European Journal of Surgical Oncology 2000; **26**: 747–750
doi:10.1053/ejso.2000.0997, available online at http://www.idealibrary.com on IDEAL®

Clinical outcome and bromodeoxyuridine-derived proliferation indices in 75 invasive breast carcinomas

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Introduction: *In vivo* labelling of human breast tumours with bromodeoxyuridine (BrdUrd) and analysis by flow cytometry (FCM) allows the labelling index (LI), S phase duration (t_s) and the potential doubling time (t_{pot}) of the tumour to be estimated.
Methods: The data for a series of tumour specimens from 75 patients with invasive breast carcinoma were reported in 1991, correlated with their lymph-node status, tumour size and grade.
Results and Conclusions: This study reports the follow-up data over 10 years in respect of time to recurrence and death from the disease. There were no significant correlations between proliferation data and outcome measures. No adverse events were identified which could be attributed to the use of the halogenated pyrimidine label *in vivo*.

Key words: **breast cancer; bromodeoxyuridine; recurrence; outcome.**

Introduction

The factors determining the biological aggressiveness of invasive breast cancer are not fully understood. Lymph-node status, tumour size, stage and grade, and receptor status provide useful additional information, but cannot predict the outcome in individual cases. The contribution of cell proliferation to clinical outcome has been discussed in previous reviews.[1,2] Although it often appears to be of prognostic significance, it has not found widespread clinical utility. Many such studies provide a static index of the proportion of dividing cells, or labelling index (LI), but they do not give information on dynamic indices *in vivo* such as the S phase duration (t_s), or an estimate of tumour potential doubling time (t_{pot}).

Flow cytometry (FCM) and *in vivo* pulse labelling with BrdUrd allows the DNA index, the S phase fraction (SPF), the total and aneuploid labelling indices, and two-time dependent indices, t_s and t_{pot}, to be calculated from single biopsy samples. Our original study[3] was the first to report such dynamic indices in breast carcinoma *in vivo*. Stanton and colleagues subsequently reported similar results using this technique.[4] The correlation of such data, including dynamic indices, with clinical outcome in breast carcinoma has not been previously reported.

This study reports the cell kinetics of a selected cohort of symptomatic breast carcinomas following *in vivo* pulse labelling with BrdUrd; their lymph-node status, tumour size and grade, and their correlates with clinical outcomes, including time to recurrence and death.[3,5]

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Patients, materials and methods

Between 1989 and 1991, 75 patients (age range 39–80 years) with breast cancer consented to receive a single intravenous dose of 250 mg bromodeoxyuridine (BrdUrd), a thymidine analogue which labels DNA in the S phase of the cycle of proliferating cells, between 1.75 and 10 hours prior to surgery.[3,5] Patients underwent simple mastectomy with axillary clearance ($n=59$), wide local excision (WLE) with axillary clearance ($n=15$) or WLE alone ($n=1$). Twelve patients were pre- or peri-menopausal, and 57 were post-menopausal. None of the cohort received pre-operative adjuvant therapy. Patients were given adjuvant therapy post-operatively according to standard protocols.

Tumour samples harvested at the time of surgical excision were subjected to multiparameter flow cytometric analysis of DNA and BrdUrd uptake. The technical details and findings have previously been reported.[3] Where multiple samples were studied from any one tumour, the mean values for the flow cytometric data for each parameter were used in the multivariate analyses.

Clinical records were reviewed for all cases, noting time to recurrence and time and cause of death where appropriate.

0748–7983/00/080747+04 $35.00/0

Table 1. Aggregate data for tumour size, grade, node status, ploidy status and proliferation parameters

		Number of patients with data available from FCM analysis ($n=69$ unless stated otherwise)	Number of patients from whole cohort ($n=75$)
Tumour size	T1	23	25
	T2	36	40
	T3	10	10
Tumour grade	G1	9	9
	G2	19	28
	G3	29	34
Lymph node status	Positive	38	40
	Negative	30	34
	Unknown	1	1
Ploidy ($n=58$)	Diploid	29	
	Aneuploid	29	
Median	Li (%)	4.2 ($n=69$)	
	t_s (h)	8.7 ($n=51$)	
	t_{pot} (days)	8.2 ($n=51$)	

Table 2. Status of the cohort to date

Status	Alive/dead at (months) and cause of death			
	Alive	Died (breast cancer)	Died (other causes)	Total
Recurrence				
Recurred	7	24	1	32
No recurrence	31	0	8	39
Total	38	24	9	71

Statistics

Kaplan–Meier plots were used to demonstrate recurrence/survival over the period of the study. The period of follow-up ranged from 6 to 134 months.

The effect on recurrence/survival of prognostic variables was assessed using Cox proportional hazards regression model. Each prognostic variable was analysed independently. The effect of each variable was measured by the change minus twice the logarithm of the maximized likelihood. The additional effect of adding the cell proliferation indices independently to a model controlled for tumour size, grade and lymph-node status was also investigated using Cox proportional hazards regression model.

Results

Six primary tumours (8.0%) failed to yield usable data on flow cytometric analysis. Of the 69 tumours that could be analysed, 38 patients were node-positive and 30 were node-negative. The node status of one tumour was not known. There were 23 T1, 36 T2 and 10 T3 tumours.

The aggregate data for ploidy status, proliferation parameters, node status and tumour size and grade are recorded in Table 1. The follow-up status was known for 71 patients. These details are given in Table 2. The Kaplan–Meier curve for time to recurrence is displayed in Fig. 1.

