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Proliferation kinetics and prognosis in gastric cancer after resection

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Abstract

The influence of proliferation and proliferation kinetics on prognosis in gastric cancer after complete resection are controversial. In a prospective study we investigated the tumour specimens of 111 patients after resection of gastric cancer, who received 200 mg intravenous (i.v.) bromodeoxyuridine (BrdU) pre-operatively. The following biological parameters were analysed in the tumour tissue using flow-cytometry: DNA ploidy, proportion of S-phase cells, BrdU labelling index (LI), DNA synthesis time (T_s), potential tumour doubling time (T_{pot}), proliferating cell nuclear antigen (PCNA) and Ki-67 LI. The median follow-up time was 40 months (range 19–62 months). Besides the established pathohistological prognostic factors, univariate analysis revealed a prognostic influence on survival for BrdU LI, T_{pot} and the proportion of S-phase cells. By multivariate Cox analysis of the completely resected cases, only tumour stage and T_{pot} had a significant, independent influence on survival. By classification and regression trees (CART) analysis, resection status, tumour stage and T_{pot} defined risk groups with significantly different outcomes. A short T_{pot} was a predictor of better survival in stage I, II and IIIA tumours. Ploidy and the other investigated proliferation-related parameters failed to demonstrate any influence on prognosis after resection of gastric cancer.

Keywords: Gastric neoplasms; Proliferation; Cell kinetics; Bromodeoxyuridine; Ki-67; PCNA; Prognostic factors

1. Introduction

Increase in tumour size, invasion and metastasis are all dependent on the multiplication of cancer cells. Theoretically, the impact of proliferation and proliferation kinetics on tumour prognosis would seem to be obvious, i.e. a tumour which rapidly proliferates should have a poorer survival. However, it has been difficult to show that proliferation has any impact on clinical outcome [1]. The results of various studies investigating proliferation-related features such as percentage of S-phase cells in the cell cycle, or the expression of proliferation-related antigens such as Ki-67 or proliferation cell nuclear antigen (PCNA) are contradictory [2]. Even the determination of tumour proliferation kinetics using *in vivo* bromodeoxyuridine (BrdU) has not led to conclusive results [3]. Parameters of cell proliferation kinetics can be assessed using the BrdU/DNA assay with flow-cytometry after *in vivo* administration of the thymidine analogue BrdU. Following injection, BrdU is incorporated into DNA during the S-phase, and can be detected subsequently by a specific, monoclonal antibody [4]. The BrdU/DNA assay provides data for the calculation of the BrdU labelling index (LI), DNA ploidy and the distribution of cells in different phases of the cell cycle. Using the pulse labelling technique, the DNA synthesis time (T_s) and the potential tumour doubling time (T_{pot}) can be calculated [5]. T_{pot} is defined as the time that would be required for the number of tumour cells to double assuming no cell loss and is a relative measure of tumour growth [6].

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The assessment of biological prognostic factors (PF) is of clinical importance, especially for a disease with a poor prognosis such as gastric cancer. The aim of investigating PF is a more biological assessment of tumour behaviour in order to stratify patients according to their individual tumour biology so that they can be treated with different treatment protocols. Despite several articles, that have dealt with PF in gastric cancer, no biological factor has been used as a PF in the clinical setting so far. The reason for the sometimes confusing data on the prognostic influence of proliferation is the fact that these studies are often retrospective and combine incompletely- and completely-resected tumours. Prognostic factors must be assessed prospectively in completely-resected tumours only, following a standardised treatment protocol and a standardised pathohistological evaluation of the resected specimen.

The purpose of the present study was to investigate the influence of different proliferation-related parameters on prognosis after resection of gastric cancer. All patients were operated upon using a strictly standardised technique, and all of the specimens were evaluated histopathologically according to a protocol based on the requirements of the World Health Organization (WHO).

2. Patients and methods

2.1. Patients, surgery and follow-up

Between February 1994 and August 1997, 115 consecutive patients with biopsy-proven adenocarcinoma of the stomach who were scheduled for surgery were enrolled in this prospective, single centre study after written informed consent. The *in vivo* application of BrdU was approved by the Ethics Committee of the Medical Faculty of the Technische Universität München. None of the patients received neoadjuvant or adjuvant treatment. 5–7 hours before resection, the patient received an intravenous (i.v.) bolus of a 200 mg BrdU solution produced by the hospital pharmacy, which had been purchased as a powder (Sigma, Deisenhofen, Germany).

