Relationships of S-Phase Fraction of Breast Carcinoma in Relapse to Duration of Remission, Estrogen Receptor Content, Therapeutic Responsiveness, and Duration of Survival¹

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ABSTRACT

The fractions of tumor cells in S phase (DNA synthesis) were measured by in vitro thymidine labeling and autoradiography in 48 breast carcinomas after relapse. The S-phase fractions (SPF's), expressed as S-phase cells/100 cells, had a lognormal distribution with a geometric mean of 6.5 and a median of 7.4. Paired SPF measurements on the primary and relapsed breast carcinomas of 14 patients showed that the SPF usually increased over time. The SPF after relapse correlated negatively with the interval between primary therapy and relapse and with duration of survival after relapse. Low SPF's were associated with older age, minimal nuclear anaplasia, and estrogen receptor positivity, but SPF was the only variable that could be shown to have independent prognostic significance. Therefore, the prognostic powers of the estrogen receptor status and nuclear grade appear to result from their correlations with the SPF. Either low SPF or presence of estrogen receptor predicted response to hormonal therapy.

INTRODUCTION

The wide variability of the interval between primary definitive treatment of breast carcinoma and subsequent relapse is well known (3, 7, 8). Routine pathological study has disclosed a number of variables that relate to the prerelapse interval. They include the number and bulkiness of axillary lymph nodal metastases, size of the primary carcinoma, characteristics of infiltrative growth, inflammatory cellular reaction, and nuclear grade (4, 5, 11). We have confirmed the observation of Tubiana et al. (31) that high thymidine labeling index in primary breast carcinoma is associated with short prerelapse intervals (18). Lack of correlation between thymidine labeling index and size of the primary carcinoma or number of axillary metastases suggested that the thymidine labeling index as a risk factor for early relapse may operate independently of the pathologic stage of the carcinoma (18). If the SPF³ as measured by thymidine labeling remains constant or changes predictably over long time intervals, this measurement on breast carcinoma in relapse should relate to the prerelapse interval as determined retrospectively. We tested this hypothesis in a study of 48 patients with relapsed breast carcinoma. In the earlier prospective study, we used a method of in vitro pulse labeling with [³H]dThd that underestimated the SPF because grain counts sometimes were low. In the current retrospective study, we blocked intracellular thymidylate synthesis with 5-fluoro-2'-deoxyuridine in order to achieve high grain counts over S phase cells and to measure the SPF more precisely.

MATERIALS AND METHODS

Patient Population. Patients entered the study from April 1974 through February 1979. Criteria for eligibility included: (a) availability of an autoradiograph from metastatic or locally recurrent breast carcinoma after short-term incubation with [³H]dThd suitable for evaluation of 1000 or more neoplastic cells for nuclear labeling; (b) prior definitive surgical therapy of primary breast carcinoma with or without adjuvant radiation therapy; (c) no other previous breast carcinoma; (d) no subsequent development of contralateral breast carcinoma unless it occurred so late in the course of the carcinoma on which the SPF measurement was made (index carcinoma) that the contralateral, possibly second primary carcinoma could be excluded with reasonable assurance as contributing to the course of the progressive carcinomatosis; and (e) no other malignant neoplasm unless the nonmammary neoplasm did not appear to have metastasized and was histologically distinct from the breast carcinoma.

One patient was excluded because of a probable second primary breast carcinoma subsequent to radical mastectomy. Another patient was excluded because of an ovarian carcinoma with abdominal metastases. Two patients who were retained in the study had contralateral breast carcinomas. One had had a radical mastectomy 16 years prior to tylectomy for the index carcinoma, and recurrence of the second breast carcinoma was limited to the tylectomy scar. The other had a contralateral carcinoma that appeared after metastatic breast carcinoma was diagnosed in lymph nodes and bones. Three patients who were retained in the study had colorectal adenocarcinoma. The first had an adenocarcinoma of the colon resected 11 years after initial relapse of the breast carcinoma in skin and bones. The second patient was found to have adenocarcinoma of the rectum at the same time as the relapse of breast carcinoma in the mastectomy scar. The third patient had a colonic carcinoma resected 2 years prior to relapse of breast carcinoma in the ipsilateral axilla. None of the 3 patients with colorectal carcinomas had evidence of metastatic carcinoma in liver or abdominal cavity, and their breast carcinomas were histologically distinguishable from ordinary colorectal adenocarcinomas.

