Reviews in Oncology 2 (1989) 1
(in Acta Oncologica Vol. 28, Fasc. 1)

FROM INSTITUT GUSTAVE-ROUSSY, F-94805 VILLEJUIF, FRANCE.

TUMOR CELL PROLIFERATION KINETICS AND TUMOR GROWTH RATE

MAURICE TUBIANA

Abstract

The present knowledge on the growth rate and the proliferation kinetics of human tumor is based on the measurement of the tumor doubling times (DT) in several hundred patients and on the determination of the proportion of proliferating cells with radioactive thymidine or by flow cytometry in large numbers of patients. The results show that the DT of human tumor varies widely, from less than one week to over one year with a median value of approximately 2 months. The DTs are significantly correlated with the histological type. They depend upon 1) the duration of the cell cycle whose mean duration is 2 days with small variations from tumor to tumor, 2) the proportion of proliferating cells and consequently the cell birth rate which varies widely among tumors and which is significantly correlated to the DT, 3) the cell loss factors which also vary widely and which are the greatest when proliferation is most intensive. These studies have several clinical implications: a) they have further increased our understanding of the natural history of human tumor, b) they have therapeutic implications since tumor responsiveness and curability by radiation and drugs are strongly influenced by the cell kinetic parameters of the tumor, c) the proportion of proliferating cells is of great prognostic value in several types of human cancers. The investigation of the molecular defects, which are correlated with the perturbation of control of cell proliferation, should lead to significant fundamental and therapeutic advances.

Key words: Tumor growth rate, tumor progression, cell kinetics, labelling index, flow cytofluorometry.

In the late 1950s, a keen interest in the growth and natural history of human tumors arose among clinicians from 2 converging types of investigation. Firstly, it was shown that 1) the growth rate of tumors could be assessed by sequential measurement of tumor size, and 2) in most patients the growth rate of the primary tumor remained constant throughout the period of observation. The caliper, an instrument for the determination of tumor size, became a powerful tool for the analysis of the natural history of human tumors (1).

Secondly, the advent of tritiated thymidine labelling provided a technique for the measurement of the duration of the cell cycle and of the proportion of proliferating cells.

In the first part of this paper, we shall review the knowledge gained through measurement of tumor doubling time. In the second part, we shall discuss the methods used for the study of the proliferation kinetics of tumor cells, the results obtained and their relationship with tumor growth rate. In the third, we shall examine the clinical implications of these studies. Finally, we shall discuss prospects for the future.

Tumor growth rate

Until 3 decades ago it was felt that growth of human tumors was both unpredictable and rapid. However, when the growth of experimental tumors was studied, it appeared that in the vast majority of cases this growth curve could be well fitted by a relatively simple equation, such as the logistic equation or the Gompertz' equation. Moreover, each type of tumor has a well defined growth curve which is specific to that tumor system. In particular a correlation was observed between the growth rate of the primary tumor and that of its metastases (1).

Collins et al. (2) were probably the first, in 1956, to study quantitatively the growth of untreated human lung metastases. They found that during the observation period, the growth rate, or the doubling time (DT), was constant, in other words that growth was exponential. These observations had a considerable impact on clinical thinking. Several hundred human tumors were studied; for the vast majority the pattern of growth was exponential during the observation period. However, progressive length-

Table

Mean kinetic parameters of various histological types of human tumors (4). (The number in parentheses indicate the number of patients for whom data were available)

Histological type	Doubling time (days)	Labelling index (%)	Growth fraction (%)	Cell loss factor (%)	Radiosensitivity (mean tumor control dose in Gy)	Chemosensitivity
Embryonal tumors	27	30	90	93	25–30	++
Malignant lymphomas	29	29	90	93	35–45	++
Mesenchymal sarcomas	41	4	11	68	85	–
Squamous cell carcinomas	58	8	25	89	60–70	+
Adenocarcinomas	83	2	6	71	60–80	±

ening of the DT, which is observed in all experimental tumors, is also found in about 15 to 20% of the human tumors followed up over a long period; available data suggest that the damping factors may manifest themselves late in the course of human tumors and for widely different tumor sizes. Therefore, in view of the relative brevity of most observation periods, seldom more than 5 DT, one cannot rule out the possibility that many tumors appear to grow exponentially only because they have been observed over too short a period. In a very small proportion of patients, clear evidence of a shortening of DT was observed, for example long dormancy followed by rapid growth; in a few patients it has been possible to relate this acceleration to change in hormonal status as at the menopause or puberty, or to the progression of the histological characteristics to a more malignant type.

Another way of approaching the study of tumor growth rate is to measure some substance secreted by neoplastic cells. The data obtained from estimations of myeloma immunoglobulin in multiple myelomas (monoclonal gammopathy) by radioimmunoassay suggest that the growth rate here is progressively decreasing, i.e. Gompertzian (3). In summary despite the occurrence of irregular growth patterns the growth rate is in most patients either constant (exponential growth) or progressively decreasing.

The DT of human tumors varies widely from one patient to another and is on average very long. Combining 530 values selected from the literature and our own figures, we found that the median value for the DT was about 2 months (4). Additional data collected since 1971 have not significantly modified this figure. The frequency distribution of the individual values for the DT is log-normal. The scatter of values for DT is such that, in the sample studied, 0.1% of the tumors had a DT of about one week or less while another 0.1% had a DT of several years. The log-normal type of distribution of the DT suggests that there is no discontinuity between cancers whose growth is very rapid and those where growth is too slow to be accurately measured (1, 5). The growth rate of tumors of the same histological type arising in different patients may differ widely, but the growth rates of separate metastases developing in the same patient are usually similar to one another.

