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THE DYNAMICS OF THE SERUM CONCENTRATION OF CEA,
CA15-3 AND CA19-9 AND SURVIVAL IN PATIENTS TREATED
FOR ADVANCED BREAST AND COLORECTAL CANCER

The Determination of the prognostic correlates of changes in tumour
markers CEA, CA15-3 and CA19-9 during chemotherapy treatment for
advanced breast and colorectal cancer

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The dynamics of the serum concentration of CEA, CA15-3 and CA19-9 and survival in patients treated for advanced breast and colorectal cancer

Keywords:

Breast neoplasms, Colorectal neoplasms, Palliative chemotherapy, Survival, CA15-3 antigen, CA19-9 antigen, Carcinoembryonic antigen

There is evidence that kinetics of tumour markers (TMs) CEA, CA15-3 and CA19-9 provide valuable information about disease state over time in patients with advanced breast and colorectal cancer but the literature contains differences in methodology so comparing findings is difficult.

By modifying criteria developed by Rustin and colleagues [1-5] in ovarian carcinoma we have retrospectively identified a subset of patients (those with progressive (P) TMs) where survival is significantly reduced compared with those with responsive (R) TMs. This is true for CEA, CA15-3 and CA19-9 at the first chemotherapy given in advanced disease (chem1) (Hazard ratios (HR) = 9.99, 8.89, 5.75, $P \leq 0.001$ in all cases) and CEA and CA19-9 at the second chemotherapy (chem2) (HR = 7.95, 9.00, $P = 0.001$ and 0.002 respectively) in patients with breast cancer. It is also true for CEA at chem1 in patients with colorectal cancer (HR = 2.51, $P < 0.001$). Further studies are necessary to see if treatment directed by these criteria can influence survival.

CEA and CA19-9 Rustin category in colorectal patients and CA15-3 Rustin category in breast patients correlated significantly with radiological category at chem1 and chem2 (CEA $r_s = 0.45$ and 0.43 , CA19-9 $r_s = 0.26$ and 0.35 , CA15-3 $r_s = 0.28$ and 0.44). CA19-9 also correlates with radiological category at chem2 ($r_s = 0.38$) in breast patients. This provides valuable information because RECIST criteria can delay radiological identification of disease progression compared with WHO criteria [6, 7] and new therapies may act to stabilise tumour growth rather than reduce it [8].

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(IV) List of Abbreviations

ACT	Alpha-1-antichymotrypsin
AFP	Alpha-fetoprotein
AIC	Akaike's information criterion
APC	Adenomatous polyposis coli
ASCO	American Society of Clinical Oncology
ATAC	Arimidex, Tamoxifen Alone or in Combination (clinical trial)
BPH	Benign Prostatic Hyperplasia
BRCA1 and BRCA2	Breast cancer 1 and 2 (genes often altered in breast cancer)
CA125	Cancer Antigen 125
CA15-3 also known as EMA	Cancer Antigen 15-3
CA19-9	Cancer Antigen 19-9
CA27.29	Cancer Antigen 27.29
CA-50	Cancer Antigen 50
CA549	Cancer Antigen 549
cAMP	Cyclic Adenosine-Monophosphate
CEA also known as CEACAM5 or CD66e	Carcinoembryonic Antigen
CMF	Chemotherapy regimen comprising cyclophosphamide, flourouracil and methotrexate
CMFP	Chemotherapy regimen comprising Cyclophosphamide, methotrexate, 5-flourouracil and prednisone
CMG	Carcinoembryonic Antigen Gene Family
CRC	Colorectal cancer
CYFRA 21-1	Cytokeratin 19 fragment
DAVTH	Chemotherapy regimen comprising Dibromodulcitol, doxorubicin, vincristine, tamoxifen and fluoxymesterone
DCC	Deleted in colon cancer (gene often altered in colorectal cancer)
DCIS	Ductal Carcinoma In Situ
DNA	Deoxyribonucleic acid
DPC4 / SMAD4	Deleted in pancreatic cancer 4 / mothers against decapentaplegic 4 (gene often altered in colorectal cancer)
ECMF	Epirubicin, Cyclophosphamide, Methotrexate, 5-Flourouracil (chemotherapy regimen)
EMA also known as CA15-3	Epithelial Membrane Antigen
ER	Oestrogen Receptor
ESR	Erythrocyte sedimentation rate
FAP	Familial adenomatous polyposis coli
FEC	Chemotherapy regimen comprising cyclophosphamide, flourouracil and epirubicin
FUFA (5-FU and FA)	Chemotherapy regimen comprising 5-Flourouracil and Folinic acid

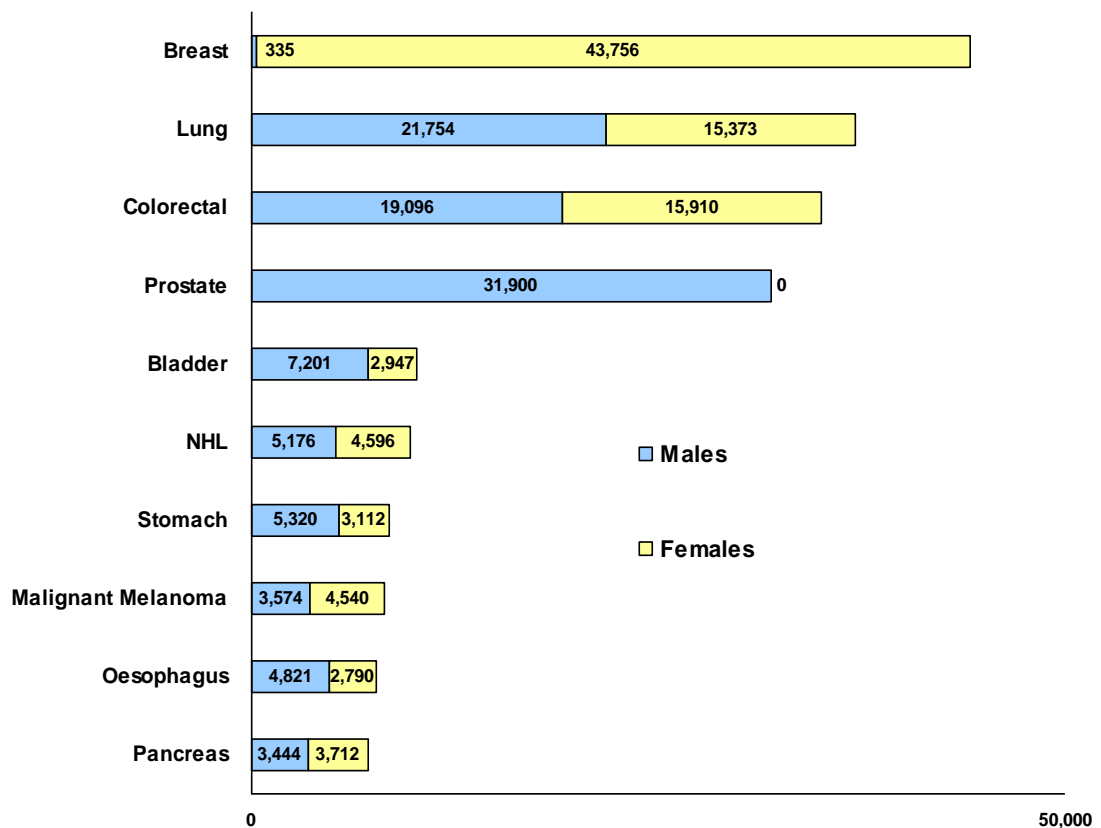
FUT2	The secretor gene
FUT3	The Lewis gene
hCG	Human Chorionic Gonadotrophin
HER-2/neu or ErbB-2	The gene for the human epidermal growth factor receptor type 2
hK3	human kallikrein 3
HNPCC	Hereditary non-polyposis colon cancer
HR / HRs	Hazard ratio / Hazard ratios
ICON	International Collaboration Ovarian Neoplasm trial
LCIS	Lobular Carcinoma In Situ
LDH	Lactate Dehydrogenase
MCA	Mucin like carcinoma associated antigen
MIC-1	Macrophage inhibitory cytokine 1
MMM	Chemotherapy regimen comprising methotrexate, mitomycin C and mitoxantrone
MUC	Mucin
MSH1 / MSH2	MutS homolog 1 and 2 (genes involved in HNPCC)
NCA also known as CEACAM6	Non-specific Cross-reacting Antigen
NED / ED	No evidence of disease / Evidence of disease
NICE	National Institute of Clinical Excellence
NPV	Negative predictive value
OPMAS	Oncology Patient Management Audit System
PBL	Peripheral Blood Lymphocytes
PEM also known as MUC1	Polymorphic Epithelial Mucin
PMS2	Postmeiotic Segregation Increased 2 (gene involved in HNPCC)
PPV	Positive Predictive Value
PR	Progesterone Receptor
PSA	Prostate Specific Antigen
PSG	Pregnancy-Specific Glycoproteins
RECIST	Response Evaluation Criteria in Solid Tumours
SCC	Squamous Cell Carcinoma Antigen
TMs	Tumour Markers
TNM	Tumour, Node, Metastasis (staging system)
TPA / TPS	Tissue Polypeptide Specific Antigen
TTP	Time to progression
UICC	International Union Against Cancer
ULN	Upper Limit of Normal
VEGF	Vascular Endothelial Growth Factor
WHO	World Health organisation

(1) Introduction

(1.1) Cancer

It is reported that approximately a quarter of people in the UK die from the diverse group of diseases collectively known as cancer, these diseases are characterised by uncontrolled cell replication. 276,678 new cancer cases were registered in the UK in 2003, and the number of cancer deaths in 2004 was recorded as just above 153,397 (these figures are excluding non-melanoma skin cancer) [10]. The 10 most commonly occurring cancers in the UK are shown in Figure 1.

Figure 1 Graph showing the incidence of the 10 most common cancers in the UK in 2003. Information from Cancer Research UK [10]



Cancer is a multi-step process, a disease caused by an accumulation of genetic faults within the genetic material of a cell leading to the disruption of normal cellular processes. Proto-oncogenes are genes whose product normally drives cell proliferation, for example growth factors, cell surface receptors, signal transduction system components, DNA binding nuclear proteins or genes involved in cell cycle control. Mutations affecting proto-oncogenes turn these genes into oncogenes and alter these processes.

The products of Tumour Suppressor genes (gatekeepers) on the other hand act to prevent unregulated cell cycle progression e.g. the APC gene on chromosome 5 which is mutated in familial adenomatous polyposis FAP a hereditary predisposition to colorectal cancer as well as in most sporadic colorectal cancers [11].

Mutation in either proto-oncogenes or tumour suppressor genes can result in unregulated cell proliferation; however, it is now thought that several mutations are required in order for cancer to develop. Some individuals are predisposed to cancer as they inherit genetic mutations which directly cause cancer or move the individual one step closer to cancer development.

Genetic changes continue to accumulate even after the development of cancer (often at an increased rate) and these mutations further increase the complexity of the disease and its treatment.

The genetic faults leading to cancer arise as a result of inherited problems passed on to the next generation via germ cell mutations or because of

environmental factors which cause somatic cell mutations, or due to a combination of both of these things. Inherited genetic predispositions can be in the form of faulty DNA repair processes, inappropriate gene expression, loss of gene function and many more. Environmental factors which may lead to cancer include such things as diet, exposure to industrial carcinogens such as asbestos and bacterial or viral infection; for example *Helicobacter pylori* has been linked with gastric cancer [12] and the human papilloma virus (HPV) has been linked with Cervical cancer [13] the second most common female cancer worldwide.

When they do arise cancers can be classified into six major categories as seen in table 1, but can also be of mixed type.

Table 1 The major cancer groups.

Category	Arises in...
Carcinoma	Epithelium
Sarcoma	Supportive and connective tissue (e.g. bones)
Lymphoma	Lymph nodes
Leukaemia	White blood cells of the Bone marrow
Myeloma	Specifically Plasma cells of the Bone Marrow
Germ Cell Tumours	Germ Cells

It is known that at least 80% of all cancers are carcinomas (the term adenocarcinoma refers to a sub group of carcinomas which arise in glandular epithelium) and more than 90% of all cancers are solid tumours i.e. carcinomas, sarcomas and lymphomas rather than blood cancers. Solid tumours are most commonly categorised using the Tumour, Node, Metastasis (or TNM) staging system which is defined in table 2.

Table 2 The TNM staging system

Category	Definition
Tumour	Usually staged T0 to T4 attempts to describe the extent of the primary tumour where T0 is carcinoma in situ and level of invasion increases from there.
Node	<p>Usually classified N0 to N3 where N0 is no lymph node metastases and N1-3 are varying degrees of lymph node metastases with 1 being involvement of the nearest lymph nodes to the primary tumour and 3 being the involvement of the most distant.</p> <p>Only the lymph nodes draining the site of the primary tumour are taken into account in this analysis. If distant lymph nodes contain metastatic tumour then this is counted as a metastasis.</p>
Metastasis	Usually classified as M0 or (MX) to M1 where M0 indicates no distant metastases and M1 indicates that a distant metastasis is present

This system is used in combination with the individual disease staging system (Table 3) in order to decide on the best treatment in each case.

Table 3 Individual disease staging systems for breast, colorectal, prostate and ovarian cancers

Stage	Cancer Type			
	Breast	Colorectal	Prostate	Ovarian
0 (carcinoma in situ)	Can be either ductal (DCIS), which is very early breast cancer, or Lobular (LCIS), which is not cancer but can be a precursor of it.	Cancer found only in the mucous membrane of the colon or rectum.		
I	Primary tumour is no larger than 2cm and has not spread.	(Duke's A) – Cancer has spread beyond the innermost lining and involves the inside wall of the colon or rectum.	(A1) – Cancer found in the prostate only and usually discovered by chance.	<p>A – Cancer in a single ovary</p> <p>B – Cancer found in both ovaries</p> <p>C – Cancer in one or both ovaries and –</p> <ul style="list-style-type: none"> • cancer found on the outside surface of the ovary / ies or • cancer has ruptured the ovary wall or • cancer cells found in the peritoneal fluid.
II	A - Primary tumour no larger than 2cm but has spread to the axillary lymph nodes, or - Primary tumour is 2-5cm but has not spread to the axillary lymph nodes.	(Duke's B) – Cancer has spread to the muscularis propria of the colon or rectum but not to the lymph nodes.	(A2, B1 or B2) – Cancer again confined to the prostate but more advanced than stage I.	A – Cancer found in one/both ovaries and the uterus and/or fallopian tubes.
	B - Primary tumour 2-5cm and has spread to the axillary lymph nodes, or - Primary tumour is > 5cm but has not spread to the axillary lymph nodes.			<p>B – Cancer found in one/both ovaries and other tissue within the pelvis.</p> <p>C – Cancer found in one/both ovaries and the uterus and/or fallopian tubes and/or other tissue in the pelvis and –</p> <ul style="list-style-type: none"> • cancer found on the outside surface of the ovary/ies or • cancer has ruptured the ovary wall or • cancer cells found in the peritoneal fluid.
III	A - Primary tumour < 5cm, has spread to the axillary lymph nodes and these are stuck together or to other structures, or - Primary tumour is > 5cm and has spread to the axillary lymph nodes which may be attached together or to other structures.	(Duke's C) – Cancer has spread to the nearby lymph nodes but there are no distant metastases.	(C) – cancer spread outside the prostate to proximal tissues.	A – Cancer confined to the pelvis but cancer cells are at the surface of the peritoneum.
	B - The primary tumour has spread to tissues near the breast, or - The primary tumour has spread to lymph nodes along the breast bone.			<p>B – Cancer has spread to the peritoneum but is < 2cm.</p> <p>C – Cancer has spread to the peritoneum but is > 2cm and/or has spread to the abdominal lymph nodes.</p>
IV	- Cancer has metastasised to other parts of the body, or to the lymph nodes in the neck.	(Duke's D) – Cancer has metastasised.	(D1, D2) – Cancer has metastasized.	Cancer has metastasised outside the abdomen and is found in the liver.

Disease stage has been found to be an effective predictor of survival in both breast and colorectal cancer [14, 15] and treatment is planned on an individual patient basis according to these measurements and many other factors such as tumour cell type, cancer differentiation and the health and preference of the patient. Practice can vary greatly but is becoming increasingly evidence based.

As well as radiological and histological results clinicians increasingly use biochemical information to aid in the diagnosis and treatment of some malignancies. Serum tumour markers are a diverse group of chemicals present in the serum of healthy individuals. Levels of these chemicals can become elevated in people with cancer but they can also increase in response to other biological or external stimulus. Unlike routine radiological examinations such as computed tomography (CT) scans and mammograms tumour marker levels are simple to obtain, relatively inexpensive (as they can be detected in a routine blood sample) and apart from minor discomfort, cause little inconvenience or risk to the patient.

(1.2) Tumour markers

Carcinoembryonic antigen or CEA was one of the first tumour markers to be identified and developed by Gold and Freedman in 1965 [16]. As with most tumour markers CEA levels can be ascertained from the serum from a simple blood test using monoclonal antibodies specific for one particular epitope. The use of certain tumour markers can aid clinical follow up, may be the first sign of the development of advanced disease and in some cases can provide

information about survival. Nicolini et. al. (2003) have shown significantly ($p = 0.0017$ at 84 months from mastectomy) increased survival in breast cancer patients who were treated in a “tumour marker guided” manner. Treatment was based upon an elevated level of one or more tumour markers within the CEA – TPA – CA15-3 (carcinoembryonic antigen – tissue polypeptide antigen – cancer antigen 15-3) tumour marker panel compared with conventional treatment based upon positive radiological or clinical diagnosis [120]. Other research suggests that in the setting of advanced disease tumour markers may give an indication of the degree to which the cancer is responding to treatment [1]. These findings indicate a potentially very important role for tumour markers to play in the setting of advanced disease.

In order for a tumour marker to be valuable it should be able to provide information which helps to diagnose the malignancy, identify recurrence or predict response to treatment or ideally all of these things. In order to be effective a tumour marker should be –

- Sensitive (correctly identify all of the individuals with the disease being tested for),
- Specific (does not incorrectly identify an individual without the disease as a sufferer),
- Detectable via a non-intrusive, inexpensive test, and
- Always representative of tumour volume and disease stage (in order to accurately measure fluctuations in disease status),

However, as yet no tumour marker is without limitations.

Now in several cases arrays of markers are being used at once to investigate to what extent combining the results of the different markers increases sensitivity and specificity. Fuzzy logic modelling used by Schneider et. al. in gastro intestinal in GI cancers [18] and lung cancer [19] aims to increase sensitivity and specificity by combining tumour markers in a panel. This approach is also being studied in a current trial using fuzzy logic in the diagnosis of lung cancer.

Potential tumour markers are continuously being identified and tested but as yet relatively few are used regularly in clinical practice those which are include squamous cell carcinoma antigen (SCC) in cervical cancer, prostate specific antigen (PSA) in prostate cancer, cancer antigen 125 (CA125) in ovarian cancer and human chorionic gonadotrophin (hCG) in gestational choriocarcinoma. The use of PSA and CA125 are described in more detail below.

(1.2.1) PSA and Prostate Cancer

31,900 men were diagnosed with prostate cancer in the UK in 2003 and in 2005 10,000 male deaths were attributed to it [10]. This makes prostate cancer the most common cancer and the second most common cause of cancer death in males in the UK [10]. Like colorectal and breast cancers, the vast majority of prostate cancers are carcinomas (epithelial in origin) and their incidence increases with age. Few cases of prostate cancer occur in the under 50s and Cancer Research UK state that “more than 60% of cases occur in men over 70” [10], they estimate the lifetime risk of a man getting prostate cancer to be

approximately 1 in 13 although it is found in its highest rates in African American populations.

Prostate cancers are staged using the TNM system (Table 2) and as shown in Table 3 and are given a Gleason score according to how well differentiated the cells within the primary tumour are. If cells are well differentiated (Gleason score <4) the tumour is less aggressive and the patient has a better prognosis than those with poorly differentiated prostate cancer (Gleason score 8 – 10).

Treatment for prostate cancer can vary greatly according to the many factors which must be taken into account in each case. Currently treatment options for localised cancer include watchful waiting (particularly in older patients), radical prostatectomy or radical radiotherapy. The outcomes of these various treatment modalities are currently being investigated as each is associated with negative effects for the patient. Bill-Axelsson et. al. (2005) compared radical prostatectomy with watchful waiting in early prostate cancer and found that radical prostatectomy reduces the risk of prostate cancer related death, development of metastases and local progression over 10 years. However these results are estimated 10 year results and the authors point out that “the absolute reduction in the risk of death after 10 years is small” [20].

Hormone treatment in the form of orchidectomy or treatment with an anti-androgen or luteinising hormone-releasing hormone agonist can be used in conjunction with these treatments. Hormone treatment aims to reduce the level of circulating testosterone and can have a dramatic effect upon the tumour.

Hormone therapy is also used in advanced disease sometimes alongside chemotherapy.

Patients can be treated with chemotherapy when hormone treatment is no longer effective. Docetaxel is usually the first line treatment since it has been shown to improve survival when compared with mitoxantrone [21]. Research is currently being done into many areas of prostate cancer treatment, one example is the ProtecT trial (Prostate testing for cancer and Treatment) run by the University of Bristol which aims to compare efficacy of the three main modalities of treatment currently used, treatment with radiotherapy, radical prostatectomy and active monitoring.

Prostate specific antigen (PSA) is a serine protease which is produced in the prostate and which acts to “dissolve the gel formed after ejaculation and thereby permit sperm movement in the female genital tract” [22] The gene encoding PSA, or as it is otherwise known human kallikrein 3 (hK3), is located on 19q. During childhood levels of PSA do not differ greatly between the sexes however this all changes at about 12 years of age when the prostate develops in males and as a result male PSA levels increase dramatically [23].

A small amount of PSA leaks into the body’s general circulation where it can be detected in the serum in all men however the level of PSA in the serum often increases as a result of the development of prostate cancer. The most commonly quoted cut off level is 4 ng / ml, a PSA level less than this is generally considered normal but this level can vary depending upon the assay used.

Although PSA can be used to screen patients for prostate cancer it is not specific enough to exclude the possibility of prostate cancer in all men who have normal PSA levels and not sensitive enough to identify all patients with prostate cancer as it is reported that approximately 45% of men with localised prostate cancer (cancer within the prostate) have a PSA of <4 ng/ml [22]. Thompson et. al. (2004) found that out of 2950 men whose PSA levels were ≤ 4 ng/ml 15.2% were diagnosed with prostate cancer and 14.9% of the cancers were Gleason score ≥ 7 [24]. These results mean that in some cases the PSA test is at best inaccurate and at worst misleading.

Other limitations of the PSA test are the fact that there is large intra-individual variation in PSA levels, with higher levels of PSA often seen in those with larger prostates and in older men (which could be attributed to the fact that prostatic volume can increase with age). It has also been speculated that race may affect PSA levels [25]. Other benign prostatic conditions found commonly in the population considered to be at risk of prostate cancer such as benign prostatic hyperplasia (BPH) and prostatic inflammation can also lead to an increased serum PSA.

Another problem with screening for prostate cancer is that there is little evidence, as yet, that early detection actually reduces the mortality rate of the disease. It is possible that this screening may prove to be ineffective in the long term particularly in older men where prostate cancer often progresses very slowly and

patients may die of other conditions before they are affected by the prostate cancer itself.

It is now known that PSA is present in the plasma as a free molecule or it is bound to one of several plasma proteins e.g. alpha-1-antichymotrypsin (ACT). The binding of some of these proteins can inhibit the recognition of PSA by the various immunoassay antibodies that are used to detect PSA levels thereby producing inconsistencies in testing. Stenman et. al. however describe how by calculating the proportion of free PSA compared to the total PSA the level of false-positive results in early diagnosis can be reduced by 20-40% (this assay is based on the fact that more free PSA is seen in men with prostate cancer than in those with benign prostatic disease) [26].

Despite problems surrounding the use of the PSA test in screening and the process of screening itself there are proven advantages to the use of the marker in patients who have been diagnosed with prostate cancer. PSA levels have been shown to increase with increasing tumour stage [27] and PSA response to treatment has been shown to correlate with patient response and survival: A fall in PSA following treatment with Strontium-89 for bony metastatic disease correlated with longer overall survival in a small cohort (30 patients) [28].

Investigations are now underway to identify novel tumour markers in prostate cancer such as macrophage inhibitory cytokine 1 (MIC-1) which has been found to predict presence of prostate cancer of Gleason score ≥ 7 and which combined with PSA improved the specificity of PSA alone [29].

(1.2.2) CA125 and Ovarian Cancer

Ovarian cancer is the fourth most common cancer in UK women, 6,906 cases were diagnosed in 2003, It is also the fourth most common cause of cancer death with 4,434 deaths recorded in 2004 [10]. The majority of cases are diagnosed at a late stage (stage III or IV) resulting in a poor survival rate. Ovarian cancers can be divided into carcinomas (approximately 80-90% of cases) and non-epithelial cancers [10], subtypes can be seen in Table 4.

Table 4 Ovarian cancer sub-groups.

Ovarian Cancer Type	Subtype	Arising from
Carcinomas	Serous	Epithelium
	Mucinous	Epithelium (resembling mucinous tissue)
	Endometrioid	Epithelium (resembling endometrial tissue)
	Clear cell	Epithelium (cells with distinctive appearance and clear cytoplasm)
	Undifferentiated	Epithelium (cells are hard to identify)
Non-Carcinomas	Germ cell	The ova
	Stromal	Connective tissue of the ovaries

The Non-epithelial cancers usually arise in younger women than epithelial ovarian cancers and, on average, are found at an earlier stage. Because of this, when treating non-epithelial ovarian cancer, maintaining fertility is often high on the list of priorities when choosing treatment options. As with breast, colorectal and prostate cancer, ovarian cancer is staged using the TNM staging system shown in Table 2 and the staging criteria in Table 3.

Treatment for epithelial ovarian cancer (ovarian carcinoma) can include surgery where appropriate for early stage disease or palliation of symptoms in advanced disease. The International Collaborative Ovarian Neoplasm trial 1 or ICON1 showed that adjuvant platinum based chemotherapy improves both recurrence

free and overall survival compared with no chemotherapy so this is now the gold standard adjuvant treatment [30]. In 2003 a systematic review and meta-analysis (including the above study) also found that there is no significant difference between chemotherapy and radiotherapy as adjuvant therapies [31]. In advanced ovarian cancer the debate is ongoing as to whether a platinum based chemotherapy is as effective alone as it is in combination with paclitaxel [32, 33].

The tumour marker cancer antigen 125 or CA125 is a glycoprotein which is identified in serum using the monoclonal antibody OC125 [34]. CA125 is expressed during development and is present at low levels in the serum of healthy individuals. CA125 level is often raised above the upper limit of normal in patients with epithelial ovarian cancer but it can also become raised benign conditions such as endometriosis, pelvic inflammatory disease, fibroids, renal failure, acute pancreatitis, peritonitis [35] and leiomyomas as well as non-ovarian malignancies [36]. In some women CA125 level can fluctuate during the menstrual cycle and pregnancy [3], this reduces its diagnostic power in premenopausal women.

Despite these limitations levels of CA125 found in benign conditions and cancers which are non-ovarian in nature are often lower than those found in patients with ovarian cancer [35, 36]. CA125 remains a powerful prognostic factor and the degree of CA125 elevation has been shown to correlate with ovarian cancer stage [37]. a rise in CA125 has been shown to predict clinical relapse by 4 months in about 70% of patients [3]. It has also been shown that nadir levels of

CA125 can predict time to disease progression and overall survival in patients with ovarian cancer [38, 39].

The power of CA125 has also been demonstrated in monitoring response to treatment and the work of Rustin et. al. has produced some reliable definitions of CA125 marker response and progression known as the “Rustin criteria of response” [40] which have been shown to correlate significantly with clinical response and progression [4, 5] These response criteria are detailed below:-

- CA125 Response

Or the “50% response criteria” [3, 4] requires that a raised level of CA125 which is double the upper limit of normal (ULN) before treatment, falls by at least 50% [3] following treatment. This response “must be confirmed and maintained for at least 28 days” [41].

In patients with measurable disease the Rustin criteria of response has a sensitivity and specificity of 92% and 72% respectively and is also able to predict time to progression [40].

- CA125 Progression

Has been defined and validated as an increase of CA125 to \geq twice the nadir level confirmed by a second sample which must also be \geq twice the nadir (provided that this value is \geq twice the ULN) [5].

Using this criterion progression can be predicted [1, 2] with a sensitivity of 82% [3] or 85.9% [5] and a specificity of 98% [3] or 91.3% [5] depending on which paper you read. Rustin et. al. (1996) also report that this definition of progression has a positive predictive value (PPV) of 94.8% and a negative predictive value (NPV) of 77.8% [5].

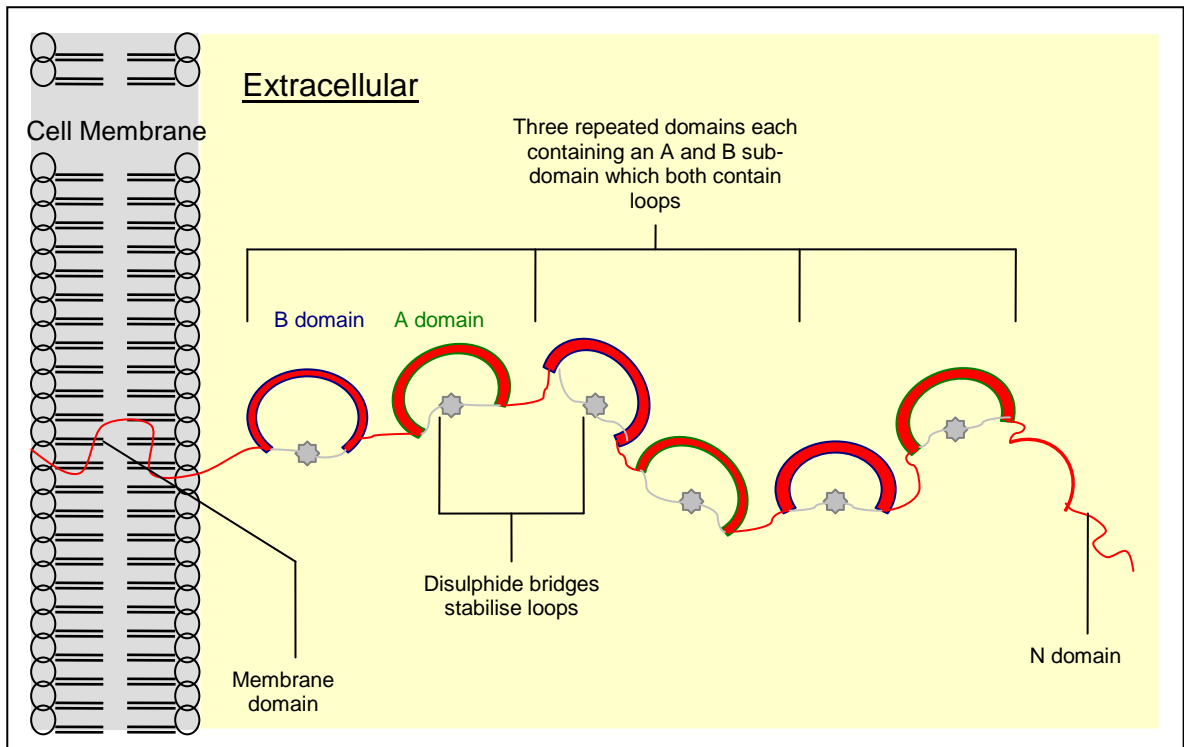
In this study I will principally investigate whether the Rustin criteria of response and progression, developed in ovarian cancer and described above, can be applied when looking at the tumour markers CEA, CA15-3 and cancer antigen 19-9 (CA19-9) in breast cancer and CEA and CA19-9 in colorectal cancer.

(1.3) CEA, CA 15-3 and CA 19-9 in Breast and Colorectal cancer

(1.3.1) CEA

The protein structure of the 180kD oncofoetal glycoprotein Carcinoembryonic Antigen (CEA also known as CEACAM5 or CD66e) can be seen in Figure 2 [42]. CEA is produced by the foetus and in smaller amounts by normal adult cells [43]. The molecular weight of the CEA protein alone is 70kD and the extra 110kD is attributable to its extensive pattern of glycosylation [44]. CEA levels are established using anti CEA antibody. CEA was one of the earliest tumour markers to be used and was originally identified in colorectal cancer in 1965 by Gold and Freedman [16] and has since been used as a marker of adenocarcinomas generally. CEA is part of the CEA gene family which in turn is part of the immunoglobulin superfamily [45].

Figure 2 CEA protein structure adapted from Hörig et. al. 2000 [42].



The CEA gene family are located on chromosome 19 the locus of CEA itself is 19q13. The CEA family consists of the pregnancy-specific glycoproteins (PSG) which are, as the name suggests, expressed in pregnancy, and the CEA subgroup [46] also known as the carcinoembryonic antigen gene family (CEA). In 1988 Zimmerman et. al. reported that there was a tissue specific variation in the transcriptional activity of CEA and non-specific cross-reacting antigen (NCA also known as CEACAM6) [47].

It is postulated that CEA acts as an intercellular adhesion molecule and in colon cancer it is thought that the cellular localisation of CEA may become dispersed from its usual location in the luminal membrane of the colonocytes [44] as a result of this it is hypothesised that inappropriate expression or localisation of CEA may play a role in tumour growth and the metastatic process. Blumenthal

et. al. reported in 2005 that by targeting either CEA or non-specific cross-reacting antigen (NCA) with monoclonal antibodies *in vitro* the processes of cell migration, invasion and adhesion are affected. The use of these monoclonal antibodies *in vivo* had an anti-metastatic effect [48] however it is unknown through which antigen (CEA or NCA) the antibodies had their effect .

CEA is shed from the surface of the cell and is found in the serum of healthy individuals but its level in the serum can increase in many carcinomas for example colorectal, breast, gastric and lung. CEA can also become elevated as a result of heavy smoking and several benign conditions such as alcoholic cirrhosis, liver abscess, obstructive jaundice, pancreatitis, inflammatory bowel disease, gastritis, diverticulitis, fibrocystic breast disease, renal failure and granulomatous cystitis [43] to name a few! Conditions affecting liver function can alter CEA level as this is where the majority of CEA clearance occurs [49].

In patients with cancer, CEA level is thought to relate to overall tumour burden and was recently shown to have the highest accuracy when distinguishing between malignant and benign pleural effusions when compared with the tumour markers CYFRA 21-1, CA15-3, CA19-9 and CA125 [50], it has also been linked with reduced cell mediated immunity [51]

(1.3.2) CA15-3 and CA19-9

Like CEA the cancer (or carbohydrate) antigens 15-3 and 19-9 (CA15-3 and CA19-9 respectively) are oncofoetal antigens. CA15-3 and CA19-9 are antigenic areas on large molecules called mucins. These areas are recognised by specific

antibodies which enable biochemical tests to establish their level in serum.

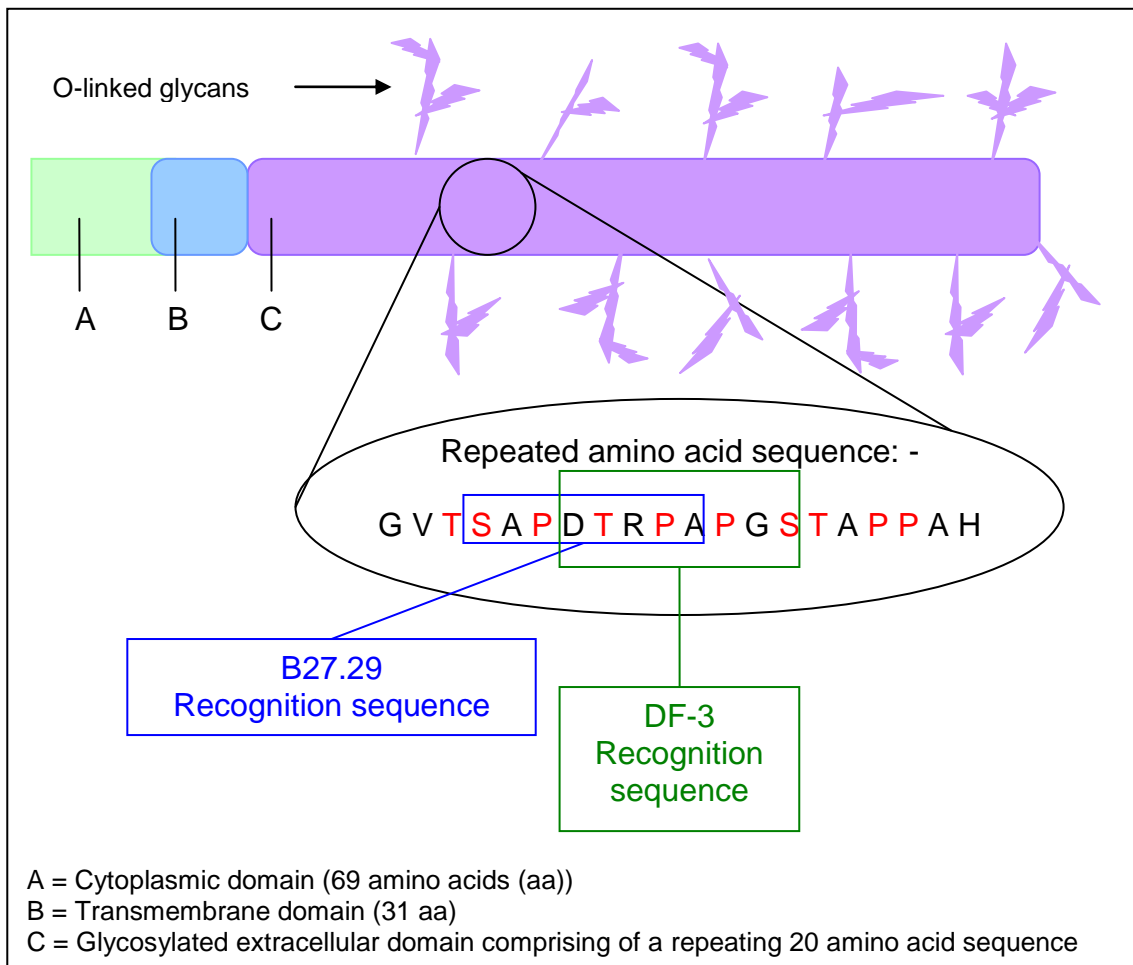
Mucins are complex glycoproteins particularly found in mucinous membrane secretions. Mucins are a family of proteins that are synthesized as membrane-bound proteins and are presented on the luminal surface of the cell where they are secreted (e.g. mucin 7 or MUC7), or remain membrane bound (e.g. mucin 1 or MUC1) [52]. Mucins have a high molecular weight and constitute part of most epithelial tissues.

The physiological role of MUC 1 is currently unknown however it is thought to be involved in cell adhesion [53] and cell protection [54] and it may be involved with lubrication, renewal, differentiation, and cell signalling. Mucins are normal constituents of many cell types and are differentially expressed and glycosylated in different tissues and disease states. MUC 1 is a product of the *MUC 1* gene located on 1q21-24 [55], as its name suggests it is membrane bound mucin.

CA15-3 is also known as epithelial membrane antigen (EMA) or episalin. It is an epitope present on the MUC 1 protein or polymorphic epithelial mucin (PEM) (see Figure 3).

The number of tandem repeats found within the extracellular domain of the MUC 1 protein varies between individuals (25 to over 125). The repeated sequence is 20 amino acid residues long, rich in serine (S), threonine (T) and proline (P) residues and is extensively O-glycosylated (see Figure 3) [56]. It is this level of glycosylation which varies between tissues and increases the rigidity of the extracellular domain as well as conferring a negative charge to the structure [53].

Figure 3 PEM / MUC 1 protein structure



The level of PEM or MUC 1 can be determined in several ways, here are three of them:-

- By determining the CA15-3 tumour marker level. This is established using a double determinant assay which uses 2 antibodies. The first is DF3 (first discovered by Kufe et. al. in 1984 [57]) which recognises an epitope within the repeated sequence in the extracellular domain of MUC1 (see figure 3). The second antibody II5-D8 (or Mam-6) first

discovered by Hilkens et. al. (1984) [58] recognises a carbohydrate epitope within the carbohydrate side chains of extracellular domain.

- A more recently developed assay uses the single monoclonal antibody B27.29. This recognises the CA27.29 antigen which comprises both an amino acid sequence present on the MUC 1 protein which overlaps with that recognised by DF3 (see Figure 3) and a carbohydrate epitope [52, 59]. The B27.29 antibody has been shown to be a “fast and reliable immunoassay for measuring PEM in serum” [55]. Results of this assay have been shown to be comparable to those of the CA15-3 assay with increased sensitivity of CA27.29 in low concentrations of antigen [60].
- A third assay uses monoclonal antibodies 695 and 552 [61].

Like CEA it is thought that malignancy results in a loss of cell polarity of MUC1 presentation which usually occurs on the apical surface of the cell, as a result this protein is also implicated in the metastatic process. It is thought that in some cancers MUC1 may act to inhibit apoptosis therefore conferring drug resistance to the tumour [62]. It has been shown by Reddish et. al. that elevated MUC1 protein levels (>40U.ml), identified using CA27.29 this time rather than CA15-3, correlate with higher CD69⁺ peripheral blood lymphocytes (PBL) and shorter survival following active specific immunotherapy ($P = 0.0093$) indicating a possible link between immunosuppression and an increased level of serum MUC 1 protein [63]. It is also known that MUC1 is targeted by cytotoxic T lymphocytes in the immune reaction to breast cancer cells [64].