After meticulous preoperative staging, including endoluminal ultrasound and laparoscopy in locally advanced tumours to exclude peritoneal carcinomatosis, the tumour resection was performed according to a standardised protocol (total or subtotal gastrectomy with extended lymphadenectomy). The lymphadenectomy was always done in a extended fashion, i.e. the so-called 'D2 lymphadenectomy' according to the Rules of the Japanese Gastric Cancer Association, avoiding a left-pancreatic resection and splenectomy, if possible. The resection strategies of the department have recently been published elsewhere [7,8]. The histopathological evaluation of the resected specimen was also carried out according to a standardised protocol [9]. The depth of infiltration and the number of resected and involved lymph nodes were reported according to the TNM classification (International Union Against Cancer (UICC), 1997) [10]. The TNM categories and stages of the patients are listed in Table 1. The male to female ratio was 1.8:1, and the average age at the time of surgery was 66 years, with a range of 26–88 years.

Immediately after resection, a tissue sample of approximately 1 cm^3 was excised from the tumour margin. The specimen was divided in two parts, one for flow cytometry, the other for histopathological evaluation as a control. The samples for flow cytometry were mechanically disintegrated and mashed through a nylon net to produce a single cell suspension. By using a suspension obtained from a large amount of tumour tissue, the problem of tumour heterogeneity was minimised [11,12]. Corresponding normal gastric tissue was also investigated in all patients.

The patients enrolled were observed for further follow-up. 4 of the 115 patients died within the first month after resection due to postoperative complications and were excluded from analysis according to the protocol. Postoperative mortality was 3%. Of the remaining 111 patients, 69 (62%) died within the follow-up period, while for the surviving patients, observation was continued for a median follow-up time of 40 months (range: 19–62 months). The median survival time of all investigated patients was 24 months and the estimated 5-year survival rate was 33%. Median survival for patients after complete resection (UICC R0, $n=66$, 59%) was 47 months and survival after incomplete resection (UICC R1 or R2, $n=45$, 41%) was 8 months ($P<0.001$).

2.2. Staining

After fixation, cells were incubated for 10 min in a 0.5% pepsin solution after which the cell nuclei were

Table 1
Stage distribution of 111 analysed resected adenocarcinomas of the stomach (International Union Against Cancer (UICC) 1997)

Stage	TNM	*n* (%)
I	T1, N0, M0 T1, N1, M0 T2, N0, M0	15 (14)
II	T1, N2, M0 T2, N1, M0 T3, N0, M0	24 (22)
IIIA	T2, N2, M0 T3, N1, M0 T4, N0, M0	16 (14)
IIIB	T3, N2, M0	10 (9)
IV	T1-4, N3, M0 T1-4, N0–3, M1	46 (41)
		111 (100)

resuspended in 2 N HCl for 10 min. Samples were incubated for 30 min at room temperature (RT) in a 1:10 dilution of the primary monoclonal anti-BrdU antibody (Becton Dickinson, Heidelberg, Germany). This was followed by incubation with a fluorescein isothiocyanate (FITC)-conjugated anti-mouse antibody (Becton Dickinson, 30 min, RT, dilution 1:50). All steps were followed by repeated washings with Mg^{++}/Ca^{++}-free phosphate buffered solution (PBS) (Sigma). DNA was stained by a 25 μg/ml propidium iodide (PI) solution (Sigma) containing 1 pg/ml of RNAse A (Sigma) for 30 min at RT. Staining for Ki-67 and PCNA (both from Dako Diagnostics, Hamburg, Germany) was performed without pepsin treatment and protein denaturation. Isotype controls were used in every experiment.