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³ The abbreviations used are: SPF, S-phase fraction (the number per 100 of neoplastic cell nuclei shown to be synthesizing DNA in in vitro exposure to [³H]thymidine); [³H]dThd, tritiated thymidine; ER, estrogen receptor; PGR, progesterone receptor.

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Forty-eight patients met the criteria for inclusion in the study. One was male, and 47 were female. Their mean age was 59.6 years with a range of 31 to 90 years. Seven were black, and 41 were white. The primary tumors had been treated by radical mastectomy, standard or modified, in 42 instances; 2 had had simple mastectomy; one had had segmental resection; and 3 had had tylectomy. One patient, who had a radical mastectomy, received adjuvant chemotherapy. Three patients received adjuvant immunotherapy with Bacillus Calmette-Guérin, 2 after radical mastectomy and one after simple mastectomy. Two additional patients received both adjuvant radiation therapy and adjuvant chemotherapy after radical mastectomy. The other 29 patients received no adjuvant therapy.

Clinical and Histopathological Data. The authors obtained clinical information from hospital records and office records of physicians. Patients who had not recently visited their physicians were contacted by mail or telephone. The pathological stage of the breast carcinoma at the time of initial treatment was determined from the patient’s hospital record and the pathologist’s report. Staging was according to rules of the American Joint Committee for Cancer Staging and End-Results Reporting (1). Pathological staging of the lymph nodes was available for 40 patients. Six patients did not have axillary dissections; therefore clinical axillary findings were used for staging. For 2 patients, staging data were not available. Microsections of the relapsed breast carcinomas stained with hematoxylin and eosin were examined and classified according to their histopathological features as previously described (16).

SPF Measurement. The SPF was measured by labeling of tissue slices with [3H]dThd in vitro and detection of nuclear uptake by autoradiography in 4-μm sections cut from paraffin blocks. Hyperbaric oxygen (3 to 4 atmospheres) and 5-fluoro-2′-deoxyuridine were used during the 2-hr period of incubation at 37° in order to obtain high grain counts over labeled nuclei. 5-Fluoro-2′-deoxyuridine inhibits thymidylate synthetase, reduces the intracellular thymidylate pool size, and enhances the uptake of the exogenous [3H]dThd. Procedural details were exactly as reported previously (17). The SPF was derived from a count of 2000 neoplastic nuclei by one of the authors (J. S. M.). The nuclei were taken in groups of 400 from 5 different areas of the microsection selected by a standard system in order to reduce bias (16). Nuclei with 5 or more overlying grains were designated as labeled with background of less than 1 grain/nucleus. In all cases, the mean grain count of nuclei designated as labeled exceeded 20, and nuclei with low grain counts were few (25). For this reason, and because of a close correspondence between in vitro and in vivo measurements of the thymidine labeling index by this method (17), the thymidine labeling index was considered to represent the SPF, which was expressed as number of S phase cells per 100 cells.

ER and PGR Assays. Both assays were performed on cytosol prepared by centrifugation at 100,000 × g, 17β-[3H]estradiol was the ligand for ER assay. Unbound ligand was removed with dextran-charcoal, and specificity of binding was assessed by saturability of binding sites with a 250-fold excess of unlabeled 17β-estradiol as previously reported (19). We designated cytosols with saturable binding of less than 10 fmol/mg cytosol protein as negative for ER, those with 10 to 49 fmol/mg cytosol protein as positive low binding, and those with 50 fmol/mg cytosol protein as positive high binding. For PGR, we followed the method of Pichon and Milgrom (20) with the additional use of [3H]promestrone (R5020; Roussel-Uclaf) (21) as a ligand run in parallel with [3H]progesterone. Duplicate samples of cytosol adjusted to 2 mg protein per ml were incubated for 4 hr at 0° with labeled ligand, and another duplicate set was incubated with 100-fold excess of unlabeled ligand and labeled ligand for saturation analysis. The steroidal reagents were added to the cytosol in ethanol, and the ethanol concentration in the incubation mixture was 4%. Unbound ligand was removed with dextran-charcoal. We designated cytosols with saturable binding of both [3H]progesterone and [3H]R5020 of 10 fmol/mg protein as positive for PGR. Those with saturable binding of less than 10 fmol/mg protein with one ligand and at most 19 fmol/mg protein with the other ligand were designated as negative for PGR. The ER and PGR assays were controlled with normal human proliferative-phase endometrium, which consistently gave strongly positive results for both receptors, and with normal kidney, which consistently gave negative results for both receptors.