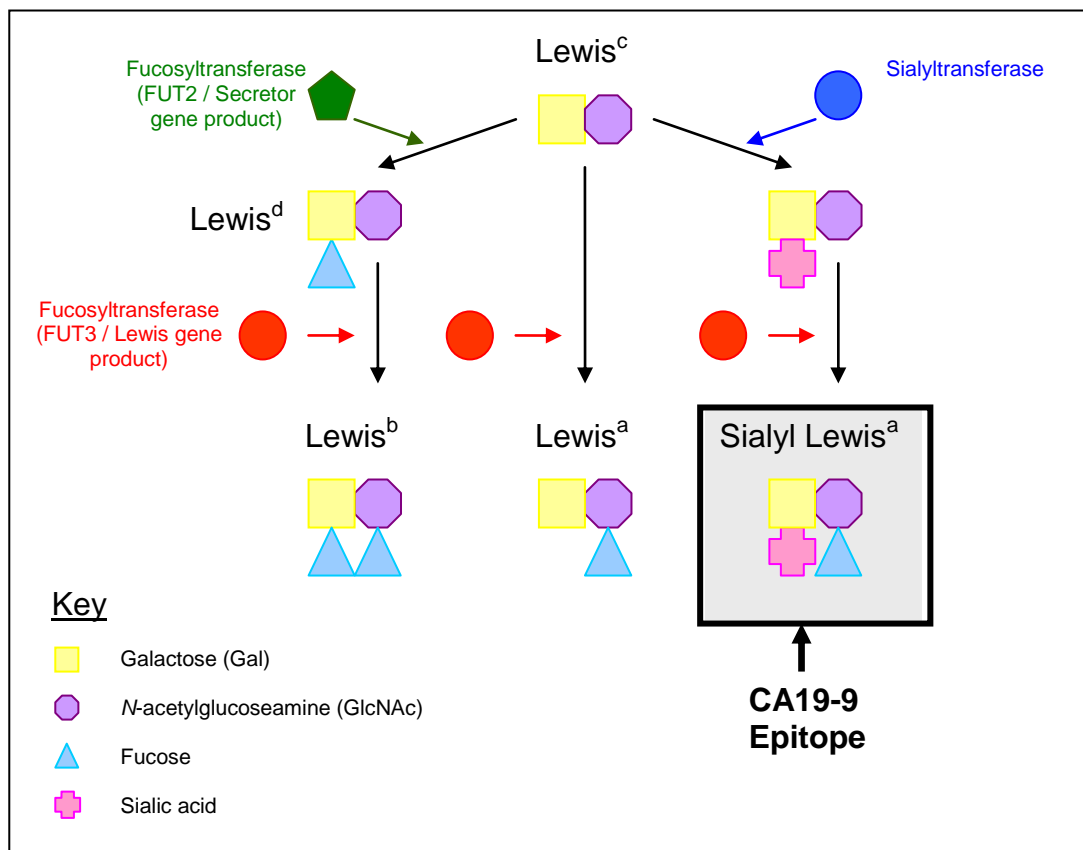
Abnormal levels of CA15-3 are found in 70 – 80% of patients with metastatic breast cancer [35, 65] and it is thought that this is as a result of the over expression of MUC1 [56]. As well as being found in breast cancer increased levels of MUC1 or CA15-3 have been found in other carcinomas such as advanced transitional cell carcinoma of the bladder [66]. CA15-3 may also become raised in patients with benign diseases such as fibrocystic disease [67] chronic hepatitis, liver cirrhosis, sarcoidosis, tuberculosis, systemic lupus erythematosus [68], and approximately 5% of people who are apparently healthy [35, 69].

CA19-9 is mucinous tumour marker of the sialyl Lewis^a epitope. This epitope is formed by the glycans of several different mucins including MUC1. Unlike CA15-3 which is recognised by antibodies which bind to the protein core and an antibody that binds a carbohydrate epitope of MUC 1, CA19-9 or sialyl Lewis^a is recognised by the 1116 NS 19-9 antibody (first derived by Koprowski et. al. in 1979 [44]) which recognises a purely carbohydrate epitope on the mucins. The amount of binding is pH dependant.

Sialyl Lewis^a is a variant of the normal Lewis blood group antigens (Le^a or Le^b) which are found on intracellular adhesion molecules and are thought to be involved in binding e-selectin. They are thought to be expressed in approximately 90-95% of the population as roughly 5-10% of people are Lewis-negative and so cannot synthesise this antigen [70, 71]. The sialyl Lewis^a or CA19-9 epitope is a product of the interaction or competition of at least three genes *FUT2* – the

secretor gene, *FUT3* – the Lewis gene (located on 19q and 19p respectively) and a sialyltransferase (see Figure 4) [72]. The synthesis and release of CA19-9 is thought to be up-regulated by cyclic adenosine-monophosphate (cAMP) [73].

Figure 4 Flow diagram illustrating the production of the Lewis epitopes Le^a, Le^b and SalyI-Le^a adapted from Vestergaard et. al. [72].



CA19-9 can become raised in patients with cirrhosis of the liver, acute pancreatitis, biliary obstruction or cirrhosis, benign obstructive jaundice, pulmonary disease [35], cystic fibrosis [74] and, as with CEA, levels can become raised as a result of smoking, however, gastro-intestinal and particularly pancreatic malignancies [75] usually produce the most grossly elevated levels.

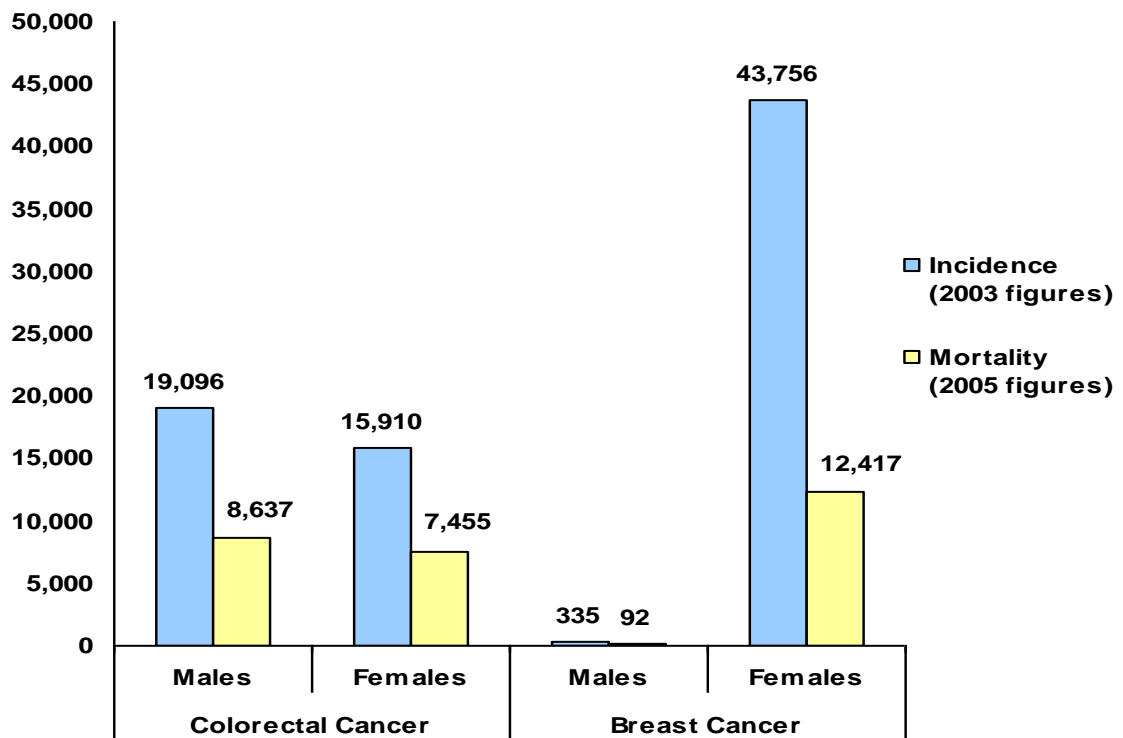
At least “80% of patients with exocrine pancreatic adenocarcinoma” [35] are said to have a raised level of CA19-9. Baseline levels of CA19-9 in patients receiving chemotherapy for pancreatic cancer have been shown to be an independent prognostic factor for survival [76] and are known to “correlate with both recurrence and survival” [77]. It is also thought that “abnormally high serum levels of CA19-9” may correlate with unresectable disease [78] and that a normal early CA19-9 level following radical surgery is “a relatively favourable prognostic index” [79]. Further to this Ziske et. al. (2003) found that a when treating inoperable pancreatic cancer, patients with a decrease in the baseline CA19-9 level of >20% had significantly better median survival than those where CA19-9 levels increased or decreased by < 20% [80], results very similar to those obtained by Maisey et. al. in 2005 [76].

CA19-9 has been studied in combination with CA-50 and CEA in the monitoring of gastric carcinoma where it is thought its measurement “may help in checking the prognosis, determining the efficacy of palliative treatment modalities, and recognising recurrences” [81] and in combination with CEA and CA125 in the study of advanced bladder cancer where the markers were shown to predict disease response to chemotherapy [82].

(1.3.3) Breast and Colorectal Cancer

29% of all cancer diagnoses in 2002 were breast or large bowel (colorectal) and in 2004 the same two cancer types made up 19% of all cancer deaths (see Figure 2) [10]. There is a reasonable, and improving, chance of cure and disease free survival in both of these cancer types as research continues to be undertaken into treatment and surveillance methods.

Figure 5 UK incidence and mortality rates in colorectal and breast cancer (information from Cancer Research UK [10])



(1.3.3.1) Breast Cancer .

Breast cancer can begin as either ductal carcinoma in situ (DCIS) which, as its name suggests is very localised early breast cancer, or lobular carcinoma in situ (LCIS) which is also non-invasive. More advanced cancers can be either invasive ductal cancers (which amount to 75% of all breast cancers) or invasive lobular cancers (approximately 10% of all breast cancers). Invasive lobular cancers are found mainly in women aged 45-55 years whereas the incidence of invasive ductal cancers increases with age. It is for this reason, together with the fact that DCIS is not always readily palpable, that women aged 50 to 70 are asked to attend for a routine mammogram every 3 years. A further form of cancer, inflammatory breast cancer, is very uncommon; in this disease the lymph ducts in the breast become blocked by cancer cells.

Very few breast cancers arise as a result of genetic predisposition however there are two very well known genes, BRCA1 and BRCA2, which when mutated in certain recognised ways indicate that the individual will develop breast cancer during their life with a high degree of certainty. As well as these extremely well known genes which may carry inherited mutations, others, which may be more commonly mutated in sporadic breast cancer are now known, for example Cyclin D and p53 [11].

Breast cancer is staged as seen in Tables 2 and 3 and treatment is assigned accordingly, however with breast cancer, other factors must also be taken into account; The hormonal status of the tumour must be considered (is it positive or negative for oestrogen and progesterone receptors, ER and PR status

respectively), as must its HER-2/neu (or ErbB-2) status and the menopausal status of the female patients which can also have a large impact upon the clinical decisions which are made.

When a patient presents with LCIS the usual treatment is surveillance, almost all other breast cancer patients are offered surgery in the first instance. Patients with DCIS are offered surgery in the form of a wide local excision which may be followed by radiotherapy. Other individuals with potentially curable disease (Stage I to III) are offered mastectomy or wide local excision depending on, amongst the other factors described above, the size and position of the tumour. Controversy still exists as to the long term efficacy of wide local excision compared with mastectomy particularly in young patients [84]. During surgery the axillary lymph nodes are also removed and analysed for local metastases in order to correctly stage the tumour. New research suggests that histology from the sentinel node could be effective in detecting local metastatic spread and therefore reduce the need to remove all of the surrounding lymph nodes in some patients [85]. This technique could reduce the number of women who go on to develop debilitating lymphadenopathy.

Neo-adjuvant chemotherapy is currently given in order to down size locally advanced tumours in order to make surgical resection possible. Recently a Cochrane Review has been published which investigated the use of neo-adjuvant versus adjuvant chemotherapy in women with operable breast cancer. This review found no difference in overall or disease free survival between the 2 groups and recommended the use of neo-adjuvant chemotherapy in order to

reduce the level of surgery required and “to evaluate chemosensitivity and to facilitate translational research” [86].

Adjuvant radiotherapy is offered to patients if there is a high chance of local recurrence and chemotherapy in the form of epirubicin (an anthracyclin) with cyclophosphamide, methotrexate and 5-fluorouracil (ECMF) is the current standard treatment given in the adjuvant setting, and was shown to confer statistically significant advantages in terms of relapse free and overall survival ($p = <0.001$ in both cases) over CMF alone [87]. ECMF is offered to patients who are ER negative and to some pre-menopausal patients who have a high risk of recurrence.

Hormone therapy is offered to all but ER negative patients. This is in the form of Tamoxifen or an aromatase inhibitor in postmenopausal patients and ovarian suppression or removal in pre-menopausal patients. It is thought by the Early Breast Cancer Trialists' Collaborative Group that “some years of adjuvant tamoxifen treatment substantially improves the 10-year survival of women with ER-positive tumours and of women whose tumours are of unknown ER status” [88] however tamoxifen can cause increased risk of thromboembolic disorders and endometrial changes. Because of this the aromatase inhibitor anastrozole was compared with tamoxifen in a randomised trial called the Arimidex, Tamoxifen Alone or in Combination trial (the ATAC trial). Results of the ATAC trial showed that Anastrozole treatment significantly reduced the occurrence of endometrial cancer, vaginal bleeding and thromboembolic events amongst others ($p=0.02$, $p<0.0001$ and $p=0.0006$ respectively), whereas treatment with

tamoxifen “was significantly better tolerated with respect to musculoskeletal disorders and fractures ($p < 0.0001$ for both)” [89]. Recent evidence suggests that third generation aromatase inhibitors (anastrozole, letrozole and exemestane) have a more favourable toxicity profile than tamoxifen [90, 91].

Roughly 20 to 30% of patients with breast cancer are found to be HER-2 positive, this has been linked with aggressive tumour biology [92]. In these patients the monoclonal antibody trastuzumab can now be used. Previously trastuzumab (or Herceptin®) treatment was only approved in the palliative setting however recent studies have shown that just one year of treatment with this drug significantly ($p < 0.001$) improves disease free survival [93] and overall survival in HER-2 positive women in the adjuvant setting [94].

Breast cancer develops into advanced disease with the presence of distant metastases in approximately 40 to 50% of cases within the first five years following primary treatment [83]. The most common sites of metastatic disease are the bones liver, lungs and brain. The development of advanced disease usually means that the disease is no longer ‘curable’ and treatment becomes palliative rather than adjuvant, however, with the development of increasingly sophisticated surgical techniques isolated metastases can occasionally be resected with curative intent.

In the palliative setting aims of treatment are mainly to improve or maintain the patient’s quality of life to alleviate disease symptoms and to prolong survival. Treatment options again depend upon many factors such as patient preference,

existing co-morbidities, the sites of metastatic disease, hormone receptor and HER-2 status and the treatments previously received by the patient.

Treatment options again include hormonal therapy for those eligible. The same considerations that apply to hormone treatment in the adjuvant setting also apply in the setting of advanced disease i.e. patients must have hormone sensitive disease. This form of treatment is often the first treatment option in advanced breast cancer in postmenopausal patients as treatment can be initiated rapidly. If hormonal therapy with one of these drugs ceases to be effective treatment can be changed to another of them as a second line hormonal therapy. In premenopausal patients ovarian suppression or removal is the usual method of hormone therapy.

Chemotherapy is another treatment option and several different individual chemotherapy drugs and combinations of drugs are used in the palliative setting. First line treatment is usually in the form of anthracycline (e.g. doxorubicin) and / or taxane (e.g. taxol) regimens. In anthracycline resistant disease taxane based treatment is often used (alone or combined with gemcitabine or capecitabine) [91]. Monoclonal antibody treatment in the form of trastuzumab can be given to patients eligible for treatment (HER-2 positive) as a single agent or in combination with non-anthracycline based chemotherapy. In metastatic breast cancer trastuzumab in combination with chemotherapy has been shown to confer a significant survival advantage to treatment with chemotherapy alone [92]. Radiotherapy can also be given if necessary for palliation of symptoms and localised disease control.

Bone is the most common site for metastatic disease in patients with breast cancer; bisphosphonates are another class of drugs which are commonly given to patients with metastatic bone involvement or tumour-induced hypercalcaemia (as well as patients without breast cancer who have osteoporosis).

Bisphosphonates are usually given as regular infusions and they work by reducing the rate of bone growth and dissolution [95]. In patients with advanced breast cancer the use of bisphosphonates has been shown to significantly reduce skeletal events such as fractures and may reduce bone pain [96]. Some side effects of bisphosphonates have recently come to light for example an increased incidence of osteonecrosis of the jaw [92].

(1.3.3.2) CEA, CA15-3 and CA19-9 in Breast Cancer

CEA and CA15-3 are the most established and studied tumour markers in breast cancer; other markers such as Tissue Polypeptide Specific Antigen (TPA) are used but less frequently. Studies into the sensitivity and specificity of these markers are often difficult to compare due to differences in assay technique, tumour marker source used (e.g. serum/tumour tissue), time of sampling, therapy investigated and disease stage. Comparison can also be hindered as tumour markers are often combined together in tumour marker panels and studies sometimes do not report the results for each marker individually.

The use of CA19-9 in patients with breast cancer is not yet well documented as it is thought that its main clinical utility lies in patients with Gastro intestinal malignancies.

Localised Disease

It is now widely accepted that neither CEA nor CA15-3 is of real value in screening for breast cancer, In 1998 Molina et. al. reported that the sensitivity of CEA and CA15-3 was 18% and 16% respectively in detecting loco-regional disease [97]. In 2003 Molina et. al. quoted similar sensitivities of 13.0% and 18.8% respectively they also reported that when the markers CEA and CA15-3 were combined sensitivity increased but remained low at 22.8% [98]. Due to the lack of both sensitivity and specificity of both CEA and CA15-3 it is thought that neither tumour marker is of value in the detection of early breast cancer [69, 83, 99]. Despite this it is thought that increased pre-operative levels of both markers in patients with loco-regional disease are thought to correlate with adverse patient outcome in the long term [69, 100]. It has been speculated that raised markers at this early stage may indicate the presence of unidentified metastatic disease [83].

CEA and CA15-3 levels have been shown to correlate with tumour size and lymph node status [98] and Sherring et. al. (1998) report that increasing CA15-3 levels were associated with greater tumour size and involvement of axillary lymph nodes but not oestrogen receptor status [101] similar findings to those of Cañizares et. al. (2001)[102]. However Duffy et. al. (2000) use multivariate analysis to show that CA15-3 is actually a prognostic marker independent of these two factors [53].

Kumpulainen et. al. (2002) found that survival varies significantly ($p < 0.001$) between patients with normal and abnormal (ULN = 29 U/ml) CA15-3 levels

(Hazard ratio = 3.36) and that raised CA15-3 is an independent prognostic factor for reduced disease specific survival [14]. This may result in part from the fact that they substituted postoperative CA15-3 levels when no preoperative levels were available, however, their findings do agree with those of Duffy et. al. (2004) [103]. Shering et. al. (1998) using multivariate analysis also report preoperative CA15-3 to be independently and significantly predictive of disease free and overall survival at 5 years ($p < 0.01$ in both cases and relative risk (RR) = 1.84 and 2.4 respectively) using 30.38 U/ml as the cut-off level [101].

Ebeling et. al. (2002) used univariate analysis to demonstrate that raised preoperative CA15-3 but not CEA correlates with early relapse ($p = 0.0003$) however both CEA and CA15-3 correlate with death from the disease ($p = 0.0001$ in both cases) [100], this confirmed their earlier results [104]. Other studies, again using univariate analysis, also found that both CEA and CA15-3 significantly correlate with overall survival but unlike Ebeling et. al. (2002) [100] found that increased levels of both markers correlated significantly with disease free survival [98, 102]. In fact Molina et. al. (2003) found that CEA and not CA15-3 was an independent prognostic factor for both DFS and overall survival [98] and Canizares et. al. (2000) [102] found that neither of the markers were predictive.

Ebeling et al (1999) found that “single **postoperative** tumour marker values alone were without prognostic value concerning relapse or death” [104] however their multivariate analyses have also found a significant link between a post-surgery

decline in pre-surgery CEA values with an increased risk of recurrence and death, the same was not found with CA15-3 [100, 104].

Despite the fact that groups such as ASCO do not recommend the use of tumour markers in routine follow up of patients after potentially curative treatment both CEA and CA15-3 are routinely used in the follow up of patients with breast cancer and in monitoring the efficacy of treatment in advanced disease but as yet, there is no clear framework for their use.

Detecting Advanced Disease

Several studies now demonstrate that increases in the levels of CEA and CA15-3 sometimes pre-date clinical and radiological evidence of advanced disease development; this is often called the tumour marker lead time.

Table 5 shows lead times as quoted by several different papers. Lauro et. al. (1999) quoted a mean lead time of 3 months for both CA15-3 and CA27-29 (range 2-7months) [99] which is to be expected as both of these tests are identifying the same molecule. Blijlevens et. al. quote the lead time for CA15-3 as double this (6 months) however this is based upon data from only 3 patients [105].

Table 5 Sensitivity of tumour markers at the time of advanced breast cancer and tumour marker lead times.

Date	Author	N° of patients / N° with advanced disease	Sensitivity % of tumour marker in advanced disease (mean lead time in months)	
			CEA	CA15-3
1991	Nicolini et. al. [106]	285 / 40	45	52 (2.7)
1993	Sölétormos, G et. al. [107]	90 / 21	10	48 (2.1 [‡])
1995	Blijlevens et. al. [105]	121 / 91	(2)**	(6)**
1996	Molina et. al. [108]	200 / 89	30 (4.9)	47 (4.8)
1996	Pectasides et.al. [109]	209 / 68	34.1	68.2
1998	Molina et. al. [97]	521 / 185	61	70
1999	Lauro et. al. [99]	220 / 70	35 (2)	79 (3)
1999	Robertson et. al. [110]	67 / 67 ^b	42	54
2001	D'Alessandro et. al. [67]	1365 / 111	-	65.7
2002	De La Lande et. al. [111]	119 / 119	-	(5.3*)
2003	Kurebayashi et. al. [112]	528 / 528	30.5	44.0
2003	Nishimura et. al.[113]	220 / 220	41.4	50.9

[§] All patients had distant metastases (not simply advanced disease)

[‡] Calculated from a lead time quoted as 64 days

* Calculated from a lead time of 162 days, range 31-765 (however these results excluded patients with a lead time of less than 30 days and those who initially developed a local recurrence).

** Calculated with data from one patient (CEA) and three patients (CA15-3)

As can be seen from the sensitivities listed in Table 5, CA15-3 is considered to be more effective than CEA as and indicator of recurrence or metastatic disease. It is also thought that a tumour markers sensitivity in detecting advanced disease is reduced when the advanced disease takes the form of local recurrence as opposed to metastatic disease, Molina et. al. (1996) illustrated this when they reported that 30% of patients had a raised CEA level before diagnosis of advanced disease compared with 47% with raised CA15-3 and that these figures increased to 33% and 56% respectively when patients with local recurrence were excluded [108]. Martoni et. al. (1995) report that during metastatic disease 45% of patients have raised levels of CEA and 71% have raised CA15-3 (77% sensitivity when CEA and CA15-3 were combined) [65]. I have not included these

figures in table 5 as the marker levels were not necessarily recorded at the time of diagnosis of advanced disease.

Kurebayashi et. al. (2003) report 55.2% of patients with advanced breast cancer have raised CEA, CA15-3 or both [112] (n=348, as only 66% of the 528 patients in table 5 had both CEA and CA15-3 measured), this is compared with 75% as quoted by both Nicolini et. al. (1991) [106] and Robertson et. al. (1999) [110].

The results of Robertson et. al. (1999) refer to a group of patients with distant metastatic disease and not simply advanced disease, it is therefore worth noting that this may give falsely high sensitivity as it is known that the sensitivity of CEA and CA15-3 to detect advanced disease are thought to be related to the site of disease development and as previously stated levels are thought to be higher in patients with metastatic disease than in those with local recurrence [105, 108].

The highest levels of CEA and CA15-3 have been associated with liver and bone metastases [97, 99, 105, 108, 113, 114] and Nishimura et. al. (2003) found that both CEA and CA15-3 were at their lowest in patients with brain metastases [113]. Tampellini et. al. (1997) found higher CA15-3 levels in patients with pleural effusions as well as the viscera [115]. Markers appear to lack the sensitivity to detect lung metastases however De La Lande et. al. (2002) found that a CA15-3 lead time was most commonly found in patients who developed lung metastases [111].

Like CA15-3, elevation of CA27.29 the alternative MUC1 antigen (identified by the B27.29 antibody described in section 1.3.2) has also been found to correlate

with the development of advanced disease with a sensitivity comparable to that of CA15-3. In a group of 166 patients where 26 patients went on to develop recurrence Chan et. al. (1997) found that the CA27.29 assay had a sensitivity of 57.7%, specificity of 97.9%, a PPV of 83.3 and a NPV of 92.6% and also that the average lead time before disease recurrence was 5.3 months which is comparable with the result for CA15-3 [116].

Colomer et. al. (1989) showed that in relapsing patients elevated CA15-3 (> 40U/ml) was the first sign of recurrence significantly more frequently than CEA (45% vs 25% respectively, $p < 0.001$). this paper also claims that the addition of CEA does not add any more information to that provided by the CA15-3 assay alone, conversely it actually increases the number of false positive results seen [68]. It is worth noting here that although tumour marker lead times are sometimes seen they are not seen in all cases for example Nicolini et. al. (1991) found that of 285 breast cancer patients, 40 patients who relapsed were evaluated and of these only 21 patients (53%) had a tumour marker lead time from one or more of the markers CEA, CA15-3 or TPA [106].

The marker TPA is often measured in conjunction with CA15-3 and CEA. It has been demonstrated that by combining CA15-3 and TPA the sensitivity and specificity of CA15-3 to disease recurrence is further increased [67, 106, 109, 117]. Sonoo et. al. (1996) used TPA, CA15-3 and CEA in a tumour marker panel and found that the changes in the levels of the markers correlated significantly with response to therapy (see Table 6) however the power of this study was low ($n = 45$) [118]

The value of early detection and earlier treatment of advanced breast cancer is widely debated, although theoretically treatment at an earlier stage would reduce tumour burden to a greater degree than later treatment this approach has not been advocated. Joseph et. al. (1998) report that in a group of 1898 patients followed up for recurrence, 129 patients developed recurrent disease. In a retrospective review of how these recurrences were detected they found no significant difference in survival ($p=0.18$) between patients where recurrence was detected by intensive follow up ($n=27$, 21%) (including LTF, CEA and CA15-3 analysis, Chest radiograph, CT and bone scan) and those where recurrence was detected by minimal follow up ($n=99$, 79%) with history, physical examination and mammography. In contrast to these findings in 1997 Nicolini et. al. [17] reported that early treatment based upon the detection of advanced disease by the CEA-TPA-CA15-3 tumour marker panel (as used above by Sonoo et. al. [118]) increased survival “from mastectomy to 72 months and from salvage treatment to 30 months” significantly ($p = 0.031$ and 0.004 respectively) [119]. In 2003 Nicolini et. al. again demonstrated that early treatment of metastatic breast cancer based upon the same tumour marker panel again resulted in a significant increase in survival from salvage treatment or mastectomy ($p = 0.015$ and 0.007 respectively) [120].

It may be that the inclusion of TPA by Nicolini et. al. in the above studies increases tumour marker sensitivity for recurrence or that the tumour marker assays done by Joseph et. al. (performed at intervals of 3 months for the first 2 years and then at 6 month intervals for the following 2 years) at were not performed frequently enough to identify the tumour marker lead times quoted in

Table 5 which precede clinical of radiological evidence of advanced disease. The power all three studies was relatively low and as a result further research into these important questions is needed.

During Advanced Disease

Molina et al (1998) found elevated levels of CEA in 61% of patients with advanced breast cancer ($n = 413$) compared with elevated CA15-3 in 70% of patients and Nishimura et al (2003) found similar results (67.3% for CEA and 76.8% for CA15-3) [113]. Lauro et al (1999) quote these figures as 79% for CA15-3, 70% for CA27-29 and a low 35% for CEA, however, the greatest sensitivity (82%) was achieved by combining all 3 markers [99]. The level of positivity is now thought to be between 70% and 80% for CA15-3 [35].

CA15-3 is thought to be more sensitive and specific than CEA in the monitoring of advanced breast cancer [65, 112] however increased sensitivity is thought to be gained by combining the 2 markers [83, 112], Molina et. al. state that “most reports indicate that by using CEA as well as CA15-3 it is possible to increase sensitivity by 7% to 20% compared to that obtained with CA15-3 alone” which they report as having a sensitivity of 55-70% in advanced breast cancer [83].

As with the development of loco-regional disease overall survival is thought to be shorter in CEA and CA15-3 positive patients who have advanced breast cancer [109], Kurebayashi et. al. (2003) found that time to progression (TTP) was significantly shorter in patients who had positive pre-treatment levels of CEA and CA15-3 but also that patients who had a fall of >20% in either marker level during

therapy had a significantly longer TTP. They also found that negative pre treatment CA15-3 levels but not CEA levels were an independent predictor of increased time to progression in patients with advanced disease [112]. Loprinzi et. al also found that CEA levels were not predictive of survival [114]. The results of Nishimura et. al. (2003) concur with these findings in terms of CEA however contradict them entirely in terms of CA15-3 stating that patients with negative CA15-3 levels after recurrence had significantly poorer survival rates ($p = 0.003$) [113].

Some studies only look at CA15-3 and again the same contradicting results are reported; Berruti et. al. (1994) report that raised CA15-3 level (>30 U/ml) at the time of disease recurrence is an independent prognostic factor for shorter overall survival ($n = 115$) [121]. Tampellini et. al. (1997) found this not to be the case and instead related CA15-3 level to disease extent which itself was found to be an independent variable in determining survival [115]. It has also been speculated that raised CA15-3 levels are linked with Oestrogen Receptor (ER) positivity [111] and Loprinzi et al (1986) also link elevated pre-treatment CEA levels with oestrogen receptor (ER) positivity [114].

Many studies aiming to compare tumour marker response and response to treatment in advanced breast cancer look at single tumour marker levels however some studies use a more dynamic approach to tumour markers by looking at marker changes over time and in relation to treatment a summary of 13 such studies can be seen in Table 6. In 1995 Martoni et. al. did this (see Table 6) and investigated change in CEA and CA15-3 (and MCA and CA549)

levels during hormone therapy and chemotherapy for metastatic disease. They defined 3 categories of tumour marker response in patients with abnormal tumour marker values at the start of treatment; $\geq 25\%$ increase = progressive disease, $\leq 25\%$ decrease = partial response, $> 25\%$ decrease and normalising of tumour marker values = complete response. This system showed that CA15-3 had a sensitivity of 67% in monitoring the clinical course of the disease in patients with an abnormal baseline level which also identified progressive disease with a sensitivity of 87% and a positive predictive value (PPV) of 100%. This was better than CEA which had a sensitivity of 53% [65].

Blijlevens et. al. (1995) also defined response as a 25% increase or decrease from the baseline; they found that in 38% of patients CEA marker response correlated with lack of progression and this figure was higher at 49% for CA15-3. The levels of false positives were recorded as 22% (CEA) and 11% (CA15-3) respectively [105].

Robertson et. al. (1991) when looking at the CEA-ESR-CA15-3 index of response and progression (see Table 6) found that combined marker changes within this index correlated with UICC response (classified as non-progression or progression) with a maximum sensitivity of 92% and specificity of 82% at 4 months after treatment start. They also found that the maximum sensitivity and specificity for CEA and CA15-3 combined (without ESR) was also at 4 months and was 85% and 82% respectively. Correlation with UICC response was found irrespective of whether 10% or 20% marker change was used to define that response [122]. Dixon et. al. (1993) examined the same index (CEA-ESR-CA15-

3) but in patients undergoing systemic chemotherapy rather than hormone therapy, they found significant correlation between the index score at 6-8 weeks after initiation of therapy and the UICC response at 3-4 months after initiation of therapy (again classified as non-progression or progression). Their results showed the response index to have a sensitivity of 89% and a specificity of 96% at this time. These results indicate that marker kinetics can reflect response to treatment [123].

Dixon et.al. (1993) also take these results one step further by treating people based upon their tumour marker movements, although their study was underpowered they found that there was a significantly clinical remission period in patients who were treated with continuous chemotherapy in a tumour marker dependant way compared with those who stopped cytotoxics after 6 months [123].

Table 6 Research into tumour marker kinetics and response to treatment in advanced breast cancer

Breast Cancer							
Author	Date	Markers & Cut-off values	P/R*	Patients	Aims	Treatment	Results / Conclusion
Loprinzi et. al. [114]	1986	CEA: > 5ng/mL	P	97 women with metastatic breast cancer	To evaluate “the use of pre-treatment and serial CEA levels “	DAVTH Vs DAVTH alternating with CMFP	Elevated pre-treatment CEA correlated significantly with ER positivity, prolonged disease-free intervals, hepatic and bone metastases and multiple sites of metastatic disease but not response rate, time to treatment failure or survival. In the first 4 months of Serial CEA measurements CEA levels declined progressively from elevated levels or “initially rose significantly (mean, 243% of pre-treatment value) and then declined” [114].
Kiang et. al. [124]	1990	CEA: > 5 ng/ml CA15-3: >25 U/ml	P	30 women with elevated CEA (n=24) and / or CA15-3 (n=12)	To examine the kinetics of CEA and CA15-3 “in terms of plasma doubling time and half-life and their relationship to therapeutic response” [124]	Various chemotherapy regimens	The authors report finding “four distinct kinetic patterns “ of CEA response to chemotherapy: <ul style="list-style-type: none"> • Marker continues to increase • Marker level declines • Marker surge followed by decline • Sharp marker decline followed by increase. They also found that “changes of CA15-3 kinetics were similar to that of CEA” [124].
Robertson et. al. [122]	1991	CEA: > 6 ng ml CA15-3: > 3 U ml (and ESR)	P	65 patients	Assess the correlation of the CEA-CA15-3-ESR tumour markers when combined with UICC assessed response	Systemic endocrine therapy	“changes in the markers at 2, 4 and 6 months showed a highly significant correlation with UICC assessed response at 6 months” [122]. They found no significant difference in survival between groups assessed (radiologically or biochemically) as progression or non-progression.

Breast Cancer

Author	Date	Markers & Cut-off values	P/R*	Patients	Aims	Treatment	Results / Conclusion
Dixon et. al. [123]	1993	CEA: >6ng ml ⁻¹ CA15-3: >33U ml ⁻¹ (ESR)	P	55 (of 67 patients treated with chemotherapy)	To test the “biochemical response index comprising ESR, CEA and CA15-3” [123] to direct continuous chemotherapy treatment.	Mitozantrone + CMF (<i>n</i> = 21) or CMF (<i>n</i> = 34).	Marker changes at 2 and 4 months correlated with the UICC assessed response at 3 and 6 months (<i>p</i> <0.001), sensitivity = 100%, specificity = 87%; PPV = 85%. The survival from first treatment and median time to biochemical progression were longer (<i>p</i> = 0.04 and 0.05 respectively) in the responsive patients who received continuous chemotherapy compared with the control group.
Blijlevens et. al. [105]	1995	CEA: 5µg/l CA15-3: 30U/l (TPS)	P	121 (43 = NED, 78 = ED)	Using CEA and CA15-3 as a reference to investigate if TPS can determine activity of the disease, location of metastases and response to treatment as assessed by UICC criteria.	29 of the ED patients received chemotherapy or hormone therapy, no further details.	TPS + CA15-3 has a sensitivity = 72% for detection of metastatic disease. Using a 25% increase or decrease in baseline tumour marker level as a tumour marker progression or response the “correlation with clinical deterioration after 6 months of therapy was 44% for TPS 33% for CEA and 28% for CA15-3” [105].
Martoni et. al. [65]	1995	CEA: 2.5ng/ml CA15-3: 30U/l (MCA, CA549)	P	71 (of 201) were monitored for relationship with response to treatment	To look at the markers ability to detect advanced disease. Study of tumour marker changes in response to treatment was also done on a subset of patients (<i>n</i> =71).	Hormone therapy or chemotherapy (either CMF, FEC or MMM)	<ul style="list-style-type: none"> From normal base line levels Sensitivity = 42% for CEA and 87% for CA15-3 (specificity = 100%). Combining markers decreased specificity of changes in marker levels to monitor clinical course (only progression was evaluable in this group). From abnormal baseline levels “Overall sensitivity of marker changes was between 53% (CEA) and 67% (CA15-3)” [65] in monitoring clinical course (As measured by WHO criteria).

Breast Cancer

Author	Date	Markers & Cut-off values	P/R*	Patients	Aims	Treatment	Results / Conclusion
Sonoo et. al. [118]	1996	CEA: 2.5ng/ml CA15-3: 30U/l (TPA)	R	45 (36 with recurrent breast cancer, 9 stage IV disease)	To investigate the "relationship between the initial changes and the kinetic patterns of the markers after therapy and the objective responses" [118].	Hormone, chemotherapy and radiotherapy	20% increase or decrease in markers was taken as significant. Initial changes in all three markers significantly correlated with the therapeutic responses ($P < 0.01$) Five distinct kinetic patterns were made by the tumour markers following the initiation of therapy. Kinetic patterns of the tumour markers were better at correlating with therapeutic response than initial marker changes.
Rubach et. al. [9]	1997	CEA: Unknown CA15-3: Unknown	R	298 patients with stage I to IV disease	"to investigate the prognostic value of CA15-3, CEA and ESR, mainly in metastatic disease" [9].	No details given	Found there to be a significant relationship (log-rank P value) between CEA and CA15-3 marker patterns and both overall survival and disease free survival when they categorised marker movements as; normal, decreasing, fluctuating or increasing.
Luaro et. al. [99]	1999	CEA: < 5 U/ml CA15-3: < 30 U/ml CA27-29: < 35 U/ml (and MCA)	R	220 patients: 180 = no evidence of disease (30 developed advanced disease later), 40 = advanced disease at diagnosis	To "define the most useful tumour marker panel in breast cancer patients' follow up and in monitoring treatment response" [99] as assessed by UICC criteria.	Various regimens of endocrine therapy, radiotherapy and chemotherapy	Both CA15-3 and CA27-29 correlated with the course of advanced disease 81% of the time compared with 40% of the time for CEA.

Breast Cancer							
Author	Date	Markers & Cut-off values	P/R*	Patients	Aims	Treatment	Results / Conclusion
Robertson et. al. [110]	1999	CEA: >6 $\mu\text{g l}^{-1}$ CA15-3: > 33 Kum l^{-1} (and ESR)	P	83	To investigate if CEA, CA15-3 and ESR can measure remission and progression in Metastatic breast cancer	Hormone or chemotherapy	A marker panel of "CA15-3 and CEA (with and without ESR) provide an objective method to guide therapy in patients with metastatic breast cancer". "Overall changes in the three tumour markers reflected UICC-defined disease progression in 34 out of 39 patients (87%)" and. "there was an excellent correlation between changes in the three tumour markers and the disease progression by UICC criteria" [110] although significance is not quantified here
De La Lande et. al. [111]	2002	CA15-3: 30 U/mL	R	119 patients and 140 matched controls	To analyse "the prognostic implications of CA15-3 kinetics before and at first metastasis" [111].	Standard treatment	Significantly better survival in:- - Patients with a CA15-3 lead time >30 days compared with those who do not. - Patients who have a longer interval (>770 compared with ≤ 770 days) between diagnosis and first elevation of CA15-3
Kurebayashi et. al.[112]	2003	CEA: 5 ng/ml CA15-3: 30 U/ml	R	348	To clarify the significance of CEA and CA15-3 in monitoring advanced breast cancer	Systemic therapy with endocrine therapy, chemotherapy or both.	CEA and CA15-3 correlate with response to therapy but only in patients with raised marker levels. There was a significant ($p < 0.01$) correlation between a 20% reduction in raised CEA and CA15-3 marker levels during therapy and a longer time to progression.

Breast Cancer							
Author	Date	Markers & Cut-off values	P/R*	Patients	Aims	Treatment	Results / Conclusion
Nicolini et. al. [120]	2003	CEA: (1981-95) >7ng ml ⁻¹ { (1995-99) 5 ng ml ⁻¹ CA15-3: (1981-95) 32mU ml ⁻¹ { (1995-99) 32U ml ⁻¹ (and TPA)	P	109 the results of which only 68 were evaluated	To see if treatment based upon the CEA-TPA-CA15-3 tumour marker panel can prolong survival	Various systemic treatments	88% sensitivity of the tumour marker panel for detecting distant metastases. Patients treated early in a tumour marker guided way had significantly longer survival from mastectomy and from salvage therapy (<i>p</i> < 0.01 in both cases) at 7 years and 3 years respectively. This is the final report of the study by Nicolini into the effect of treatment based upon the CEA-CA15-3-TPA tumour marker panel following publication of their preliminary findings in 1997 [17]. They found that “tumour marker guided” salvage treatment significantly prolongs disease-free and overall survivals” [120].

CA27.29	Cancer antigen 27.29
CA549	Cancer Antigen 549
CMF	Chemotherapy regimen comprising cyclophosphamide, fluorouracil and methotrexate
CMFP	Cyclophosphamide, methotrexate, 5-fluorouracil and prednisone
DAVTH	Dibromodulcitol, doxorubicin, vincristine, tamoxifen and fluoxymesterone
ER	Oestrogen receptor
ESR	Erythrocyte sedimentation rate
FEC	Chemotherapy regimen comprising cyclophosphamide, fluorouracil and epirubicin
MCA	Mucin like Carcinoma Associated Antigen
MMM	Chemotherapy regimen comprising methotrexate, mitomycin C and mitoxantrone
NED/ED	Evidence of disease / No evidence of disease
P/R*	Study type – prospective or retrospective
TPS/TPA	Tissue polypeptide specific antigen
UICC	International Union Against Cancer

CA15-3 level is considered to be an effective predictor of outcome and is thought to correlate with both disease free survival (DFS) and overall survival (OS). Kumpulainen et. al. (2002) found that CA15-3 level correlated with OS with a greater degree of significance than tumour stage [14]. Kurebarashi et al (2003) demonstrated that a decrease in raised levels of CEA and CA15-3 correlate well with disease “response to therapy” [112] and increased levels are shown to correlate with shorter survival times [98]. Rubach et. al. (1997) have already shown that patient survival (both relapse free and overall) and tumour marker kinetics (CEA and CA15-3) during post-surgical follow up and/or therapy are significantly linked ($p < 0.05$ in all cases), see table 6. A summary of their results from 298 patients can be seen in table 7 below

Table 7 Results of Rubach et. al. (1997) [9].

	Ten Year Overall / relapse-free survival rates (%)	
	CEA	CA15-3
Within normal range	63.0 / 13.9	71.9 / 20.7
Decreased	56.9 / 12.7	57.5 / 12.9
Fluctuating	40.2 / 11.7	24.7 / 0.0
Increasing	31.4 / 6.2	27.9 / 4.8

As only log rank p values are quoted in this paper it is impossible to comment on the exact nature of the relationship between marker movement and survival but the results indicate that there may be a survival advantage for patients if their tumour markers never rise above the upper limit of normal. The results also suggest that patients with decreasing markers may have better survival than patients whose tumour markers fluctuate or increase; this would seem intuitive if tumour marker levels correlate with tumour burden. The difference between

patients whose markers fluctuate and those whose markers increase is less clear as patients with fluctuating CEA levels appear to have improved survival when compared with patients with increasing tumour markers but this is not the case with CA15-3.

As well as monitoring response to treatment there is now increasing evidence that the use of CEA and CA15-3 can be used to successfully select patients who are responsive to treatment, Dixon et al (1993) demonstrated that by using CEA and CA15-3 (along with ESR) levels to select patients to be treated with continuous rather than conventional chemotherapy regimens overall survival and time to biochemical progression significantly increased [123].

