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Proliferation Kinetics of Malignant Non-Hodgkin's Lymphomas Related to Histopathology of Lymph Node Biopsies*

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Summary. Since lymphomas of high and low malignancy have been differentiated by the Kiel-classification, the cell kinetics of malignant lymphomas have become an interesting subject. In 20 non-Hodgkin's lymphomas we have determined the initial labelling index, the mitosis index, the proliferation rate and the durations of DNA-synthesis and mitosis. The kinetic variables have been estimated by using an in vitro-incubation of fresh biopsies and double-labelling with radioactive DNA-precursors.

In 8 lymphomas of low malignancy we found an initial labelling index of 7.8%, a mitosis index of 0.3% and a potential tumour doubling time of 100.2 h for a cell production rate without regard to cell loss. On the other hand, lymphomas of high malignancy showed a labelling index of 16.7%, a mitosis index of 0.7% and a potential tumour doubling time of 40.5 h. All these values could be differentiated with high statistical confidence. DNA-synthesis time and mitosis time of both lymphoma groups did not show any significant differences.

From these findings we obtained confirmation of the cyto-morphological principles used in classifying lymphomas.

Key words: Non-Hodgkin's lymphomas – Cell kinetics – In-vitro labelling – Double labelling.

Introduction

The proliferation kinetics of malignant lymphomas have been paid little attention when compared with morphological, histochemical and immunological research. Not until the investigations of Cooper et al. (1966), Cooper et al. (1968), Trepel and Schick (1976), Bremer and Fliedner (1979) and Murphy et al. (1979) was significant understanding of the growth behaviour of lymphomas obtained.

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Knowledge of proliferation kinetics is not only of academic interest, but provides important data in prognosis.

The classification of malignant non-Hodgkin's lymphomas by the old Rappaport system (1966), has been enlarged since well and poorly differentiated lymphomas have been graded in groups of low and high malignancy (Gérard-Marchant et al. 1974; Lennert et al. 1975) although kinetic data are missing. Clinical follow-up studies will reveal the usefulness and justification of the Kiel-classification. Initial reports are favorable (Musshoff et al. 1976; Brittinger et al. 1977; Meugé et al. 1978). These results prompt the question whether this classification mainly based on cyto-morphological criteria and immunological observations, does indeed imply differences in cell kinetics.

In lymphomas of low malignancy reduced cell elimination seems to be mainly responsible for the steady growth of tumour mass. Cell production does not necessarily have to be higher than in normal or hyperplastic lymph nodes (Craddock and Nakai, 1962; Bremer and Fliedner, 1979). On the other hand, in those highly malignant lymphomas comparable with experimental tumours or other rapidly growing human tumours, the cell production rate is definitely increased. In addition to this the death rate of lymphoma cells is also increased but clearly remains below that of the cell growth rate (Bremer and Fliedner, 1979).

The published results on kinetics of malignant lymphomas are certainly not sufficient in number to make assessments since the spectrum of subtypes of these tumours has not been recorded as systematically as their histopathology. For the time being, until an adequate number of each subtype of lymphomas are available, the data and findings of 20 lymphoma biopsies, 8 from low and 12 from high malignant tumours are presented here. We thus cover only a rather crude differentiation of two grades of malignancy. Preconditions of our kinetic research were a clear histo- and cytomorphological diagnosis according to the Kiel-classification and restriction of investigations to untreated lymphomas.

Material and Methods

Isotopes. (6-3H)-thymidine with specific activity of 5 Ci/mmol and (2-14C)-thymidine with specific activity of 50–60 mCi/mmol were used from Radiochemical Centre Amersham/England.

Human Malignant Non-Hodgkin's Lymphomas. Only untreated lymphomas excised for primary diagnosis were used for examination. Most of them were part of an additional study within the "lymphoma study group", Hannover (Georgii et al. 1979). Patients bearing the lymphomas were admitted to the clinic for abdominal surgery (director: Prof. Dr. R. Pichlmayr) and to the oto-rhino-laryngological-clinic (director: Prof. Dr. Dr. E. Lehnhardt) at the Medical School, Hannover, between June 1976 and May 1980.

