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Clinical Relevance of Markers of Cell Proliferation in Human Lymphoid Malignancies: A Concise Review

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Abstract—*The major advances being made in the understanding of the biology of human lymphoid malignancies have shown these to be a heterogenous group of tumours with respect to a variety of biological markers. The cell proliferative rate, an important determinant of tumor aggressiveness and response to therapy, is one of the biological phenomena currently being investigated in the lymphoid malignancies, particularly in the non-Hodgkin's lymphomas. In this paper we describe the techniques used in the analysis of cell proliferation in the lymphoid malignancies, and review the patterns of cell proliferation found in the various types of these tumours and the clinical relevance of these findings. We indicate that differences in cell proliferative rate are an important determinant of the response of non-Hodgkin's lymphomas to current therapeutic modalities and may explain the paradox that a significant number of reputed unfavorable tumors are now curable. Finally, we suggest that inherent differences in the proliferative rate of the neoplastic cell(s) of the so-called favorable histological types of non-Hodgkin's lymphomas may determine histologic progression as well as therapeutic responsiveness.*

INTRODUCTION

IN THE past decade, major advances have been made in our understanding of the pathogenesis and pathology of lymphoid malignancies, particularly of the non-Hodgkin's lymphomas [1, 2]. The advances have been associated with the development of a number of classifications of the non-Hodgkin's lymphomas, and several schemes are currently in use [3]. Despite the overall clinical value of the various classifications, uncertainties such as the reproducibility of histopathologic diagnoses and the heterogeneity in biological behavior of tumors of the same subgroup have led to the proposal of other prognostic parameters. These have included subtle histological features, tumor size, clinical stage, specific organ involvement, immunologic markers, serum lactate dehydrogenase and plasma glycosyl transferase activities [3–9]. However, the clinical relevance of the majority of these parameters remains to be clearly established. Furthermore, a paradox is evident when recent results of the treatment of non-Hodgkin's lymphomas are considered, as the clinically aggressive large-cell lymphomas have been found to be highly responsive to therapy, with a significant number of apparent cures, while complete remission is unusual for histological types associated with an indolent clinical course [2].

Cell proliferative rate is thought to be an important determinant of the response of tumors to therapy [10, 11]. Rapidly growing tumors are associated with an aggressive clinical course but sensitive to therapy, while slowly-growing tumors generally have an indolent clinical course and are less responsive to therapy [11]. Assessment of cell proliferation in the lymphoid malignancies has largely been based upon tumor doubling times [11], and histological criteria such as cell size and the presence of mitotic figures [12–14]. Recently, a number of different probes of cell proliferation, including (i) thymidine radiolabeling of deoxyribonucleic acid, (ii) flow

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cytometric analysis of cell size and deoxyribonucleic acid profile and (iii) enzyme markers, have been applied to the analysis of cell proliferation in these diseases.

In this review, the data obtained from these studies will be examined and, where appropriate, related to current pathologic and immunologic observations of the lymphoid malignancies, particularly the non-Hodgkin's lymphomas. In addition, emphasis will be given to the implications these new insights may have for the design of therapeutic regimes for these diseases.

THYMIDINE RADIOLABELING OF DEOXYRIBONUCLEIC ACID

The incorporation of radiolabels [^{14}C]- and [^{3}H]-thymidine into deoxyribonucleic acid during the S-phase (deoxyribonucleic acid synthesis) of the cell cycle has been widely used as a marker of cell proliferation [10]. Early studies of cell proliferation in non-Hodgkin's lymphomas used autoradiography to measure this parameter in single-cell suspensions and showed considerable variation in the percentage (range 5.4–48.0) of radiolabeled cells (labeling index), but clinicopathologic correlates were not evident [15, 16].

