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Carbohydrate Antigen 549 in Metastatic Breast Cancer during Cytostatic Treatment and Follow-up

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This study was designed to investigate whether the serum tumour marker CA 549 gave early and reliable information about disease activity among metastatic breast cancer patients during cytostatic treatment and follow-up. 50 females with metastatic breast cancer were monitored clinically and with the tumour marker CA 549. Response evaluation was based upon clinical (World Health Organization) and elaborated CA 549 criteria, respectively. In 113 blindly and matched evaluations, concordance appeared in 73/113 and discordance in 40/113 evaluations. In 27, discordance concerned degree of response, in 2 clinical progression followed marker progression after the end of the study, and in 11 progressive disease was established by clinical investigation alone. CA 549 response excluded clinical progression in bone or viscera and reversed. Clinical progression within 2 months in viscera and bone was predicted among 91% by marker progression. Clinical progression was excluded among 93% without marker progression. In conclusion, monitoring of metastatic breast cancer patients could include CA 549 if standardised criteria for marker evaluation are used.
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INTRODUCTION

THE MAIN issue with tumour marker monitoring of metastatic cancer is to provide early and reliable information about disease activity. Early information about the response could contribute to the treatment being continued despite serious complications or adverse effects, and in patients progressing, an inefficient therapy could be stopped and an alternative treatment instituted.

Several biochemical compounds have been tested as potential tumour markers in breast cancer. None have, however, proved reliable alone to define clearly whether a patient was responding or progressing [1]. Recently, carbohydrate antigen 549 (CA 549), a breast-cancer-associated glycoprotein, was characterised and a sensitive immunological assay developed for serum measurements [2]. The difference between normal, benign and malignant breast diseases was not qualitative but quantitative, as higher serum concentrations were observed more frequently in patients with breast cancer compared with normal or benign conditions and other malignancies [2–6] (Table 1). Clinical investigations, as yet sparse, concurrently revealed a low sensitivity of the CA 549 test for screening purposes [3–6]. In patients with metastatic disease, however, concordance between clinical disease activity and CA 549 concentration was found in the few cases examined [3, 5, 7].

The purpose of this study was to investigate whether conventional clinical evaluation or serum CA 549 levels supplied first information about changed disease activity and to determine the predictive value of CA 549.

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PATIENTS AND METHODS

Patients' characteristics, treatments, and clinical response evaluation

67 consecutive patients were admitted to the study, all with histologically proven advanced progressive breast cancer and measurable or evaluable disease. The median age was 50 years, range 28–69. 50 patients had a performance status below 3, no evidence of renal dysfunction, and no prior chemotherapy for advanced disease. They received a first-line therapy in a randomised study comparing epirubicin 70 mg/m^2 day 1 and 8 every 4 weeks with epirubicin 60 mg/m^2 day 1 and 8 plus cisplatin 100 mg/m^2 day 1 every 4 weeks. Treatment with cisplatin was discontinued after 6 cycles whereas epirubicin was continued until disease progression or until a maximum cumulative dose of 1000 mg/m^2. 17 patients received epirubicin alone due to performance status 4, renal dysfunction, or refused treatment with cisplatin. Patients with complete (CR) or partial response (PR) were followed without further treatment until progressive disease (PD). Patients with no change (NC) or PD received second-line hormonal or cytostatic treatment.

Clinical response criteria were defined according to WHO [8]. CR was defined as disappearance for at least 4 weeks of all evidence of disease. In patients with bone metastases, complete disappearance of all lesions on X-ray was required. PR required a 50% decrease in measurable/evaluable lesions persisting for a minimum of 4 weeks, no appearance of new lesions, and no individual lesion increasing by 25% or more in size. NC designated patients where a 50% decrease in tumour size could not be established or where a 25% increase in the size of one or more measurable or evaluable lesions could not be demonstrated. PD required a 25% increase in measurable lesions or development of new lesions. Patients with early death (before 4 weeks) were registered as having PD.

