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ASSESSMENT OF HUMAN TUMOUR PROLIFERATION USING BROMODEOXYURIDINE—CURRENT STATUS

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Abstract

The study of human tumour proliferation has been facilitated by the development of monoclonal antibodies recognizing halogenated pyrimidines. Flow cytometry can be used to detect the incorporation of bromodeoxyuridine (BUdR) into DNA simultaneously with the measurement of total DNA content. We have studied in excess of 600 tumours using in vivo administration of BUdR. The cell kinetic information generated from this approach is more complete than can be obtained from in vitro incubation with DNA precursors such as tritiated thymidine (^{3}HTdR) or by single parameter DNA analysis. The duration of S-phase (T_s) can be estimated in addition to the labelling index (LI). From these two parameters, the potential doubling time (T_{pot}) can be calculated. Our data show a wide variation in T_s as well as LI, making both these parameters important variants in determining overall proliferation. Although there is tremendous variation in T_{pot} between tumours of the same and different types, the median values are surprisingly short at around 5 days. A close relationship exists between the presence of DNA aneuploidy and the proliferation parameters. The clinical relevance of T_{pot} is currently being assessed independently in two trials of accelerated versus conventional fractionation.

Key words: Bromodeoxyuridine, proliferation, tumours, aneuploidy.

The rate at which human tumours proliferate has long been thought to be related to clinical outcome. In radiotherapy, proliferation is now recognised as one of the major factors contributing to the inability of conventional fractionation to cure some human tumours (1, 2). This has stimulated interest in the use of altered fractionation schedules designed to exploit differences in the response of early and late-responding tissues and tumours (3). The most obvious method to overcome the repopulation of surviving clonogenic tumour cells during a course of conventional radiotherapy would be to shorten the overall treatment time. Accelerated treatment would be of particular benefit to patients whose tumours are capable of rapid proliferation.

In order to identify patients who may benefit from an altered treatment schedule, it is necessary to be able to assess proliferation rapidly and quantitatively. The most commonly used techniques to measure proliferation have been in vitro incorporation of tritiated thymidine (^{3}HTdR) and autoradiography (4, 5) to calculate a labelling index (LI) or flow cytometry-derived S-phase fractions (SPF) from DNA profiles (6, 7). Both of these techniques have drawbacks; the former is laborious to quantify and not applicable in vivo, the latter is more rapid and applicable universally but is dependent upon computer models to extract the SPF data. Neither of these techniques measure a cell kinetic parameter in the strictest sense of the word; both LI and SPF measure only the number of cells in a particular phase of the cell cycle at a particular time. The most exciting development in the field of cell kinetics has been the development of monoclonal antibodies recognising halogenated pyrimidines incorporated into DNA (8) and of flow cytometric methods to simultaneously measure the uptake of bromodeoxyuridine (BUdR) or iododeoxyuridine (IUdR) and total DNA content (9). The use of a monoclonal antibody negated the need for BUdR to be radiolabelled as detection could be achieved by flow cytometry or immunohistochemistry. This, in conjunction with a technique devised at the Gray Laboratory by Begg et al. (10) to measure the potential doubling time (T_{pot}) from a single biopsy, has provided the opportunity to study in vivo cell kinetics. The in vivo administration of

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BUdR to patients generates potentially more biologically meaningful cell kinetic data, rapidly and quantitatively using procedures which are amenable to multicentre study and with the potential to be used predictively to determine treatment options.

This paper will review our experience with BUdR administration to patients and report on the preliminary data from clinical trials in which cell kinetics are being assessed using BUdR.

Material and Methods

Patient selection. A total of 380 patients with a variety of tumours from different sites have been studied at Mount Vernon Hospital. In addition, 200 patients with colorectal, gastric, urinogenital or breast cancer have been studied at St Mary's Hospital, Portsmouth, and Southampton General Hospital in collaboration with Mr. D. A. Rew. In accessible sites, the biopsy was carried out under local anaesthesia, otherwise the biopsy was removed at the time of staging under anaesthesia or at surgical resection. All patients gave their informed consent.

BUdR administration. In the initial studies, 500 mg of BUdR was dissolved in 200 ml of 0.9% saline immediately prior to intravenous infusion over 30 min. The majority of patients have received 200 mg of BUdR, administered as a single push in 20 ml of 0.9% saline without loss of sensitivity in detection of BUdR. Several sources of BUdR have been used in these studies. Initially, the preparation suitable for human use was obtained from the NCI Investigational Drugs Branch (Bethesda, Maryland, USA). Secondly, a commercial preparation was obtained from Takeda Chemical Industries Ltd. (Osaka, Japan). More recently, BUdR has been prepared at the Department of Pharmacy, University of Strathclyde, Glasgow. The biopsy or surgical resection was usually performed 4–8 h after injection of BUdR. No discernable toxicity has been observed clinically associated with the injection of BUdR. A portion of each biopsy or surgical resection was fixed in 70% ethanol for flow cytometry and an adjacent portion fixed in formal saline for histopathological examination and immunohistochemistry.