Criteria for Assessment of Response to Therapy. Results of therapy were classified according to the definitions of the Breast Cancer Task Force Treatment Committee (6) with slight modifications. Patients were considered eligible for evaluation if they had progressive disease, received either hormonal or cytotoxic therapy (but not both together) for at least 1 month, and did not receive radiation therapy at the same time. Only therapeutic trials following SPF measurement were evaluated. Complete response was defined as complete disappearance of all known disease for at least 8 weeks, and partial response was a shrinkage in total tumor size of at least 50% for at least 8 weeks with no increase in any single lesion. Stable disease was defined as no measurable growth of lesions but less than 50% shrinkage lasting at least 8 weeks, and no response was the continued growth of tumor or appearance of any new lesion.

Analysis of Data. We were interested in 4 intervals: (a) prerelapse interval (primary therapy to diagnosis of relapse); (b) pre-SPF interval (primary therapy to time of biopsy from which SPF was measured); (c) postrelapse interval (diagnosis of relapse to death or final contact); and (d) total duration (prerelapse interval plus postrelapse interval). The various intervals for patients with below and above median SPF’s on their relapsed tumors were plotted in the survival curve format according to Cutler and Ederer in Benedetti and Yuen (2). The Breslow modification of the generalized Wilcoxon test was used to compare the resulting curves (2). Multivariate analyses were done according to the methods of Cox (9) and Draper and Smith (10).

RESULTS

SPF Distribution, Clinical and Histological Features, and Relapse Pattern of Breast Carcinoma. The frequency distribution of SPF’s of relapsed breast carcinoma, like that of the SPF’s of primary breast carcinomas (16), was skewed positively in a manner consistent with a lognormal distribution (Chart 1). For this reason, in SPF was used for statistical analysis. The mean SPF was 8.7 with the median, 7.4; the
geometric mean, 6.5; and the range, 1.0 to 28.8. Corresponding values for a series of 170 primary breast carcinomas were 6.0, 4.2, 3.9, and 0.05 to 29.3, respectively (16). The mean age of the patients at the time relapse of breast carcinoma was diagnosed was 59.6 years, and the range was 31 to 90. Corresponding figures for the series of 170 patients with primary breast carcinoma at the time of diagnosis were 58.8 and 27 to 89. At the time of initial treatment, 6 patients were diagnosed as Stage I (4 with positive axillary lymph nodes), 30 were Stage II (14 with positive axillary nodes), 7 were Stage III (6 with positive axillary nodes), and 3 were Stage IV (one with positive axillary nodes). Data for staging were not available for 2 patients. The initial stage did not correlate with In SPF of the relapsed tumor \( r = 0.158; p = 0.29 \). The age of the patient at the time of diagnosis of relapse and In SPF of the relapsed tumor were negatively correlated \( r = 0.448; p = 0.0014 \), and the mean age at relapse of 22 patients with above median SPF’s was 52 years compared to 65 years for 26 patients with SPF’s at or below the median of 7.4 \( p = 0.001 \) by \( t \) test.

