 104 \times 10 \times 35 \times 5 \times 0.45/0.35$   
 $= 0.00079$

APPENDIX 3: Patients excluded for unacceptable combinations of treatment

| Hospital reference | Treatment                                       |
|--------------------|-------------------------------------------------|
| 78/5457            | Oophorectomy + Adriamycin/cyclophosphamide      |
| 80/4829            | Tamoxifen + Adriamycin/cyclophosphamide         |
| A03591             | Tamoxifen + CMF                                 |
| C74587             | Tamoxifen + AC                                  |
| C97492             | Tamoxifen + cyclophosphamide/prednisolone       |
| D21401             | Tamoxifen followed by XRT (No progression)      |
| D28057             | Tamoxifen + cyclophosphamide/prednisolone       |
| D29793 *           | Tamoxifen + CMF                                 |
| D50138 *           | Tamoxifen + AC + prednisolone                   |
| D55033 *           | Tamoxifen followed by excision (No progression) |
| D66438 *           | Tamoxifen + AC                                  |
| D66463 *           | Tamoxifen + CMF                                 |
| D68029             | Tamoxifen followed by XRT (No progression)      |
| D71126 *           | Tamoxifen + AC                                  |
| D79893 *           | Tamoxifen + AC                                  |
| E11453 *           | Oophorectomy + CMF                              |
| E12811             | Tamoxifen + VAP/cyclophosphamide                |
| E15247 *           | Tamoxifen + CMF                                 |
| E19481 *           | Oophorectomy + VAP/cyclophosphamide             |

\*Receptor status known

APPENDIX 4: Patients excluded from analysis of  
probability of relapse because of no progression of  
disease before treatment

| Hospital<br>reference | Treatment                  | (Previous<br>treatment) |
|-----------------------|----------------------------|-------------------------|
| 80/1601 *             | Tamoxifen & norethisterone | (CT)                    |
| A10632 *              | Tamoxifen                  | (CT, XRT)               |
| A95264 *              | "                          | (XRT)                   |
| B62927 *              | "                          | (CT)                    |
| C25903 *              | "                          | (CT)                    |
| C48075                | "                          | (XRT)                   |
| C65751 *              | "                          | (CT)                    |
| D02874 *              | X-ray menopause            | (XRT)                   |
| D07211 *              | Tamoxifen                  | (CT)                    |
| D28324 *              | "                          | (XRT)                   |
| D51637 *              | "                          | (XRT)                   |
| D57806                | "                          | (XRT)                   |
| E00291 *              | "                          | (CT)                    |
| E00395 *              | "                          | (XRT)                   |
| E24508                | "                          | (CT)                    |

## Breast Cancer Follow-up

Name: \_\_\_\_\_

Hospital

1-Christie ☐  
2-Withington ☐Consultant's  
initials

Hospital Number (left justified)

Menopausal Status

1-Pre (LMP<6/12)  
2-Peri (LMP 6/12 - 3yrs) ☐  
3-Post (LMP >3yrs)

Date of Birth

Age at Presentation

## Primary Disease

Predominant Site

1 - UO  
2 - LO ☐  
3 - UI ☐  
4 - LI  
5 - Subareolar

Side

1 - Right  
2 - Left ☐

Maximum Clinical Diameter

1 - <2  
2 - 2-5cms ☐  
3 - 5cms +  
9 - NK

## Clinical Findings

## Attachment

Skin

1 - None or tethered  
2 - (Infiltration  
(Ulceration  
(Peau D'Orange  
(Satellite Nodule) ☐

Deep

1 - None  
2 - Pectoral ☐  
3 - Chest wall

## Nodes

Axilla

1 - None palpable  
2 - Palpable mobile ☐  
3 - Palpable fixed

SCF

1 - Absent  
2 - Present ☐

Lymphoedema

1 - Absent  
2 - Present ☐

## Clinical Stage

T

0 - T<sub>0</sub>  
1 - T<sub>1</sub>  
2 - T<sub>2</sub>  
3 - T<sub>3</sub>  
4 - T<sub>4</sub>

N

0 - N<sub>0</sub>  
1 - N<sub>1</sub>  
2 - N<sub>2</sub>  
3 - N<sub>3</sub>

M

0 - M<sub>0</sub>  
1 - M<sub>1</sub>

Manchester

## Date of Primary Treatment

## Surgery

1 - None  
2 - Incision biopsy  
(inc. Trucut) ☐  
3 - Excision biopsy  
4 - Simple Mastectomy  
5 - Radical MastectomyAdjuvant  
Radiotherapy (primary site &/or regional nodes)1 - No  
2 - Yes ☐

## Adjuvant Rx

0 - None  
1 - CMF  
2 - Melphalan ☐  
3 - Tamoxifen  
4 - XRAM  
5 - Oophorectomy  
6 - Cyclophosphamide  
7 - Tamoxifen &  
cyclophosphamide  
8 - Other, state:  
\_\_\_\_\_  
\_\_\_\_\_

## Adjuvant Trial

0 - None  
1 - CMF  
2 - Tamoxifen (Christie) ☐  
3 - Tamoxifen (ICI)  
4 - Other, state:  
\_\_\_\_\_  
\_\_\_\_\_

## Histology - predominant

1 - Infiltrating duct ☐  
2 - Inf. Lobula  
3 - In situ  
4 - Other - State  
\_\_\_\_\_  
\_\_\_\_\_

## Nodes Involved

 99 - NK

## Nodes Removed

 99 - NK

(98 if diffuse involvement (nodes cannot be counted))

## Multifocal Disease

1 - No  
2 - Yes ☐  
9 - NK

## Extranodal Spread

1 - No  
2 - Yes ☐  
9 - NK

## Pectoral Infiltration

1 - No  
2 - Yes ☐  
9 - NK

## Skin Infiltration

1 - No  
2 - Yes ☐  
9 - NK

## Path. Size

1 - <2  
2 - 2-5cms ☐  
3 - 5cms+  
9 - NK

## Receptors (primary) for stages 1, 2 and 3 only

Oestrogen  
Cytosol

Progesterone

## Eventual Outcome

Date of death or last seen alive

Oestrogen  
Nuclear

Protein

9999 - Not done  
8888 - +ve, level  
not known

## Status

1 - Alive NED  
2 - Alive + other cancer  
3 - Alive with disease ☐  
4 - Dead non-cancer  
5 - Dead, other cancer  
6 - Dead, breast cancer

# Breast Cancer Follow-up

## Advanced Disease

Name: \_\_\_\_\_ Hospital 1-Christie ☐ 2-Withington ☐ Consultant's initials ☐☐☐☐ Hospital Number ☐☐☐☐☐☐☐☐☐☐

Date of objective progression (or presentation of Stage 3/4) \_\_\_\_\_ Date of start of treatment \_\_\_\_\_ Karnofsky Performance score at this time \_\_\_\_\_

☐☐☐☐☐☐ ☐☐☐☐☐☐ 999-NK

Clinical Findings 1 - Normal; 2-Abnormal (not due to cancer); 3-Abnormal due to cancer

☐ Breast or chest wall ☐ Opp. Breast ☐ Axilla ☐ Ipsi. SCF ☐ Opp. SCF ☐ Liver ☐ Other; state: \_\_\_\_\_

X-rays 1 - Normal; 2 - Abnormal (definitely malignant); 9 - Not done

☐ Bone ☐ Lung ☐ Pleura ☐ CNS (CAT scan)

Radiological (scan) 1 - Normal; 2 - Abnormal; 9 - Not done Menopausal Status

☐ Bone ☐ Liver (isotope) ☐ Liver (ultrasound) ☐ Brain 1- Pre 2- Peri 3- Post ☐

## Receptors

Oestrogen cytosol ☐☐☐☐ 9999 - Not done 8888, +ve level not known

Oestrogen nuclear ☐☐☐☐

Progesterone ☐☐☐☐

Protein ☐☐☐☐

## Treatment

☐☐☐ ☐☐☐ ☐☐☐ ☐☐☐

## Response

Overall assessment Soft Tissue Bone Liver Pleura Lung Nodes

1-Progression ☐ ☐ ☐ ☐ ☐ ☐  
2-Static ☐  
3-Partial ☐  
4-Complete ☐  
5-Not applicable ie no disease ☐  
6-Present but not assessable ☐  
9-Not known ☐

Date of progression \_\_\_\_\_

☐☐☐☐☐☐

Best Karnofsky performance score

☐☐☐ 999 - NK

APPENDIX 6: VARIATION OF RECEPTORS - MULTIPLE SYNCHRONOUS BIOPSIES

| Sample reference | ER  | Kd   | PR  | Kd   | pro | ER   | Kd    | PR  | Kd   | pro |
|------------------|-----|------|-----|------|-----|------|-------|-----|------|-----|
| BC 502,3         | -ve |      | -ve |      | 1.0 | -ve  |       | -ve |      | 0.8 |
| BC 1181,2        | -ve |      | -ve |      | 2.3 | -ve  |       | -ve |      | 2.9 |
| BC 1021,2        | -ve |      | -ve |      | 1.0 | -ve  |       | -ve |      | 1.4 |
| BC 264a,b        | -ve |      | -ve |      | 1.5 | -ve  |       | -ve |      | 2.5 |
| BC 476,7         | -ve |      | -ve |      | 2.2 | -ve  |       | -ve |      | 2.8 |
| BC 1304,5        | -ve |      | -ve |      | 1.4 | -ve  |       | -ve |      | 1.1 |
| BC 1828,9        | -ve |      | -ve |      | 2.7 | -ve  |       | -ve |      | 1.7 |
| BC 1150,1        | -ve |      | -ve |      | 2.3 | -ve  |       | -ve |      | 3.2 |
| BC 708,9         | -ve |      | -ve |      | 2.1 | -ve  |       | -ve |      | 1.1 |
| BC 1322,3        | -ve |      | -ve |      | 3.4 | -ve  |       | -ve |      | 2.9 |
| BC 1307,8        | -ve |      | -ve |      | 0.8 | -ve  |       | -ve |      | 0.7 |
| BC 1524,5        | -ve |      | -ve |      | 1.4 | -ve  |       | -ve |      | 1.2 |
| ID 9,10          | -ve |      | -ve |      | \$  | 10   | (IEF) | -ve |      | 10  |
| BC 437a,b        | -ve |      | -ve |      | 2.1 | 149  | 1.1   | 170 | 2.6  | 4.3 |
| BC 547,8         | -ve |      | -ve |      | 1.8 | 29   | 2.3   | 193 | 4.8  | 3.2 |
| BC 921,3         | 12  | 4.7  | 36  | 18   | 3.3 | 35   | 1.6   | 162 | 8    | 3.7 |
| BC 965,6         | 17  | 3.2  | -ve |      | 3.2 | 31   | 0.7   | -ve |      | 3.2 |
| BC 1478,9        | 24  | 1.9  | 366 | 11.4 | 1.5 | -ve  |       | -ve |      | 0.7 |
| BC 922,4         | 33  | 1.6  | 95  | 12   | 4.4 | 29   | 4.8   | 85  | 8.7  | 3.3 |
| BC 1577a,b       | 45  | 1.5  | -ve |      | 1.6 | 123  | 0.7   | -ve |      | 1.1 |
| BC 381a,b        | 48  | 3.0  | -ve |      | 0.5 | 21   | 1.8   | -ve |      | 2.3 |
| ID38/BC1507      | 41  | NS   | -ve |      | 3.6 | 1055 | 3.4   | -ve |      | 3.4 |
| BC 1759,60       | 57  | 7.2  | 119 | 2.1  | 2.1 | 34   | 6.8   | 40  | 12.2 | 2.7 |
| BC 840,1         | 65  | 11.3 | 15  | 11.4 | 2.6 | 33   | 4.6   | 11~ | NS   | 1.8 |



9

|            |     |     |     |      |     |     |     |     |      |     |
|------------|-----|-----|-----|------|-----|-----|-----|-----|------|-----|
| BC 310a,b  | 66  | 1.8 | 200 | 3.3  | 0.6 | 150 | 2.7 | 139 | 5.3  | 9.0 |
| BC 723,4   | 28  | 4.6 | -ve |      | 1.3 | 52  | 4.2 | -ve |      | 1.9 |
| BC 1861,2  | 95  | 1.3 | -ve |      | 3.6 | 390 | 3.1 | -ve |      | 2.4 |
| BC 634a,b  | 116 | 6.4 | -ve |      | 1.8 | 194 | 5.2 | -ve |      | 3.3 |
| BC 891,2   | 139 | 1.7 | 94  | 8.1  | 1.8 | 354 | 2.0 | 83  | 15.4 | 2.4 |
| BC 980,1   | 143 | 0.7 | -ve |      | 1.4 | 133 | 2.6 | -ve |      | 0.9 |
| BC 325a,b  | 155 | 0.9 | -ve |      | 1.6 | 285 | 0.5 | -ve |      | 2.1 |
| BC 1295,6  | 218 | 1.2 | 25  | 2.6  | 1.7 | 607 | 3.6 | 11~ | 4.4  | 2.8 |
| BC 1503,4  | 295 | 0.9 | -ve |      | 1.6 | 279 | 2.7 | 17  | 8.5  | 3.1 |
| BC 846,7   | 310 | 1.7 | 431 | 12.5 | 2.9 | 93  | NS  | 171 | NS   | 2.8 |
| BC 509,10  | 367 | 1.5 | 555 | 7    | 1.8 | 267 | NS  | 100 | 10   | 1.2 |
| BC 1319,20 | 587 | 1.6 | 329 | 2.4  | 0.7 | 70  | 2.5 | 50  | 4.2  | 0.5 |
| BC 1470,1  | 36  | 4.7 | 651 | 10.5 | 3.0 | 30  | 2.9 | 135 | 7.1  | 4.5 |
| BC 1540,1  | 831 | 6.9 | 521 | 30   | 2.1 | 735 | 7.5 | 422 | 14.7 | 1.8 |

~Negative result at conventional lower limit of detection

\$Heavily blood-stained cytosol

# APPENDIX 7: VARIATION OF RECEPTORS - ASYNCHRONOUS SAMPLES

| Sample ref.   | --- First sample ---- |     |      |     |     | --- Second sample --- |      |     |     |     | Int  |
|---------------|-----------------------|-----|------|-----|-----|-----------------------|------|-----|-----|-----|------|
|               | RE                    | Kd  | RP   | Kd  | pro | RE                    | Kd   | RP  | Kd  | pro |      |
| BC 1011, 1449 | -ve                   |     | 24   | 1.8 | 1.0 | -ve                   |      | -ve |     | 0.9 | 392  |
| BC 149, 1770  | 346                   | 6.5 | -ve  |     | 0.7 | 61                    | 10.2 | -ve |     | 4.0 | 207  |
| BC 61, 149    | 94                    | 0.6 | -ve  |     | 1.6 | 57                    | NS   | 17  | NS  | 1.8 | 146  |
| BC 12, 567    | 17                    | 4.9 | NK   |     | 0.7 | 234                   | 0.6  | 260 | 10  | 2.3 | 1211 |
| BC 1080, 953  | -ve                   |     | -ve  |     | 2.2 | -ve                   |      | -ve |     | 1.6 | 133  |
| BC 787, 906   | 24                    | 2.8 | 17   | 14  | 3.3 | -ve                   |      | -ve |     | 0.9 | 86   |
| BC 152, 483   | 260                   | NS  | 530  | NS  | 1.9 | 84                    | 0.5  | -ve |     | 2.4 | 588  |
| BC 689, 914   | 28                    | 0.8 | -ve  |     | 0.8 | -ve                   |      | -ve |     | 1.3 | 246  |
| BC 914, 1239  | -ve                   |     | -ve  |     | 1.3 | 56                    | 7.1  | -ve |     | 1.8 | 342  |
| BC 689, 1239  | 28                    | 0.8 | -ve  |     | 0.8 | 56                    | 7.1  | -ve |     | 1.8 | 588  |
| BC 279, 978   | 63                    | 5.2 | 100  | 2.6 | 1.6 | 63                    | 7.1  | -ve |     | 1.2 | 960  |
| BC 1053, 1298 | -ve                   |     | -ve  |     | 1.2 | 30                    | 5.0  | -ve |     | 0.7 | 215  |
| BC 620, 980   | 370                   | 4.0 | 58   | 1.2 | 0.7 | 143                   | 0.7  | -ve |     | 1.4 | 441  |
| BC 403, 1073  | 35                    | 2.6 | 394  | 4.3 | 1.3 | 190                   | 2.4  | 93  | 15  | 2.9 | 836  |
| BC 308, 1224  | 85                    | 2.3 | -ve  |     | 0.7 | -ve                   |      | -ve |     | 2.0 | 1117 |
| BC 411, 874   | 8                     | 2.4 | 71   | 7.7 | 2.8 | -ve                   |      | -ve |     | 1.6 | 324  |
| BC 434, 1002  | -ve                   |     | -ve  |     | 0.8 | -ve                   |      | -ve |     | 1.1 | 744  |
| BC 3, 255     | 37                    | 3.9 | NK   |     | 0.9 | -ve                   |      | -ve |     | 0.8 | 796  |
| BC 668, 849   | -ve                   |     | -ve  |     | 1.9 | -ve                   |      | -ve |     | 2.8 | 226  |
| BC 462, 963   | 52                    | 1.9 | -ve  |     | 1.8 | 75                    | 3.3  | -ve |     | 1.8 | 662  |
| BC 393, 1028  | -ve                   |     | -ve  |     | 0.7 | -ve                   |      | -ve |     | 1.2 | 561  |
| BC 1148, 1613 | -ve                   |     | -ve  |     | 3.7 | -ve                   |      | -ve |     | 1.3 | 383  |
| BC 485, 1051  | 163                   | 1.8 | 1474 | 1.6 | 1.9 | -ve                   |      | 233 | 1.2 | 1.2 | 724  |
| BC 603, 901   | 70                    | 1.0 | 704  | NS  | 3.4 | 33                    | 11.5 | -ve |     | 0.9 | 371  |
| BC 525, 1050  | 312                   | 1.8 | -ve  |     | 0.8 | NK                    |      | 75  |     | 1.2 | 645  |
| BC 471, 798   | -ve                   |     | 73   | 9.2 | 1.2 | 27                    | 2.3  | -ve |     | 0.7 | 496  |