Among 72 biopsies from lymph node tumours there were 30 non-Hodgkin's lymphomas. Out of them 20 lymphomas were acceptable for autoradiographic evaluation. 10 tumours had insufficient labelling or consisted only of necrotic material, some had to be excluded from further investigation because of pretreatment of patients with cytostatics, made known after incubation. The lymphomas were examined histologically at our institute by conventional staining techniques for haematologic tumours, and by additional immunologic cell characterization and electron microscopy. These

examinations were performed with further parts of the same lymph nodes which were used for cell kinetic preparations.

In-Vitro Incubation. Specimens measuring 5 to 10 mm in diameter and 2 to 3 mm thickness were excised from the lymph node tumours in the operating room and cut into pieces of 1 mm in maximum diameter. For transport the material was kept in Eagle's Medium M 199 (Fa. Serva, Heidelberg) with constant temperature of 37° C. Time elapse between excision and incubation was restricted to 10 min.

Incubation of tissue was performed in a shaking water bath under high pressure (2.2 atm) of carbogen gas with a constant temperature of 37° C and shaking frequency of 120/min. The incubation apparatus was a modification of that used by Helpap and Stiens (1974). For details see Kienzle (1980).

The tissue was incubated for the first hour with 2 µCi 14C-thymidine/4 ml Eagle's Medium. Thereafter it was carefully washed with fresh medium and then reincubated with 20 µCi 3H-thymidine/4 ml medium for another 2 h. After this procedure the tissue was again washed and fixed in buffered formalin for 48 h.

The incubation method including double-labelling with radioisotopes was established in earlier experiments (Lang et al. 1980, in press) using 23 Polyoma-virus induced renal sarcomas of rats for comparative in-vivo and in-vitro examinations.

Histoautoradiography. After the usual histological preparation and embedding in paraplast the small tissue pieces were cut into 12 to 15 step sections which had a thickness of 3 to 4 µ each and were mounted with an AR 10 stripping film (fa. Kodak, Stuttgart). Following a 21-day exposure the slides were developed, stained with an aqueous solution of Haemalaun and mounted with Cedax and commercial cover slips.

Evaluation of Histoautoradiograms. Microscopic examination was done using 100-fold objective-magnification with oil-immersion and 10-fold ocular-magnification with an ocular grid (fa. Zeiss). Details of subtraction of non-specific back-ground labelling and counting procedure was described by Kienzle (1980). 1.000 cells per tumour were counted as a minimum by two observers, independently. Within the lymphomas the following characteristics were registered: total number of lymphoid cells separated into small lymphocytes and blasts; cells labelled with 14C-thymidine together with or without 3H-thymidine; cells exclusively labelled with 3H-thymidine; mitoses.

Method of Calculating Kinetic Variables. From the results of cell counting the following variables were calculated (Pilgrim and Maurer 1965):

1. Labelling index (LI): $\mathrm{LI} = \frac{N(14C)}{N(\mathrm{total})}$

2. Mitotic index (MI): $\mathrm{MI} = \frac{N(M)}{N(\mathrm{total})}$

3. DNA-synthesis time (T_S): $T_S = \frac{N(14C) \times \Delta T}{N(3H)}$

4. Duration of mitosis (T_M): $T_M = \frac{N(M) \times \Delta T}{N(3H)}$

5. Potential tumour doubling time (T_{dpot}): $T_{dpot} = \frac{T_C}{PF} = \frac{N(\mathrm{total}) \times T_S}{N(14C)}$

Explanation of abbreviations:

N (14C): number of cells labelled with 14C-thymidine with or without 3H-thymidine
N (3H): number of cells labelled exclusively with 3H-thymidine
N (total): all cells counted with or without label
$N(M)$: number of mitosis
PF: proliferative fraction
ΔT: time interval between 14C and 3H-labelling.

Table 1. 30 non-Hodgkin's lymphomas were received for evaluation with double labelling in-vitro. 20 tumours had sufficient label, 10 tumours showed too much necrosis or had insufficient label. Accurate histological diagnosis was made in different specimens of same lymph node tumours according to the Kiel-classification

Histology of lymphomas	Double labelling	
	Evaluated	Not evaluable
Lymphocytic	3	1
Immunocytic	–	1
Centrocytic	1	–
Centroblastic/centrocytic	4	2
Centroblastic	2	3
Burkitt-type	2	1
Lymphoblastic	4	1
Immunoblastic	4	1
Total	20	10

Table 2. Kinetic parameters of the low malignant group of non-Hodgkin's lymphomas by evaluation of an in-vitro double labelling technique.