The In SPF and the maximum diameter of the recurrent tumor from which tissue was taken for SPF measurement showed a low order of correlation \( r = 0.30; p = 0.039 \). The SPF increased with increasing nuclear grade (increasing nuclear anaplasia). For Grades 1, 2, and 3, respectively, the geometric means of the SPF’s were 3.5 (15 cases), 7.6 (25 cases), and 12.6 (8 cases) \( p < 0.001 \) by analysis of variance. Occurrence of SPF’s of 16.7, 13.8, and 10.2 in the Grade 1 group and of 7.1 and 7.7 in the Grade 3 group indicates that the SPF cannot be inferred from the nuclear grade with much confidence in a given case. An attempt to predict the SPF of relapsed carcinomas by histological classification failed. Fourteen carcinomas were classified as differentiated (glandular and ductular structures present), and 34 were undifferentiated (no glandular or ductular differentiation). Their geometric mean SPF’s were 6.3 and 7.1, respectively \( p = 0.6 \). Seven relapsed carcinomas had patterns that were recognizable as specially named types. Five were lobular (small cell) with SPF’s ranging from 1.3 to 7.4. The latter and another of 6.8 are higher than the range into which primary lobular carcinomas usually fall (16, 18). A tubular carcinoma (SPF 2.0) and a medullary carcinoma (SPF 11.3) were typical of our experience with primary tumors of those types (16, 18).

At initial diagnosis of relapse, the tumor was confined to the chest wall (including breast following tylectomy in one patient) in 16 patients (mean age, 57 years; geometric mean SPF, 6.3), was present only in lymph nodes with or without chest wall involvement in 12 patients (mean age, 65 years; geometric mean SPF, 7.6), was present in bone with or without other nonvisceral sites in 6 patients (mean age, 64 years; geometric mean SPF, 4.1), and was present in viscera including pleura and peritoneum in 14 patients (mean age, 56 years; geometric mean SPF, 7.2). Ranges of age and SPF overlapped extensively, and analysis of variance showed no significant differences among the mean ages or geometric mean SPF’s of the relapsed carcinomas of the 4 groups.

**SPF, ER’s, and PGR’s.** ER assays were performed on 39 specimens of relapsed breast carcinoma, and the level of saturable 17\( \beta \)-estradiol binding by the tumor cytosol was inversely related to the SPF of the carcinoma (Table 1). PGR assays were done on cytosols from 9 tumors and were negative in 8 (geometric mean SPF, 12.1; range, 6.8 to 28.8). The SPF of the PGR-positive tumor was 2.6.

**SPF and the Course of Breast Carcinoma over Time.** The frequency distribution of prerelapse intervals was positively skewed with the mean, 3.7 years; median, 2.5 years; and range, 0.1 to 20.0 years. The biopsy from which the SPF was measured was not always taken at the time of initial diagnosis of relapse, and the pre-SPF intervals had a mean of 5.1 years, a median of 3.5 years, and a range of 0.1 to 21.0 years. The postrelapse interval had a mean of 2.1 years, a median of 1.5 years, and a range of 0.0 to 13.0 years. The total duration from diagnosis of the primary breast carcinoma to death or time of last contact had a mean of 5.8 years, a median of 4.0 years, and a range of 0.4 to 23.1 years. The SPF correlated inversely with the prerelapse interval \( r = -0.320; p = 0.017 \) and the pre-SPF interval \( r = -0.415; p = 0.003 \). Similar negative correlations of SPF with the postrelapse interval and the total duration were obtained. However, these must be interpreted with caution because 62.5% of the patients were still alive at the time of last contact. Charts 2 to 4 show the prerelapse and postrelapse intervals and their sums (total duration) treated according to the cumulative survival format of Cutler and Ederer in Benedetti and Yuen (2) with division into a group with SPF’s at or below the median and another with SPF’s above the median. In each comparison, shorter intervals which indicated more rapid progression of disease were associated with above median SPF’s.

At the close of the study, 30 patients were alive, and 18 were dead. Analysis of the latter as a separate group showed decreasing intervals between initial treatment and relapse and between relapse and death as the SPF’s increased (Table 2). These relationships could not be attributed to correlations of the initial stage of the disease with SPF.

**Table 1**

<table>
<thead>
<tr>
<th>ER assay result</th>
<th>No. of observations</th>
<th>Geometric mean</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>18</td>
<td>9.6</td>
<td>2.0—28.8</td>
</tr>
<tr>
<td>Positive (low binding)</td>
<td>8</td>
<td>5.2</td>
<td>1.3—13.8</td>
</tr>
<tr>
<td>Positive (high binding)</td>
<td>13</td>
<td>3.8</td>
<td>1.0—15.4</td>
</tr>
</tbody>
</table>

* The mean In SPF’s of the 3 ER classifications are significantly different by analysis of variance; \( p < 0.01 \).