Clinical examination included history, physical examination, blood tests (haemoglobin, leucocytes, platelets, ionised serum

Table 1. Frequency of elevated (> 10 kU/l) CA 549 concentrations among healthy controls, patients with benign breast disease, early breast cancer, advanced breast cancer and other malignancies as reported by references [2–6]

Reference	Healthy controls	Benign breast disease	Early breast cancer	Advanced breast cancer	Ovarian cancer	Prostate cancer	Lung cancer	Colon cancer
[2]	3/257 (1%)	3/79 (4%)	1/30 (3%)	40/80 (50%)	—	5/29 (17%)	4/19 (21%)	2/40 (5%)
[3]	4/100 (4%)	0/69 (0%)	10/88 (11%)	83/94 (88%)	30/60 (50%)	12/30 (40%)	13/40 (32%)	7/41 (18%)
[4]	7/70 (10%)	0/16 (0%)	—	56/123 (46%)	10/26 (39%)	—	4/22 (18%)	4/42 (10%)
[5]	5/100 (5%)	1/69 (1%)	10/88 (11%)	83/94 (88%)	30/60 (50%)	12/30 (40%)	13/40 (33%)	7/41 (17%)
[6]	1/100 (1%)	0/31 (0%)	6/48 (13%)	38/46 (83%)	—	—	—	—

calcium, serum bilirubin, alkaline phosphatase, aspartate aminotransferase and lactate dehydrogenase), ultrasonic scanning of the liver, chest X-rays, radiographic bone survey, bone scintigraphy, and in a few patients computed tomography, depending on metastatic site.

CA 549 assay

Serum specimens were sampled (median 18/patient, range 1–37), and frozen at −85°C in a serum bank until assayed in duplicate by HybriBREScan IRMA CA 549 supplied by Hybritech Europe SA, Liège, Belgium. The assay uses the BC4N 154 as the capture antibody covalently linked to polystyrene beads and BC4E 549 labelled with ^{125}I as the tracer. Briefly, the CA 549 in the serum or calibrators (20 µl), are incubated with 300 µl of assay buffer and the antibody-coated beads for 2 h (with continuous shake-up on a rotator to bind the CA 549 to the immobilised capture antibody). After repeated washing, 200 µl of tracer antibody is added and incubated for 2 h at room temperature. After additional washing and aspiration the radioactivity bound to the beads is counted. 6 calibrators, 0, 5, 10, 25, 50, and 75 kU/l are used to construct a calibration curve [9].

Calculation of significant changes in CA 549 concentrations

It is well established in clinical chemical practice that a change in 2 serial results obtained from specimens from any individual is due to analytical variation as well as deterioration or amelioration of the patient's condition. For a change to be statistically significant at $P \leq 0.05$ the difference must exceed $1.96 \sqrt{2} \times$ the total analytical variation (CV%) [10, 11] (1.96 = Student's t). The precision profile (Fig. 1) of the total analytical variation was based upon the within-assay precision calculated from deviations between duplicates cumulated from all the assay runs (assay standards as well as controls, and patient samples were used for cumulation) and the between-assay precision calculated from 3 controls included in each assay run: (i) mean 52.7 kU/l, S.D. 4.1, n 24, (ii) mean 12.7 kU/l, S.D. 0.9, n 24, and (iii) mean 6.0 kU/l, S.D. 0.5, n 22. According to common laboratory practice an acceptable performance for an IRMA assay implied CV% < 10 [12]. The precision profile of the total analytical variation (Fig. 1) revealed CV% well below 10% in most of the measuring range, 1–100 kU/l (1 kU/l = minimal detectable concentration corresponding to response of zero calibrator + 3 S.D.; 100 kU/l = upper range of the standard curve). CV% increased rapidly at concentrations below 10 kU/l and this value was selected as cut-off, leaving lower marker concentrations non-evaluable. As most measurements were between 10 and 80 kU/l, a pooled CV% of 7.8% was used to calculate differences between two concentrations. If the difference exceeded $1.96 \sqrt{2} \times 7.8\% = 22\%$, it was considered significant at the 5% level.