Some of the papers on this subject produce interesting results but closer inspection of the methodology within some of these papers shows inconsistencies which probably greatly influence the accuracy of the results obtained. Nicolini et al (2003) [120] is a final report of a study from which data was preliminarily published in 1997 [17] and the main findings of Nicolini et al (2003) can be seen in table 6. In the preliminary study data was collected between 1977 and 1993 [17] and in the later study the dates during which patients were recruited is quoted as 1981 to 1999 [120]. No information is provided regarding the reasons for the different start dates and the authors do not clarify how patients were selected for the study from the available population. The long study duration results in the possible influence of other variables upon the results because factors such as available treatments and imaging techniques will improve over time.

In both of these papers the authors describe the way that patients were allocated to the two different treatment groups. The groups consisted of “early” treatment based upon the findings of the tumour marker panel, or conventional treatment based upon the findings of other clinical tests such as radiology. The patients were not allocated randomly to these groups but in some cases patients refused early treatment and were therefore allocated to the conventional treatment group. Others were included in the conventional treatment group because they did not have an increased tumour marker level at the time of relapse, or because they had an increase in tumour marker level at the same time as their relapse which was also otherwise diagnosed. These patients could not have been included in the “early” treatment group as they did not have an increase in tumour marker prior to their relapse or because they did not consent to early treatment. This failure to randomise results in the comparison of two groups with different characteristics and makes it impossible to draw any conclusions from results which are obtained when the two groups are compared [17,120].

(1.3.3.3) Colorectal Cancer

Incidence and mortality of colorectal cancers in the UK can be seen in Figure 5. Colon cancer accounts for 62% and rectal cancer 38% of the 35,006 recorded colorectal incidences in 2003. However colon cancer makes up 64% of the colorectal deaths in 2005 whereas rectal cancer accounts for 36% of deaths [10]. It is possible that this difference is due to increased detection of rectal cancers at an early stage, this could be due potentially to earlier development of symptoms from a tumour at this site.

Most colorectal cancers are sporadic adenocarcinomas which arise in an existing adenoma (a benign growth with a glandular structure) as a result of an accumulation of genetic mutations over time [49], for example, imbalanced DNA methylation is thought to be an early stage in colorectal cancer formation as is a deletion in 18q affecting the genes DPC4 (also known as SMAD4) and DCC, and 17q losses. It is thought that both copies of the adenomatous polyposis coli (APC) gene are inactivated in up to 80% of cases of sporadic colorectal cancer [10].

It is thought that only approximately 3-6% of colorectal cancers are hereditary of which there are two main forms, both are autosomal dominant disorders.

1. Familial Adenomatous Polyposis (FAP) is the least common of the two predispositions. It is a disease characterised by the development of benign polyps throughout the large bowel. One or more of these polyps will almost certainly become malignant over time. FAP is caused by

loss of function of one copy of the APC (adenomatous polyposis coli) gene of 5q21. The APC gene product is involved in the degradation of β -catenin which in turn is part of the protein complex which activates proto-oncogenes e.g. *c-myc* [10].

2. Hereditary non-polyposis colorectal cancer (HNPCC) as the name suggests has no preceding polyps. It is nearly always caused by a mutation in MSH1 or MSH2 (and less commonly the PMS2 gene); this affects the process of DNA mismatch repair rendering the cell more vulnerable to DNA damage.

Both of these predispositions are associated with early onset of colorectal cancers and HNPCC is also associated with increased incidence of other malignancies.

The colorectal cancer staging system is defined in Tables 2 and 3. The most important aspect of early treatment for Dukes A, B and C colorectal cancers is surgery to resect the tumour; this may be undertaken in Dukes D colorectal cancer in order to palliate symptoms. Despite surgery half of patients who have undergone resection of colorectal cancer will go on to relapse and die of the disease within 5 years, decline in survival is not as steep after this point and 42% of patients survive to ten years post surgery [125].

The current National Institute for Clinical Excellence (NICE) guidelines state that chemotherapy should be offered post surgery to patients with Dukes C colorectal

cancer as this confers a proven survival advantage. For these patients the current recommended adjuvant treatment is 5-Flourouracil (5-FU) and Folinic acid (FA) with oxaliplatin, or capecitabine alone [126] and guidelines concerning the use of irinotecan in this setting are currently in development. There is little evidence to support or reject the use of chemotherapy in patients with Dukes B colon cancer at present however the QUASAR trial aims to answer this question [127].

In the case of rectal cancer the advantage of surgery followed by chemotherapy over surgery alone is not known, however some detail can be found concerning the impact of radiotherapy with cancer at this site. It is known that the addition of radiotherapy to a chemotherapy regimen does not increase survival significantly but does reduce the incidence of loco-regional recurrence at the 5 year point [128], and also that chemotherapy with early radiotherapy confers a significant disease free but not overall survival advantage over patients who receive chemotherapy with late radiotherapy [129].

Following curative resection of localised colorectal disease (Duke's A, B and C) approximately 50% of patients will go on to develop disease recurrence [49], most recurrences will occur within the first 3 years, and nearly all will have occurred within the first 5 years following the original diagnosis and resection. Although a great deal of treatment for patients who present with, or develop, metastatic disease is palliative, re-resection of local recurrence or metastatic disease is becoming more available and effective as surgical techniques and

diagnostic techniques such as imaging technologies and biochemical tests become more sophisticated.

Where resection of metastatic disease is not possible palliative chemotherapy has been shown to confer a 35% (95% confidence interval of 24%-44%) reduction in the risk of death and an “improvement in median survival of 3.7 months” [130]. NICE guidelines recommend the use of irinotecan or oxaliplatin combined with 5-FU/FA as first line chemotherapy followed by Oxaliplatin – 5-FU/FA or irinotecan alone in subsequent cycles if appropriate [131].

(1.3.3.4) CEA and CA19-9 in Colorectal Cancer

A great deal of work has been done on the use of CEA and CA19-9 in colorectal cancer in the adjuvant setting and in predicting relapse. CEA and CA 19-9 are the most commonly used tumour markers in this setting.

Localised disease

CEA is thought to be elevated in more cases of localised colorectal cancer than CA19-9 and no correlation between raised levels of one of these tumour markers and raised levels of the other has been found [70]. Serum levels of the markers have been observed to increase significantly along with increased Duke’s stage as has their sensitivity [132].

In patients with localised rather than advanced colorectal cancer the percentage of patients who have raised CEA can be seen in the Table 8. It is thought that “CA19-9 contributes little to the early diagnosis of colorectal cancer” as elevated

CA19-9 levels are only found in 20-30% of newly diagnosed colorectal cancer patients [35] as can also be seen from Table 8.

Table 8 Sensitivity of tumour markers to detect localised colorectal cancer.

			% of Patients with raised tumour marker at detection of localised colorectal cancer	
Date	Author	N° of Patients analysed	CEA	CA19-9
1997	Nakayama et. al. [70]	121	39.5	20.6
1998	Andicoechea et. al. [133]	214	31.3	-
2000	Díez et. al. [134]	174	34.4	-
2001	Zheng et. al. [135]	97	30.9	25.8
2004	Morita et. al. [136]	114	45.3	29.8
2005	Chen et. al. [137]	574	42.3	16.9

As with CEA and CA15-3 in breast cancer increased levels of both CEA [133, 138] and CA19-9 [70] prior to curative surgery for colorectal cancer have been linked with reduced survival [15, 135, 139, 140]. Zheng et. al. (2001) found the same results but their analysis also included some individuals with Dukes stage D disease [135]. These findings are disputed by other studies [137, 141]. Morita et. al. (2004) found that raised preoperative CEA and CA19-9 looked as though they correlated with decreased overall survival but this decrease only reached significance with CEA [136] and Gebauer et al (2001) found that increased CEA and CA19-9 correlated significantly with reduced overall survival but only increased CEA correlated with reduced disease free survival [139]. Chen et. al. (2005) studied 574 colorectal cancer patients who had undergone a potentially curable resection, they found significantly poorer prognosis in stage II colorectal cancer patients who had increased preoperative levels of both CEA and CA19-9 compared to patients who had normal levels of both [137].

CA19-9 is thought to be less sensitive than CEA but despite the lack of sensitivity of CA19-9 Reiter et. al. (2000) found that when they evaluated “the prognostic value of preoperative serum levels of CEA and/or CA 19-9“ in a retrospective study they found that Only Dukes’ classification and CA 19-9 level correlated significantly with prognosis [142]. When looking at risk of recurrence rather than prognosis the findings of Zheng et. al. (2001) were similar to this. They found that elevated pre-operative CA19-9 was associated with higher Dukes’ stage as well as number of positive lymph nodes and increased risk of recurrence [135] this study found that CEA did not correlate well with increased rate of recurrence. In contrast to these studies Weissenberger et al (2005) found that raised preoperative CA19-9 at this time was not prognostic of shorter survival at all [143].

Several studies have found that increased levels of CEA correlate with greater tumour stage [133, 135, 144, 145] and higher rate of recurrence, Díez et al (2000) found that this relationship changed over time with raised CEA only correlating with increased risk of recurrence in the first two years of follow up, after this time no significant correlation was seen [134]. Wiratkapun et. al. saw correlation between raised CEA and the later appearance of distant metastases but not local recurrence [138]. It is postulated that poor prognosis, higher rate of recurrence and high levels of CEA itself may all be due to increased tumour stage therefore there appears to be a relationship between high CEA and poor prognosis when actually both of these factors occur as a result of a third prognostic indicator. Chapman et. al. (1998) showed that if tumour stage has not

been controlled for raised preoperative CEA level correlates significantly ($p = 0.001$) with survival, however, if tumour stage is controlled for this correlation is no longer significant [146].

Although our study will look at the tumour markers in the serum many studies have been done which look at the expression levels of these markers in the tissues themselves [139, 147, 148]. These studies also associate increased levels of CEA and CA19-9 [149, 150] within the primary tumour with reduced survival. Nakayama et al (1997) also showed that both pre-operative and post-operative CA19-9 levels correlate significantly with expressed CA19-9 levels [70]. Nakagoe et. al. (2001) found that increased preoperative CEA is not an independent predictor for survival but that high CEA within the tumour tissue itself was an independent predictor of shorter survival [144]. In contrast to this Gebauer et al (2001) looked at CEA and CA19-9 levels in tumour tissue (at the time of resection with curative intent) and found that raised levels of either marker did not have a significant effect on survival [139], however, this was a small study ($n = 41$, 20 of whom had tumour recurrence in comparison to Nakagoe et. al. where $n = 79$ [144]) which may account for the different findings. Gebauer et. al. (2001) did find that a raised CEA (but not CA19-9) levels in the supposedly normal mucosa adjacent to the resected tumour did correlate significantly with reduced survival ($p = 0.0385$) [139].

Elevated pre-operative levels of CEA have also been linked with poor tumour differentiation [146] and metastasis to the liver, it is postulated that this could be due to the uptake of CEA by the liver facilitating the metastatic process.

Most studies refer to preoperative levels of CEA and CA19-9 being related to poor prognosis in colorectal cancer however Weissenberger et. al. (2005) relate pre-radiotherapy values (in rectal cancer alone) to poor outcome. It may be that raised levels at any point indicate poor prognosis. This study also showed significant correlation ($p < 0.001$) between CEA levels increasing or decreasing during radiotherapy and overall survival [143], an indication that tumour marker response to treatment may correlate with prognosis. This study included 203 rectal cancer patients with stage II and III disease and excluded smokers of >40 cigarettes per day increasing the accuracy of the results but this obviously does not help to determine the impact of raised CEA levels in patients who are heavy smokers.

Post-operative raised levels of CEA have been shown to correlate significantly disease free and overall survival [139] and with adjusted survival [151] as have post-operatively raised levels of CA19-9 [70].

Detecting Advanced Disease

Increasing CEA and CA19-9 levels are often detected prior to clinical or radiological detection of advanced disease. Examples of some of the tumour marker lead times quoted can be seen in table 9:-

As can be seen from this table, CEA is thought to be more sensitive at detecting disease recurrence or the development of distant metastases during follow-up than CA 19-9. CEA is also thought to become raised earlier than CA19-9 during

the development of advanced disease. Nicolini et. al. (1995) found that sensitivity of CEA in detecting early recurrence was 43% and its specificity was 96%, the lead time of CEA was found to be 5 ± 3.2 (mean \pm standard deviation) [152]. McCall et. al. (1994) quote similar figures for CEA sensitivity and specificity in detecting recurrence - 58% and 93% respectively [153].

Table 9 Sensitivity of tumour markers at the time of advanced colorectal cancer and tumour marker lead times.

			Sensitivity % of tumour marker at recurrence / ADD / first palliative chemotherapy (and mean lead time (LT) in months)	
Date	Author	N ^o of patients / N ^o evaluated	CEA	CA19-9
1990	Quentmeier et. al. [154]	179 / 179, 70 ^(LT)	82.1(4.7)	-
1991	Chu et. al. [151]	425 / 132	79	-
1993	Kouri et. al. [155]	105 / 102 ^(CEA) 94 ^(CA19-9)	71	46
1994	McCall et. al. [153]	311 / 98	58 (6*)	-
1994	Fiella et. al. [156]	370 / 96	84	48 (3.7 / 3*)
1995	Nicolini et. al. [152]	90 / 18, 14 ^{‡(LT)}	61 (5)	-
2001	Hanke et. al. [157]	90 / 85	51	39
2002	Berglund et. al. [158]	87 / 87	87	-
2004	Morita et. al. [136]	155 / 40	80.0 (3*)	42.5 (2*)

* lead times given as median rather than mean

‡ 14 patients but 16 relapses analysed

In the postoperative monitoring of CRC patients CEA appears to be better at predicting disease recurrence or metastases than CA19-9. Filella et. al. (1994) looked at both CEA and CA19-9 in 370 patients with colorectal cancer to investigate how effective CA19-9 is at detecting CRC recurrence compared with CEA. They found that the “Sensitivity of CA 19-9 in the early diagnosis of recurrence was much lower than that obtained for CEA (75%). Only one patient had elevated CA 19-9 levels and normal CEA” [156], similar findings have also been reported elsewhere [136]. Fiella et al (1994) state that combining CEA and

CA19-9 does not usefully improve sensitivity [156]. One more possible advantage of combining CA19-9 with CEA in colorectal cancer is that CA19-9 is found at increased levels in fewer benign diseases than CEA [35].

Another interesting point raised by Fiella et. al. is that when progressive rise of CA19-9 was taken into account “the concordance between kinetic CA 19-9 serum levels and disease status (99.65%) was similar to CEA concordance (100%)” [156] in patients without clinical evidence of disease.

The main advantage of early detection of advanced disease is the increased potential for curative resection of recurrence in patients where recurrence/advanced disease is detected by CEA or computed tomography (CT) compared with recurrence/advanced disease detected by symptoms. Quentmeier et al (1990) found that respectability of recurrence was significantly ($p = 0.01$) related to earlier diagnosis [154] and Chau et. al. (2004) found that 3.1% of pts with symptomatic recurrent CRC underwent curative resection (for liver or lung metastases) compared with 23.8% of patients where recurrence was detected by surveillance CT and/or CEA [159] although this appears to be an impressive difference Wolf et. al. (1997) estimated that CEA directed curative re-resection would confer a survival advantage of less than 5% [160].

During Advanced Disease

At the time of development of advanced disease CEA and CA19-9 are often raised but the quoted percentage of patients with raised tumour markers at this time varies greatly as illustrated by Table 9. Trillet-Lenoir et. al. (2004) found

raised levels of CEA and CA19-9 in 85.7% and 67.5% of patients respectively in a group of (n=91) patients with metastatic colorectal carcinoma undergoing first or second line chemotherapy [161] (this data is not in Table 9 as patients may have been undergoing second-line chemotherapy).

As with work done in breast cancer, a lot of studies look at the prognostic implications of single marker levels in advanced colorectal cancer [162] [163] often producing contradictory results. As well as aiding the diagnosis of advanced disease there is evidence that raised pre-treatment levels of both CEA [164, 165] and CA19-9 [166] are **independent** prognostic indicators of poor survival. Some studies do not support these findings [158], Mitry et. al. (2004) found that although raised CEA was significantly associated with survival in univariate analysis it was not in multivariate analysis [167].

Tomasevic et. al. 2003 looked at single CEA levels determined before the start of chemotherapy in advanced disease and found that median survival in 114 patients decreased significantly ($p = 0.006$) with increased CEA levels [168] and other studies have found similar results [160, 163]. Park et. al. (2005) divided patients undergoing curative resection into four groups depending on their preoperative CEA levels (<3, 3-6, 6-17, and >17ng/mL), but they only found a significant difference in survival between these groups in patients with stage II tumours [169].

Webb et. al. (1995) looked at CEA and CA19-9 levels taken prior to chemotherapy given in advanced disease and concluded that raised CA19-9 had

“no prognostic significance” [170] whereas CEA was found to be an independent predictor of poor prognosis (Hazard Ratio (HR) = 1.8, 95% Confidence Interval (CI) = 2.8-1.2). Again other studies dispute this, finding CA19-9 the better predictor of survival [166, 171] for example Wang et. al. (2002) and Kouri et al (1993) used multivariate analysis to show that raised CA19-9 but not CEA was predictive of survival in patients with metastatic colorectal cancer ($p < 0.001$ in both cases) [155, 171].

As in localised disease, in Advanced disease raised CEA has been found to correlate significantly with both poor performance status and poorly differentiated tumours [170]. Other studies have found that raised CEA levels correlate with the presence of liver metastases [145, 153, 166, 172, 173] and Kouri et. al. (1993) found that both raised CA19-9 and CEA levels occur significantly more frequently in patients with liver metastases [155]. In patients with liver metastases raised levels of CEA and CA19-9 correlate with the number of metastases, and raised CEA but not CA19-9 correlates with the size of the liver metastases [174]. Again there is a link with reduced survival in these individuals because in patients with liver metastases raised CEA has been associated with depressed cell-mediated immunity which in turn is associated with shorter survival [51]. In contrast to this Sasaki et. al. (2005) used multivariate analysis to show that raised CA19-9 and not CEA correlated with shorter extra-hepatic disease free time in patients with liver metastases [175].

In patients undergoing hepatic resection for colorectal metastases Aldrighetti et. al. (2005) found **pre-operative** CEA levels ($> 5\text{ng/ml}$) were one of the

independent prognostic factors for poor survival [176]. In contrast Hohenberger et. al. (1994) reported raised **post**-operative CEA but not **pre**-operative CEA correlated significantly with prognosis (both disease free and overall survival). Hohenberger et. al. also found that a reduction in an abnormal pre-operative CEA level to a normal post-operative level confers significantly better survival than a persistently elevated CEA marker level [177].

This finding, as in breast cancer, raises the possibility that a change in marker level over time (marker kinetics) may confer important prognostic information about disease state and survival as absolute tumour marker levels have been shown to do but with the added possibility that tumour marker kinetics may provide more detailed information about fluctuations in disease status and treatment efficacy. Several studies have investigated this possibility. Korenaga et. al. (1997) found a significant relationship ($p = 0.001$) between a shorter individual CEA doubling time and reduced overall survival [178] however this study included both gastric and colorectal carcinoma patients. The major findings of 10 more studies who have investigated this possibility can be seen in Table 10.

Table 10 Research into tumour marker kinetics and response to treatment in advanced colorectal cancer

Colorectal Cancer							
Author	Date	Markers & Cut-off values	P/R*	Patients	Aims	Treatment	Results / Conclusion
Lawton et. al. [179]	1980	CEA: >30ng/ml	P	43 patients following palliative resection of colonic cancer	To see if changes in CEA levels relate "to the clinical status of the patient and coincident therapy"[179]	27 received chemotherapy treatment with 5-FU and MCCN	Showed "CEA level increase with the expected progression of the residual tumour but that the rise is slower in patients receiving chemotherapy" [179] and that irrespective of treatment, in patients where CEA rose quickly, prognosis was worse.
Aabo et. al. [173]	1986	CEA: >8ng/ml	R	175 patients with progressive colorectal cancer	"To elucidate the relationship between the level of plasma CEA and survival of the patients" [173] in progressive disease.	1*	Rise in CEA from the ULN "indicates with a high degree of certainty relapse or disease progression in colorectal patients" [173]. But that CEA does not reliably predict clinical response to chemotherapy and "an increase of CEA is little prognostic value concerning survival" [173]
Allen-Mersh et. al. [180]	1987	Any decrease in CEA to below the pre-treatment level.	R	329 patients	To assess correlation between a fall in CEA following treatment and prolonged survival	Chemotherapy (various regimens)	A fall in CEA following treatment correlated significantly with increased survival ($p < 0.001$). In radiological non-responders there was a significant improvement ($p = 0.04$) in survival in those whose CEA level fell following treatment.
Quentmeier et. al. [181]	1989	Individual reference level of CEA developed for each patient	R	35 patients who had hepatic metastases only	to find out if serial CEA measurements" yield reliable data on the therapeutic progress and the individual prognosis" [181] of the patients	Intra-hepatic chemotherapy. 5-FU first line (n=35), Mitomycin C second line (n=8)	Individual reference level of CEA was set in the 3-month reference period at the beginning of chemotherapy. Survival of patients whose CEA level never decreased below the reference level following the reference period was significantly ($p < 0.001$) shorter than the other patients.

Colorectal Cancer

Author	Date	Markers & Cut-off values	P/R*	Patients	Aims	Treatment	Results / Conclusion
Kouri et. al. [166]	1992	CEA: >5 µg/l CA19-9: >37U/ml	R	85 patients from a phase II chemotherapy trial.	“to analyse the value of CEA and CA19-9 determinations in predicting the tumour response” [166] compared to the UICC criteria.	Epirubicin, sequential methotrexate and 5-flourouracil.	Response (R) and progression (P) defined as ≥ 35% decrease or increase respectively in a raised marker level. CEA (R): sensitivity 84%, specificity 77%, PPV 67% CA19-9 (R): sensitivity 88%, specificity 67%, PPV 50% CEA (P): 75% correlated with clinical progression CA19-9 (P): 60% correlated with clinical progression
Hamm et. al. [182]	1998	CEA: change in level of ≥ 36% in patient with at least on elevated level (3.5 ng/mL)	R	81 patients, all the patients from 1 centre seen in a year who had CEA (above ULN) measured.	“to determine the reliability of CEA level in determining tumour response to chemotherapy in the metastatic setting” [182]	Chemotherapy (various regimens)	Sensitivity of CEA = 54% (95% CI 0.37-0.75) Specificity of CEA = 53% (95% CI 0.42-0.63) Power was very low for this part of the analysis. There was no significant difference in survival between patients who had a “significant drop in the CEA level in the first 6 months of treatment” [182] and those who did not (this part of the study was correctly powered).

Colorectal Cancer

Author	Date	Markers & Cut-off values	P/R*	Patients	Aims	Treatment	Results / Conclusion
Hanke et. al. [157]	2001	CEA: ≥ 10 ng/ml CA19-9: ≥ 50 IE/ml	P	85 patients on 1st line treatment (primary tumour resected).	"to examine the diagnostic accuracy of monitoring of palliative chemotherapy by means of CEA and CA19-9" [157] compared this the WHO criteria.	First line chemotherapy consisting of high dose 5-FU and FA	CEA rise of $> 50\%$ differentiated between PD and NC/PR/CR with sensitivity = 76% and Specificity = 90%. A CEA decrease of $\geq 30\%$ excluded progression in 99% of cases They concluded - "A CEA or CA19-9 rise is only conditionally appropriate for recording progressions. A progression however, can be excluded with falling levels with high diagnostic accuracy, in which CEA offers a greater degree of certainty than CA19-9" [157]
Berglund et. al. [158]	2002	CEA: < 3.1 $\mu\text{g/l}$ (TPS, VEGF, bFGF)	R	87 patients	"To evaluate the reliability and validity of serum ...CEA.....in monitoring palliative chemotherapy in advanced colorectal cancer" [158].	First line 5-FU and leucovorin	A decrease in $>25\%$ of CEA level from a raised baseline level had sensitivity of 45% for an objective response and 46% for a subjective response, specificity was 88%. CEA decrease of $>25\%$ was found significantly more in patients with response than patients with no response BUT they conclude that "Repeated measurements of CEAare of limited value in monitoring chemotherapy in ACRC" [158].
Ito et. al. [183]	2002	CEA: ≥ 5 ng/ml	P	22	To investigate if the doubling time and half-life of CEA could predict progression or prognosis	Surgery and no other details given	Of 14 patients assessed for CEA half life the group who developed metastases after curative surgery (n=9) had a significantly longer (p < 0.001) CEA half life of 8.01 ± 2.07 days [median \pm SD] compared to those (n=5) who did not develop metastases (half life 4.33 ± 1.11 days).

Colorectal Cancer							
Author	Date	Markers & Cut-off values	P/R*	Patients	Aims	Treatment	Results / Conclusion
Trillet-Lenoir et. al. [161]	2004	CEA: > 5µg/l CA19-9: > 30 kU/l	P	91 patients (Karnofsky index ≥50% and presence of ≥1 measurable hepatic/pulmonary metastasis)	To evaluate the usefulness of CEA and CA19-9 in evaluating response to chemotherapy in metastatic CRC.	Chemotherapy (regimen at discretion of the investigators)	<p>“Meaningful PPV values (>90%) for progression of an increase of the marker levels were only obtained using the 200% increase threshold for CEA alone“ or in combination with CA19-9 [161].</p> <p>To assess the response of metastatic CRC to chemotherapy CEA alone or in combination with CA19-9 (in addition to CT) “should be used with caution in common practice“ [161].</p>

- 1* Adjuvant treatment = methylcyclohexylchloroethylnitrosourea and –FU Vs no treatment or Palliative treatment = 5-FU Vs furanidyl-5-FU
- 5-FU 5-Flourouracil
- ACRC Advanced colorectal cancer
- bFGF Basic fibroblast growth factor
- CI Confidence interval
- CRC Colorectal cancer
- CT Computed tomography
- MCCN Methyl chloethyl cyclohexyl nitrosourea
- P/R* Study type – prospective or retrospective
- PD / NC / PR / CR progressive disease / no change / partial remission / complete remission
- PPV Positive predictive value
- SD Standard deviation
- TPS Tissue polypeptide specific antigen
- UICC International Union Against Cancer
- ULN Upper limit of normal
- VEGF Vascular endothelial growth factor
- WHO World Health Organisation

As can be seen from this table Hamm and Cripps (1998) found no correlation between change in CEA after treatment and overall survival [182] whereas Allen-Mersh et. al. (1987) found that tumour shrinkage (of > 25% measured clinically or radiologically) and fall in CEA level following the start of chemotherapy both correlated with increased survival ($p < 0.00001$ and < 0.0002 respectively) in disseminated colorectal cancer. However tumour shrinkage was found to be the stronger predictor of survival [180]. Wang et. al. (2001) found similar results in terms of survival when they classified response as a 50% drop in CEA for >4 weeks following chemotherapy for metastatic colorectal cancer. They found that patients who had a CEA response survived significantly longer than those who did not and that CEA marker response had a sensitivity of 72% and 81% and a PPV of 53% and 85% when detecting true responses and progressive disease respectively [184].

Hanke et. al. (2001) found that CEA was more accurate at demonstrating response (compared with WHO criteria) than CA19-9 and that patients who had an initial fall of $\geq 50\%$ in CEA level had a significantly higher probability (relative risk (RR) = 2.9, $p = 0.002$) of achieving a (partial or complete) response with further treatment [157]. Hanke et. al. (2001) do not link response to treatment with increased survival and possibly quality of life however it would seem intuitive to do this. Preketes et. al. added further evidence to support the theory that marker movement following therapy can be predictive of survival when they showed a significant association between

the percentage fall in patients (n=33) CEA levels following cryo- and chemo therapy for colorectal liver metastases and their survival [185].

CEA, CA15-3 and CA19-9 are frequently used in the diagnosis and treatment of both localised and advanced breast and colorectal cancers (CEA and CA19-9) as indicators of survival and tumour burden. A great deal is known about these biological markers to date however their role in indicating the efficacy of treatment in advanced disease is not clear. Several studies have investigated the use of sequential tumour marker measurements to track the course of advanced disease in response to treatment in both breast and colorectal cancer however the findings of these studies are difficult to compare and limited in their findings for several reasons:

- Few studies use comparable definitions of tumour marker response and progression.
- Studies are mainly retrospective.
- Definitions of tumour response and progression vary.

In 1996 the first guidelines regarding the use of tumour markers in breast and colorectal cancer were adopted by the American Society of Clinical Oncology (ASCO), these guidelines were published in 1997. In patients with breast cancer ASCO found insufficient evidence to recommend the routine use of either CEA, CA15-3 or CA27.29 in screening, diagnosis, staging or surveillance but stated that “in the absence of readily measurable disease, CA15-3 and CEA levels can be used to document treatment failure” [186].

The authors also acknowledged that there was evidence to support the ability of CA15-3 and CA27.29 to detect the development of advanced disease but they found no robust evidence in the literature of this early detection leading to clinical benefit for the patient. An update to these recommendations was published in 2001 which recommended no change to the previous guidelines in terms of the use of any of these three markers [187].

In the ASCO colorectal cancer tumour marker guidelines published in 1997 it was recommended that CEA “be measured preoperatively if it would change surgical management” [186] and that the levels of CEA are measured postoperatively each 2-3 months for ≥ 2 years in patients with stage II and III disease if there was a possibility of liver resection should metastatic disease develop. The panel found insufficient evidence in the literature reviewed to recommend the routine use of CA19-9 in the screening, diagnosis, staging or surveillance of colorectal cancer [186]. Once again these guidelines were not changed following the updates published in 2001 [187].

In a more recent update to these colorectal cancer guidelines published in 2006 no change was made to the guidelines for the use of CA19-9 in colorectal cancer but it was recommended that CEA levels be measured every 3 months postoperatively for at least 3 years with a confirmed increase in level being used to instigate further investigations [188]. It was also reported that that adjuvant chemotherapy may produce transient false elevations of CEA. The largest change to the recommendations for CEA measurement was in monitoring response to therapy in advanced disease

where it is now recommended that CEA should be measured at 1 to 3 monthly intervals throughout treatment with systemic therapy and “persistently rising values should prompt restaging but suggest progressive disease even in the absence of corroborating radiographs” [188]. The authors of these recommendations warn that caution should be taken in interpreting results in the first 4-6 weeks of a new therapy as transient and misleading rises in CEA may be seen (particularly during treatment with oxaliplatin) [188] these findings are similar to those of the recent report of the European Group on Tumour Markers who conclude the report with their topics for future work one of which states that “the clinical use of existing markers should be optimised” [189] citing the use of CEA in monitoring treatment for advanced disease as an illustration of this [189].

In patients who have received adjuvant therapy for either breast or colorectal cancer in the form of surgery it is standard practice to see the patient for follow up visits and to undertake routine radiotherapy and biochemical testing. These visits serve to highlight possible areas of concern relating to anomalous test results, symptoms or side effects from treatment, and to identify potential disease recurrence at the earliest possible stage, however, until recently there was little evidence to support the fact that this early detection had any effect upon enhanced survival or quality of life in patients who developed advanced disease.

In 2002 a large scale systematic review (5 trials and 1342 patients) and meta-analysis showed significantly earlier detection of recurrence and improved

survival in patients followed up intensively after curative intent colorectal cancer resection compared with standard follow up [190], several other studies corroborate these findings [191, 192] whereas some do not [193, 194] (the results of Secco et. al. (2000) concern rectal cancer alone).

In some cases the advantages of intensive follow up are less straight forward for example; Chau et. al. (2004) found that a significantly larger number of patients underwent resection for hepatic or pulmonary metastatic disease (from a colorectal primary) when relapses were detected by CT or CEA levels as opposed to symptoms [159]. In addition to these findings Bonthuis et. al. (2004) discovered that although routine follow up (not intense follow up) did not correlate significantly with survival, significantly ($p = 0.006$) longer survival was seen in patients who had curative re-resection where recurrence was detected in routine follow up compared with other methods of detection [195]. Also Rodríguez-Moranta et. al. (2006) found that in stage II and III colorectal cancers where curative resection has been carried out intensive follow up resulted in significantly higher overall survival in patients with stage II tumours and patients with rectal tumours (the reason for this is thought to be the increased possibility of re-resection if such tumours recur), no difference in survival was seen in the group of patients as a whole [196] suggesting that intensive follow up may be beneficial only in certain patient groups.

A Cochrane review into follow up strategies for patients with stage I to III breast cancer suggested in terms of detection of recurrence, survival and quality of life there was that “follow-up programmes based on regular

physical examinations and yearly mammography alone are as effective as more intensive approaches based on regular performance of laboratory and instrumental tests” [197]. Duffy (2006) identifies limitations often found with studies which look at survival for example the fact that many of the studies are becoming out of date as they use “older and less sensitive biochemical tests rather than the newer tumour markers such as CA 15-3” [69] as well as the use of older imaging procedures and older treatment regimens [69] and this is indeed an issue which is highlighted by the authors of the Cochrane review above [197].

Currently advanced disease and disease progression in response to treatment is most commonly monitored radiologically. Various criteria of response and progression of the disease, based upon the imaging techniques, have been proposed and validated over time in order to standardise the reporting of disease extent and response to treatment. The definitions of response and progression are based upon the premise that “overall cancer burden can be characterised by a quantitative evaluation of tumour lesions, which are measurable, and a qualitative evaluation of tumour lesions, which are not measurable” [198] and also that the change in this tumour burden over time is an estimate of the efficacy of treatment, unfortunately these assumptions do not always hold true.

Previously the main criteria of response were the UICC (International Union Against Cancer) [199] and the WHO (World Health Organisation) criteria [200]. The UICC criteria were originally proposed in 1977 [199]. They were

subsequently integrated into the WHO criteria [198] which was developed “on the initiative of the World Health Organisation” [200] and published in 1981, the WHO criteria are defined as follows:-

WHO Criteria

Based upon radiological measurement of lesions two-dimensionally (longest diameter (LD) and greatest perpendicular diameter are measured and multiplied). The sum of the products of tumour measurement provides a baseline measurement and changes from this baseline are measured over time (≥ 4 weeks), response is categorised as follows:

- Complete Response – all target lesions disappear
- Partial Response – $\geq 50\%$ decrease from baseline
- No Change – Partial response or Progressive disease criteria are not met.
- Progressive Disease - $\geq 25\%$ increase of one or more lesions, or the appearance of new lesions [200].

This system has since been replaced by the RECIST criteria (Response Evaluation Criteria in Solid Tumours) proposed by Therasse et. al. (2000) in an attempt to standardise tumour measurement following advances in imaging technology and particularly for use in clinical trials [201]. RECIST criteria of response are subtly different from the WHO criteria despite also being based upon the detection and measurement of measurable disease

and recording the presence of non-measurable disease. The RECIST criteria can be simplistically defined in the following way:-

RECIST Criteria

RECIST is based upon identification and one-dimensional measurement of at least one target lesion (≥ 10 mm as measured by spiral CT scan). Small lesions and other disease for example bone lesions and pleural effusions cannot be assessed using these criteria. The longest diameter (LD) of each target lesion and the sum of all LDs is recorded and change over time (≥ 4 weeks) in this figure is assessed. Response of target lesions is classified as follows:-

- Complete Response – all target lesions disappear
- Partial Response – $\geq 30\%$ decrease in the sum of all LDs
- Stable Disease – $< 30\%$ decrease or $< 20\%$ increase in sum of all LDs
- Progressive Disease - $\geq 20\%$ increase in the sum of all LDs

Although successful in its attempt to standardise and simplify disease measurement there are several problems with the RECIST criteria. Although it is generally accepted that disease measurement by WHO and RECIST are comparable [202] the RECIST criteria can be associated with some problems; it can be difficult to apply to all disease sites, anatomical changes in tumours may be detected later than they would otherwise be [6], definitions of response and progression are arbitrary (change from a baseline) and observer variation may affect the consistency of measurement [8]. In addition to this Therasse et. al. (2006) conclude that use of RECIST criteria can delay

identification of disease progression when compared with WHO criteria. They state that a 20% increase in one dimension (the minimum increase required by RECIST to define progression) is “approximately equivalent to a 44% increase in bidimensional product” [6] when WHO criteria require a minimum of 25% increase in bidimensional product to classify the disease status as progression, a difference in sensitivity of approximately 19%.

When Mazumdar et. al. (2004) retrospectively tested concordance between WHO and RECIST criteria using data from 130 patients they found that 32-35% of patients who had been classified as having progressive disease by WHO criteria were classified differently using RECIST criteria, this is compared with the much smaller values of 8-16% and 3-12% for patients where disease was classified as partial response and stable disease respectively [7].

Another limitation of this approach to response evaluation generally is that with the advent of new therapies (such as tyrosine kinase inhibitors in renal cancer [203]) which act on cellular processes and often stabilise tumour growth initially rather than reduce it [8]) the phrase “overall cancer burden can be characterised by a quantitative evaluation of tumour lesions” [198] is becoming increasingly inaccurate.

It is because of these limitations that it would be extremely beneficial if tumour markers could provide additional, reliable information about response to

treatment in advanced disease. This would be especially important in cases of disease progression where the limitations of RECIST are most apparent.

(1.4) Aims of the Project

We hope to be able to determine within the study populations of breast and colorectal cancer patients –

- The sensitivity and specificity of each individual tumour marker in detecting the development of advanced disease.
- The relative levels of each tumour marker at the time of and prior to the development of advanced disease and at the time of each chemotherapy regimen.
- Tumour marker lead times prior to the diagnosis of advanced disease.

The main aim of this project, however, is to assess the efficacy of our own modified tumour marker response criteria (see table 11). This is based upon criteria developed by Rustin et. al. using CA125 in ovarian cancer studies (see section 1.1.2 CA125 and Ovarian Cancer). We use our modified criteria on CEA, CA15-3 and CA19-9 during treatment for advanced breast cancer, and CEA and CA19-9 during treatment for advanced colorectal cancer. It is vital that we test both the sensitivity and specificity of the modified response criteria as the Rustin criteria of response and progressions have been shown to have a specificity of >98%.

We aim to establish if the response to chemotherapy, as measured by the modified response criteria, correlates with response to chemotherapy as

measured radiologically and overall patient survival. This modified response criteria should also assess whether tumour marker changes at lower levels, within the statistically defined normal range convey potentially valuable information about disease response and survival.

If there is time we will also investigate how soon after initiation of chemotherapy treatment marker movement can be considered to indicate response to treatment.

This will enable us to answer our main study questions

1. Are tumour markers better than radiology at showing response to chemotherapy?
2. Which is the best marker for each disease site and what do the other tumour markers and radiology results add to this?
3. What is the best way to monitor patients undergoing chemotherapy?

(2) Patients and Methods

This retrospective study was given Management approval by the Chair of the Airedale Research Ethics Committee.

Data collection was done retrospectively by looking through patients notes and where data was missing by looking on the radiology and pathology databases of Airedale NHS Trust.

Provisional analysis and results were presented as a poster in 2006 at the National Cancer Research Institute Cancer Conference in Birmingham.

(2.1) Patients

Patients were identified from the Oncology Patient Management Audit System (OPMAS) of Airedale NHS Trust. The details of all patients who were registered onto the hospital chemotherapy prescribing database were obtained by Dr M Crawford. For breast cancer patients this was all patients who were registered onto this database from June 1992 until June 2003 (12 patients had no registration date entered so the above dates are the best guess excluding these patients) and for colorectal cancer patients this was all patients who had been registered onto the database between June 1992 and February 2004.

Patients were excluded from the analysis if their hospital case notes could not be located, if sections (volumes) of the case notes were missing, or if the case notes had been destroyed or microfilmed. Patients were also excluded if they had no chemotherapy treatment at Airedale General Hospital or developed another malignancy which precluded the detailed analysis of their tumour markers at the time of advanced disease.

(2.2) Methods

Data was collected retrospectively from patients hospital notes and stored on individual data collection sheets (Appendices A and B) which enabled marker kinetics to be identified more clearly. Data from each set of case-notes was collected to death or to the time of data collection, dates of death were updated at the time of analysis to make the survival curves more robust however no further information was added to the data collection sheets concerning the patients who had subsequently died.

The chemotherapy regimens studied here include cytotoxic chemotherapy only (not hormone therapy). Chemotherapy treatment intent (adjuvant or palliative) was usually recorded in the hospital notes, if it was not recorded explicitly then treatment intent was elucidated from the hospital notes.

Clinician's advice was sought on this if necessary.

There was no restriction on the number of chemotherapy cycles within a regimen that a patient had to have to be included in the study, if chemotherapy treatment had been commenced then they were included.

Tumour Marker Measurement

Only tumour marker levels measured in Airedale General Hospital were used in this project as assays and techniques vary between locations and therefore results obtained may not have been comparable.

CA15-3 was routinely recorded in breast cancer patients and not in colorectal cancer patients as it is not a recognised tumour marker for this disease site therefore analysis in the breast patients includes the three tumour markers CEA, CA15-3 and CA19-9 whereas analysis in the colorectal patients only includes CEA and CA19-9. Apart from this methods of analysis and data collection were the same in both groups.

In 1993 the upper limits of normal for CEA and CA19-9 were changed from 10 ng/ml and 33 U/ml respectively (CA15-3 was not in regular use within the hospital until after this point). From this point levels of CEA and CA15-3 were assayed using the Abbot AxSYM (Abbot Diagnostics) system. CA19-9 was assayed differently initially but then in during April 1995 also started to be assayed using this system (this assay change occurred without a change of normal ranges for CA19-9). From the change of assay in 1993 the upper

limits of normal (ULN) for the markers remained at the following levels until April 2004:-

- CEA = 3 ng/ml
- CA15-3 = 28 U/ml and
- CA19-9 = 37 U/ml.

In April 2004 there was a change in laboratory analyser to the TOSOH AIA 1800 System analyser which used the antibodies ST AIA-PACK CEA, 19-9 and 27.29 (TOSOH Bioscience inc.). As a result of this the normal ranges of the markers were altered to:-

- CEA = 0-10 ng/ml
- CA19-9 = 0-30 U/ml (the upper limit of normal for CA19-9 was altered to 35 U/ml later in 2004).
- CA 27.29 = 6-23.5 U/ml. It was felt that because CA15-3 and CA27.29 have overlapping epitopes on the MUC1 glycoprotein (see figure 3 – Introduction) and have >0.95 correlation coefficients [63] this change in assay was acceptable in the context of this study.

Marker values measured and reported as outside the ranges quoted above are regarded as being abnormal. Tumour marker levels of <1 and <2 were regarded as 1 and 2 respectively for the purposes of this study, if more than one tumour marker level was recorded on one day then the highest value was used.

If two tumour marker measurements were taken on the same day then the highest measurement was used in the analysis. If two tumour marker measurements were taken on the same day using different assay techniques then the marker level generated using the newer technique was quoted.