Histological type	Kinetic parameters of non-Hodgkin's lymphomas – low malignancy –				
	LI_{14C} (%)	MI (%)	T_S (h)	T_M (h)	T_{dpot} (h)
Lymphocytic	3.1[a]	0.1	7.0	0.2	227.4
	4.0[a]	0.2	6.5	0.3	162.8
	5.4[a]	0.2	5.7	0.2	106.2
Centrocytic	7.2[a]	0.3	5.8	0.2	81.4
Centroblastic/centrocytic	9.7	0.4	4.5	0.2	46.2
	10.4	0.6	6.7	0.4	64.3
	11.5	0.5	5.8	0.3	50.2
	10.8	0.3	6.8	0.2	62.9

[a] Over-all labelling index

LI_{14C}: Initial labelling index. In lymphocytic and centrocytic lymphomas an "over-all" index was given including all unlabelled small lymphocytes. In centroblastic-centrocytic lymphomas a "tumour" index was evaluated regarding only lymphoblasts. MI: mitotic index. T_M: mitotic time. T_S: DNA-synthesis time. T_{dpot}: potential tumour doubling time

Results

The 20 evaluable lymphomas consisted of two groups, the first one containing 8 lymphomas of low malignancy and a second group with 12 highly malignant tumours following the Kiel-classification (see Table 1). In Tables 2 and 3 the

Table 3. Kinetic parameters of the high malignant group of non-Hodgkin's lymphomas by evaluation of an in-vitro double labelling technique

Histological type	Kinetic parameters of non-Hodgkin's lymphomas – low malignancy –				
	LI_{14C} (%)	MI (%)	T_S (h)	T_M (h)	T_{dpot} (h)
Centroblastic	16.8	0.8	5.9	0.3	36.4
	14.1	1.0	6.0	0.5	42.7
Burkitt-type	22.6	1.2	6.8	0.4	30.2
	20.8	0.7	8.8	0.3	42.2
Lymphoblastic	19.3	0.5	6.5	0.3	34.4
	10.4	0.7	5.2	0.3	52.0
	17.6	0.7	6.7	0.3	40.0
	18.8	0.5	8.2	0.2	43.7
Immunoblastic	15.1	0.7	5.9	0.3	39.1
	13.5	0.6	5.9	0.3	46.5
	13.3	0.5	5.6	0.2	44.5
	18.8	0.7	6.4	0.3	34.0

LI_{14C}: initial labelling index regarding only lymphoblasts excluding small lymphocytes. MI: mitotic index. T_M: mitotic time. T_S: DNA-synthesis time. T_{dpot}: potential tumour doubling time

results of the initial labelling index (14C-index), the mitotic index, the calculated DNA-synthesis time, the mitotic time and the potential tumour doubling time for the individual lymphomas separated into classes corresponding to the cytomorphological diagnosis are given. For the lymphocytic and centrocytic lymphomas the initial labelling index (3.1 to 7.2%) is given as an "over-all" index which means the labelled blast-cells in relation to the total number of lymphocytic cells. For all other lymphomas the 14C-index (9.7 to 22.6%) means a "tumour cell index" regarding only the population of blasts. The mitotic index was found to be roughly 20% of the labelling index in most cases with an absolute range between 0.1 and 1.2%. The duration of DNA-synthesis showed no remarkable spread of values within the total number of lymphomas, ranging between 4.5 and 8.2 h. The mitotic time amounted to 0.2 up to 0.5 h. For both DNA-synthesis time and the duration of mitosis no significant dependence was observed on the type of lymphoma. In contrast, the duration of the potential tumour doubling time showed an obvious tendency towards prolongation in lymphomas with low labelling index. The total range was found between 227.4 and 30.2 h.

Table 4 shows the kinetic variables given as mean values for the 8 lymphomas of low malignancy and the 12 lymphomas of high malignancy. Within the first group the results were calculated additionally after excluding lymphocytic lymphomas with their extremely low proliferative activity. The mean value of the initial labelling index was 7.8% for the low-malignancy group, the mitotic index 0.3%. The DNA-synthesis time was 6.1 h, the mitotic time 0.2 h. Assuming no cell loss the tumour doubling time amounted to 100.2 h for all 8 lymphomas and

Table 4. Mean values and standard deviations (sd) of kinetic parameters in 8 lymphomas of low malignancy and 12 lymphomas of high malignancy. First column in the low malignant group includes lymphocytic, centrocytic and centroblastic-centrocytic lymphomas. Second column excludes values of lymphocytic lymphomas. The difference of values was statistically significant for initial labelling index (LI_{14C}), mitotic index (MI) and potential tumour doubling time (T_{dpot}) with a high confidence limit. DNA-synthesis time and duration of mitosis did not show a statistically significant difference