BUdR staining. The ethanol fixed tumours were dissected into fragments and nuclei extracted using 0.4 mg/ml pepsin (Sigma Chemical Co., Poole, England) in 0.1 mol/l HCl (pH 1.5) by incubation at 37°C for 30–45 min with constant agitation. After filtration through 35 μm nylon mesh, the nuclei were centrifuged at 2000 rpm and the pellet resuspended in 2.5 ml of 2 mol/l HCl for 12 min at room temperature to partially unwind DNA and allow access of the anti-BUdR monoclonal antibody. After washing twice in phosphate-buffered saline (PBS), a 1:25 dilution of a rat anti-BUdR monoclonal antibody (Sera Lab, Crawley, England) was added in 0.5 ml PBS containing 0.5% Tween-20 (Sigma Chemical Co., Poole, England) and 0.5% normal goat serum (NGS) (Sigma Chemical Co.) and incubated for 1 h at room temperature. After washing with PBS, the second antibody was added. This consisted of 1:25 dilution of a goat anti-rat 1 gG (whole molecule) FITC conjugate (Sigma Chemical Co.) in 0.5 ml of the PBS/Tween/NGS solution. After incubation for 30 min at room temperature, the suspensions were washed in PBS and resuspended in 2 ml of PBS containing 10 μg/ml of propidium iodide (PI) (Sigma Chemical Co.).

Flow cytometry. The specimens were analysed on an Ortho Systems 50-H Cytofluorograph with 2150 computer. Excitation was at 488 nm from a 5-Watt argon ion laser (Coherent, Cambridge, England) operating at 200 mW. Green fluorescence (FITC/BUdR) was collected between 510 and 560 nm and red fluorescence (PI/DNA) was collected above 620 nm. At least 10000 events were collected in list mode and cell doublets, etc. were excluded by gating on the red fluorescence peak versus area signal.

Data analysis. The calculations of LI, T_s and T_{pot} have been described in detail elsewhere (10, 11). Briefly, the LI was defined as the percentage of cells exhibiting significant BUdR uptake making a simple correction for those cells which had divided in the time period between injection and biopsy. This was achieved by setting a computer-generated region around all BUdR labelled nuclei and around those which had G1 DNA content. The number of events in the latter region was halved; this number was subtracted from the total BUdR labelled and the total number of events and the LI recalculated. The lower limit delineating nuclei with significant BUdR uptake was determined by staining nuclei not exposed to the monoclonal antibody. In diploid tumours, the LI could only be calculated for all cells, both normal and tumour, in the population. In aneuploid tumours, the LI could be calculated for both the total cell population and also the aneuploid cells only by setting regions according to DNA content.

The duration of S-phase was calculated according to the method of Begg et al. (10). The relative movement technique relies on making two assumptions. The first concerns the distribution of the bivariate cytogram obtained immediately after labelling with BUdR. The method assumes a uniform distribution throughout S-phase such that the mean DNA content of the population would give a value in mid-S. In order to quantify relative movement (RM), the mean DNA content of the G1 and the G2 populations was also calculated. The RM at time zero would be calculated by subtracting the mean DNA of G1 cells from that of the BUdR labelled population and dividing by the G1 DNA content subtracted from the G2. This would give a starting value of 0.5. The second assumption is that the progression of cells through S-phase is linear. Thus, the RM value of the cohort of BUdR-labelled cells progressing through S-phase (excluding those cells which divide and enter G1) will increase with time. The RM will eventually reach a value of 1.0 when the only BUdR labelled cells,

which have not divided, now reside in G2. Assuming linearity, these cells represent the cells which were in early S-phase at the time of injection. Thus, the increase in RM from 0.5 to 1.0 describes T_s. The T_s can then be calculated from a single observation; for minimum error, this should be made close to $0.5T_s$ for that particular tumour. In practice, a time delay of 4–8 h between injection and biopsy provides suitable BUdR/DNA profiles.

From these two parameters, the potential doubling time (T_{pot}) was calculated. This term is the shortest possible time a cell population can double its number, taking into account the presence of dividing and non-dividing cells but in the absence of cell loss (12). It is calculated from the formula:

$$T_{pot} = \lambda \frac{T_s}{LI}$$

where λ is a correction factor for the age distribution of the tumour population. λ can vary between 0.693 and 1.38, but a value of 0.8 has been assumed in our studies based on observations in experimental tumour systems.