Using either autoradiography or liquid scintillation counting to measure the radiolabeling of deoxyribonucleic acid, several recent studies have confirmed the initial observation that the proliferative rate of non-Hodgkin's lymphomas varies considerably [17–25]. In addition, these studies have also detailed differences in thymidine radiolabeling between the categories of current histologic classifications, thus enabling clinicopathologic correlations to be made. Histological types of non-Hodgkin's lymphoma known to be of high-grade malignancy consistently showed a range of high uptakes of thymidine [17–24], with the highest values being found for Burkitt's tumor [17, 18, 20, 21, 24]. By contrast, there was heterogeneity in the radiolabeling of tumors included in the category of low-grade malignancy, with values ranging from low (labeling index less than 4%) to high levels [18, 19, 21–24]. The clinical relevance of differences in the thymidine radiolabeling of non-Hodgkin's lymphomas was emphasized by one study which showed that within the established prognostic groups of nodular and diffuse histology, an inverse relationship exists between labeling index and survival [23]. Furthermore, this finding was not significantly related to other prognostic factors such as the pathological stage or the absence or presence of systemic symptoms. Of particular interest was that this independent prognostic capability of the labeling index included tumor types of reputedly favorable prognosis, with high values being associated with a significant decrease in survival. In a study of the cell kinetics of bone marrow blasts [25] from childhood lymphoid malignancies, the labeling index was found to have clinicopathological relevances. The marked variability in the labeling index (range 0.9–60.0%) related to immunologic markers of cell origin, with neoplastic cells of B cell origin having the highest proliferative rate (mean labeling index 42%), while non-T, non-B acute lymphoblastic leukemia blast cells had the lowest labeling index (mean 4.8%). The mean labeling index of T leukemic blasts was intermediate (11.6%). These findings correlate with the known clinical behavior of the various immunologic tumor types as B cell tumors have the worst prognosis of the childhood lymphoid malignancies, while in contrast, non-T, non-B acute lymphoblastic leukemia has the longest survival and T cell tumors have an intermediate prognosis [25]. These data suggest that in childhood lymphoid malignancies neoplastic cell kinetic findings are of prognostic relevance.

Similar findings to these from childhood lymphoid malignancies have been recorded for adult acute lymphoblastic leukemia [26]. Bone marrow leukemic blast cells exhibit a wide range of labeling indices (2–52%), with B cell tumors having the highest proliferative rate and non-T, non-B cells the lowest. The mean proliferative rate of T leukemic blasts was just above that of non-T, non-B cells. Furthermore, the labeling index has been found to be an important determinant of the response to chemotherapy, with lymphoblasts having a high labeling index (greater than 9%) responding more favorably to chemotherapy than those with low values [26, 27]. However, paradoxically, those with low labeling indices have a longer period of remission [27], suggesting that current antileukemic regimes are inadequate for more rapidly proliferating lymphoblasts.

The prognostic value of the labeling index in non-Hodgkin's lymphomas have been amplified by the recent findings in chronic lymphocytic leukemia and multiple myeloma of a close relationship between the labeling index of the neoplastic cells and prognosis, with increased thymidine radiolabeling being an independent marker of reduced survival [28–30].

FLOW CYTOMETRIC ANALYSIS OF CELL SIZE AND DEOXYRIBONUCLEIC ACID CONTENT

Measurement of cell volume by the Coulter volume method and flow cytometric analysis of cell deoxyribonucleic acid content have been used in several recent studies of cell proliferation in

single-cell suspensions derived from non-Hodgkin's lymphomas [24, 31–36].

Cell size is thought to reflect the proliferative state of lymphocytes [33] and is a major determinant of the various categories of current classifications of non-Hodgkin's lymphomas [37, 38]. In keeping with cytological features, Coulter volume studies have found in the main low cell volumes ($<250\ \mu m^3$) for tumors of favorable histological type and generally elevated values for non-Hodgkin's lymphomas of unfavorable type [24, 31]. However, within the various types of unfavorable tumors considerable heterogeneity was found in the modal Coulter volume values which was most prominent amongst the diffuse large-cell lymphomas [24]. These data suggest that cell volume determination may have clinical value, particularly in identifying the subtypes of diffuse large-cell lymphomas, which are known to have different clinical patterns [39, 40].

Flow cytometric analysis of cellular deoxyribonucleic acid content have produced results consistent with those of Coulter volume studies, with tumors of favorable prognosis generally containing a small proportion of proliferating cells while those of unfavorable prognosis possess a larger fraction of cells in S-phase, but with a wide range of individual values in both groups [31–34].

In one study [33] concurrent flow cytometric analysis of Coulter volume and cell deoxyribonucleic acid content supported a direct relationship between cell size and proliferative rate for non-Hodgkin's lymphomas of B lymphocyte origin, and amongst kinetically different subpopulations of the same tumor. Burkitt's tumor diverged from this correlation, as although several studies have shown the cells of this tumor to have the highest proliferative rate [17, 18, 20, 21, 24], mean G1 Coulter volumes were found to be less than more slowly proliferating large-cell lymphomas [33]. Other biological factors also appear to influence cell size in T cell lymphomas, as although a relationship between cell size and proliferative rate was found within individual tumor samples, a direct correlation was absent for this group of lymphomas [33].