Despite a wide range of DT within any particular histological type of tumor, there are statistically significant differences between the mean DTs of a number of tumor types. We have collected values for the DT of 389 patients with pulmonary metastases which were classified into 5 histological categories. The following order of doubling time was found: embryonal tumors (27 days), malignant lymphomas (29 days), mesenchymal sarcomas (41 days), squamous cell carcinomas (58 days) and adenocarcinomas (82 days) (Table). Within the same histological group, the distribution of individual values is log-normal; furthermore, the spread of individual values is comparable in the different groups, as evidenced by the parallelism of the cumulative plots in probit log scale. For each tumor type the less differentiated tumors have the fastest growth rate (5).

Tumor cell proliferation kinetics

In 1951, it was shown that in mammalian cells DNA synthesis takes place during only a part of the intermitotic time. This observation was based on the use of a labelled precursor of DNA which is incorporated into DNA when the cell is synthesizing DNA. Thymine is the only one of the four bases of DNA which is not present in RNA. Thymidine is a precursor of thymine and is therefore a specific precursor of DNA. Thymidine is usually labelled with tritium (^{3}H-Th). On autoradiography, the cells which have incorporated ^{3}H-Th are easily identified. The cell cycle has been extensively studied in malignant and normal cells (Fig. 1). The term 'cell cycle' refers to the 4 subsequent phases of cellular proliferation from one mitosis to the next: postmitotic phase (G_1) during which the cell prepares the next phase of DNA synthesis, synthesis of DNA (S), and premitosis (G_2) to mitosis (M). Most cells, but not all, after mitosis enter a phase, termed G_0, which is quiescent from the point of view of proliferation. During this phase they may have a functional activity, but they do not prepare the next mitosis. They can be trig-

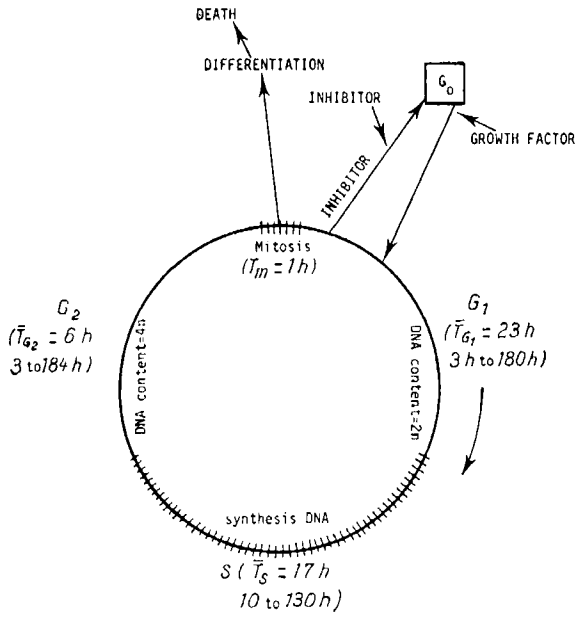

Fig. 1. Cell cycle. Schematic description. For each phase of the cell cycle, the mean duration and the limits of the 95% confidence interval are given (for human tumors). After a mitosis, the new cell can either enter a new cell cycle, or differentiate which is often associated with loss of proliferative potential and the differentiated cell dies after completion of its life-span, or can become temporarily quiescent (G_0 phase). Under appropriate stimulus, quiescent cells can be triggered into proliferation and progress into G_1 and throughout the cell cycle.

gered into a proliferative state by an appropriate stimulus, usually a specific growth factor.

Methods

Labelling with tritiated thymidine. ^{3}H-Th can be administered in vivo. In experimental animals, ^{3}H-Th is usually injected intra-peritoneally or intravenously. In view of the long half-life of tritium, intravenous administration has only been used in a few series of patients and these investigations are permissible only in patients with a short life expectancy. The determination of the cell cycle time (Tc) requires a large number of samples and such investigations are therefore performed on large tumors which are easily accessible. These problems explain why the number of human tumors, in which Tc has been measured, is relatively small.

The most reliable data on cell cycle duration have been obtained by the percentage of labelled mitoses method (4) which permits the measurement of the time interval taken by a labelled cell to progress from the S phase throughout the G_2 phase till mitosis and thereafter to return for the next mitosis after a full cell cycle. However, this technique is time-consuming and simpler but less accurate methods have been introduced. The rate of reduction of the mean grain count per labelled mitoses after continuous labelling enables the measurement of the mean time interval between 2 mitoses since during each mitosis this number is halved. In the stathmokinetic method, the cells are blocked in mitosis and the rate of accumulation of mitotic cells is proportional to the rate of progression throughout the cell cycle. The duration of the S phase has also been measured by double labelling, using 2 different precursors of DNA (for example, ^{3}H-thymidine and ^{14}C-thymidine), and more recently, by cytofluorometry (6).