Flow cytometric analysis was performed with a FACScan flow cytometer (Becton Dickinson). The data of approximately 10 000–15 000 cells per sample were collected and stored in a list mode. The data were analysed using a CONSORT 30 data analysis system and FACScan Research software (both from Becton Dickinson). Duration of the S-phase (T_s) was determined from a Relative Movement (RM) analysis as described in Ref. [5]. The analysis was done by placing computer drawn windows around the different populations of the cell cycle in the dual parameter BrdU/DNA histogram, assuming that the RM was 0.5 at time 0. The mean red fluorescence of the labelled cells (F_L) relative to the means of G1 (F_{G1}) and G2 (F_{G2}) was determined first. Then, T_s could be calculated since the time between the injection of BrdU and resection (t) was known. The equations used for calculating T_s were: (1) $RM=(F_L-F_{G1})/F_{G2}-F_{G1}$ and (2) $T_s=0.5\times t/RM-0.5$. T_{pot} was calculated as the ratio $(T_s/LI)\times\lambda$, assuming that the age factor (p) = 1 in rapidly proliferating tissue [6].

2.3. Statistics

The correlation among tumour-biological factors were described by Spearman's rank-correlation coefficient together with the corresponding value of the Chi^2 test. To study the association with established pathohistological prognostic factors, levels were determined for the various groups and compared using the Mann–Whitney U-test. The difference between the overall levels in gastric cancer tissue and benign gastric tissue was analysed using the Wilcoxon test. S-phase, BrdU LI, T_S and T_{pot} were then coded as binary variables by employing log-rank statistics to determine optimal cut-off points for discrimination of low- and high-risk patients.

Survival rates were calculated according to Kaplan–Meier for various groups and compared by applying the log rank test [13]. The prognostic impact of all analysed factors on survival time after resection was assessed by the Cox's Proportional Hazard Model using the Statistical Package for the Social Sciences (SPSS) software package (SPSS Inc. Chicago, IL, USA) which provides estimates of the relative risk together with 95% confidence intervals (CIs) [14]. For comparison of prognostic strength of the various factors, covariate selection was carried out by multivariate regression by a stepwise-forward procedure which chooses factors with the highest additional prognostic information. In addition, classification and regression trees (CART) were constructed, analysing the impact of every factor in subgroups and resulting in a hierarchical system for classification of patients into different risk-groups [15]. CART was performed by the statistical programming environment software S-Plus (MathSoft, 1998). All survival analyses were carried out using binary coded variables and all test decisions were performed at a significance level of $P=0.05$.

3. Results

The DNA-index could be determined in 99% ($n=110$) of the tumour specimens, 47.3% were diploid and 52.7% ($n=58$) aneuploid. Evaluation of the BrdU LI and the calculation of T_s and T_{pot} from the DNA histogram were possible in 61% ($n=68$) of the patients, 46 of them were completely resected. The difference of the medians between normal and tumour tissues were significant for all biological factors investigated (Table 2), however the range of values showed a large overlap. If the samples of a single patient were compared, the values of tumour versus normal tissue differed significantly. There was no association between the investigated tumour biological variables and established pathohistological prognostic features such as TNM/R category, tumour stage, lymph node ratio or grading. Significant differences were found only between diploid and aneuploid tumours. Aneuploid tumours had a higher percentage of S-phase cells compared with

Table 2
Medians and ranges of the biological variables analysed in gastric adenocarcinomas and normal gastric tissues

Parameter	Tumour tissues Median (range)	Normal tissues Median (range)
S-Phase (%)	19.2 (7.1–50.9)	11.3 (4.2–34.0)
BrdU LI (%)	13.0 (3.0–33.9)	6.0 (0.5–26.5)
T_s (h)	11.9 (3.9–50.0)	9.3 (5.0–70.0)
T_{pot} (day)	4.1 (1.3–18.4)	6.5 (1.6–80.8)
PCNA LI (%)	64.1 (9.5–93.5)	54.7 (8.6–92.8)
Ki-67 LI (%)	41.0 (4.2–86.8)	31.5 (1.0–92.1)

BrdU LI, bromodeoxyuridine labelling index; T_s, duration of S phase; T_{pot}, potential tumour doubling time; PCNA LI, proliferating cell nuclear antigen labelling index; Ki-67 LI, Ki-67 labelling index.