For the purposes of this study tumour marker levels which were taken during a time period when the ULN was anything other than 3ng/ml for CEA, 28U/ml for CA15-3 or 37U/ml for CA19-9 will be indicated in the text. If assessment of a Rustin response to a chemotherapy regimen occurred at a time when a marker assay change occurred this will be stated in the text. The only exception to this concerns patients who have Rustin response measured at the time of the CA19-9 assay change in April 1995 because this change in assay was not associated with a change in the normal ranges.

(2.2.1) Tumour Marker Levels Leading up to and at the Time of Advanced Disease Diagnosis

Date of advanced disease diagnosis was defined retrospectively as the date when metastatic or unresectable locally recurrent disease was first seen radiologically, histologically or cytologically, if this date was ambiguous and difficult to identify clinician's advice was sought. Some patients with colorectal cancer went on to have potentially curative surgery for example for liver metastases following this date. If this is the case then we also recorded the date following this resection when advanced disease re-occurs.

Tumour marker levels were included in this analysis if they were recorded within 60 days of this date. The nearest tumour marker level to the date of advanced disease diagnosis (ADD) was used and if there were two tumour marker levels recorded equidistant from the date of ADD then the marker prior to ADD was used.

Patients were included in the tumour marker doubling and lead time analysis if they had a raised marker at the time of advanced disease diagnosis (within 60 days of this date) and a previous tumour marker level was recorded which was > the upper limit of normal (ULN). Only patients whose tumour markers increased prior to advanced disease diagnosis were used in this analysis.

In order to ascertain tumour marker doubling times the following equation was used:-

$$\text{Doubling Time} = d / \log_2 (y / x)$$

Where;

- **x** = level of the first raised value > the upper limit of normal (ULN)
- **y** = level of the raised value at the time of the clinical advanced disease diagnosis
- **d** = the time interval between the two marker levels.

Tumour marker lead times were expressed as the number of days between the date of the first increase of the tumour marker to a level above the ULN (following the final tumour marker level \leq the ULN) and the date of advanced disease diagnosis.

(2.2.2) Tumour Marker Levels at the Time of the First and Second Chemotherapy Regimens given in Advanced Disease

Tumour marker levels were included in this analysis if they were recorded within 30 days of the chemotherapy start date. The nearest tumour marker level to the chemotherapy start date was used and if there were two tumour marker levels recorded equidistant from the date of chemotherapy initiation then the marker prior to this was used.

(2.2.3) Tumour Marker Response to Chemotherapy Given in Advanced Disease

The principal aim of this study was to investigate the effect that tumour marker kinetics in response to treatment have upon survival, this was investigated firstly by survival analysis (using WinSTAT ®). For this analysis and throughout all other analyses tumour marker response to treatment was defined according to the Rustin Criteria of Response which were developed in ovarian cancer and which we have modified for use in this setting (Table 11).

In order to be included in the analysis patients were required to have had at least 3 tumour marker levels recorded; the first marker level must have been within 30 days of chemotherapy start date and there must have been at least two further tumour marker levels taken within the 100 days following the chemotherapy start date.

If two tumour marker levels were recorded equidistant from the chemotherapy start date then the marker prior to initiation of chemotherapy was used as the initial tumour marker level.

Table 11 Classification of patients according to marker response to chemotherapy based on the Rustin criteria.

*Categories not defined by the original Rustin Criteria of Response

Category	Inclusion Criteria
Response (R)	Defined as a decrease of $\geq 50\%$ in a raised first marker level within 100 days of the start of chemotherapy for advanced disease, which is confirmed by a following sample also $\geq 50\%$ lower than the first marker level.
Always Normal (AN) *	A marker level which remains below the ULN from the first marker level, and throughout the 100 day period following the first chemotherapy for advanced disease.
Stable (S)	A marker level which is not always within the normal range during the time between recording of the first marker level, and throughout the 100 day period following the first chemotherapy for advanced disease, but which does not increase or decrease by $\geq 50\%$ (confirmed by a following sample) within this time period.
Progression (P)	A marker level which increases to twice that of the first marker level within 100 days following the first chemotherapy for advanced disease, providing that this value is \geq twice the ULN e.g. in CA15-3 ≥ 56 U/ml. This value must be confirmed by a following sample unless the patient dies before one is taken.
Progression from Within the Normal Range (PN)*	A marker level which increases to twice the first marker level within 100 days following the first chemotherapy for advanced disease, but to a level which is $>$ the ULN but $<$ twice that of the ULN. Again this increase must be confirmed by a following sample unless the patient dies before one is taken.

Response was regarded any raised level which fell by $\geq 50\%$. Patients who responded from a level which was raised but not raised to \geq twice the ULN were noted.

Date of response or progression was the date when the marker first reached the required level. It was important that this change in marker level was confirmed and so this was done by looking at the following tumour marker

level. If this did not corroborate the result then this marker movement was not considered significant and was not included (the only exception to this rule is when tumour marker progression is seen but not confirmed as the patient dies before a confirmatory sample is taken, in this case tumour marker progression is recorded). The confirmatory marker level did not have to fall within the 100 day period after the first chemotherapy was given.

Transient rises in a tumour marker or spiking as it is sometimes known [110] is a phenomenon which has been seen in previous studies and it is thought to occur as a result of tumour lysis in response to treatment. For the purpose of this analysis tumour lysis was defined as a rapid doubling or more of a tumour marker concentration (to a level \geq twice the upper limit of normal) within the first 45 days following the initiation of a chemotherapy regimen, followed by falling tumour marker levels. On occasions when tumour marker lysis was thought to have occurred the original dramatic increase in tumour marker was discounted and the trend in the tumour marker following this point was used to assess overall tumour marker response.

Survival was recorded in days and if the actual date of death was not available then the date of death notification was used instead. If the patient was known to have evidence of advanced breast or colorectal cancer then it was assumed that the patient died of this disease. Death dates were updated before survival analysis was done in order to make the analysis more robust. Median Survival data was calculated using the Kaplan-Meier graphs, if the survival curve was horizontal at 50% survival then a mean was calculated

using the time point when survival fell to 50% and the time point that survival fell below 50%.

Further analysis was performed using regression analysis with the kind help of Andy Scally Lecturer and Statistician at the University of Bradford. For this regression analysis data on the following factors was collected (if available) and taken into account using the analysis:-

Sex

Was graded 0 = Male and 1=Female

Age

The patient's age at the time of the commencement of the cycle of chemotherapy under investigation was recorded.

Tumour Differentiation

Was graded 0 = well differentiated, 1 moderately differentiated, 2 = poorly differentiated if two possible differentiations were quoted in the notes e.g. 'moderately to poorly differentiated' than the worst case scenario (in this case poorly differentiated) was recorded. 'Moderately well differentiated' was classed as moderately differentiated.

Tumour Grade

Was recorded as it is reported in histology reports as a grade of 1 to 3 if the report stated two different grades e.g. 'grade II or III' then the highest grade

quoted (in this case grade 3) was recorded. If conflicting grades were quoted on two separate histology reports then the grade from the definitive histology where the tumour was excised was used.

Tumour Stage

Tumour stage was categorised into an ordinal scale by using the Duke’s classification system for the majority of the colorectal cancer patients so Duke’s A =0 Duke’s B = 1, Duke’s C = 2 and Duke’s D = 3. For the breast cancer patients and those with colorectal cancer whose tumours had been staged using the TNM system the following table (table 12) was used to convert the TNM stage into a single disease stage.

TN (Tumour and Node) stage was often quoted without an M (metastasis) status. In this situation the M status was elucidated from the radiology reports and treatment type (i.e. adjuvant or palliative). If either the T (tumour) or the N (node) status was missing then the disease stage was considered unavailable and the information was omitted from the analysis.

Table 12 Converting TNM stage to an ordinal scale

	N0	N1	N2	N3	M1
T1	I	II	III	III	IV
T2	I	II	III	III	IV
T3	II	III	III	III	IV
T4	III	III	III	III	IV
M1	IV	IV	IV	IV	IV

Nodal Status

The total number of lymph nodes found to be involved in the malignant process was recorded and categorised into a four point ordinal scale using the following key:-

0 Involved nodes = 0

1-3 Involved nodes = 1

4-9 Involved nodes = 2

>9 Involved nodes = 3

The involvement or not of the highest, apical or high tie node was also taken into account in the analysis with uninvolved = 0 and involved = 1. If for example it was stated in the histology report that 0 of 20 nodes were involved then it would be assumed for this analysis that the apical node was uninvolved, equally if 17 of 17 or all of the 17 nodes were said to be involved it was assumed for the purpose of this analysis that the apical node was involved.

Tumour Location (colorectal cancer patients only)

Was categorised as Colon = 0 and Rectum = 1, for the purpose of this study tumours of the caecum and sigmoid were placed in the colon tumour group and tumours of the recto-sigmoid were grouped with the rectal tumours.

Hormone Receptor Status (breast cancer patients only)

Oestrogen receptor, progesterone receptor and HER2 status were all categorised in the same way – Negative = 0, Positive = 1. If any of these results were not recorded at the time of diagnosis but were later tested when advanced disease developed we accepted the results of this analysis to be true of the primary tumour.

Involvement of Liver, Lungs, Bone or Brain

Involvement of each Organ in the malignancy was recorded separately as 0 = not involved, 1 = involved. Only true parenchymal metastases were used for this analysis.

If a patient developed metastatic disease in one of these organs but then went on to have a successful resection of the metastatic disease and no further metastatic disease was seen in that organ at the time of chemotherapy then the organ was recorded as not being involved in the malignant process.

Radiological Response

Was graded to be a response = 0, stable disease = 1 or progressive disease = 2. This grading was done by me based upon the information recorded from the radiology reports. If disease progression was seen in one area and disease response was seen in another then the disease status was recorded as stable, similarly if stable disease was seen in one area and response in another this was classed as responsive disease and likewise if stable disease in one area and was seen on a scan with progression in another area then

this was classed as progression. In cases where this classification was unclear I referred to the hospital notes and sought advice from a clinician.

Radiological response could only be elucidated from a CT, Bone or MRI scan and this had to be within 120 days of the start of chemotherapy regimen being studied.

Tumour Marker Response

For the purposes of the regression analysis the Rustin categories described in Table 12 were arranged into an ordinal scale as follows:-

Always Normal = 0

Response = 1

Stable = 2

Progression from Within the Normal Range = 3

Progression = 4

If synchronous tumours were resected then the characteristics of the larger of the two tumours was recorded for the purpose of this analysis.

(3) Results

(3.1) Breast Cancer Patients

Of 540 patients who had been registered to the Oncology Patient Management Audit System (OPMAS) between June 1992 and June 2003, complete sets of hospital case-notes could be collected and examined for evidence of advanced breast cancer for 490 (91%) of the patients. Of the 50 sets of case-notes which could not be examined, 45 sets (90%) could not be located in medical records (many of these had been destroyed in line with hospital policy, and in 4 cases some volumes of the case-notes could be found but not the complete set) and 5 sets (10%) had been microfilmed.

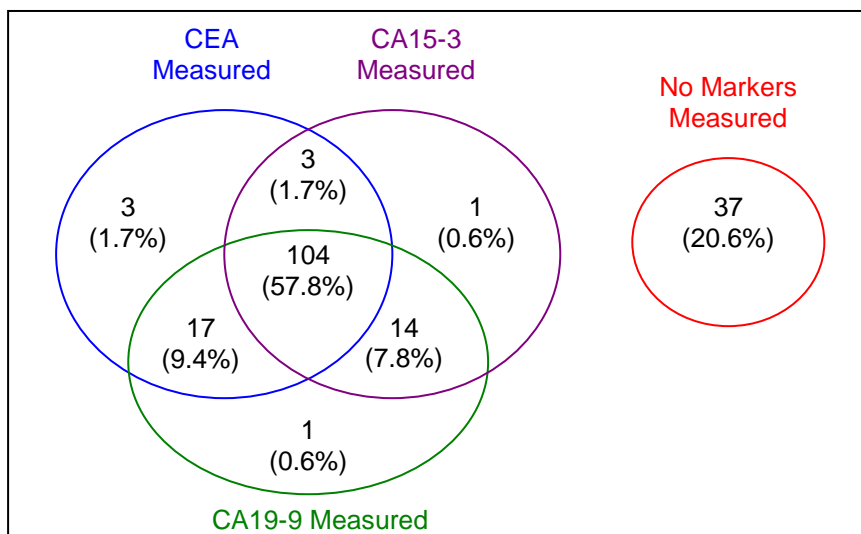
Of the 490 patients whose notes were located, 7 (1.4%) were excluded from the study because of previous or coexisting malignancies however as long as it did not interfere with the treatment of their advanced breast cancer patients who had previously had other malignancies such as basal cell carcinoma, cervical cancer, endometrial adenocarcinoma, Hodgkin's disease, and Liposarcoma were included in the study. Evidence of the later development of advanced breast cancer or the presence of advanced disease at presentation was found in 180 or 36.7% of the women (37.3% of the remaining 483 women after exclusions).

(3.1.1) Tumour Marker Levels at the Time of, and Leading up to, Advanced Disease Diagnosis

(3.1.1.1) At the time of Advanced Disease Diagnosis

Of the 180 breast cancer patients with advanced disease 143 (79.4%) had one or more markers recorded within 60 days of the date of advanced disease diagnosis, 127 (88.8%) had CEA level recorded, 122 (85.3%) had CA15-3 recorded and 136 (95.1%) had CA19-9 recorded at this time, the proportions can be seen in Figure 6 and details of the marker levels can be seen in Table 13 including how many patients had tumour marker levels recorded with the new assay technique which was implemented in April 2004.

Figure 6 Markers measured at the time of advanced disease diagnosis in 180 women with advanced breast cancer (within 60 days of this date).



The data in Table 13 shows that the distribution of marker levels in all cases was positively skewed and many of the distributions could not be transformed logarithmically, reciprocally or by square rooting the data so the Mann-Whitney U test was used to get a measure of the difference between values

obtained pre- and post-tumour marker assay change. Median marker levels and interquartile ranges at this time are shown in Figure 7.

Table 13 Tumour marker levels at advanced disease diagnosis in 143 women with advanced breast cancer (within 60 days of this date).

MC = Marker assay change (April 2004)

Tumour Marker		Measured at ADD (% of 143 women with one or more markers measured)	Raised at ADD (% of those where marker was measured)	Range	Skew	Mann-Whitney <i>U</i> test result <i>z</i> (<i>P</i>) for markers measured
CEA	Pre-MC	115 (80.4%)	62 (53.9%)	1-45406	10.70	2.46 (0.01)
	Post-MC	12 (8.4%)	7 (58.3%)	1-5326	2.63	
CA15-3	Pre-MC	110 (76.9%)	75 (68.2%)	9-13083	5.17	-0.15 (0.88)
	Post-MC	12 (8.4%)	8 (66.7%)	12-3676	2.64	
CA19-9	Pre-MC	125 (87.4%)	35 (28.0%)	1-71940	7.78	1.78 (0.07)
	Post-MC	11 (7.7%)	5 (45.5%)	8-123	1.45	

At the time of the diagnosis of advanced disease 143 women had one or more tumour marker levels recorded and 104 (72.7%) of these women had all 3 tumour markers recorded. CEA was raised in 53.5% of all women where it was measured (69 of 127), CA15-3 was raised in 68.0% of women (83 of 122) and CA19-9 was raised in 29.4% (40 of 136).

Figure 8 shows the concordance between the raised marker levels in the 104 women who had all three marker levels measured at this time.

Figure 7 Box and whisker plot showing markers measured at the time of advanced breast cancer diagnosis (within 60 days of this date). Change in marker assay was April 2004 change.

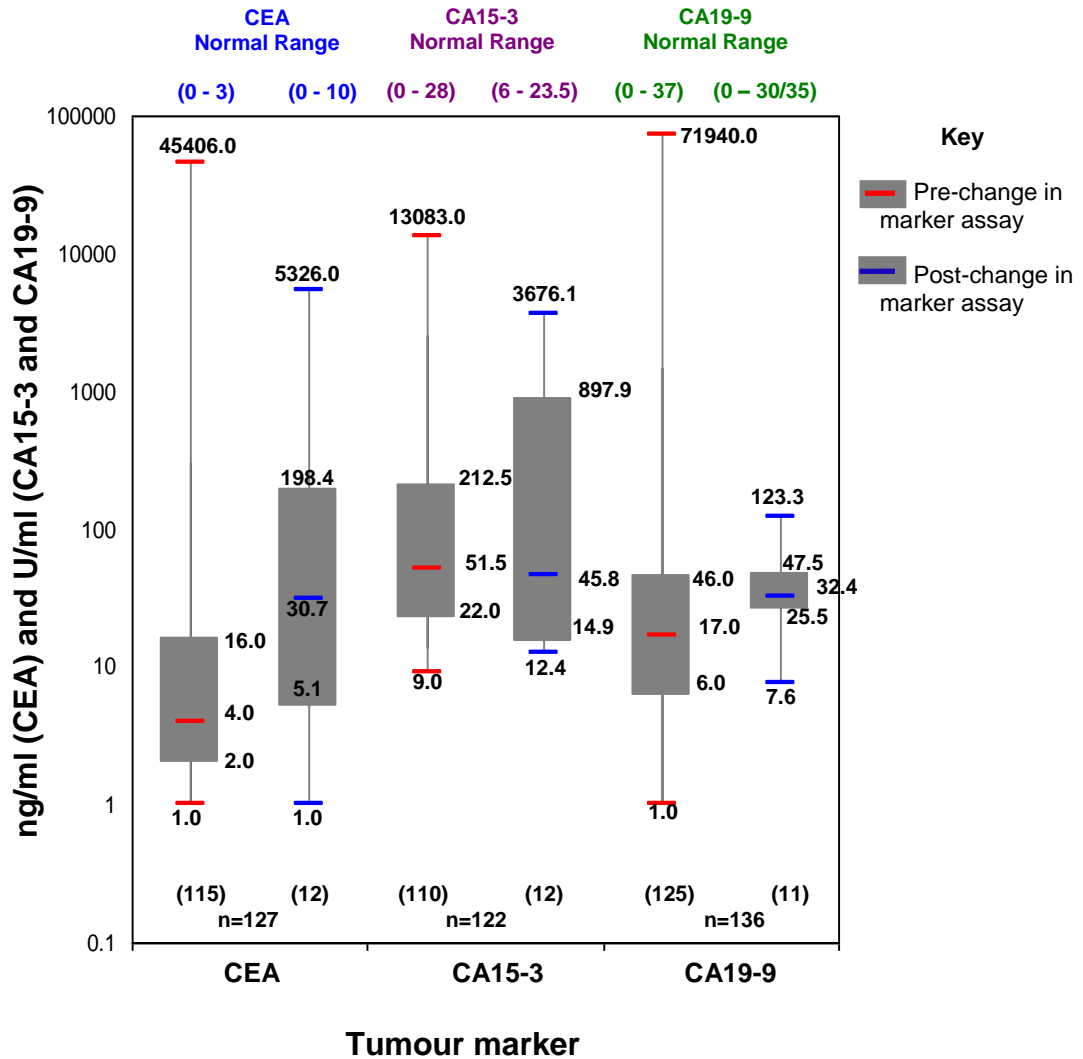


Figure 8 Concordance of raised markers in the 104 women who had CEA, CA15-3 and CA19-9 measured at advanced breast cancer diagnosis (within 60 days of this date).

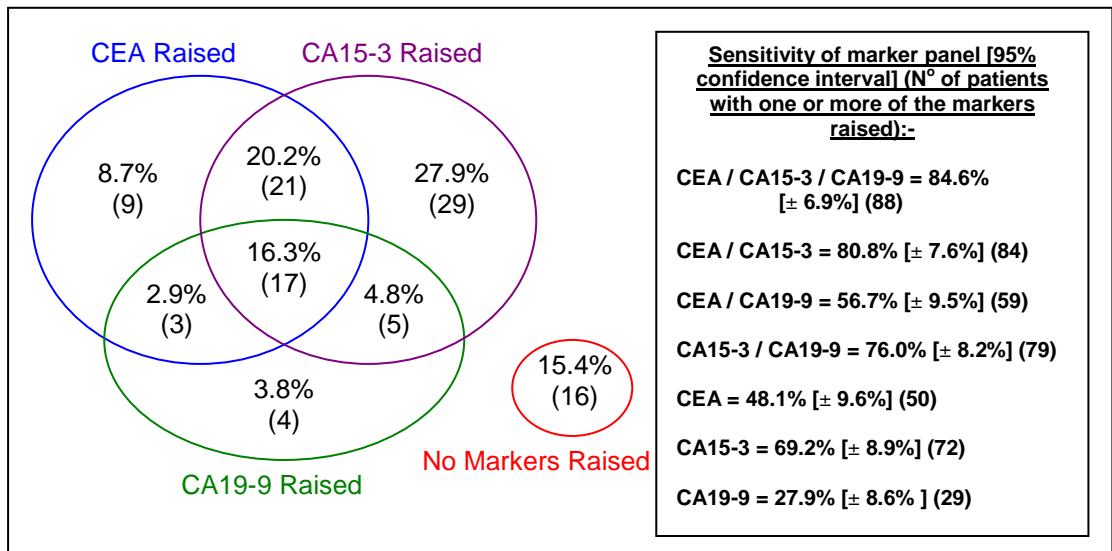


Figure 15 and Table 16 show the sensitivities in more detail and compares them with sensitivities of each marker panel at the start of chemotherapy one and two.

(3.1.1.2) Leading up to Advanced Disease Diagnosis

Of the patients who had CEA, CA15-3 and CA19-9 level recorded at the time of advanced disease diagnosis (127, 122 and 136 patients respectively) 17 patients (7, 12, and 3 respectively) were eligible for analysis of tumour marker doubling times. Reasons for the exclusion of the other patients can be seen in Table 14 as can the calculated median marker doubling and lead times prior to the diagnosis of advanced disease.

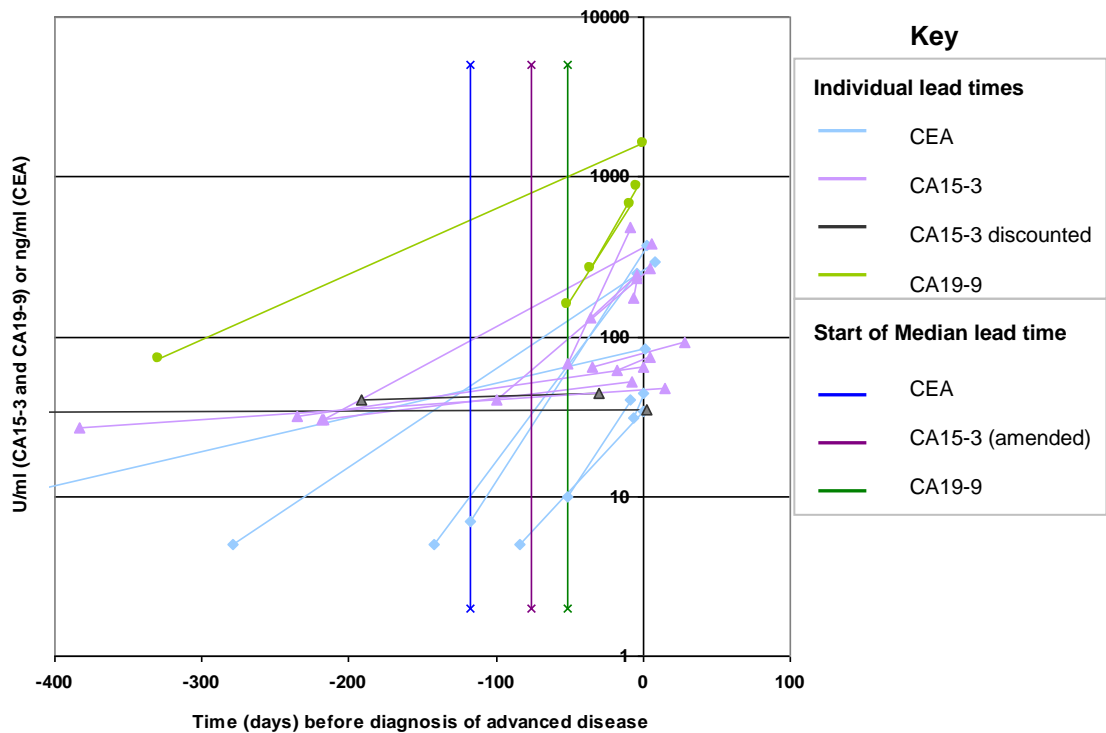
In two of the cases where CA15-3 lead time was measured (n=12) the increase in marker level did not appear to be exponential (increase in marker level both by 4U/ml in 23 weeks in one case and in the other case in 211 weeks). Lead and doubling times were calculated to include these patients initially but amended results for CA15-3 lead and doubling times with these two individuals excluded can also be seen in Table 14. Individual tumour marker increases and median lead times can be seen in Figure 9.

Table 14 Results of marker doubling and lead time analysis in women with advanced breast cancer.

ADD = Advanced disease Diagnosis
 ULN = Upper Limit of Normal
 * = With or without the category below

	CEA n=127 (%)	CA15-3 n=122 (%)	CA19-9 n=136 (%)
Excluded because:-			
Marker level at ADD not raised *	60 (47)	39 (32)	95 (70)
No previous tumour marker measurement	35 (28)	37 (30)	25 (18)
No previous normal value	9 (7)	7 (6)	1 (1)
Previous level ≤ ULN	12 (9)	24 (20)	12 (9)
Previous level unknown	1 (1)	0	0
Marker kinetics do not show an increase in level	1 (1)	3 (2)	0
Change of assay between 2 marker levels	2 (2)	0	0
Eligible for doubling time analysis	7 (6)	12 (10)	3 (2)
Marker lead time in months – Mean / Median	6.1 / 3.9	8.1 / 4.8	4.5 / 1.7
<i>Amended lead time</i>	-	4.3 / 2.5	-
Marker doubling time in months – Mean / Median	1.4 / 0.8	29.7 / 3.3	1.2 / 0.7
<i>Amended lead time</i>	-	4.4 / 2.4	-

Figure 9 Tumour marker lead times up to the diagnosis of advanced breast cancer (CA15-3 median lead time quoted is the amended lead time n=10).



(3.1.2) Tumour Marker Levels at the Time of the First and Second Chemotherapy Regimens given in Advanced Disease

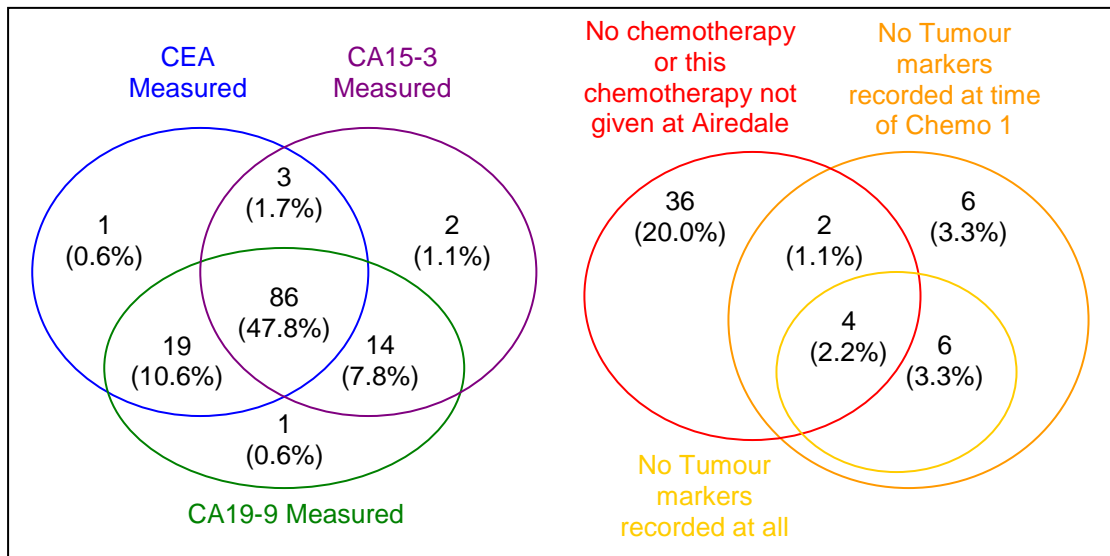
36 (20.0%) of the 180 patients who had advanced breast cancer did not have evidence of palliative chemotherapy at all (4 of whom (2.2%) also had no tumour markers recorded) and 3 (1.7%) had palliative chemotherapy but not

at Airedale, all 39 (21.7%) were discounted from the analysis leaving 141 women who received palliative chemotherapy at Airedale Hospital for advanced breast cancer.

(3.1.2.1) At First Chemotherapy

A further 3 women had some palliative chemotherapy at Airedale but not course one, and 12 of the remaining women had no markers recorded within 30 days of the beginning of the first course of palliative chemotherapy (n=6) or no tumour markers recorded at all (n=6) in the hospital notes. This left 126 (70.0% of the original 180) who had one or more tumour markers recorded at the beginning of the first palliative chemotherapy treatment. The distribution of markers measured can be seen in Figure 10.

Figure 10 Tumour markers measured at the time of the beginning of the first palliative chemotherapy regimen in the 180 women with advanced breast cancer (within 30 days of this date).



As can be seen from Figure 10, of the 126 women with one or more of the three tumour markers recorded at this time, 86.5% (n=109) had CEA measured, 83.3% (105) had CA15-3 measured and 95.2% (120) had CA19-9

measured, a breakdown of which can be seen in Table 15. Of the 126 women who had one or more markers measured at the start of the first chemotherapy regimen 68.3% (86) had all three markers measured at this point (8.1% (7) of these women had their markers measured after the change of assay).

At the start of the first chemotherapy CEA was raised in 58.7% of all women where it was measured (64 of 109) similarly for CA15-3 was raised in 77.1% (81 of 105) of patients and CA19-9 was raised in 32.5% (39 of 120). Figure 11 shows the concordance between the raised marker levels in the 86 women who had all three marker levels measured at this time. The distribution of marker levels at this time was skewed positively by varying degrees and several of the distribution curves could not be transformed logarithmically, reciprocally or by square rooting the data so the Mann-Whitney U test was used. Details can be seen in Table 15 and Figure 13.

Figure 11 Concordance of raised markers in the 86 women who had CEA, CA15-3 and CA19-9 measured at the time of the first chemotherapy given in advanced breast cancer (within 30 days of this date).

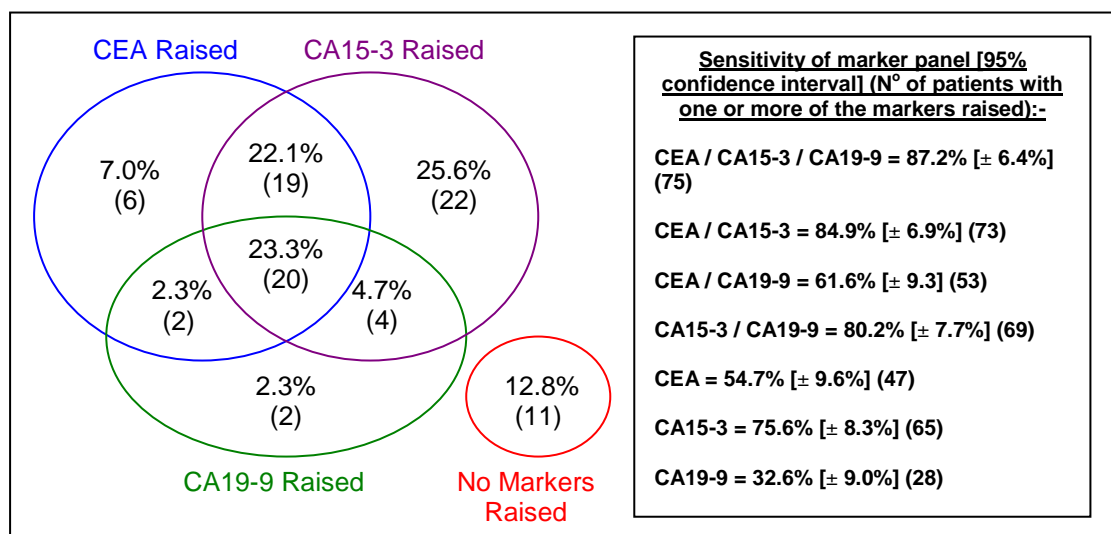


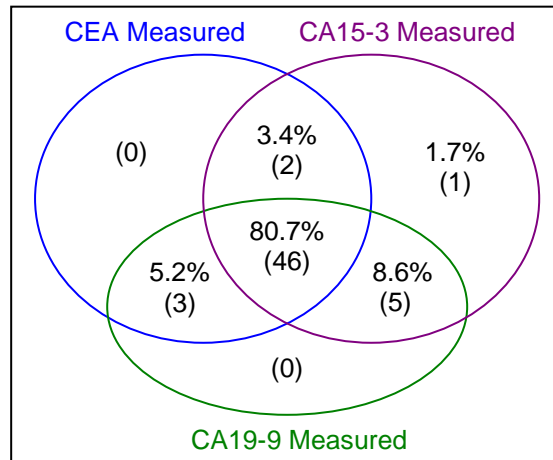
Figure 15 and Table 16 show the sensitivities in more detail and compare them with the sensitivities of each marker panel at advanced disease diagnosis and the start of chemotherapy two.

(3.1.2.2) At Second Chemotherapy

Of the 141 women who had advanced breast cancer and received palliative chemotherapy at Airedale Hospital 51.8% (73) had only one course of palliative chemotherapy and so could not be included in this analysis (three of the 73 patients also had no tumour markers recorded, and one woman changed chemotherapy regimens after just one cycle and began a second regimen immediately so for the purposes of this study was classed as only having one course of chemotherapy). Of the remaining 68 women 2 had no tumour markers recorded in the hospital case-notes and 8 did not have tumour markers recorded sufficiently close to the start of the second chemotherapy course to be included in this study. The final lady had been previously treated elsewhere and the regimen number was not known, she was therefore also excluded from the analysis.

The remaining 57 women (40.4% of the original 141) had one or more tumour markers recorded at the beginning of the second palliative chemotherapy treatment. The distribution of markers measured can be seen in Figure 12. Of all women who had a tumour marker measured at this time (n=57) 89.5% (51) had CEA measured and 94.7% (54) had CA15-3 measured with the same proportion also having CA19-9 measured.

Figure 12 Tumour markers measured at the time of the beginning of the second palliative chemotherapy regimen in 57 women with advanced breast cancer (within 30 days of this date).



Further details of tumour marker levels at the start of the second chemotherapy given in advanced breast cancer can be seen in Table 15 and a comparison of marker levels at the start of the first and second chemotherapy regimens can be seen in Figure 13.

Table 15 Tumour marker levels at the time of initiation of the first (n=126) and second (n=57) chemotherapy regimens given to patients with advanced breast cancer (within 30 days of this date).

MC = Marker assay change (April 2004)

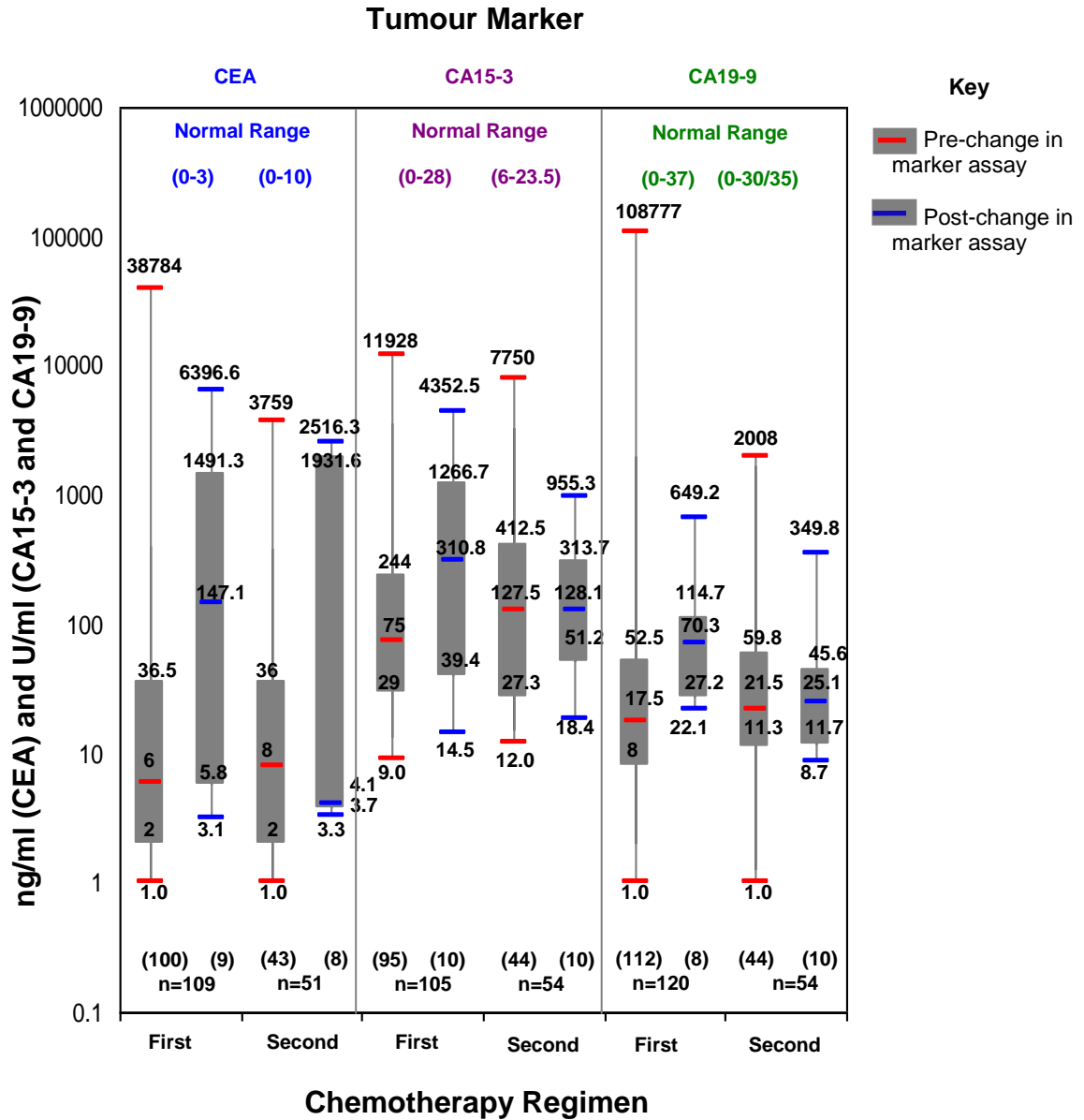
Chemotherapy Regimen	Tumour Marker		Measured at Initiation of Therapy (% of 126 or 57 women with one or more markers measured)	Raised at Initiation of Therapy (% of those where marker was measured)	Range	Skew	Mann-Whitney U test result z (P) for markers measured
1	CEA	Pre-MC	100 (79.4%)	58 (58.0%)	1-38784	9.93	2.65 (<0.01)
		Post-MC	9 (7.1%)	6 (66.7%)	3-6397	2.28	
	CA15-3	Pre-MC	95 (75.4%)	73 (76.8%)	9-11928	4.69	1.46 (0.14)
		Post-MC	10 (7.9%)	8 (80.0%)	15-4353	2.07	
	CA19-9	Pre-MC	112 (88.9%)	34 (30.4%)	1-108777	8.38	2.27 (0.02)
		Post-MC	8 (6.3%)	5 (62.5%)	22-649	2.61	
2	CEA	Pre-MC	43 (75.4%)	32 (74.4%)	1-3759	6.36	0.56 (0.58)
		Post-MC	8 (14.0%)	3 (37.5%)	3-2516	1.29	
	CA15-3	Pre-MC	44 (77.2%)	32 (72.7%)	12-7750	4.16	0.20 (0.84)
		Post-MC	10 (17.5%)	9 (90.0%)	18-955	1.89	
	CA19-9	Pre-MC	44 (77.2%)	15 (34.1%)	1-2008	3.61	-0.02 (0.98)
		Post-MC	10 (17.5%)	2 (20.0%)	9-350	2.84	

Again as can be seen from Table 15, marker level distribution was skewed positively by varying degrees and several of the distribution curves could not be transformed logarithmically, reciprocally or by square rooting the data so the Mann-Whitney U test was used.

There was no significant difference ($P > 0.3$ in all cases) between the CEA, CA15-3 or CA19-9 levels of patients who were beginning chemotherapy one and those beginning chemotherapy two either with or without the inclusion of post assay change marker levels.

Of the 57 women who had one or more markers measured at the start of the second chemotherapy regimen 80.7% (46) had all three markers measured at this point (17.4% (8) of these women had their markers measured after the change of assay which occurred in 2004).

Figure 13 Box and whisker plot showing markers measured at the time of first and second chemotherapy regimens given in advanced breast cancer (within 30 days of start date).



At the start of the second chemotherapy regimen CEA was raised in 68.6% of all women where it was measured (35 of 51), CA15-3 was raised in 75.9% (41 of 54) of women and CA19-9 was raised in 31.5% (17 of 54). Figure 14 below shows the concordance between the raised marker levels in the 46 women who had all three marker levels measured at this time.

Figure 14 Concordance of raised markers in the 46 women who had CEA, CA15-3 and CA19-9 measured at the time of the second chemotherapy given in advanced breast cancer (within 30 days of this date).

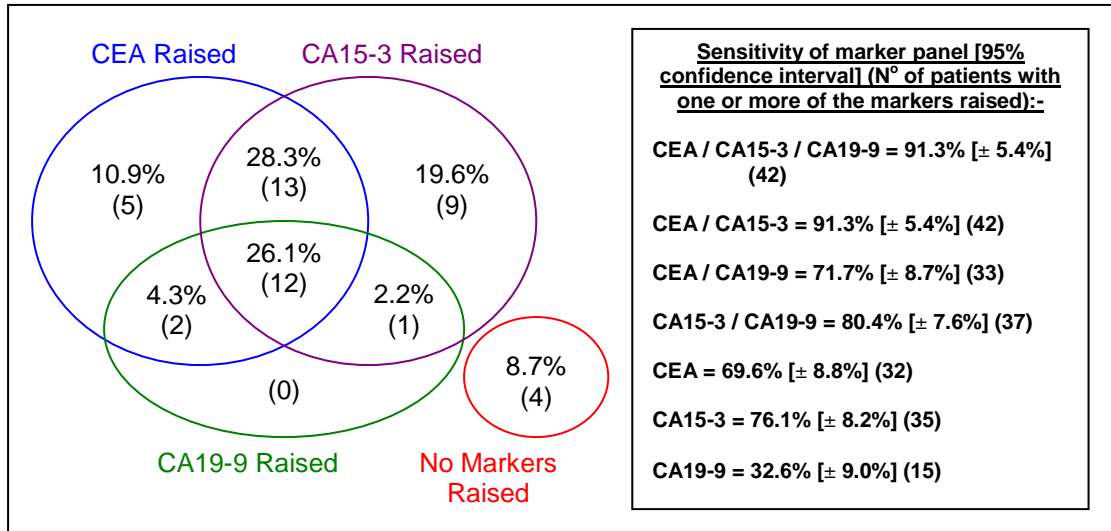


Figure 15 and Table 16 show the sensitivities in more detail and compares them with sensitivities of each marker panel at advanced disease diagnosis and the start of chemotherapy one.