The potential clinical value of deoxyribonucleic acid histograms produced by flow cytometry has been emphasized by a study which correlated the proliferative rate of non-Hodgkin's lymphoma cells with clinicopathologic findings and response to treatment [34]. Advanced non-Hodgkin's lymphomas of unfavorable prognosis with 5% or more cells synthesizing deoxyribonucleic acid showed a significantly greater number of complete responses to chemotherapy than similar tumors with a lower fraction of proliferating cells. However, complete responses obtained in the latter group were more durable, which may reflect in part the regime of chemotherapy used. This study could not adequately assess the prognostic relevance of proliferative activity in the favorable tumors.

In three recent studies [33, 35, 36] flow cytometric analysis of cell deoxyribonucleic acid has demonstrated aneuploidy in almost 50% of large cell lymphomas of B cell type, and in a significantly lesser percentage of T cell lymphomas and favorable histological types. Aneuploid cell populations were most commonly hyperdiploid and usually mixed with diploid populations [33, 35, 36]. Aneuploid cells exhibited features associated with clinical aggressiveness in that they were larger than associated diploid cells and had a higher proliferative rate [33]. These data suggest that aneuploidy as detected by flow cytometry may be a useful biological marker of aggressive disease. Preliminary clinical data is consistent with this hypothesis as the median survival of patients with non-Hodgkin's lymphomas of unfavorable histology exhibiting aneuploidy is markedly less than similar tumours containing only diploid cell populations [36].

Flow cytometric analysis of multiple myeloma cells has also shown a correlation between aneuploidy and aggressive disease, but this finding is not consistently associated with increased thymidine radiolabeling [41, 42]. This suggests that aneuploidy is a prognostic marker that is independent of the proliferative rate of the myeloma cells.

ENZYME MARKERS

Enzyme studies in lymphoid malignancies have in the main been directed towards the cytochemical identification of the origin of the neoplastic cell [13]. A number of enzymes, including thymidine kinase, dihydrofolate reductase, ribonucleotide reductase, deoxyribonucleic acid polymerase, adenosine deaminase and glycolytic enzymes such as lactate dehydrogenase, are known to exhibit a specific increase in activity during the S-phase of the mammalian cell cycle [43, 44] and some recent studies have exploited these subtle changes in cellular biochemistry to analyse the proliferative behavior of non-Hodgkin's lymphoma cells.

Thymidine kinase is the rate-limiting enzyme for the incorporation of thymidine into cell deoxyribonucleic acid and is thought to have an important regulatory role in cell proliferation [45]. Thymidine kinase is a particularly useful marker of cell proliferation as the marked increase in enzyme activity that occurs during cell deoxyri-

bonucleic acid synthesis is due to the appearance of an isoenzyme (TK1), which is readily distinguishable from the relatively constant TK2 activity [46].

Early studies of thymidine kinase activity in lymphoproliferative diseases reported low enzyme levels in normal lymphocytes and chronic leukemic cells, while high enzyme activity was found in lymphoblasts [47, 48]. More recently, determination of both total enzyme activity and the predominant isoenzyme type have demonstrated a correlation between the profile of thymidine kinase activity and the histopathology of non-Hodgkin's lymphomas [46]. In keeping with a mature cytological appearance, normal lymphoid cells, chronic lymphocytic leukemia cells and the solid tissue counterpart, diffuse, well-differentiated lymphocytic lymphoma, exhibited only TK2 activity. Thymidine kinase TK1 was the predominant isoenzyme found in solid tumors composed of less-differentiated cells and peripheral blood lymphoblasts of the leukemic phase of non-Hodgkin's lymphomas. Moreover, there was a stepwise increase in the mean TK1 activity with progressive degrees of morphological cellular dedifferentiation expressed in the Rappaport classification. Of major interest was the considerable variation found in the TK1 activity of the tumors of each category of this scheme, suggesting that this biochemical probe for biological aggressiveness may apply not only for histopathologic types but also for individual patients within these categories. Support was given to this concept by the recent finding that the presence of peripheral blood plasma and/or lymphocyte TK1 activity is an independent marker of clinically aggressive non-Hodgkin's lymphomas [49, 50].