The labelling index (LI) measured on autoradiography determines the proportion of cells in S phase. In most studies, the LI was measured in vitro by incubating fresh tumor specimens with ^{3}H-Th. Experimental studies have shown good consistency between LI measured in vivo or in vitro. LI assay is simple but should be performed with precise methodology. Two problems are critical a) the technique of autoradiography and film processing, and b) the method used for background subtraction. In order to avoid large statistical fluctuations and to insure reproducibility of the assay, a large number of cells should be counted. There are a few other pitfalls. Some cells can have a slow cycle and are thus insufficiently labelled resulting in an underestimation of the LI. It has been shown that an uneven distribution of labelled cells in a tumor may arise from the pattern of ^{3}H-Th concentration throughout the tumor. One of the main advantages of LI is that during counting, normal cells can be distinguished from tumor cells, therefore non-malignant cells present in the tumor can be excluded (6).

Measurement of cell DNA content by image cytometry (IC). Normal cells are diploid when they are quiescent or in G_1 phase of the cell cycle. Their nuclei have a 2 n DNA content. In G_2 or during prophase they have a 4 n DNA content, and an intermediate content in S phase. When the assay of the DNA content is sufficiently precise, it is possible to measure the proportion of cells in S phase.

The assay is based on Feulgen staining. It is accurate on smears because the cells do not overlap and the nuclei are not damaged; moreover, it is easy to select the intact tumor cells.

On slides from a solid tumor the DNA content of a section of the nucleus is measured which is only a part of the total DNA content of this nucleus. Algorithms based on stereological methods have been used to compute the distribution of the total DNA content of the nuclei knowing the distribution of DNA content of the nuclear sections.

Image cytometry is tedious but has two advantages: a) one can select under the microscope the cells in which the DNA content is measured; b) if the fixation has not damaged the DNA molecules, it can be performed on paraffin-embedded sections allowing retrospective studies (6).

Flow cytometry (FCM). Currently this is the most widely used technique. The DNA is labelled in vitro with ethydium bromide, or propidium iodide. Flow cytometry

enables a rapid and precise assay of a large number of cells, allowing the distribution of the DNA content to be known accurately (6).

For solid tumors, one of the problems is to obtain a good cell suspension since the disruption of tumors into single cells is difficult to achieve. The presence of cellular debris or microaggregates of cells may constitute a source of artefact. In order to avoid that, several authors isolate the cell nuclei or extract them from tissue blocks. This technique allows retrospective studies on paraffin-embedded tissues. However, when the isolated nuclei are studied, it is very difficult to distinguish a normal cell from a tumor cell. A large number of normal cells are often intermingled within the tumor; usually the percentage of S phase cells is lower in normal cells than in tumor cells which results in an underestimation of the proportion of cells in S phase, especially in diploid tumors. When a suspension of undamaged cells is studied, normal cells can be distinguished from tumor cells using as a discriminant cell size membrane markers or other cell characteristics. However, in most studies the results are given for a cell population which includes normal cells.

The main problem in FCM or image cytometry is related to tumor cell ploidy. When the tumors are diploid, the determination of the proportion of cells with an intermediate DNA content is relatively simple but still requires a correction since there is an overlapping between: 1) the G_1 cells and those which have just entered into S phase, and 2) those which have nearly completed the S phase and G_2 cells. Software of varying complexity is used for these corrections and the precise determination of the percentage in S phase depends upon the accuracy of the instrument and the effectiveness of the computer program.

When the tumor is aneuploid, the determination of the percentage of cells in S phase becomes less precise and sometimes impossible, in particular when there is a mixture of diploid and aneuploid cells or if multiple aneuploid peaks are present. For example, in the study of Hedley et al. (7) out of 490 patients with breast cancer a valid estimation of the percentage of cells in S phase was obtained in only 60% of the patients.

Labelling with non-radioactive precursor of DNA. Bromodeoxyuridine (BrdU) and iododeoxyuridine (IudR) are analogs of thymidine, which are incorporated into DNA during S phase. Labelling with BrdU can be performed in vitro or in vivo. Subsequently labelled cells are recognized by a monoclonal antibody against BrdU or IudR.

When BrdU is injected to patients prior to the biopsy, this method gives information on the duration of S phase and the potential DT (T_{pot}).

Two other techniques have been used for the measurement of the proportion of proliferative cells. Primer-dependent α-DNA polymerase can identify the cells which are engaged in a cell cycle independently of the cycle phase. The Kiel group has reported that a monoclonal antibody, Ki-67, specifically stains cycling cells. This antibody reacts with a nuclear antigen which is expressed throughout the whole cycle but is absent in quiescent cells. It has been used for measuring the tumor growth fraction (6).

Data

The first important finding was the identification of *quiescent tumor cells,* that is of cells which are not engaged in a cell cycle. Most of them have a DNA content equivalent to that of cells in G_1 phase. In normal tissues, there are 2 types of quiescent cells: the so-called G_0 cells which can be triggered into proliferation by specific growth factors, and the differentiated cells which have become unable to divide. The same 2 categories are present in differentiated human tumors; there is also a third category, that of tumoral cells, not cycling due to lack of oxygen or nutrients.