Figure 15 Tumour marker panel sensitivity at the time of the diagnosis of advanced disease and at the start of the first and second chemotherapy regimens given in advanced breast cancer (in patients who had all tumour markers recorded).

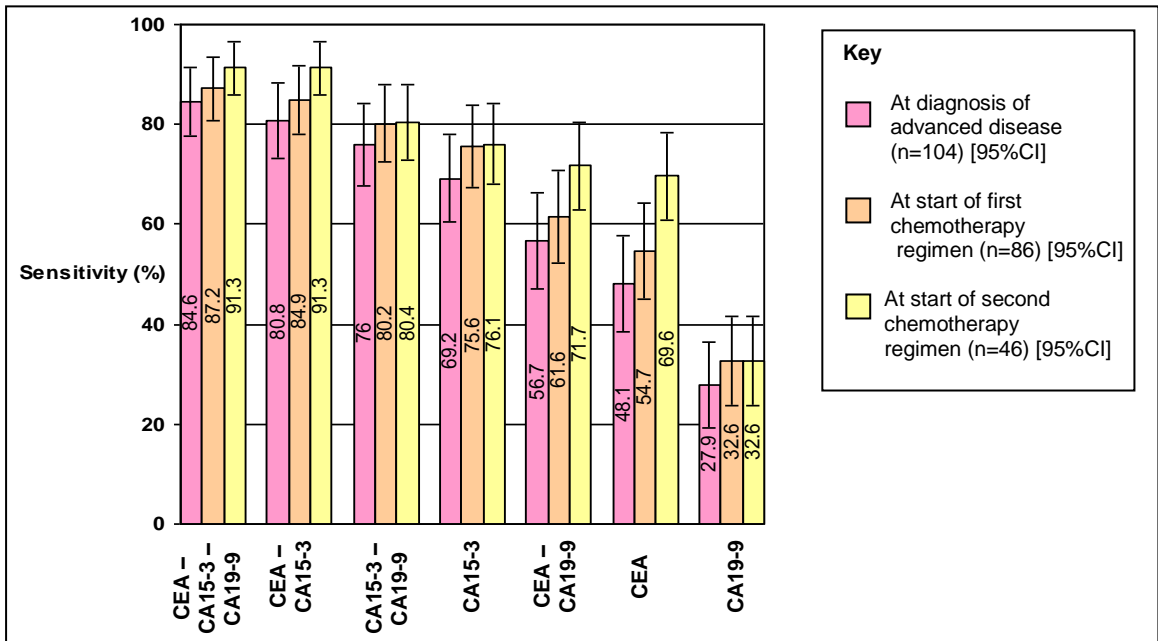


Table 16 Differences in between the sensitivity of each tumour marker panel at the time of the diagnosis of advanced disease, the start of the first, and the start of the second chemotherapy regimens given in advanced breast cancer (in patients who had all tumour markers recorded).

Note: Using Table 16 and Table 21.

From Figure 15 we know that the sensitivity of the tumour marker panel CEA, CA15-3 and CA19-9 at the time of advanced disease diagnosis is 84.6% and the sensitivity of CA15-3 alone at this time is 69.2%. The table can be used to compare the two sensitivities and see how different they are and whether this difference is statistically significant.

1. The sensitivities of the tumour marker panel CEA, CA15-3 and CA19-9 is compared with the sensitivity of CA15-3 alone in the following square of the table.
2. The results in the selected square of the table show that the 95% confidence interval for the difference in the sensitivities of the two tumour marker panels is 3.9 to 26.9 (a positive confidence interval which indicates that the sensitivity of the tumour marker panel on the left hand side of the table is greater than that on the top of the table)
3. The *p* value in this case is 0.009 and is therefore significant. This indicates that there is a significant possibility that there is a difference between the sensitivity of the two tumour marker panels being compared.

		Advanced disease diagnosis						
		CEA-CA15-3-CA19-9	CEA-CA15-3	CEA-CA19-9	CA15-3-CA19-9	CEA	CA15-3	CA19-9
Advanced Disease Diagnosis	CEA-CA15-3-CA19-9		-6.5 to 14.1 (0.5)	15.5 to 40.3 (<0.001)	-2.2 to 19.4 (0.1)	23.7 to 49.3 (<0.001)	3.9 to 26.9 (0.009)	43.2 to 70.2 (<0.001)
	CEA-CA15-3			11.5 to 36.7 (<0.005)	-6.4 to 16.0 (0.4)	19.7 to 45.7 (<0.001)	-0.2 to 23.4 (0.05)	39.4 to 66.4 (<0.001)
	CEA-CA19-9				-32.1 to -6.5 (<0.005)	-5.0 to 22.2 (0.2)	-25.6 to 0.6 (0.06)	15.4 to 42.2 (<0.001)
	CA15-3-CA19-9					14.7 to 41.1 (<0.001)	-5.3 to 18.9 (0.3)	34.5 to 61.7 (<0.001)
	CEA						-34.5 to -7.7 (<0.005)	7.0 to 33.4 (<0.005)
	CA15-3							27.7 to 54.9 (<0.001)
	CA19-9							

Key

- p* value is not significant
- p* value has borderline significance
- p* value is significant

4. The table can be used to compare the sensitivity of different tumour marker panels at the same time point or at different time points e.g at advanced disease diagnosis compared with at the start of chemotherapy one or two.

Differences in between the sensitivity of each tumour marker panel at the time of the diagnosis of advanced disease, the start of the first, and the start of the second chemotherapy regimens given in advanced breast cancer (in patients who had all tumour markers recorded).

		Difference in Percentage of patients with raised markers - 95% Confidence Interval (p)																					
		Advanced disease diagnosis						Chemotherapy One						Chemotherapy Two									
		CEA-CA15-3-CA19-9	CEA-CA15-3	CEA-CA19-9	CA15-3-CA19-9	CEA	CA15-3	CA19-9	CEA-CA15-3-CA19-9	CEA-CA15-3	CEA-CA19-9	CA15-3-CA19-9	CEA	CA15-3	CA19-9	CEA-CA15-3-CA19-9	CEA-CA15-3	CEA-CA19-9	CA15-3-CA19-9	CEA	CA15-3	CA19-9	
Advanced Disease Diagnosis	CEA-CA15-3-CA19-9		-6.5 to 14.1 (0.5)	15.5 to 40.3 (<0.001)	-2.2 to 19.4 (0.1)	23.7 to 49.3 (<0.001)	3.9 to 26.9 (0.009)	43.2 to 70.2 (<0.001)	-12.6 to 7.4 (0.6)	-10.6 to 10.0 (1.0)	10.5 to 35.5 (<0.005)	-6.4 to 15.2 (0.4)	16.9 to 42.9 (<0.001)	-2.3 to 20.3 (0.1)	38.1 to 65.9 (<0.001)	-18.5 to 5.1 (0.3)	-18.5 to 5.1 (0.3)	-0.8 to 26.6 (0.07)	-8.7 to 17.1 (0.5)	1.1 to 28.9 (0.03)	-4.8 to 21.8 (0.2)	35.9 to 68.1 (<0.001)	
	CEA-CA15-3			11.5 to 36.7 (<0.005)	-6.4 to 16.0 (0.4)	19.7 to 45.7 (<0.001)	-0.2 to 23.4 (0.05)	39.4 to 66.4 (<0.001)	-17.0 to 4.2 (0.2)	-14.9 to 6.7 (0.5)	6.4 to 32.0 (<0.005)	-10.7 to 11.9 (0.9)	12.9 to 39.3 (<0.005)	-6.5 to 16.9 (0.4)	34.1 to 62.3 (<0.001)	-23.2 to 2.2 (0.1)	23.2 to 2.2 (0.1)	-5.3 to 23.5 (0.2)	-13.3 to 14.1 (1.0)	-3.3 to 25.7 (0.1)	-9.3 to 18.7 (0.5)	31.8 to 64.6 (<0.001)	
	CEA-CA19-9				-32.1 to -6.5 (<0.005)	-5.0 to 22.2 (0.2)	-25.6 to 0.6 (0.06)	15.4 to 42.2 (<0.001)	-43.5 to -17.5 (<0.001)	-41.4 to -15.0 (<0.001)	-19.0 to 9.2 (0.5)	-36.9 to -10.1 (<0.005)	-12.2 to 16.2 (0.8)	-32.5 to -5.3 (0.006)	9.9 to 38.3 (<0.001)	-50.9 to -18.3 (<0.001)	-50.9 to -18.3 (<0.001)	-31.9 to 1.9 (0.08)	-40.4 to -7.0 (0.005)	-29.9 to 4.1 (0.1)	-36.2 to -2.6 (0.02)	6.7 to 41.5 (<0.01)	
	CA15-3-CA19-9					14.7 to 41.1 (<0.001)	-5.3 to 18.9 (0.3)	34.5 to 61.7 (<0.001)	-22.4 to -0.01 (0.05)	-20.3 to 2.5 (0.1)	1.2 to 27.6 (0.03)	-16.1 to 7.7 (0.5)	7.8 to 34.8 (<0.005)	-11.8 to 12.6 (1.0)	29.2 to 57.6 (<0.001)	-29.0 to -1.6 (0.03)	-29.0 to -1.6 (0.03)	-10.8 to 19.4 (0.6)	-18.9 to 10.1 (0.6)	-8.8 to 21.6 (0.4)	-14.9 to 14.7 (1.0)	26.6 to 60.2 (<0.001)	
	CEA						7.0 to 33.4 (<0.005)	-34.5 to -7.7 (<0.005)	7.0 to 33.4 (<0.005)	-52.7 to -25.5 (<0.001)	-50.4 to -23.2 (<0.001)	-27.7 to 0.7 (0.4)	-45.9 to -18.3 (<0.001)	-20.9 to 7.7 (0.4)	-41.5 to -13.5 (<0.005)	1.4 to 29.6 (0.03)	-60.1 to -26.3 (<0.001)	-60.1 to -26.3 (<0.001)	-40.9 to -6.3 (0.007)	-49.4 to -15.2 (<0.001)	-38.8 to -4.2 (0.01)	-45.2 to -10.8 (<0.005)	-1.7 to 32.7 (0.08)
	CA15-3							27.7 to 54.9 (<0.001)	-30.0 to -6.0 (<0.005)	-27.8 to -3.6 (0.01)	-6.0 to 21.2 (0.3)	-23.5 to 1.5 (0.09)	0.7 to 28.3 (0.04)	-19.2 to 6.4 (0.3)	22.3 to 50.9 (<0.001)	-36.9 to -7.3 (<0.005)	-36.9 to -7.3 (<0.005)	-18.4 to 13.4 (0.8)	-26.7 to 4.3 (0.2)	-16.4 to 15.6 (1.0)	-22.6 to 8.8 (0.4)	19.5 to 53.7 (<0.001)	
	CA19-9								-73.5 to -45.1 (<0.001)	-71.2 to -42.8 (<0.001)	-47.8 to -19.6 (<0.001)	-66.6 to -38.0 (<0.001)	-40.8 to -12.8 (<0.005)	-62.0 to -33.4 (<0.001)	-17.8 to 8.4 (0.5)	-80.7 to -46.1 (<0.001)	-80.7 to -46.1 (<0.001)	-60.9 to -26.7 (<0.001)	-69.7 to -35.3 (<0.001)	-58.7 to -24.7 (<0.001)	-65.4 to -31.0 (<0.001)	-20.5 to 11.1 (0.6)	
Chemotherapy One	CEA-CA15-3-CA19-9											18.9 to 46.1 (<0.001)	-0.03 to 23.2 (0.05)	40.0 to 69.2 (<0.001)	-15.5 to 7.3 (0.5)	-15.5 to 7.3 (0.5)	1.7 to 29.3 (0.03)	-6.0 to 19.6 (0.3)	3.6 to 31.6 (0.01)	-2.2 to 24.4 (0.1)	37.9 to 71.3 (<0.001)		
	CEA-CA15-3											16.5 to 43.9 (<0.001)	-2.6 to 21.2 (0.1)	-18.4 to 5.6 (0.3)	-18.4 to 5.6 (0.3)	-1.0 to 27.4 (0.07)	-8.8 to 17.8 (0.5)	0.9 to 29.7 (0.04)	-5.0 to 22.6 (0.2)	35.4 to 69.2 (<0.001)			
	CEA-CA19-9											-32.2 to -5.0 (0.007)	-7.8 to 21.6 (0.4)	-27.9 to -0.1 (0.05)	14.1 to 43.9 (<0.005)	-45.8 to -13.6 (<0.005)	-45.8 to -13.6 (<0.005)	-27.2 to 7.0 (0.2)	-35.5 to -2.1 (0.03)	-25.1 to 9.1 (0.4)	-31.4 to 2.4 (0.09)	11.1 to 49.9 (<0.005)	
	CA15-3-CA19-9												11.5 to 39.5 (<0.005)	-7.8 to 17.0 (0.5)	32.8 to 62.4 (<0.001)	-24.2 to 2.0 (0.3)	-24.2 to 2.0 (0.3)	-6.5 to 23.5 (0.3)	-14.4 to 14.0 (1.0)	-4.6 to 25.8 (0.2)	-10.5 to 18.7 (0.6)	30.4 to 64.8 (<0.001)	
	CEA													-35.1 to -6.7 (<0.005)	7.3 to 36.9 (<0.005)	-53.4 to -19.8 (<0.001)	53.4 to -19.8 (<0.001)	-34.5 to 0.5 (0.06)	-42.9 to -8.5 (<0.001)	-32.4 to 2.6 (0.1)	-38.8 to -4.0 (0.02)	4.2 to 40.0 (0.02)	
	CA15-3														28.1 to 57.9 (<0.001)	-29.7 to -1.67 (0.03)	-29.7 to -1.67 (0.03)	-11.8 to 19.6 (0.1)	-19.8 to 10.2 (0.5)	-9.8 to 21.8 (0.5)	-15.8 to 14.8 (1.0)	25.5 to 60.5 (<0.001)	
	CA19-9															-76.6 to -40.8 (<0.001)	-76.6 to -40.8 (<0.001)	-57.0 to -21.2 (<0.001)	-65.7 to -29.9 (<0.001)	-54.8 to -19.2 (<0.001)	-61.4 to -25.6 (<0.001)	-16.8 to 16.8 (1.0)	
Chemotherapy Two	CEA-CA15-3-CA19-9															-11.5 to 11.5 (1.0)	3.7 to 35.5 (0.02)	-3.3 to 25.1 (0.1)	5.5 to 37.9 (0.009)	0.1 to 30.3 (0.05)	38.9 to 78.5 (<0.001)		
	CEA-CA15-3																3.7 to 35.5 (0.02)	-3.3 to 25.1 (0.1)	5.5 to 37.9 (0.009)	0.1 to 30.3 (0.05)	38.9 to 78.5 (<0.001)		
	CEA-CA19-9																	-26.1 to 8.7 (0.3)	-16.5 to 20.7 (0.8)	-22.3 to 13.5 (<0.005)	18.7 to 59.5 (<0.005)		
	CA15-3-CA19-9																			-6.9 to 28.5 (0.2)	-12.6 to 21.2 (0.6)	27.5 to 68.1 (<0.001)	
	CEA																			-24.7 to 11.7 (0.5)	16.6 to 57.4 (<0.005)		
	CA15-3																				23.1 to 63.9 (<0.001)		
	CA19-9																						

(3.1.3) Tumour Marker Response to Chemotherapy Given in Advanced Disease

(3.1.3.1) At First Chemotherapy

Of the 126 women who had advanced disease, the first course of palliative chemotherapy at Airedale and a tumour marker level recorded at the start of the first chemotherapy regimen a further 26 women (20.6%) were excluded at this stage as they did not have enough tumour marker levels recorded for analysis of any marker movement to take place (at least one marker level recorded within 30 days of the chemotherapy start date and a further 2 of the same marker levels recorded within the 100 days following this date). This left 100 patients (55.6% of the original 180 women with advanced breast cancer) who could be analysed for Rustin response to chemotherapy one which is defined by Table 11 (Methods section).

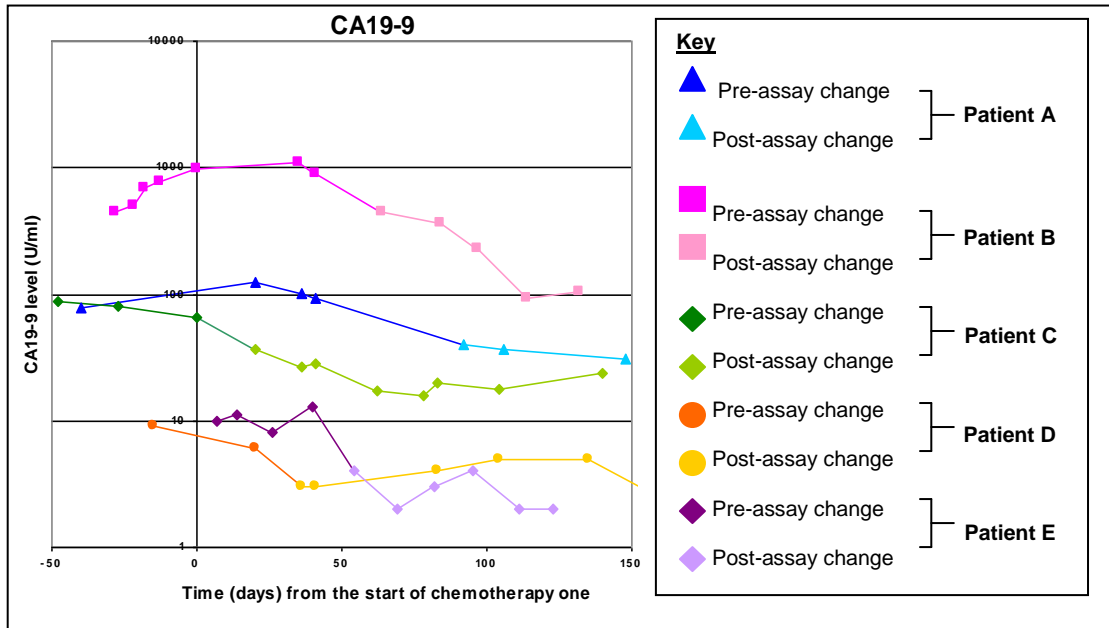
Of these patients, one had CA19-9 Rustin response ascertained at the time when the 1995 change in this marker occurred (Figure 16A, patient A) as the trajectory of this marker did not change dramatically following the marker assay change the change was discounted and the category of Rustin response was categorised in the normal way. Patients B, C, D and E shown in Figure 16A represent the marker movements of colorectal cancer patients who this change in marker assay also affected. As with the patient A, the trajectories of the marker responses for patients B, C and E were not thought to be affected by the marker assay change and Rustin category was established in the normal way. The trajectory of marker movement in patient

D did seem to be affected by the marker change but as this all occurred within the normal marker range this marker was categorised as Always Normal.

One further patient had CA15-3 and CA19-9 Rustin response ascertained at the time of the 2004 change of assay (Figure 16B, patient F) as the trajectory of CA15-3 level did not change dramatically the marker assay change was discounted and Rustin response was again categorised in the normal way. For CA19-9 response in this patient the marker remained below the upper limit of normal throughout the marker change and so was categorised as Always Normal.

Figure 16 Marker levels following the initiation of the first chemotherapy regimen in patients where marker assay change occurred during the 100 days following chemotherapy start date.

A. CA19-9 at the time of 1995 marker assay change.



B. CA15-3 at the time of 2004 marker assay change.

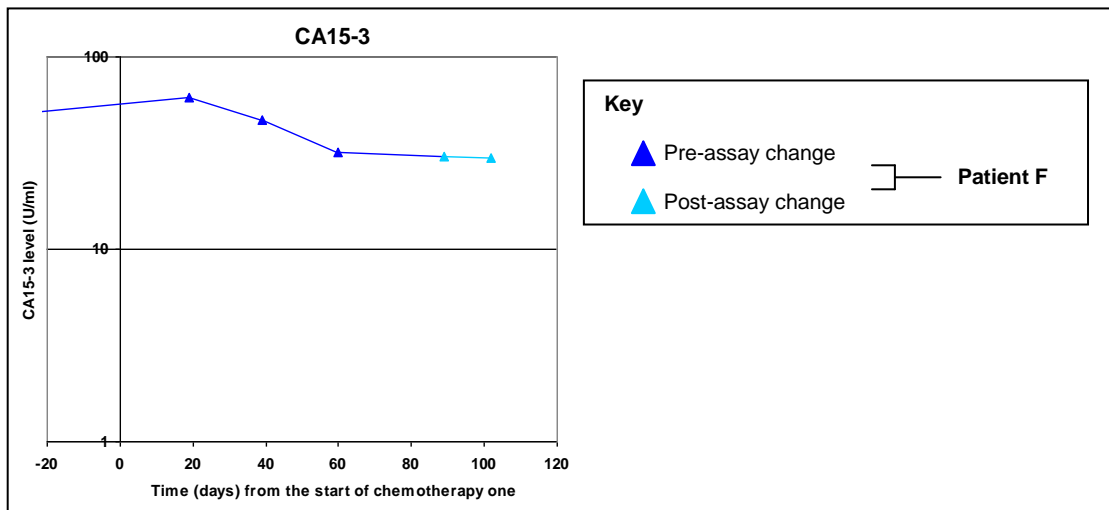
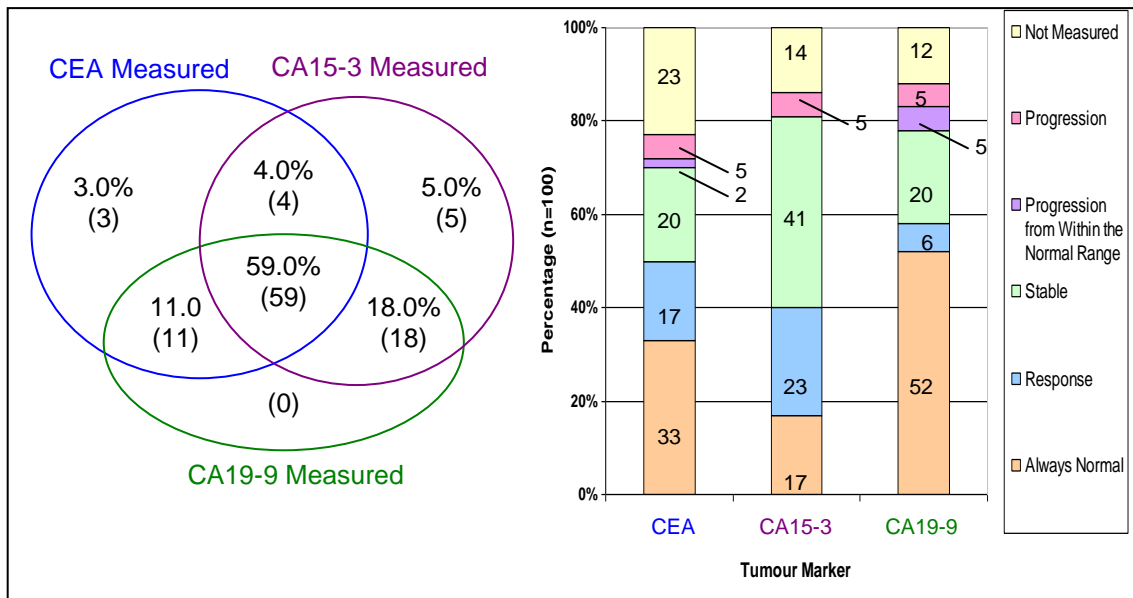


Figure 17 shows the distribution of the measurement of markers among these 100 patients and illustrates the proportions of each which fell into each Rustin category of response.

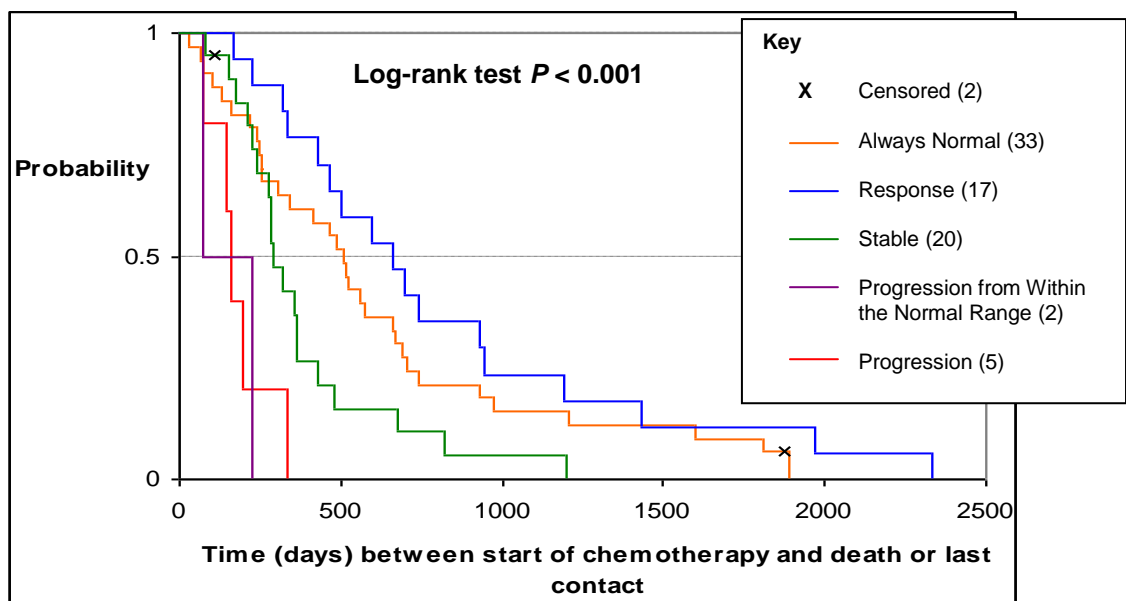
Figure 17 Tumour markers measured and Rustin category of response in the 100 women whose tumour markers could be analysed following the initiation of chemotherapy one.



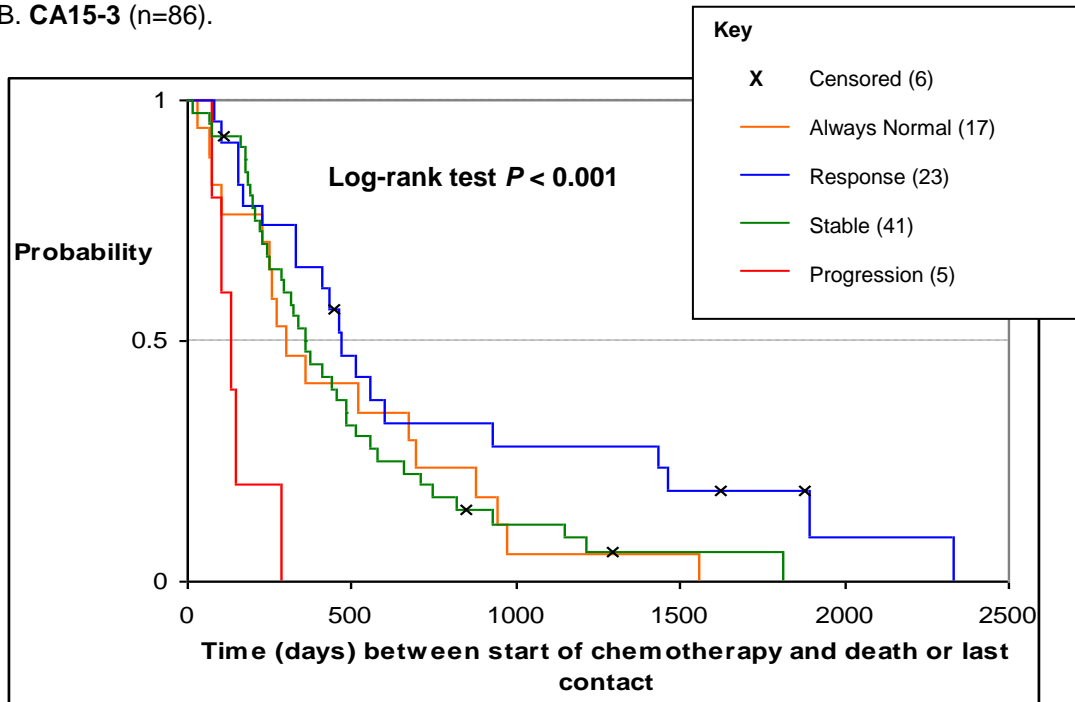
The following Kaplan-Meier graphs (Figure 18A, B and C) show overall survival from the start of the first chemotherapy given in advanced disease according to tumour marker and Rustin response category.

Figure 18 Rustin response category and survival from the start of the first chemotherapy given in advanced breast cancer.

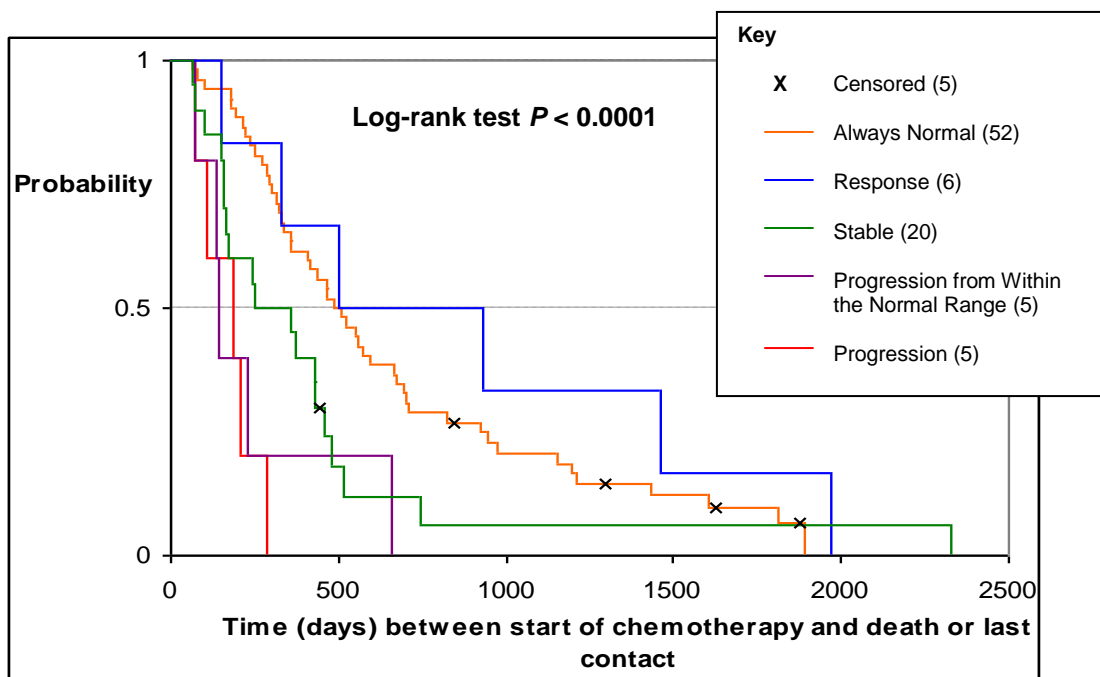
A. CEA (n=77).



B. CA15-3 (n=86).



C. CA19-9 (n=88).



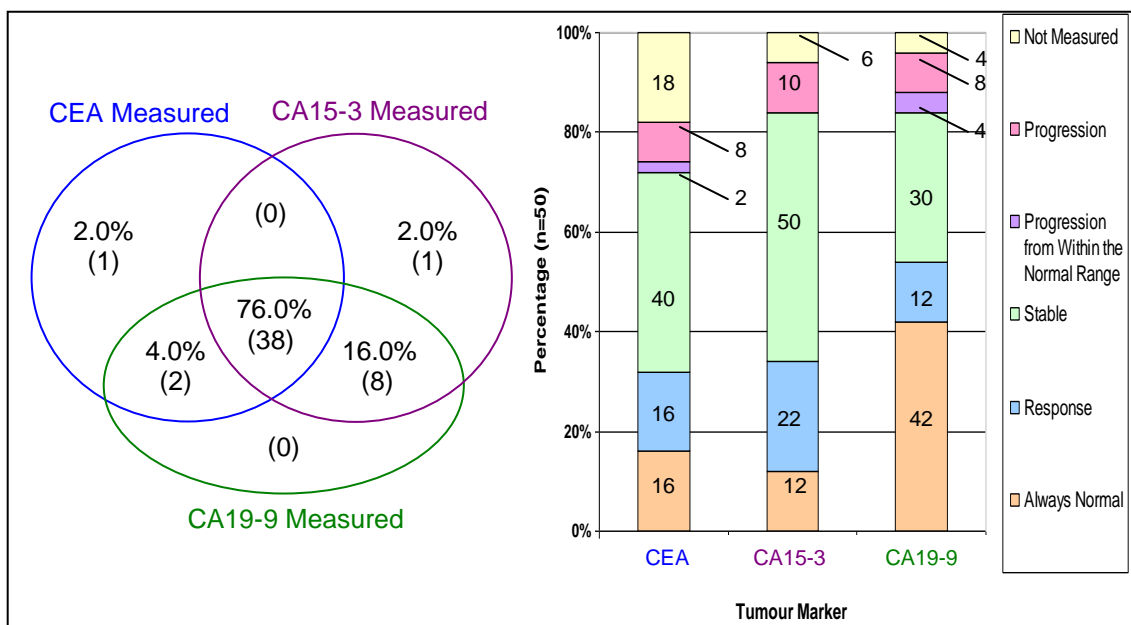
(3.1.3.2) At Second Chemotherapy

Of the 57 women who had advanced disease, the second course of palliative chemotherapy at Airedale and a tumour marker level recorded at the start of the second chemotherapy regimen (within 30 days of this date) a further 7

women (12.3%) were excluded at this stage as they did not have enough tumour marker levels recorded for analysis of marker movement to take place. This left 50 patients (27.6% of the original 181 women with advanced breast cancer) who could be analysed for Rustin response to chemotherapy two.

One patient had a transient rise in CEA level within 45 days of the initiation of the second chemotherapy regimen, as a result this rise was discounted and the CEA level was considered to remain stable, it was analysed as such for the purpose of this study. The Venn diagram below (Figure 19) shows the measurement of markers and marker responses among all 50 women whose markers could be analysed at this point in time.

Figure 19 Tumour markers measured and Rustin category of response in the 50 women whose tumour markers could be analysed for Rustin response to chemotherapy two.



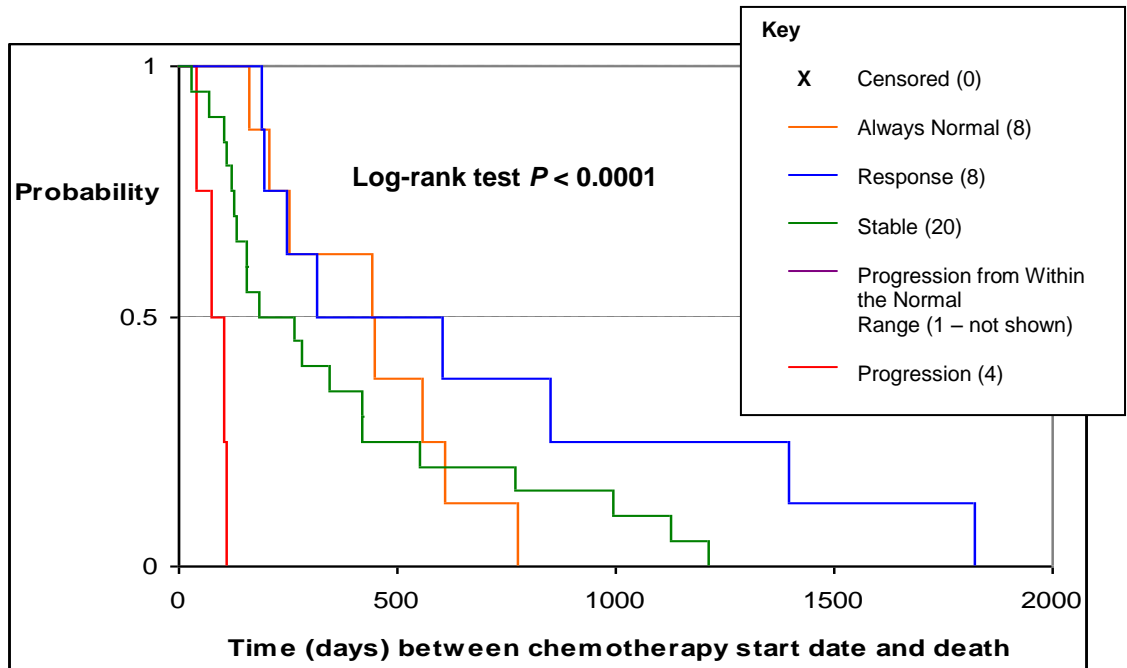
The following four Kaplan-Meier graphs (Figure 20A, B, C and D) show overall survival from the start of the second chemotherapy given in advanced disease according to tumour marker and Rustin response category.

Only one patient had a CEA level which showed progression from within the normal range, as this could not be used to generate a curve on the graph shown in Figure 20A, this patient was removed from this analysis. Two patients had CEA level which responded (i.e. fell by $\geq 50\%$) but from a level which was originally less than twice the upper limit of normal (ULN). In Figure 20A these patients are categorised as having stable disease according to the modified Rustin criteria of response and the survival curve in Figure 20B shows the amended Kaplan-Meier graph which is generated if these two patients were categorised separately.

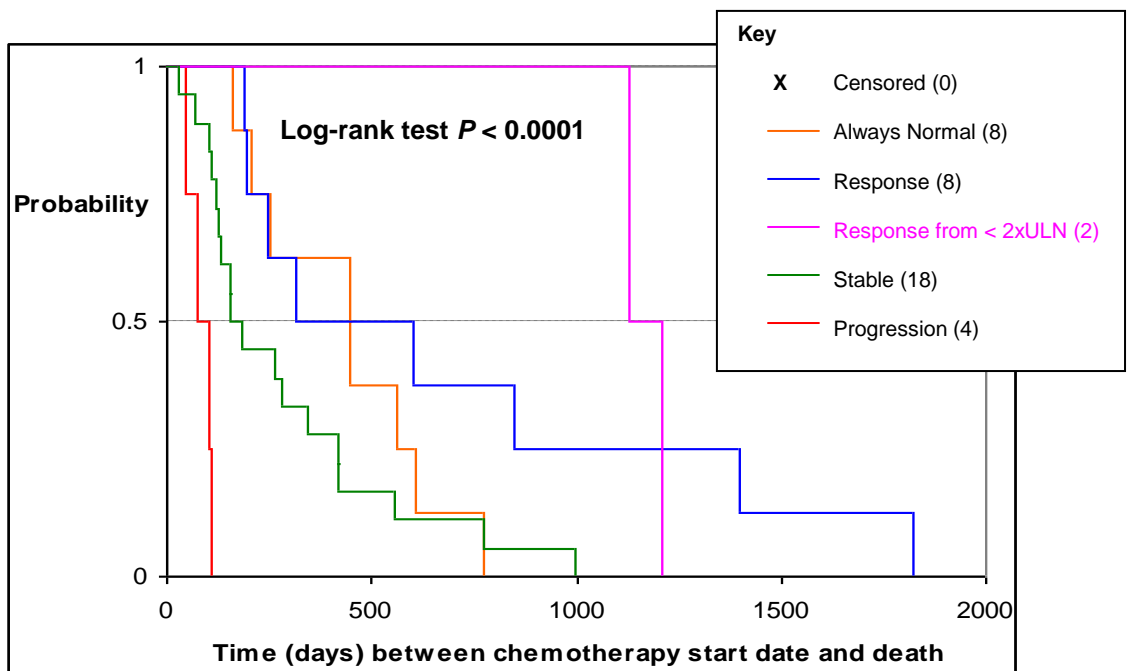
Of the 47 patients analysed for CA15-3 Rustin response to the second chemotherapy regimen, one patient had a response from a level which was < twice the ULN, this was classed as stable in Figure 20C and because only one patient displayed this marker movement a separate curve could not be generated on the Kaplan-Meier graph in this case. No patients had such a response when CA19-9 kinetics were examined.

Figure 20 Rustin response category and survival from the start of the second chemotherapy given in advanced breast cancer.

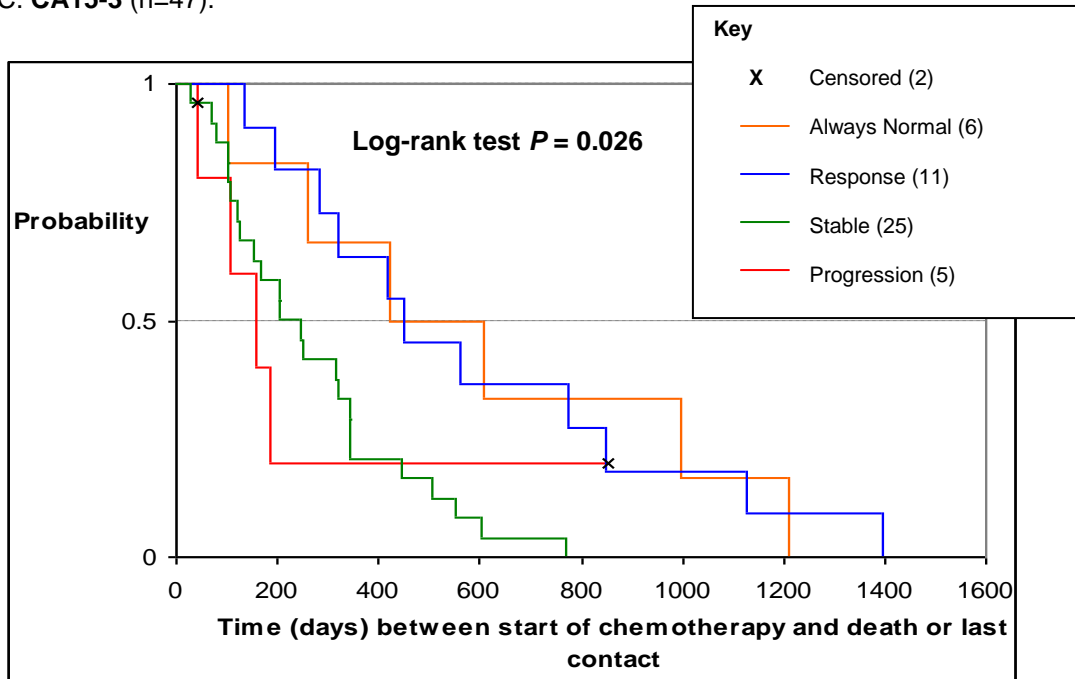
A. CEA (n=41).