|               |     |     |      |     |     |      |      |      |      |     |      |
|---------------|-----|-----|------|-----|-----|------|------|------|------|-----|------|
| BC 175, 975   | 139 | NS  | 305  | NS  | 3.6 | 158  | 0.7  | 313  | 2.0  | 2.4 | 1164 |
| BC 358, 1278  | 11  | 1.5 | 154  | 4.5 | 1.2 | 375  | 1.4  | 142  | 0.8  | 2.4 | 1122 |
| BC 1278, 1533 | 375 | 14  | 142  | 0.8 | 2.4 | 32   | 12.9 | 113  | 2.5  | 0.6 | 190  |
| BC 358, 1533  | 11  | 1.5 | 154  | 4.5 | 1.2 | 32   | 12.9 | 113  | 2.5  | 0.6 | 1312 |
| BC 672, 1006  | 48  | 1.8 | -ve  |     | 1.4 | 26   | 4.4  | -ve  |      | 1.2 | 375  |
| BC 765, 1392  | -ve |     | -ve  |     | 1.8 | -ve  |      | -ve  |      | 4.3 | 595  |
| BC 325, 1402  | 155 | 0.9 | -ve  |     | 1.2 | 482  | 3.1  | -ve  |      | 2.8 | 1281 |
| BC 557, 1295  | 43  | 2.7 | -ve  |     | 0.7 | 607  | 3.6  | 11~  | 4.4  | 2.8 | 1008 |
| BC 128, 544   | -ve |     | -ve  |     | 1.8 | -ve  |      | -ve  |      | 2.8 | 729  |
| BC 361, 1021  | -ve |     | -ve  |     | 1.2 | -ve  |      | -ve  |      | 1.0 | 868  |
| BC 1021, 1183 | -ve |     | -ve  |     | 1.0 | -ve  |      | -ve  |      | 4.7 | 163  |
| BC 361, 1183  | -ve |     | -ve  |     | 1.2 | -ve  |      | -ve  |      | 4.7 | 1031 |
| BC 526, 688   | -ve |     | -ve  |     | 1.0 | 28   | 0.5  | -ve  |      | 1.5 | 255  |
| BC 609, 740   | -ve |     | -ve  |     | 2.1 | -ve  |      | -ve  |      | 1.2 | 203  |
| BC 707, 786   | -ve |     | -ve  |     | 2.9 | -ve  |      | -ve  |      | 3.4 | 108  |
| BC 205, 501   | -ve |     | 818  | 6.8 | 0.7 | -ve  |      | 187  | 7.3  | 1.5 | 485  |
| BC 395, 1017  | -ve |     | -ve  |     | 1.4 | 59   | 2.0  | -ve  |      | 1.1 | 167  |
| BC 65, 1201   | 350 | NS  | 1218 | NS  | 2.2 | 889  | 0.9  | 1167 | 15   | 0.9 | 1619 |
| BC 257, 430   | 48  | 6.6 | 68   | 5.9 | 2.5 | 15   | 3.1  | 254  | 3.7  | 2.2 | 282  |
| BC 430, 1447  | 15  | 3.1 | 254  | 3.7 | 2.2 | 18   | 1.2  | 28   | 10.8 | 1.0 | 1120 |
| BC 257, 1447  | 48  | 6.6 | 68   | 5.9 | 2.5 | 18   | 1.2  | 28   | 10.8 | 1.0 | 1402 |
| BC 674, 1066  | 186 | 1.0 | -ve  |     | 1.4 | 1000 | 3.1  | 144  | 4.2  | 0.9 | 420  |
| BC 276, 900   | 229 | 0.8 | NK   |     | 0.4 | 724  | NS   | 1034 | 2.9  | 2.9 | 870  |
| BC 212, 502   | 53  | 0.9 | -ve  |     | 0.6 | -ve  |      | -ve  |      | 1.0 | 468  |
| BC 92, 256    | 48  | 2.3 | -ve  |     | 2.6 | 76   | 0.8  | 80   | 4.5  | 2.5 | 353  |
| BC 312, 649   | NK  |     | -ve  |     | 1.0 | -ve  |      | -ve  |      | 2.9 | 524  |
| BC 1233, 1494 | 84  | 1.0 | 16   | 1.5 | 1.6 | -ve  |      | -ve  |      | 0.9 | 215  |
| BC 706, 1203  | 136 | 2.8 | 156  | 2.0 | 0.4 | 100  | 3.4  | 180  | 8.1  | 1.0 | 528  |
| BC 348, 1284  | 195 | 3.6 | 1800 | 6.5 | 0.4 | 82   | 6.4  | 56   | 6.8  | 0.9 | 1137 |

|               |     |     |     |      |     |      |      |     |      |     |      |
|---------------|-----|-----|-----|------|-----|------|------|-----|------|-----|------|
| BC 183, 419   | 245 | NS  | -ve |      | 1.1 | -ve  |      | -ve |      | 2.5 | 438  |
| BC 84, 976    | 33  | 2.2 | 43  | 10   | 2.0 | 37   | 1.8  | 30  | 3.1  | 2.7 | 1334 |
| BC 552, 1665  | -ve |     | -ve |      | 1.6 | -ve  |      | 76  | 2.2  | 1.9 | 1123 |
| BC 645, 1547  | -ve |     | -ve |      | 1.8 | -ve  |      | -ve |      | 1.9 | 884  |
| BC 628, 1378  | 46  | 3.8 | 250 | 22   | 4   | -ve  |      | -ve |      | 1.5 | 793  |
| BC 919, 1350  | 365 | 1.7 | 500 | 4.5  | 0.9 | 875  | 1.0  | 900 | 5.1  | 0.4 | 419  |
| BC 635, 1007  | 186 | 1.6 | -ve |      | 1.5 | 100  | 2.8  | -ve |      | 1.2 | 441  |
| BC 857, 1482  | 195 | NS  | 704 | 10.6 | 2.7 | 43   | 1.3  | -ve |      | 2.8 | 559  |
| BC 1058, 1792 | 100 | 1.3 | 83  | 2.1  | 1.5 | 38   | 16.2 | 40  | 4.2  | 1.8 | 584  |
| BC 686, 1548  | 26  | 3.0 | -ve |      | 1.0 | 39   | 1.3  | -ve |      | 1.3 | 830  |
| BC 896, 1518  | -ve |     | -ve |      | 2.3 | -ve  |      | -ve |      | 1.3 | 574  |
| BC 675, 1456  | -ve |     | 92  | 2.8  | 2.8 | 7    | 7.8  | -ve |      | 2.3 | 752  |
| BC 1014, 1404 | 561 | 0.5 | -ve |      | 0.9 | 258  | NS   | 17  | 9.4  | 2.8 | 352  |
| BC 1026, 1507 | 301 | NS  | 524 | 7.3  | 2.1 | 1055 | 3.4  | -ve |      | 3.4 | 424  |
| BC 1401, 1626 | -ve |     | -ve |      | 1.1 | -ve  |      | -ve |      | 1.0 | 175  |
| BC 1556, 1754 | -ve |     | -ve |      | 3.1 | 12   | 9.9  | -ve |      | 3.5 | 234  |
| BC 973, 1700  | -ve |     | -ve |      | 3.3 | -ve  |      | -ve |      | 3.3 | 605  |
| BC 607, 1349  | 48  | 6   | -ve |      | 3.5 | 30   | 8.0  | -ve |      | 0.4 | 425  |
| BC 533, 961   | 36  | 2.7 | 182 | 1.7  | 2.2 | -ve  |      | -ve |      | 1.2 | 548  |
| BC 408, 667   | 120 | 2.4 | 26  | 4.0  | 5.0 | 312  | 0.2  | 50  | 9.2  | 2.2 | 386  |
| BC 775, 1445  | 108 | 3.0 | 248 |      | 2.0 | 80   | 0.8  | 267 | 12.4 | 2.7 | 608  |
| BC 907, 1748  | 14  | 4.5 | 67  | 4    | 1.8 | 43   | 1.7  | 5~  | 3.3  | 5.1 | 753  |
| BC 1428, 1746 | 469 | 2.7 | -ve |      | 3.2 | 23   | NS   | -ve |      | 8.3 | 234  |
| BC 1366, 1387 | -ve |     | -ve |      | 0.7 | -ve  |      | -ve |      | 0.9 | 793  |
| BC 1124, 1617 | -ve |     | -ve |      | 1.7 | -ve  |      | -ve |      | 1.6 | 418  |
| BC 1285, 1659 | -ve |     | -ve |      | 5.1 | -ve  |      | -ve |      | 1.4 | 294  |
| BC 1633, 1781 | -ve |     | -ve |      | 0.8 | -ve  |      | -ve |      | 2.0 | 98   |
| BC 215, 1585  | 15  | NS  | -ve |      | 0.4 | 199  | 1.6  | -ve |      | 1.6 | 1605 |
| BC 236, 1848  | -ve |     | -ve |      | 1.0 | -ve  |      | 26  | 11.9 | 2.2 | 1763 |

|               |      |     |     |      |     |     |      |                |      |     |      |
|---------------|------|-----|-----|------|-----|-----|------|----------------|------|-----|------|
| BC 891, 1568  | 139  | 1.7 | 94  | 8.1  | 1.8 | 258 | 1.2  | 5 <sup>~</sup> | 12.5 | 3.3 | 615  |
| BC 1514, 1833 | -ve  |     | -ve |      | 1.8 | 6   |      | -ve            |      | 4.8 | 248  |
| BC 899, 1910  | 39   | 5.8 | 96  | 12.3 | 1.4 | NK  |      | 1000           | NS   | 4.0 | 819  |
| BC 342, 1778  | 748  | 2.2 | 75  | 6.5  | 3.3 | 622 | 14   | 9 <sup>~</sup> | 14.6 | 5.1 | 1207 |
| BC 720, 1892  | 1000 | 6.4 | -ve |      | 0.6 | 331 | 7.1  | NK             |      | 0.1 | 1395 |
| BC 444, 1851  | -ve  |     | 63  | NS   | 1.6 | 26  | 13.8 | -ve            |      | 1.6 | 1434 |
| BC 647, 1394  | -ve  |     | -ve |      | 1.3 | -ve |      | -ve            |      | 0.9 | 752  |

RE & RP expressed as fmol/mg cytosol protein.

<sup>-10</sup>

Kd x 10

pro = cytosol protein concentration (g/l)

Int = Interval in days between samples

NS = Not stated      NK = not known

<sup>~</sup>Negative result at conventional limit of detection



APPENDIX 8: Details of patients whose receptor status was unknown

| Hospital reference | A    | B   | C  | T | N | M | Res | CW | OB | Ax | Cx | Ot | Li | Bo | Lu | Pl |
|--------------------|------|-----|----|---|---|---|-----|----|----|----|----|----|----|----|----|----|
| 42753              | 11   | 11  | 0  | 3 | 1 | 0 | ED  | x  | x  | x  |    |    |    |    |    |    |
| 717113             | 123  | 44  | 17 | 2 | 1 | 0 | CR  |    |    |    | x  |    |    |    |    |    |
| 766009             | 5    | 1   | 1  | 4 | 1 | 0 | PD  | x  | x  |    | x  |    |    |    |    |    |
| 773694             | 33   | 33  | 4  | 2 | 0 | 1 | PD  |    |    |    |    |    |    | x  |    |    |
| 783911             | 42   | 39  | 3  | 3 | 0 | 0 | CR  |    |    | x  |    |    |    |    |    |    |
| 786016             | 15   | 15  | 4  | 4 | 3 | 1 | PD  | x  |    | x  | x  |    |    | x  |    |    |
| 786996             | -46  | -2  | -2 | 2 | 1 | 0 | TS  |    |    |    |    |    |    |    | x  |    |
| 792357             | -59  | -44 | 6  | 3 | 0 | 0 | PR  |    |    |    |    |    |    | x  |    |    |
| 793711             | -37  | -0  | 0  | 3 | 0 | 0 | PD  |    |    |    |    |    |    |    | x  |    |
| 795631             | -34  | -4  | 1  | 2 | 0 | 0 | PD  |    |    |    |    | x  |    | x  |    |    |
| 806485             | 11   | 11  | 9  | 4 | 2 | 1 | PR  | x  | x  |    |    |    | x  | x  | x  | x  |
| 808405             | 171  | 43  | 22 | ? | ? | ? | NA  |    |    |    |    |    |    | x  |    |    |
| 815728             | -66  | -16 | -9 | 1 | 0 | 0 | ND  | x  |    |    |    | x  |    |    | x  |    |
| 818430             | -15  | -5  | 1  | 2 | 0 | 0 | PD  |    |    |    | x  |    |    |    |    |    |
| 823510             | -40  | -21 | 6  | 2 | 1 | 0 | SD  | x  |    | x  | x  |    |    |    |    |    |
| A14638             | -58  | -25 | 13 | 2 | 0 | 0 | PR  |    |    | x  |    |    |    |    | x  |    |
| A16720             | 31   | 31  | 1  | 0 | 2 | 0 | PD  |    |    | x  |    |    |    |    |    |    |
| A78196             | 37   | 21  | 4  | 2 | 0 | 0 | PD  | x  |    |    |    |    |    |    |    |    |
| A93978             | -73  | -73 | 6  | 4 | 3 | 0 | PD  |    |    |    |    |    |    | x  |    |    |
| B20541             | 48   | 17  | 5  | 2 | 1 | 0 | PD  |    |    |    |    |    |    | x  | x  |    |
| B31194             | 37   | 37  | 37 | 4 | 2 | 0 | PR  | x  |    | x  |    |    |    |    |    |    |
| B34460             | -154 | -15 | 1  | 2 | 0 | 0 | PD  | x  |    |    |    |    |    |    |    |    |
| B37033             | -83  | -49 | 26 | 3 | 0 | 0 | ND  | x  |    |    |    |    |    | x  |    |    |
| B38139             | 26   | 26  | 4  | 1 | 1 | 1 | PD  |    |    |    | x  |    |    | x  |    |    |
| B50483             | -22  | -5  | 2  | 2 | 0 | 0 | PD  |    |    |    |    |    |    |    | x  |    |
| C19114             | -7   | -7  | -7 | 4 | 1 | 0 | NA  | x  |    |    |    |    |    |    |    |    |

| Ref No | A    | B    | C   | T | N | M | Res | CW | OB | Ax | Cx | Ot | Li | Bo | Lu | Pl |
|--------|------|------|-----|---|---|---|-----|----|----|----|----|----|----|----|----|----|
| C21027 | -84  | -39  | 13  | 2 | 0 | 0 | PR  |    |    |    |    |    |    | x  | x  | x  |
| C33212 | -73  | -52  | 23  | 2 | 1 | 0 | PR  |    |    |    |    |    |    |    | x  |    |
| C35654 | -57  | -16  | 1   | 2 | 1 | 0 | PD  |    |    |    |    | x  |    |    |    | x  |
| C41640 | -97  | -70  | 24  | 4 | 1 | 0 | NA  | x  |    |    |    |    |    |    |    |    |
| C55025 | -126 | -78  | -5  | 2 | 1 | 0 | NA  |    |    | x  |    |    |    |    |    |    |
| C63084 | -37  | -8   | -6  | 3 | 0 | 0 | TS  | x  | x  |    |    |    |    |    |    |    |
| C65524 | 107  | 60   | 0   | 1 | 0 | 0 | PD  | x  |    |    |    |    |    | x  |    |    |
| C65818 | -117 | -117 | 26  | 4 | 1 | 0 | PR  | x  |    |    |    |    |    |    |    |    |
| C73246 | -107 | -55  | 46  | 1 | 1 | 0 | NA  |    |    | x  |    |    |    |    |    |    |
| C77851 | -104 | -34  | -32 | 3 | 1 | 0 | PR  |    |    |    |    |    |    |    | x  |    |
| C84272 | 101  | 21   | 6   | 2 | 0 | 0 | ND  | x  |    |    |    |    |    |    |    |    |
| C88164 | -103 | -47  | 5   | 2 | 0 | 0 | PR  |    |    |    |    |    |    | x  | x  |    |
| C94525 | -166 | -100 | 53  | 1 | 1 | 0 | NA  |    |    |    |    |    |    |    |    | x  |
| C99420 | -95  | -95  | 68  | 4 | 0 | 0 | ND  | x  |    |    |    |    |    |    |    |    |
| C99957 | 92   | 92   | 18  | 4 | 0 | 0 | PR  |    |    |    |    |    |    | x  |    |    |
| D03670 | -90  | -74  | 27  | 1 | 0 | 0 | CR  | x  |    |    |    |    |    |    |    |    |
| D08646 | 32   | 32   | 7   | 4 | 1 | 0 | PR  |    |    |    |    |    |    | x  |    |    |
| D11081 | 27   | 12   | 1   | 1 | 0 | 0 | ED  | x  |    |    | x  |    |    |    | x  |    |
| D16072 | 57   | 42   | 7   | 2 | 0 | 0 | PD  | x  |    |    |    |    |    | x  |    |    |
| D19984 | -115 | -79  | -48 | 1 | 0 | 0 | NA  | x  |    |    |    |    |    |    |    |    |
| D34674 | 28   | 28   | 1   | 4 | 1 | 1 | PD  | x  |    | x  |    |    |    | x  |    |    |
| D36164 | -71  | -38  | -35 | 2 | 0 | 0 | NA  | x  |    |    |    |    |    |    |    |    |
| D38402 | 28   | 28   | 4   | 4 | 0 | 0 | PD  | x  |    |    |    |    |    |    |    |    |
| D39907 | -62  | -62  | -26 | 4 | 1 | 0 | PR  |    |    |    |    |    |    |    | x  |    |
| D50119 | 53   | 38   | 2   | 2 | 1 | 0 | ED  |    |    |    |    | x  |    |    | x  | x  |
| D52595 | 18   | 18   | 1   | 4 | 0 | 0 | PD  | x  |    | x  |    |    |    |    | x  |    |
| D61165 | -3   | -3   | 0   | 4 | 1 | 0 | PD  | x  |    |    |    |    |    |    |    |    |
| D62258 | 17   | 17   | 8   | 3 | 3 | 1 | PR  | x  |    | x  | x  |    |    | x  |    |    |



| Ref No  | A    | B   | C   | T | N | M | Res | CW | OB | Ax | Cx | Ot | Li | Bo | Lu | Pl |
|---------|------|-----|-----|---|---|---|-----|----|----|----|----|----|----|----|----|----|
| D68749  | 45   | 45  | 3   | ? | ? | 1 | ED  |    |    |    |    |    |    | x  |    |    |
| D74940  | -52  | -48 | 27  | 2 | 1 | 0 | CR  |    |    |    | x  |    |    |    |    |    |
| D77116  | -51  | -51 | 19  | 4 | 0 | 0 | ND  | x  | x  |    |    |    |    |    |    |    |
| D80763  | 41   | 41  | 3   | 4 | 2 | 0 | PD  | x  |    |    |    |    |    |    |    |    |
| D81515  | 171  | 23  | 4   | 1 | 0 | 0 | PD  | x  |    |    |    | x  |    | x  |    |    |
| D81539  | 20   | 20  | 1   | 4 | 0 | 0 | PD  | x  |    |    |    |    |    |    |    |    |
| D82854  | 2    | 2   | 2   | 4 | 2 | 1 | ED  | x  |    | x  |    |    | x  |    |    |    |
| D85108  | 74   | 74  | 18  | 4 | 2 | 0 | CR  | x  |    |    |    |    |    | x  |    |    |
| D86205  | -48  | -29 | 2   | 2 | 1 | 0 | PD  |    |    |    |    |    |    |    | x  |    |
| D86206  | -118 | -97 | 11  | 1 | 0 | 0 | ND  | x  |    |    |    |    |    |    |    |    |
| D88059  | -43  | -43 | 17  | 4 | 0 | 0 | PR  | x  |    |    |    |    |    |    |    |    |
| D89628  | -26  | -26 | 2   | 4 | 0 | 1 | PD  | x  |    |    |    |    |    | x  |    |    |
| D90780~ | 6    | 6   | 3   | 2 | 0 | 0 | PD  | x  |    |    |    |    |    |    |    |    |
| E01041  | 38   | 38  | 18  | 4 | 0 | 0 | PR  | x  |    |    |    |    |    |    |    |    |
| E02369~ | -15  | -15 | 10  | 3 | 0 | 0 | ND  | x  |    |    |    |    |    |    |    |    |
| E03999  | -75  | -20 | -18 | 1 | 0 | 0 | NA  | x  |    |    |    |    |    |    |    |    |
| E33316  | -147 | -22 | 3   | ? | ? | 0 | PD  |    |    |    |    |    |    | x  |    |    |
| E38704  | -127 | -25 | 6   | 2 | 1 | 0 | PD  |    | x  |    |    |    | x  | x  |    |    |
| E38316  | -6   | -6  | -6  | 4 | 1 | 0 | NA  | x  | x  |    |    |    |    |    |    |    |
| E39511  | 7    | 7   | 3   | 4 | 3 | 1 | PD  | x  |    | x  | x  |    |    | x  |    |    |
| E40037  | -95  | -21 | 13  | 2 | 1 | 0 | PR  | x  |    |    | x  |    | x  |    |    |    |
| E56838  | -7   | -7  | -7  | 4 | 1 | 0 | ND  | x  |    | x  |    |    |    |    |    |    |

A = Survival from presentation      (- = uncensored )

B = Survival from 'first relapse'      "

C = Time to progression      "

~Primary treatment with tamoxifen

For classification of response, and sites of disease, see Appendix 9.