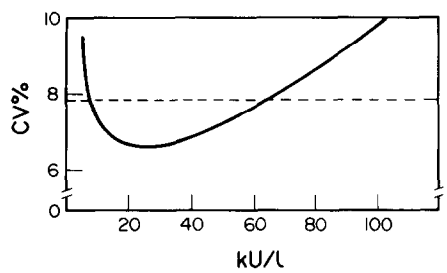

Fig. 1. The total analytical precision (CV%) for the CA 549 assay at different marker concentrations (kU/l). As most concentrations were measured between 10 and 85 kU/l, 7.8% was chosen as a pooled CV (– – –) for calculating significant differences between concentrations.

CA 549 response criteria

Evaluation of CA 549 data could not be based on a significant fall or rise of its concentrations alone, as single spikes and fluctuating concentrations occurred without relation to infections, blood transfusions or analytical imprecision. Consequently the following arbitrary definitions were chosen. Spikes were defined as having a duration < 6 weeks and an increment < 66%. Rising concentrations were confirmed during two time intervals. An increment of > 66% entailed relapse regardless of interval between measurements.

The magnitude and duration of significant changes were taken into account in the guidelines for marker evaluation as follows. CA 549 complete response (CA 549 CR): decrement > 22% and concentrations below cut-off ≥ 6 weeks; CA 549 partial response (CA 549 PR): decrement > 22% and observation period ≥ 3 weeks; CA 549 no change (CA 549 NC): marker concentrations fluctuated and fulfilled neither response nor progression criteria; CA 549 spike: 22% < increment < 66% and observation period ≤ 6 weeks; CA 549 progression (CA 549 PD): 22% < increment < 66% and observation period ≥ 6 weeks or increment > 66% and observation period ≥ 3 weeks; CA 549 early death: elevated pretreatment concentration and death within ≤ 4 weeks.

Matching clinical and marker evaluations, calculation of concordance, lead time, discordance, and predictive values for CA 549

The patients were monitored for a median 358 days (range 3–698) and both clinical and CA 549 evaluations were performed approximately every 3 weeks during therapy and every 6 weeks during control periods. Evaluations were performed blindly and matched.

In case of concordance, lead time was calculated. Positive lead

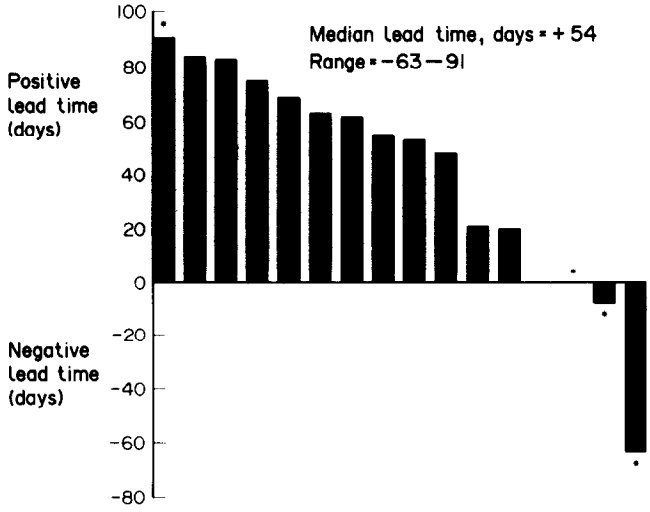

Fig. 2. Lead time (days) in 16 of 113 concordant evaluations with CA 549 and clinical response. Each bar represents 1 patient.* Clinical evaluation was based on response in lymph node metastases.

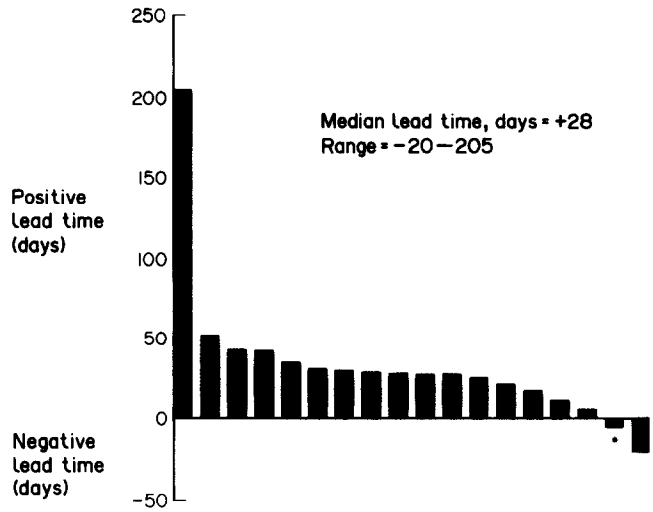

Fig. 3. Lead time (days) in 18 of 113 concordant evaluations with CA 549 and clinical progression. Each bar represents 1 patient.* Clinical evaluation based on progression in skin metastases.