Of the other enzymes, only adenosine deaminase and lactate dehydrogenase activities have been examined in any detail for the non-Hodgkin's lymphomas. The intracellular level of adenosine deaminase is differentially elevated in T cell lymphoid malignancies as opposed to those of B cell origin [51, 52], a finding which in part may be due to differences in cell proliferative rate [52]. Total serum lactate dehydrogenase activity has been found to be an independent prognostic marker in American Burkitt's lymphoma, diffuse histiocytic lymphoma and acute lymphoblastic leukemia, with in general a serum enzyme activity in excess of 400 IU/l associated with a poorer prognosis [53–55]. However, the relationship of these findings to cell proliferation is at present unclear.

DISCUSSION

Using a number of different methods to analyse cell proliferation, recent studies have shown a correlation between clinicopathology and the proliferative rate of lymphoid malignancies.

Histological types of non-Hodgkin's lymphomas composed of less-undifferentiated cells and associated with a favorable prognosis were found in the main to have a low proliferative rate. Tumors composed of larger undifferentiated cells exhibited almost uniformly a high cell proliferative rate, which is in keeping with the associated clinical aggressiveness. However, heterogeneity was found in the cell proliferative rates of both the favorable and unfavorable prognostic categories of non-Hodgkin's lymphomas. A significant number of elevated values occurred in the former category, while a wide range of individual proliferative rates was found in the latter category. The clinical implications of high proliferative rates in non-Hodgkin's lymphomas of reputedly favorable prognosis are unclear, but some data suggest that this phenomenon may be clinically relevant. Recently, a subtype of the favorable tumor nodular poorly differentiated lymphocytic lymphoma has been described which differs from the classical clinicopathologic features of this tumor by the less differentiated appearance of the cells, a starry sky pattern, an increased mitotic index and a tendency to evolve into a diffuse pattern growth and an aggressive leukemic phase [14]. These clinicopathologic features are consistent with a high cell proliferative rate, indicating that cell kinetic studies would be a value adjunct in the identification of this subtype. More generally, studies of the cell proliferative rate of tumors with a reputed favorable prognosis may enable the early identification of these non-Hodgkin's lymphomas at risk of evolving to unfavorable histologies or to have divergent histologies at presentation. Both these phenomena are thought to adversely alter the clinical course of favorable-type non-Hodgkin's lymphoma [56–58]. The range of proliferative rates found in tumors of unfavorable prognosis was most marked in the diffuse large-cell lymphomas, confirming the known heterogeneity of this type [39, 40], but the clinical significance of this interesting finding remains to be determined. Limited data is available on the influence of individual tumor proliferative rates on the response to therapy and survival in the lymphoid malignancies. However, a recent review [11] of the relationship between the growth rates of human tumors and responsiveness to therapy suggests that the proliferative rate of the neoplastic cell is a significant determinant of responsiveness to therapy and curability. The review indicated that while rapidly proliferating tumors are clinically aggressive, they are more sensitive to therapy and a significant proportion of durable remissions can be achieved. In contrast, slowly

growing tumors respond poorly to therapy and responses are usually not sustained. These data suggest that differences in neoplastic cell proliferation could provide an explanation for the paradox that while new therapeutic regimes have obtained long-term disease-free survival in a significant proportion of non-Hodgkin's lymphomas of unfavorable histological type, tumors of favorable histological type have a limited response to these regimes and an indolent clinical course [59, 60].

Other factors must also determine the response and survival in lymphoproliferative disorders, as intensive treatment of acute lymphoblastic leukemia only confers marginal survival advantage when the blast cells have a high proliferative rate [26]. Furthermore, in multiple myeloma the presence of a more rapidly proliferating clone of neoplastic cells confers resistance to a wide range of anticancer agents [30, 41].

The future clinical role of cell kinetic studies in the lymphoid malignancies remains to be determined. However, it seems possible that the fraction of proliferating neoplastic cells will be a significant parameter in determining optimum therapy for this heterogenous group of tumors. In particular, the measurement of cell proliferative rates in non-Hodgkin's lymphomas may identify subtypes within the so-called favorable and unfavorable histological types that are likely to respond to intensive chemotherapy regimes. This would help ameliorate present controversies over the treatment of the non-Hodgkin's lymphomas.