## APPENDIX 9: DATA FOR LOGRANK ANALYSIS

A= Survival from presentation (- = uncensored)

B= Survival from first relapse "

C= Time to progression (1st hormone treatment) "

D= Primary tumour receptor status (o not known, - negative, + < 60 fmol/mg, ++ >60 fmol/mg)

E= Receptor status as assessed at the time of treatment (o not known, - negative, + positive).

F= Overall receptor status (assessed at or before treatment) (o not known, - negative, + positive)

Res = Assessment of response (PD = progression, SD = stable disease, PR= partial regression, CR = complete regression, ED = early death, NA = not assessable, ND = not documented, TS = too soon for assessment)

Sites of disease (CW = chest wall/breast, OB = opposite breast, Ax = axilla, Cx = cervical nodes, Ot = other, Li = liver, Bo = bone, Lu = lung, Pl = pleura, Br = brain).

| Hospital<br>reference | A   | B   | C   | D  |    |    | E  |    |    | F  |    |    | T | N | M | Res | Sites of disease |    |    |    |    |    |    |    |    |    |
|-----------------------|-----|-----|-----|----|----|----|----|----|----|----|----|----|---|---|---|-----|------------------|----|----|----|----|----|----|----|----|----|
|                       |     |     |     | ER | En | PR | ER | En | PR | ER | En | PR |   |   |   |     | CW               | OB | Ax | Cx | Ot | Li | Bo | Lu | Pl | Br |
| 200644                | -2  | -2  | -2  | ++ | o  | ++ | +  | o  | +  | +  | o  | +  | 4 | 3 | 1 | TS  | x                |    | x  | x  | x  |    | x  |    |    |    |
| 4957                  | 16  | 4   | 1   | +  | o  | -  | +  | o  | +  | +  | o  | -  | 2 | 1 | 0 | PD  | x                |    | x  |    |    |    | x  |    | x  |    |
| 772724                | -62 | -50 | -3  | ++ | o  | ++ | o  | o  | o  | +  | o  | +  | 2 | 1 | 0 | NA  |                  |    |    |    |    |    |    | x  |    |    |
| 807820                | -25 | -1  | -1  | -  | o  | -  | -  | o  | -  | -  | o  | -  | 1 | 0 | 0 | TS  |                  |    |    |    | x  |    | x  |    |    |    |
| 812455                | 17  | 9   | 3   | ++ | +  | +  | o  | o  | o  | +  | +  | +  | 3 | 1 | 0 | PD  | x                |    |    |    | x  |    |    | x  |    |    |
| 817852                | 10  | 10  | 1   | o  | o  | o  | o  | o  | o  | -  | +  | -  | 2 | 3 | 1 | PD  | x                |    |    |    | x  |    |    |    |    |    |
| 822389                | -28 | -1  | 2   | o  | o  | o  | +  | +  | -  | +  | +  | -  | 1 | 0 | 0 | PD  |                  |    | x  |    |    |    | x  | x  |    |    |
| A17298                | -25 | -13 | 0   | o  | o  | ++ | o  | o  | o  | o  | o  | +  | 2 | 1 | 0 | NA  |                  |    |    |    |    | x  | x  | x  |    |    |
| A21049                | -49 | -12 | -4  | +  | +  | ++ | o  | o  | o  | +  | +  | +  | 1 | 0 | 0 | PR  |                  | x  |    |    |    |    | x  |    |    |    |
| A26124                | -47 | -13 | -13 | -  | -  | -  | o  | o  | o  | -  | -  | -  | 2 | 0 | 0 | PR  |                  |    |    |    |    |    |    | x  |    |    |
| A26742                | -9  | -9  | 6   | -  | -  | -  | -  | -  | -  | -  | -  | -  | 4 | 1 | 0 | PD  | x                |    | x  |    |    |    | x  |    |    |    |
| A35899                | 37  | 37  | 14  | +  | +  | ++ | +  | +  | +  | +  | +  | +  | 2 | 0 | 1 | NA  |                  |    |    |    |    |    | x  |    |    |    |
| A42956                | -26 | -16 | -14 | ++ | +  | ++ | o  | o  | o  | +  | +  | +  | 2 | 0 | 0 | PR  |                  |    |    |    |    |    | x  | x  |    |    |
| A43051                | 27  | 17  | 2   | o  | o  | o  | o  | o  | o  | +  | o  | -  | 3 | 1 | 0 | PD  |                  |    |    |    |    |    | x  | x  |    |    |
| A47759                | -22 | -12 | 3   | ++ | +  | -  | +  | +  | -  | +  | +  | -  | 2 | 1 | 0 | PD  | x                |    |    |    |    |    |    |    |    |    |
| A57699                | -4  | -4  | -4  | -  | o  | -  | -  | o  | -  | -  | o  | -  | 3 | 2 | 0 | TS  | x                |    | x  |    |    |    |    |    |    |    |

| Ref.   | A   | B   | C   | D  |   |    | E |   |   | F |   |   | T | N | M | Res | CW | OB | Ax | Cx | OT | Li | Bo | Lu | Pl |
|--------|-----|-----|-----|----|---|----|---|---|---|---|---|---|---|---|---|-----|----|----|----|----|----|----|----|----|----|
| A58024 | -49 | -49 | 12  | o  | o | o  | o | o | o | + | + | + | 4 | 0 | 1 | PR  | x  | x  | x  |    | x  |    | x  |    |    |
| A62038 | 17  | 13  | 5   | -  | o | -  | o | o | o | - | o | - | 3 | 1 | 0 | PD  | x  |    | x  |    |    |    |    |    |    |
| A6340  | 30  | 10  | 6   | -  | - | -  | o | o | o | - | - | - | 3 | 0 | 0 | PD  |    |    |    |    |    |    | x  |    |    |
| A65491 | -79 | -40 | 17  | o  | o | o  | + | o | + | + | o | + | 2 | 0 | 0 | CR  |    |    | x  |    |    |    |    |    |    |
| A77196 | -67 | -50 | -42 | o  | o | ++ | o | o | o | o | o | + | 2 | 0 | 0 | NA  | x  |    |    |    |    |    |    |    |    |
| A80410 | -49 | -41 | 5   | -  | - | -  | o | o | o | - | - | - | 1 | 1 | 0 | PD  |    |    |    |    |    |    |    | x  |    |
| A95579 | -56 | -26 | 6   | ++ | + | ++ | o | o | o | + | + | + | 2 | 1 | 0 | PD  |    |    | x  |    |    |    | x  |    |    |
| A96306 | 54  | 11  | 11  | +  | o | -  | o | o | o | + | o | - | 2 | 0 | 0 | NA  |    |    |    |    |    |    | x  |    |    |
| B29809 | -36 | -36 | -34 | ++ | + | +  | + | + | + | + | + | + | 4 | 2 | 0 | SD  |    |    | x  |    |    |    | x  |    |    |
| B29939 | -17 | -2  | -2  | -  | o | -  | o | o | o | - | o | - | ? | 0 | 0 | NA  |    |    |    |    |    |    | x  | x  |    |
| B46523 | -69 | -31 | 5   | ++ | o | ++ | + | + | + | + | + | + | 2 | 1 | 0 | PR  | x  |    | x  |    |    |    | x  |    |    |
| B49468 | -22 | -18 | 5   | -  | - | -  | - | - | - | - | - | - | 2 | 3 | 0 | PD  |    |    | x  | x  |    |    |    | x  |    |
| B55184 | -64 | -5  | 3   | -  | o | ++ | o | o | o | - | o | + | 2 | 1 | 0 | PD  |    |    |    |    |    |    | x  |    |    |
| B69616 | 2   | 2   | 2   | -  | o | -  | - | o | - | - | o | - | 4 | 0 | 1 | PD  | x  |    |    |    |    | x  |    |    |    |
| B73501 | -81 | -6  | -4  | o  | o | o  | + | o | + | + | o | + | 2 | 0 | 0 | CR  | x  |    |    |    |    |    |    |    |    |
| B74398 | 12  | 5   | 1   | o  | o | o  | + | + | + | + | + | + | 3 | 0 | 0 | PD  |    |    | x  |    |    |    | x  |    |    |
| B83448 | -38 | -35 | -34 | +  | + | +  | - | - | - | + | + | + | 2 | 0 | 0 | NA  |    |    | x  |    |    |    |    |    |    |

| Ref.   | A    | B   | C  | D  |   |    | E |   |   | F |   |   | T | N | M | Res | CW | OB | Ax | Cx | OT | Li | Bo | Lu | Pl |
|--------|------|-----|----|----|---|----|---|---|---|---|---|---|---|---|---|-----|----|----|----|----|----|----|----|----|----|
| C04401 | -29  | -7  | -5 | ++ | + | +  | o | o | o | + | + | + | 2 | 1 | 0 | TS  |    |    |    |    |    |    |    | x  |    |
| C07949 | -57  | -28 | 0  | -  | o | -  | o | o | o | - | o | - | 2 | 0 | 0 | PD  |    |    |    |    |    |    | x  | x  | x  |
| C23013 | 31   | 25  | 13 | ++ | o | ++ | o | o | o | + | o | + | 2 | 1 | 0 | NA  |    | x  | x  |    |    |    |    |    |    |
| C29620 | 103  | 94  | 6  | o  | o | o  | - | o | + | - | o | + | 2 | 1 | 0 | PR  | x  |    |    |    |    |    |    |    |    |
| C33217 | -7   | -7  | -7 | +  | - | ++ | + | - | + | + | - | + | 3 | 1 | 0 | PR  | x  |    | x  |    |    |    |    |    |    |
| C35913 | -20  | -15 | 7  | o  | o | o  | o | o | o | - | - | - | 2 | 0 | 0 | PD  | x  |    |    |    |    |    | x  |    |    |
| C40135 | -70  | -54 | 11 | -  | o | ++ | o | o | o | - | - | + | 2 | 0 | 0 | ND  |    |    |    |    |    |    | x  |    |    |
| C4093  | 35   | 21  | 3  | -  | o | -  | o | o | o | - | o | - | 2 | 1 | 0 | PD  |    |    |    |    |    |    |    | x  |    |
| C50654 | 4    | 4   | 1  | -  | o | -  | - | o | - | - | o | - | 2 | 1 | 1 | PD  | x  |    | x  |    |    |    | x  |    |    |
| C55110 | 97   | 47  | 7  | o  | o | o  | o | o | o | - | - | - | ? | 0 | 0 | NA  | x  |    |    |    |    |    |    |    |    |
| C55354 | 65   | 28  | 6  | o  | o | o  | - | - | - | - | - | - | 2 | 0 | 0 | PD  |    |    | x  |    | x  |    | x  |    |    |
| C55956 | -127 | -14 | 2  | o  | o | o  | o | o | o | + | + | + | 2 | 0 | 0 | PD  | x  |    |    |    | x  |    |    |    |    |
| C57770 | 36   | 20  | 13 | o  | o | o  | o | o | o | - | - | - | 1 | 1 | 0 | PD  |    |    |    |    |    |    | x  | x  |    |
| C58005 | 45   | 16  | 4  | -  | - | -  | - | - | - | - | - | - | 1 | 1 | 0 | PD  |    |    |    |    |    | x  | x  |    |    |
| C59732 | 18   | 18  | 2  | ++ | o | ++ | + | o | + | + | o | + | 3 | 1 | 1 | PD  | x  |    | x  |    |    |    | x  | x  |    |
| C60611 | 36   | 36  | 2  | +  | o | o  | + | o | o | + | o | o | 4 | 0 | 1 | PD  | x  |    | x  |    |    |    | x  |    |    |
| C60825 | -40  | -40 | -1 | +  | o | +  | + | o | + | + | o | + | 2 | 2 | 0 | TS  |    |    | x  |    | x  |    | x  | x  |    |

| Ref.   | A    | B   | C   | D      | E     | F     | T | N | M | Res | CW | OB | Ax | Cx | OT | Li | Bo | Lu | Pl |
|--------|------|-----|-----|--------|-------|-------|---|---|---|-----|----|----|----|----|----|----|----|----|----|
| C66669 | -52  | -52 | -24 | o o o  | o o o | + + + | 4 | 0 | 0 | PR  | x  |    |    |    |    |    |    |    |    |
| C70742 | -116 | -6  | -3  | o o o  | + + + | + + + | 2 | 1 | 0 | TS  | x  |    |    |    |    |    |    |    |    |
| C73787 | -17  | -17 | 9   | + o ++ | + o + | + o + | 4 | 1 | 0 | SD  | x  |    | x  |    |    |    |    |    |    |
| C74384 | 4    | 4   | 1   | - - -  | - - - | - - - | 4 | 3 | 1 | PD  | x  |    | x  |    |    |    |    |    |    |
| C79694 | 44   | 20  | 2   | - - -  | o o o | - - - | 1 | 0 | 0 | ED  |    |    |    |    |    |    | x  | x  |    |
| C81803 | 85   | 49  | 6   | o o o  | + o - | + o - | 2 | 1 | 0 | PD  | x  | x  |    |    |    |    |    |    |    |
| C84327 | -96  | -13 | 10  | o o o  | + + - | + + - | 3 | 0 | 0 | SD  |    |    | x  |    | x  |    |    |    |    |
| D00712 | -82  | -45 | -41 | - - -  | o o o | - - - | 2 | 1 | 0 | NA  |    |    | x  |    |    |    |    |    |    |
| D07239 | 79   | 61  | 4   | o o o  | + + + | + + + | 2 | 0 | 0 | PD  |    |    | x  |    |    |    | x  | x  |    |
| D08497 | -90  | -38 | 17  | o o o  | + + + | + + + | 4 | 2 | 0 | CR  | x  |    |    |    |    |    |    |    |    |
| D12839 | -49  | -13 | 10  | - - -  | o o o | - - - | 2 | 1 | 0 | PR  |    |    |    | x  |    |    |    |    |    |
| D13430 | 17   | 9   | 3   | ++ o - | o o o | + o - | 2 | 1 | 0 | NA  |    |    |    |    |    |    | x  |    |    |
| D15384 | 75   | 57  | 2   | o o o  | + - - | + - + | 4 | 0 | 0 | PD  | x  |    |    |    |    |    |    |    |    |
| D17972 | 9    | 9   | 3   | - o o  | - o o | - o o | 4 | 1 | 0 | PD  | x  |    | x  |    |    |    |    |    |    |
| D18039 | 29   | 1   | 1   | - o o  | o o o | - o o | 2 | 1 | 0 | ED  |    |    |    |    |    |    | x  |    |    |
| D19484 | 21   | 13  | 2   | + + -  | + + - | + + - | 1 | 0 | 0 | ED  |    |    | x  |    |    |    |    |    |    |
| D21417 | -54  | -17 | -4  | o o o  | - - + | - - + | 3 | 0 | 0 | TS  | x  |    | x  |    |    |    |    |    |    |

| Ref.   | A   | B   | C   | D  |   |    | E |   |   | F |   |   | T | N | M | Res | CW | OB | Ax | Cx | OT | Li | Bo | Lu | Pl |
|--------|-----|-----|-----|----|---|----|---|---|---|---|---|---|---|---|---|-----|----|----|----|----|----|----|----|----|----|
| D23511 | -24 | -24 | 2   | -  | - | -  | - | - | - | - | - | - | 4 | 2 | 0 | PD  | x  |    |    |    |    |    |    |    |    |
| D24434 | 46  | 28  | 6   | o  | o | o  | o | o | o | + | o | + | 2 | 0 | 0 | PD  |    |    |    |    |    |    | x  |    |    |
| D24640 | -75 | -48 | -48 | ++ | o | ++ | o | o | o | + | o | + | 2 | 0 | 0 | CR  |    |    |    |    |    |    |    | x  |    |
| D24780 | -71 | -71 | 9   | ++ | o | ++ | + | o | + | + | + | + | 4 | 1 | 0 | ND  | x  |    |    |    |    |    |    |    |    |
| D25699 | -70 | -17 | 12  | o  | o | o  | + | + | + | + | + | + | 2 | 0 | 0 | CR  |    |    | x  |    |    |    |    |    |    |
| D26629 | 50  | 13  | 2   | -  | o | -  | o | o | o | - | o | - | 2 | 0 | 0 | PD  | x  |    |    |    | x  |    | x  | x  |    |
| D27398 | 56  | 42  | 10  | +  | o | -  | o | o | o | + | o | - | 2 | 1 | 0 | PR  |    |    | x  |    |    |    |    |    |    |
| D28518 | -66 | -30 | 28  | o  | o | o  | + | - | - | + | - | - | 2 | 0 | 0 | PR  |    |    |    |    |    |    | x  |    |    |
| D30545 | -60 | -11 | 2   | o  | o | o  | - | o | - | - | o | - | ? | ? | ? | PD  | x  | x  | x  | x  |    |    |    |    |    |
| D31400 | 30  | 15  | 1   | +  | o | ++ | o | o | o | + | o | + | 2 | 1 | 0 | PD  | x  |    |    |    |    |    | x  |    |    |
| D31809 | 45  | 27  | 2   | -  | o | -  | o | o | o | - | o | - | 2 | 0 | 0 | PD  |    |    | x  | x  |    |    |    |    |    |
| D32011 | -74 | -74 | -2  | ++ | o | ++ | + | o | + | + | o | + | 4 | 0 | 0 | TS  | x  |    |    |    |    |    | x  |    |    |
| D33476 | 65  | 47  | 28  | ++ | o | ++ | o | o | o | + | + | + | 2 | 1 | 0 | CR  |    |    |    |    |    |    |    | x  |    |
| D33785 | -20 | -20 | -8  | ++ | + | +  | + | + | + | + | + | + | 4 | 1 | 0 | ND  |    |    |    |    |    |    | x  |    |    |
| D34795 | 34  | 24  | 6   | -  | o | -  | o | o | o | - | o | - | 2 | 1 | 0 | ED  |    |    |    |    |    |    | x  |    |    |
| D35892 | 42  | 42  | 5   | -  | o | -  | - | o | - | - | o | - | 4 | 2 | 1 | PD  | x  |    | x  | x  |    |    | x  |    |    |
| D36299 | 25  | 25  | 10  | -  | o | -  | - | o | - | - | o | - | 3 | 0 | 1 | ND  | x  |    |    |    |    |    | x  |    |    |