Mendelsohn in 1962 defined the *growth fraction* (GF) as the ratio between the number of proliferating (P) cells and the total number of cells including quiescent (Q) cells, i.e.

$$GF = \frac{P}{P+Q}$$

Continuous labelling can provide an approximate measure of the percentage of proliferating cells. If a population of cells is labelled continuously over a period corresponding to the duration of the cycle (minus that of the DNA synthesis phase) then all the cells which are engaged in the cycle should be labelled if the GF is 1. This method requires a perfusion of ^{3}H-Th.

The method originally advocated by Mendelsohn is based on the percent labelled mitosis curve and is even more difficult to perform in patients. A simpler but approximate method is based on the relationship: $LI \times T_c/T_s$ = growth fraction, where T_s = duration of the S phase, T_c = duration of the cell cycle.

The growth rate of a tumor depends upon 3 main parameters: 1) the duration of the cell cycle T_c, 2) the proportion of proliferating cells (growth fraction), and 3) the cell loss factor (8).

The measurement of the duration of the human tumor T_c unexpectedly revealed that it was not significantly different from that of normal cells with the same morphology and tissue specificity as the corresponding tumor cells. It is of similar duration (2 to 4 days) in rapidly growing and slowly growing tumors. There is a striking discrepancy between the short duration and the relative constancy of T_c in human tumors on one hand, and the long duration and the wide spread of DT (1 week to more than 1 year) on the other. The in vivo or in vitro measurement of LI after pulse labelling in several hundred human tumors has shown that it varies widely, from $<0.1\%$ to $>50\%$. In each histological group of tumors the individual values of the LIs also vary widely and their distribution is usually log-normal. Nevertheless there are significant differences between the mean values of the various groups.

The highest mean LIs were found in the lymphomas and the embryonal tumors, the lowest in adenocarcinomas (4) (Table).

The LIs are higher in the undifferentiated tumors. For example, in breast tumors the proliferation rate is higher in tumors without estrogen or progesterone receptors.

A positive correlation between cell density and LI has been reported in human tumors. This suggests that the control mechanism which regulates cell proliferation in normal tissues still operates in tumors with a low cell density and that in these tumors the cell-to-cell interactions are less disturbed than in tumors in which the cell density is high.

In a survey carried out in 1976 on the 53 human tumors in which the parameters of the cell cycle had been measured, the mean duration of T_c was 2 days. In more than 80% of the tumors, T_c varied from 1 to 4 days (4). The spread of individual T_c duration within a human tumor is generally relatively wide, e.g. from 15 h up to several days; this spread is less wide in experimental tumors. The mean duration, T_s, of the S phase is 18 h with a ratio of 3 between the extreme values of the confidence interval. T_c and T_s do not vary with the histological type of the tumor, and the mean ratio of T_s/T_c is 0.4. It varies from 0.5 when T_c equals to 1 day, to 0.3 for a T_c of 2 days and to 0.2 for a T_c of 5 days (5). Therefore the LI is correlated with the proportion of cells engaged in the proliferative cycle, i.e. the growth fraction.

The *cell birth rate* and therefore the *potential DT* (DT_{pot}) can be calculated knowing the LI and T_s: The DT_{pot} is always much shorter than the actual DT. Since DT_{pot} assumes that all that are produced remain in the tumor, the difference between DT and DT_{pot} is due to *cell loss* (9). The greater the discrepancy between the DT and the DT_{pot}, the greater the cell loss; the cell loss factor can be calculated according to the following formula:

$$\text{cell loss factor} = 1 - \frac{DT_{pot}}{DT}$$

Other ways of expressing cell loss can be more relevant, for example the proportion of cells which die per 10^9 cells per day, or the cell turnover rate which takes into account both the GF and the cell loss factor.

In human tumors, the daily cell loss seems to be the smallest in those tumors of histological type where the growth is the slowest and the LI the lowest. It appears that the probability of cell death is the greatest when the proliferation is most intense. This correlation observed between the mean values of DT, LI and cell loss factors in various histological groups of tumors was found to be valid in a group of breast cancer in which the DT and LI were measured for each patient. The discrepancy between potential DT and actual DT increases with the LI, again showing that the higher the LI the higher the cell loss. The cell loss factor is higher in tumors with a high growth fraction and both contribute to a high cell turnover rate. This explains why: a) the range of DT (one week to one year) is smaller than the range of LI (0.01% to 35%) and b) there is a correlation between LI and DT. In summary, the cell losses from human solid tumors are considerable and help to explain why the DTs are on the average 30–90 times longer than the duration of the cell cycle but the differences between the mean durations of the DTs in different histological groups are mainly due to differences in GF. Differences in GF also explain why the DTs of human tumors are much longer than the DTs of experimental tumors.

Another significant advance was the recognition of the impact of vascularization on cell proliferation in experimental tumors (11). The same influence probably exists also in human tumors.

Ascites tumors and leukemia. The comparison of the duration of the cell cycle of the same cell line growing either as a solid tumor or as an ascites emphasizes the role of intercellular contacts. In ascitic tumors T_c is longer and lengthens during the growth of the tumor, conversely to what is observed in solid tumors. The duration of T_s and T_{G1} is slightly longer than the corresponding phases in solid tumors (5). In leukemia, it it possible to distinguish 2 types of leukemic cells, large and small. By studying variations of the LI of these 2 types of cell, as a function of time, it can be shown that large cells correspond to cells in cycle while the small cells are quiescent. However, this quiescence is reversible and small cells, appropriately stimulated, will re-enter the mitotic cycle and proliferate. Studies using continuous perfusion of tritiated thymidine have shown that despite an initially low LI, nearly all the cells can eventually be labelled.