Results

Human tumour staining profiles. Fig. 1 shows examples of BUdR/DNA staining profiles obtained from human tumours. Both tumours are examples of squamous cell carcinomas (SCC) from the head and neck. Fig. 1a and b shows the DNA profile and bivariate cytogram obtained from a biopsy of a diploid tumour from the retromolar area removed 5.6 h after the injection of BUdR. The DNA profile (Fig. 1a) shows the presence of one DNA stemline, the BUdR/DNA profile (Fig. 1b) clearly shows the redistribution of BUdR-labelled cells. One population has divided and resides in G1 at channel 30 whilst the BUdR-labelled cells, still progressing through S-phase, have increased their DNA and are skewed towards the G2 DNA content. The T_s, calculated from the latter population, was estimated to be 8.6 h and the corrected LI was 7.5%. The T_{pot} value, computed from T_s and LI, was estimated to be 3.8 days. The situation is often more complex in solid tumours due to the presence of abnormal DNA stemlines (Fig. 1c and d). In this biopsy, taken 6 h after the injection of BUdR, the DNA index was 1.85. It is clear from the BUdR/DNA profile (Fig. 1d) that the majority of proliferation was associated with the aneuploid cells although some proliferating cells were present in the diploid population indicated by the BUdR labelled cells which had divided at channel 16. In the aneuploid population, the redistribution of BUdR labelled cells around the cell cycle is again clear. Those cells which had divided reside in G1 at channel 33, whilst those cells still progressing through S-phase had increased their DNA and the resultant distribution was skewed towards G2. The LI, in this specimen, was 8.4% for the total cell population and 11.8% for the aneuploid cells alone. The T_s was calculated for the aneuploid cells and was estimated to be 9.9 h. These two parameters, for the aneuploid population, gave a short T_{pot} of only 2.8 days.

Proliferation parameters of human tumours. Fig. 2 summarises the data on LI, T_s and T_{pot} for six of the main groups of tumours we have studied. The bars represent the median value for each particular parameter for each group and the number to the right of the bar is the range for that particular parameter. It is evident that both LI and T_s are important variables in determining proliferation. There is a wide range for both of these parameters in each of the tumour groups. It has been shown many times in the past that the LI shows wide heterogeneity between tumours, but these studies are among the first to demonstrate that T_s is equally as variable. We have measured T_s values as short as 4.0 h to over 37 h. Interestingly, there was some relationship between T_s and the tissue from which the tumour originated. It would appear that T_s increases throughout the upper aerodigestive tract with tumours from the oral mucosa, having a shorter median T_s (9.9 h) than those derived from the oesophageal mucosa (12.4 h) or the bronchial mucosa (15.1 h). Tumours from the cervix tended to have a longer T_s (15.8 h), whilst melanomas had a short T_s (10.7), possibly reflecting proliferation in skin.

The variation in both LI and T_s was reflected in the values obtained for T_{pot}. A measure of the intraspecimen heterogeneity of T_{pot} can be deduced from the coefficient of variation (CV). The CVs obtained for head and neck, lung, oesophagus, cervix, melanoma and colorectal tumours were 101%, 167%, 156%, 61%, 82% and 68% respectively. It was evident that a slightly different order of proliferative activity exists when T_{pot} was considered in comparison with LI alone. This discrepancy is explained by the tumour type specific variation in T_s.

Proliferation parameters and DNA aneuploidy. It is becoming increasingly apparent from our studies that there is a clear relationship between DNA index and proliferation parameters. This relationship is demonstrated in Fig. 3 in which the T_s and LI of head and neck tumours have been plotted according to whether the tumour was diploid or aneuploid. The dotted lines represent median values of both T_s and LI for all the tumours. Ninety-four per cent of the tumours with the shorter than median T_s and lower than median LI were diploid, whereas only 18% of tumours with the longer T_s and higher LIs were diploid. This relationship appears to hold for all the major groups of tumours we have studied (Table). In each group (except melanoma), the median T_s is longer in tumours which are aneuploid by an average of 25% compared to diploid tumours. The LI, however, is also higher in aneuploid tumours. The increase in LI was of greater magnitude, ranging from 1.3 to 4.3 times the diploid LI. This difference is partly due to the inability of FCM to discriminate normal from tumour cells in diploid tumours whereas, in