**Chart 1.** Frequency distribution of SPF’s of relapsed breast carcinomas.
Multivariate analyses were done to determine which of several variables might have acted independently to influence the course of the disease. Because the prerelapse interval was lognormally distributed, its logarithms were used in the analyses. A stepwise regression analysis (10) was done to assess the relationship between In prerelapse interval and the following variables: age; initial stage; nuclear grade; ER; and In SPF. The analysis included variables progressively from most highly correlated to least correlated with In prerelapse interval. The criterion for inclusion was correlation significant at the 0.10 level. The In SPF was the most highly correlated variable, and nuclear anaplasia (advanced versus slight to moderate) was the second most highly correlated variable, but when the nuclear grade was considered concomitantly with the other variables in the regression model, it demonstrated no prognostic significance. No other variable met the inclusion criterion. The relationship is given by:

\[ y = 1.93 - 0.56 \, (\ln \text{SPF}) \]

where \( y \) = In prerelapse interval in years.

Because of the presence of censored data, stepwise Cox regression analyses (9) were done to assess the relationships between the postrelapse interval or the total duration of the clinical course and the same set of variables (age, initial stage, nuclear grade, ER status, and ln SPF). The analyses listed variables progressively from most highly to least correlated with the time interval. The criterion for inclusion was that the correlation was significant at the 0.10 level. The ln SPF correlated best with both the postrelapse interval and the total duration. No other variable met the inclusion criterion.

Generalized Wilcoxon tests showed no significant differences in total duration of the clinical course for different nuclear grades although lesser degrees of anaplasia were associated with longer prerelapse intervals (\( p < 0.06 \)) and postrelapse intervals (\( p < 0.10 \)).

Among the patients at risk for at least 2 years after relapse, only 1 of 8 with SPF’s of 10 or more survived beyond 2 years, whereas 15 of 19 with SPF’s of less than 10 survived more than 2 years after relapse. The one long-term survivor (5.2+ years) with a high SPF had metastatic carcinoma limited to a left axillary lymph node 1.1 years after right radical mastectomy for Stage II carcinoma and was treated by excision of the lymph node and radiation therapy. Although no evidence of tumor was found in the left breast by palpation or mammography, the axillary metastasis may have come from an occult lesion in the left breast that was subsequently included in the radiation therapy field.

SPF of the Primary Breast Carcinoma Compared with SPF of Relapsed Breast Carcinoma. We had measured the SPF of the primary breast carcinoma of 14 patients at the time of initial diagnosis. The intervals between the SPF measurements on primary and relapsed carcinomas averaged 1.7 years with a range of 0.1 to 4.0 years. The paired values (primary lesion listed first) are as follows: 1.0, 2.6; 2.3, 12.0; 2.4, 12.4; 3.4, 7.7; 3.6, 7.4; 3.7, 6.8; 10.1, 9.1; 10.2, 15.1; 10.6, 18.9; 11.9, 12.8; 12.8, 7.4; 13.2, 15.5; 25.2, 28.8; and 25.4, 22.9. The geometric mean SPF of the primary carcinomas was 6.7, and that of the relapsed carcinomas was 10.9. The difference between ln SPF’s of the primary and relapsed carcinomas was significant by paired \( t \) test (\( p = 0.013 \)). Two patients had 2 SPF measurements on relapsed carcinoma separated by 0.1 and 0.6 years. Both showed an increase, and these paired relapse values were: 7.1, 18.6; and 4.1, 10.8.
ER, SPF, and Response to Hormonal and Cytotoxic Therapy. Twenty patients had evaluable trials of hormonal therapy with the following results: oophorectomy in 3 premenopausal women, 2 no responses and one stabilization; oophorectomy and adrenalectomy in a premenopausal woman, no response; adrenalectomy in a postmenopausal woman, complete response; aminoglutethimide and dexamethasone in a postmenopausal woman, partial response; additives (diethylstilbestrol with or without prednisone or fluoroxymesterone in 10 women and megestrol acetate in 1 woman), 2 complete responses, 3 partial responses, and 6 no responses; and the antiestrogenic tamoxifen in 3 women, no response. Altogether, 3 complete responses, 3 partial responses, and 6 no responses; and the antiestrogenic tamoxifen in 3 women, no response. Typically, 3 complete responses (mean duration, 16+ months), 4 partial responses (mean duration, 8+ months), one stabilization (24 months), and 12 no responses resulted.