Kinetic parameters		Non-Hodgkin's lymphomas			
		Low malignancy ($N=8$)		– Difference –	High malignancy ($N=12$)
LI_{14C}	(%) mean	7.8	9.7[a]	significant	16.7
	(sd)	(3.3)	(1.8)	$P<0.005$	(3.54)
MI	(%) mean	0.3	0.3[a]	significant	0.7
	(sd)	(0.16)	(0.08)	$P<0.005$	(0.21)
T_S	(h) mean	6.1	5.9[a]	not significant	6.5
	(sd)	(0.83)	(0.92)		(1.04)
T_M	(h) mean	0.2	0.2[a]	not significant	0.3
	(sd)	(0.06)	(0.07)		(0.06)
T_{dpot}	(h) mean	100.2	61.0[a]	significant	40.5
	(sd)	(63.8)	(13.8)	$P<0.025$	(6.07)

[a] Lymphomas of low malignancy without lymphocytic lymphomas

61.0 h without lymphocytic lymphomas. On the other hand, the highly malignant lymphomas had an initial labelling index of 16.7%, mitotic index of 0.7%, DNA-synthesis time of 6.5 h and a mitotic time of 0.3 h. The potential tumour doubling time was 40.5 h. A statistical test showed highly significant differences between labelling index, mitotic index and doubling time of both groups. Even if lymphocytic lymphomas were separated the differences remained significant. There was no statistical difference between DNA-synthesis time and mitotic time of both groups of lymphomas.

Discussion

The intention of our studies on non-Hodgkin's lymphomas was to explore whether support for the prognostic implications of the Kiel-classification with its two main groups of low and high malignancy, could be found by comparing their proliferation kinetics. Our results seem to confirm this view: significant differences among the main variables of cell proliferation could be seen between the two lymphoma groups. In contrast, DNA-synthesis time and mitosis time did not differ significantly. This latter finding was in close agreement with the constancy of these cycle-phase times reported for many tumours growing at different growth rates (Steel 1977; Bremer and Fliedner 1979).

A detailed interpretation of the autoradiographic findings and their kinetic indications which were obtained by the double-labelling method will be given in a separate communication (Lang et al. 1980, in press). It will also be demon-

strated that the incubation method of biopsies yields reliable results, as shown by the comparison of in-vivo and in-vitro labelling in an experimental tumour used in our laboratory (Zobl et al. 1975; Lang et al. 1978).

Ignoring the proliferative fraction of lymphoma cells, which may lie between 50 and 100% in highly malignant lymphomas (Trepel 1972; Ivesen et al. 1974; Trepel and Schick 1976; Bremer and Fliedner 1979) an "over-all" generation time or potential tumour doubling time (Steel and Bensted 1965) was calculated from the values of labelling index and DNA-synthesis time. This means a cell production rate which may be regarded as an important kinetic variable for two reasons:

1. It characterizes the degree of proliferation of a lymphoma which may, therefore, also reflect its malignancy in some respect.

2. It allows the estimation of cell loss, if it is related to the actual tumour growth rate (as far as the increasing size of a lymphoma may be directly observed).

The basic variable measured initially by most authors is the "initial" labelling index, which is equivalent to a pulse labelling index in-vivo (Hilscher and Maurer 1962; Rajewski 1965; Helpap and Maurer 1969). These labelling indices are registered either as tumour- or over-all indices. With the exception of lymphocytic and purely centrocytic lymphomas the quoted values are usually tumour indices and relate to the population of blast-cells which are assumed to be the potentially proliferative cells (Craddock and Nakai 1962; Clarkson et al. 1965; Gavosto et al. 1967; Saunders et al. 1967; Schiffer 1971; Bremer and Fliedner 1979) and disregards small lymphocytic cells. In highly differentiated lymphomas of lymphocytic or centrocytic type such a tumour labelling index relating only to the relatively small subpopulation of blasts might give a realistic idea of the proliferative rate of these blast-cells. However, it would not allow an estimation of the potential tumour doubling time of the entire population.