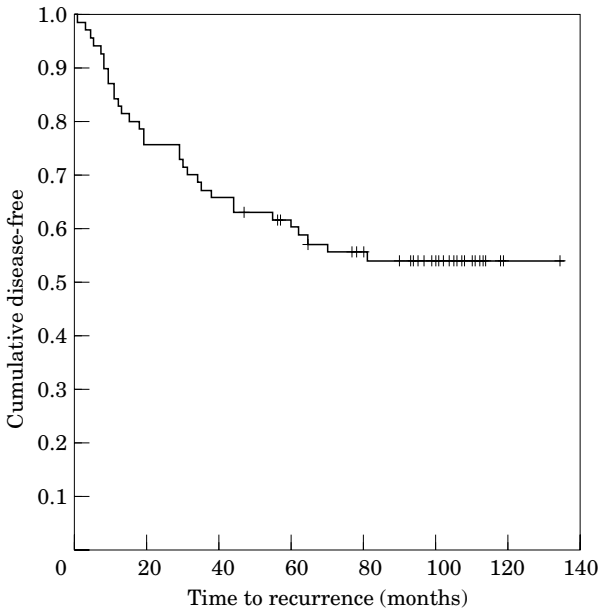

Fig. 1. Kaplan–Meier estimates of time to recurrence of 71 breast cancer patients.

No untoward features were found on clinical review or

Table 3. Cox proportional hazards regression model to determine significant independent predictors of mortality and recurrence from breast cancer

Explanatory variable		*n*	*P*-value	Hazard ratio	95% CI
Significant (10% level)					
Tumour size (cm)	Death	71	0.028	1.29	1.04–1.60
	Recurrence	71	0.006	1.35	1.11–1.64
Non-significant (10% level)					
Labelling index (LI)	Death	65	0.432	0.96	0.85–1.07
	Recurrence	65	0.616	1.03	0.92–1.16
Potential doubling time (t_{pot})	Death	49	0.455	0.98	0.94–1.03
	Recurrence	49	0.407	0.98	0.92–1.04
S phase duration (t_s)	Death	49	0.766	1.01	0.93–1.10
	Recurrence	49	0.360	1.04	0.95–1.14

in the pattern of results to suggest any short- or long-term toxicity (or benefit) from the subclinical dose of the hypothetically genotoxic substituted pyrimidine used for tumour labelling.

The size of tumour was found to be a significant independent predictor of recurrence and death from breast cancer (Table 3), but tumour grade and lymph-node status were not. None of the cell proliferation indices were independently significant predictors of recurrence or death from breast cancer, in models that controlled for tumour size, grade and lymph-node status. The models looking at the additional effects of S phase duration and aneuploid LI failed to compute because of the low number of patients available for analysis.

Discussion

This is a small study of patients presenting through the symptomatic breast service. There was a selection bias for tumours over 1.5 cm in diameter for administrative reasons. For practical considerations the sampling time ranged from 1.75 to 10 h. There is some evidence from *in vitro* studies that this might affect the estimate of t_s,[6] but the contribution of this effect is difficult to assess *in vivo*, and it has not affected the overall consistency of the results. The series is not representative of the spectrum of DCIS and invasive tumours, nor were numbers of cases chosen to attain statistical significance in survival studies. The study was undertaken to assess the utility of the FCM/BrdUrd technique *in vivo*, and the quality and spectrum of data so obtained.

The limitations of the technique in respect of the data obtained, sampling variation and the difficulties of disaggregating breast tumours for flow cytometric analysis have been reviewed in detail elsewhere.[1,7]

The number of evaluable patients was small and highly selected, which may account for the lack of correlation between node status and outcome measures. The lack of correlation in this study between proliferation parameters, including dynamic indices, and survival is consistent with the findings in a larger cohort of colorectal cancers studied in a similar fashion with *in vivo* pulse labelling.[8] We know of no other studies that correlate clinical outcome with *in vivo* dynamic indices of cellular proliferation in breast tumours. Studies of static indices of cellular proliferation and outcome variously report a correlation with clinical outcome.[2,9,10]

It is apparent that apoptosis also plays a significant role in the balance between cell proliferation and cell loss.[11,12] This can also be inferred from our data, as the observed growth rates of tumours are considerably less than the potential doubling times we obtained. Measures of cell proliferation rates in the primary tumour are thus only a single component of the biological aggressiveness of breast carcinoma, which will also be strongly influenced by metastatic potential and invasive characteristics.

The relatively small number of cases, and the incomplete data for S phase duration and aneuploid index, oblige that the study findings should be treated with caution. We nevertheless submit that this study has been a valuable pilot for a technique which uniquely allows the quantitative study of time vectored cell proliferation parameters of clinical tumours *in vivo* in a safe and simple way. It represents a generational advance over earlier techniques of tumour proliferation analysis using tritiated thymidine labelling.

Acknowledgements

We thank Dr G. D. Wilson of The Gray Laboratory, Northwood, Middlesex, for technical help in the primary study, Professor I. Taylor and Mr P. C. Weaver for permission to study patients under their care, and the ladies who kindly agreed to participate in the study. D. A. R. was funded between 1989 and 1991 by the Cancer Research Campaign and by the Wessex Regional Health Authority.

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Accepted for publication 19 July 2000

World Wide Web Site Reviews

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—the content of the site, including quality, reliability, accuracy, scope and depth;
—design and aesthetics, layout, interactivity, presentation, appeal, graphics and use of media;
—disclosure of authors, sponsors and developers;
—currency of information and maintenance of the site;
—the authority of the source;
—the ease of use, including facility to download, navigability and functionality;
—quality of links to other sites;
—attribution and documentation;
—appropriateness for intended audience;
—contact addresses and feedback mechanisms;
—user support;
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