Table 3
Univariate analysis of prognostic factors for all 111 patients; only significant factors are listed

Variable	5-year survival (%)	RR (95% CI)	*P* value
R0	56		
R1	0	8.5 (4.9–14.6)	<0.001
Stage I, II, IIIA	73		
Stage IIIB, IV	10	7.7 (3.4–14.4)	<0.001
pT 1, 2	82		
pT 3, 4	20	7.3 (2.6–20.0)	<0.001
pN0	71		
pN+	16	5.5 (2.6–11.5)	<0.001
LN ratio ⩽0.2	55		
LN ratio >0.2	0	5.1 (3.0–8.4)	<0.001
M0	47		
M1	0	4.4 (2.7–7.3)	<0.001
T_{pot} ⩽3.8 days	53		
T_{pot} >3.8 days	29	1.9 (1.0–3.8)	0.039
S-Phase ⩽14.4%	48		
S-Phase >14.4%	28	1.8 (1.0–3.3)	0.046
BrdU LI ⩽14.6%	33		
BrdU LI >14.6%	56	0.5 (0.3–1.0)	0.044

R, resection status; LN-ratio, ratio of invaded to removed lymph nodes; BrdU LI, Bromodeoxyuridine labelling index; T_{pot}, potential tumour doubling time; RR, relative risk; 95% CI, 95% confidence interval.

diploid tumours (22.7% versus 14.3%, median values) and a longer T_s (13.7 h versus 9.15 h). BrdU LI did not correlate with the S-phase fraction of the cell cycle calculated from the DNA-histogram. The grade of the tumours had no influence on BrdU LI, T_{pot} or survival. However, only 12% of the specimens (13/111) were Grade 2, the remaining specimens were all Grade 3 lesions.

Log-rank statistics found a significant discrimination between low- and high-risk patients only for S-phase, BrdU LI and T_{pot}. The following cut-off values were determined: S-phase: 14.4%, BrdU LI 14.6% and T_{pot} 3.8 days.

Besides the histopathological prognostic factors, univariate Cox regression analysis demonstrated that the

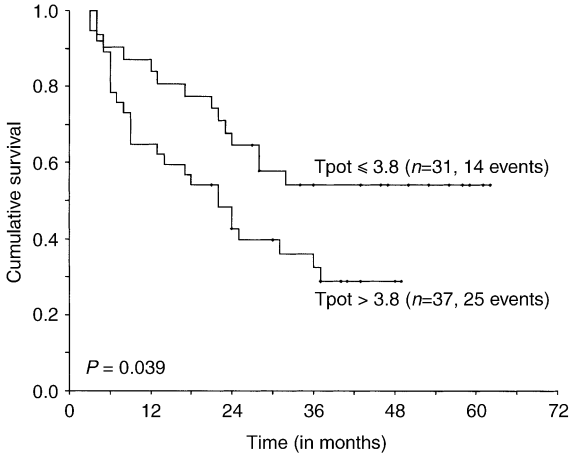


Fig. 1. Influence of potential tumour doubling time (T_{pot}) on survival.

biological parameters T_{pot}, proportion of S-phase cells in the cell cycle and BrdU LI had a significant influence on survival (Table 3). In particular, tumours with a longer T_{pot} (>3.8 days) had a worse survival (Fig. 1). DNA ploidy or the expression of the proliferation-associated antigens PCNA and Ki-67 did not show a statistically significant influence in the analysis. In stepwise multivariate analysis of the whole investigated group, only resection status and tumour stage were found to have an independent prognostic value ($P<0.001$).

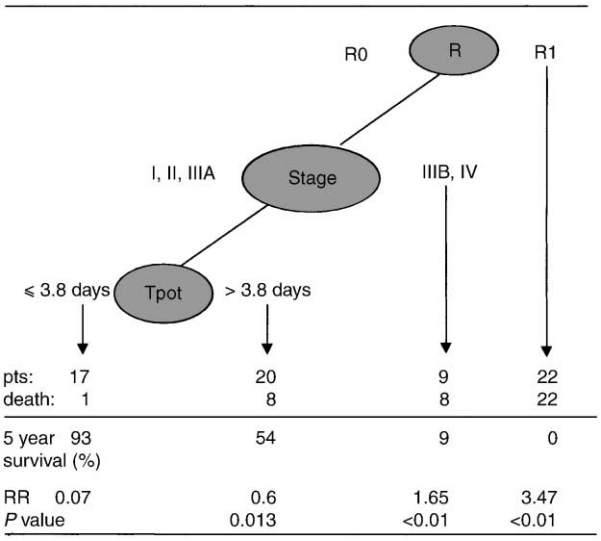
In order to identify new prognostic factors on a standardised basis, the R0 resected patients ($n=66$) were analysed separately [2]. In this subgroup, tumour stage and T_{pot} were the only significant, independent prognostic factors according to a stepwise multivariate Cox analysis (Table 4). The finding that a longer T_{pot} correlates with worse survival, was even more striking when aneuploid tumours were analysed separately.