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REFERENCES

1. STREULI RA, ULTMANN JE. Non-Hodgkin's lymphomas: historical perspective and future prospects. *Semin Oncol* 1980, **7**, 223–233.
2. BERARD CW, GREENE MH, JAFFE ES, MAGRATH IT, ZIEGLER JL. A multi-disciplined approach to understanding non-Hodgkin's lymphomas. *Ann Intern Med* 1981, **94**, 218–235.
3. GARVIN AJ, SIMON R, YOUNG RC, DEVITA VT, JR, BERARD CW. The Rappaport classification of non-Hodgkin's lymphomas: a closer look using other proposed classifications. *Semin Oncol* 1980, **7**, 234–243.
4. CABANILLAS F, BURKE JS, SMITH TL, MOON TE, BUTLER JJ, RODRIGUEZ V. Factors predicting the response and survival in adults with advanced non-Hodgkin's lymphoma. *Arch Intern Med 1978*, **138**, 413–418.
5. FISHER RI, DEVITA VT, JR, JOHNSON BL, SIMON R, YOUNG RC. Prognostic factors for advanced diffuse histiocytic lymphoma following treatment with combination chemotherapy. *Am J Med* 1977, **63**, 177–182.
6. BLOOMFIELD CD, KERSEY JH, BRUNNING RD, GAJL-PECZALSKA KJ. Prognostic significance of lymphocyte surface markers in adult non-Hodgkin's malignant lymphoma. *Lancet* 1976, **ii**, 1330–1333.
7. BLOOMFIELD CD, GAJL-PECZALSKA KJ, FRIZZERA G, KERSEY JH, GOLDMAN AI. Clinical utility of lymphocyte surface markers combined with the Lukes–Collins histologic classification in adult lymphoma. *N Engl J Med* 1979, **301**, 512–518.
8. FERRARIS AM, GIUNTINI P, GAETANI GF. Serum lactic dehydrogenase as a prognostic tool for non-Hodgkin's lymphomas. *Blood* 1980, **54**, 928–932.
9. KHILANANI P, CHOU T-H, RAFANTHARATHORN V, KESSEL D. Evaluation of two plasma fucosyltransferases as marker enzymes in non-Hodgkin's lymphoma. *Cancer* 1978, **41**, 701–705.
10. GORDON STEEL G. *Growth Kinetics of Tumours. Cell Population Kinetics in Relation to the Growth and Treatment of Cancer.* Oxford, Clarendon Press, 1977.
11. SHACKNEY SE, MCCORMACK GW, CUCHURAL GJ, JR. Growth rate patterns of solid tumors and their relation to responsiveness to therapy. *Ann Intern Med* 1978, **89**, 107–121.
12. EVANS HL, BUTLER JJ, YOUNESS EL. Malignant lymphoma, small lymphocytic type. A clinicopathologic study of 84 cases with suggested criteria for intermediate lymphocytic lymphoma. *Cancer* 1977, **41**, 1440–1455.
13. MANN RB, JAFFE ES, BERARD CW. Malignant lymphomas—a conceptual understanding of morphologic diversity. *Am J Pathol* 1979, **94**, 103–192.
14. COME SE, JAFFE ES, ANDERSEN JC *et al.* Non-Hodgkin's lymphomas in leukemic phase: clinicopathologic correlations. *Am J Med* 1980, **69**, 667–674.
15. COOPER EH, PECKHAM MJ, MILLARD RE, HAMLIN IIIE, GERARD-MARCHANT R. Cell proliferation in human malignant lymphomas. Analysis of labelling index and DNA content in cell populations obtained by biopsy. *Eur J Cancer* 1968, **4**, 287–296.