In the relevant literature over-all indices for lymphocytic and centrocytic lymphomas are reported between 0.05 and 25% (Craddock and Nakai 1962; Peckham and Cooper 1970; Silvestrini et al. 1977; Silvestrini et al. 1978; Nishikori et al. 1978; Foadi et al. 1979) and our own results are within this range (3.1 to 5.4%). An explanation for the wide spread of values according to our observation may be that at least two of the four highly differentiated lymphomas showed a very irregular distribution of label, so we had to count very high cell numbers to get a valid over-all index.

The indices are generally higher in the centroblastic-centrocytic lymphomas where the proportion of centroblasts seems to be decisive for the rate of proliferation (Silvestrini et al. 1977; Silvestrini et al. 1978). Yet even for the centroblastic subpopulation the indices may lie between 5 and 27% (Peckham and Cooper 1970). Nevertheless, an increased proliferation rate may be assumed for those centroblastic-centrocytic lymphomas in contrast with highly differentiated lymphomas. This was also supported by a relatively high tumour labelling index (10 to 12%) in our 4 cases.

The results in the group of high malignancy show a greater uniformity when the published data – including our own values – are compared. This is understandable because the cell population under study consists almost exclu-

sively of blast-cells. Therefore, there are far fewer difficulties in the interpretation of autoradiograms. Our results agree very well with those of Klein et al. (1972) in lymphoblastic lymphomas, and those of Ivesen et al. (1974) in Burkitt-lymphomas.

The statement that highly malignant lymphomas have, on average, a substantially shorter potential doubling-time (40 h) than lymphomas of low malignancy (100 h) is crucial. This difference stays significant even if the lymphocytic lymphomas are excluded from calculation.

Comparison of published data on kinetics of lymphomas demands a series of critical reflections on the subject. Varying histological classifications often hamper a clear determination of the lymphoproliferative disease under consideration. Often it is not specified whether the results include treated lymphomas, whose kinetics may change considerably in comparison with the untreated tumours (Saunders et al. 1967; Sheehy et al. 1975). The methodical conditions used to examine a lymphoma also have a decisive influence on its rate of proliferation. The growth kinetics of solid lymphomas cannot be compared easily with those of the cell cultures which were studied by Smith et al. (1970), Burk et al. (1978), Drewinko et al. (1978) and Drewinko et al. (1979). Moreover, it seems doubtful whether preparing a cell suspension from a biopsy before incubation with radionuclids (Cooper et al. 1968; Silvestrini et al. 1977; Silvestrini et al. 1978) will provide similar results to a direct incubation of fresh biopsy material. Experience with experimental tumours definitely points to the fact that reliable in-vitro labelling is only obtained under strict observation of clearly defined conditions (Rajewski 1966; Lennartz et al. 1971; Klein et al. 1972; Helpap and Stiens 1974). From this it is undestandable that a lower mitotic index was found after incubation of biopsy particles without hyperbaric oxygen pressure and temperature regulation, compared with tissue examined immediately after excision (Camplejohn and Aherne 1974). Extremely short incubation times (Cooper et al. 1968) might also influence the labelling index artificially.

The observed differences of proliferation within the same type of lymphoma may be explained by several facts:

1. Some lymphomas put into one group by cyto-morphological classification actually consist of different cell populations. This is especially true for immunocytomas (Silvestrini et al. 1977; Silvestrini et al. 1978) and centroblastic-centrocytic lymphomas (Clarkson et al. 1965; Peckham and Cooper 1970; Nishikori et al. 1978).

2. Growth rate seems to be dependent on tumour size. Therefore, bigger lymphomas show a lower labelling index than smaller ones (Drewinko et al. 1974; Iversen et al. 1974; Sheehy et al. 1975). Here one may see an analogy with other solid human tumours whose growth rate declines with increasing tumour mass (Steel and Lamerton 1966).

3. Proliferative activity of tumour cells and labelling index respectively may vary within the same lymphoma as in most other solid tumours (Klein et al. 1972; Denekamp and Kallman 1973; Livingston et al. 1974). In recent years

this phenomenon has been shown in renal carcinomas in man (Rabes et al. 1978).

4. Often the labelling index is given as an over-all index in blastic lymphomas and thus does not only refer to the production rate of blasts but also to the extent of infiltration of neoplastic cells within the lymph node (Cooper et al. 1968; Saunders et al. 1967).