For further structural analysis within the subgroup of all tumours, in which T_{pot} could be calculated, we set up a hierarchical prognosis model using CART analysis using those variables which had shown significant prognostic impact by univariate analysis. Three variables were selected: resection status, stage and T_{pot},

Table 4
Univariate and stepwise multivariate Cox analysis of prognostic factors in R0 resected patients

Variable	Category	Univariate analysis		Multivariate analysis	
		RR (95% CI)	*P* value	RR (95% CI)	*P* value
Stage	I, II, IIIA/IIIB, IV	5.2 (2.0–13.9)	0.003	10.6 (3.6–31.6)	<0.001
Infiltration	pT1, 2/pT3, 4	5.0 (1.5–16.9)	0.003	Not selected	
Nodal-status	pN0/N+	3.7 (1.5–9.2)	0.002	Not selected	
Distant metastases	M0/M1	3.2 (1.0–4.5)	0.024	Not selected	
S-phase (%)	⩽14.4/>14.4	2.8 (1.0–7.6)	0.034	Not selected	
T_{pot} (day)	⩽3.8/>3.8	2.3 (0.8–6.3)	0.080	4.25 (1.45–12.4)	0.008
BrdU LI (%)	⩽14.6/>14.6	2.0 (0.6–4.5)	0.091	Not selected	

RR, relative risk; 95% CI, 95% confidence interval; T_{pot}, potential tumour doubling time; BrdU LI, Bromodeoxyuridine labelling index.



pts:	17	20	9	22
death:	1	8	8	22
5 year survival (%)	93	54	9	0
RR	0.07	0.6	1.65	3.47
P value		0.013	<0.01	<0.01

Fig. 2. Prognostic model following classification and regression trees (CART) analysis of all 68 patients in which doubling time (T_{pot}) could be calculated. Three variables, resection status, tumour stage and T_{pot} define risk-groups with significantly different outcomes. In stage I, II and IIIA tumours, a long T_{pot} is a significant indicator for a worse outcome. RR, relative risk; pts, patients; R, resection status.

which thus classified the patients into significantly different risk groups. The group with tumours stage I, II and IIIA could be divided further with respect to T_{pot}. The 17 patients with a shorter T_{pot} ($\leqslant$3.8 days) had a 5-year survival rate of 93%, and only 1 of these patients died of a tumour recurrence. Of the 20 patients in these stages with a long T_{pot} ($>$3.8 days), 8 patients died of recurrent gastric cancer, and the 5-year survival was 54% ($P=0.013$, Fig. 2).

4. Discussion

All procedures in this prospective, single centre study on the effect of proliferation and proliferation kinetics on survival after gastric adenocarcinoma resection were strictly standardised. This study confirms that the well-known and accepted patho-histological factors such as resection status, tumour stage according to the TNM system and lymph node ratio have a prognostic impact [16,17]. Of the proliferation-associated biological features we investigated, only T_{pot} had a significant impact on survival in the R0 resected group by multivariate and CART analysis. DNA ploidy, proportion of S-phase cells of the cell cycle, BrdU LI, DNA synthesis time T_s, or the expression of the proliferation-related antigens PCNA and Ki-67, measured by flow cytometry, failed to demonstrate a prognostic impact in this study.

However, T_{pot} could be calculated in this study in only 61% of cases from the DNA histogram. This agrees with the literature, which reports detection rates of *in vivo* BrdU varying between 62 and 100% in gastric cancer [12,18–20]. Especially problematic was the interpretation of the BrdU/DNA histograms in the non-intestinal type carcinomas according to the Lauren classification [21]. Diffuse and mixed-type carcinomas (non-intestinal) are made up of scattered solitary or small clusters of cells. Even in more solid cellular areas, the individual cells are only loosely attached to each other and connective tissue proliferation is marked [22]. The contamination of the sample with connective tissue might have prevented accurate evaluation of the tumour BrdU incorporation in the subgroup of non-intestinal tumours. BrdU detection rates of 100% have only been reported by Japanese groups, perhaps since the intestinal type is still much more common than non-intestinal in Japan. In this type, we also had no difficulty detecting BrdU in intestinal-type tumours. However, this limitation for detecting BrdU may be an obstacle for routine clinical use in Western patients.