16. PECKHAM MJ, COOPER EH. The pattern of cell growth in reticulum cell sarcoma and lymphosarcoma. *Eur J Cancer* 1970, **6**, 453–463.
17. MEYER JS, HIGA E. S-Phase fractions of cells in lymph nodes and malignant lymphomas. *Arch Pathol Lab Med* 1979, **103**, 93–97.
18. SILVESTRINI R, PIAZZA R, RICCARDI A, RILKE F. Correlation of cell kinetic findings with morphology of non-Hodgkin's malignant lymphomas. *J Natl Cancer Inst* 1977, **58**, 499–504.
19. SCARFFE JH, CROWTHER D. Pretreatment cell kinetic studies in human lymphoid malignancies as possible prognostic factors. In: LUTZ D, ed. *Pulse-cytophotometry*. Ghent, European Press, 1977, part iii, 675–682.
20. SILVESTRINI R, COSTA A, DAIDONE MG, RILKE F. Prognostic significance of labeling index in non-Hodgkin's human malignant lymphomas. *Antibiot Chemother* 1978, **24**, 105–111.
21. QUAGLINO D, DE PASQUALE A, PICCININI L, TONELLI M, MUGNAINI M. Autoradiographic studies on lymph node populations from non-Hodgkin B lymphomas. *Acta Haematol* 1980, **64**, 148–153.
22. KVALOY S, GODAL T, MARTON PF, STEEN H, BRENNHOVD IO, ABRAHAMSEN AF. Spontaneous (^{3}H)-thymidine uptake in histological subgroups of human B-cell lymphomas. *Scand J Haematol* 1981, **26**, 221–234.
23. COSTA A, BONADONNA G, VILLA E, VALAGUSSA P, SILVESTRINI R. Labeling index as a prognostic marker in non-Hodgkin's lymphomas. *J Natl Cancer Inst* 1981, **66**, 1–5.
24. HANSEN H, KOZINER B, CLARKSON B. Marker and kinetic studies in the non-Hodgkin's lymphomas. *Am J Med* 1981, **71**, 107–123.
25. MURPHY SB, MELVIN SL, MAUER AM. Correlation of tumor cell kinetics with surface markers results in childhood non-Hodgkin's lymphoma. *Cancer Res* 1979, **39**, 1534–1538.
26. CLARKSON BD. The elusive goal: presidential address. *Cancer Res* 1981, **41**, 4865–4884.
27. HART JS, LIVINGSTON RB, MURPHY WK, BARLOGIE B, GEHAN EA, BODEY GP. Neoplasia, kinetics and chemotherapy. *Semin Oncol* 1976, **3**, 259–270.
28. MOAYERI H, SOKAL JE. *In vitro* leukocyte thymidine uptake and prognosis in chronic lymphocytic leukemia. *Am J Med* 1979, **66**, 773–778.
29. SIMONSSON B, NILSSON K. ^{3}H-Thymidine uptake in chronic lymphocytic leukemia cells. *Scand J Haematol* 1980, **24**, 169–173.
30. HOFMANN V, SALMON SE, DRURIE GM. Drug resistance in multiple myeloma associated with high *in vitro* incorporation of ^{3}H-thymidine. *Blood* 1981, **58**, 471–476.
31. BRAYLAN RC, FOWLKES BJ, JAFFE ES, SANDERS SK, BERARD CW, HERMAN CJ. Cell volumes and DNA distributions of normal and neoplastic human lymphoid cells. *Cancer* 1978, **41**, 201–209.
32. DIAMOND LW, BRAYLAN RC. Flow analysis of DNA content and cell size in non-Hodgkin's lymphoma. *Cancer Res* 1980, **40**, 703–712.
33. SHACKNEY SE, SKRAMSTAD KS, CUNNINGHAM RE, DUGAS DJ, LINCOLN TL, LUKES RJ. Dual parameter flow cytometry studies in human lymphomas. *J Clin Invest* 1980, **66**, 1281–1294.
34. SCARFFE JH, CROWTHER D. The pre-treatment proliferative activity of non-Hodgkin's lymphoma cells. *Eur J Cancer* 1981, **17**, 99–108.
35. SHACKNEY SE, CUNNINGHAM RE, SCHVETTE WS, SMITH CA, NICHOLS PW, LUKES RJ. Patterns of cell proliferation in relation to aneuploidy by flow cytometry in the non-Hodgkin's lymphomas. *Proc Am Soc Clin Oncol* 1981, **22**, 337 (abstract).
36. HAGEMEISTER FB, RABER M, BARLOGIE B, MADDOX AF, CABANILLAS F. Flow cytometry analysis in lymphoma: effect of abnormal DNA content. *Proc Am Assoc Cancer Res* 1981, **22**, 42 (abstract).
37. DORFMAN RF. Pathology of the non-Hodgkin's lymphomas: new classifications. *Cancer Treat Rep* 1977, **61**, 945–951.
38. NATHWANI BN. A critical analysis of the classification of non-Hodgkin's lymphomas. *Cancer* 1979, **44**, 347–384.
39. STRAUCHEN JA, YOUNG RC, DEVITA VT, JR, ANDERSON T, FANTONE JC, BERARD CW. Clinical relevance of the histopathological sub-classification of diffuse "histiocytic" lymphoma. *N Engl J Med* 1978, **299**, 1382–1384.
40. WARNKE R, MILLER R, GROGAN T, PEDERSON M, DILLEY J, LEVY R. Immunologic phenotype in 30 patients with diffuse large-cell lymphoma. *N Engl J Med* 1980, **303**, 293–300.
41. LATREILLE J, BARLOGIE B, JOHNSTON D, DREWINKO B, ALEXANIAN R. Ploidy and proliferative characteristics in monoclonal gammopathies. *Blood* 1982, **59**, 43–51.