time was defined as the number of days marker information preceded clinical information. When clinical information preceded marker information, lead time was negative.

A model was developed for assessing (i) the ability of CA 549 to predict and exclude clinical response in bone and viscera: TP = positive lead time regarding response, FN = negative lead time regarding response, FP = marker response without clinical response, TN = neither marker nor clinical response; (ii) the ability of CA 549 to predict and exclude clinical PD in bone and viscera, using different cut points for acceptable positive lead time: TP = acceptable positive lead time regarding progression, FN = negative lead time regarding progression, FP = limits for acceptable positive lead time regarding progression exceeded, TN = neither marker nor clinical progression. TP and FP denote a true and false positive result, respectively, and TN and FN a true and false negative result, respectively. PVpos and PVneg denote predictive value of a positive and negative result, respectively.

Statistics

Confidence intervals for frequencies were calculated with MEDSTAT [13].

RESULTS

Because of high imprecision (CV > 10%) at concentrations below 10 kU/l, marker profiles from 17 patients were regarded as non-evaluable. 50 were evaluable: 47 with pretreatment CA 549 concentrations above 10 kU/l and 3 with low pretreatment concentrations but rising to above 10 kU/l. 113 matchings between clinical and marker evaluations were performed, there was concordance in 73/113 (65%, 95% confidence limits 55–73%), discordance in 40/113 (35%, 95% confidence limits 27–45%).

Among the concordant matchings, lead time was not calculable for 39, in 9 matchings due to missing blood samples, in 8 to lacking clinical investigations, in 5 to early deaths, and in 17 to no change in previous evaluations (1 clinical and marker CR in steady state, 12 clinical and marker NC, 4 continuous clinical and marker PD). Lead time was calculable for 34 matchings with concordance. Among these, marker information preceded clinical information in 30 matchings but was delayed in 4 (Figs 2 and 3). The remaining 40 matchings were discordant (Table 2); in 27 there was discordance with regard to degree of response, in 2 matchings clinical evaluation showed PR but CA 549 PD; however, both clinical PR evaluations were actually PR in a steady state. In 11 matchings PD was established by clinical investigation alone.

CA 549 substantiated clinical information in 98 of the 113 evaluations (87%, 95% confidence limits 79–92%), namely 39 with concordance but non-calculable lead time, 30 with positive lead time, 27 with agreement about no PD, and 2 discordant evaluations where, however, clinical PD followed CA 549 PD after the end of the study (lead time 41 and 291 days).

Of special interest were 15 of the 113 evaluations where CA 549 information was delayed. These comprised 4 matchings with negative lead time (Figs 2 and 3), and 11 where clinical PD appeared without marker PD (Table 2). For 11 of these matchings clinical investigation showed changes in skin or lymph node metastases and for 4 changes in bone or viscera. When change in disease status occurred in skin or lymph nodes only, 14 of 15 matchings (93%) gave a negative or no lead time.

Table 2. Distribution of discordant evaluations in 40 of 113 matchings between CA 549 and clinical evaluation

Clinical evaluation		Marker evaluation			
		No change	Partial response	Complete response	Progression
No change	18		15	3	
Partial response	9	3		4	2*
Complete response	2	2†			
Progression	11	9‡	2§		

* Clinical partial response was in a steady state in both evaluations.

† Clinical complete response was based on disappearance of lymph node metastases in both evaluations.

‡ 7/9 evaluations with clinical progression were based on new skin metastases.

§ 1 clinical progression was based on new skin metastases (corresponding marker partial response was in a steady state) and 1 marker evaluation was based on partial response in a steady state.