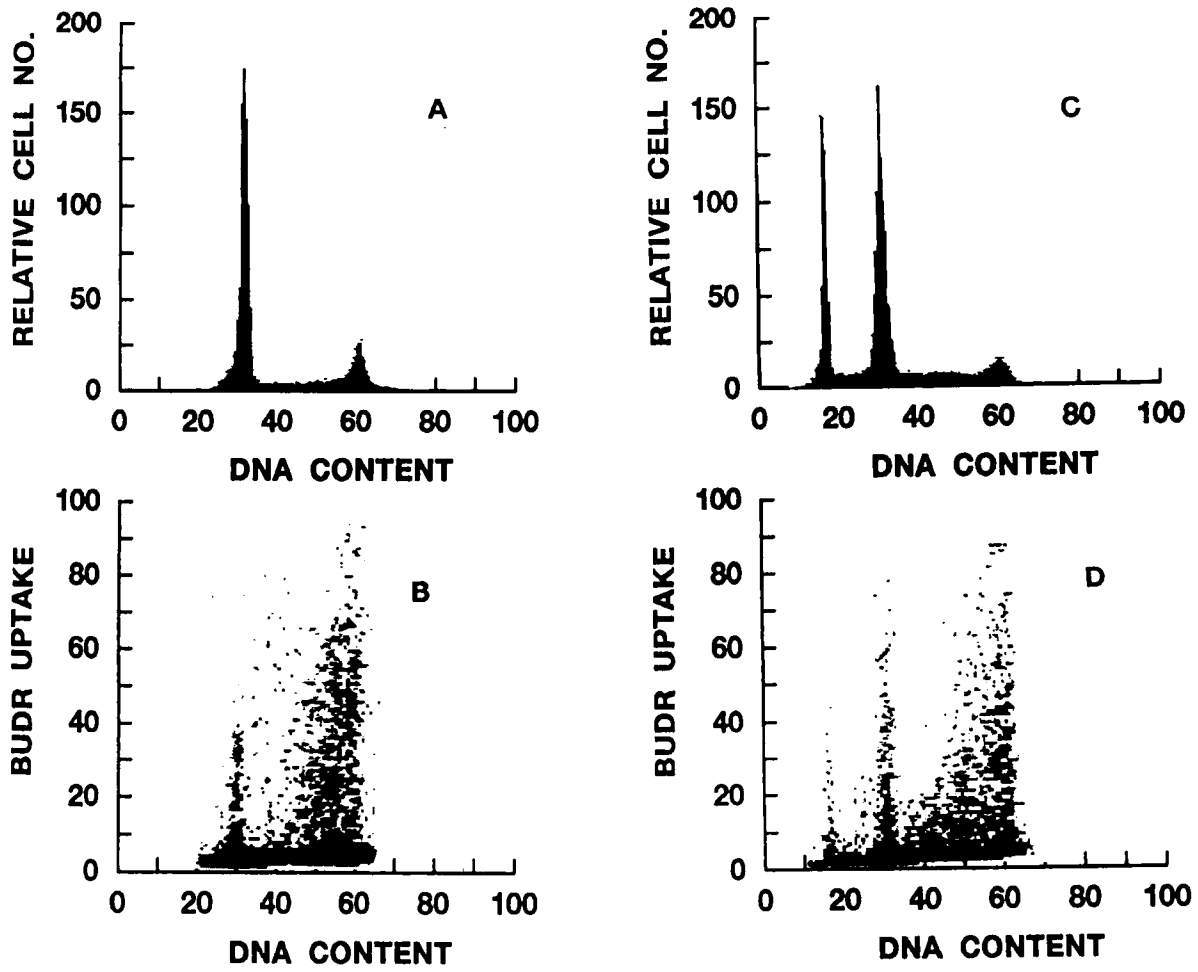

Fig. 1. Examples of BUdR/DNA staining profiles obtained from human tumours. a) DNA profile of a diploid squamous cell carcinoma from the retromolar region and b) the BUdR versus DNA cytogram showing redistribution of labelled cells 5.6 h after injection. c) The DNA profile of an aneuploid squamous cell carcinoma of the tonsil and d) the BUdR versus DNA cytogram obtained 6 h after injection of BUdR showing cell cycle redistribution of BUdR mainly associated with aneuploid cells.

aneuploid tumours, discrimination can be based on DNA content. Although these two parameters, LI and T_s, are inversely correlated in the computation of T_{pot}, the LI is dominant in determining the overall proliferation. Aneuploid tumours tend to have shorter T_{pots} than diploid tumours, due mainly to their high LI. The discrepancy in the FCM's ability to estimate LI efficiently in diploid tumours is not a problem when calculating T_s. In some of the tumour groups, particularly cervix, the T_s value, in the aneuploid tumours, increases with increasing DNA index. In addition, the LI increases with increasing T_s. This might suggest that DNA abnormalities may be important in determining the duration of S-phase and that T_s may be a dominant determinant of LI.