42. BUNN PA, JR, KRASNOW S, MAKUCH RW, SCHLAM ML, SCHECHTER GP. Flow cytometric analysis of DNA content of bone marrow cells in patients with plasma cell myeloma: clinical implications. *Blood* 1982, **59**, 528–535.
43. BASERGA R. The cell cycle. *N Engl J Med* 1981, **304**, 453–459.
44. HOVI T, SMYTH JF, ALLISON AC, WILLIAMS SC. Role of adenosine deaminase in lymphocyte proliferation. *Clin Exp Immunol* 1976, **23**, 395–403.
45. KIT S. Thymidine kinase, DNA synthesis and cancer. *Mol Cell Biochem* 1976, **11**, 161–182.
46. ELLIMS PH, VAN DER WEYDEN MB, MEDLEY G. Thymidine kinase isoenzymes in human malignant lymphoma. *Cancer Res* 1981, **41**, 691–95.
47. BRESNICK E, KARJALA RJ. End-product inhibition of thymidine kinase activity in normal and leukemic leukocytes. *Cancer Res* 1964, **24**, 841–846.
48. RABINOWITZ Y, WILHITE BA. Thymidine salvage pathway in normal and leukemic leukocytes with effects of ATP on enzyme control. *Blood* 1969, **33**, 759–771.
49. ELLIMS PH, GAN TE, MEDLEY G, VAN DER WEYDEN MB. Prognostic relevance of thymidine kinase isozymes in adult non-Hodgkin's lymphomas. *Blood* 1981, **58**, 926–931.
50. ELLIMS PH, GAN TE, VAN DER WEYDEN MB. Thymidine kinase isoenzymes in chronic lymphocytic leukaemia. *Br J Haematol* 1981, **49**, 479–481.
51. BLATT J, REAMAN G, POPLACK DG. Biochemical markers in lymphoid malignancy. *N Engl J Med* 1980, **303**, 918–922.
52. VAN DER WEYDEN MB, ELLIMS PH, GAN TE. Enzymic markers in lymphoproliferative disorders. *Am J Haematol* Submitted.
53. ARSENEAU JC, CANELLOS GP, BANKS PM, BERARD CW, GRALNICK HR, DEVITA VT, JR. American Burkitt's lymphoma: a clinicopathologic study of 30 cases. 1. Clinical factors relating to prolonged survival. *Am J Med* 1975, **58**, 314–321.
54. SCHNEIDER RJ, SEIBERT K, PASSE S *et al.* Prognostic significance of serum lactate dehydrogenase in malignant lymphoma. *Cancer* 1980, **46**, 139–143.
55. KEATING MJ, SMITH TL, GEHAN EA *et al.* Factors related to length of complete remission in adult acute leukemia. *Cancer* 1979, **45**, 2017–2029.
56. HUBBARD SM, CHABNER BA, DEVITA VT, JR *et al.* Histologic progression in non-Hodgkin's lymphoma. *Blood* 1982, **59**, 258–264.
57. FISHER RI, JONES RB, DEVITA VT, JR *et al.* Natural history of malignant lymphomas with divergent histologies at staging evaluation. *Cancer* 1981, **47**, 2022–2025.
58. OSBORNE CK, NORTON L, YOUNG RC *et al.* Nodular histiocytic lymphoma: an aggressive nodular lymphoma with potential for prolonged disease-free survival. *Blood* 1980, **56**, 98–103.
59. ANDERSON T, BENDER RA, FISHER RI *et al.* Combination chemotherapy in non-Hodgkin's lymphoma: results of long-term follow up. *Cancer Treat Rep* 1977, **61**, 1057–1066.
60. ROSENBERG SA. Non-Hodgkin's lymphoma—selection of treatment on the basis of histologic type. *New Engl J Med* 1979, **301**, 924–928.