Comparison between the parameters of cell kinetics of primary tumors, metastases, and recurrence. A few studies have compared the cell kinetics of primary tumors and of metastases. For instance, for breast cancers the mean DT of the primary tumors measured on sequential mammography is about 4 months, whereas the DT of pulmonary metastases is about 70 days and that of skin metastases 40 days. For all the adenocarcinomas studied, the mean DT is 166 days in the primary tumors and 82 days in the lung metastases (4). For the squamous cell carcinomas the respective values are 82 and 58 days (4).

In 3 patients with cancer of the larynx, the LIs of the lymph node metastases were higher than the LIs of the primary tumor (11.7% and 6% respectively) (5).

Bresciani et al. compared the PLM curves of the primary and local recurrence in 2 patients. The mean T_c was shorter in the recurrences and the distribution of T_c less wide (5).

These data suggest that the progeny of the original metastasizing cells have a more rapid growth rate than cells in the primary tumor as a result of clonal selection, analogous to that seen in successive grafts of experimental tumors or in relapses of human tumors.

Clinical implications

The natural history of human tumors

Till the last decade, the natural history of human cancers was poorly known. From the knowledge which has accumulated, a clearer picture now emerges (8).

In experimental tumors the growth of small tumors before they reach a measurable volume is either equal to that of bulky tumors or more rapid. Thus a backward extrapolation, assuming a constant growth rate (exponential growth pattern), gives the maximal duration of the subclinical growth. For example, if the volume doubling time of the tumor at the time at which it is clinically detectable is 2 months, the duration of the subclinical growth, from a single malignant cell to 1 g (10^9 cells), is equal to 30 doubling times or 60 months if the duration of the doubling times remains constant (exponential growth pattern). This duration would be shorter if the growth pattern is Gompertzian as the doubling time lengthens with time (8).

For all tumor types the duration of the tumor growth during this occult phase always appears to be much shorter than the interval between the carcinogenic stimulus and the appearance of the tumor. There are 2 possible explanations: either a long phase of dormancy of the first neoplastic cells before they start proliferating or the existence of several stages of precancerous lesions which precede the development of the cancerous clone. The second explanation is the most likely. This long delay emphasizes the possibility of using antipromoters (retinoids, vitamine C, etc. ...) in patients at high risk of developing second cancers, such as those with a cured cancer of the upper respiratory and digestive tract, or patients with Hodgkin's disease treated by multiple chemotherapy. This concept of chemoprevention is currently tested in several controlled trials.

Knowing the probability distribution of the DT of a given type of tumor, one can build a model of its natural history. By extrapolating back to the origin of the metastases it was shown in the early 70s that most metastases, even if they become clinically detectable several years or decades after the treatment of the primary tumor, were already present at the time of the initial treatment. This was the rationale of the development of adjuvant therapy.

Fig. 2 shows that the time intervals between detection of primary tumor to detection of metastases, and detection of metastases to death are both related to the growth rate of the tumor; therefore they should show a correlation. This has been observed in breast cancer and osteosarcoma (Fig. 3). Moreover in a study on 203 patients whose metastases had been untreated, Malaise et al. (12) observed a correlation between the time interval between detection of first metastases and death, and the DT of the metastases; this correlation was significant within each of the histological types studied as well as for the whole group. The distribution of survival of the patients is log-

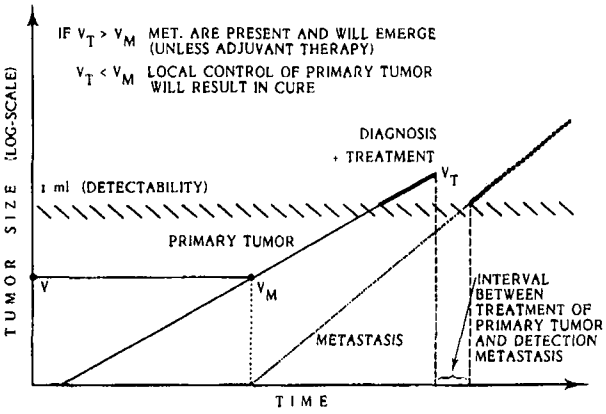

Fig. 2. Schematic representation of the natural history of a tumor and of metastatic dissemination. When the volume of a given tumor reaches a critical mass (V_m), variant cells which are able to migrate and to seed in another tissue appear in the tumor and initiate a distant metastasis. The growth rate of the metastasis is always more rapid than that of primary tumor (shorter DT). If the size of the tumor, when it is detected and treated (V_T), is smaller than V_M, the local treatment of the tumor will cure the patient. If, conversely, $V_T > V_M$ and if no adjuvant therapy is carried out, the metastasis will emerge clinically at some time, generally a few months or years after the treatment of the primary tumor. The time interval between the treatment of the primary tumor and the clinical emergence of the metastasis depends upon the duration of the metastasis doubling time and the time at which the primary tumor reached the volume V_M (from 14).

