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THE CLINICAL APPLICATIONS OF CELL KINETICS IN CANCER THERAPY

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INTRODUCTION

The study of cell kinetics has been of interest to clinicians involved in the care of cancer patients for several reasons. An initial hypothesis which motivated this interest was that, using tools like the FLM (fraction of labeled mitoses) curve, major differences would be demonstrated between cancer cells and normal cells in terms of duration of the phases of cell cycle. Except that cancer cells in general show some prolongation in G_2, this has not been found to be the case (1). A second early hypothesis was that, in general, cancer cell populations would have a much higher proliferating fraction than normal cell populations: a major rationale for the design of S-phase active antimetabolites. In fact, human leukemic blasts usually have a *lower* level of proliferation, as measured by tritiated thymidine (^{3}HTdR) labeling, than corresponding normal bone marrow precursors (2). Most human solid tumors have a relatively low growth fraction, compared to normal host tissues like the bone marrow or gastrointestinal mucosa.

A third hypothesis, which remains viable though unresolved, is that tumor cells can be efficiently and differentially synchronized or recruited, by appropriate pretreatment with some agent, so that more of them are in a phase of the cell cycle (such as S) where they can be killed by a subsequently administered "executor" agent. A related hypothesis, now supported by several studies, is that a direct correlation exists between pretreatment proliferative state and clinical response: that patients with higher growth fraction tumors are more likely to respond than those with lower

growth fraction tumors because most chemotherapeutic agents preferentially affect proliferating cells.

Recently, work from our laboratory and others has supported a hypothesis initially put forward by Sky-Peck (3): that a decrease in tumor-cell labeling index (LI) predicts for subsequent clinical response.

This brief review focuses on developments related to the viable hypotheses in cell kinetics, particularly with reference to their real or potential application in clinical cancer therapy.

ATTEMPTS AT SYNCHRONIZATION AND RECRUITMENT

Experimental Tumors

Dethlefsen et al (4) recently reviewed the use of the S-phase–specific inhibitors, cytosine arabinoside and hydroxyurea, in attempts to block cell populations in S with subsequent release of a synchronized cohort. In a variety of rodent systems, they conclude that "the published data strongly suggest that the drugs . . . which can cause good synchronization in vitro, can exert only a mild synchronizing effect in vivo and then only in tissues that have a relatively high growth fraction and short cell cycle-transit time." However, the work of Gibson & Bertalanffy (5) suggested that repeated injections of cytosine arabinoside (about 40 mg/m^2 every 2 hr for a total of 8 injections, or 16 hr: roughly twice the mean duration of S) results in marked synchronization of the B16 melanoma, a solid murine tumor with an intermediate baseline LI (19%) and growth fraction (53%).

Vinca alkaloids have been shown to arrest both tumor and normal cells in mitosis (6). Klein et al (7) demonstrated that Ehrlich ascites tumor cells in vivo could escape from the mitotic arrest produced by vincristine and travel as a synchronized cohort through at least two successive waves of mitosis. As demonstrated by labeling experiments, the second peak consisted almost entirely of cells that had been arrested by vincristine in the first peak. Thus, vincristine here produced synchronization and not recruitment of cells from a "nonproliferating pool."

It has been widely postulated that administration of a cell cycle nonspecific agent to a sensitive tumor, with resultant reduction in tumor volume, leads to an increase in growth fraction of the remaining tumor and resultant heightened sensitivity to administration of cell cycle specific therapy (8). One would expect, if this hypothesis is correct, to see an increase in LI and mitotic index (MI) at some time after administration of the first drug, presumably after it had produced tumor reduction, at least in terms of viable cell mass. There is a surprising dearth of data to support or refute this concept, from experimental systems. Schenken et al (9) recently presented preliminary data in one mouse system to support this conclusion. A number of empirical studies have reported increased therapeutic efficacy from scheduling of an alkylator followed by an antimetabolite (10–12). However, the appropriate control of simultaneous alkylator and antimetabolite was not reported, and recent work by Valeriote et al (13) suggests that, at least for phenyalanine mustard (L-PAM) and 5-FU, the reverse sequence of antimetabolite followed by alkylator may be most effective.

Work by Capizzi (14) and by Chlopkiewiez et al (15) demonstrated an apparent increase in the therapeutic index of methotrexate when it was preceded by L-asparaginase given several days before. This may be due to shutting-off of protein-dependent initiation of DNA synthesis secondary to asparaginase, with a resultant piling up of cells at the G_1-S interface which are then released when intracellular drug levels decline to a point sufficient to allow renewed DNA synthesis, resulting in progression of a temporarily synchronized cohort of tumor cells through S.