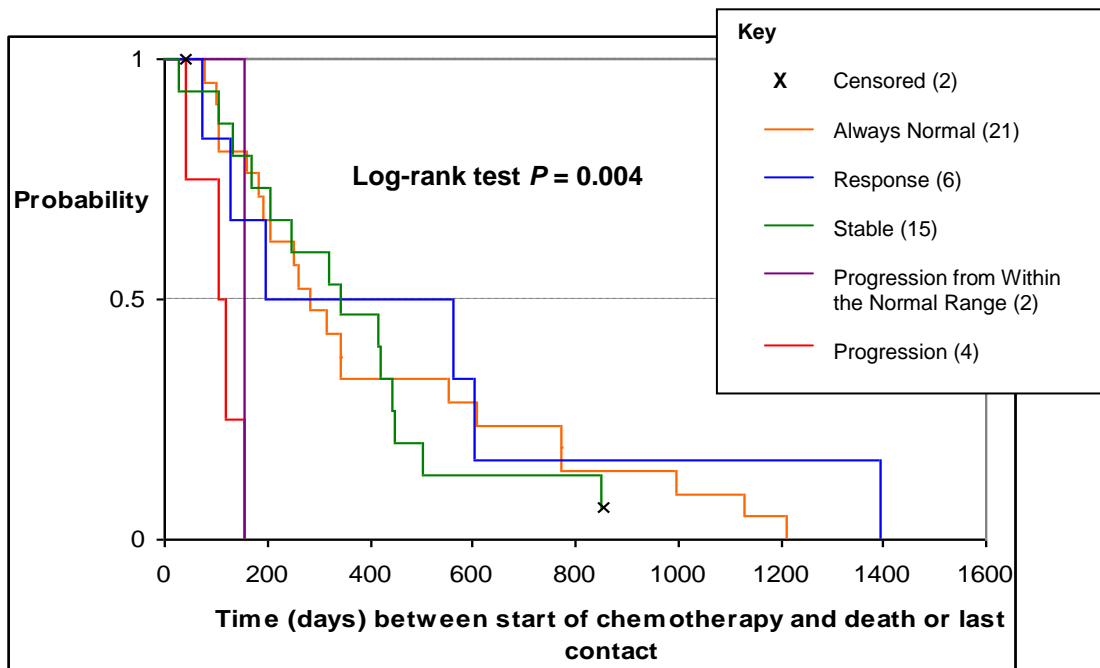
B. CEA - Survival of the 2 patients who responded from $< 2x$ ULN as a separate curve (n=41).



C. CA15-3 (n=47).



D. CA19-9 (n=48).



Median survivals could be calculated from the graphs in Figures 18 and 20; these are displayed in Table 22 and Figure 35.

The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the Rustin response criteria at identifying

radiological response to the first and second chemotherapy regimens are shown in Table 17 and survival according to radiological response can be seen in Figure 21A and 21B. Results of Spearman rank correlation and Cox's proportional hazards regression for both breast and colorectal cancer patients can be seen in Table 24. The Cox's regression was also performed taking into account other possible confounding factors such as lymph node status. The results of this analysis can be seen in Table 25.

All breast cancer patients who had a tumour stage quoted had this quoted using the TNM system, which was converted to a single stage using the conversion chart (Table 12, Patients and Methods section).

Table 17 Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of Rustin progression at identifying radiological progression and Rustin response at identifying radiological response from chemotherapy initiation in patients with advanced breast cancer.

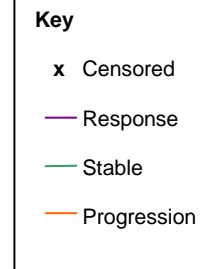
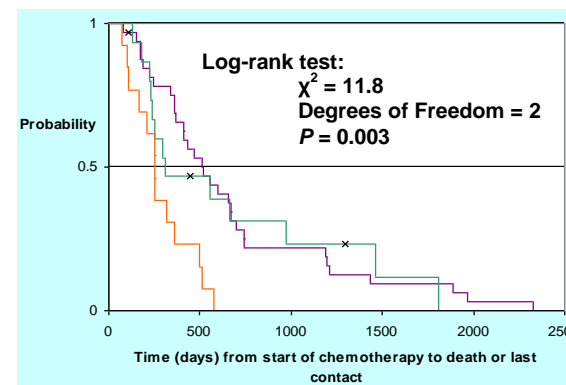
P only = Only patients categorised as having Rustin progression considered to show tumour marker progression

P and PN = Patients with both Rustin progression and progression from within the normal range considered to show tumour marker progression.

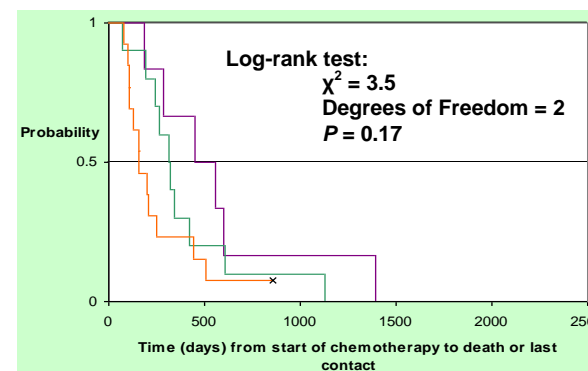
Breast					
First Chemotherapy (N° of patients eligible for analysis)		Sensitivity % (N° of patients)	Specificity % (N° of patients)	PPV % (N° of patients)	NPV % (N° of patients)
CEA (48)	P only	8.3 (1/12)	100 (36/36)	100 (1/1)	76.6 (36/47)
	P and PN	8.3 (1/12)	97.2 (35/36)	50.0 (1/2)	76.1 (35/46)
	Response	35.7 (10/28)	80.0 (16/20)	71.4 (10/14)	47.1 (16/34)
CA15-3 (51)	P only	9.1 (1/11)	97.5 (39/40)	50.0 (1/2)	79.6 (39/49)
	P and PN	As above	As above	As above	As above
	Response	40.7 (11/27)	83.3 (20/24)	73.3 (11/15)	55.6 (20/36)
CA19-9 (53)	P only	25.0 (3/12)	97.6 (40/41)	75.0 (3/4)	81.6 (40/49)
	P and PN	25.0 (3/12)	90.2 (37/41)	42.9 (3/7)	80.4 (37/46)
	Response	6.9 (2/29)	91.7 (22/24)	50.0 (2/4)	44.9 (22/49)
Second Chemotherapy (N° of patients eligible for analysis)					
CEA (24)	P only	33.3 (3/9)	100 (15/15)	100 (3/3)	71.4 (15/21)
	P and PN	33.3 (3/9)	93.3 (14/15)	75.0 (3/4)	70.0 (14/20)
	Response	33.3 (2/6)	77.8 (14/18)	33.3 (2/6)	77.8 (14/18)
CA15-3 (28)	P only	23.1 (3/13)	93.3 (14/15)	75.0 (3/4)	58.3 (14/24)
	P and PN	As above	As above	As above	As above
	Response	66.7 (4/6)	90.9 (20/22)	66.7 (4/6)	90.9 (20/22)
CA19-9 (28)	P only	7.7 (1/13)	100 (15/15)	100 (1/1)	55.6 (15/27)
	P and PN	15.4 (2/13)	100 (15/15)	100 (2/2)	57.7 (15/26)
	Response	50.0 (3/6)	86.4 (19/22)	50.0 (3/6)	86.4 (19/22)

Figure 21 Radiological response category and survival in advanced breast cancer from:-

A. The first chemotherapy regimen (n=61)



B. The second chemotherapy regimen (n=29)



(3.2) Colorectal Cancer Patients

Complete sets of hospital case-notes could be located and examined for 86% (or 313) of the 366 patients who had been registered to OPMAS as receiving chemotherapy for colorectal cancer between June 1992 and February 2004.

Of the 53 sets of case-notes which could not be examined, 48 sets (91%) could not be located in medical records (many of these had been destroyed in line with hospital policy, in 4 cases some of the case-notes could be found but not the complete set of volumes) and 5 sets (9%) had been microfilmed.

Of the 313 patients whose notes were located 14 (4.5%) were excluded from the study because of previous or coexisting malignancies or because the site of the primary tumour was unclear (e.g. Lung or oesophageal cancer), or the possibility that the advanced disease present was not colorectal in origin.

Evidence of advanced colorectal cancer was found in 199 or 63.6% of these patients (66.6% of the remaining 299 women after exclusions). Patients who had previously sarcoma, Squamous cell carcinoma, transitional cell carcinoma of the bladder, prostate cancer, basal cell carcinoma and others were included in the study if the advanced disease was treated as advanced colorectal cancer.

(3.2.1) Tumour Marker Levels at the time of, and Leading up to, Advanced Disease Diagnosis

(3.2.1.1) At the time of Advanced Disease Diagnosis

Of the 199 colorectal cancer patients with advanced disease, 22 further patients were discounted, 19 (9.5%) had no tumour markers recorded within 60 days of the date of advanced disease diagnosis and 3 patients (1.5%) had an unknown date of diagnosis of advanced disease. The remaining 177 patients (88.9%) had one or more markers recorded within 60 days of the date of advanced disease diagnosis, 176 (99.4%) of these patients had CEA level recorded and 175 (98.9%) had CA19-9 recorded at this time. 174 patients (98.3%) had both CEA and CA19-9 measured within 60 days of advanced disease diagnosis, one patient had CA19-9 alone measured and 2 patients had CEA alone measured.

For 11 patients the tumour marker recorded at this time was measured following the change of assay which occurred in April 2004. The data for the marker levels at advanced disease diagnosis shows that the distributions were positively skewed and many of them could not be transformed logarithmically, reciprocally or by square rooting the data so the Mann-Whitney U test was used to compare marker levels at the time of advanced disease diagnosis before and after the change in marker assay. Details of the marker levels can be seen in Table 18 and Figure 22.

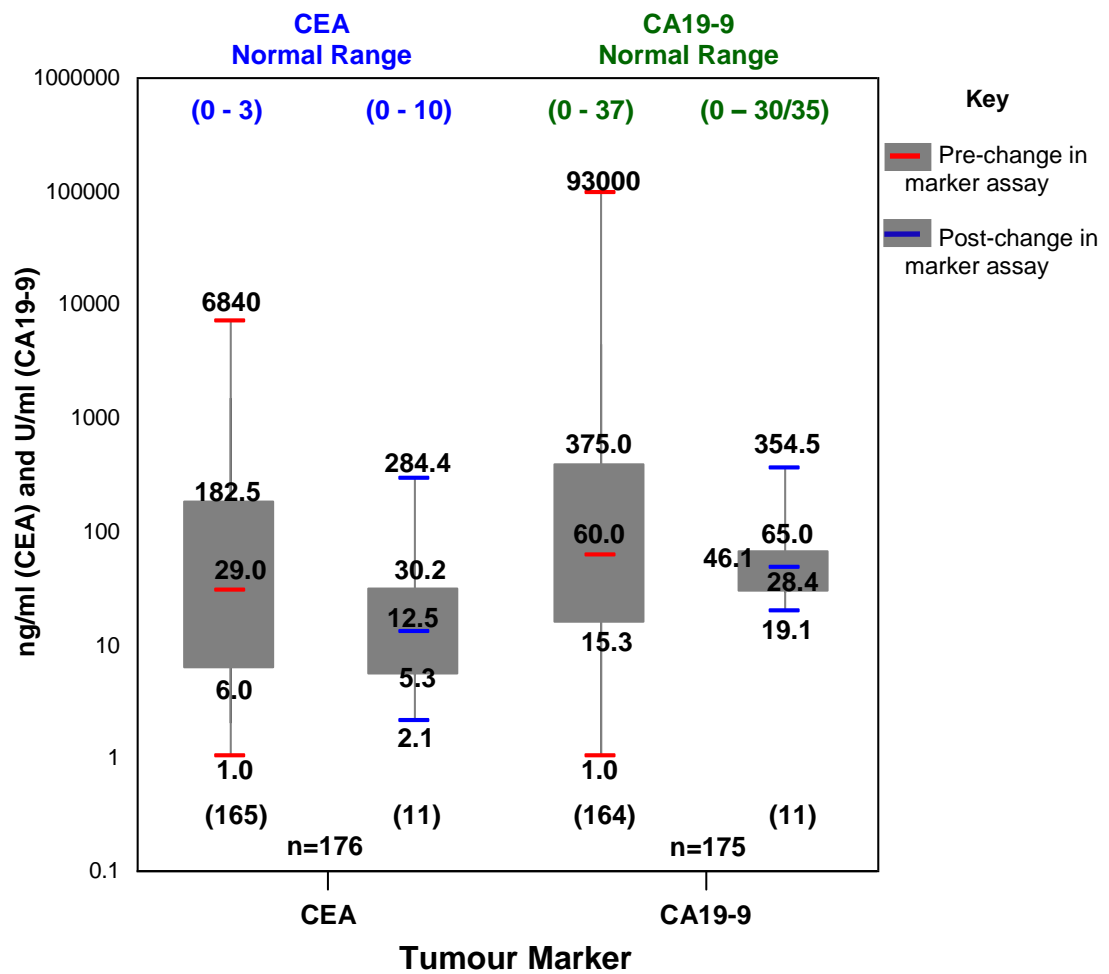
Two patients had CEA and CA19-9 levels at advanced disease diagnosis which were taken before the assay change which occurred in 1993. The details in Table 18 and Figure 22 include these patients in the Pre-marker assay change group, however, if the data is excluded from this group the tumour marker details in Table 18 change very little (range does not change at all, skew is altered to 5.59 for CEA and 11.48 for CA19-9 and Mann-Whitney results are altered to -1.12 (0.26) and -0.44 (0.66) respectively). If these patients were to be excluded the data from Figure 22 is also changed very slightly from a 25th Percentile of 15.3 to 14.8 (CA19-9) , a median of 29 to 27 (CEA) and 60 to 58 (CA19-9) a 75th percentile of 182.5 to 181 (CEA) and 375 to 362 (CA19-9).

Table 18 Tumour marker levels at advanced disease diagnosis in 177 patients with advanced colorectal cancer (within 60 days of this date).

MC = Marker assay change (April 2004)

Tumour Marker		Measured at ADD (% of 177 women with one or more markers measured)	Raised at ADD (% of those where marker was measured)	Range	Skew	Mann-Whitney <i>U</i> test result <i>z</i> (<i>P</i>) for markers measured
CEA	Pre-MC	165 (93.2%)	136 (82.4%)	1-6840	5.62	-1.15 (0.25)
	Post-MC	11 (6.2%)	7 (63.6%)	2-284	2.92	
CA19-9	Pre-MC	164 (92.7%)	97 (59.1%)	1-93000	11.2	-0.50 (0.62)
	Post-MC	11 (6.2%)	8 (72.7%)	19-355	3.01	

Figure 22 Box and whisker plot showing markers measured at the time of advanced colorectal cancer diagnosis (within 60 days of this date). Assay change was the 2004 assay change



At the time of the diagnosis of advanced disease CEA was raised in 81.8% of all patients (144 of 176) where it was measured and CA19-9 was raised in 60.0% (105 of 175). Figure 23 shows the concordance between the raised marker levels in the 174 patients who had both marker levels measured at this time.

Figure 23 Concordance of raised markers in the 174 patients who had CEA and CA19-9 measured at advanced colorectal cancer diagnosis (within 60 days of this date).

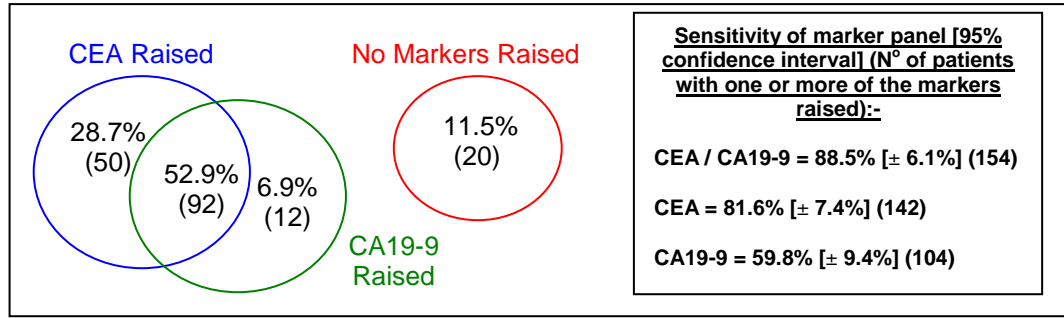


Figure 28 and Table 21 show the sensitivities in more detail and compare them with sensitivities of each other marker panel at the start of chemotherapy one and two.

(3.2.1.2) Leading up to Advanced Disease Diagnosis

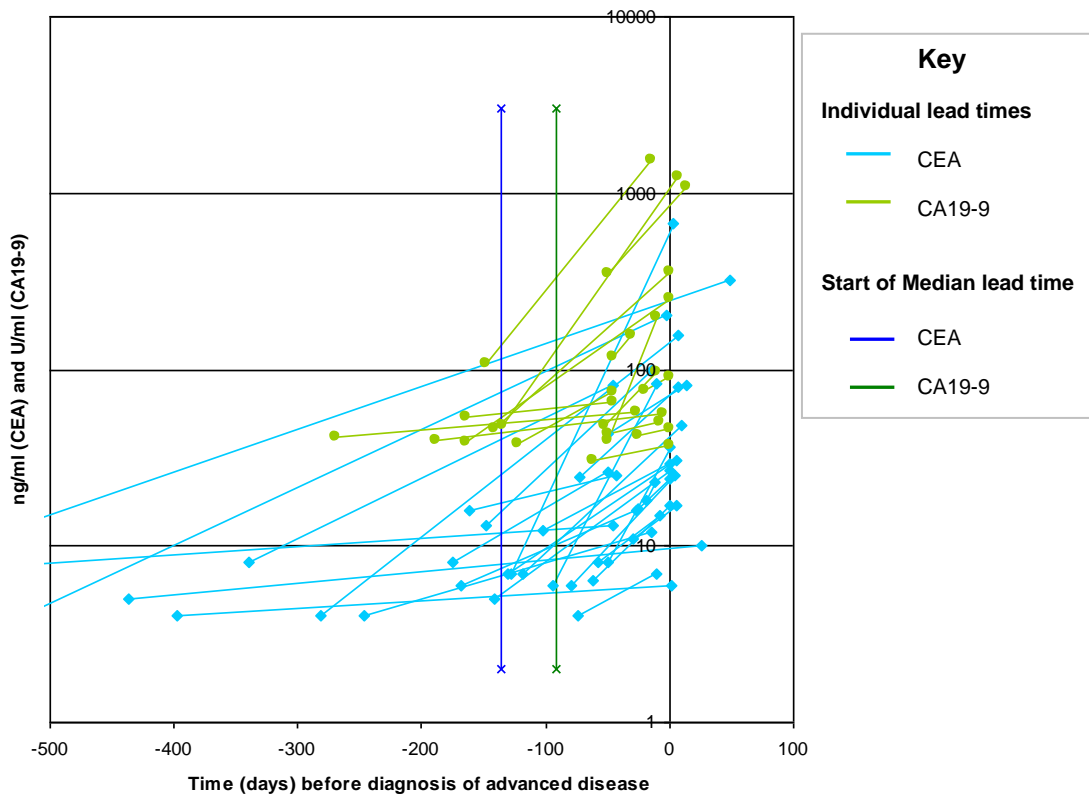
Of the patients who had CEA and CA19-9 level recorded at the time of advanced disease diagnosis (176 and 175 patients respectively) 36 patients (28 and 16 respectively) were eligible for analysis of tumour marker doubling times, reasons for the exclusion of the other patients can be seen in Table 19 as can the calculated median marker doubling and lead times prior to the diagnosis of advanced disease. Individual tumour marker increases and lead times can be seen in Figure 24.

Table 19 Results of marker doubling and lead time analysis in patients with advanced colorectal cancer

ADD = Advanced disease Diagnosis
 ULN = Upper Limit of Normal
 * = With or without the category below

	CEA n=176 (%)	CA19-9 n=175 (%)
Excluded because:-		
Marker level at ADD not raised *	33 (19)	70 (40)
No previous tumour marker measurement	70 (40)	57 (33)
No previous normal value	22 (13)	18 (10)
Previous level ≤ ULN	18 (10)	12 (7)
Previous level unknown	0	0
Marker kinetics do not show an increase in level	4 (2)	1 (1)
Change of assay between 2 marker levels	1 (1)	1 (1)
Eligible for doubling/lead time analysis	28	16
Marker lead time in months - Mean / Median	7.0 / 4.5	3.5 / 3.0
Marker doubling time in months - Mean / Median	4.1 / 2.0	4.9 / 2.3

Figure 24 Tumour marker lead times up to the diagnosis of advanced colorectal cancer.



(3.2.2) Tumour Marker Levels at the Time of the First and Second Chemotherapy Regimens given in Advanced Disease

29 (14.6%) of the 199 patients who had advanced colorectal cancer did not have evidence of palliative chemotherapy at all (7 of whom also had no further evidence of advanced disease after re-resection and in some cases adjuvant chemotherapy). A further 4 (2.0%) patients had palliative chemotherapy but not at Airedale, all 33 (16.6%) were discounted from the analysis leaving 166 patients who received palliative chemotherapy at Airedale Hospital for advanced colorectal cancer.

(3.2.2.1) At First Chemotherapy

From the remaining 166 patients two patients had some palliative chemotherapy at Airedale but not course one, and 2 further patients had no

markers recorded in the hospital case-notes within 30 days of the beginning of the first course of palliative chemotherapy. This left 162 (81.4% of the original 199) patients with tumour markers recorded at the beginning of the first palliative chemotherapy treatment, all of these patients had both CEA and CA19-9 recorded at this time. CEA was raised in (138) 85.2% and CA19-9 was raised in (106) 65.4% of these patients. Figure 25 below shows the concordance between the raised marker levels in the 162 patients.

Figure 25 Concordance of raised markers in the 162 patients who had CEA and CA19-9 measured at the time of the first chemotherapy given in advanced colorectal cancer (within 30 days of this date).

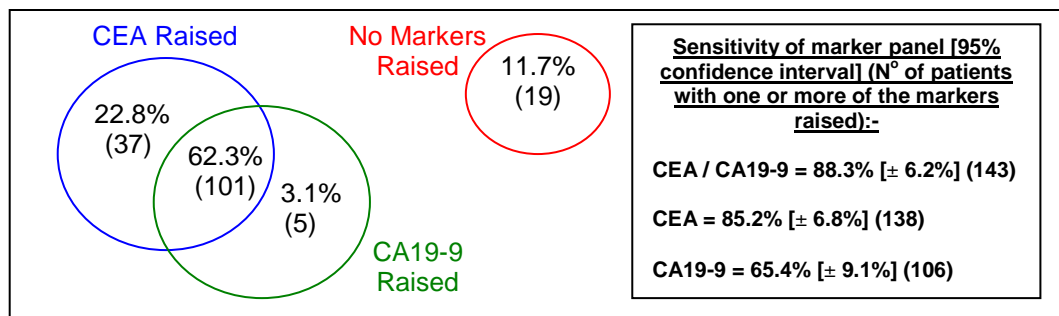


Figure 28 and Table 21 show the sensitivities in more detail and compare them with sensitivities of each other marker panel at advanced disease diagnosis and the start of chemotherapy two.

As with marker levels at the time of the diagnosis of advanced disease marker level distribution was skewed positively by varying degrees. It was not possible to transform all of the distribution curves logarithmically, reciprocally or by square rooting the data so the Mann-Whitney U test was used to compare levels from patients measured before the change in marker assay with those taken after. Details of marker distribution at this time can be seen in Table 20 and Figure 26.

Two patients had CEA and CA19-9 levels at the start of the first chemotherapy regimen which were taken before the assay change which occurred in 1993. The details in Table 20 and Figure 26 include these patients in the Pre-marker assay change group, however, if the data is excluded from this group the tumour marker details in Table 20 change very little (range does not change at all, skew is altered to 4.15 for CEA and 9.06 for CA19-9 and Mann-Whitney results are altered to -1.65 (0.10) and -1.03 (0.31) respectively). If these patients were excluded the data from Figure 26 would also change very slightly from an unchanged 25th Percentile for both CEA and CA19-9, an unchanged median for CEA and a change from 103 to 89 for CA19-9 and a change in 75th percentile of 282 to 303 (CEA) and 837 to 818.5 (CA19-9).

(3.2.2.2) At Second Chemotherapy

Of the 166 patients who received palliative chemotherapy at Airedale Hospital for advanced colorectal cancer 54.8% (91) had only one course of palliative chemotherapy and so could not be included in this analysis and two patients (1.2%) received their second chemotherapy elsewhere and so were excluded.

Of the remaining 73 patients one patient had an unknown start date for chemotherapy two and so was excluded from the analysis. This left 72 patients (43.4% of the original 166) who had one or more tumour markers recorded at the beginning of the second palliative chemotherapy treatment.

All of the 72 patients had CA19-9 measured and 71 of them had CEA measured at this time. Table 20 shows the tumour marker levels at the

beginning of the first and second chemotherapy regimens and Figure 26 enables comparison between these two time points in more detail. As can be seen from Table 20 marker distribution was, in all cases, skewed positively by varying degrees. Several of the distribution curves could not be transformed logarithmically, reciprocally or by square rooting the data so the Mann-Whitney U test was used to test the difference between the pre- and post-2004 marker assay change groups

At the start of chemotherapy two only a single patient had CEA and CA19-9 levels which were taken before the assay change which occurred in 1993. The details in Table 20 and Figure 26 include this patients in the Pre-marker assay change group, however, if the data from this patient is excluded from this group the tumour marker details in Table 20 again change very little (range does not change at all, skew is altered to 3.39 for CEA and 7.40 for CA19-9 and Mann-Whitney results are altered to -1.91 (0.06) and -0.99 (0.32) respectively). If these patients were excluded the data from Figure 26 would also changed very slightly from an unchanged 25th Percentile for CEA and a change from 36.8 to 36.5 for CA19-9, a change in median CEA from 169 to 171 and a change from 184.5 to 176 for CA19-9 and a change in 75th percentile of 439.5 to 441.8 (CEA) and 546.3 to 472 (CA19-9).

There was no significant difference ($P > 0.4$ in all cases) between the CA19-9 tumour marker levels of patients who were beginning chemotherapy one and those beginning chemotherapy two, either with or without the inclusion of post assay change marker levels. CEA levels however were significantly different

between the two groups ($z = 2.60$ and 2.68 respectively and $P < 0.01$ both with and without the inclusion of post assay change marker levels).

Table 20 Tumour marker levels at the time of initiation of the first (n=162) and second (n=72) chemotherapy regimens given to patients with advanced colorectal cancer (within 30 days of these dates).

MC = Marker assay change (April 2004)

Chemotherapy Regimen	Tumour Marker		Measured at initiation of therapy (% of 162 or 72 patients with one or more markers measured)	Raised at initiation of therapy (% of those where marker was measured)	Range	Skew	Mann-Whitney <i>U</i> test result <i>z</i> (<i>P</i>) for markers measured
1	CEA	Pre-MC	151 (93.2%)	130 (86.1%)	1-6686	4.18	
		Post-MC	11 (6.8%)	8 (72.7%)	2-592	3.21	
	CA19-9	Pre-MC	151 (93.2%)	99 (65.6%)	1-93000	8.80	
		Post-MC	11 (6.8%)	7 (63.6%)	14-4850	3.28	
2	CEA	Pre-MC	61 (84.7%)	59 (96.7%)	1-6204	3.42	-1.93 (0.05)
		Post-MC	10 (13.9%)	9 (90.0%)	4-826	2.96	
	CA19-9	Pre-MC	62 (86.1%)	46 (74.2%)	1-127000	7.35	
		Post-MC	10 (13.9%)	7 (70.0%)	23-1012	1.77	

Of the 72 patients who had one or more markers measured at the start of the second chemotherapy regimen 98.6% (71) had both CEA and CA19-9 measured (10 of these patients had their markers measured after the change of assay).

At the start of the second chemotherapy regimen CEA was raised in 95.8% (68 of 71) and CA19-9 was raised in 73.6% (53 of 72) of all patients where it was measured. Figure 27 shows the concordance between the raised marker levels in the 71 individuals who had both CEA and CA19-9 measured at this time.

Figure 26 Box and whisker plot showing markers measured at the time of first and second chemotherapy regimens given in advanced colorectal cancer (within 30 days of start date).

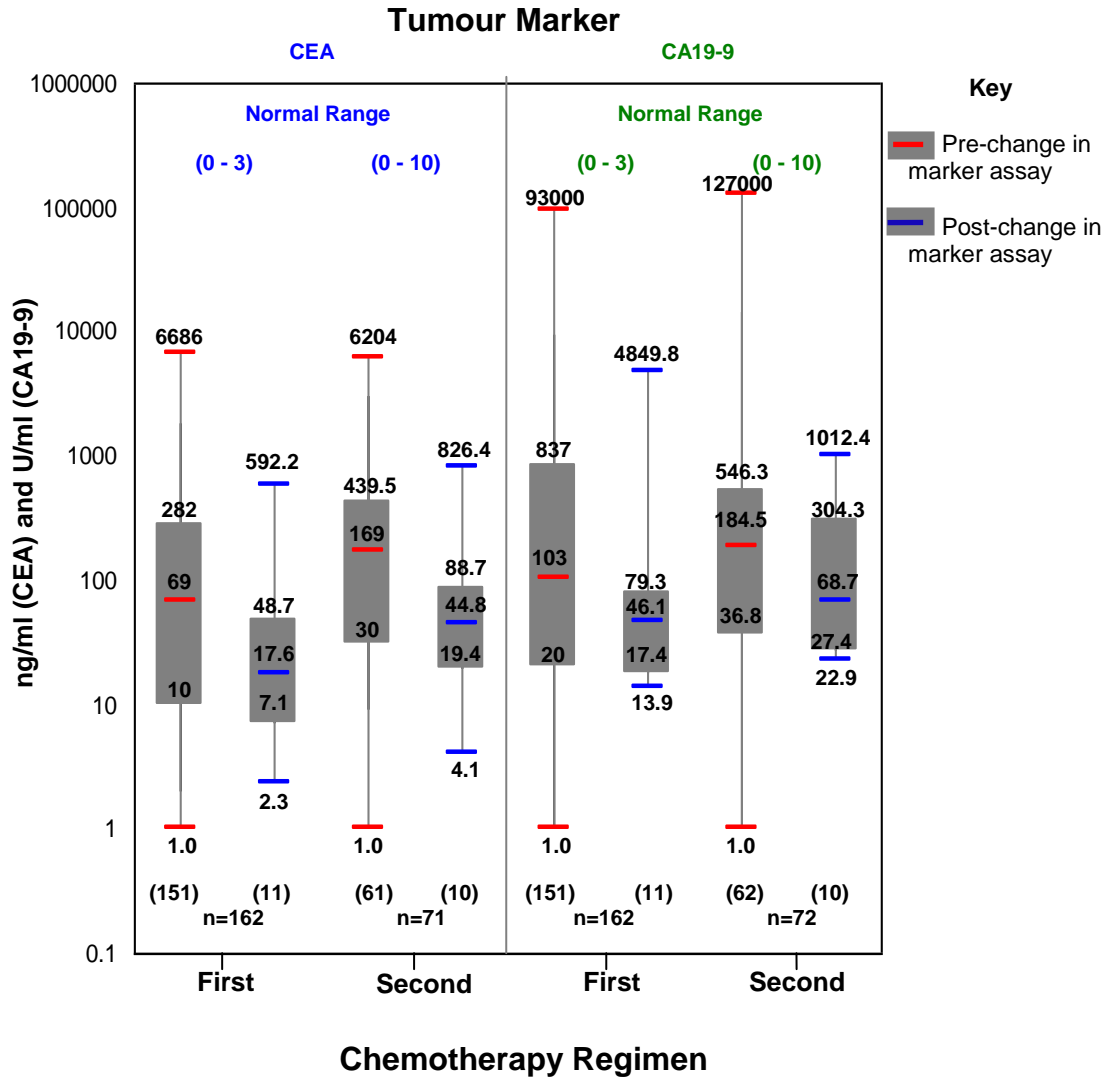


Figure 27 Concordance of markers in the 71 patients who had CEA and CA19-9 measured at the time of the second chemotherapy given in advanced colorectal cancer (within 30 days of this date).

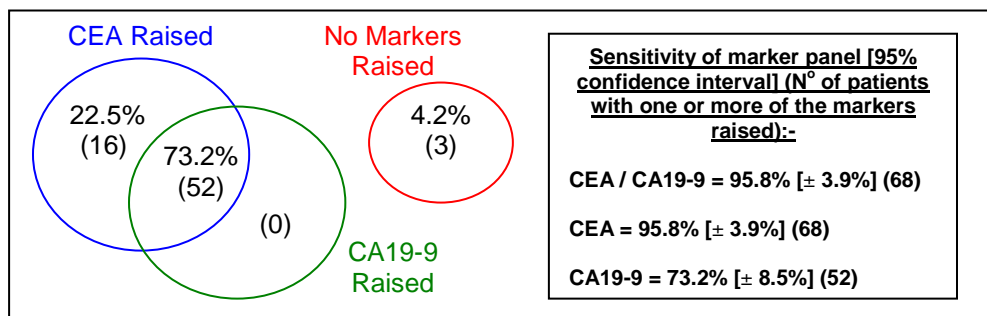


Figure 28 and Table 21 show the sensitivities in more detail and compares them with sensitivities of each other marker panel at advanced disease diagnosis and the start of chemotherapy one.

Figure 28 Tumour marker panel sensitivity at the time of the diagnosis of advanced disease and at the start of the first and second chemotherapy regimens given in advanced colorectal cancer (in patients who had all tumour markers recorded).

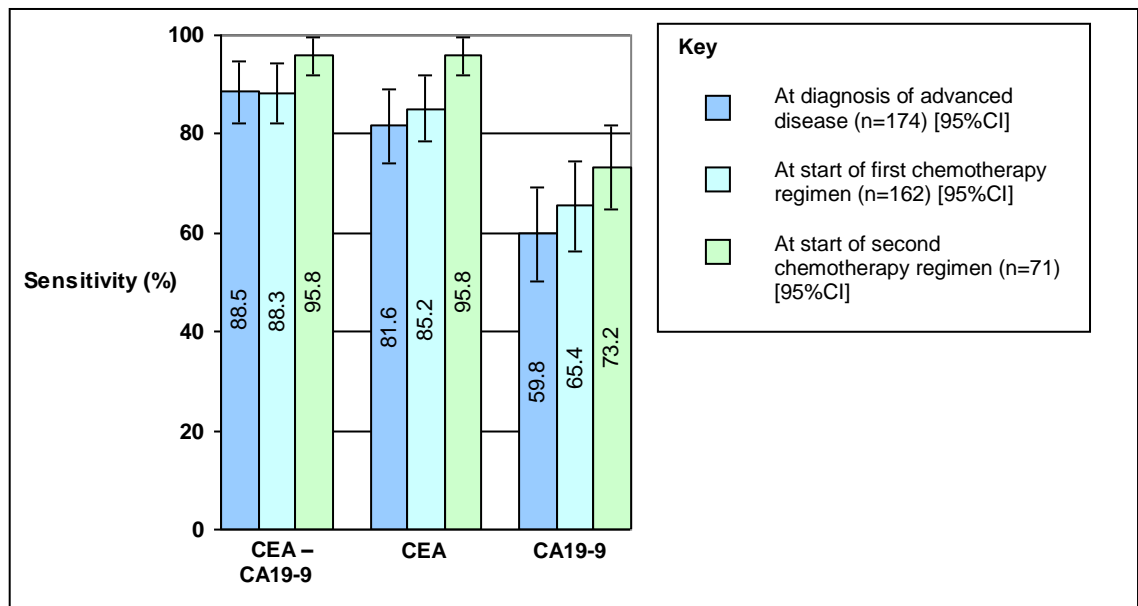


Table 21 Differences between the sensitivity of each tumour marker panel at the time of the diagnosis of advanced disease, the start of the first, and the start of the second chemotherapy regimens given in advanced colorectal cancer (in patients who had all tumour markers recorded). Instructions for using this table can be found on page 114.

		Difference in Percentage of patients with raised markers - 95% Confidence Interval (p)								
		Advanced disease diagnosis			Chemotherapy One			Chemotherapy Two		
		CEA-CA19-9	CEA	CA19-9	CEA-CA19-9	CEA	CA19-9	CEA-CA19-9	CEA	CA19-9
Advanced Disease Diagnosis	CEA-CA19-9		-0.6 to 14.4 (0.07)	19.5 to 37.9 (<0.001)	-6.7 to 7.1 (1.0)	-3.9 to 10.5 (0.4)	14.1 to 32.1 (<0.001)	-15.3 to 0.7 (0.08)	-15.3 to 0.7 (0.08)	5.2 to 25.4 (<0.005)
	CEA			12.2 to 31.4 (<0.001)	-14.4 to 1.0 (0.09)	-11.6 to 4.4 (0.4)	6.8 to 25.6 (<0.005)	-23.9 to -4.5 (<0.005)	-23.9 to -4.5 (<0.005)	-2.8 to 19.6 (0.1)
	CA19-9				-37.9 to -19.1 (<0.001)	-35.0 to -15.8 (<0.001)	-16.0 to -4.8 (0.3)	-48.6 to -23.4 (<0.001)	-48.6 to -23.4 (<0.001)	-26.7 to -0.1 (0.05)
Chemotherapy One	CEA-CA19-9					-4.3 to 10.5 (0.4)	13.7 to 32.1 (<0.001)	-15.6 to 0.6 (0.07)	-15.6 to 0.6 (0.07)	4.8 to 25.4 (<0.005)
	CEA						10.4 to 29.2 (<0.001)	-19.5 to -1.7 (0.02)	-19.5 to -1.7 (0.02)	1.2 to 22.8 (0.03)
	CA19-9							-42.5 to -18.3 (<0.001)	-42.5 to -18.3 (<0.001)	-20.8 to 5.2 (0.2)
Chemotherapy Two	CEA-CA19-9							-6.6 to 6.6 (1.0)	-6.6 to 6.6 (1.0)	10.7 to 34.5 (<0.005)
	CEA									10.7 to 34.5 (<0.005)
	CA19-9									

Key

- p value is not significant
- p value has borderline significance
- p value is significant

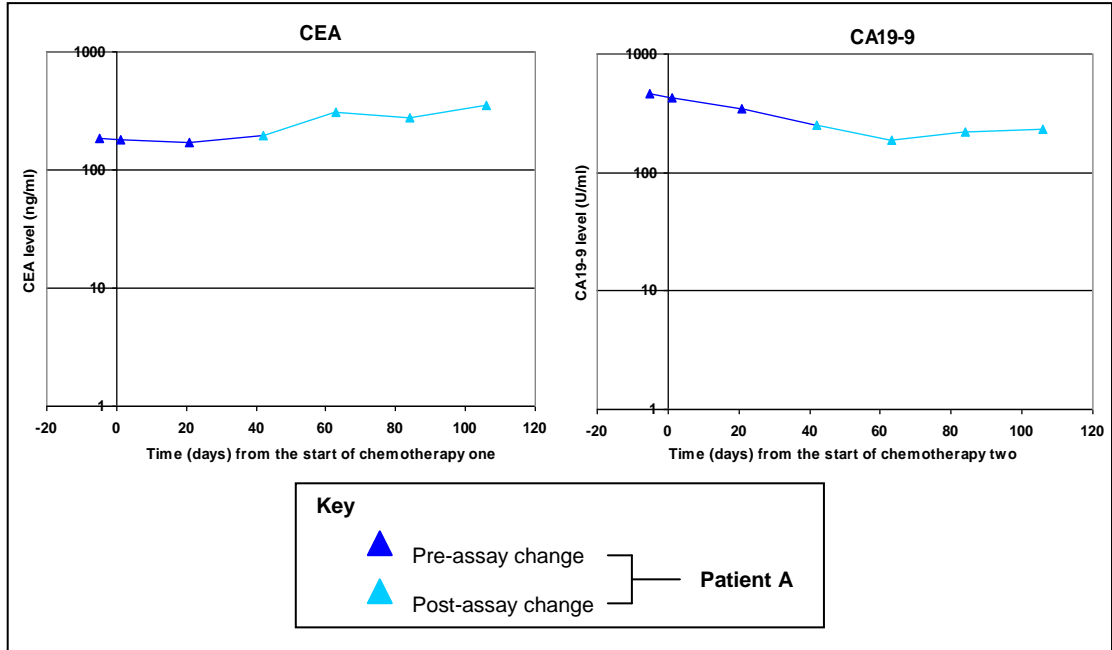
(3.2.3) Tumour Marker Response to Chemotherapy Given in Advanced Disease

(3.2.3.1) At First Chemotherapy

Of the 162 patients who had advanced disease, the first course of palliative chemotherapy at Airedale and a tumour marker level recorded at the start of the first chemotherapy regimen a further 12 patients (7.4%) were excluded at this stage as they did not have enough tumour marker levels recorded for analysis of marker movement to take place (at least one marker level recorded within 30 days of the chemotherapy start date and a further 2 marker levels recorded within the 100 days following this date). This left 150 patients (75.4% of the original 199 patients with advanced colorectal cancer) who could be analysed for Rustin response to chemotherapy one which is defined by Table 11 (Methods section).

For one of the patients the change in assay of markers which occurred in 1993 fell in the period of assessment of Rustin response following initiation of the first chemotherapy regimen. CEA and CA19-9 levels for this patient before and after the assay change can be seen in Figure 29. As the markers post-assay change appear to be on a similar slope to those pre-assay change in this figure I have considered them to be continuous and therefore have considered patient A to show Stable CEA and CA19-9 levels.

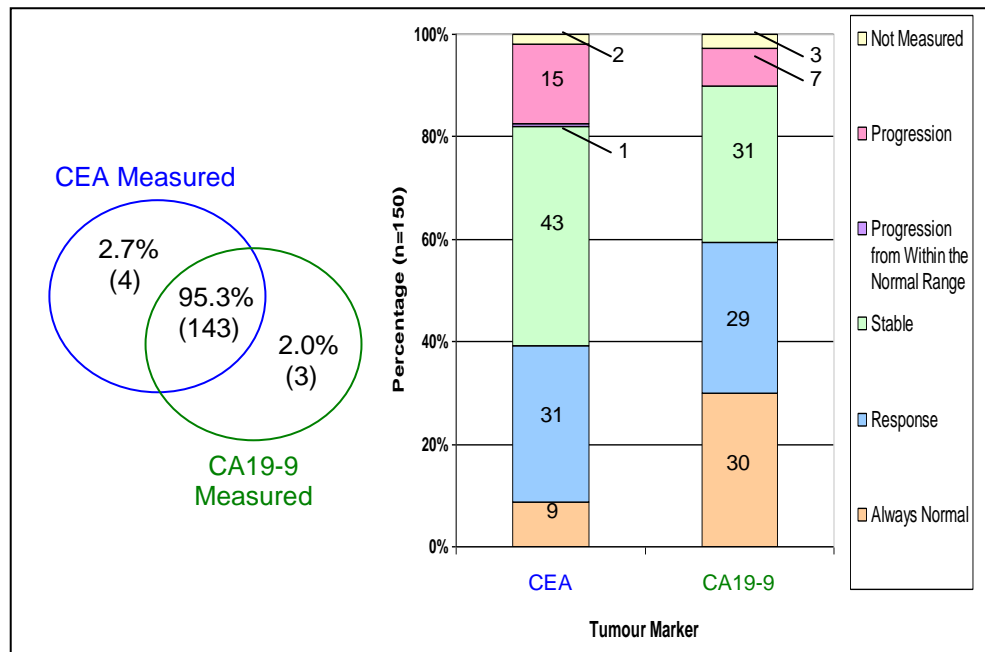
Figure 29 CEA and CA19-9 marker levels following the initiation of the first chemotherapy regimen in the patient where marker assay changed during the 100 days following chemotherapy start date.