A problem with any study of tumour biology is the inherent heterogeneity of any tumour. In this study, we used a relatively large portion of tissue from the tumour edge and adjacent central areas in order to minimise this problem. Necrotic areas, which are often present in the central areas of advanced tumours, were excluded from the analysis. Several studies have shown that inter-patient variability is much greater than intratumoral heterogeneity when assessing T_{pot} [11,12,23], suggesting that differentiation among patients is possible. However, the biological variation within a tumour has been shown to be as high as 30% (coefficient of variation) for T_{pot}, indicating that for tumours with T_{pot} values close to the median, it is not possible to unambiguously classify these as fast- or slowly-growing tumours [11]. To date, no study has included enough patients to be able to divide the tumours into three subgroups, i.e. to assess individual tumours as being fast, slow or intermediate. A strict division into only two groups, as was done in this study, might simplify the problem, but should be used with caution to assess tumour biology.

The most interesting finding of the present study was the adverse effect of proliferation kinetics on survival; namely that a prolonged potential tumour doubling time was associated with a poorer survival. Although the levels of significance for cell kinetic parameters were not very high (Tables 3 and 4), this fact was supported by the highly significant CART analysis (Fig. 2), which included all patients in whom T_{pot} could be assessed. In particular, in the group of patients with a tumour which was completely resected with a sufficient safety margin for both the primary tumour and the lymphatic system (stage I, II and IIIA), a long T_{pot} was associated with a worse outcome. Until now, no study showing an impact of T_{pot} on survival in gastric cancer has been published. A few Japanese groups have addressed the role of *in vivo* and *in vitro* BrdU LI in this context, but the correlation of T_{pot} or T_s to survival after resection was not reported

[24–26]. These studies focused more on the influence of the S-phase fraction, calculated by the incorporation of BrdU, on survival. Furthermore, the study groups in these publications were somewhat heterogeneous and included incompletely-resected patients, as well as patients with intraperitoneal and/or adjuvant chemotherapy.

In 1998, Palmqvist and colleagues presented for the first time a modified view of the influence of proliferation kinetics on survival [27]. His group analysed patients with colorectal cancer after *in vivo* administration of iododeoxyuridine (IdUrd). They found a survival advantage for patients with fast proliferating tumours in tumour stage Duke's B. In addition, Gamel and colleagues reported an overall survival advantage for patients with breast cancer with a high BrdU LI after a 10-year survival analysis [28]. Furthermore, in some haematological malignancies, such as acute non-lymphoblastic leukaemia, the proliferative activity of the malignant cell population is lower than their normal equivalents [29].

What could be the biological basis for this surprising result, which is contradictory to the unproved clinical tenet, that fast proliferating tumours are more aggressive '*per se*'? There is increasing evidence that the crucial step in oncogenesis is the mutation of proto-oncogenes and/or tumour suppressor genes [30]. Their proteins regulate the cell cycle via so-called check-points. It can be speculated, that following this event, two types of mutated cells might be defined: rapidly or slowly proliferating cells. During malignant progression, the slowly proliferating cells have more 'time' to accumulate genetic alterations than rapidly proliferating cells. We found a higher proportion of the slowly proliferating cells are arrested in the S-phase of the cell cycle. In some these tumours, a proportion of up to 30% of so-called 'silent S-phase cells' was detected in our study. These are cells which are in the S-phase of the cell cycle, but are not stained by BrdU, indicating that they do not participate in the proliferation process [31], but are able to enter the replication process again. Cells in the S-phase are particularly sensitive to genetic alteration. In addition, in a separate set of experiments, we have found evidence that rapidly proliferating tumours undergo apoptosis at a much higher rate than slowly proliferating tumours. Therefore, rapidly proliferating tumours could remove genetically altered cells via this pathway.