5. The labelling index is known to be dependent on the localization of tumour cells. The highest values have been found by most investigators within the lymph nodes or within the bone marrow (Clarkson et al. 1965; Drewinko and Alexanian 1977; Foadi et al. 1968; Keiser et al. 1968; Mauer and Fisher 1962; Murphy et al. 1979).

Despite these limitations our findings show clearly that the differentiation of lymphomas into groups of high and low malignancy can be achieved by a simple determination of the initial labelling index. In this we completely agree with Silvestrini et al. (1977), Silvestrini et al. (1978) and Durie et al. (1980).

Arguments against the diagnostic value of the initial labelling index (Cooper et al. 1968) are based on the fact that equally high indices occur in blast-cells of normal lymph nodes to those found in malignant lymphomas. The blast-indices in normal lymph nodes or in the spleen (Pabst and Reinecke 1980) may even be higher than in blastic lymphoma cells. This only means that on grounds of the labelling index alone, no differentiation can be made between hyperplasia and malignancy. From a kinetic point of view the diagnosis of malignancy would only be achievable with the knowledge of cell loss or rate of elimination, in addition to the cell production rate.

An increase in the number of cases available for kinetic research and prospective evaluation of clinical follow-up will decide the relevance of these preliminary results.

References

Bremer K, Fliedner TM (1979) Kinetik normaler und pathologischer Lymphocyten. In: Stacher A, Höcker P (eds) Lymphknotentumoren. Urban und Schwarzenberg, Wien, p 13–19

Brittinger G, Bartels H, Bremer K, Burger A, Duhmke E, Gunzer U, König E, Stacher A, Stein H, Theml H, Waldner R (1977) Retrospektive Untersuchungen zur klinischen Bedeutung der Kiel-Klassifikation der malignen Non-Hodgkin-Lymphome. Strahlentherapie 153:222–228

Burk KH, Drewinko B, Trujillo JM, Ahearn MJ (1978) Establishment of a human plasma cell line in vitro. Cancer Res 38:2508–2513

Camplejohn RS, Aherne WA (1974) In vitro labelling of childhood cancers with tritiated thymidine. Br J Cancer 29:487–489

Clarkson B, Ota K, Ohkita T, O'Connor A (1965) Kinetics of proliferation of cancer cells in neoplastic effusions in man. Cancer 18:1189–1212

Cooper EH, Frank GL, Wright DH (1966) Cell proliferation in Burkitt tumours. Eur J Cancer 2:377–384

Cooper EH, Peckham MJ, Millard RE, Hamlin IME, Gérard-Marchant R (1968) Cell proliferation in human malignant lymphomas. Eur J Cancer 4:287–296

Craddock C, Nakai G (1962) Leukemic cell proliferation as determined by in-vitro deoxyribonucleic acid synthesis. J Clin Invest 41:360–369

Denekamp J, Kallman RF (1973) In-vitro and in-vivo labelling of animal tumours with tritiated thymidine. Cell Tissue Kinet 6:217–227

Desselberger U, Georgii A, Zobl H, Ostertag H (1972) Zur onkogenen Aktivität des Polyomavirus bei der Ratte. Zentralbl Bakt I Abt Orig A 219:28–38

Drewinko B, Brown BW, Humphrey R, Alexanian R (1974) Effect of chemotherapy on the labelling index of myeloma cells. Cancer 34:526–531

Drewinko B, Alexanian R (1977) Growth kinetics of plasma cell myeloma. J Natl Cancer Inst 58:1247–1253

Drewinko B, Bobo B, Roper PR, Malahy MA, Barlogie B, Jansson B (1978) Analysis of the growth kinetics of a human lymphoma cell line. Cell Tissue Kinet 11:177–191

Drewinko B, Barlogie B, Mars W, Malahy MA, Jansson B (1979) Proliferative characteristics of ARH-77 cells, an established human IgG-producing myeloma cell line. Cell Tissue Kinet 12:675

Durie BGM, Salmon SE, Moon TE (1980) Pretreatment tumor mass, cell kinetics, and prognosis in multiple myeloma. Blood 55:364–372

Foadi MD, Cooper EH, Hardisty RM (1968) Proliferative activity of leukaemic cells at various stages of acute leukaemia of childhood. Br J Haematol 15:269–281

Foadi MD, Slater AM, Pegrum GD (1979) Two distinctive lymphocyte populations in chronic lymphocytic leukaemia. Eur J Cancer 15:1295–1304

Gavosto F, Pileri A, Gabutti V, Masera P (1967) Cell population kinetics in human acute leukaemia. Eur J Cancer 3:301–307