The relapsed tumors of 17 patients who had definitive trials of hormonal therapy had been assayed for ER. Seven were ER negative, and all were nonresponsive. Their mean SPF was 11.1 with a range of 2.1 to 25.4. Of the 10 ER-positive tumors, 3 showed complete response, 3 showed partial response, 1 showed stabilization, and 3 were nonresponsive (p < 0.05 by x2 test with Yates’ correction, and p = 0.02 by Fisher’s exact test). The SPF’s of the 6 objective responders were all less than 7.5, and the SPF of the patient who stabilized was 5.7. The 3 ER-positive patients who were nonresponders to hormonal therapy had SPF’s of 3.3, 12.4, and 13.8. PGR assays were done on tumors of only 2 patients with definitive hormonal therapy trials. PGR’s were negative in both cases, and neither patient responded to therapy.

The patterns of response to hormonal therapy for patients with carcinomas below and above the median SPF are shown in Table 3. Only one patient with above the median SPF had a relapsed breast carcinoma that responded to hormonal therapy. This SPF was 7.42, barely above the median of 7.40. Three responses occurred among the 7 tumors with Grade 1 nuclei, 4 responses and one stabilization occurred among the 10 with Grade 2 nuclei, and no responses occurred among the 3 with Grade 3 nuclei. x2 tests with the data grouped for either 3 or 2 degrees of freedom showed no significant relationship between nuclear grade and response to hormonal therapy.

Twenty-two patients received evaluable trials of cytotoxic chemotherapy after SPF measurement on their relapsed carcinoma. A variety of different regimens, usually with multiple cytotoxic drugs, was used. Two complete responses, 2 partial responses, 4 stabilizations, and 14 no responses were observed. The response patterns for the ER-positive and ER-negative groups (21 evaluable patients) were not significantly different, nor did the response patterns of the groups with below median and above median SPF differ significantly.

DISCUSSION

The relapsed breast carcinomas in this report were selected by accessibility of tumor for biopsy and may underrepresent patients with osseous and visceral metastases who comprised 17% and 29% of initial relapses. The degree to which selection may affect the applicability of our results to relapsed breast carcinoma is uncertain. No effort was made to persuade clinicians to biopsy patients who otherwise would not have had biopsies of their relapsed tumor for histological confirmation and receptor assays. Results of SPF measurements were not available to the clinicians and could not have influenced therapy.

The SPF’s of these relapsed breast carcinomas were high in comparison to a large series of primary breast carcinomas (16). However, our observations on both primary and relapsed breast carcinomas agree closely with those of Schiffer et al. (22) who reported lognormal distributions and median thymidine labeling indices of 4.7 in 67 primary carcinomas and 7.8 in 17 metastatic carcinomas. We previously noted a tendency toward higher thymidine labeling indices in carcinomas with axillary nodal metastases (15, 18), but the SPF in a more recent study did not correlate significantly with the number of axillary metastases (16). The thymidine labeling indices of primary tumors measured without thymidylate synthetase blockade, and therefore not fully representative of the SPF,
correlated positively with early relapse of breast carcinoma (18). The high SPF’s of the relapsed carcinomas relative to primary carcinomas could be explained either by an association between high SPF and potential for relapse or by increase of the SPF during the course of the disease. Our observation of a significant increase in the SPF from the primary to relapsed carcinoma in 14 patients provides evidence for the latter explanation. An increase in SPF would probably, but not necessarily (28), be accompanied by an increase in the growth rate of the tumor. This change may in part account for the rapid growth of breast carcinoma that sometimes is observed after long periods of quiescence.