When skin and lymph node metastases were excluded from the calculation, the sensitivity of marker response (CA 549 CR or CA 549 PR) for clinical response (CR or PR) in bone or viscera was 85% (95% confidence limits 62–97%, 17 TP, 3 FN). Specificity of no marker response (CA 549 NC or CA 549 PD) for no clinical response (NC or PD) was 68% (95% confidence limits 54–80%, 38 TN, 18 FP). Among the patients with marker response 49% (95% confidence limits 31–66%, 17 TP, 18 FP) showed a clinical response in bone or viscera (PVpos), whereas lack of clinical response in bone or viscera was identified in 93% of patients (95% confidence limits 80–98%, 38 TN, 3 FN) without marker response (PVneg). Marker response excluded clinical PD in bone or viscera but not clinical NC. Conversely clinical response in bone or viscera excluded CA 549 PD but not CA 549 NC.

When skin and lymph node metastases were excluded from the calculation, positive lead time for PD congregated in three main groups: (i) lead time 1 month ($\leq$ 31 days, n = 15, range 0–31 days), (ii) lead time between 1 and 2 months ($>$ 31 days and $\leq$ 60 days, n = 5, range 35–52 days), and (iii) lead time more than 2 months ($\geq$ 60 days, n = 2, range 205–291 days). The ability of CA 549 to signal changed disease activity in bone or viscera was calculated using 31 days, 52 days and 291 days as arbitrarily set cut-off points for acceptable positive lead time (Table 3). Obviously, specificity and PVpos was 100% if positive lead time was always regarded as a true positive result. If acceptable lead time, however, was restricted to 1 month, percentages diminished with relatively wide confidence intervals, but if acceptable lead time was extended to 2 months, percentages increased and the confidence intervals narrowed. CA 549 correctly identified 83% (95% confidence limits 63–95%, 20 TP, 4 FN) of the patients with clinical PD in bone or viscera within approximately 2 months and 96% (95% confidence limits 87–99%, 50 TN, 2 FP) without clinical PD. According to the 95% confidence limits for PVpos (71–99%) at least 71% will experience clinical PD in bone or viscera within 2 months following CA 549 PD (20 TP, 2 FP). Clinical PD was excluded with a high and narrow 95% confidence interval for PVneg (82–98%) among patients without CA 549 PD (50 TN, 4 FN). If PD identified by physical examination (skin and lymph nodes) was added to PD identified by CA 549 (bone, viscera), 90% of clinical PD was disclosed (95% confidence limits 76–97%, 35 TP, 4 FN). Furthermore, 95% of the patients with PD in skin and/or lymph nodes or CA 549 PD were identified at examination or within approximately 2 months (95% confidence limits 82–99%, 35 TP, 2 FP).

DISCUSSION

A new approach for investigating a tumour marker was implemented. This implied a homogeneous patient population, well-defined intervals for marker sampling, estimation of the quality of the tumour marker assay, independent criteria for marker and clinical response evaluation, matching of blinded evaluations, and calculation of the clinical applicability of the tumour marker to response and progression in terms of predictive values supplied with probability levels for confidence limits.

The purpose of the present study was to determine the value of the tumour marker CA 549 in monitoring metastatic breast cancer patients during cytostatic treatment and follow-up. When skin and lymph nodes were excluded from the calculation, no patient experienced clinical progression in bone or viscera while developing marker response (CA 549 complete or partial response) or conversely developed marker progression while responding clinically (completely or partially). Among the patients with CA 549 progression, 91% developed clinical progression in bone or viscera within 2 months; 93% of the patients without CA 549 progression had no clinical progression in bone or viscera.