As could be seen in Figure 16A (breast section) several of the colorectal patients (Patients B, C, D and E) also had Rustin response analysed over the CA19-9 assay change which occurred in 1995 as was stated with this figure the assay change was not considered to affect the Rustin categories assigned in these cases.

Figure 30 shows the distribution of the measurement of markers among the 150 patients with colorectal cancer and illustrates the proportions of each which fell into each Rustin category of response.

Figure 30 Tumour markers measured and Rustin category of response in the 150 patients whose tumour markers could be analysed following initiation of chemotherapy one.

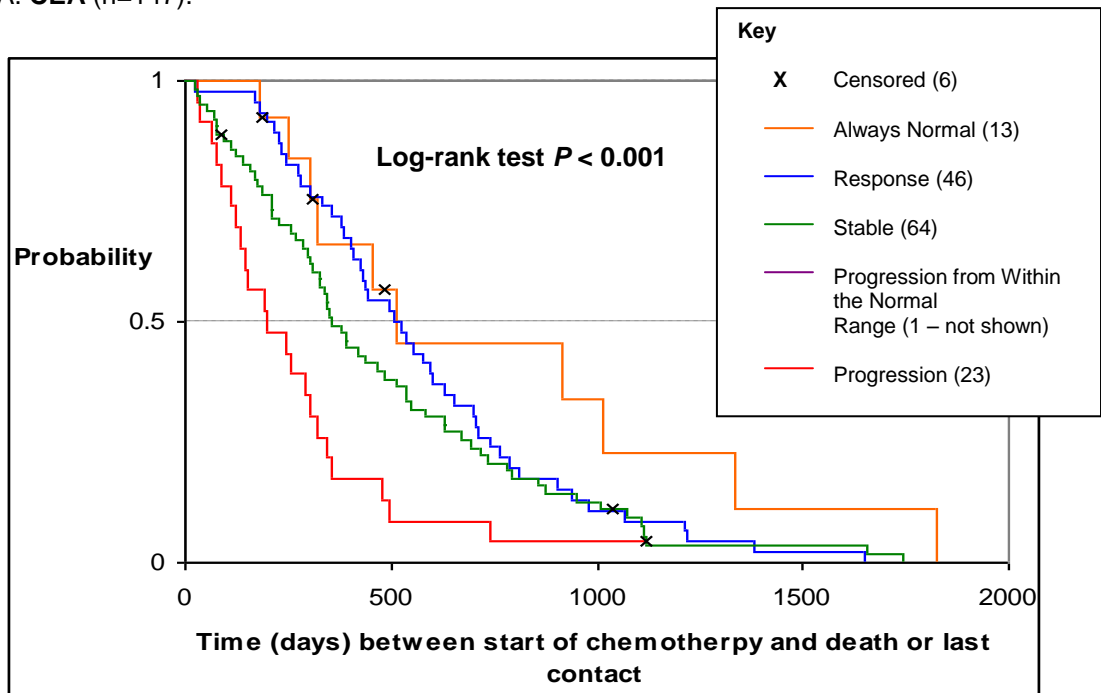


The four Kaplan-Meier graphs in Figure 31 show overall survival from the start of the first chemotherapy given in advanced disease according to tumour marker and Rustin response category. The log rank values for CEA and CA19-9 shown in Figures 31A and 31C were significant whether the patient shown in Figure 29 was included in the analysis or not ($P < 0.001$ in all cases).

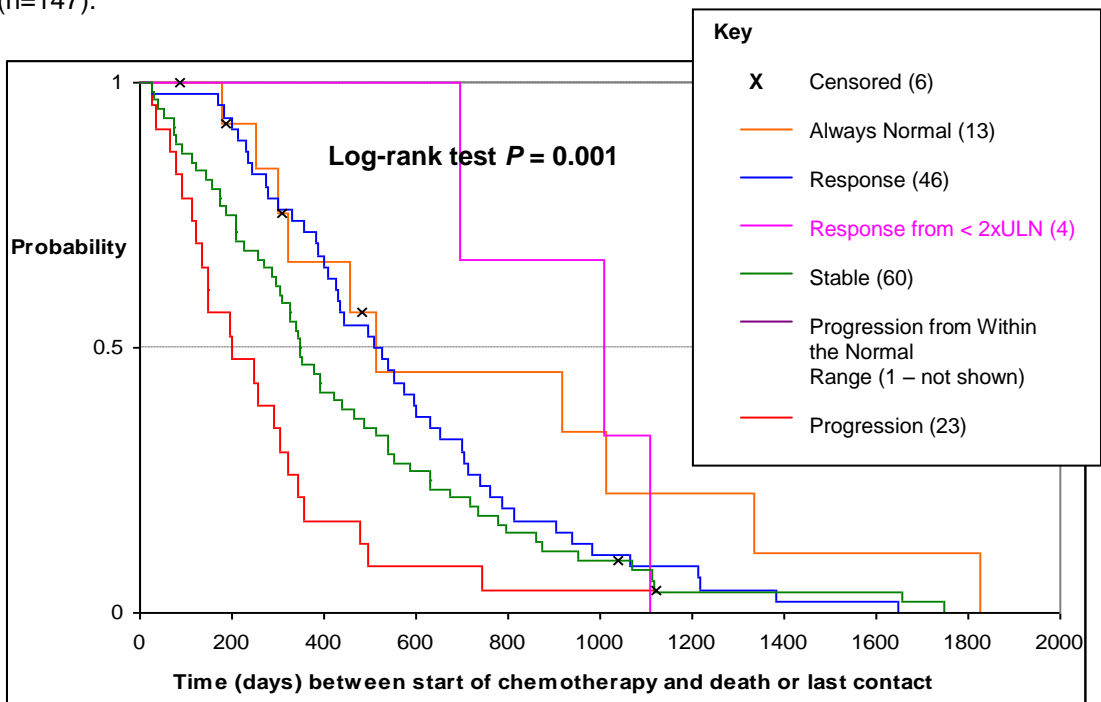
The data used to produce Figure 31A has one patient removed as the patient had a progression from within the normal range and this data cannot be analysed on its own. Four of the patients in Figure 31A and six of the patients in Figure 31C who are classified (according to the Rustin response criteria) as having stable tumour markers had a $\geq 50\%$ fall in a tumour marker level but from a level that was not \geq twice the ULN, these patients are analysed as a separate group in Figure 31B and 31D respectively.

Figure 31 Rustin response category and survival from the start of the first chemotherapy given in advanced colorectal cancer

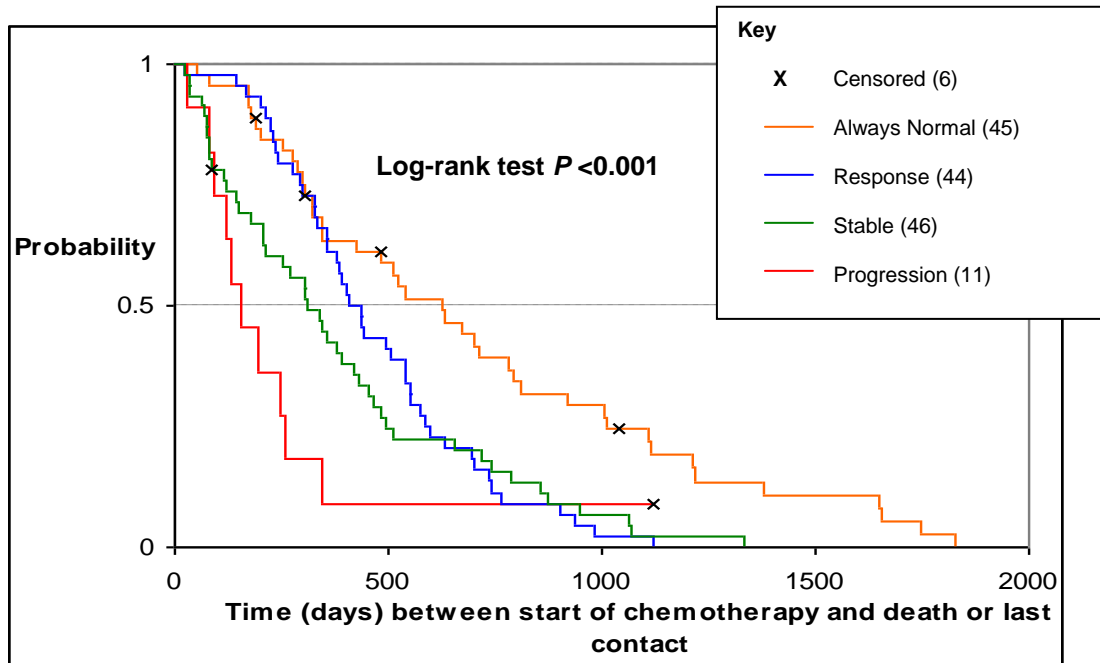
A. CEA (n=147).



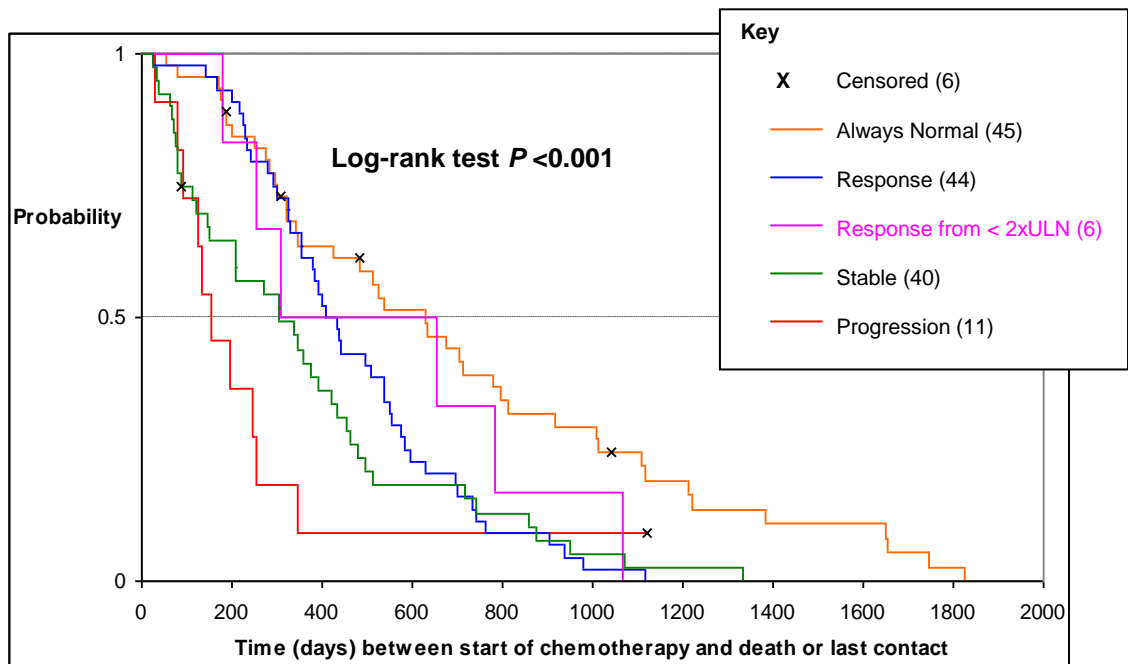
B. CEA - Survival of the 4 patients who responded from $< 2x$ ULN as a separate curve (n=147).



C. CA19-9 (n=146).



D. CA19-9 (n=146).



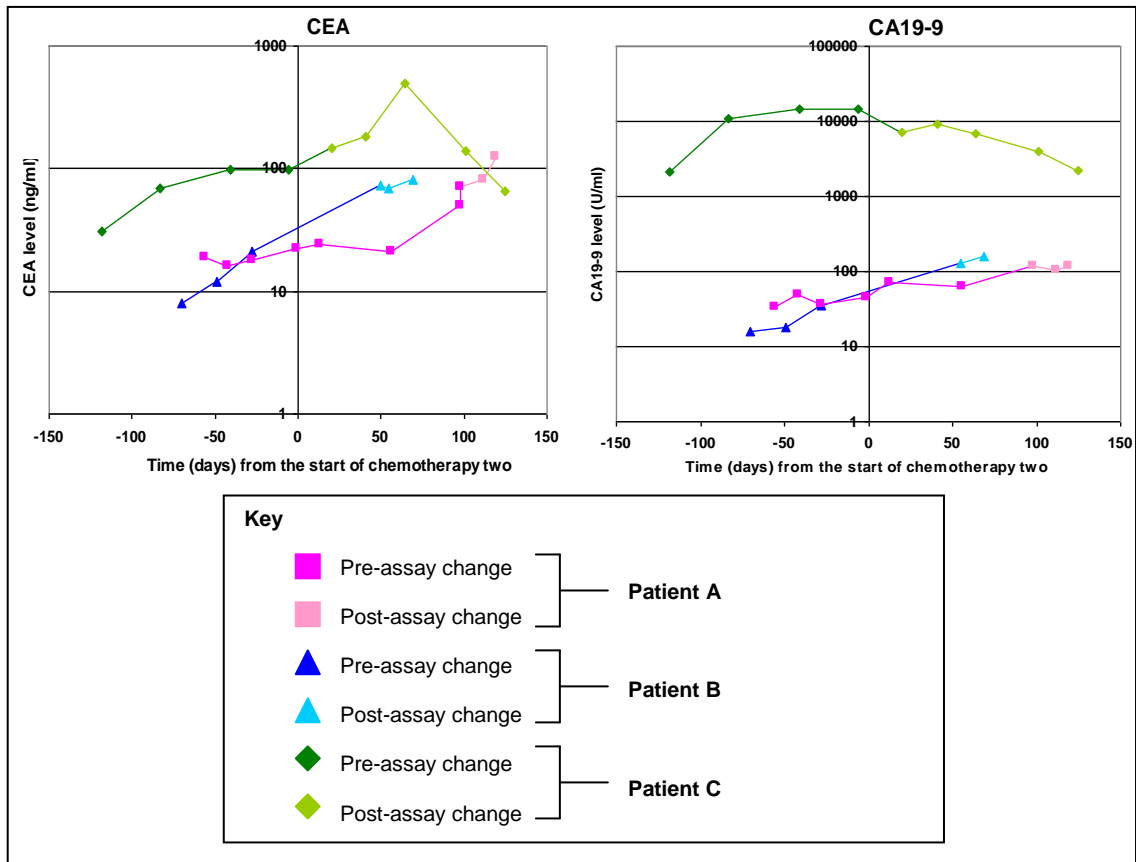
(3.2.3.2) At Second Chemotherapy

Of the 72 patients who had advanced disease, the second course of palliative chemotherapy at Airedale and a tumour marker level recorded at the start of the second chemotherapy regimen (within 30 days of this date) a further 6

(8.3%) were excluded at this stage as they did not have enough tumour marker levels recorded for analysis of marker movement to take place. This left 66 patients (33.2% of the original 199 patients with advanced colorectal cancer) who could be analysed for Rustin response to chemotherapy two.

For three of the patients the change in assay of markers which occurred in 1993 (Figure 32, Patient A) and April 2004 (Figure 32, Patients B and C) fell in the period of assessment of Rustin response following initiation of the second chemotherapy regimen. CEA and CA19-9 levels for these patients before and after the assay change can be seen in Figure 32. This made assessment of the Rustin Response to chemotherapy more difficult in these patients. As the post-assay change markers appear to be on a similar slope to the pre-change marker levels I have considered them to be continuous and therefore have considered patient A to show CEA progression and CA19-9 to show progression from within the normal range, patient B to progression for both CEA and CA19-9 and patient C to show stable CEA and response for CA19-9.

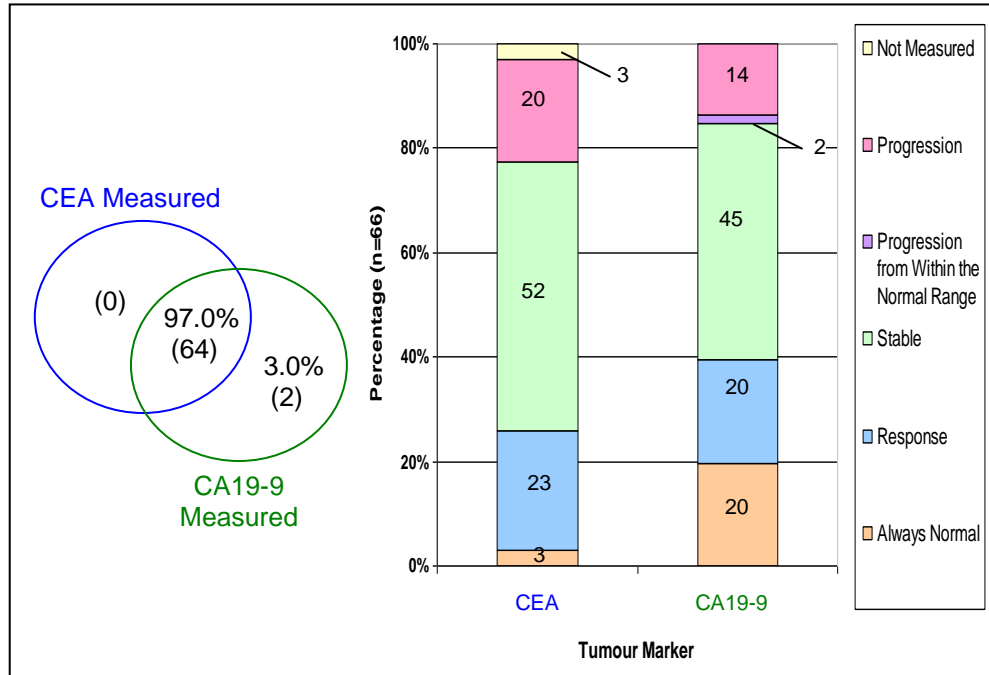
Figure 32 CEA and CA19-9 marker levels following the initiation of the second chemotherapy regimen in 3 patients where marker assay changed during the 100 days following chemotherapy start date.



A further patient had a doubling of CEA from 435 ng/ml to a level of >1000 ng/ml at 48 days post chemotherapy start date, this level then fell to a stable level, for the purpose of the following analyses this was regarded as a case of prolonged tumour lysis and was categorised as stable in terms of Rustin response to chemotherapy two.

Figure 33 shows the distribution of the measurement of markers among the 66 patients and the proportions of each which fell into each Rustin category of response.

Figure 33 Tumour markers measured and Rustin category of response in the 66 patients whose markers could be analysed following initiation of chemotherapy two.



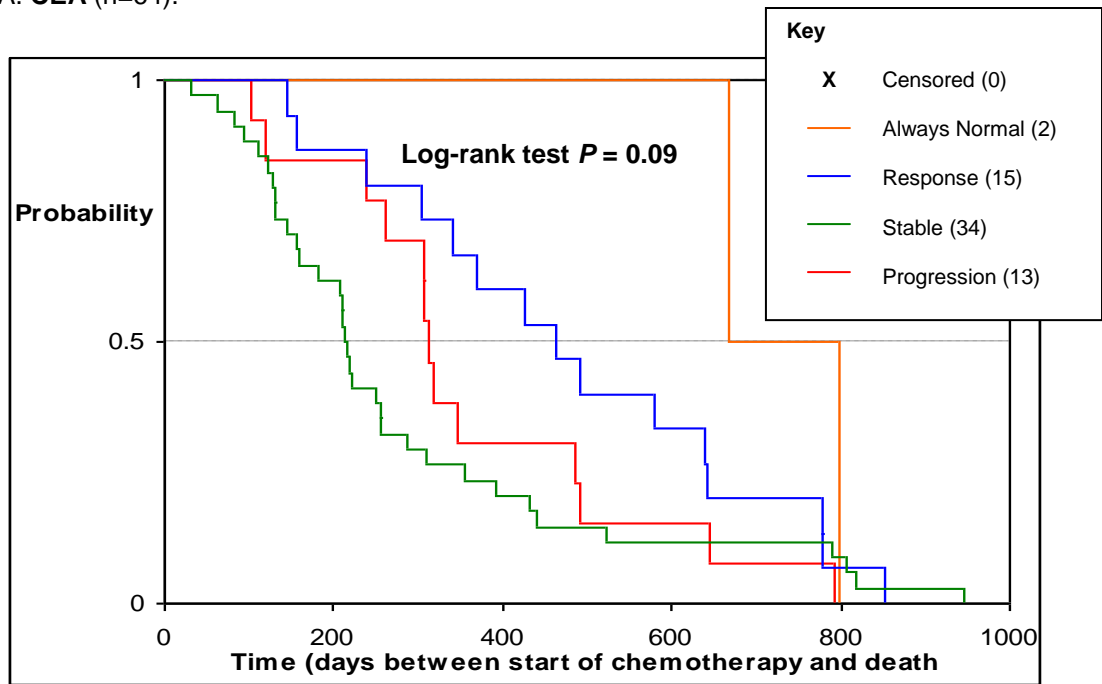
The Kaplan-Meier graphs in Figure 34 show survival from the start of the second chemotherapy given in advanced disease according to tumour marker Rustin response category. The log rank values for CEA and CA19-9 were not significant whether the three patients shown in Figure 32 were included in the analysis or not ($P = 0.1$ for CEA, and $P = 0.4$ for CA19-9 in both cases) and in the case of CEA regardless of the inclusion or exclusion of the patient with prolonged tumour lysis.

Figure 34(A, B and C) shows graphs with all eligible patients included. There were 2 patients who had $\geq 50\%$ a fall in CA19-9 but from a level which was originally less than twice the upper limit of normal (ULN), in Figure 34B these patients are categorised as having stable disease according to the modified Rustin criteria of response however the survival curve in Figure 34C shows

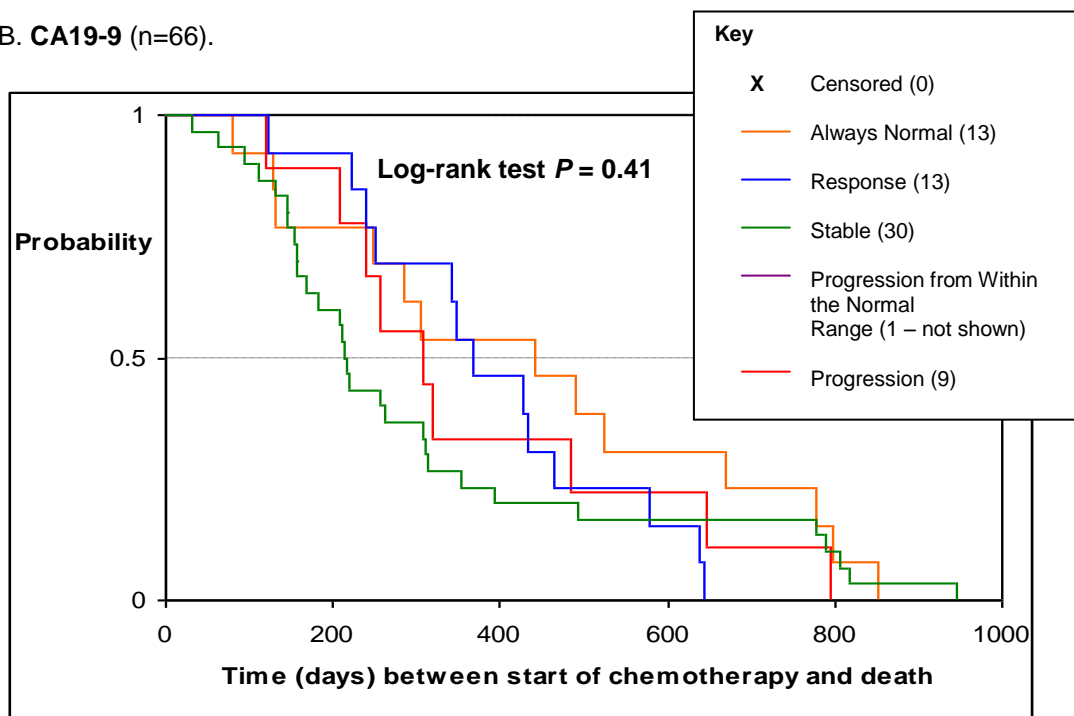
the amended Kaplan-Meier graph which is generated if these two patients were categorised as a separate group.

Figure 34 Rustin response category and survival from the start of the second chemotherapy given in advanced colorectal cancer

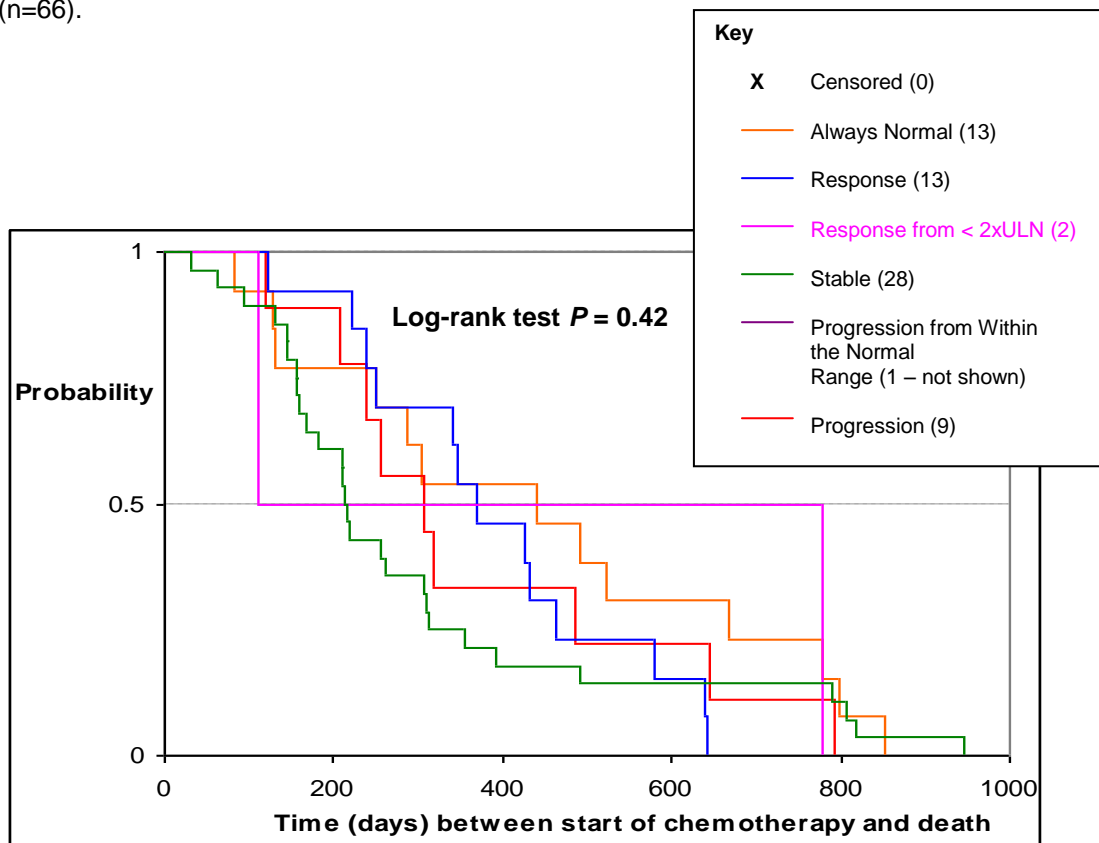
A. CEA (n=64).



B. CA19-9 (n=66).



C. **CA19-9** - Survival of the 2 patients who responded from < 2x ULN as a separate curve (n=66).



Median survivals could be calculated from the graphs in Figures 31 and 34; these are displayed in Table 22 and Figure 35.

The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the Rustin response criteria at identifying radiological response to the first and second chemotherapy regimens are shown in Table 23 and survival according to radiological response can be seen in Figure 36A and B.

Results of Spearman rank correlation and Cox's proportional hazards regression analysis for both breast and colorectal cancer patients can be seen in Table 24. In order to do these analyses the precise ordering of the

Rustin categories of response had to be specified. This order was unclear from the Kaplan-Meier graphs shown in Figures 18, 20, 31 and 34, because of this, and after discussion with a statistician, the categories were ordered as follows:-

1. Response (R)
2. Always Normal (AN)
3. Stable (S)
4. Progression from Within the Normal Range / Progression (PN / P)

AN was placed between R and S on the basis that it was unclear which category it most closely resembled (i.e. which side of R it sat on) and so it was felt that the best guess would be to place it between the two. PN and P were grouped together because often there were very few individuals who had markers categorised as PN so very little could be said about this group alone.

Tests for interaction were performed on the results of the breast and colorectal cancer patients again using the Cox's proportional hazards regression model, results of this analysis can be seen in Table 25. Because of time limitations not all possible confounding factors (which data was collected on originally) could be taken into account in this analysis.

Table 22 Median Survival in weeks according to tumour marker response to the first and second chemotherapy regimens given in advanced disease.

A. Breast cancer

		CEA		CA15-3		CA19-9	
Chemotherapy Regimen (n° of patients)		1 (77)	2 (41)	1 (86)	2 (47)	1 (88)	2 (48)
Survival in weeks (n° of patients) according to Marker Response Category	Always Normal	73.1 (33)	63.6 (8)	43.1 (17)	60.1 (6)	69.3 (52)	40.4 (21)
	Response	94.9 (17)	45.1 (8)	66.7 (23)	64.1 (11)	71.3 (6)	28.1 (6)
	Stable	41.9 (20)	26.3 (20)	51.6 (41)	35.0 (25)	35.7 (20)	49.3 (15)
	Progression from Within the Normal Range combined with Progression	23.0 (7)	14.9 (5)	19.0 (5)	22.3 (5)	20.6 (10)	17.3 (6)
Log-rank Test	χ^2 (Degrees of Freedom)	24.0 (3)	11.5 (3)	17.1 (3)	9.3 (3)	24.0 (3)	15.0 (3)
	P	< 0.001	0.009	< 0.001	0.026	< 0.001	0.002

B. Colorectal cancer

		CEA		CA19-9	
Chemotherapy Regimen (N° of patients)		1 (147)	2 (64)	1 (146)	2 (66)
Survival in weeks (n° of patients) according to Marker Response Category	Always Normal	73.3 (13)	95.6 (2)	89.9 (45)	63.0 (13)
	Response	72.6 (46)	66.1 (15)	58.3 (44)	52.6 (13)
	Stable	50.6 (64)	30.4 (34)	44.3 (46)	30.4 (30)
	Progression from Within the Normal Range combined with Progression	27.7 (24)	44.6 (13)	22.3 (11)	36.7 (10)
Log-rank Test	χ^2 (Degrees of Freedom)	18.1 (3)	6.4 (3)	18.2 (3)	2.6 (3)
	P	< 0.001	0.09	<0.001	0.4

Figure 35 Median Survival in days according to tumour marker response, error bars indicate the 95% confidence interval and * indicates where the 95% confidence interval cannot be calculated

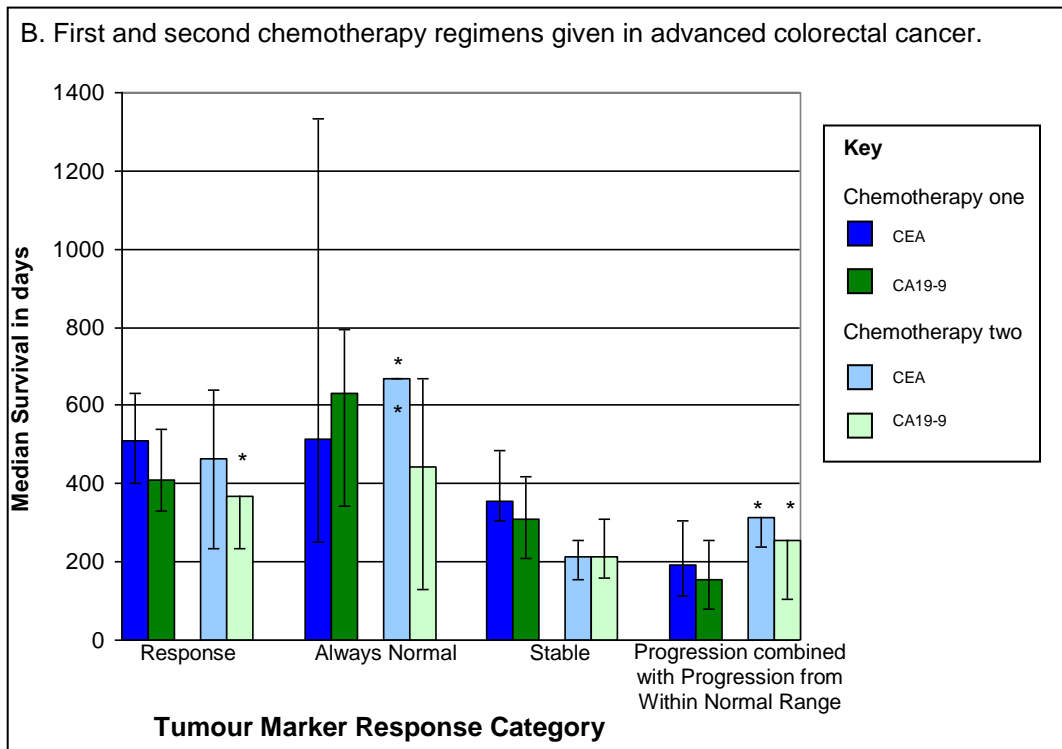
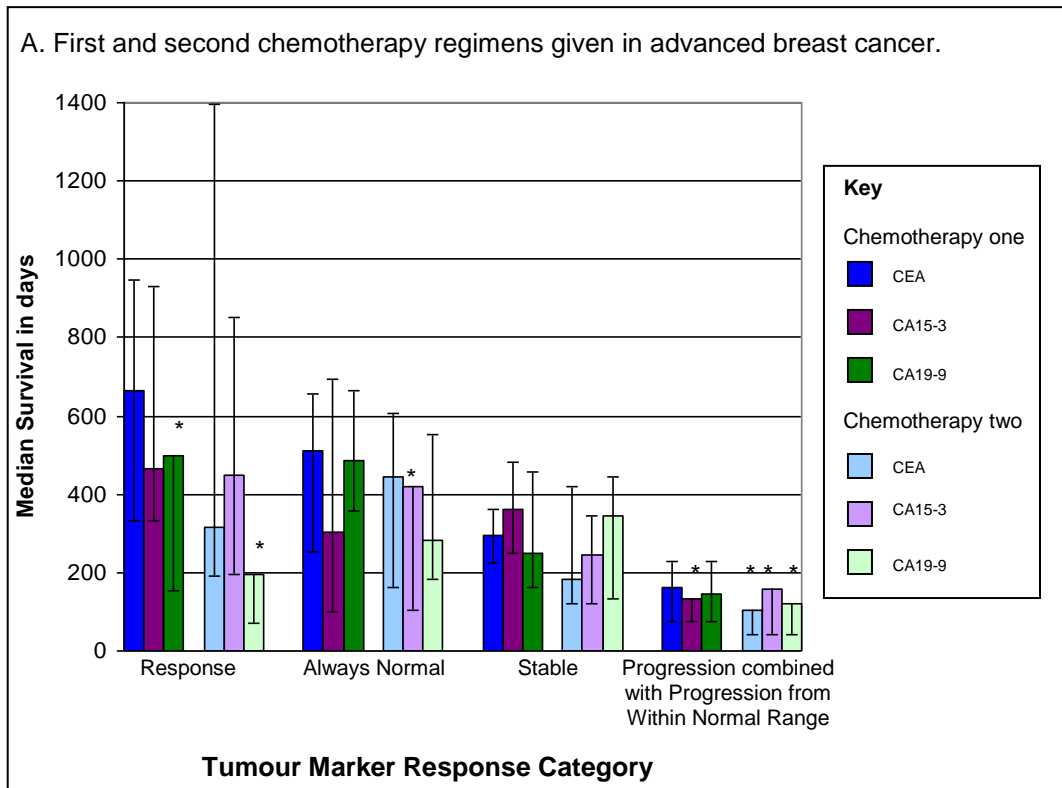


Table 23

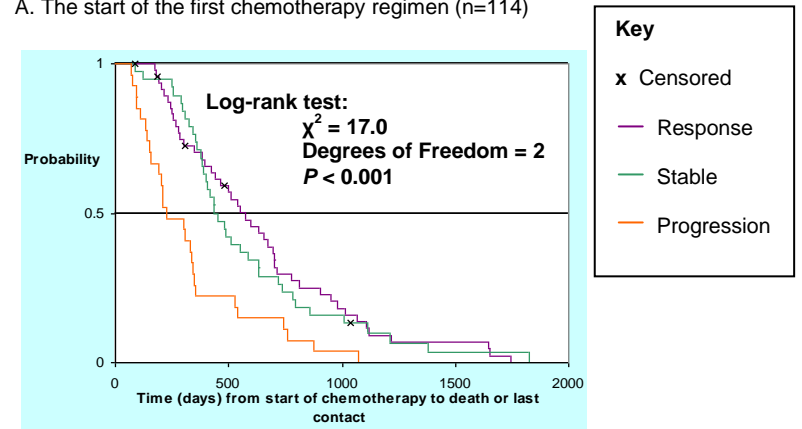
Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of Rustin progression at identifying radiological progression and Rustin response at identifying radiological response from chemotherapy initiation in patients with advanced colorectal cancer.

P only = Only patients categorised as having Rustin progression considered to show tumour marker progression
P and PN = Patients with both Rustin progression and progression from within the normal range considered to show tumour marker progression.

Colorectal					
First Chemotherapy (N° of patients eligible for analysis)		Sensitivity % (N° of patients)	Specificity % (N° of patients)	PPV % (N° of patients)	NPV % (N° of patients)
CEA (113)	P only	33.3 (9/27)	93.0 (80/86)	60.0 (9/15)	81.6 (80/98)
	P and PN	As above	As above	As above	As above
	Response	51.0 (25/49)	81.3 (52/64)	67.6 (25/37)	68.4 (52/76)
CA19-9 (112)	P only	20.0 (5/25)	96.6 (84/87)	62.5 (5/8)	80.8 (84/104)
	P and PN	As above	As above	As above	As above
	Response	36.7 (18/49)	73.0 (46/63)	51.4 (18/35)	59.7(46/77)
Second Chemotherapy (N° of patients eligible for analysis)					
CEA (50)	P only	36.8 (7/19)	93.5 (29/31)	77.8 (7/9)	70.7 (29/41)
	P and PN	As above	As above	As above	As above
	Response	50.0 (7/14)	83.3 (30/36)	53.8 (7/13)	81.1 (30/37)
CA19-9 (51)	P only	15.0 (3/20)	93.5 (29/31)	60.0 (3/5)	63.0 (29/46)
	P and PN	25.0 (4/20)	93.5 (29/31)	66.7 (4/6)	64.4 (29/45)
	Response	28.6 (4/14)	86.5 (32/37)	44.4 (4/9)	76.2 (32/42)

Figure 36 Radiological response category and survival in advanced colorectal cancer from

A. The start of the first chemotherapy regimen (n=114)



B. The start of the second chemotherapy regimen (n=51)

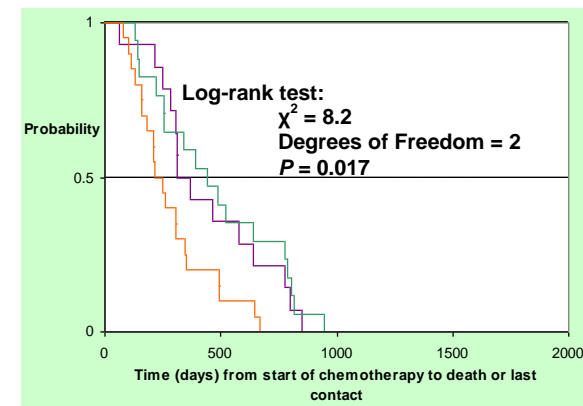


Table 24

Table 24 A and B shows:

1. Results of Spearman rank correlation analysis (of Rustin response categories which were ordered in the following way:- R (response) – AN (always normal) – S (stable) – P/PN (progression / progression from within the normal range), and radiological staging which were ordered:- R – S – P)

2. Unadjusted Hazard Ratio calculated using Cox's Proportional Hazards regression model for survival of breast and colorectal cancer patients. Survival according to Rustin response category using Response as defined by these criteria as baseline (radiological response category used response as defined by radiological response criteria as baseline).

The regression analysis was carried out both with and without the patients with Always Normal (AN) tumour markers included.

Sometimes other groups were compared within the analysis e.g. The hazard ratio for survival of AN compared with that of S this is shown in the table in this way. The significance of the difference between the survivals of the two groups is also shown here.