A consistent, inverse relationship is evident between the SPF of the relapsed breast carcinoma and the various measured intervals that characterize the course of the disease. Multivariate analyses failed to demonstrate that other variables had any effect independently of the SPF on the intervals. This was true of initial stage of the disease, which showed only a weak relationship to SPF (15, 16), as well as of patient age, nuclear grade, and ER status, all of which have proved to be significantly correlated with SPF (16, 24). Therefore, the SPF appears to be the prime determinant of the rapidity of progression of breast carcinoma that relapses after primary therapy. The predictive powers of nuclear grade (4, 5, 11) and of ER status (13) appear to derive largely or entirely from their correlations with SPF. Our failure to observe a relationship between stage of the carcinoma at initial diagnosis and course of the disease is not inconsistent with the well-established relationship between stage and prognosis of breast carcinoma. In our research, we studied only patients whose carcinomas had relapsed after initial therapy, and their stages were in general higher than would be expected for an unselected group of patients. It may be of interest, however, that in our group of relapsed patients, the prerelapse interval was not correlated with initial stage, and a Kruskal-Wallis nonparametric test (23) did not reveal any interdependence of the 2 variables. Therefore, we are led again to the conclusion that among breast cancers that will relapse, the SPF is the primary determinant of the relapse-free interval.

The equation derived from the regression analysis that relates lognormal relapse-free interval to lognormal SPF fits the data for short and moderate relapse-free intervals better than for long relapse-free intervals, which it underestimates. The SPF’s of the tumors with long relapse-free intervals are not sufficiently low to account for the long intervals unless one assumes a rate of tumor cell loss during the latent period that approaches 100%. On the other hand, it is possible that the SPF during latency was actually much lower than after relapse.

The ultimate postrecurrence survival intervals of all patients with high SPF’s are not available yet, but the observations at this time are consistent with an incompatibility between high SPF and long-term survival after dissemination of breast carcinoma. Survival beyond 2 years following relapse of the breast carcinoma appears to be unlikely when the SPF is high (10 or more). Two explanations can be offered. (a) High SPF is associated with rapid cellular replication. Although the SPF is proportional to the duration of DNA synthesis (T S), the T S of breast carcinomas has not varied nearly as widely as the SPF as measured by thymidine labeling (22, 27, 29, 30, 32), and Schiffer et al. (22) reported a tendency for the T S to shorten as the thymidine labeling index increased. Rapid cellular proliferation is a prerequisite for rapid growth of the tumor although the growth rate is modified by the rate of loss of cells. The positive correlation between SPF and size of the biopsied relapsed tumor is consistent with rapid growth of tumors with high SPF and reinforces our belief that the rapidly growing breast carcinomas are found among those with high SPF’s. (b) Relapsed breast carcinomas with high SPF’s usually lack ER (16, 24) and do not respond to hormonal therapy.

Among the 10 ER-positive tumors subjected to evaluable trials of hormonal therapy, the group of 3 that did not respond objectively or stabilize during hormonal therapy contained the 2 ER-positive tumors with the highest SPF’s. This observation suggests that the group of ER-positive tumors that does not respond to hormonal therapy can be identified by SPF measurements and that further investigation along this line would be worthwhile. Although none of the 3 evaluable patients with Grade 3 nuclei responded to hormonal therapy, the nearly equal response rates between the groups with Grade 1 and Grade 2 nuclei indicate that the nuclear grade will not be very useful in predicting response.

The observation that presence of ER in breast carcinoma is associated with low SPF led to studies that related ER content to cytotoxic responsiveness (12, 14). A recent survey of results of cytotoxic therapy after ER assay based on 14 studies showed that responses occurred in 56% of 334 patients with ER-positive tumors and 50% of 407 patients with ER-negative tumors.4 Relative response rates for ER-positive and ER-negative tumors varied widely from one study to another. We could not relate rates of response to cytotoxic chemotherapy to either ER status of SPF. Breast carcinomas have a wide range of SPF’s, and cytotoxic drugs that are specific for cycling cells or cells in certain phases of the replicative cycle may be more effective against tumors with high SPF’s than those with low SPF’s as indicated by experimental models (26). Our material gave us no opportunity to study this question because the patients were treated with various combinations of cytotoxic agents containing both cycle phase-nonspecific and cycle phase-specific drugs.

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