Georgii A, Krmpotic E, Stünkel K, Thiele J, Vykoupil K-F, Diehl V (1979) Verwendung der Kiel-Klassifikation unter gleichzeitigem Einsatz von Zytogenetic, Membranmarkern und Elektronenmikroskopie. In: Stacher A, Höcker P (eds) Lymphknotentumoren. Urban & Schwarzenberg, München-Berlin-Wien, p 82–90

Gérard-Marchant R, Hamlin I, Lennert K, Rilke F, Stansfeld AG, Unnik JAM van (1974) Classification of non-Hodgkin's lymphomas. Lancet II:406–408

Helpap B, Maurer W (1969) Autoradiographische Untersuchung zur Frage der Vergleichbarkeit des Einbaus von markiertem Thymidin unter in vivo-Bedingungen und bei Inkubation von Gewebsproben. Virchows Arch Abt B Zellpath 4:102–118

Helpap B, Stiens R, Grouls V (1973) Autoradiographische Untersuchungen zum Thymidin-Einbau nach mehrstündiger Inkubation von Rattenlebern. Histochemie 34:305–316

Helpap B, Stiens R (1974) Die Bedeutung der Zellkinetik bei der Behandlung maligner Tumoren. Dtsch Med Wochenschr 99:1815–1820

Hilscher W, Maurer W (1962) Autoradiographische Bestimmung der Dauer der DNS-Verdopplung und ihres zeitlichen Verlaufs bei Spermatogonien der Ratte durch Doppelmarkierung mit C-14- und H-3-Thymidin. Naturwissenschaften 49:352

Hirst DG, Denekamp J (1979) Tumour cell proliferation in relation to the vasculature. Cell Tissue Kinet 12:31–42

Iversen U, Iversen OH, Bluming AZ, Ziegler JL, Kyalwasi S (1972) Cell kinetics of African cases of Burkitt lymphoma. Eur J Cancer 8:305–308

Iversen OH, Iversen U, Ziegler JL, Bluming AZ (1974) Cell kinetics in Burkitt lymphoma. Eur J Cancer 10:155–163

Keiser G, Wagner HP, Frey-Wettstein M (1968) Cytokinetic studies in two acute lymphoblastic leukemias. Helv Med Acta 34:155–169

Kienzle S (1980) Proliferationskinetische Untersuchung mit radioaktiver Doppelmarkierung unter in-vitro Inkubation an humanen Non-Hodgkin-Lymphomen. Dissertation, Hannover

Klein HO, Lennartz KJ, Eder M, Gross R (1970) In vitro-Verfahren zur autoradiographischen Bestimmung der Zellkinetik der Erythroblasten bei Tier und Mensch. Histochemie 21:369–382

Klein HO, Lennartz KJ, Gross R, Eder M, Fischer R (1972) In-vivo- und in-vitro-Untersuchungen zur Zellkinetik und Synchronisation menschlicher Tumorzellen. Dtsch Med Wochenschr 97:1273–1282

Lang W, Zobl H, Georgii A (1978) The effect of neonatal thymectomy on the growth kinetics of a virus-induced renal sarcoma of the rat. Eur J Cancer 14:431–437

Lennartz KJ, Klein HO, Féaux de Lacroix W, Klein PJ (1971) Vergleichende in vivo- und in vitro-Untersuchung der Zellkinetik experimenteller Tumoren und die Bestimmung des Generationszyklus von Tumorzellen des Menschen in vitro. Verh Dtsch Ges Pathol 55:591–596

Lennert K, Mohri N, Stein H, Kaiserling E (1975) The histopathology of malignant lymphoma. Br J Haematol 31 (Suppl. II):29–43

Livingston RB, Ambus U, George SL, Freireich EJ, Hart J (1974) In-vitro determination of thymidine-3H-labelling index in human solid tumours. Cancer Res 34:1376–1380

Mauer AM, Fisher V (1962) Comparison of the proliferative capacity of acute leukaemia cells in bone marrow and blood. Nature 193:1085–1086

Maurer W, Schultze B (1968) Überblick über autoradiographische Methoden und Ergebnisse zur Bestimmung von Generationszeiten und Teilphasen von tierischen Zellen mit markiertem Thymidin. Acta Histochem. (Suppl.) 8:73–87