The effect of proliferation kinetics on survival becomes apparent only in patients with tumour stages in which a complete resection with sufficient safety margins could be achieved. In tumour stages IIIB and IV, macroscopic and microscopic tumour spread is already so advanced that differences in outcome are obscured. Since the fate of the patient is determined by the extensive spread of the disease, the effect of various tumour-biology-related features is no longer evident.

With this background, the impact of proliferation on the response to adjuvant therapy should also be reassessed. Although chemotherapy and radiotherapy act primarily on proliferating tissues, proliferation-related factors and proliferation kinetics have not yet been correlated with response to therapy. This lack of correlation was recently reported by the final analysis of an European Organization for Research and Treatment of Cancer (EORTC) trial of the correlation between proliferation kinetics (T_{pot}) and the response to radiotherapy in head and neck tumours [32]. Interestingly, in this multicentre investigation, a long DNA synthesis time (T_s) was an independent predictor of worse survival. Following our investigation, it becomes even more questionable whether response to therapy can be assessed by measuring tumour proliferation. Both modalities — chemotherapy or radiotherapy — destroy rapidly proliferating cells, however, the fate of the patient is determined by the tumour cells which are resistant to therapy. Not the proliferation rate *per se*, but the accumulation of underlying genetic changes, which do not affect the 'growth' of a tumour, might be the basis for 'non-response' after conventional tumour therapy. Proliferation itself, although a prerequisite, does not seem to be the major characteristic of malignancy, but might pave the way for the development of malignant features such as the ability to metastasise, to invade, or develop the resistance to non-operative treatments [33].

Besides the fact that the potential tumour doubling time, T_{pot}, was identified as a independent prognostic factor in completely-resected gastric cancer, our results indicate that proliferation kinetics might have a much different role in tumour progression than previously recognised. It can therefore be hypothesised that the impact of the determinant 'time' might be fundamental to the whole process of clinically detectable cancer development.

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References

1. Schipper DL, Wagemans MJM, Peters WHM, Wagener DJT. Significance of cell proliferation measurement in gastric cancer. *Eur J Cancer* 1998, **34**, 781–790.
2. Hermanek P, Maruyama K, Sobin LH. Stomach carcinoma. In UICC, Hermanek P, Gospodarowicz MK, Henson DE, Hutter RVP, Sobin LH, eds. *Prognostic Factors in Cancer*. Berlin, Heidelberg, New York, Springer-Verlag, 1995, 47–63.