| Ref.   | A   | B   | C   | D  |   |    | E |   |   | F |   |   | T | N | M | Res | CW | OB | Ax | Cx | OT | Li | Bo | Lu | Pl |
|--------|-----|-----|-----|----|---|----|---|---|---|---|---|---|---|---|---|-----|----|----|----|----|----|----|----|----|----|
| D37729 | -69 | -57 | -21 | o  | o | o  | o | o | o | + | + | + | 3 | 0 | 0 | CR  | x  |    |    |    |    |    |    |    |    |
| D37934 | 17  | 17  | 14  | -  | o | -  | - | o | - | - | o | - | 4 | 1 | 0 | NA  | x  |    | x  |    |    |    |    |    |    |
| D38875 | -70 | -37 | 15  | +  | o | -  | o | o | o | + | o | - | 1 | 1 | 0 | PR  |    |    |    |    |    |    |    | x  | x  |
| D50259 | -60 | -46 | -43 | +  | o | -  | - | - | - | + | - | - | 3 | 0 | 0 | NA  | x  |    |    |    |    |    |    |    |    |
| D51378 | -50 | -34 | -34 | ++ | o | o  | o | o | o | + | o | o | 2 | 0 | 0 | NA  | x  |    |    |    |    |    |    |    |    |
| D55582 | 19  | 8   | 4   | -  | o | -  | o | o | o | - | o | - | 3 | 1 | 0 | PD  | x  |    |    |    |    |    |    |    |    |
| D55660 | -65 | -54 | 10  | ++ | o | ++ | o | o | o | + | o | + | 2 | 1 | 0 | ND  |    |    |    |    |    |    |    | x  |    |
| D56559 | 54  | 45  | 8   | +  | o | ++ | o | o | o | + | + | + | 2 | 1 | 0 | NA  |    |    |    |    |    |    |    |    | x  |
| D57028 | -52 | -21 | 4   | +  | o | ++ | o | o | o | + | o | + | 2 | 0 | 0 | PD  |    |    |    |    |    |    |    | x  |    |
| D57547 | -65 | -31 | -31 | ++ | o | ++ | o | o | - | + | o | + | 2 | 0 | 0 | CR  |    | x  |    |    |    |    | x  | x  |    |
| D57621 | -16 | -16 | 2   | -  | + | -  | - | + | - | - | + | - | 4 | 2 | 0 | PD  | x  |    | x  |    |    |    |    |    |    |
| D57839 | -65 | -49 | 3   | ++ | o | ++ | o | o | o | + | o | + | 2 | 0 | 0 | NA  |    |    |    |    |    |    | x  | x  |    |
| D58766 | 5   | 5   | 5   | +  | + | -  | + | + | - | + | + | - | 4 | 2 | 1 | PD  | x  |    | x  |    |    | x  |    |    |    |
| D59809 | 46  | 26  | 8   | -  | o | ++ | o | o | o | - | o | + | 2 | 1 | 0 | PD  | x  |    |    |    |    |    | x  |    |    |
| D60379 | 39  | 22  | 0   | o  | + | o  | o | o | o | - | + | - | 2 | 0 | 0 | PD  |    |    |    | x  |    |    | x  |    |    |
| D60727 | 52  | 2   | 2   | -  | - | -  | o | o | o | - | - | - | 2 | 0 | 0 | NA  |    |    |    |    |    |    |    |    | x  |
| D62855 | 26  | 2   | 0   | -  | - | -  | o | o | o | - | - | - | 1 | 0 | 0 | ED  | x  |    |    |    |    | x  | x  | x  |    |







| Ref.    | A   | B   | C   | D  | E |    |   | F |   |   | T | N | M | Res | CW | OB | Ax | Cx | OT | Li | Bo | Lu | Pl |
|---------|-----|-----|-----|----|---|----|---|---|---|---|---|---|---|-----|----|----|----|----|----|----|----|----|----|
| E01446  | 33  | 14  | 1   | +  | + | +  | o | o | o | + | + | + | 2 | 0   | 0  | PD |    |    |    |    |    | x  | x  |
| E04222  | -38 | -18 | -2  | ++ | + | ++ | o | o | o | + | + | + | 2 | 1   | 0  | TS |    | x  |    |    |    | x  |    |
| E07884  | -5  | -5  | -5  | ++ | + | ++ | + | + | + | + | + | + | 4 | 1   | 1  | TS | x  | x  |    |    |    | x  |    |
| E09503  | 13  | 3   | 2   | -  | - | -  | o | o | o | - | - | - | 2 | 0   | 0  | NA |    |    |    |    |    |    | x  |
| E10696  | -16 | -16 | 1   | -  | - | -  | - | - | - | - | - | - | 4 | 1   | 0  | PD | x  |    |    |    |    |    |    |
| E11426  | -37 | -22 | -20 | o  | o | o  | - | - | - | - | - | - | 3 | 1   | 0  | SD |    |    |    |    | x  |    |    |
| E11581  | 10  | 10  | 2   | ++ | o | o  | + | o | o | + | o | o | 4 | 0   | 0  | PD | x  |    |    |    | x  |    | x  |
| E12027  | -31 | -31 | -3  | +  | + | ++ | + | + | + | + | + | + | 3 | 1   | 1  | PR | x  |    | x  |    |    |    |    |
| E12573  | 99  | 22  | 19  | o  | o | o  | + | + | + | + | + | + | ? | ?   | ?  | NA |    | x  |    |    | x  |    |    |
| E12760  | -35 | -7  | 0   | +  | + | +  | o | o | o | + | + | + | 3 | 1   | 0  | PD | x  |    |    |    |    | x  |    |
| E12830  | -35 | -26 | -8  | ++ | + | ++ | o | o | o | + | + | + | 3 | 1   | 0  | NA |    |    |    |    |    |    | x  |
| E13198  | 34  | 19  | 5   | o  | o | o  | + | + | - | + | + | - | 2 | 0   | 0  | PD | x  |    |    | x  | x  | x  |    |
| E14266~ | -31 | -31 | -31 | ++ | + | ++ | + | + | + | + | + | + | 2 | 1   | 0  | CR | x  | x  |    |    |    |    |    |
| E15589  | -21 | -6  | -6  | ++ | + | +  | + | o | + | + | + | + | 2 | 1   | 0  | PR | x  |    |    |    | x  |    |    |
| E16199  | -31 | -3  | -3  | ++ | + | -  | + | + | - | + | + | - | 3 | 0   | 0  | TS | x  |    |    |    | x  |    |    |
| E16623  | -33 | -21 | -4  | o  | o | o  | o | o | o | - | - | - | 2 | 0   | 0  | TS |    |    |    |    |    | x  |    |
| E16655  | -23 | -23 | 12  | ++ | + | ++ | + | + | + | + | + | + | 2 | 2   | 1  | SD |    |    |    |    | x  |    |    |

| Ref.   | A   | B   | C   | D  |   |    | E |   |   | F |   |   | T | N | M | Res | CW | OB | Ax | Cx | OT | Li | Bo | Lu | Pl |
|--------|-----|-----|-----|----|---|----|---|---|---|---|---|---|---|---|---|-----|----|----|----|----|----|----|----|----|----|
| E17140 | -32 | -2  | -1  | -  | - | -  | o | o | o | - | - | - | 3 | 1 | 0 | TS  |    |    |    | x  |    |    | x  | x  |    |
| E18184 | -32 | -18 | 15  | ++ | + | ++ | + | - | - | + | + | + | 4 | 0 | 0 | NA  |    |    | x  |    |    |    | x  |    |    |
| E2116  | -40 | -7  | 5   | ++ | - | -  | + | + | - | + | - | - | 3 | 1 | 0 | CR  | x  |    | x  |    |    |    |    | x  |    |
| E21241 | -23 | -8  | 4   | ++ | + | +  | o | o | o | + | + | + | 2 | 1 | 0 | PD  | x  |    |    |    |    |    | x  |    |    |
| E21246 | -26 | -26 | 14  | ++ | + | ++ | o | o | o | + | + | + | 4 | 3 | 0 | PR  | x  |    | x  | x  |    |    |    |    |    |
| E24208 | -28 | -28 | 12  | ++ | + | -  | + | + | - | + | + | - | 4 | 0 | 1 | PR  |    |    |    |    |    |    |    | x  |    |
| E26059 | -16 | -16 | 8   | ++ | + | +  | + | + | + | + | + | + | 4 | 0 | 0 | SD  | x  |    |    |    |    |    | x  |    |    |
| E26362 | -15 | -15 | 11  | ++ | o | o  | + | o | o | + | o | o | 3 | 0 | 0 | PR  | x  |    |    |    |    |    |    |    |    |
| E27007 | -27 | -11 | 3   | ++ | + | +  | - | o | + | + | + | + | 2 | 0 | 0 | PD  | x  |    |    |    |    |    | x  |    |    |
| E28636 | -5  | -5  | -1  | ++ | o | +  | + | o | + | + | o | + | 4 | 3 | 1 | TS  | x  |    | x  | x  |    |    | x  |    |    |
| E29390 | -21 | -21 | 15  | ++ | + | +  | + | + | + | + | + | + | 4 | 0 | 0 | PR  | x  |    |    |    |    |    |    |    |    |
| E29672 | -23 | -23 | -6  | ++ | + | ++ | + | + | + | + | + | + | 4 | 1 | 0 | NA  |    |    |    |    |    |    | x  |    | x  |
| E31893 | -22 | -22 | -22 | ++ | + | ++ | + | + | + | + | + | + | 4 | 0 | 0 | SD  | x  |    |    |    |    |    |    |    |    |
| E34160 | 6   | 6   | 3   | +  | + | ++ | + | + | + | + | + | + | 4 | 3 | 1 | PD  | x  |    | x  | x  |    |    | x  | x  |    |
| E34204 | -15 | -15 | 9   | ++ | + | ++ | + | + | + | + | + | + | 4 | 2 | 0 | SD  | x  |    | x  |    |    |    |    |    |    |
| E34704 | 12  | 7   | 2   | +  | + | ++ | o | o | o | + | + | + | 2 | 1 | 0 | PD  |    |    |    |    | ?  |    | x  |    |    |
| E34780 | -21 | -10 | -5  | ++ | + | ++ | + | o | + | + | + | + | 2 | 0 | 0 | TS  | x  |    |    |    | x  |    |    | x  |    |



| Ref.   | A    | B   | C  | D      | E  |   |   | F |   |   | T | N | M | Res | CW | OB | Ax | Cx | OT | Li | Bo | Lu | Pl |
|--------|------|-----|----|--------|----|---|---|---|---|---|---|---|---|-----|----|----|----|----|----|----|----|----|----|
| E35138 | -10  | -10 | 7  | ++ +   | ++ | + | + | + | + | + | + | 1 | 1 | 0   | SD |    |    | x  |    |    |    |    |    |
| E36386 | -13  | -13 | 4  | + - +  | +  | - | + | + | - | + | + | 4 | 2 | 0   | NA | x  |    | x  |    |    |    |    |    |
| E37176 | -77  | -21 | 10 | o o o  | +  | o | + | o | o | o | ? | ? | ? | SD  | x  |    |    | x  |    |    | x  |    |    |
| E38675 | -17  | -3  | -1 | + - ++ | o  | o | o | + | - | + | 2 | 0 | 0 | TS  |    |    |    |    |    |    | x  |    |    |
| E38747 | 4    | 4   | 4  | ++ +   | +  | + | + | + | + | + | 4 | 2 | 1 | PR  | x  |    | x  |    |    |    | x  |    |    |
| E39221 | -19  | -19 | 2  | + o +  | +  | o | + | + | o | + | 3 | 1 | 0 | PD  | x  |    | x  |    |    |    |    |    |    |
| E40274 | -12  | -4  | 1  | ++ +   | -  | o | o | o | + | + | - | 4 | 0 | 0   | PD |    |    |    |    |    | x  |    |    |
| E43253 | -377 | -17 | -5 | o o o  | +  | - | + | + | - | + | ? | ? | ? | PR  | x  |    |    |    |    |    |    |    |    |
| E44160 | -13  | -13 | 4  | + - -  | +  | - | - | + | - | - | 4 | 1 | 1 | PD  | x  |    | x  |    |    |    | x  |    |    |
| E46035 | -14  | -14 | 8  | + + +  | +  | + | + | + | + | + | 4 | 2 | 0 | SD  | x  |    | x  |    |    |    |    |    |    |
| E46292 | 1    | 1   | 1  | ++ +   | ++ | + | + | + | + | + | 4 | 2 | 1 | ED  | x  |    | x  |    |    |    | x  |    |    |
| E47659 | 11   | 2   | 2  | + + +  | o  | o | o | + | + | + | 2 | 0 | 0 | ED  |    |    |    |    |    |    | x  |    |    |
| E47728 | 3    | 3   | 3  | + - -  | +  | - | - | + | - | - | 4 | 2 | 1 | PD  | x  |    | x  |    |    |    | x  | x  |    |
| E55310 | -16  | -2  | 0  | o o o  | -  | o | - | - | o | - | ? | ? | ? | PD  |    |    | x  |    |    |    |    |    |    |
| E57497 | -8   | -4  | -4 | - - -  | o  | o | o | - | - | - | 2 | 1 | 0 | NA  |    |    |    |    |    |    | x  |    | x  |
| E58565 | -4   | -4  | -3 | ++ +   | ++ | + | + | + | + | + | 4 | 2 | 0 | TS  | x  |    | x  |    |    |    |    |    |    |
| E59327 | -6   | -2  | -1 | ++ +   | ++ | o | o | o | + | + | + | 3 | 1 | 0   | TS | x  |    |    |    |    | x  | x  |    |

| Ref.    | A  | B  | C  | D  |   |    | E |   |   | F |   |   | T | N | M | Res | CW | OB | Ax | Cx | OT | Li | Bo | Lu | Pl |
|---------|----|----|----|----|---|----|---|---|---|---|---|---|---|---|---|-----|----|----|----|----|----|----|----|----|----|
| E59330  | -6 | -6 | -6 | +  | o | -  | + | o | - | + | o | - | 4 | 1 | 0 | PR  | x  |    | x  |    |    |    |    |    |    |
| E59863  | -6 | -6 | -6 | +  | o | +  | + | o | + | + | o | + | 4 | 3 | 1 | PR  | x  |    |    | x  |    |    |    | x  |    |
| E60810~ | 4  | 4  | 4  | +  | o | ++ | + | o | + | + | o | + | 2 | 1 | 0 | ED  | x  |    | x  |    |    |    |    |    |    |
| E61372  | -5 | -5 | -5 | o  | o | ++ | o | o | + | o | o | + | 4 | 0 | 0 | TS  | x  |    |    |    |    |    |    |    |    |
| E61820  | 6  | 6  | 2  | ++ | o | -  | + | o | - | + | o | - | 4 | 1 | 0 | PD  | x  |    | x  |    |    |    |    |    |    |
| E64961  | -1 | -1 | -1 | ++ | o | ++ | + | o | + | + | o | + | 4 | 3 | 1 | TS  | x  |    | x  | x  |    |    |    | x  |    |

\*Hormone treatment started before date of confirmation of relapse

£Receptors assayed between primary treatment and date of start of treatment

~Primary treatment with tamoxifen

? Not known

# APPENDIX 10: PROGESTERONE RECEPTOR - COMPARISON OF IEF AND

## DCC ASSAYS

| Sample<br>Ref | DCC<br>(fmol/ml) | Kd   | IEF<br>(fmol/ml) | pI       |
|---------------|------------------|------|------------------|----------|
| BC 1507a      | 60               | 4.6  | 41 £ *           | 5.2      |
| "             | 60               |      | 27 £ *           | 5.3      |
| "             | 60               |      | 7 £ *            | 5.6      |
| Pool 6        | 15               | 2.27 | 10 £ *           |          |
| " 1           | -ve              |      | 14 £             |          |
| " 0           | -ve              |      | 33 *             | 5.4      |
| " 2           | -ve              |      | 27               |          |
| " 3           | -ve              |      | -68              |          |
| " 4           | -ve              |      | -25              |          |
| " 5           | -ve              |      | -19              |          |
| BC 1457       | 150              | 6    | 126              | 5.5, 7.0 |
| BC 1593       | -ve              |      | 4 £              |          |
| BC 1594a      | 56               | 8    | 19 £ *           | 5.2      |
| BC 1594b      | 160              | 11   | 33               | 5.8      |
| BC 1471a      | 800              | 4.0  | 138              | 5.4      |
| "             | 800              |      | 308              | 5.2      |
| BC 1474       | 420              | 3.0  | 240              | 5.7      |
| "             | 420              |      | 130              | 5.6      |
| BC 1450a      | 310              | 3.6  | 154              | 6.0      |
| "             | 310              |      | 164              | 5.0      |
| BC 1477       | 700              | 7    | 254              | 5.2      |
| "             | 700              |      | 275              | 5.7      |
| BC 1470       | 1500             | 6.7  | 284              | 5.2      |
| "             | 1500             |      | 450              | 5.5      |
| BC 1471b      | 250              | NS   | 195              | 5.0      |
| "             | 250              |      | 249              | 5.2      |

|          |      |      |      |           |
|----------|------|------|------|-----------|
| BC 1635a | 767  | NS   | 93   | 5.0       |
| BC 1635b | 1834 | NS   | 231  | 5.2       |
| "        | 1834 |      | 182  | 5.1       |
| "        | 1834 |      | 106  | 5.2       |
| BC 1507b | 190  | 14   | 145  | 6.3       |
| BC 1619  | 450  | 5.6  | 198  | 6.0       |
| BC 1451  | -ve  |      | -3   |           |
| BC 1478  | 250  | 3.9  | 98   | 6.6       |
| BC 1506  | 866  | 6.9  | 524  | 5.2       |
| Belfast4 | -ve  |      | -5   |           |
| Belfast1 | 57   | NS   | 67   | 5.5       |
| Belfast2 | 254  | NS   | 329  | 6.2       |
| BC 1471c | 1400 | 3.5  | 373  | 5.9       |
| "        | 1400 |      | 462  | 5.8       |
| BC 1567  | -ve  |      | -18  |           |
| BC 1464  | -ve  |      | -10  |           |
| BC 1714  | -ve  |      | 2 £  |           |
| BC 1667  | -ve  |      | -20  |           |
| Belfast5 | -ve  |      | 61   | *         |
| Belfast3 | -ve  |      | 53   | * No peak |
| BC 1640  | -ve  |      | -16  |           |
| BC 1635  | 392  | 2.8  | 199  | 5.8       |
| ID 75    | -ve  |      | 27 £ |           |
| BC 1450  | 1002 | 16.7 | 209  | 4.5       |
| ID 86    | -ve  |      | 26 £ |           |
| BC 1772  | 2826 | NS   | 644  | 6.5, 5.2  |
| ID 35    | 202  | 10.6 | 36   | 5.6       |
| BC 1769  | 508  | NS   | 49   | 5.2       |
| BC 1616  | 2112 | NS   | 543  |           |

|         |      |       |        |      |
|---------|------|-------|--------|------|
| ID 19   | 540  | 3.0   | 201    | 5.3  |
| "       | 540  |       | 245    | 5.3  |
| "       | 540  |       | 136    | 5.3  |
| BC 1588 | 800  | 6     | 433    | 5.2  |
| ID 38   | -ve  |       | 15 £   |      |
| BC 1666 | -ve  |       | 18 £   |      |
| Pool 12 | -ve  |       | 35 *   | 5.0  |
| " 13    | -ve  |       | 22 £   |      |
| " 14    | -ve  |       | 10 £   |      |
| " 15    | -ve  |       | 21 £   |      |
| ID 19   | -ve  |       | 80 *   | 5.3  |
| ID 100  | -ve  |       | -42    |      |
| ID 102  | -ve  |       | -68    |      |
| "       | -ve  |       | -181   |      |
| BC 1635 | 938  | 9.36  | 118    | 5.1  |
| "       | 938  |       | 92     | 5.1  |
| "       | 938  |       | 190    | 5.0  |
| BC 1946 | 727  | 3.79  | 300    | 5.6  |
| "       | 727  |       | 240    | 5.6  |
| BC 1942 | 228  | 16.32 | 103    | 5.1  |
| "       | 228  |       | 41     | 5.75 |
| BC 1911 | 114  | 4.07  | 22 £ * | 5.6  |
| "       | 114  |       | 103    | 5.6  |
| BC 2004 | -ve  |       | 20 £   |      |
| "       | -ve  |       | -31    |      |
| BC 1945 | 1729 | 1.96  | 393    | 5.6  |
| "       | 1729 |       | 361    | 5.6  |
| BC 1914 | 1356 | 3.74  | 429    | 5.75 |
| "       | 1356 |       | 388    | 5.75 |