Proliferation and clinical outcome. The ultimate test of T_{pot}, or the parameters which determine it, is to prove prognostic or diagnostic significance. In the major groups of tumours we have studied, we have seen no relationship between proliferation and histopathological differentiation status or grading (13). In addition, there was no relationship with Dukes' classification in the colorectal adenocarcinomas (14). Proliferation has also been independent of site, age, sex and tumour size.

We have recently made a preliminary evaluation of patients who were treated with accelerated fractionation and whose pretreatment cell kinetics were assessed using BUdR. A more detailed report of this study has been submitted elsewhere (Lochrin et al., unpublished report). The patients studied were a cohort of the pilot study of continuous, hyperfractionated, accelerated radiation treatment (CHART). This treatment schedule has been described previously in detail (15) and consists of administering 3 fractions of 1.5 Gy per day, with a 6-h gap between fractions, for 12 continuous days. The patients described in Fig. 4 all had primary tumours from various sites in the head and neck region. This particular subset of head and neck tumours was more aggressive than the group as a whole as they were chosen for accessibility of

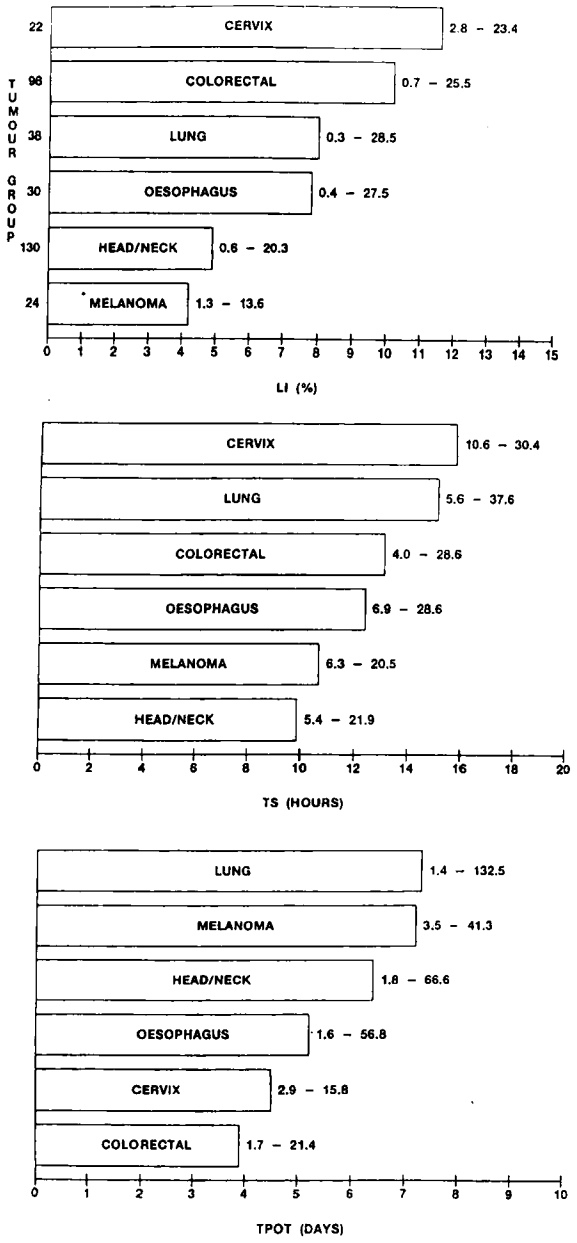

Fig. 2. Proliferation parameters of tumours from different sites. The bars represent median values for each parameter in each tumour group. The numbers to the right of each bar represent the range for that particular parameter. The numbers shown to the left of each bar in the upper panel are the number of tumours studied.

biopsy. This feature was reflected in the median values for LI, T_s and T_{pot}, which were 7.9%, 9.8 h and 3.9 days respectively. Fig. 4 presents the clinical outcome in terms of freedom from local recurrence for each proliferation parameter and histological grade. The open symbols represent successes, i.e. those patients who achieved complete local tumour clearance and remained free from local regrowth of tumour. This group represented 23 of the 38 patients (61%). The closed symbols denote failures, either