3. **AIC** = Akaike's information criterion – A smaller value for this is indicative of a better model

A. Breast cancer patients

Tumour Marker	Chemotherapy one		Chemotherapy Two			
	Spearman rank correlation (ρ)	Unadjusted Hazard Ratio (ρ)	Spearman rank correlation (ρ)	Unadjusted Hazard Ratio (ρ)		
CEA	[n=48] 0.1413 (0.3)	<u>With AN Included</u> [n=77] R = 1.00 AN = 1.49 (0.2) S = 2.77 (0.004) P/PN = 9.99 (<0.001) ? AN = S ($p = 0.04$) AIC = 496	<u>Without AN</u> [n=44] R = 1.00 S = 2.74 (0.006) P/PN = 11.68 (<0.001) AIC = 228	[n=24] 0.3436 (0.1)	<u>With AN Included</u> [n=41] R = 1.00 AN = 1.87 (0.2) S = 2.32 (0.07) P/PN = 7.95 (0.001) AIC = 225	<u>Without AN</u> [n=33] R = 1.00 S = 2.19 (0.1) P/PN = 6.43 (0.004) AIC = 166
		CA15-3	[n=51] 0.2785 (0.048)	<u>With AN Included</u> [n=86] R = 1.00 AN = 1.80 (0.09) S = 1.76 (0.06) P/PN = 8.89 (<0.001) AIC = 562	<u>Without AN</u> [n=69] R = 1.00 S = 1.72 (0.07) P/PN = 10.32 (<0.001) AIC = 413	[n=28] 0.4399 (0.02)
CA19-9	[n=53] 0.1262 (0.4)	<u>With AN Included</u> [n=88] R = 1.00 AN = 1.30 (0.5) S = 2.22 (0.1) P/PN = 5.75 (0.001) AIC = 585	<u>Without AN</u> [n=36] R = 1.00 S = 1.92 (0.2) P/PN = 4.10 (0.01) AIC = 184	[n=28] 0.3845 (0.04) (Fishers exact = 0.394)	<u>With AN Included</u> [n=48] R = 1.00 AN = 1.37 (0.5) S = 1.50 (0.5) P/PN = 9.00 (0.002) AIC = 267	<u>Without AN</u> [n=27] R = 1.00 S = 1.37 (0.6) P/PN = 7.94 (0.007) AIC = 118
Radiology		[n=61] R = 1.00 S = 1.11 (0.8) P = 3.18 (0.01) ? S = P ($p = 0.01$) AIC = 362			[n=29] R = 1.00 S = 1.69 (0.3) P = 2.81 (0.06) ? S = P ($p = 0.2$) AIC = 140	

B. Colorectal cancer patients

Tumour marker	Chemotherapy one		Chemotherapy two			
	Spearman rank correlation (ρ)	Unadjusted Hazard Ratio (ρ)	Spearman rank correlation (ρ)	Unadjusted Hazard Ratio (ρ)		
CEA	[n=113] 0.4453 (<0.001)	<u>With AN Included</u> [n=147] R = 1.00 AN = 0.61 (0.2) S = 1.19 (0.4) P/PN = 2.51 (<0.001) ? AN = S ($p = 0.07$) AIC = 1124	<u>Without AN</u> [n=134] R = 1.00 S = 1.18 (0.4) P/PN = 2.49 (<0.001) AIC = 1026	[n=50] 0.4280 (0.002)	<u>With AN Included</u> [n=64] R = 1.00 AN = 0.58 (0.5) S = 1.75 (0.08) P/PN = 1.43 (0.4) AIC = 411	<u>Without AN</u> [n=62] R = 1.00 S = 1.78 (0.07) P/PN = 1.41 (0.4) AIC = 394
CA19-9	[n=112] 0.2601 (0.006)	<u>With AN Included</u> [n=146] R = 1.00 AN = 0.53 (0.006) S = 1.20 (0.4) P/PN = 1.82 (0.09) ? AN = S ($p < 0.001$) AIC = 1111	<u>Without AN</u> [n=101] R = 1.00 S = 1.19 (0.4) P/PN = 1.81 (0.1) AIC = 728	[n=51] 0.3465 (0.01) (Fishers exact = 0.386)	<u>With AN Included</u> [n=66] R = 1.00 AN = 0.78 (0.5) S = 1.14 (0.7) P/PN = 1.13 (0.8) AIC = 432	<u>Without AN</u> [n=53] R = 1.00 S = 1.23 (0.6) P/PN = 1.15 (0.7) AIC = 324
Radiology		[n=114] R = 1.00 S = 1.09 (0.7) P = 2.58 (<0.001) ? S = P ($p < 0.001$) AIC = 808			[n=51] R = 1.00 S = 0.83 (0.6) P = 2.16 (0.03) ? S = P ($p = 0.008$) AIC = 301	

Table 25

Results of Cox's Proportional Hazards regression model for survival of breast and colorectal cancer patients at chemotherapy one adjusted according to various factors. Rustin response categories were ordered in the following way:- R (response) – AN (always normal) – S (stable) – P/PN (progression / progression from within the normal range) and Response was used as the baseline for these calculations.

* Stage 0 (Dukes' A) and 1 (Dukes' B) combined as baseline with a HR of 1.00.

All but two of the colorectal patients who had a tumour stage for their tumour had a Dukes' stage which was used. One of the 2 patients who just had a TNM stage had distant metastases at diagnosis so was categorised as Dukes' D the other patient had a TNM stage (T2 N2) which was converted to a single stage and classed as stage III (Dukes' C) using Table 12 (Patients and Methods Section).

Tumour Site	Tumour Marker	Adjusted Hazard Ratio ⁺ (p)				
Breast	CEA	<u>For Lymph node status [n=54]</u> R = 1.00 AN = 1.56 (0.3) S = 2.54 (0.03) P/PN = 11.42 (<0.001) 0 involved = 1.00 1-3 involved = 1.34 (0.5) 4-9 involved = 1.10 (0.8) >9 involved = 2.13 (0.1) ? AN = S (p = 0.2)	<u>For Lymph node status and Liver Involvement [n=50]</u> R = 1.00 AN = 1.50 (0.3) S = 2.38 (0.05) P/PN = 16.61 (<0.001) 0 involved = 1.00 1-3 involved = 1.66 (0.3) 4-9 involved = 1.28 (0.6) >9 involved = 2.93 (0.04) Liver Involved = 0.95 (0.9)			
	CA15-3	<u>For Lymph node status [n=62]</u> R = 1.00 AN = 1.24 (0.6) S = 1.42 (0.3) P/PN = 8.30 (0.001) 0 involved = 1.00 1-3 involved = 1.01 (1.0) 4-9 involved = 0.98 (1.0) >9 involved = 1.43 (0.3)	<u>For Lymph node status and Liver Involvement [n=60]</u> R = 1.00 AN = 1.53 (0.4) S = 1.69 (0.2) P/PN = 9.45 (0.001) 0 involved = 1.00 1-3 involved = 0.94 (0.9) 4-9 involved = 0.88 (0.8) >9 involved = 1.29 (0.5) Liver Involved = 1.24 (0.6)			
	CA19-9	<u>For Lymph node status [n=64]</u> R = 1.00 AN = 2.32 (0.1) S = 3.71 (0.04) P/PN = 20.54 (<0.001) 0 involved = 1.00 1-3 involved = 0.92 (0.8) 4-9 involved = 1.07 (0.9) >9 involved = 1.43 (0.4)	<u>For Liver Involvement [n=83]</u> R = 1.00 AN = 1.57 (0.3) S = 3.07 (0.03) P/PN = 8.05 (<0.001) Involved = 1.59 (0.07)	<u>For Lymph node status and Liver Involvement [n=60]</u> R = 1.00 AN = 2.17 (0.2) S = 3.87 (0.04) P/PN = 26.17 (<0.001) 0 involved = 1.00 1-3 involved = 0.97 (0.9) 4-9 involved = 1.15 (0.8) >9 involved = 1.85 (0.2) Liver Involved = 1.02 (1.0)		
Colorectal	CEA	<u>For Stage* [n=128]</u> R = 1.00 AN = 0.60 (0.2) S = 1.19 (0.4) P/PN = 2.67 (0.001) Dukes' C = 1.64 (0.05) Dukes' D = 3.22 (0.001) ? AN = S (p = 0.06)	<u>For Stage* and Liver Involvement [n=123]</u> R = 1.00 AN = 0.57 (0.2) S = 1.14 (0.5) P/PN = 2.64 (0.001) Dukes' C = 1.59 (0.06) Dukes' D = 3.19 (0.002) Liver Involved = 0.95 (0.8)	<u>For Lymph node status and Liver Involvement [n=127]</u> R = 1.00 AN = 0.69 (0.4) S = 1.43 (0.1) P/PN = 3.03 (<0.001) 0 involved = 1.00 1-3 involved = 1.07 (0.8) 4-9 involved = 1.87 (0.02) >9 involved = 3.71 (>0.001) Liver Involved = 0.83 (0.4)		
	CA19-9	<u>For Age [n=146]</u> R = 1.00 AN = 0.50 (0.004) S = 1.19 (0.4) P/PN = 1.88 (0.08) For one year = 0.99 (0.4)	<u>For Stage* [n=125]</u> R = 1.00 AN = 0.43 (0.001) S = 0.96 (0.9) P/PN = 2.14 (0.06) Dukes' C = 1.77 (0.03) Dukes' D = 4.74 (<0.001) ? AN = S (p = 0.001)	<u>For Liver Involvement [n=140]</u> R = 1.00 AN = 0.48 (0.003) S = 1.14 (0.5) P/PN = 1.66 (0.2) Liver Involved = 0.95 (0.8)	<u>For Stage* and Liver Involvement [n=120]</u> R = 1.00 AN = 0.37 (<0.001) S = 0.90 (0.7) P/PN = 2.06 (0.08) Dukes' C = 1.77 (0.03) Dukes' D = 4.89 (<0.001) Liver Involved = 0.91 (0.7)	

(4) Discussion

(4.1) Tumour Markers Leading up to Advanced Disease

Diagnosis

(4.1.1) Breast Cancer

When looking at tumour marker lead times results are often limited by small sample sizes [105, 109] and our results are no exception. Lead times could be measured in relatively few ladies (17) in all cases the three main reasons for the exclusions were that marker level at ADD was not raised (Table 14), that there was no previous tumour marker measurement and finally that the previous marker level was \leq the upper limit of normal (ULN). When we look at the results in Table 14 and Figure 9 we can see that CEA appears to have the longest lead time (if the patients where CA15-3 increase does not appear to be exponential are discounted) followed by CA15-3 and lastly CA19-9. If amended lead time was used then CA15-3 lead time was found to be the similar to that found by Molina et. al. in 1996 [108] (4.3 months compared with 4.8 months – Table 5) which are much more reliable results as they are derived from a sample of 89 individuals who developed advanced breast cancer rather than 12. The results obtained for CEA also appear comparable to those quoted in Table 5 but I could find no previous results concerning CA19-9 lead times in breast cancer patients. As there are so few individuals in all 3 of our analyses these results cannot be relied upon with any degree of certainty.

(4.1.2) Colorectal Cancer

Lead times could be measured in 36 individuals; the 3 main reasons for exclusion remained the same as in the breast cancer patients and are shown in Table 19. When we look at the results in Table 19 and Figure 24 we can see that CEA again appears to have a longer median lead time (4.5 months) than CA19-9 (3 months) a result which tallies with the lead times of other studies which are shown in Table 9 [136, 152-154, 156] however these are mean lead times and when we compare them with our mean lead times (again in Table 19), these appear to be generally longer than those quoted in Table 9. Despite this CEA and CA19-9 appear to have similar doubling times (2 months) but again the numbers used in this study are relatively small compared to those in Table 9.

Other studies have shown that CA19-9 is less effective than CEA at early identification of colorectal cancer recurrence [156] and our results support this, as can be seen from Table 19. Despite analysing almost the same number of patients, more patients were excluded from the CA19-9 analysis than from the CEA analysis as a result of having normal marker levels at the time of advanced disease diagnosis.

(4.2) At the Time of Advanced Disease Diagnosis and First and Second Chemotherapy Regimens

(4.2.1) Breast Cancer

At advanced disease diagnosis only CEA was found to have significantly different marker levels measured before and after the marker assay change of 2004 (Table 13). The same was seen for CEA and CA19-9 at chemotherapy one but none of the markers at chemotherapy two. This could be because the marker ranges shown in Figures 7 and 13 undergo the largest change for CEA, the results could also be affected by the relatively small numbers in the post-marker change groups. Disregarding the post-marker change results the sensitivity of CEA and CA15-3 at advanced disease diagnosis was 54% and 68% respectively (Table 13), these results were comparable, although towards the higher end of the figures quoted by other studies in Table 5 (Introduction). This could be because the time when sensitivity was measured in these studies was not uniform, Molina et. al. [97] recorded raised markers before advanced disease was diagnosed whereas the time of measurement was not stated by Luaro et. al. [99], however the sensitivity of CA15-3 correlates quite closely with estimates made by other reviews [35, 83]. The Sensitivity of CA19-9 was the lowest at 28% which again could not be compared with other results as none were found.

As can be seen from Figure 15 and Table 16 a panel of all 3 tumour markers appeared to be the most sensitive in terms of proportion of patients with a tumour marker raised at advanced disease diagnosis (ADD) but the sensitivity

was not significantly different to that of CEA and CA15-3 without CA19-9 which was 80.8% (95% CI for the difference = - 6.5% to 14.1%, $p=0.5$). This sensitivity is higher than the 55.2% reported one study [112] and 75% quoted by two others [106, 110] despite the fact that one of these studies involves only patients with distant metastases [110] and so sensitivity here would be expected to be high.

Both of these marker combinations (CEA - CA15-3 and all three markers) were significantly more sensitive than the best single tumour marker CA15-3 ($p=0.05$ and 0.009 respectively) although for CEA-CA15-3 this significance was borderline. We found that the most sensitive single marker in those we looked at was CA15-3 (69.2% [95% CI = 60.4%-78.1%]) which was raised in significantly more people than had raised CEA at this time (95% CI for the difference -34.5% to -7.7%, $p < 0.005$) and this fits with previous results displayed in Table 5 [112, 113]. CA19-9 alone was the least sensitive marker (27.9%) and this was found to be significant (95% CI for the difference between CA19-9 alone and CEA alone = 7.0% to 33.4%, $p < 0.005$).

Guadagni et. al. (2001) found that overall in a study of 2191 patients with breast disease (in 1453 cases this disease was malignant) adding CEA to CA15-3 only increased the overall sensitivity of these two markers by 1.4% [204], in contrast to this Molina states that “most reports indicate that by using CEA as well as CA15-3 it is possible to increase sensitivity by 7% to 20% compared to that obtained with CA15-3 alone” [83]. We found that by adding CEA to CA15-3 the sensitivity of CA15-3 increased by 11.5% from 69.2% to 80.8% (95% CI for

the difference = -0.2% to 23.4%, $p=0.05$), a borderline significant increase but one which was more compatible with the findings of Molina et. al. (2006) [83].

Tumour marker panels at the start of chemotherapy one and two share the same pattern of sensitivity as the tumour markers measured at the time of advanced disease diagnosis. As we would expect the sensitivity of the marker panels shown in Figures 8, 11, 14 and the summary graph in Figure 15 appears to rise between advanced disease diagnosis and the start of chemotherapy one and again between the start of chemotherapy one and the start of chemotherapy two and this is the case in all of the panels apart from CA19-9 alone where the sensitivity is the same at chemotherapy one and two. Despite this apparent trend Table 16 shows that there was no significant difference between the proportion of patients with raised tumour markers at diagnosis of advanced disease and the proportion with raised markers in the same tumour marker panel at the start of chemotherapy regimen. The only exception was CEA alone where the difference was 21.5% (95% CI for the difference - 38.8% to - 4.2%, $p=0.01$), no significant difference in the sensitivity of this marker was found between the start of chemotherapy one and two.

Again looking at Figure 15 but this time for chemotherapy one and two a panel of all 3 tumour markers appeared to be the most sensitive in terms of proportion of patients with a tumour marker raised at advanced disease diagnosis (ADD) but by looking at Table 16 we can see that in each case this sensitivity was not significantly affected by excluding CA19-9 from the marker panels ($p=0.7$ and 1.0 respectively). At the time of chemotherapy one only the tumour marker

panel containing all 3 tumour markers was found to be significantly more sensitive than CA15-3 alone (which was again the most sensitive single tumour marker) and this was only borderline significance. The difference between the panel containing CEA and CA15-3 and that containing only CA15-3 did not reach significance. However at the time of chemotherapy two, as with advanced disease diagnosis, both of these tumour marker panels contained a raised marker significantly more often than CA15-3 alone (the significance of these results was borderline in both cases). At chemotherapy two it is also interesting to note that CA15-3 alone was no longer found to be significantly more sensitive than CEA (95% CI for the difference -24.7% to 11.7%, $p = 0.5$) possibly as a result of the smaller sample size ($n=46$) meaning that a larger difference in proportion was needed before significance was reached. CA19-9 alone was always the least sensitive marker and this was significant in all cases.

(4.2.2) Colorectal Cancer

Neither of the markers had significantly different marker levels pre- and post-marker assay change at advanced disease diagnosis (Table 18). At the start of chemotherapy one and two the only marker where a borderline significant difference was found was CEA at the time of chemotherapy two (Table 20).

The sensitivities of the markers at each time point can be seen in Figures 23, 25, 27 and they are combined in Figure 28. At advanced disease diagnosis the sensitivity of CA19-9 which we calculated appeared to be a little higher than those quoted by other studies and shown in Table 9 (Introduction) however our

CEA results appear comparable with those quoted in this table [136, 151-158]. At all three time points (advanced disease, chemotherapy one and chemotherapy two) the sensitivity of CA19-9 was significantly lower than CEA alone and CEA and CA19-9 combined (Table 21, Figure 28). At all time points the addition of CA19-9 to CEA appeared to increase its sensitivity only marginally however this increase never reached a significant level. This finding is similar to that of Fiella et. al. (1994) [156].

Tumour marker panels at the start of chemotherapy one and two share the same pattern of sensitivity as the tumour markers measured at the time of advanced disease diagnosis in that there is a gradual increase in sensitivity between a marker panel at advanced disease diagnosis and the same panel at the start of chemotherapy one and a further increase in sensitivity from this time point to the start of chemotherapy two (Figure 28). Significant differences in the proportion of patients with raised tumour markers at any of these time points were seen with CEA alone and CA19-9 alone where the proportion with a raised marker level at advanced disease diagnosis was not significantly different to the proportion at chemotherapy one but for CEA alone both of these were significantly less than the proportion of patients with a raised marker at chemotherapy two (95% CI for the difference between chemotherapy one and two = -19.5% to -1.7%, $p=0.02$). For CA19-9 alone the sensitivity was significantly greater at chemotherapy two than at advanced disease diagnosis but no difference was found between chemotherapy one and two. This progressive increase of the proportion of patients with raised markers as the disease progresses from identification, to chemotherapy one, and then

chemotherapy two is expected in patients with both breast and colorectal cancer if tumour marker levels relate to tumour burden, because tumour burden would also be expected to increase between these time points. More patients are needed in all cases to see if these differences do reach significance.

(4.3) Response to Chemotherapy Given in Advanced Disease

(4.3.1) Breast Cancer

CA15-3 had the fewest, and CA19-9 had the most patients, whose tumour markers were categorised as Always Normal (AN) and this was the case at chemotherapy one and chemotherapy two (Figure 17 and 19). This indicates that CA15-3 has more potential to provide useful information about response to treatment than CA19-9 as AN markers do not provide any indication of disease state over time.

We found that survival varied significantly across Rustin categories of tumour marker response for all three tumour markers following the first chemotherapy, as the Kaplan-Meier graphs in Figure 18 and the results of the log-rank tests in Table 22A demonstrate, this finding is similar to those of Rubach et. al. (1997) who found significant differences in survival between patients whose CEA and CA15-3 marker movements were categorised as normal, decreasing, fluctuating or increasing [9] (Table 7, Introduction). The results of the Cox's proportional hazards regression model are shown in Table 24, this model picks apart the difference in survivals according to tumour marker response category or radiological staging producing a hazard ratio (HR) for survival for each

category. Following chemotherapy one, hazard ratios (HRs) for individuals with stable (S) CEA and those with progressive (P) CEA (either progression or progression from within the normal range) were 2.8 times and 10.0 times respectively that of individuals with responsive (R) markers. Individuals with markers always within the normal range (AN) did not have a significantly different hazard ratio to responders however it was significantly ($p= 0.04$) different to those with stable (S) markers. Unlike CEA the difference in the hazard ratios (HRs) of CA15-3 and CA19-9 only reached significance when comparing R and P (HR=8.89 and 5.75 respectively and $p \leq 0.001$ in both cases). This was also the case for radiological response where P had a significantly worse HR than S or R. In all tumour markers AN appears, although not significant, to have a greater HR than R indicating that this may be a worse prognostic indicator in patients with breast cancer. This finding is echoed by the median survivals shown in Table 22A and Figure 35A but it is impossible to confirm from our results as the wide confidence intervals of Figure 35A show.

Following chemotherapy two, Cox's analysis for CEA and CA19-9 (Table 24) showed that in both cases only P now showed a significantly different HR from R (HR=7.95, $p =0.001$ and HR=9.00, $p =0.002$ respectively). This was no change for CA19-9 but a change for CEA as at chemotherapy one S was also significantly different from R, this may reflect a real change in the significance of having stable markers at this point but the HRs at the two time points are similar so it may or may have arisen as a result of the fewer patients available for analysis at chemotherapy two. CA15-3 marker response now showed that S alone had a significantly greater HR than the patients whose CA15-3 showed R

(HR=2.88). I would have expected patients whose markers showed progression to also have a significantly increased HR over the responders however this anomaly may be due to the anomalous length of survival of one of the patients who had progressive CA15-3 (Figure 20C). The HRs for radiological response at chemotherapy two did not reach statistical significance (P had a borderline significant HR at 2.81) but were similar to HRs at the time of chemotherapy one. The lack of statistical significance at this time is not surprising as the result of the log-rank test shown in Figure 21B was also not significant possibly as a result of the small numbers of people (n=29) where survival and radiological response could be measured at this time.

By looking at the results of Akaike's information criterion (AIC) (a test of how well a statistical model fits the data) shown in green in Table 24 we can see that at chemotherapy one the radiological response model has the best fit when we include AN in the tumour marker models. However when this group is removed CEA and CA19-9 response become better models than the radiology. The same pattern is seen at chemotherapy two with radiological response fitting the data better than tumour marker response until AN is excluded resulting in the CA19-9 response model alone becoming a slightly better model. In all cases CA19-9 goes from being the worst model to the best, possibly because as previously stated so many patients in this group have AN markers and when these are excluded the markers which respond one way or another reflect the disease state well. The removal of the AN from CA15-3 had little effect on this model. This is an unexpected finding as in most other studies CA15-3 appears to correlate with clinical course better than CEA [65, 99, 112]

(although the opposite was found by Blijlevens et. al. In 1995 [105]), however this correlation with the course of the disease does not appear to translate into better correlation with the survival model as shown by the AIC scores of CA15-3 compared with CEA (Table 24).

Following adjustment according to possible confounding factors (Table 25) lymph node status had no significant effect on the results of the Cox's analysis in Table 24 for CEA or CA15-3 but it did for CA19-9 where, following adjustment, patients with stable (S) as well as P marker changes for CA19-9 now had significant HRs compared with P alone in the unadjusted analysis (S was not found to be statistically different from AN, $p = .0.2$). None of the HRs for lymph node status reached statistical significance so we cannot say that the original nodal status of the tumour had any detectable influence upon the survival according to the Rustin category of response of any of the markers. These results could have occurred because lymph node status has little bearing on the reaction of tumour markers to chemotherapy or could reflect the change in nodal testing over time, the variation in nodes tested varies greatly between individuals possibly making this a less accurate measure of disease extent, there is also the possibility that by grouping nodal status into an ordinal scale as we did some small effects have not become apparent. Adjusting for Liver involvement alone and liver involvement and lymph node status together we concluded that liver involvement also had no significant effect upon any of the marker responses however had the same effect upon the CA19-9 S patients as lymph node status alone.

As has been shown by previous studies Marker changes can reflect the clinical course of advanced breast cancer (Table 6) some of those use a 20 or 25% definition of response and progression [65, 105, 112] some less than this [122] (Robertson et. al. Used both 10 and 20% changes as the basis for a scoring system which they related to response [110]), some use more complex measures such as “the inter-assay coefficient of variation” [123] and in other studies the measures of response evaluation are not clear [99]. When we compared how Rustin response corresponded with radiological response (Table 17) CA19-9 had the lowest sensitivity at chemotherapy one and CEA had the lowest at chemotherapy two, CA15-3 had the greatest sensitivity at both chemotherapy one and two (40.7% and 66.7% respectively) but this did not reach the sensitivity of the original Rustin response criteria in ovarian cancer which is quoted as having a sensitivity of 92%, the same paper also quotes a specificity of 72% [40] and we found CEA, CA15-3 and CA19-9 to have a greater specificity than this at chemotherapy one and two in patients with breast cancer. CA15-3 also had the greatest PPV and NPV at these time points.

Table 17 also shows the concordance of tumour marker progression and radiological progression, Rustin progression reflects radiological progression with a specificity of >90% for all three tumour markers following chemotherapy one and >93% following chemotherapy two which is comparable to the Rustin response categories in ovarian cancer which have been shown to have specificities of $\geq 98\%$ [3]. The sensitivities however, are not as comparable and the highest reached is 33.3% by CEA at chemotherapy two compared with 82%

[3] or 85.9% [5] for the original Rustin response criteria in epithelial ovarian cancer. These sensitivities are also lower than those quoted by Robertson et. al. (1991) however they combined markers in a panel with ESR and, unlike our results they also found no significant difference in survival between progressors and non-progressors [122]. Our sensitivities are also lower than those reported by Dixon et. al. (1993) who used the same marker panel but who also used a complex method scoring and of categorisation that would be difficult to use clinically [123]. Some other papers are difficult to compare as they divide patients into those with normal and abnormal baseline levels [65].

In order to get a better feel for the correlation between Rustin response and radiological staging results of the Spearman rank correlation can be seen in Table 24. The only marker where Rustin response showed a significant correlation with radiological stage following chemotherapy one was CA15-3 which compares favourably with the findings of Guadagni et. al. (2001) who (unlike us) found that both CEA and CA15-3 paralleled response to treatment but this was significantly more powerful in CA15-3 [204]. We found that the correlation for CA15-3 was not strong ($r_s = 0.28$) and the significance was borderline $p=0.048$, however a stronger correlation between these two factors was seen following the second chemotherapy regimen ($r_s = 0.44$, $p=0.02$) and at this point in time a moderate correlation was also seen between CA19-9 response and radiological staging ($r_s = 0.38$, $p=0.04$), a correlation was also seen between CEA category and radiological stage ($r_s = 0.34$) but this did not reach significance. Radiological stage was assessed as described in the Materials and methods section and as such may have been subject to error of

interpretation. It is also worth noting that there appears to be some difference in survival according to radiological response at chemotherapy one but no significant difference following chemotherapy two (log-rank test $p=0.003$ and 0.2 respectively) as can be seen Figure 21 possibly as a result of the reduced number of patients eligible for analysis at this point ($n=29$ compared with 61).

It is worth noting that like other studies we found one breast cancer (and one colorectal cancer) patient where there was a marker surge followed by a decline [118, 124] (other discrete kinetic patterns of tumour markers were also seen by Sonoo et al (1996)) this phenomenon is though to be attributable to tumour lysis and was discounted.

(4.3.2) Colorectal Cancer

As Table 10 shows (Introduction) there is already a body of evidence which indicates that CEA progression correlates with disease progression [157, 166, 173] and that CEA kinetics relate to differences in survival [180, 181, 185] but fewer papers include CA19-9 [157, 161, 166]. As is the case with breast cancer studies there appears to be little consistency in the measures of response and progression within these articles, some studies simply look at a rise [173] or fall [180] in the marker level whilst others used a 10% [161], 25% [158], 35% [166] or 36% [182] increase or decrease. Most papers are very guarded in their recommendation of the use of tumour markers to monitor response to treatment, for example Trillet-Lenoir et. al. (2004) state that to assess the response of metastatic CRC to chemotherapy, CEA alone or in combination with CA19-9 (in addition to CT) “should be used with caution in common

practice“ [161] and Hanke et. al. (2001) who state that “a CEA or CA19-9 rise is only conditionally appropriate for recording progressions“ [157].

Our results show that, as was the case in patients with breast cancer, for both chemotherapy one and two CA19-9 was the marker where the greatest number of patients were classified as having Always Normal (AN) tumour markers (Figure 30 and 33). For both CEA and CA19-9 survival was found to vary significantly across Rustin categories of tumour marker response following the first chemotherapy as the Kaplan-Meier graphs and results of the log-rank tests demonstrate in Figure 31 and Table 22B. This was not the case at the time of chemotherapy two where no significant difference in survival was detected (Figure 34 and Table 22B).

Results of the Cox's regression analysis (Table 24) for CEA suggested that an AN response may be better in terms of survival than R but this did not reach statistical significance (HR=0.61, $p=0.2$). The only HR which was significantly different from R was that for P (HR=2.51, $p<0.001$). The difference between AN and S was not found to be significant ($p=0.07$).

It was found that for CA19-9 AN markers appear to be better in terms of survival than R and this time results did reach significance (HR=0.53, $p=0.006$). This finding in patients with colorectal cancer is reinforced by Table 22B which appears to show that median survival in colorectal cancer patients was longer in those whose markers are AN as opposed to the breast cancer patients (Table 22A) which appear to have longer mean survival if there is tumour

marker response (R) following chemotherapy one. Despite this as Figure 35 A and B show because of the relatively small numbers and large variation within the samples these findings cannot be confirmed.

Cox's regression analysis for CA19-9 was the only instance following chemotherapy one where P was not found to have a significantly different HR from R (Table 24), this could be due to the long survival of a patient who had progression according to CA19-9 (this outlier can be seen in Figure 31C). This, combined with the fact that the Cox's proportional hazards model assumes constant ratio of hazard to outcome over time and this may not be strictly true for the Kaplan-Meier graph in Figure 31C, may have affected the results in this case. Radiological response follows the usual trend where the HR for survival for P was significantly different from the HR for both S and R (which were not significantly different from each other).

Following chemotherapy two no significantly different hazard ratios could be found for any of the responses in either of the tumour markers, P did however have a significantly worse HR for survival than S or R when radiological response was analysed)and this analysis was based on the data from fewer patients). As was seen with the results for chemotherapy one, and although significance was not reached, in both cases the HRs for AN were less than those of R. This indicates that in patients with colorectal cancer, unlike those with breast cancer, having a CEA or CA19-9 level which remains AN may confer a survival advantage, even over patients who have responsive tumour markers. This is an interesting finding particularly as it correlates with the

findings of Rubach et. al. (1997) whose results indicated (but did not confirm) that there may be a survival advantage in having marker levels within the normal range compared to markers which decrease [9] (Table 7, Introduction). It is also important to note that in our results although at chemotherapy one AN breast cancer patients had greater HRs than the HRs in responsive patients (non of these reached significance), following chemotherapy two median survivals were longer for AN patients than they were for responders in the case of CEA and CA19-9.

By looking at the results of Akaike's information criterion (AIC) in Table 24 we can see that at chemotherapy one the radiological response model has the best fit when we include AN in the tumour marker models (as with the breast cancer patients) and the markers are similar to each other in their level of fit. However when the AN group is removed CA19-9 response becomes a slightly better model than the radiology. At Chemotherapy two radiological response is the best fitting model both before and after removal of the AN groups. Although removal of the AN group in all cases (breast and colorectal cancer) improves the AIC score and therefore the 'fit' of the model it is not possible yet to predict which patients will fall into this category of response and so it can only be applied following the tumour marker response, this may be a useful tool retrospectively rather than clinically.

In both breast and colorectal cancer patients the 'goodness of fit' of the models (both radiological and tumour marker) as assessed by AIC is better following the second chemotherapy than following the first possibly because the

individuals included at chemotherapy two go on to have fewer further chemotherapy regimens, compared to those included at chemotherapy one, which can influence their survival, therefore the difference in survival which is seen could reflect the changes in disease state which occur at the time of this chemotherapy regimen more accurately.

When the results of the Cox's analysis of CA19-9 Rustin response following chemotherapy one was adjusted (Table 25) for age this was found to have no significant effect on the results, however when tumour stage was taken into account a significant result was seen for both CEA and CA19-9, this is not a total surprise as raised levels of both CEA [133, 135, 144, 145] and CA19-9 [135] have been shown to correlate with tumour stage in localised disease. A greater original tumour stage was associated with an increased HR from Dukes' A/B to Dukes' C - HR=1.64, $p=0.05$ and from Dukes' A/B to Dukes' D HR=3.22, $p=0.001$ for CEA, and Dukes' A/B to Dukes' C - HR=1.77, $p=0.03$ and from Dukes' A/B to Dukes' D HR=4.74, $p<0.001$ for CA19-9. Despite this the significance of the findings did not greatly affect the results of the Cox's analysis (the HR of AN remained significantly below R for CA19-9 and the HR of P remained significantly above R for CEA).

In the past studies have found that raised CEA levels correlate with the presence [145, 153, 166, 172, 173], or size [174], of liver metastases and some studies have also found a link between them and raised CA19-9 [155]. It is also known that CA19-9 can become raised in benign liver disorders such as cirrhosis and benign obstructive jaundice [35] as can CEA [43] as clearance of

this tumour marker occurs in the liver [49] (CA15-3 may also become raised in liver cirrhosis [68]), and we wanted to ensure that any significant correlation between tumour marker response and survival was not being influenced by the presence of liver metastases so our results were tested to see if liver involvement could have any effect upon the results. As was the case with the breast cancer patients, liver involvement had no significant effect on the results.

In contrast to liver involvement, increased Lymph node status did appear to be associated with significantly larger HRs in patients with 4-9 or >9 involved nodes, this test was only done in CEA at this time. This is an interesting finding as CEA and CA15-3 levels have been shown to correlate with lymph node status in patients with breast cancer [98] and yet as can be seen from Table 25 they had no bearing upon CEA or CA15-3 response in breast cancer patients but a link was seen in CEA marker response in colorectal cancer patients.

When comparing Rustin category and radiological staging, tumour marker response reflected radiological response as can be seen in Table 23 we found that our CEA response criteria had a lower sensitivity than that of Wang et. al. (2001) who also used a 50% reduction criteria and found that this had a sensitivity of 72% when detecting true imaging responses and a PPV of 53% [184] compared to 51% and 67.6% respectively in our results following chemotherapy one, this may have been because Wang et. al. required that the fall in CEA to be maintained for at least 4 weeks whereas we did not. We found CEA to be more sensitive at identifying radiological response than CA19-9 at both chemotherapy one and two but sensitivities for both were considerably

lower than the sensitivity of the original Rustin response criteria (92% [40]). The lowest specificity was 73.0% (CA19-9 at chemotherapy one) which was comparable to the 72% quoted for the original response criteria in ovarian cancer [40].

Progression as defined by our modified Rustin criteria correspond with radiological progression with a specificity of $\geq 93\%$ for both tumour markers following chemotherapy one and chemotherapy two which is comparable to the original Rustin criteria (specificities $\geq 98\%$ [3]). As with the breast cancer patients, the sensitivities however, are not as comparable. The highest reached is 36.8% by CEA at chemotherapy two compared with 82% [3] or 85.9% [5] for the original Rustin criteria in epithelial ovarian cancer.

At chemotherapy one and two survival varied significantly according to radiological response (Figure 36A and B) unlike marker response in breast cancer, CEA and CA19-9 Rustin response also showed a significant correlation with radiological staging as can be seen by the results of the Spearman rank correlation in Table 24. At both points in time this correlation was stronger for CEA ($r_s = 0.45$ and 0.43 respectively) than for CA19-9 ($r_s = 0.26$ and 0.35 respectively). This leaves a paradox within our results at the time of chemotherapy two because Figure 36B shows that survival differs significantly according to radiological staging but Figures 34A and B and Table 24 show that these differences do not reach significance according to Rustin categories and yet there is a significant correlation between radiological response and Rustin response categories at this time (Table 24). It is possible that with the inclusion

of more individuals a significant association between tumour marker response and survival may have been found because the HRs following chemotherapy one are similar to those following chemotherapy two but these calculation are based upon more than twice the number of individuals.

(4.4) Limitations of the Analysis

Many reviews point out flaws in the existing literature implicating poor study design leading to confounders and reliance on P values [205] in the lack of consistency in the evidence for the efficacy of tumour markers. In the updated ASCO guidelines published in 2006 looking at markers in gastrointestinal malignancies the authors state that “the literature is characterised by studies that included small patient numbers, studies that were retrospective, and studies that commonly performed multiple analyses until one revealed a statistically significant result” [188]. Some parts of our analysis were underpowered and all were retrospective, which can lead to best fit results, both of which could have lead to errors. Human error in copying figures (e.g. to and from data collection sheets) and in performing calculations could also have lead to some inaccuracies although checking aimed to minimise this wherever possible. In some areas such as Tables 16 and 21 there has also been multiple testing.

The analysis of tumour marker response and survival was vulnerable to inconsistencies from four main areas: firstly it was based heavily upon the first marker level, if this marker level happened to be an anomalous result then this

could have a profound effect upon the perceived tumour marker response. Secondly, marker levels were not measured at uniform intervals so could have all been clustered together in the first 3 weeks of analysis. This could result in the recorded tumour marker response differing from the true nature of the tumour marker change. Thirdly, according to our criteria of response and progression definition of progression does not require a confirmatory marker level if patient dies before a further marker level is taken whereas definition of response does require a confirmatory marker level. Fourthly, the change of marker assays may have introduced a further source of error, however by graphing and looking at each marker change individually (in Figures 16, 29 and 32) this has hopefully been minimised.

The definition and categorisation of radiological stage within this study was subjective and not comparable to the way radiological response is categorised in clinical practice. In this study if radiological disease progression was seen in one area and disease response was seen in another I categorised the disease status as stable. However, clinically the lesion which showed the worst response to treatment would be used and this case would be classed as progressive disease. The information regarding radiological response was purely included in this study to give an indication of the correlation between both radiological changes and tumour marker movement in response to chemotherapy treatment.

Within our methods there were slight deviations from the true Rustin response criteria which state that the for Rustin response to be confirmed the 50% fall in

tumour markers “must be confirmed and maintained for at least 28 days” [41] and of course the inclusion of the “progression from within the normal range” patients with truly Progressive patients

Although Data about factors such as involvement of the apical/highest node, lung, brain or bone involvement, ER/PR/Her2 status in breast cancer patients and position of the original tumour in colorectal cancer patients were collected they were not included in the Cox’s proportional hazards regression model analysis due to time pressures. Data on other factors such as performance status and disease free interval was not collected and so it is possible that there are confounding factors which have not been identified by this analysis. These potential confounding factors could also be a further source of error because the properties of the primary tumour were usually taken from the original histology and in some cases there had been neo-adjuvant therapy given prior to this which may have down staged the tumour.

(4.5) Further Work

It would be valuable to conduct a study prospectively to see if tumour marker kinetics still correspond with survival, the true extent of this correspondence and how tumour marker kinetics relate to clinical course as defined by RECIST criteria.

It is important to establish how soon after initiation of chemotherapy tumour marker kinetics can be shown to be indicative of response to treatment.

Depending upon the results of this analysis treatment could be modified

according to tumour marker kinetics to investigate if this can affect tumour marker movement in the first instance, and because it has been shown that kinetics do relate to survival [157], if this can affect survival as has been indicated by other studies using small samples e.g. Dixon et al (1993) who found that directing chemotherapy according to tumour marker response was “associated with a significant lengthening of remission duration and an improved quality of life and survival” [123]. Treatment in a tumour marker directed way such as this is currently being investigated in the OVO5 phase III clinical trial in recurrent ovarian cancer which aims to investigate if there is a difference in patient survival in patients whose chemotherapy treatment is initiated when clinically indicated compared to patients where chemotherapy is initiated in a tumour marker dependant manner CA125. This trial is currently closed to recruitment but still in the follow up phase, preliminary results have not yet been published [206].

Adding other possible confounding factors to the Cox’s proportional hazards regression model to see which factors do appear to be linked with survival would also provide valuable information however there may need to be more patients included in this analysis before it produces accurate results.

Crawford, Peace et al (2005) have already investigated the significance of CA125 nadir levels in ovarian cancer and found that patients whose CA125 reaches a lower nadir level survive longer on average than those patients whose CA125 does not drop as low [39]. It would be interesting to how the nadir of each of the markers studied here relate to survival.

(5) Conclusion

Many studies have small sample sizes [207] and the literature contains differences and inconsistencies in methodology which make it difficult to compare findings between studies and in some cases, makes their conclusions misleading. During this study I have further improved my analytical skills and have realised the importance of approaching the existing literature critically and thoroughly. Despite these issues there is an increasing body of evidence which suggests that although looking at “single and dichotomised” [111] tumour marker values in metastatic breast and colorectal cancer can provide some prognostic information, tumour marker kinetics may provide valuable information about disease response to therapy [118] which has also been associated with significant differences in survival [9]. Some work has been done in other disease sites, for example the work of Yamao et. al. (1999) in gastric cancer [208] and of course the work of Rustin and colleagues in ovarian cancer [1-5] and some criteria have been laid down by the EGTM who in their recent report into tumour markers in colorectal cancer conclude that “ the clinical use of existing markers should be optimised” [189] citing the use of CEA in monitoring treatment for advanced disease as an illustration of this [189]. Because tumour markers provide a relatively low cost way of providing clinical information this is particularly important in the current financial situation within the NHS.

By using modified Rustin response criteria in patients with advanced breast and colorectal cancer this study has identified a subset of patients, those with

progressive markers, where survival is significantly reduced compared with those with responsive markers. This is true for CEA, CA15-3 and CA19-9 at chemotherapy one and CEA, CA19-9 at chemotherapy two in breast cancer patients, and CEA at chemotherapy one in colorectal cancer patients. Using the same modified response criteria CEA and CA19-9 kinetics in colorectal cancer and CA15-3 kinetics in breast cancer have been found to correlate significantly with radiological response at chemotherapy one and two (CA19-9 response also correlates with radiological staging at chemotherapy two but not one in breast cancer). This may be particularly valuable clinically as it has been found that using RECIST criteria to interpret radiology results can delay the identification of disease progression when compared with WHO criteria [6, 7] and also with the advent of new therapies which may stabilise tumour growth initially rather than reduce it [8]. Despite this, radiological staging does appear to fit the statistical model better than any of the markers when patients with markers which are always normal are included.

Appendices

Appendix A – Breast Cancer Data Collection Sheet

Patient		Tumour									
Patient Number		Tumour Differentiation								Previous malignancy/ies and date/s of diagnosis/es	
Age at First Diagnosis		Tumour Grade (0 – IV)									
Trial		Tumour Stage (T N M)									
Death due to Breast Ca?		No of lymph nodes involved									
		Apical Node (free / Involved)									
		Oestrogen receptor status									
		Progesterone receptor status									
		Her 2 status									
Date	Tumour marker levels							Dates and types of Chemotherapy/Radiot herapy/Hormone therapy Regimen (first and last dates, number of cycles, and if adjuvant or palliative) and dates and types of surgical procedure	Test - Radiology/Histology	Histology Number	Results
	CEA	CEA*	CA15-3	CA15-3*	CA19-9	CA19-9*	CA125				
Continued ↓											

* = Tumour marker levels following change of assay

Appendix B – Colorectal Cancer Data Collection Sheet

Patient		Tumour									
Patient Number	Age at First Diagnosis	Tumour Differentiation		Tumour Stage		No of lymph nodes involved		Highest Node (free / Involved)		Previous malignancy/ies and date/s of diagnosis/es	
Trial	Death due to Colorectal Ca?	0 - IV		T N M							
Date	Tumour marker levels					Dates and types of Chemotherapy/Radiotherapy/Hormone therapy Regimen (first and last dates, number of cycles, and if adjuvant or palliative) and dates and types of surgical procedure	Test - Radiology/Histology	Histology No	Results		
	CEA	CEA*	CA19-9	CA19-9*	CA125						
Continued											



* = Tumour marker levels following change of assay

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