# APPENDIX 11: ISOELECTRIC FOCUSING OF SERUM WITH 3H-R5020

Total dpm focussed

| A: Serum<br>+3H-R5020 | B: Serum<br>+3H-R5020<br>+NEA | B/A (%) | pI<br>(A, B) |
|-----------------------|-------------------------------|---------|--------------|
| 11753                 | 12752                         | 108.5   | *            |
| 4224                  | 4035                          | 95.5    | *            |
| 26627                 | 13210                         | 49.6    | *            |
| 28400                 | 27212                         | 95.8    | 5.4, 5.4     |
| 28903                 | 27629                         | 94.3    | 4.9, 4.9     |
| 28151                 | 29189                         | 103.7   | 4.9, 4.9     |
| 26040                 | 26025                         | 99.9    | 4.9, 4.9     |
| 7534                  | 12686                         | 168.4   | 4.9, 4.9     |
| 30692                 | 44169                         | 143.9   | 5.4, 4.9     |
| 67101                 | 69814                         | 104     | 4.9, 4.9     |
| 57151                 | 54469                         | 95.3    | 5.4, 4.6     |
| 38445                 | 68005                         | 176.8   | 4.6, 5.4     |
| 7873                  | 9070                          | 115.2   | 5.5, 5.5     |
| 3345                  | 3513                          | 105     | 4.8, 4.4     |
| 526                   | 575                           | 109.3   | 4.9, 4.4     |
| 16292                 | 17592                         | 108     | 4.8, 4.8     |
| 33555                 | 16062                         | 47.2    | *            |
| 42090                 | 34818                         | 81.2    | 4.9, 4.6     |
| 48877                 | 46946                         | 96      | 4.9, 4.9     |
|                       |                               | -----   | -----        |
|                       | Mean                          | 105.7   | 4.95         |
|                       | SD                            | 33.7    | 0.3          |
|                       | SEM                           | 7.7     | 0.06         |

\*pH gradient not measured

APPENDIX 12: EFFECT OF MOLYBDATE ON IEF OF PROGESTERONE  
RECEPTOR

| Sample<br>reference | With molybdate         |                        | Without molybdate      |                        |   |
|---------------------|------------------------|------------------------|------------------------|------------------------|---|
|                     | DCC assay<br>(fmol/ml) | IEF assay<br>(fmol/ml) | DCC assay<br>(fmol/ml) | IEF assay<br>(fmol/ml) |   |
| Belfast 1           | 57                     | 67                     | 92                     | -36                    | * |
| Belfast 2           | 254                    | 329                    | 208                    | 44                     | * |
| Belfast 3           | -ve                    | 53                     | -ve                    | 35                     |   |
| Belfast 4           | -ve                    | -5                     | -ve                    | 10 (-ve)               |   |
| Belfast 5           | -ve                    | 61                     | -ve                    | 24 (-ve)               |   |
| BC 1769             | 508                    | 49                     | 72                     | 6.3                    | * |
| BC 1471             | 55                     | 63                     | 150                    | -31                    | * |
| BC 1757             | -ve                    | -10                    | -ve                    | 23 (-ve)               |   |
| BC 1471             | 1400                   | 373                    | 800                    | -19                    | * |
|                     |                        | 462                    |                        | 52                     |   |
| BC 1471             | 250                    | 195                    | 800                    | 62                     | * |
|                     |                        | 249                    |                        | 69                     | * |
| BC 1635             | 767                    | 93                     | 766                    | 8                      | * |
| BC 1772             | 2826                   | 644                    | 1631                   | 97                     | * |
| BC 1451             | -ve                    | -3                     | -ve                    | 29 (-ve)               |   |

\* Used for calculation of mean (+ve by DCC assay).



APPENDIX 13: AGAROSE vs ACRYLAMIDE GEL FOR IEF OF  
PROGESTERONE RECEPTOR

| Sample            | DCC  | AGAROSE |                   |     | ACRYLAMIDE |                   |          |
|-------------------|------|---------|-------------------|-----|------------|-------------------|----------|
|                   |      | IEF     | IEF/DCC<br>(mean) | pI  | IEF        | IEF/DCC<br>(mean) | pI       |
| BC 2204           | -ve  | 20      |                   |     | -33        |                   |          |
|                   |      | -32     |                   |     | -15        |                   |          |
| BC 1945           | 1729 | 361     | 0.22              | 5.6 | 301        | 0.19              | 6.2      |
|                   |      | 393     |                   | 4.7 | 345        |                   | 5.6      |
| BC 1914           | 1356 | 429     | 0.3               | 5.6 | 198        | 0.11              | 6.4      |
|                   |      | 388     |                   | 4.7 | 108        |                   | 6.8      |
| BC 1946           | 727  | 240     | 0.37              | 5.6 | 352        | 0.49              | 6.5      |
|                   |      | 300     |                   | 4.7 | 360        |                   | 7.2      |
| BC 1942           | 228  | 41      | 0.28              | 5.6 | 66         | 0.48              | 6.4, 7.2 |
|                   |      | 104     |                   | 4.8 | 151        |                   | 6.4, 5.8 |
| BC 1911           | 114  | 22      | 0.6               | 5.6 | 65         | 0.26              | -        |
|                   |      | 103     |                   | 5.6 | -6         |                   | -        |
| BC 1635<br>(14/9) | 1834 | 231     | 0.09              | 5.2 | 148        | 0.06              | 6.6      |
|                   |      | 182     |                   | 5.1 | 82         |                   | 6.6      |
|                   |      | 106     |                   | 5.2 | 72         |                   | -        |
| BC 1635           | 938  | 130     | 0.15              | 5.1 | 61         | 0.08              | 6.9      |
|                   |      | 92      |                   | 5.1 | 99         |                   | -        |
|                   |      | 191     |                   | 5.1 | 62         |                   | 5.1      |
| BC 1507           | 60   | 41      | 0.42              | 5.2 | 59         | 0.7               | -        |
|                   |      | 27      |                   | 5.3 | -4         |                   |          |
|                   |      | 7       |                   | 5.6 | 71         |                   |          |

APPENDIX 14: IEF OF FREE 3H-R5020

| Fraction No.    | Experiment 1 |      | 2     |      | 3     |      |
|-----------------|--------------|------|-------|------|-------|------|
|                 | dpm          | %max | dpm   | %max | dpm   | %max |
| 1               | 13398        | 100  | 33012 | 100  | 33905 | 100  |
| 2 (Application) | 9247         | 69   | 11221 | 34   | 15694 | 46   |
| 3               | 2481         | 19   | 2269  | 7    | 3564  | 11   |
| 4               | 1951         | 15   | 2766  | 8    | 2596  | 8    |
| 5               | 868          | 6    | 1054  | 3    | 1251  | 4    |
| 6               | 254          | 2    | 226   | <1   | 356   | 1    |
| 7               | 262          | 2    | 221   | <1   | 351   | 1    |
| 8               | 177          | 1    | 238   | <1   | 236   | <1   |
| 9               | 310          | 2    | 167   | <1   | 377   | 1    |
| 10              | 150          | 1    | 85    | <1   | 180   | <1   |
| 11 {pI 5.8      | 147          | 1    | 163   | <1   | 188   | <1   |
| 12 {            | 114          | <1   | 197   | <1   | 140   | <1   |
| 13 {            | 101          | <1   | 297   | <1   | 229   | <1   |
| 14 {pI 5.0      | 132          | 1    | 556   | 2    | 308   | <1   |
| 15              | 216          | 2    | 804   | 2    | 1024  | 3    |
| 16              | 461          | 3    | 1833  | 6    | 2623  | 8    |
| 17              | 578          | 4    |       |      |       |      |
| 18              | 1625         | 12   |       |      |       |      |

APPENDIX 15: PRECISION OF ASSAY OF PROGESTERONE RECEPTORS

| Sample<br>reference | Result (fmol/ml) |      |     |     |     |     | Mean  |
|---------------------|------------------|------|-----|-----|-----|-----|-------|
|                     | 1                | 2    | 3   | 4   | 5   | 6   |       |
| BC 1635a            | 210              | 264  | 215 | 159 | 123 | 142 | 185.5 |
| BC 1635b            | 231              | 182  | 106 |     |     |     | 173   |
| BC 1507             | 41               | 27   | 7   |     |     |     | 25    |
| BC 1635c            | 118              | 92   | 190 |     |     |     | 133   |
| BC 1471a            | 249              | 195  |     |     |     |     | 222   |
| BC 1471b            | 138              | 308  |     |     |     |     | 223   |
| BC 1474             | 240              | 130  |     |     |     |     | 185   |
| BC 1450             | 154              | 164  |     |     |     |     | 159   |
| BC 1477             | 254              | 275  |     |     |     |     | 265   |
| BC 1470             | 284              | 450  |     |     |     |     | 367   |
| BC 1471c            | 373              | 462  |     |     |     |     | 418   |
| ID 19               | 201              | 245  | 136 |     |     |     | 194   |
| BC 1945             | 393              | 361  |     |     |     |     | 377   |
| BC 1914             | 429              | 388  |     |     |     |     | 409   |
| BC 1911             | 22               | 103  |     |     |     |     | 62    |
| BC 1942             | 41               | 104  |     |     |     |     | 73    |
| BC 1946             | 300              | 240  |     |     |     |     | 270   |
| ID 192              | 35               | 50   | 33  | 44  | 43  |     | 41    |
| ID 222              | 4006             | 3688 |     |     |     |     | 3847  |
| ID 216              | 140              | 256  |     |     |     |     | 198   |
| ID 201              | 1918             | 2348 |     |     |     |     | 2133  |
| ID 203              | 99               | 136  |     |     |     |     | 118   |
| ID 199              | 4265             | 4395 |     |     |     |     | 4330  |
| ID 171              | 2496             | 2505 |     |     |     |     | 2500  |
| ID 170              | 1563             | 567  |     |     |     |     | 1065  |
| ID 180              | 438              | 622  |     |     |     |     | 530   |
| ID 177              | 1679             | 2336 |     |     |     |     | 2007  |

APPENDIX 16: OESTROGEN RECEPTOR - COMPARISON OF IEF AND THE DCC ASSAY

| SAMPLE   | DCC<br>(fmol/ml) | Kd   | IEF<br>(fmol/ml) | pI       |
|----------|------------------|------|------------------|----------|
| BC 1635a | 200              | 7.87 | 53               | 7.6, 6.1 |
| "        | 200              |      | 59               | 7.4, 6.1 |
| "        | 200              |      | 61               | 7.4, 6.1 |
| "        | 200              |      | 98               | 7.4, 5.7 |
| "        | 200              |      | 177              | 7.6, 6.4 |
| BC 1471  | 14               | NS   | -1               | *        |
| BC 1615  | -ve              |      | 17               | £        |
| BC 1457  | 20               | 1.85 | 33               | 7.6, 5.6 |
| BC 1593  | -ve              |      | 10               | £        |
| BC 1594a | -ve              |      | 11               | £        |
| BC 1588  | 60               | 3.3  | -3               | *        |
| BC 1635b | 130              | 4.98 | 104              | 6.8, 5.4 |
| "        | 130              |      | 58               | 6.4, 5.4 |
| "        | 130              |      | 87               | 6.4, 5.4 |
| "        | 130              |      | 81               | 6.4, 5.4 |
| "        | 130              |      | 86               | 6.4, 5.4 |
| "        | 130              |      | 60               | 6.4, 5.4 |
| BC 1635c | 171              | NS   | 80               | NS       |
| BC 1451  | -ve              |      | 2                | £        |
| BC 1478  | -ve              |      | -20              |          |
| Pool 8   | -ve              |      | 12               | £        |
| BC 1664  | -ve              |      | -9               |          |
| BC 1669  | -ve              |      | 14               | £        |
| BC 1711  | -ve              |      | 5                | £        |
| Pool 5   | -ve              |      | -13              |          |

|          |      |      |        |          |
|----------|------|------|--------|----------|
| BC 1506  | 660  | 10.3 | 198    | 6.8, 5.1 |
| BC 1363  | -ve  |      | -48    |          |
| Pool 4   | 40   | 3.41 | 24     | 6.8, 5.9 |
| Pool 11  | -ve  |      | 7 £    |          |
| Pool 1   | -ve  |      | 9 £    |          |
| Pool 2   | -ve  |      | -15    |          |
| BC 1635d | 270  | 12   | 123    | 7.5, 6   |
| BC 1594b | 15   | 3    | 6 £ *  |          |
| Pool 0a  | -ve  |      | 9 £    |          |
| Pool 0b  | -ve  |      | -4     |          |
| Pool 0c  | -ve  |      | -4     |          |
| Pool 0d  | -ve  |      | -4     |          |
| Pool 0e  | -ve  |      | -16    |          |
| ID 204   | 16   | NS   | 13 £ * |          |
| ID 38    | 150  | NS   | 51     | NS       |
| Yates    | 335  | NS   | 170    | 6.6, 5.4 |
| BC 1450  | 808  | 1.1  | 287    | 6.6, 4.8 |
| BC 1616  | 21   | NS   | -15 *  |          |
| BC 1507  | 2000 | 38   | 1550   | 6.8, 5.6 |
| "        | 2000 |      | 1542   | 6.5, 5.1 |
| "        | 2000 |      | 1649   | 6.5, 5.1 |
| ID 19a   | 1350 | NS   | 1621   | NS       |
| BC 1635e | 574  | 2.67 | 142    | 6.5, 5.2 |
| "        |      |      | 117    | 6.3, 5.2 |
| "        |      |      | 160    | 6.3, 5.2 |
| "        |      |      | 215    | 6.3, 5.2 |
| "        |      |      | 264    | 6.3, 5.2 |
| "        |      |      | 214    | 6.4, 5.2 |

£ -ve result

\* Discordant assessment of receptor status

APPENDIX 17: Wt & cytosol protein concentration of  
needle biopsies

| Sample Wt.<br>(mg) | Protein<br>(g/l) | Sample Wt.<br>(mg) | Protein<br>(g/l) |
|--------------------|------------------|--------------------|------------------|
| 15                 | 8.4              | 30                 | 7                |
| 16                 | 0.8              | 30                 | 20               |
| 16                 | 2.6              | 30                 | 20.4             |
| 17                 | 2.1              | 32                 | 5.6              |
| 19                 | 3.3              | 33                 | 14.2             |
| 20                 | ND               | 34                 | 9.2              |
| 20                 | 2.2              | 35                 | 1.2              |
| 20                 | 4.2              | 35                 | 13.6             |
| 20                 | 12.8             | 35                 | 17.4             |
| 22.6               | 7.2              | 35                 | 30               |
| 23                 | 0.7              | 37.2               | 3.5              |
| 24                 | 4                | 40                 | 5.6              |
| 25                 | 4.2              | 40                 | 6.0              |
| 25                 | 4.8              | 40                 | 10               |
| 25                 | 5.4              | 40                 | 13.6             |
| 25                 | 6.8              | 45                 | 12.8             |
| 25                 | 7                | 51                 | 5.6              |
| 25                 | 8.4              | 60                 | 10               |
| 25                 | 9.6              | 60                 | 15.4             |
| 25                 | 12.8             | 65                 | 10               |
| 25                 | 13.4             |                    |                  |
| 26                 | 1.6              |                    |                  |
| 27.5               | 1.25             |                    |                  |
| 28                 | 8                |                    |                  |

APPENDIX 18: Induction study - Clinical & biochemical data

{ SUMMARY OF DATA AT END OF APPENDIX }

FORMAT

Patients initials              Hospital number              Age  
Previous treatment for cancer of the breast  
First biopsy: Date. Site. Type of biopsy  
Histology: Result. (Report reference number)  
Cytology : Result. (Report reference number)  
Assay reference number. (Type of assay). Sample weight  
Oestrogen receptor result              Kd or pI  
Progesterone receptor result      Kd or pI  
protein concentration  
  
Treatment: Date & dosage  
  
Second biopsy: Date. Site. (Type of biopsy)  
Histology: Result. (Reference number)  
Cytology : Result. (reference number)  
Assay reference number  
Oestrogen receptor  
Progesterone receptor  
Protein concentration  
  
Sites of disease: Assessable (Not assessable)  
Assessment of response: Formal result  
Results: Date of progression or last follow-up. Whether  
alive  
Comment: Heterogeneity of response. Medical conditions

Patient: A. A.

Number: D78554

Age: 61

Previous treatment: Mastectomy + radiotherapy

Biopsy 1: 11/9/81. Skin nodule, chest wall (open biopsy)

Histology: Adenocarcinoma (81/6624)

Cytology : not done

Assay reference BC 1665 (DCC)

REc -ve

RPc 76 fmol/mg       $Kd = 2.17 \times 10^{-10}$

protein concentration = 1.7 g/l

Treatment: Tamoxifen 10 mg b.d. from 22/9/81.

Biopsy 2: 29/9/81. Skin nodule, chest wall (open biopsy)

Histology: Recurrent carcinoma (81/7078)

Cytology : Not done

Assay reference BC 1695 (DCC)

REc -ve

RPc 23 fmol/mg       $Kd = 2.39 \times 10^{-10}$

Areas of disease: Skin infiltration of chest wall, Ribs (2), pubis (2).

Results: Progression of disease on 23/2/82 (increased soft tissue disease)

Assessment of response: Regression

Comment: Initial regression of bulk of soft tissue disease. Slow increase in some nodules. Bone disease remained unchanged over the period of treatment.



Patient: M. Ba-.      Number: B34460      Age: 72

Previous treatment: Tamoxifen from 3/9/79 to 3/7/80.

(Response unknown, no objective disease present).

Biopsy 1: 28.7.81 Skin nodule, chest wall (open biopsy)

Histology: Well-differentiated duct carcinoma (81/5611)

Cytology : Not done

Assay reference ID 35 (IEF)      Sample wt>200

REc -ve \*

RPc 36 fmol/ml = >72 fmol/mg.    pI 5.6

protein content <0.5 g/l

\*simultaneous Scatchard analysis indicated REc = 16

fmol/ml

Treatment: Tamoxifen 10 mg b.d. from 28/7/81

Biopsy 2: 11/8/81. Skin nodule, chest wall (open biopsy)

Histology: Poorly differentiated duct carcinoma  
(81/5888)

Cytology : Not done

Assay reference ID 36 (IEF)      Sample wt = 78 mg

REc -ve

RPc = 69 fmol/ml = 30 fmol/mg      pI 5.6

protein concentration = 2.2 g/l

Sites of disease: Skin nodules (left chest wall),  
(cytologically -ve pleural effusion present)

Assessment of response: Progression

Result: Progression of disease at 25/8/81 (new skin  
nodule, increase in existing lesions).

Patient: J. B.                      Number: 82/3510                      Age: 56

Previous treatment: Radiotherapy & oophorectomy  
(Response unknown)

Biopsy 1: 8/9/82. Primary tumour (needle biopsy)

Histology: Not done

Cytology : Malignant cells (NCS 14952)

Assay reference ID 202 (IEF)                      Sample wt = 28 mg

REc = 156 fmol/ml = 19.5 fmol/mg                      pI 6.5, 5.6

RPc = 431 fmol/ml = 58 fmol/mg                      pI 5.0

Protein concentration = 8 g/l

Treatment: Tamoxifen 160 mg on 9/9/82. then tamoxifen 20  
mg daily

Biopsy 2: 15/9/82. Primary tumour (needle biopsy)

Histology: Not done

Cytology : Malignant cells (NCS 14984)

Assay reference ID 203 (IEF)                      Sample wt = 25 mg

REc not done

RPc(a) = 99 fmol/ml = 24 fmol/mg                      pI 5.9, 5.3

RPc(b) = 136 fmol/ml = 32 fmol/mg                      pI 5.3

Protein concentration = 4.2 g/l

Areas of disease: Primary tumour (T4), Infiltrate of  
skin of chest wall, axillary node, cervical nodes (5).