Meungé A, Hoerni B, de Mascarel A, Durand M, Richand P (1978) Non-Hodgkin's malignant lymphomas. Clinico-pathologic correlations with the Kiel-classification. Eur J Cancer 14:587–592

Murphy SB, Melvin SL, Mauer AM (1979) Correlation of tumor cell kinetic studies with surface marker results in childhood non-Hodgkin's lymphoma. Cancer Res 39:1534–1538

Musshoff K, Schmidt-Wollmer H, Leimert K, Sandritter W (1976) Preliminary clinical findings on the Kiel-classification of malignant lymphomas. Z Krebsforsch 87:229

Nishikori M, Fukuhara S, Tatsumi E, Domae N, Takiuchi Y, Uehara N, Shirakawa S, Uchino H (1978) The pattern of cell growth in human malignant lymphoma – Determination of labelling index and surface membrane markers as a indicator of cell proliferation. Acta Haematol Jap 41:867–873

Pabst R, Reinecke G (1980) Lymphocyte proliferation in isolated perfused human spleens. Cell Tissue Kinet 13:209

Peckham MJ, Cooper EH (1970) The pattern of cell growth in reticulum cell sarcoma and lymphosarcoma. Eur J Cancer 6:453–463

Pilgrim Ch, Maurer W (1965) Autoradiographische Untersuchung über die Konstanz der DNS-Verdopplungs-Dauer bei Zellarten von Maus und Ratte durch Doppelmarkierung mit 3H- und 14C-Thymidin. Exp Cell Res 37:183–199

Rabes HM, Carl P, Meister P (1978) Kartierung der Wachstumsstruktur menschlicher Nierencarcinome. Verh Dtsch Ges Pathol 62:336

Rajewski MF (1965) Thymidin-Inkorporation und O_2-Konzentration in Explantaten normaler und maligner Gewebe "in vitro". Naturwissenschaften 52:341–342

Rajewski MF (1966) Zellproliferation in normalen und malignen Geweben: 3H-Thymidin-Einbau in vitro unter Standardbedingungen. Biophysik 3:65–93

Rappaport H (1966) Tumors of the hematopoietic system. Atlas of tumor pathology, Section 3 Fasc 8. Armed Forces Institute of Pathology, Washington, DC

Saunders EF, Lampkin BD, Mauer AM (1967) Variation of proliferative activity in leukemic cell populations of patients with acute leukemia. J Clin Invest 46:1356–1363

Schiffer LM (1971) Observations on the in-vitro measurement of human lymphocyte DNA synthesis time. Cell Tissue Kinet 4:597–599

Sheehy PF, Fried J, Dowling MD, Clarkson BD (1975) Studies of cellular proliferation in human leukemia. Cancer 36:203–210

Silvestrini R, Piazza R, Riccardi A, Rilke F (1977) Correlation of cell kinetic findings with morphology of non-Hodgkin's malignant lymphomas. J Natl Cancer Inst 58:499–504

Silvestrini R, Costa A, Daidone MG, Rilke F (1978) Prognostic significance of the labeling index in non-Hodgkin's human malignant lymphomas. Antibiotics Chemother 24:105–111

Smith H, Murray D, Gökcen M (1970) Life cycle of lymphoid cells in vitro. Europ J Cancer 6:269–271

Steel GG, Bensted JPM (1965) In vitro studies of cell proliferation in tumours – I. Eur J Cancer 1:275–279

Steel GG, Lamerton LF (1966) The growth rate of human tumours. Br J Cancer 20:74–86

Steel GG (1968) Cell loss from experimental tumours. Cell Tissue Kinet 1:193–207

Steel GG (1977) Growth kinetics of tumors. Clarendon Press, Oxford, p 146–150

Trepel F (1972) Zellproliferation in malignen Lymphomen. In: Stacher A (ed) Leukämien und maligne Lymphome. Urban & Schwarzenberg, München-Berlin-Wien, p 212–218

Trepel F, Schick P (1976) Proliferation und Wachstum von lymphatischen Zellpopulationen bei malignen Lymphomen. Hämatologie und Bluttransfusion 18 (Sonderband zu Blut), 33–47

Tsirimbas AD, Wittmann K, Dörmer P (1970) Zellkinetik in Lymphdrüsen – I. Klin. Wochenschr. 48:923–929

Zobl H, Lang W, Georgii A (1975) Proliferation kinetics of a virus-induced sarcoma in the rat kidney. Eur J Cancer 11:159–167 (1975)

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