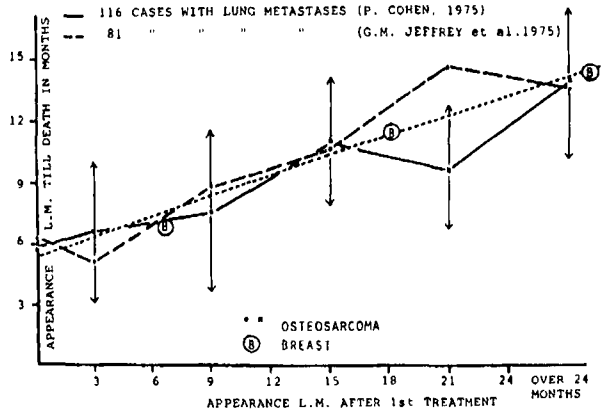

Fig. 3. Correlation between mean survival after appearance of lung metastases and time at which the metastases were discovered after the treatment of the primary tumor. For osteosarcoma, data from Cohen and Jeffrey et al. were collected by Breur. For breast carcinoma, data from Cutler et al. There is a striking consistency between the 3 sets of data (from 1).

normal and the spread of individual values is approximately comparable to that of the DT.

The time interval between treatment of the primary tumor and detection of first metastasis of several types of tumors is related to the probability distribution of DT of these tumors. However, this time interval depends also

upon the size of the primary tumor at the time of the metastatic spread (13, 14). The model predicts that in the subsets of patients, in whom metastatic spread occurs early, the occult metastases will be large at the time of the treatment of the primary tumor and therefore adjuvant chemotherapy will be less effective. This is in keeping with clinical data (8).

A model of tumor growth can be used to predict the proportion of patients in whom metastatic dissemination could be avoided by an earlier diagnosis. For breast cancers, the predictions are in good agreement with the results of screening programs. Such models can also determine the size distribution of the subclinical metastases at the time of treatment of the primary tumor (8).

These examples show that mathematical models are now available for the quantification of the natural history of human tumors. Their predictions are in accordance with clinical data; however, their use necessitates reliable data on the distribution of tumor growth rate and the metastasis appearance curves as a function of the size of the primary tumor at initial treatment. Till now such data have been produced only for breast cancers.

Therapeutic implications

The treatment of a solid tumor with radiation or cytotoxic drugs provokes disturbances in the proliferative status of both neoplastic and normal tissues. It is well known that cell sensitivity to radiation or drugs varies throughout the cell cycle. Moreover, both agents temporarily inhibit the progression of proliferating cells through the various phases of the cell cycle. Preferential killing and reversible blocking change the age distribution of the cell population and increase the proportion of cells in one of the phases of the cell cycle, thereby producing a cycling variation of the sensitivity of surviving cells. The prospects for exploiting this cell *reassortment*, or synchronization, in the treatment of cancer raised great hopes which have not been fulfilled. The aim was to schedule therapy so that tumor cells in a vulnerable phase would be killed during the first course, leaving a partially synchronized population of tumor cells which could be eradicated by additional courses administered with proper scheduling. However, in human tumors it was found impossible to take advantage of this cell reassortment and the trials which were designed to investigate the validity of this concept did not support it.

Another effect caused by both radiation and drugs is *repopulation*. Damage to a tissue triggers a recruitment of previously quiescent cells into the proliferative pool. A number of studies have well documented repopulation in normal tissues with a rapid cell turnover, such as intestinal epithelium or bone marrow. Malaise & Tubiana discovered in 1966 that, in the mouse, fibrosarcoma tumors regrew earlier after irradiation than would have been expected if the surviving cells maintained the doubling time of the original tumor. In 1968 to 1970, Barendsen et al. (15), and Hermens & Barendsen, demonstrated in an irradiated rhabdomyosarcoma that the viable clonogenic cells had a doubling time only one-third of that of unirradiated tumors. Griswold et al. found in 1970 a similar acceleration of the proliferation rate of the clonogenic cells of a plasmocytoma after administration of a cytotoxic drug. Rockwell et al. showed that the clonogenic tumor cell recruitment occurs within one day. Several other investigators have since confirmed the occurrence of a phase of rapid repopulation in treated experimental tumors (16).

In patients, a rapid regrowth of cutaneous or pulmonary metastases has been observed (16). The acceleration can be characterized by the ratio of the doubling time before treatment to that during the repopulation phase. In 31 human metastases in which it was measured the value of this ratio ranged from 2.5 to 5.

The practical importance of repopulation was overlooked for over one decade, for example in the Ellis' NSD formula the tumor repopulation component was considered negligible (17). Recently a renewed interest in tumor repopulation was caused by the research on unconventional fractionation and by efforts made for optimizing the timing in combination of radiotherapy and chemotherapy (16, 18). However, the mechanisms of tumor repopulation remain largely unknown. Experiments carried out at Villejuif have shown that treated experimental tumors release stimulating factors which are able to recruit tumor cells in vitro. This mechanism has not yet been documented for human tumors; its role is probable, but remains to be demonstrated.

The clinical use of repopulation requires the identification of those tumors in which repopulation is likely to be of importance. A significant positive correlation between the pretreatment and the posttreatment doubling time has been reported but is weak. Trott & Kummermehr (19) have concluded from experimental data that the pretreatment potential doubling time is the best guide to tumor proliferation rate during treatment. Conversely among human tumors with a low cell density and a low pretreatment labelling index a great elevation of the labelling index has been sometimes observed following treatment. This would suggest that the control mechanisms which regulate the cell proliferation in normal tissues still operate in these tumors (16). The debate remains open.