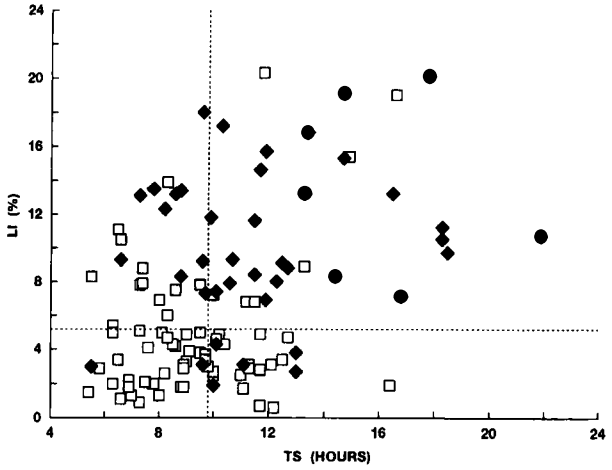

Fig. 3. The relationship between duration of S-phase and labelling index in head and neck cancer. The LI values are the total LI for diploid tumours and the aneuploid cell LI for aneuploid tumours. The dotted lines represent median values for both parameters for this particular group of patients. The open symbols represent diploid tumours, whilst the closed symbols are aneuploid tumours.

those patients who failed to achieve complete tumour clearance (26%) or those who achieved clearance but subsequently recurred in the treatment field (13%).

There was no significant influence of any of the parameters measured on local tumour control. Similar numbers of successes or failures were observed above or below the median value for each parameter (dotted lines in Fig. 4). In contrast to the cell kinetic data, there was a trend for the low-grade tumours to do better with CHART. In grades 1 and 2, 83% and 73% of tumours remained disease-free, whilst only 44% of grade 3 tumours were in this category. The trend does not reach statistical significance with the numbers available.

Discussion

The measurement of human tumour proliferation using BUdR administration and flow cytometry has proven to be a practical and feasible technique. With the development of the tissue preparation and staining procedures, small amounts of tissue (less than 100 mg) have been adequate to yield results, provided they contain viable tumour cells. This, and the requirement for no specialist procedures or facilities at the hospital where the biopsy was taken, has opened up possibilities for multicentre studies. In Britain, a multicentre randomised trial of CHART versus conventional fractionation in which cell kinetics are being assessed is under way. The success rate for obtaining results from specimens is in excess of 90%.

The data generated by the BUdR technique in vivo provides more information on proliferation than was possible with existing methods. The major advance is the

Table

The influence of DNA aneuploidy on cellular proliferation parameters

Tumour group	Aneuploidy %		Median values		
			LI	T_s	T_{pot}
Head/neck		Overall	4.9	9.9	6.4
	38	Diploid	3.9	8.9	8.0
		Aneuploid	9.3	11.5	4.2
Lung		Overall	8.0	15.1	7.3
	77	Diploid	2.2	12.9	17.0
		Aneuploid	9.5	15.8	4.7
Oesophagus		Overall	7.8	12.4	5.2
	79	Diploid	8.0	11.2	5.4
		Aneuploid	10.2	14.4	4.1
Cervix		Overall	11.6	15.8	4.5
	76	Diploid	6.1	13.8	6.9
		Aneuploid	16.9	16.9	4.4
Melanoma		Overall	4.2	10.7	7.2
	41	Diploid	2.7	10.9	9.4
		Aneuploid	6.6	10.4	5.0
Colorectal		Overall	9.0	13.1	3.9
	52	Diploid	8.5	11.1	5.4
		Aneuploid	12.0	15.0	3.5