The total analytical variation supplied the basis for quantitating differences between CA 549 concentrations. At clinical partial response, a significant decrement (22%) of CA 549 level during 1 time interval (3 weeks) proved a reliable signal for changed disease activity. With CA 549 progression, either larger increments (66%) or longer intervals (6 weeks) were necessary because spikes and fluctuating CA 549 concentrations caused false interpretations of marker progression. Spikes and fluctuating concentrations have also been observed using other markers and in other malignancies [14–22]. The biological background appears unknown, although tumour cell death and release of intracellular substance to the circulation accounted for some cases [18, 22]. Two different patterns of rise of CA 549 level at progression were in accordance with a fast and a slow rise pattern during carcinoembryonic antigen monitoring of metastatic colon cancer during progression [23]. Pretreatment concentrations below 10 kU/l do not imply that CA 549 monitoring should not proceed, because 3 patients with multiple metastases at diagnosis, prior to clinical progression, became CA 549 producers. Combining different markers in a surveillance programme might further enhance the percentage of patients eligible for monitoring [24]. 1 breast-cancer-associated marker included in a tumour marker panel appeared sufficient. Supplementing CA 549 with CA 15.3 measurements did not increase the frequency of elevated pretreatment concentrations. The percentage of patients eligible for monitoring was enhanced only if other types of markers, i.e. carcinoembryonic antigen or tissue polypeptide antigen, were combined with CA 549 or CA 15.3. One of the next steps in our investigation will be to analyse whether concordant evaluations during monitoring will be increased by combining different types of markers.

The CA 549 rose late in 15 of the 113 evaluations primarily when disease changed in skin and lymph nodes (11 of 15 evaluations). Furthermore, among all changes in tumour burden occurring in skin or lymph nodes (15 evaluations) a positive lead time appeared in only 1 evaluation. The delay in information from CA 549 in the other evaluations probably was due to the skin and lymph nodes being easily accessible for physical examination.

CA 549 substantiated or preceded clinical information in the rest of the evaluations: 39 evaluations with concordance but non-calculable lead time, and 59 evaluations with either concordance and positive lead time, or discordance but agreement about no progression, or discordant evaluations where, however, clinical progression followed marker progression after the end of the study. In 55/59 evaluations disease changed in bone or viscera (in 4/59 in lymph nodes, 1 with positive lead time, 3 with no lead time). Especially metastases to the liver were associated with elevated CA 549 concentrations (data not presented) and therefore suitable for marker monitoring. Early marker information at liver metastases is explained by the easy access of the marker to the circulation. Delayed clinical information is explained by difficulties to monitor metastases to the liver with ultrasonic scanning or by traditional blood tests. If unanimous about no progression, discordant evaluations, however, supplied clinically useful information. Probably several mechanisms might account for the discordance (Table 2). With CA 549 no change and clinical complete and partial

Table 3. Ability of CA 549 to identify clinical progression/no progression (CR, PR, NC) or to predict and exclude clinical progression within different time limits in patients with metastatic breast cancer

	Cut-off point 31 days		Cut-off point 52 days		Cut-off point 291 days	
	TP: lead time ≤ 31 days	15	TP: lead time ≤ 60 days	20	TP: positive lead time	22
	FN: negative lead time	4	FN: negative lead time	4	FN: negative lead time	4
	FP: lead time > 31 days	7	FP: lead time > 60 day	2	FP: marker progression	0
	TN: no progression	50	TN: no progression	50	TN: no progression	50
Sensitivity	79% [54–94%]		83% [63–95%]		85% (65–96%]	
Specificity	88% [76–95%]		96% [87–99%]		100% [93–100%]*	
PVpos	68% [45–86%]		91% [71–99%]		100% [85–100%]*	
PVneg	93% [82–98%]		93% [82–98%]		93% [82–98%]	

[ ] 95% confidence limits, [ ]* 97.5% confidence limits.
Excluded were patients with progression in skin and lymph nodes only.
TP: true positives; FN: false negatives; FP: false positives; TN: true negatives.

response, undetected metastases might produce CA 549. Lower detection limit of CA 549 may explain the discordance between CA 549 partial response and clinical no change. With CA 549 complete response and clinical no change and partial response, either scar tissue or emerging cell clones not producing CA 549 might constitute a reasonable explanation for the discordance. Two evaluations were discordant because clinical progression followed CA 549 progression after the end of the study. Lower detection limit of CA 549 may explain these discordances too.