Repopulation has also been assessed by the influence upon local control of the overall treatment time. A few studies comparing split-course therapy and continuous irradiation are of particular interest. In head and neck tumors, the labelling index of the neoplastic cells is relatively high and split-course irradiation has given a percentage of local control smaller than that obtained with continuous courses. Carcinoma of the prostate have a low labelling index and in these patients continuous irradiation and split-course radiotherapy gave similar results. These 2 results are consistent with the hypothesis that the pre-

treatment labelling index is correlated with the extent of repopulation (19). However, in other types of tumors in which the labelling index is relatively high, such as bladder carcinoma, the results of continuous or split-course irradiation were similar although the total doses were equal. Thus the predictive value of the pretreatment labelling index or potential doubling time requires further investigation (16).

The few data available for human metastases in lung or skin suggest that the phase of accelerated growth rate has a duration of a few weeks or a month and is longer than the regeneration time of bone marrow or of other critical tissues. The differences in timing of repopulation in tumors and normal tissues provide opportunities for a differential effect and with a proper time interval it might be possible to preferentially hit tumor cells while they are still rapidly proliferating. An alternating radiotherapy–chemotherapy regimen has been advocated which shortens the time interval between the various agents acting on the tumor (18).

Tumor cell kinetics and responce to treatment. A few data suggest that tumors with a long proliferative index before treatment have a good chance of being radioresistant. Breur showed a correlation between growth rate of human lung metastases and their radiosensitivity. Later, other investigations confirmed that lung metastases with a short DT, whatever their histologic type, have a more rapid regression rate and a longer specific growth delay (1, 6).

Among major histopathological types of neoplasm there is a significant correlation between the proliferative fraction and/or the cell loss, the chemosensitivity, and the radiation dose necessary for tumor control (4). The only tumors that have been successfully controlled by drugs (malignant lymphoma and embryonic tumors) belong to the 2 histological types with a high proliferative fraction and a high amount of cell loss, that is, a high cell turnover rate. For experimental tumors a correlation between radiosensitivity and growth rate has also been reported.

The higher chemosensitivity of tumors with a large proportion of proliferating cells is consistent with the greater effectiveness of most cytotoxic drugs on cycling cells. However, notable exceptions have been reported. For example, in the acute phase of chronic myelogenous leukemia the cells proliferate much more rapidly yet the leukemia becomes much less chemosensitive than during the chronic phase (16).

Radioresistance of tumors with a large proportion of quiescent cells may be due to a greater amount of repair of potentially lethal damage. In fact Hermens & Barendsen, after irradiation of a rat rhabdomyosarcoma, found that the surviving clonogenic cells comprised a larger fraction of initially quiescent cells than did the total population of tumor cells (16).

Immediate tumor response depends upon 2 factors: the percentage of surviving cells and tumor shrinkage rate. A correlation has been observed in experimental tumors between the spontaneous rate of cell loss and the rate of tumor regression after treatment. The shrinkage rate may be just the expression of the daily turnover rate. In turn, shrinkage might influence reoxygenation and availability of nutrients as well as many other biological events. Courdi & Malaise (20) carried out a survey in which they found that in one-third of the studies which had investigated the correlation between the percentage of tumor cells in S phase and the immediate clinical response, there was a positive correlation between the two, whereas negative correlation had never been observed.

In conclusion, a more precise knowledge of cell kinetics during and after treatment could help to determine the optimal time intervals between the administration of drugs or ionizing radiations and to adjust treatment more precisely to the particular biological characteristics of the patient's tumor (10, 16).

Prognostic studies: relation of cell proliferation kinetics to probability of metastatic dissemination and long-term survival (5, 6). For several decades it has been suspected that a rapid tumor growth rate was associated with a poor prognosis. In these initial studies, the estimation of the growth rate was based on the answers of the patient regarding the time course of tumor size. Despite the subjectivity and the inaccuracy of these estimations, the data suggested that in patients with breast cancer, slow-growing tumors had a lower incidence of local recurrence and distant metastases than the more rapidly growing tumors. Subsequently a few studies showed that a long DT is associated with a good prognosis (1, 6, 21).

Since the discovery of the cell cycle, a large number of studies have investigated the relationship between the long-term survival and the percentage of tumor cells in S phase in various types of human tumors. The survey of the results clearly shows that the S phase fraction is of high prognostic significance in several types of cancer, in particular in breast cancer, non-Hodgkin lymphoma, leukemia, ovarian cancer, neuroblastoma, bladder cancer and lung cancer. It was found of high independent significance in 10 out of the 11 studies in which multivariate analyses of prognostic factors had been carried out. Proliferation appears generally to be of higher prognostic significance than ploidy which characterizes the DNA content of the cell in G_1 phase (all normal cells are diploid in G_1 phase and tetraploid in G_2 phase; many but not all neoplastic cells are aneuploid, e.g. their DNA content is slightly or markedly altered. Aneuploidy has been shown to be a pointer of poor prognosis) (6). In view of the wide differences in the biological characteristics of the tumors studied, it is likely that the association between a high proliferation rate and the degree of tumor aggressiveness is a general feature of human solid tumors. However, a high proliferative rate in tumor cells is probably not the cause of tumor biological aggressiveness but a variable associated with it (6).