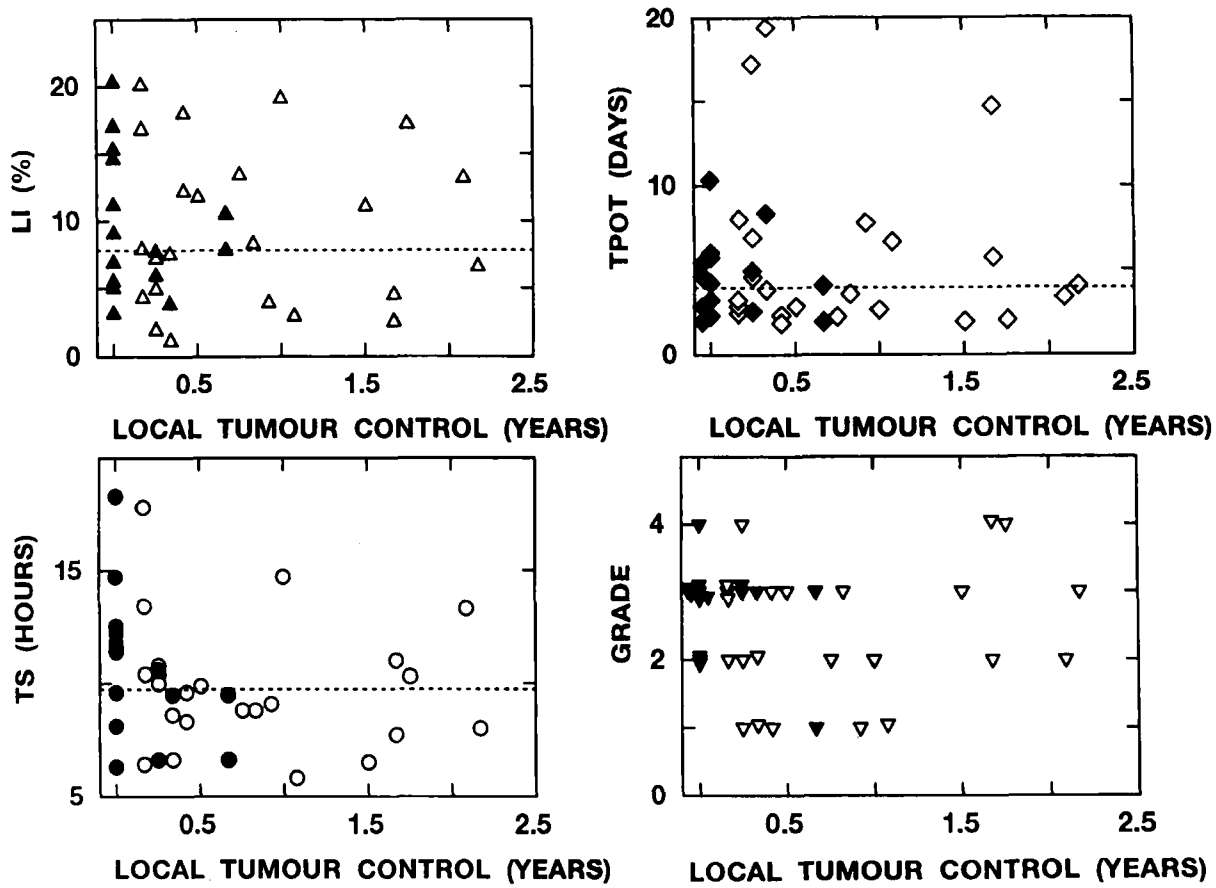

Fig. 4. The influence of proliferation on local tumour control of patients treated with CHART. Open symbols represent successes, i.e. those patients who achieved local tumour control and were free from local recurrence at the last time of observation. Filled symbols represent patients who failed to achieve local tumour control (those symbols at time zero) or those who achieved local tumour control but subsequently recurred within the treated site. The dotted lines represent median values for each proliferation parameter for this particular subset of head and neck tumours.

introduction of a time factor, the T_s, which enables tumour doubling times to be calculated. It remains to be established whether T_s or T_{pot} will be an improvement on LI as a prognostic parameter. The fact that T_s appears to be almost as variable as LI would suggest that it may be an important parameter. The differences in T_s observed between diploid and aneuploid tumours of the same type are of interest in understanding the relationship between proliferation and biological aggressiveness. It is generally thought that the primary alterations in chromosomes associated with the malignant phenotype are structural defects followed by secondary non-random changes that occur during tumour progression. These changes may confer proliferative advantage, partial suspension of growth control, intrinsic genetic instability and thus pave the way for the rapid genetic, phenotypic and biological diversification observed during progression. The duration of S-phase is under the genetic control of cell cycle proteins, whereas the LI (the number of cells in S-phase) will be determined by T_s relative to the cell cycle time and the growth fraction. Prolongation of T_s in aneuploid tumours appears to be associated with a large increase in LI. This results in T_{pot} values which are more rapid than those observed in diploid tumours. In studies of heterogeneity, it would appear that T_s is less variable in different sites from the same tumour than LI (unpublished observations). These findings infer that changes in T_s may be a characteristic of individual tumour progression. Large variations in T_s within a tumour may reflect a polyclonal origin of that tumour cell population.

Although treatment will undoubtedly perturb the proliferation of surviving tumour cells, it is our belief that the pretreatment T_{pot} may be the best indication for the potential for proliferation during treatment. Some support for this hypothesis has come from analysis of split-course radiotherapy schedules (16, 17). In these studies, it is possible to estimate the effective doubling time of the clonogenic cells by making assumptions about the loss of local control or the extra dose required to achieve the same local control compared to a continuous course of radiotherapy. Fowler (1) has calculated that, assuming a 10–15% increase in local control is equivalent to a 10% increase in total dose and that a log increase in dose represents 3.3 cell doublings, head and neck tumours have effective doubling times in the region of 3–6 days. These values are very similar to the estimates of T_{pot} made by the BUdR method and reinforce the hypothesis that repopulating tumour cells proliferate more rapidly due mainly to the decreasing influence of cell loss.