Assessment of response: Progression of disease  
(increased soft tissue disease)

Results: Progression of disease on 11.1.83

Patient: M. B.                      Number: B49468                      Age: 55

Previous treatment: Radiotherapy

Biopsy 1 : 4/12/80, Primary tumour & Cervical node (open biopsy under GA)

Histology: Primary infiltrating duct carcinoma (80/8711) & secondary carcinoma (80/8710)

Cytology : Not done

Assay reference BC 1304 (DCC)                      Large sample

REc -ve

RPc -ve

protein concentration =

Treatment: Tamoxifen 40 mg qds on 21/4/81. then tamoxifen 20 mg o.d..

Biopsy 2 : Cervical node, 28/5/81

Histology: Secondary carcinoma (81/3995)

Cytology : Not done

Reference BC 1527 (DCC)

REc -ve

RPc -ve

protein concentration = 1.4 g/l

Sites of disease: Left axilla, cervical node, lung metastasis

Results: Progression noted on 8/9/81

Assessment of response: Progression (new lung metastasis)

Comment: All areas of disease increased

Patient: E. B.            Number: E64961            Age: 62

Previous treatment: None

Biopsy 1 : 8/9/82, Primary tumour (needle biopsy)

Histology: Infiltrating duct carcinoma (82/6217)

Cytology : Not done

Assay reference ID 200 (IEF)            Sample wt = 25 mg

REc = 984 fmol/ml = 144 fmol/mg            pI 6.8

RPc = 431 fmol/ml = 63 fmol/mg            pI 5.8

protein concentration = 6.8 g/l

Treatment: Tamoxifen 20 mg b.d. from 24/9/82 to 27/9/82  
then tamoxifen 20 mg od.

Biopsy 2 : 5/10/82, Primary tumour (needle biopsy).

Histology: Not done

Cytology : Malignant cells (NCS 15109)

Assay reference ID 215 (IEF)            Sample wt = 40 mg

REc not done

RPc(a) -ve

RPc(b) -ve

protein concentration 5.6 g/l

Sites of disease: Primary tumour, cervical nodes,  
ischium & L2 vertebra, (diffuse infiltration of the  
axilla)

Assessment of response: Progressive disease

Results: Progression noted on 1/2/83 (New skin  
infiltration, primary increased). Alive 3/1/1984

Patient: A. B.                      Number: E61372                      Age 74

Previous treatment: None

Biopsy 1: 25/5/82. Primary tumour (needle biopsy)

Histology: Infiltrating ?lobular carcinoma (82/3913)

Cytology : Malignant cells (NCS 14384)

Assay reference ID 155 (IEF)                      Sample wt = 34 mg

REc = 117 fmol/ml = 12.7 fmol/mg.    pI 6.8, 5.8

RPc = 58 fmol/ ml = 6.3 fmol/mg.    pI 5.8

protein concentration = 9.2 g/l

Treatment: Tamoxifen 40 mg from 26/5/82. then tamoxifen  
20mg o.d.

Biopsy 2: 1/6/82. Primary tumour (needle biopsy)

Histology: Not done

Cytology : Malignant cells (NCS 14396)

Assay reference ID 170 (IEF)                      Sample wt = 45 mg

REc not done.

RPc(a) = 567 fmol/ml = 44 fmol/mg.                      pI 5.2

RPc(b) = 1562 fmol/ml = 122 fmol/mg                      pI 5.2

protein concentration = 12.8 g/l

Sites of disease: Primary tumour (T4) with skin  
infiltration

Results: Last seen: 10/1/84, disease unchanged

Assessment of response: Unchanged

Comment: Sustained but small reduction in size of  
tumour, with improved mammographic appearance.

Patient: M.B.                      Number: E34792                      Age 70

Previous treatment: Mastectomy & radiotherapy

Biopsy 1: 25.6.82. Parasternal mass (needle biopsy)

Histology: Metastatic lobular carcinoma (82/4656)

Cytology : Malignant cells (NCS 14544)

Assay reference ID 173 (IEF)                      Sample wt = 12 mg

REc = 724 fmol/ml = 71 fmol/mg                      pI 6

RPc = 478 fmol/ml = 47 fmol/mg                      pI 5.9, 5.2

protein concentration = 10.2 g/l

Treatment: Tamoxifen 40 mg qds on 26/6/82 then tamoxifen  
20 mg o.d.

Biopsy 2: 2/7/82. Parasternal mass (needle biopsy)

Histology: Not done

Cytology : Malignant cells (NCS 14590)

Assay reference ID 180 (IEF)                      Sample wt = 28 mg

REc not done

RPc(a) = 438 fmol/ml = 31 fmol/mg                      pI 5.5

RPc(b) = 622 fmol/ml = 44 fmol/mg                      pI 5.4

protein concentration = 14 g/l

Sites of disease: Parasternal mass, scar nodule, scalp  
nodules (3), lung metastasis.

Result: Progression noted on 21/9/82 (New lung  
metastasis, new cervical nodes)

Assessment of response: Progression of disease

Comment: Mixed response. The lesion which was biopsied  
regressed. She developed a new lung metastasis &

cervical nodes, which have remained stable since then.

Patient: B. B.                      Number: 82/3468                      Age 58

No previous treatment

Biopsy 1: 12/5/82. Skin infiltration (open biopsy)

Histology: Infiltrating duct carcinoma (2635/82).

Cytology : Not done

Reference not allocated (IEF)                      Sample wt 450mg

REc -ve

RPc = 54 fmol/ml = 3.3 fmol/mg                      pI 5.2

protein concentration = 16 g/l

Treatment: Tamoxifen 40 mg qds on 13/5/82 then 20 mg  
o.d.

Biopsy 2: 19/5/82. Skin infiltration (open biopsy)

Histology: Infiltrating duct carcinoma (2811/82).

Cytology : Not done

Reference not allocated (IEF)                      Sample wt 750 mg

REc not done

RPc(a) = 362 fmol/ml = 13 fmol/mg                      pI 5.5

RPc(b) = 409 fmol/ml = 15 fmol/mg                      pI 5.5

protein concentration = 27.6 g/l

Sites of disease: Primary tumour (T4), axillary  
infiltration, cervical nodes (2).

Assesment of disease: Partial regression

Result: Progression noted on 5/1/83.

Comment:



Patient: M. Bow.(a)                      Number: D74643                      Age 46

Previous treatment: Mastectomy

Biopsy 1: 5/2/81. i) subcutaneous nodule, chest wall (open biopsy)  
ii) metastasis, right iliac crest (trephine)

Histology: i) adenocarcinoma (81/1000)  
ii) metastatic carcinoma (81/999)

Cytology: Not done

Assay reference (i) BC 1378 (DCC)                      Sample wt >200 mg

REc -ve  
RPC -ve  
protein concentration = 1.5 g/l

Assay reference (ii) Not allocated (IEF)                      wt = 40 mg

REc -ve  
RPC -ve  
protein not measured

Treatment: From 6/2/81. Tamoxifen 10 mg b.i.d until biopsy 2, and then oophorectomy.

Biopsy 2: 19/2/81. Right iliac crest (trephine)

Histology: Metastatic infiltrating duct carcinoma (81/1371,2)

Cytology : Not done

Assay reference not allocated (IEF)                      Sample wt = 68 mg

REc -ve  
RPC = 69 fmol/ml                      pI 5.8  
Protein not measured

Sites of disease: Right iliac crest, spine (L4, L1, D12).

Result: Progression @ 6/4/82 (New soft tissue disease).

Assessment of response: Unchanged

Comment: Some sclerosis and slight reduction in activity on bone scan was noted, but not amounting clearly to regression.

Patient: M. Bow.(b)

Number: D74643

Age 47

Previous treatment: Mastectomy. Oophorectomy (see M. Bow(a)).

Biopsy 1: 14/4/82. Subcutaneous metastasis, chest wall  
(needle biopsy)

Histology: Not done

Cytology : Malignant cells (NCS 14178)

Assay reference ID 133 (IEF)

Sample wt = 33 mg

REc = 203 fmol/ml = 14.3 fmol/mg

pI 5.8, 6.6

RPc -ve

protein concentration = 14.2 g/l

Treatment: Tamoxifen 40 mg q.d.s. on 15/4/82 and then  
tamoxifen 20 mg o.d.

Biopsy 2: 27/4/82 Subcutaneous metastasis, chest wall  
(needle biopsy).

Histology: Not done

Cytology : Malignant cells (NCS 14232)

Assay reference ID 144 (IEF)

Sample wt = 20 mg

RPc = 34 fmol/ml = 8.1 fmol/mg

pI 5.0

protein concentration = 4.2 g/l

Sites of disease: Chest wall (2), (right iliac crest & spine  
not assessable due to sclerosis).

Results: Last seen 10/1/84, in remission.

Assessment of response: Partial regression

Comment : Complete regression of soft tissue disease, with  
persistent evidence of sclerotic bone metastases.

Patient: I. B.

Number: E31893

Age 67

Previous treatment: None

Biopsy 1: 11/12/80. Primary tumour (open biopsy)

Histology: Infiltrating duct carcinoma (80/8905,6)

Cytology : Not done

Assay reference BC 1311 (DCC)

Sample wt > 200 mg  
-10

REc = 300 fmol/ml = 214 fmol/mg

Kd =  $0.9 \times 10^{-10}$

RPc = 700 fmol/ml = 500 fmol/mg

Kd =  $2.6 \times 10^{-10}$

protein concentration = 1.4 g/l

Treatment: Tamoxifen 10 mg b.i.d from 23/12/80

Biopsy 2: 21/1/81. Primary tumour (open biopsy)

Histology: Infiltrating lobular carcinoma (81/0570)'

Cytology : Not done

Assay reference BC 1348 (DCC)

Sample wt > 200 mg

REc -ve

RPc = 900 fmol/ml = 1285 fmol/mg

Kd =  $1.6 \times 10^{-10}$

protein concentration = 0.7 g/l

Sites of disease: Primary tumour, (L1).

Assessment of response: Unchanged

Result: Last seen 25/10/83, no progression.

Comment : Sustained but small reduction in clinical measurements.

Patient: E. C.

Number: E28636, 81/8195

Age 67

No previous treatment.

Biopsy 1: 30/10/81. Bilateral primary tumours (open biopsy under GA. Excision biopsy on right side, incision biopsy on left side)

Histology: Left, infiltrating duct carcinoma (6039/81).  
Right, infiltrating ?metastatic carcinoma (6040/81)

Cytology : Not done

Assay reference BC 1742 (DCC)

Large sample

RE = 503 fmol/ml = 95 fmol/mg

Kd not given

RP = 127 fmol/ml = 15 fmol/ mg

Kd =  $7.7 \times 10^{-10}$

protein = 5.3 g/l

Treatment: Tamoxifen 40 mg qds on 14/11/81, then tamoxifen 10 mg b.i.d

Biopsy 2: 24/11/81. Left breast primary tumour, needle biopsy.

Histology: Not done

Cytology : Malignant cells (NCS 13534)

Assay reference ID 61 (IEF)

27.5 mg

RE = 25 fmol/ml = 20 fmol/mg

pI 7, 5.4

RP = 127 fmol/ml = 102 fmol/mg

pI 5.5

protein content = 1.25 g/l

Sites of disease: Left primary, axillary node, cervical nodes (2)

Assessment of response: Progression

Result: Progression of disease on 19/1/82 (new cervical node)

Comment : The lesion which was biopsied had remained unchanged.

Patient: C. C.

Number: E61820

Age: 84

No previous treatment

Biopsy 1: 26/5/82. Primary tumour (needle biopsy)

Histology: Infiltrating carcinoma (82/3938)

Cytology : Smear unsatisfactory (NCS 14397)

Assay reference ID 156 (IEF) 25 mg

RE = 2329 fmol/ml =242 fmol/mg pI 5.8, 5.0

RP -ve

protein content = 9.6

Treatment: Tamoxifen 10 mg b.i.d from 8/6/82

Biopsy 2: 29/6/82. Primary tumour (needle biopsy)

Histology: Not done

Cytology : Malignant cells (NCS 14566)

Assay reference ID 174 (IEF) 30 mg

RP(a) -ve

RP(b) -ve

protein content = 19.6 g/l

Sites of disease: Primary tumour (T4), axillary node, skin nodules (2)

Assessment of response: Progression

Result: Progression of disease on 31/8/82 (new skin nodules)

Patient: A. E.

Number: E60810

Age: 82

Biopsy 1: 25/5/82. Primary tumour (needle biopsy)

Histology: Papillary carcinoma (82/3921)

Cytology : Malignant cells (NCS 14383)

Assay reference ID 157 (IEF) 24 mg

RE = 84 fmol/ml = 21 fmol/mg pI 6.8, 5.7

RP = 1042 fmol/ml = 260 fmol/mg pI 5.5

protein content = 4.0 g/l

Treatment: Tamoxifen 10 mg b.i.d from 26/5/82

Biopsy 2: 15/6/82. Primary tumour (needle biopsy)

Cytology : Malignant cells (NCS 14497)

Assay reference ID 171 (IEF) 35 mg

RP(a) = 2804 fmol/ml = 161 fmol/mg pI = 5.5

RP(b) = 2494 fmol/ml = 143 fmol/mg pI = 5.5

protein = 17.4 g/l

Sites of disease: Primary tumour (T2), axillary node

Assessment of response: Not assessable

Result: Last seen 10/8/82, disease unchanged. Died 4/9/82.

Comment: An elderly lady with insulin dependent diabetes, and gross cardiac failure. Death was due to ischaemic heart disease, with diabetes contributing.

Patient: D. F.

Number: E31460

Age 78

No previous treatment.

Biopsy 1: 17/12/80. Skin infiltration, chest wall (open biopsy)

Histology: Infiltrating duct carcinoma (80/9064)

Cytology : Not done

Assay reference BC 1316 (DCC)

Large sample

RE = 26 fmol/ml = 52 fmol/ mg

Kd =  $1.6 \times 10^{-10}$

RP = 340 fmol/ ml = 680 fmol/mg

Kd =  $4.0 \times 10^{-10}$

protein = 0.5 g/l

Treatment: Tamoxifen 10 mg b.i.d from 6/1/81. Tamoxifen increased to 20 mg b.i.d on 27/1/81 and Prednisolone 5 mg added.

Biopsy 2: 27/1/81. Skin infiltration, chest wall (open biopsy)

Histology: Infiltrating duct carcinoma (81/0756)

Cytology : Not done

Assay reference BC 1376 (DCC)

Large sample

RE -ve

RP -ve

protein content = 0.9 g/l

Sites of disease: Infiltration of skin of chest wall, primary tumour (T4), axillary nodes (N2), cervical nodes, infiltration of lung.

Assessment of response: Progression

Result: Progression of disease at 17/2/81, died 11/7/81

Comment:

Patient: E. F.

Number: E47728

Age 69

No previous treatment

Biopsy 1: 29.9.81. Skin nodule (open biopsy)

Histology: Metastasis from breast carcinoma (81/7077)

Cytology : Not done

Assay reference BC 1698 (DCC)

Large sample

RE = 22 fol/ml = 15 fmol/mg

Kd =  $3.9 \times 10^{-10}$

RP -ve

protein content = 1.5 g/l

Treatment: Tamoxifen 10mg b.i.d from 29/9/81

Biopsy 2: 6/10/81 Skin nodule (open biopsy)

Histology: Undifferentiated carcinoma (81/7280)

Cytology : Not done

Assay reference BC 1705 (DCC)

Large sample

RE -ve

RP -ve

protein content = 1.0 g/l

Sites of disease: Multiple nodules over chest wall, primary tumour (T4), lung infiltration.

Assessment of response: Progression

Result: Progression on 1/12/81 (increased lung infiltration, increased soft tissue disease).

Comment:



Patient: C. F.

Number: E38797

Age: 76

No previous treatment

Biopsy 1: 26/3/81. Primary tumour (open biopsy)

Histology: Infiltrating duct carcinoma (81/2389)

Cytology : Not done

Assay reference BC 1500 (DCC)

Large sample

RE = 775 fmol/ml = 267 fmol/ mg

Kd =  $3.26 \times 10^{-10}$

RP = 101 fmol/ml = 28 fmol/ mg

Kd =  $18.3 \times 10^{-10}$

protein content = 3.5 g/l

Treatment: Tamoxifen 10 mg b.i.d. from 8/4/81

Biopsy 2: 30/4/81. Primary tumour.

Histology: Infiltrating duct carcinoma (81/3248)

Assay reference BC 1501 (DCC)

Large sample

RE = 41 fmol/ml = 12 fmol/mg

Kd =  $8.5 \times 10^{-10}$

RP = 549 fmol/ml = 161 fmol/mg

Kd =  $9.9 \times 10^{-10}$

protein content = 3.4 g/l

Sites of disease: Primary tumour (T4), axillary node,  
(diffuse bone disease)

Assessment of response: Partial regression

Result: Patient died 2/7/81, after sudden cardiovascular  
collapse & cardiac arrest, following a small traumatic  
haemopneumothorax. Regression noted before death and  
confirmed by pathologists measurements at post-mortem  
(Report no. 81/201).

Comment: An elderly lady on treatment for cardiac failure  
and angina. Death was due to ischaemic heart disease, with  
trauma and diffuse rib metastases as contributing factors.

Patient: E.G

E15589

Age 68

Previous mastectomy + radiotherapy

Biopsy 1: 26/2/82. Skin nodule (open biopsy)

Histology: Infiltrating duct carcinoma (82/1653)

Cytology : Not done

Assay reference ID 111 (IEF) wt = 195 mg

RE = 94 fmol/ml = 8 fmol/mg pI 6.4

RP = 460 fmol/ml = 39 fmol/mg pI 6.3, 5.5

protein = 11.8 g/l

Treatment: Tamoxifen 40 mg qds on 27/2/82, then 20 mg o.d.

Biopsy 2: 5/3/82. Skin nodule (open biopsy)

Histology: Carcinoma of breast origin (82/1864)

Cytology : Nt done

Assay reference ID 112 (IEF) wt = 239 mg

RE = 142 fmol/ml = 9 fmol/mg pI 6.4

RP = 1590 fmol/ml = 103 fmol/mg pI 6.3, 5.6

protein = 15.2 g/l

Sites of disease: skin nodules (3), L4, (other bone lesions)

Assessment of response: Partial regression

Results: Progression 2/2/83

Comment: 'Flare' of bone pain occurred with treatment.

Patient: E. G.

Number E10696

Age: 86

Previous treatment: Radiotherapy

Biopsy 1: 12/1/82. Primary tumour (needle biopsy)

Histology: Not done

Cytology : Malignant cells (NCS 13770))

Assay reference ID 87 (IEF)

Sample wt = 51 mg

REc -ve

RPc -ve

protein concentration 5.6 g/l

Treatment: Tamoxifen 40 mg q.d.s on 13/1/81 followed by  
tamoxifen 10mg b.i.d.

Biopsy 2: Primary tumour (needle biopsy).

Histology: Not done

Cytology : Malignant cells (NCS 13838)

Assay reference ID 97

Sample wt = 26 mg

REc -ve

RPc -ve

protein concentration = 1.6 g/l

Sites of disease: Primary tumour (T4).