Prospects

In several types of human cancer, autonomous growth is either the results of the synthesis of excessive amounts of growth factors or growth factor receptors, or is due to an excessive amplification of the signal generated by the growth factor receptor. Some of the growth factors which play a role in the proliferation of human cancers have been identified, for example bombesin in small cell lung cancer, or TGF-α in breast cancer. Several oncogenes and their products either act as a growth factor or a membrane receptor of growth factor, or play a role in the signal transduction from the membrane receptor to the nucleus. Some inhibitors reduce the proliferation rate of human tumors, such as TGF-β in breast cancers.

Emancipation from the mechanisms which control cell proliferation in normal tissues is one of the main features of malignancy and the degree of emancipation varies widely among human tumors even when they belong to the same histological type. A high proliferation rate appears to be a pointer of tumor malignancy. Proliferation rate is higher in metastases than in the primary tumor. This suggests that spread has been initiated by cells belonging to rapidly proliferating subclones. Moreover, the positive correlation observed between tumor cell density and proliferation rate suggests that a high proliferation rate is associated with a disturbance of cell-to-cell interactions and of cell contact inhibition. Likewise a positive correlation is observed between high proliferation rate and in vitro clonogenicity; and a negative correlation between the degree of tumor cell differentiation and cell proliferation rate.

The investigation of the molecular defects which are correlated with the perturbance of the control of cell proliferation should lead to significant fundamental and therapeutic advances.

Request for reprints: Prof. Maurice Tubiana, Institut Gustave-Roussy, F-94805 Villejuif, France.

REFERENCES

1. Tubiana M. L.H. Gray Medal Lecture: Cell kinetics and radiation oncology. Int J Radiat Oncol Biol Phys 1982; 8: 1471–89.
2. Collins VP, Loeffler RK, Tivey H. Observations on growth rates of human tumours. AJR 1956; 76: 988–1000.
3. Salmon SE. Expansion of the growth fraction in multiple myeloma with alkylating agents. Blood 1975; 45: 119–29.
4. Tubiana M, Malaise EP. Comparison of cell proliferation kinetics in human and experimental tumors: response to irradiation. Cancer Treat Rep 1976; 60: 1887–95.
5. Tubiana M, Malaise EP. Growth rate and cell kinetics in human tumours: some prognostic and therapeutic implications. In: Symingon T, Carter RL, eds. Scientific foundation of oncology. London: William Heineman, 1976: 126–36.
6. Tubiana M, Courdi A. Cell proliferation kinetics in human solid tumours. Relation to probability of metastatic dissemination and long-term outcome. Radiother Oncol (In press).
7. Hedley DW, Rugg CA, Gelber RD. Association of DNA index and S-phase fraction with prognosis of nodes positive early breast cancer. Cancer Res 1987; 47: 4729–35.
8. Tubiana M. The growth and progression of human tumors: implications for management strategy. Radiother Oncol 1986; 6: 167–84.
9. Steel GG. Cell loss as a factor in the growth rate of human tumours. Eur J Cancer 1967; 3: 381–7.
10. Silvestrini R, Molinari R, Costa A, Volterrani F, Gardani G. Short-term variation in labelling index as a predictor of radiotherapy response in human oral cavity carcinoma. Int J Radiat Oncol Biol Phys 1984; 10: 965–70.
11. Tannock IF. The relation between cell proliferation and the vascular system in a transplanted mouse mammary tumor. Br J Cancer 1968; 22: 258–73.
12. Malaise EP, Chavaudra N, Charbit A, Tubiana M. Relationship between the growth rate of human metastases, survival and pathological type. Eur J Cancer 1974; 10: 451–9.
13. Koscielny S, Lê MG, Tubiana M. The natural history of human breast cancer. The relationship between involvement of axillary lymph nodes and the initiation of distant metastases. Br J Cancer (Accepted for publication).
14. Koscielny S, Tubiana M, Lê MG, et al. Breast cancer: relationship between the size of the primary tumour and the probability of metastatic dissemination. Br J Cancer 1984; 49: 709–15.
15. Barendsen GW, Broerse H, Hermens AF, Madhuisen HJ, Van Peperzeel HA, Rutgers DH. Clonogenic capacity of proliferating and non proliferating cells of a transplantable rat rhabdomyosarcoma in relation to its radiosensitivity. J Natl Cancer Inst 1973; 51: 1521–6.
16. Tubiana M. Repopulation in human tumors. A biological background for fractionation in radiotherapy. Acta Oncol 1988; 27: 83–8.
17. Ellis F. Dose time and fractionation: clinical hypothesis. Clin Radiol 1969; 20: 1–7.
18. Tubiana M, Arriagada R, Cosset JM. Sequencing of drugs and radiation. Cancer 1985; 55: 2131–9.
19. Trott KR, Kummermehr K. What is known about tumor proliferation rates to choose between accelerated fractionation or hyperfractionation? Radiother Oncol 1985; 3: 1–9.
20. Courdi M, Malaise E. Prognostic factors of the percentage of S-phase cells. Fifth meeting of the European Society for Therapeutic Radiology and Oncology. (Abstract). Baden-Baden 1986; 177.
21. Tubiana M, Koscielny S. Cell kinetics, growth rate and the natural history of breast cancer. Eur J Cancer Clin Oncol 1988; 24: 9–14.