3. Dolbeare F. Bromodeoxyuridine: a diagnostic tool in biology and medicine, Part II: oncology, chemotherapy and carcinogenesis. *Histochem J* 1995, **27**, 923–964.
4. Gratzner HG. Monoclonal antibody to 5-bromo- and 5-iododeoxyuridine: a new reagent for detection of DNA replication. *Science* 1982, **218**, 474–475.
5. 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**, 620–626.
6. Steel GG. *The Growth Kinetics of Tumours*. London, Oxford University Press, 1977.
7. Siewert JR, Sendler A. The current management of gastric cancer. *Adv Surg* 1999, **33**, 69–93.
8. Stein HJ, Sendler A, Fink U, Siewert JR. Multidisciplinary approach to esophageal and gastric cancer. *Surg Clin North Am* 2000, **80**, 659–682.
9. Watanabe H. Gastric tumors. In Watanabe H, Jass JR, Sobin LH, eds. *Histological Typing of Oesophageal and Gastric Tumours, WHO International Histological Classification of Tumors,* 2nd edn. Berlin, Heidelberg, New York, Springer, 1990.
10. UICC. *TNM Classification of Malignant Tumours*, 4th edn. Berlin, Heidelberg, New York, Springer, 1997.
11. Wilson MS, West CM, Wilson GD, Roberts SA, James RD, Schofield PF. Intra-tumoural heterogeneity of tumour potential doubling times (Tpot) in colorectal cancer. *Br J Cancer* 1993, **68**, 501–506.
12. Haustermans K, Vanuytsel L, Geboes K, *et al*. In vivo cell kinetic measurements in human oesophageal cancer: what can be learned from multiple biopsies? *Eur J Cancer* 1994, **30A**, 1787–1791.
13. Kaplan EL, Meier P. Nonparametric estimation form incomplete observations. *J Am Stat Assos* 1958, **53**, 157–181.
14. Cox D. Regression models and life-tables. *Stat Soc Br* 1972, **34**, 187–220.
15. Breimann L, Friedman J, Olshen R, Stone C. *Classification and Regression Trees (CART)*. New York, Chapman and Hall, 1984.
16. Siewert JR, Böttcher K, Stein H, Roder JD, the German Gastric Carcinoma Study Group. Relevant prognostic factors in gastric cancer. *Ann Surg* 1998, **228**, 449–461.
17. Sendler A, Nekarda H, Böttcher K, Fink U, Siewert JR. Prognosefaktoren beim Magencarcinom. *Dtsch Med Wschr* 1997, **122**, 794–800.
18. Riccardi A, Danova M, Ascari E. Bromodeoxyuridine for cell kinetic investigations in humans. *Haematologica* 1988, **73**, 423–430.
19. Kamata T, Yonemura Y, Sugiyama K, *et al*. Proliferative activity of early gastric cancer measured by in vitro and in vivo bromodeoxyuridine labeling. *Cancer* 1989, **64**, 1665–1668.
20. Ohyama S, Yonemura Y, Miyazaki I. Proliferative activity and malignancy in human gastric cancers. Significance of the proliferation rate and its clinical application. *Cancer* 1992, **69**, 314–321.
21. Laurén P. The two histological main types of gastric carcinoma: diffuse and so-called intestinal type carcinoma: an attempt at a histo-clinical classification. *Acta Pathol Microbiol Scand* 1965, **64**, 31–43.
22. Morson BC, Dawson IMP. *Gastrointestinal Pathology*, 2nd edn. Oxford, London, Edinburgh, Blackwell Scientific, 1979.
23. Haustermans K, Hofland I, Pottie G, Ramaekers M, Begg AC. Can measurements of potential doubling time (Tpot) be compared between laboratories? A quality control study. *Cytometry* 1995, **19**, 154–163.
24. Ohyama S, Yonemura Y, Miyazaki I. Prognostic value of S-phase fraction and DNA ploidy studied with in vivo administration bromodeoxyuridine on human gastric cancers. *Cancer* 1990, **65**, 116–121.
25. Yonemura Y, Ohoyama S, Kimura H, Kamata T, Yamaguchi A, Miyazaki I. Assessment of tumor cell kinetics by monoclonal antibody Ki-67. *Eur Surg Res* 1990, **22**, 365–370.
26. Yonemura Y, Ohyama S, Sugiyama K, *et al*. Retrospective analysis of the prognostic significance of DNA ploidy patterns and S-phase fraction in gastric carcinoma. *Cancer Res* 1990, **50**, 509–514.
27. Palmqvist R, Oberg A, Bergstrom C, Rutegard JN, Zackrisson B, Stenling R. Systematic heterogeneity and prognostic significance of cell proliferation in colorectal cancer. *Br J Cancer* 1998, **77**, 917–925.
28. Gamel JW, Meyer JS, Province MA. Proliferative rate by S-phase measurement may affect cure of breast carcinoma. *Cancer* 1995, **76**, 1009–1018.
29. Giordano M, Danova M, Mazzini G, Gobbi P, Riccardi A. Cell kinetics with in vivo bromodeoxyuridine assay, proliferating cell nuclear antigen expression, and flow cytometric analysis. Prognostic significance in acute nonlymphoblastic leukemia. *Cancer* 1993, **71**, 2739–2745.
30. Cahill DP, Lengauer C, Yu J, *et al*. Mutations of mitotic checkpoint genes in human cancers. *Nature* 1998, **19**, 300–303.
31. Zölzer F, Speer A, Pelzer T, Streffer C. Evidence for quiescent S- and G2-phase cells in human colorectal carcinomas: a flow cytometric study with the Ki-67 antibody. *Cell Prolif* 1995, **28**, 313–327.
32. Begg AC, Haustermans K, Hart AA, *et al*. The value of pretreatment cell kinetic parameters as predictors for radiotherapy outcome in head and neck cancer: a multicenter analysis. *Radiother Oncol* 1999, **50**, 13–23.
33. Iversen OH. Is a high rate of cell proliferation carcinogenic in itself? *Hum Exp Toxicol* 1992, **11**, 437–441.