To find out whether CA 549 measurements provide early information about response after starting treatment was a major objective of this study. If response was regarded strictly as either complete or partial clinical response or complete or partial CA 549 response, only approximately 50% of the patients with CA 549 response showed clinical response. This approach, therefore, was of no clinical value. If clinical no change and CA 549 no change were included in the response group, as progression had been deferred by therapy, no patient experienced clinical progression in bone or viscera while developing marker response, or conversely developed CA 549 progression while responding clinically. This information, however, could be of clinical value, documenting that a therapy was effective and could be continued in spite of adverse effects.

Whether CA 549 measurements can provide information about incipient clinical progression was a second important question. A long lead time added no further information, as 10–16 months of response following combination therapy was expected [25, 26], eventually concordance between clinical and marker evaluation therefore would always prevail. Probabilities for developing clinical progression within a short time interval (approximately 2 months) following CA 549 progression, provided accurate quantitative information not supplied by other investigative procedures (PVpos 91%, 95% confidence limits 71–99%) (Table 3). Conversely, clinical progression was excluded with high accuracy if clinical examination revealed no (new) lymph node or skin involvement and CA 549 did not fulfil progression criteria (PVneg 93%, 95% confidence limits 82–98%). Clinical progression was disclosed with higher accuracy if the diagnostic strategy for monitoring was based upon both physical examination and CA 549 measurements. Thus, 95% of the patients showed progression either at examination or within approximately 2 months (95% confidence limits 82–99%). Also from a cost–benefit point of view the possibility of biochemical monitoring as an alternative/supplement to other investigative procedures appears tempting.

In conclusion, using elaborated evaluation criteria, the tumour marker CA 549 substantiated clinical information and provided a potentially useful approach to monitoring metastatic breast cancer patients. For monitoring patients with skin/lymph node metastases, however, physical examination gave more information. CA 549 together with physical examination either identified or predicted clinical progression, and excluded present clinical progression with high accuracy. CA 549 measurements and physical examination therefore may constitute a base for monitoring metastatic breast cancer patients during cytostatic treatment and follow up.

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Phase II Study of 5-Fluorouracil plus Leucovorin and Interferon alpha 2_b in Advanced Colorectal Cancer

A. Sobrero, M.T. Nobile, A. Guglielmi, A. Mori, C. Aschele, E. Bolli, L. Tixi, L. Gallo, G.C. Parodi, P. Bruzzi, F. Brema and R. Rosso

15 untreated patients with advanced measurable colorectal cancer along with other 29 patients in progression after failing first line chemotherapy with fluoropyrimidines received 5-fluorouracil (5FU) 500 mg/m² given as a weekly bolus at mid-infusion of leucovorin (LV), 500 mg/m² administered intravenously over 2 h and interferon alpha 2_b (IFN) 3×10^6 U given intramuscularly every other day. All patients had their previous chemotherapy at least 4 weeks prior to 5FU-LV-IFN. 5 patients discontinued the three drug regimen due to toxicity (intense weakness, fever and influenza-like symptoms in 4 patients; diarrhoea in 1 patient) however no grade IV toxicity was observed. IFN administration was reduced to twice/weekly in 5 patients due to influenza-like symptoms. 1 complete response and 5 partial responses were observed (13.6% response rate); the complete response was obtained in a patient resistant to 5FU: the response rate was only twice as much in untreated patients (3/15 patients, 20%) compared with that in patients previously treated with fluoropyrimidines (3/29 patients, 10.3%). Therefore, modulation of 5FU with LV plus IFN at the doses and schedules employed in this study may rarely overcome clinical resistance to the fluoropyrimidine and the addition of IFN does not appear to enhance the activity of 5FU plus LV.
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INTRODUCTION

THE LACK of active agents against colorectal cancer has long drawn the attention to the possibility of enhancing the efficacy of 5-fluorouracil (5FU) that remains the only marginally active drug available against this disease. Continuous infusion appears more active than the standard 5 days a month schedule, although efficacy was comparable for the 2 administration schedules in a recent large phase III study [1]. Several randomised comparisons demonstrated that biochemical modulation with either high dose leucovorin (LV) [2–7] or methotrexate [8] produces higher response rates than those obtained with 5FU alone. In addition, the combination of the fluoropyrimidine with immunomodulatory agents such as levamisol [9, 10] and interferon [11] are particularly interesting. The former combination has produced