Human tumours certainly present a wide variation in their capacity to proliferate. All groups of tumours we have studied include between 25% and 60% with the potential to proliferate rapidly based on a cut-off value of 5 days. This represents a large population of tumours which may benefit from shortened overall treatment times in radiotherapy.

In our study of patients treated with CHART, there was no relationship of cell kinetic parameters with local tumour control. There have been several reports (5, 7) of the prognostic significance of LI or SPF in head and neck tumours and most have associated a high LI with an impaired survival. In our study, the median values of LI, T_s and T_{pot} were similar for successes or failures as was the distribution of DNA aneuploidy. Our results are compatible with preliminary observations made by Begg (personal communication) of patients treated in an EORTC trial of accelerated versus conventional radiotherapy and assessed using IUdR. Although the accelerated arm of the schedule is quite different (overall time 5 weeks), there appears to be no difference in local tumour control between fast and slow proliferating tumours. In the conventional arm, fast proliferation tumours show loss of local tumour control compared to slowly proliferating tumours and to tumours treated by acceleration.

Both CHART and the EORTC regimen would appear to overcome the problem of tumour cell repopulation so that management of both slow and fast tumours are similar. However, the numbers are few in both studies and the results should be regarded with some caution. The incorporation of cell kinetic measurements into controlled trials is a major breakthrough and represents an important advance. The prognostic and diagnostic future of BUdR measurements can be properly assessed within the next few years.

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REFERENCES

1. Fowler JF. Potential for increasing the differential response between tumours and normal tissues: Can proliferation rate be used? Int J Radiat Oncol Biol Phys 1986; 12: 641–5.
2. Withers HR, Taylor JMG, Maciejewski B. The hazard of accelerated tumor clonogen repopulation during radiotherapy. Acta Oncol 1988; 27: 131–46.
3. Thames HD, Peters LJ, Withers HG, Fletcher GH. Accelerated fractionation vs. hyperfractionation: Rationales for several treatments per day. Int J Radiat Oncol Biol Phys 1983; 9: 127–38.
4. Meyer JS, Friedman E, McCrate M, Bauer WC. Prediction of early course of breast carcinoma by thymidine labelling. Cancer 1983; 51: 1879–86.

5. Chauvel P, Courdi A, Gioanni J, Vallicioni J, Santini J, Demard F. The labelling index: a prognostic factor in head and neck carcinoma. Radiother Oncol 1989; 14: 231–7.
6. Hedley DW. Flow cytometry using paraffin-embedded tissue: five years on. Cytometry 1989; 10: 229–41.
7. Tubiana M, Courdi A. Cell proliferation kinetics in human solid tumours; relation to probability of metastatic dissemination and long term survival. Radiother Oncol 1989; 15: 1–18.
8. Gratzner HG. Monoclonal antibody to 5-bromo and 5-iododeoxyuridine: a new reagent for detection of DNA replication. Science 1982; 218: 474–5.
9. Dolbeare F, Gratzner HG, Pallavicini M, Gray JW. Flow cytometric measurement of total DNA content and incorporated bromodeoxyuridine. Proc Soc Natl Acad Sci 1983; 80: 5573–7.
10. Begg AC, McNally NJ, Shrieve DC, Karcher H. A method to measure the duration of DNA synthesis and the potential doubling time from a single sample. Cytometry 1985; 6: 620–6.
11. Wilson GD, McNally NJ, Dische S, et al. Measurement of cell kinetics in human tumours in vivo using bromodeoxyuridine incorporation and flow cytometry. Br J Cancer 1988; 58: 423–31.
12. Steel GG. Growth kinetics of tumours. Oxford: Clarendon Press, 1977.
13. Dische S, Saunders MI, Bennett MH, Wilson GD, McNally NJ. Cell proliferation and differentiation in squamous cancer. Radiother Oncol 1989; 15: 19–23.
14. Rew DA, Wilson GD Taylor I, Weaver PC. Proliferation characteristics of human colorectal carcinomas measured in vivo. Br J Surg 1991; 77: (in press).
15. Saunders MI, Dische S, Fowler JF, et al. Radiotherapy employing three fractions on each of twelve consecutive days. Acta Oncol 1988; 27: 163–7.
16. Amdur RJ, Parsons JT, Mendenhall WM, Million RR, Cassisi NJ. Split-course versus continuous-course irradiation in the post-operative setting for squamous cell carcinoma of the head and neck. Int J Radiat Oncol Biol Phys 1989; 17: 279–85.
17. Taylor JMG, Withers HR, Mendenhall WM. Dose-time considerations of head and neck squamous cell carcinomas treated with irradiation. Radiother Oncol 1990; 17: 95–102.