Assessment of response: Progression

Result: Progression of disease at 22/2/82 (Increase in size  
of primary tumour, with ulceration & haemorrhage leading to  
mastectomy).

Patient: A. Ham.

Number: B73501

Age: 72

Previous treatment: Mastectomy

Biopsy 1 24/3/82. Infraclavicular mass (needle biopsy)

Histology: Carcinoma, consistent with breast primary (82/2383)

Cytology : Malignant cells (NCS 14107)

Assay reference ID 124 (IEF)                      Sample wt = 25 mg

REc = 623 fmol/ml = 89 fmol/mg                      pI 7, 5.9

RPc = 55 fmol/ml = 8 fmol/mg                      pI 5.9

protein concentration = 7 g/l

Treatment: Tamoxifen 40 mg qds on 6/4/82, then Tamoxifen 10 mg bid

Biopsy 2: Infraclavicular mass (needle biopsy)

Histology: Not done

Cytology : Malignant cells (NCS 14179)

Assay reference ID 134 (IEF)                      wt = 40 mg

RE = 210 fmol/ml = 35 fmol/mg                      pI 7.6, 5.2

RP = 101 fmol/ml = 17 fmol/mg                      pI 5.3

Sites of disease: Infraclavicular mass

Assessment of response: Complete regression

Results: No progression of disease when last seen

Comment:

Patient: B. H.                      Number: E59327                      Age: 59  
 Previous treatment: Mastectomy & radiotherapy for T3,N1 carcinoma  
 Biopsy 1: 28/9/82. Skin nodule (open biopsy)  
 Histology: Carcinoma (82/6685)  
 Cytology : ND  
 Assay reference ID 216      (IEF)      Sample wt = 80 mg  
 REc not done.  
 R<sub>Pc</sub>(a) = 256 fmol/ml = 22 fmol/mg                      pI 5.9  
 R<sub>Pc</sub>(b) = 140 fmol/ml = 12 fmol/mg                      pI 5.2  
 protein concentration = 11.6 g/l.  
  
 Treatment: Tamoxifen 40 mg qds on 29/9/82, then tamoxifen 20  
 mg od  
  
 Biopsy 2: 5/10/82. Skin nodule, chest wall (open biopsy)  
 Histology: Metastatic carcinoma (82/6852)  
 Cytology : Not done  
  
 Assay reference ID 222      (IEF)      Sample wt = 135 mg  
 REc not done.  
 R<sub>Pc</sub>(a)= 3688 fmol/ml = 233 fmol/mg                      pI 6  
 R<sub>Pc</sub>(b)= 4006 fmol/ml = 254 fmol/mg                      pI 6  
 protein concentration = 15.8 g/l  
  
 Sites of disease: Infraclavicular mass, (diffuse  
 infiltration of scar), (cervical node).  
 Assessment of response: Progression of disease  
 Results: Progression of disease noted on 18/1/83. Died  
 11.8.83  
 Comment: Local disease progressed also.

Patient: A. Har.                      Number: D21417                      Age: 65

Previous treatment: Left mastectomy. Tamoxifen 22/2/78 to 29/8/79 as adjuvant after local excision of a carcinoma from the right breast. Right mastectomy for progression of right sided tumour.

Biopsy 1: 19/4/82. Skin nodule, right chest wall (open biopsy)

Histology: Infiltrating lobular carcinoma (82/2949)

Assay reference BC 1929 (DCC)      Sample wt > 200 mg

REc -ve

RPc = 156 fmol/mg                      Kd =  $5.48 \times 10^{-10}$

protein concentration = 1.8 g/l

Treatment: Tamoxifen 40 mg qds on 27/4/82, then tamoxifen 10 mg bid.

Biopsy 2: 4/5/82. Skin nodule, right chest wall (open biopsy)

Histology: Carcinoma (82/3352)

Assay reference ID 150 (IEF)      Sample wt = 245 mg

REc = 270 fmol/ml = 9.2 fmol/mg                      pI n/a

RPc = 2541 fmol/ml = 87 fmol/mg                      pI n/a

\*  
Converted RPc = 274

protein concentration = 29.2 g/l

Sites of disease: Skin nodules right chest wall (2), diffuse infiltration of axilla.

Assessment of response: Progression of disease (new lung metastasis)

Results: Progression of disease noted on 5/10/82

Comment: Small reduction in measurements of soft tissue disease.

\* See Methods, p81

Patient: E. H.                      Number: A57699                      Age: 67

No previous treatment

Biopsy 1: 7/7/82. Primary tumour (needle biopsy)

Histology: Duct carcinoma (82/4895)

Cytology : Malignant cells (NCS 14627)

Assay reference ID 179    (IEF)    Sample wt = 15 mg

REc -ve

RPc -ve

protein concentration = 8.4 mg/ml

Treatment: Tamoxifen 40 mg qds on 13/7/82, then tamoxifen 10 mg bid.

Biopsy 2: 23/7/82. Primary tumour (needle biopsy)

Histology: Not done

Cytology : Malignant cells (NCS 14728)

Assay reference ID 190    (IEF)    Sample wt = 60 mg

REc not done

RPc(a) -ve

RPc(b) -ve

protein concentration = 15.4 g/l

Sites of disease: Primary tumour (T4), Axillary nodes (N2).

Assessment of response: No change

Results: Progression of disease on 15/2/83 (increase in primary tumour)

Comment:

Patient: M. H.                      Number: C10358                      Age: 86

No previous treatment.

Biopsy 1: 14/6/82. Primary tumour (needle biopsy)

Histology: Ductal carcinoma (82/4355)

Cytology : Malignant cells (NCS 14474)

Assay reference ID 176      (IEF)      Sample wt = 20 mg

REc = 512 fmol/ml = 40 fmol/mg      pI 6.3

RPc = 1035 fmol/ml = 81 fmol/mg      pI 5.4

protein concentration = 12.8 g/l

Treatment: Tamoxifen 10 mg b.i.d from 15/6/82

Biopsy 2: 6/7/82. Primary tumour (needle biopsy)

Histology: Not done

Cytology : Malignant cells (NCS 14604)

Assay reference ID 177      (IEF)      Sample wt = 30 mg

RPc(a) = 1679 fmol/ml = 82 fmol/mg                      pI 6

RPc(a) = 2609 fmol/ml = 128 fmol/mg                      pI 5.6

protein concentration = 20.4 g/l

Sites of disease: Primary tumour (T4), axillary node.

Assessment of response: Partial regression

Results: Last seen 20/9/83 (no progression of disease)

Comment: Also had untreated basal cell carcinoma (confirmed histologically) of right axilla and right side of neck.



Patient: K. K.

Number: 81/5264

Age: 62

Previous mastectomy & radiotherapy

Biopsy 1: 23/6/82. Subcutaneous scar recurrence (needle biopsy)

Histology: Not done

Cytology : Malignant cells (NCS 14530)

Assay reference ID 181 (IEF) Sample wt = 30 mg

REc -ve

RPC -ve

protein concentration = 20 g/l

Treatment: Tamoxifen 40 mg qds on 24/6/82, then tamoxifen 20 mg od.

Biopsy 2: 14/7/82. Subcutaneous scar recurrence (needle biopsy)

Histology: Not done

Cytology : Malignant cells (NCS 14668)

Assay reference ID 182 (IEF) Sample wt = 25 mg

REc -ve

RPC -ve

protein concentration = 13.4 g/l

Sites of disease: Scar recurrence.

Assessment of response: Progression

Results: Progression of disease on 14/7/82 (increased soft tissue disease).

Comment: New lung and liver metastases confirmed one week after local progression noted.

Patient: G. L.

Number: E39221

Age: 81

No previous treatment:

Biopsy 1: 31/3/81. Primary tumour (open biopsy)

Histology: Grade I infiltrating duct carcinoma (81/2516)

Cytology : Not done

Assay reference BC 1455 (DCC)      Sample wt > 200 mg  
REc = 20 fmol/ml = 9 fmol/mg       $Kd = 7.1 \times 10^{-10}$   
RPc = 44 fmol/ml = 20 fmol/mg       $Kd = 23 \times 10^{-10}$   
protein concentration = 2.2 g/l

Treatment: Tamoxifen 40 mg qds on 1/4/81, then 10 mg bid

Biopsy 2: 30/4/81. Primary tumour (needle biopsy)

Histology: Not done

Cytology : Not done

Assay reference ID 122 (IEF)      Sample wt = 40 mg  
REc = 110 fmol/ml = 11 fmol/mg      pI 5.9  
RPc = 59 fmol/ml = 6 fmol/mg      pI 6.4, 5.0  
\*  
Converted RPc = 24 fmol/mg  
protein concentration = 10 g/l

Sites of disease: Primary tumour (T4), axillary nodes (N2)

Assessment of response: Progression of disease.

Results: Progression of disease on 5/6/81 (increased primary & axillary disease). Alive 20/12/83

\* See Methods, p 81

Patient: S. L.                      Number: 80/0874                      Age: 52  
Previous treatment: Mastectomy. Radiotherapy & x-ray menopause  
for local recurrence  
Biopsy 1: 16/12/81. Axillary mass (needle biopsy)  
Histology: Not done  
Cytology : Malignant cells (NCS 13663)

Assay reference ID 78            (IEF)            Sample wt = 22.6 mg  
REc = 1601 fmol/ml = 222 fmol/mg                      pI 6.9, 5.8, 5.2  
RPc = 35 fmol/ml = 5 fmol/mg                      pI 6  
protein concentration = 7.2 g/l                      (poor pH gradient)

Treatment: Tamoxifen 40 mg qds on 30/12/81, then 10 mg bid.

Biopsy 2: 13/1/82. Axillary mass (needle biopsy)  
Histology: Not done  
Cytology : Malignant cells (NCS 13772)

Assay reference ID 89            (IEF)            Sample wt = 17 mg  
REc -ve  
RPc -ve  
protein concentration = 2.1 g/l

Sites of disease: Axillary mass.

Assessment of response: Progression

Results: Progression of axillary mass confirmed on 1/7/82.

Comment: Progression was suggested by measurements on 10/3/82,  
but follow-up in May & June was poor, and no measurements were  
taken until July, when progression was confirmed. The later date  
was used in analysis.

Patient: J. M.                      Number: 81/0712                      Age: 62

Previous treatment: Radiotherapy & x-ray menopause for Stage III  
tumour

Biopsy 1: 16/12/81. Skin nodule (open biopsy)

Histology: Carcinoma (6969/81)

Cytology : Not done

Assay reference ID 80                      (IEF)                      Sample wt = 134 mg

REc -ve

RPc -ve

protein concentration = 4.65 g/l

Treatment: Tamoxifen 40 mg qds on 16/12/81, then 10 mg bid.

Biopsy 2: 23/12/81. Skin nodule (open biopsy)

Histology: Adenocarcinoma (7105/81)

Cytology : Not done

Assay reference ID 81                      (IEF)                      Sample wt = 101 mg

REc -ve

RPc -ve

protein concentration = 1.45 g/l

Sites of disease: Skin nodules, confluent infiltration of chest  
wall, (marrow trephine +ve)

Assessment of response: Progression

Results: Progression of disease noted on 13/1/82 (increase soft  
tissue disease)

Comment:

Patient: F. M.

Number: C84327

Age: 65

Previous treatment: Mastectomy.

Biopsy 1: 20/7/82. Axillary mass (open biopsy)

Histology: metastatic carcinoma (81/5393)

Cytology : Not done

Assay reference BC 1597 (DCC)      Sample wt > 200 mg  
-10

REc = 1115 fmol/mg

Kd =  $8.1 \times 10^{-10}$

RPc -ve

protein concentration = 2.7 g/l

Treatment: Tamoxifen 10 mg bid from 4/8/81

Biopsy 2: 18/8/81. Axillary mass (needle biopsy)

Histology: not done

Cytology : Malignant cells (UHSM C81/0814)

Assay reference ID 41 (IEF)      Sample wt = 37.2 mg

REc = 61 fmol/ml = 17.5 fmol/mg

pI 6.4

RPc -ve

protein concentration = 3.5 g/l

Sites of disease: Axillary mass, (Bone marrow trephine +ve,  
with anaemia)

Assessment of response: No change

Results: Progression of disease confirmed on 15/6/82

(increased soft tissue disease, with new lesions)

Comment: Minor reduction in measurements of axillary mass,  
with improvement of anaemia. Died 28/10/82

Patient: R. M.

Number: E53175

Age: 49

Previous treatment: Mastectomy

Biopsy 1: 1/3/82. Axillary mass (needle biopsy)

Histology: Infiltrating duct carcinoma (82/1651)

Cytology : Malignant cells (NCS 13988)

Assay reference ID 110 (IEF) Sample wt = 20 mg

REc -ve

RPc -ve

protein concentration = 2.2 g/l

Treatment: Tamoxifen 40 mg qds on 16/3/82, then 10 mg bid.

Biopsy 2: 23/3/82. Axillary mass (needle biopsy)

Histology: Not done

Cytology : Malignant cells (NCS 14097)

Assay reference ID 126 (IEF) Sample wt = 35 mg

REc not done

RPC(a) = 2197 fmol/ml = 73 fmol/mg pI 6.3, 5.2

RPc(b) = 2218 fmol/ml = 74 fmol/mg pI 6.3, 5.2

protein concentration = 30 g/l

Sites of disease: Axillary mass

Assessment of response: Classed as 'No progression' (see Comment)

Results: Progression of disease confirmed 11/3/83 (new skin nodules). Last seen 11/10/83

Comment: Follow-up carried out at another institution, without formal measurements but the mass was said to be 'reduced', with normal bone scan 6 months after start of treatment.

Patient: M. M.

Number: D32011

Age: 60

Previous treatment: Mastectomy

Biopsy 1: 17/8/82. Metastasis of chest wall (needle biopsy)

Histology: Not done

Cytology : Malignant cells (NCS 14819)

Assay reference ID 197 (IEF) Sample wt = 32 mg

REc = 121 fmol/ml = 22fmol/mg pI -

RPc = 272 fmol/ml = 49fmol/mg pI -

protein concentration = 5.6 g/l

Treatment: Tamoxifen 40 mg qds on 18/8/82, then 20 mg od

Biopsy 2: 24/8/82. Metastasis of chest wall (needle biopsy)

Histology: Not done

Cytology : Malignant cells (NCS 14857)

Assay reference ID 201 (IEF) Sample wt = 25 mg

REc not done

RPc(a) = 1918 fmol/ml = 228 fmol/mg pI 6.2

RPc(b) = 2352 fmol/ml = 280 fmol/mg pI 6.2

protein = 8.4 g/l

Sites of disease: Chest wall metastasis , cervical node,  
(sclerotic bone lesions in pelvis and proximal femora)

Assessment of response: No change

Results: Progression noted on 12/7/83.

Comment:

Patient: A. O'N.      Number: C50654      Age: 36

No previous treatment

Biopsy 1: 20/4/82. Primary tumour (needle biopsy)

Histology: Infiltrating carcinoma (82/3024)

Cytology : Malignant cells (NCS 14195)

Assay reference ID 136      (IEF)      Sample wt = 16 mg

REc -ve

RPc -ve

protein concentration = 0.8 g/l

Treatment: Tamoxifen 40 mg qds on 27/4/82, then 20 mg bid

Biopsy 2: 4/5/82. Primary tumour (needle biopsy)

Histology: Not done

Cytology : Malignant cells (NCS 14273)

Assay reference ID 149      (IEF)      Sample wt = 16 mg

REc -ve

RPc -ve

protein concentration = 2.6 g/l

Sites of disease: Primary tumour (T2), axillary nodes, lytic lesions of pelvis, (irradiated lytic lesions of spine)

Assessment of response: Progression

Results: Progression of disease noted on 27/5/82 (increased soft tissue disease, new lytic bone lesions, increased existing bone lesions). Died

Comment: Pre-menopausal.



Patient: M. O.                      Number: E59330                      Age: 67

No previous treatment

Biopsy 1: 7/4/82. Primary tumour (needle biopsy)

Histology: Infiltrating duct carcinoma (82/2739)

Cytology : Malignant cells (NCS 14161)

Assay reference ID 131      (IEF)                      Sample wt = 19 mg

REc = 39 fmol/ml = 12 fmol/mg                      pI 6.6, 5.6

RPc -ve

protein concentration = 3.3 g/l

Treatment: Tamoxifen 40 mg qds on 21/4/82, then 10 mg bid.

Biopsy 2: 27/4/82. Primary tumour (needle biopsy)

Histology: Not done

Cytology : Malignant cells (NCS 14233)

Assay reference ID 142      (IEF)                      Sample wt = 35 mg

REc not done

RPc = 60 fmol/ml = 8.5 fmol/mg                      pI 5.4

protein concentration = 7 g/l

Sites of disease: Primary tumour (T4), axillary nodes

Assessment of response: Partial regression

Results: Last seen 10/1/84, no progression of disease.

Patient: M. P.                      Number: D21401                      Age: 76

Previous treatment: Tamoxifen from 6/2/1976 to 10/2/79  
(Disease stable, but no substantial regression. Radiotherapy  
added in April 1977). Stilboestrol from 2/10/79 to  
15/2/1980. Adriamycin & cyclophosphamide from 15/2/1980 to  
6/1/81. CMF from 13/1/81 to 2/11/82.

Biopsy 1: 2/11/82. Primary tumour (needle biopsy)

Histology: No evidence of carcinoma (82/7585)

Cytology : Malignant cells (NCS 15239)

|                                   |                   |
|-----------------------------------|-------------------|
| Assay reference ID 228      (IEF) | Sample wt = 30 mg |
| REc = 457 fmol/ml = 65 fmol/mg    | pI 6.4, 5.6       |
| RPc = 589 fmol/ml = 84 fmol/mg    | pI 5.7            |
| protein concentration = 7 g/l     |                   |

Treatment: Tamoxifen 20 mg o.d. from 3/11/82

Biopsy 2: 23/11/82. Primary tumour (needle biopsy)

Histology: Not done

Cytology : Malignant cells (NCS 15340)

|                                    |                   |
|------------------------------------|-------------------|
| Assay reference ID 238      (IEF)  | Sample wt = 25 mg |
| REc not done                       |                   |
| RPc(a) = 718 fmol/ml = 133 fmol/mg | pI 5.2            |
| RPc(b) = 568 fmol/ml = 105 fmol/mg | pI 5.2            |
| protein content = 5.4              |                   |

Sites of disease: Primary (T4) tumour, skin nodules (4), (pleural  
effusion)

Assessment of response: No change

Results: Last seen 13/12/82, no progression of disease.

Patient: E. P.                      Number: E59863                      Age: 68

No previous treatment

Biopsy 1: 26/4/82. Primary tumour (needle biopsy)

Histology: Infiltrating carcinoma (82/2898)

Cytology : Malignant cells (NCS 14266)

Assay reference ID 147      (IEF)                      Sample wt = 35 mg

REc = 551 fmol/ml = 41 fmol/mg                      pI -

RPc = 224 fmol/ml = 16 fmol/mg                      pI 5

protein concentration = 13.6 g/l

Treatment: Tamoxifen 40 mg qds on 27/4/82, then 10 mg bid

Biopsy 2: Primary tumour (needle biopsy)

Histology: Not done

Cytology : Malignant cells (NCS 14272)

Assay reference ID 152      (IEF)                      Sample wt = 40 mg

REc -ve

RPc -ve

protein concentration = 13.6 g/l

Sites of disease: Primary tumour (T4), cervical node, pelvis (4),  
right femur, skull, right humerus, (pathological fractures of  
ribs)

Assessment of response: Partial regression (sclerosis in  
bones, minor reduction in size of soft tissue disease)

Results: Progression of disease noted on 12/7/83 (Increased  
soft tissue disease, new lytic metastases). Alive 3/1/84.

Comment:

Patient: L. R.                      Number: E12756                      Age: 74

Previous treatment: Mastectomy

Biopsy 1: 2/11/82. Metastasis of chest wall (needle biopsy)

Histology: Secondary carcinoma (82/4021)

Cytology : Malignant cells (NCS 15240)

Assay reference ID 229                      (IEF)                      Sample wt = 65 mg

REc -ve

RPc = 131 fmol/ml = 13 fmol/mg                      pI 5.2

protein concentration = 10 g/l

Treatment: Tamoxifen 20 mg od from 2/11/82

Biopsy 2: Metastasis of chest wall (needle biopsy)

Histology: not done

Cytology : Malignant cells (NCS 15341)

Assay reference ID 239                      (IEF)                      Sample wt = 25 mg

REc not done

RPc -ve

protein concentration = 4.8 g/l

Sites of disease: Chest wall metastasis.

Assessment of disease: Progression

Results: Progression of disease noted on 8/2/83 (new lung lesion)

Comment: Chest wall disease also increased

Patient: M. S.                      Number: D57621                      Age: 66

No previous treatment

Biopsy 1: 11/6/81. Primary tumour (open biopsy)

Histology: Infiltrating duct carcinoma (81/4413)

Cytology : Not done

Assay reference BC 1545

Sample >200 mg

REc -ve

RPc -ve

protein concentration = 4.5 g/l

Treatment: Tamoxifen 10 mg bid from 19/6/81

Biopsy 2: 17/7/81. Primary tumour (needle biopsy)

Histology: Not done

Cytology : Not done

Assay reference ID 11                      (IEF)

Sample wt = 20 mg

REc not done

RPc -ve

protein concentration not measured.

Sites of disease: Primary tumour (T4), axillary nodes (N2)

Assessment of response: Progression of disease

Results: Progression of disease noted on 15/9/81 (increased soft tissue disease)

Comment: Also developed erythema of breast during tamoxifen treatment, to give the appearance of inflammatory carcinoma

Patient: M. S.            Number: Park Hosp. 200644    Age:

No previous treatment

Biopsy 1: 10/2/82. Primary tumour (needle biopsy)

Histology: Not done

Cytology : Malignant cells (NCS 13905)

Assay reference ID 103    (IEF)                      Sample wt = 35 mg

REc = 117 fmol/ml = 98 fmol/mg                      pI 6.8, 5.6

RPc = 354 fmol/ml = 294 fmol/mg                      pI 5.4

protein concentration = 1.2 g/l

Treatment: Tamoxifen 10 mg bid from 11/2/82

Biopsy 2: 24/2/82. Primary tumour (needle biopsy)

Histology: Infiltrating duct carcinoma (Christie 1100/82)

Cytology : Malignant cells (NCS 13974)

Assay reference ID 104    (IEF)                      Sample wt = 23 mg

RE not done

RPc(a) = 226 fmol/ml = 332 fmol/mg                      pI 5.4

RPc(b) = 107 fmol/ml = 157 fmol/mg                      pI 5.4

protein concentration = 0.68 g/l

Sites of disease: Primary tumour (T4), axillary node,  
(unmeasurable cervical nodes), (ascites), (sclerotic lumbar  
metastases).

Assessment of response: Progression of disease

Results: Progression of disease noted on 16/7/82 (new skin  
nodules)

Comment: Patient also presented with gross myxoedema, and  
was started on thyroxine at the same time. Her general state  
improved on this combined treatment.

Patient: E. S.                      Number: B37033                      Age: 53

Previous treatments: Mastectomy, tamoxifen from 17/11/78 to 30/1/81 (response not known), then no treatment.

Biopsy 1: 7/12/82. Skin nodule (open biopsy)

Histology: Consistent with ductal carcinoma (82/8521)

Cytology : Not done

Assay reference BC 2180 (DCC)                      Sample wt not recorded  
-10

REc = 36 fmol/ml = 45 fmol/mg                      Kd =  $0.44 \times 10^{-10}$

RPc = 72 fmol/ml = 90 fmol/mg                      Kd =  $13.2 \times 10^{-10}$

protein concentration = 0.8 g/l

Treatment: Tamoxifen 40 mg qds on 8/12/82, then tamoxifen 20 mg od for 6 days, then medroxyprogesterone acetate 100 mg qds for 14 days, then no treatment for 7 days, repeated as monthly cycles.

Biopsy 2: 14/12/82. Skin nodule (open biopsy)

Histology: Adenocarcinoma, consistent with ductal carcinoma

Cytology: Not done

Assay reference BC 2182 (DCC)                      Sample wt not recorded

RE -ve

RP = 210 fmol/ml = 262 fmol/mg                      Kd =  $3.6 \times 10^{-10}$

protein concentration = 0.8 g/l

Sites of disease: Skin nodules over chest wall (3), scalp nodules (5), skin nodule on back, subcutaneous nodule on arm, (diffuse sclerotic bone metastases)

Assessment of response: No change

Results: Progression of disease noted on 13/9/83 (increase in sclerotic disease)

Comment: Reduction of soft tissue disease occurred.

Patient: R. T.                      Number: E46035                      Age: 84

No previous treatment

Biopsy 1: 17/8/81. Primary tumour (open biopsy)

Histology: Mucoid carcinoma (81/60129)

Cytology : not done

Assay reference BC 1634 (DCC)                      large sample

REc = 9 fmol/ml = 23 fmol/mg                      Kd =  $5.4 \times 10^{-10}$

RPc = 10 fmol/ml = 25 fmol/mg                      Kd =  $7.2 \times 10^{-10}$

protein concentration = 0.4 g/l

Treatment: Tamoxifen 10 mg bid from 25/8/81

Biopsy 2: 1/9/81. Primary tumour (open biopsy)

Histology: Not done

Cytology : Not done

Assay reference BC 1650 (DCC)                      large sample

REc -ve

RPc = 43 fmol/ml = 36 fmol/mg                      Kd =  $1.3 \times 10^{-10}$

pro = 1.2 g/l

Sites of disease: Primary tumour (T4), axillary node

Assessment of response: No change

Results: Progression of disease noted on 27/4/82 (increased existing soft tissue lesions)

Comment: A small but consistent reduction in measurements occurred.



Patient: S. Y.            Number: E34204            Age: 82

No previous treatment

Biopsy 1: 23/1/81. Primary tumour (open biopsy)

Histology: Infiltrating duct carcinoma (81/0629)

Cytology : Not done

Assay reference BC 1363 (DCC)

Wt not recorded

REc = 1500 fmol/ml = 833 fmol/mg

No Kd. Too strongly positive

RPc = 220 fmol/ml = 122 fmol/mg

$K_d = 2.6 \times 10^{-10}$

protein concentration = 1.8 g/l

Treatment: Tamoxifen 40 mg qds on 27/8/81, then 10 mg bid

Biopsy 2: 16/2/81. Primary tumour (open biopsy)

Histology: Not done

Cytology : Not done

Assay reference not allocated (IEF)

Large sample

REc = 171 fmol/ml = 88 fmol/mg

pI 6.6, 5.4

RPc = 1307 fmol/ml = 670 fmol/mg

pI 5.4

protein content = 1.95 g/l

Simultaneous DCC assay, RPc ~1900 fmol/mg (poor Scatchard plot)

Sites of disease: Primary tumour (T4), axillary nodes

Assessment of response: No change

Results: Progression of disease noted on 20/10/81 (increase in existing soft tissue lesions)

Comment:

# SUMMARY OF INDUCTION DATA

| Pt              | RE | RP1 | As | Dose | Int | RP2              | As | Resp | Dur   | Inc? | Ratio |
|-----------------|----|-----|----|------|-----|------------------|----|------|-------|------|-------|
| KK              | -  | -   | IE | L    | 21  | -                | IE | PD   | 20    |      |       |
| JM              | -  | -   | IE | L    | 7   | -                | IE | PD   | 28    |      |       |
| MB              | +  | >72 | B  | S    | 14  | 30               | IE | PD   | 28    |      |       |
| AO <sup>N</sup> | -  | -   | IE | L    | 14  | -                | IE | PD   | 30    |      |       |
| EG              | -  | -   | IE | L    | 7   | -                | IE | PD   | 41    |      |       |
| DF              | +  | 680 | DC | S    | 21  | -                | DC | PD   | 42    |      |       |
| EF              | +  | -   | DC |      | 7   | -                | DC | PD   | 63    |      |       |
| GL              | +  | 20  | DC | L    | 28  | 24 <sup>~</sup>  | IE | PD   | 65    | *    | 1.2   |
| EC              | +  | 15  | DC | L    | 10  | 102              | IE | PD   | 66    | *    | >2    |
| SL              | +  | 5   | IE | L    | 14  | -                | IE | PD   | 70    |      |       |
| CC              | +  | -   | IE | S    | 21  | -                | IE | PD   | 84    |      |       |
| CF              | +  | 28  | DC | S    | 21  | 161              | DC | PR   | -85   | *    | >2    |
| MB              | +  | 47  | IE | L    | 7   | 44               | IE | PD   | 87    |      |       |
| MS              | -  | -   | DC | S    | 28  | -                | IE | PD   | 88    |      |       |
| LR              | -  | 13  | IE | S    | 21  | -                | IE | PD   | 98    |      |       |
| AE              | +  | 260 | IE | S    | 21  | 143              | IE | ED   | -102  |      |       |
| BH              | +  | 12  | IE | L    | 7   | 254              | IE | PD   | 112   | *    | >2    |
| EB              | +  | 63  | IE | L    | 11  | -                | IE | PD   | 116   |      |       |
| JB              | +  | 58  | IE | L    | 7   | 32               | IE | PD   | 125   |      |       |
| MB              | -  | -   | DC | L    | 35  | -                | DC | PD   | 140   |      |       |
| AA              | -  | 76  | DC | S    | 7   | 26               | DC | PR   | 154   |      |       |
| MS              | +  | 294 | IE | S    | 14  | 334              | IE | PD   | 156   | *    | 1.1   |
| AH              | -  | 156 | DC | L    | 7   | 274 <sup>~</sup> | IE | PD   | 169   | *    | 1.8   |
| EH              | -  | -   | IE | L    | 10  | -                | IE | NC   | 217   |      |       |
| BB              | -  | 3   | IE | L    | 7   | 15               | IE | PR   | 238   | *    | >2    |
| RT              | +  | 26  | DC | S    | 7   | 36               | DC | NC   | 245   | *    | 1.4   |
| SY              | +  | 122 | BO | L    | 21  | 670              | BO | NC   | 266   | *    | >2    |
| ES              | +  | 90  | DC | L    | 7   | 262              | DC | NC   | 279   | *    | >2    |
| FM              | +  | -   | DC | S    | 14  | -                | IE | NC   | 315   |      |       |
| MM              | +  | 50  | IE | L    | 7   | 280              | IE | NC   | 328   | *    | >2    |
| RM              | -  | -   | IE | L    | 7   | 72               | IE | [NC] | 337   | *    | >2    |
| EG              | +  | 39  | IE | L    | 7   | 105              | IE | PR   | 340   | *    | >2    |
| MB              | -  | -   | BO | S    | 21  | 69               | IE | NC   | 383   | *    | >2    |
| MP              | +  | 84  | IE | S    | 21  | 119              | IE | NC   | -406  | *    | 1.4   |
| EP              | +  | 17  | IE | L    | 7   | -                | IE | PR   | 441   |      |       |
| MH              | +  | 81  | IE | S    | 21  | 128              | IE | PR   | -462  | *    | 1.6   |
| AB              | +  | 6   | IE | S    | 7   | 122              | IE | NC   | -594  | *    | >2    |
| AH              | +  | 8   | IE | L    | 7   | 17               | IE | CR   | -598  | *    | >2    |
| MO              | +  | -   | IE | L    | 7   | 8                | IE | PR   | -629  | *    | >2    |
| MB              | +  | -   | IE | L    | 7   | 8                | IE | PR   | -635  | *    | >2    |
| IB              | +  | 500 | DC | S    | 28  | 1285             | DC | NC   | -1036 | *    | >2    |

<sup>~</sup>corrected value      []see details

As = Assay (IE = IEF, DC = DCC, BO = both methods)

Dose (S = standard (20 mg daily), L = loading dose)

Dur = interval to progression in days

Inc? (\* = PR increased)

# APPENDIX 19: INTRATUMOUR VARIATION OF PROGESTERONE RECEPTOR

## Ref EC (IEf only)

|         |     |      |      |
|---------|-----|------|------|
| fmol/ml |     |      |      |
| -ve     | -ve | -ve  | 76   |
| protein |     |      |      |
| 3.15    | 3.3 | 3.95 | 2.35 |
| fmol/mg |     |      |      |
| 0       | 0   | 0    | 32   |

## Ref VC (IEf only)

|         |     |     |     |
|---------|-----|-----|-----|
| fmol/ml |     |     |     |
| -ve     | -ve | -ve | -ve |
| protein |     |     |     |
| 5.25    | 2.8 | 4.9 | 4.2 |

## Ref LLo

|          |      |      |                  |          |     |
|----------|------|------|------------------|----------|-----|
| IEF.     |      |      | DCC.             |          |     |
| fmol/ml  |      |      |                  | fmol/mg. |     |
| 73       | 861  | 906  | 292              | 266      | 297 |
| 465      | 87   | 622  | 142              | 160      | 221 |
| 332      | 238  | 209  | 109              | 221      | 53  |
| 382      | 1098 | 21.4 | 369              | 391      | 11  |
| protein. |      |      |                  |          |     |
| 2.1      | 4.8  | 4.2  |                  |          |     |
| 4.3      | 5.1  | 6.6  |                  |          |     |
| 5.1      | 7.1  | 11.4 |                  |          |     |
| 5.1      | 7.1  | 6.6  |                  |          |     |
| fmol/mg  |      |      | Ave (DCC) = 211. |          |     |
| 35       | 179  | 215  | sd= 120          |          |     |
| 108      | 17   | 94   | CV= 0.57         |          |     |
| 65       | 34   | 18   | Ave (IEF) = 83   |          |     |
| 75       | 148  | 3    | sd = 69          |          |     |
|          |      |      | CV = 0.83        |          |     |

Ref MG

IEF

DCC

fmol/ml

All -ve

|         |          |           |
|---------|----------|-----------|
| 61      | -ve (26) | -ve(14)   |
| -ve(12) | -ve(23)  | -ve(7)    |
| -ve(25) | -ve(0)   | -ve(16)   |
| -ve(20) | -ve(11)  | -ve(29.6) |

protein

wet wt (mg).

|      |      |      |     |     |     |
|------|------|------|-----|-----|-----|
| 4.96 | 7.6  | 4.96 | 75  | 110 | 125 |
| 6.64 | 7.92 | 9.2  | 140 | 165 | 175 |
| 11.5 | 5.74 | 7.44 | 210 | 115 | 90  |
| 9.76 | 9.6  | 3.54 | 130 | 120 | 120 |

fmol/mg

Ave (DCC) = 0

|    |   |   |               |
|----|---|---|---------------|
| 12 | 0 | 0 | Ave (IEF) = 1 |
| 0  | 0 | 0 | sd = 3.46     |
| 0  | 0 | 0 | CV = 3.46     |
| 0  | 0 | 0 |               |

Ref HB

IEF

DCC

fmol/ml

fmol/mg

|     |     |     |     |    |   |
|-----|-----|-----|-----|----|---|
| 227 | -ve | -ve | 146 | 34 | 0 |
| -ve | -ve | 40  | 0   | 0  | 0 |
| -ve | -ve | -ve | 0   | 0  | 0 |
| 82  |     |     | 46  |    |   |

protein

|      |      |      |
|------|------|------|
| 9.68 | 7.92 | 7.15 |
| 6.35 | 7.15 | 7.15 |
| 3.52 | 5.5  | 4.84 |
| 6.6  |      |      |

fmol/mg

Ave (DCC) = 19

|      |   |     |                           |
|------|---|-----|---------------------------|
| 23.4 | 0 | 0   | sd = 46                   |
| 0    | 0 | 5.6 | CV = 2.42                 |
| 0    | 0 | 0   | Ave (DCC, +ves only) = 75 |
| 12.4 |   |     | sd= 61 CV = 0.81          |

Ave IEF = 4.14

sd = 7.86

CV = 1.9

Ref SM

## IEF

fmol/ml

|     |     |     |
|-----|-----|-----|
| 353 | 356 | 173 |
| 49  | 168 | 37  |
| 487 | -ve | 30  |
| 66  | 126 | -ve |

protein

|      |      |      |
|------|------|------|
| 10   | 11   | 5.2  |
| 7    | 4.8  | 4.8  |
| 11.4 | 11.4 | 15   |
| 16   | 10   | 15.6 |

fmol/mg

|    |    |    |
|----|----|----|
| 35 | 51 | 33 |
| 7  | 35 | 8  |
| 43 | 0  | 2  |
| 4  | 13 | 0  |

## DCC

fmol/mg

|     |     |    |
|-----|-----|----|
| 60  | 115 | 73 |
| 39  | 83  | 94 |
| 114 | 9   | 11 |
| 23  | 30  | 9  |

wet wt(mg)

|     |     |     |
|-----|-----|-----|
| 180 | 170 | 90  |
| 120 | 90  | 70  |
| 140 | 177 | 155 |
| 180 | 150 | 150 |

Ave (DCC) = 55

sd = 40.2

CV = 0.73

Ave (IEF) = 19.25

sd = 18.7

CV = 0.97

Ref NA

## IEF

fmol/ml.

|      |     |     |
|------|-----|-----|
| 385  | 185 | 71  |
| 1613 | 298 | 185 |
| 285  | 348 | 869 |
| 269  | 927 | 63  |

protein

|       |      |      |
|-------|------|------|
| 5.36  | 5.12 | 4.48 |
| 12.16 | 8.96 | 6.88 |
| 6.24  | 4.46 | 14.4 |
| 4.16  | 4.64 | 3.92 |

fmol/mg

|     |     |    |
|-----|-----|----|
| 72  | 36  | 16 |
| 133 | 33  | 27 |
| 46  | 70  | 60 |
| 65  | 200 | 16 |

## DCC

fmol/mg

|     |     |     |
|-----|-----|-----|
| 278 | 135 | 67  |
| 323 | 163 | 127 |
| 162 | 153 | 137 |
| 173 | 413 | 25  |

wet wt(mg)

|     |     |     |
|-----|-----|-----|
| 95  | 90  | 75  |
| 200 | 140 | 130 |
| 190 | 100 | 180 |
| 90  | 140 | 95  |

ave (DCC) = 180

sd = 108

CV = 0.6

Ave (IEF) = 65

sd = 53.4

CV = 0.83

Ref DC

DCC

fmol/mg

|     |     |    |
|-----|-----|----|
| 61  | 27  | 33 |
| 142 | 130 | 42 |
| 85  | 118 | 63 |
| 94  | 101 | 84 |

protein

|     |     |     |
|-----|-----|-----|
| 4.4 | 7.5 | 4.6 |
| 3.8 | 6.6 | 3.3 |
| 7   | 5   | 3.4 |
| 7.3 | 7.4 | 7.8 |

IEF

fmol/mg

|    |    |    |
|----|----|----|
| 9  | 15 | 5  |
| 76 | 65 | 9  |
| 9  | 65 | 25 |
| 53 | 76 | 44 |

Ave (DCC) = 83

sd = 37

CV = 0.45

Ave (IEF) = 37

sd = 28

CV = .75