All work with drugs in synchronizing schedules, in which a second, systemically administered agent serves as the "executor," shares one problem: Synchrony of normal cell populations may occur as well, resulting in increased host toxicity. Further work is greatly needed to delineate the time sequence of synchronization and loss of synchrony in tumor versus sensitive host tissues; it may be that a sufficient differential exists to allow for therapeutic exploitation with appropriate scheduling. A different approach involves administration of a synchronizing agent, followed by *local* therapy aimed at the tumor that kills the synchronized cohort of cells most efficiently; an example is administration of radiation (most effective at killing cells in G_2 and M) after synchronization with 5-FU, as reported by Ganzer & Nitze (16). Vincristine pretreatment might be effective as well in this setting.

Human Tumors

Lampkin et al were among the first to suggest that cell synchronization techniques could increase therapeutic efficacy in acute leukemia (17). They reported that partial synchronization could be achieved with pulse doses of cytosine arabinoside in acute lymphocytic leukemia (ALL), and that "a greater therapeutic advantage can be achieved by a second cycle-dependent drug after synchronization than after the second drug alone." The time between administration of cytosine arabinoside and the peak in LI varied from 24 to 96 hr, with no synchronization observed in 4 of 20 serial studies. Lampkin et al (18) recently reported their results in 21 patients with acute myelogenous leukemia (AML) who received ara-C as an i.v. push injection of 5 mg/kg (100–180 mg/m^2). Of 14 patients in whom consecutive LI and MI determinations were performed, 7 showed a significant increase in LI and 12 in MI at 18 to 24 hr after the ara-C. The patients then received 12-hr continuous infusions with ara-C 5 mg/kg every 12 hr, started at the time of maximal S-phase accumulation (usually 18–24 hr after the initial ara-C), and continued until complete remission, with escalations in ara-C dose if the patient failed to respond to lower doses. A complete remission was achieved in 12 out of 16 children and all 5 adults (81%).

Other investigators have not found evidence of synchronization after push doses of ara-C in AML (19), possibly because of lower doses, or differences in sampling time or patient population. Kremer et al (20) found an increase in LI in 11 out of 14 patients with remission or "anti-leukemic effect" who received either 1 or 3 daily push doses of ara-C, followed 48 hr after the last ara-C dose by LI determination and treatment with vincristine and methotrexate, while only 3 out of 9 patients with no response or early death demonstrated such an increase. (Five out of ten responders sampled at 24 hr and four out of eleven "nonresponders" had a similar in-

crease.) MacKinney et al (21) used the "Lampkin AML schedule" in 23 adults with acute leukemia: They achieved complete marrow remissions in only six.

Buchner et al (22) reported a continuous infusion schedule of ara-C in patients with acute leukemia: 100 mg/m^2 per day for 48 hr. They found in 8 out of 11 patients a 1.3–4.0 fold increase in cells in the S-phase range, by DNA histogram, at the conclusion of the infusion. They employed ifosfamide as the executor drug.

More work is needed to clarify the role of ara-C as a synchronizing agent in acute leukemia, both in clinical studies and experimental models. In a homogeneous tumor cell population, the use of pulse cytophotometry to provide rapid DNA histogram analysis should allow the clinician to determine more accurately at what point synchronization is (or is not) achieved (23), and allow for more precise timing of executor agents.

Capizzi has extended his work with asparaginase and methotrexate to clinical studies in ALL (24); in several patients studied sequentially after asparaginase (dose, 40,000 units/m^2), he found evidence of initial depression in leukemic cell DNA synthesis, followed by recovery and "overshoot" at days 7–10; a similar decline in LI of normal bone marrow precursors was followed by more rapid recovery, "overshoot," and return to baseline. He has observed complete remission in 9 out of 11 adults with ALL, all of whom were "refractory" to prior methotrexate, with a median response duration of 6 months.

Marshall et al (25) reported results of LI determination after a single intravenous dose of prednisone (30–20 mg) in 10 normal and 11 acute leukemic patients: 8 out of 10 normals had a significant increase at 6 hr with a return to baseline levels at 24 and 48 hr; the leukemic marrows demonstrated peak DNA synthesis at 24 hr; with a return to baseline at $>$ 48 hr. This provocative observation awaits confirmation and/or extension to clinical trials; that steroids produce a G_1-S block is well established (26).

In solid tumors, clinical protocols have been designed along synchronization lines using a variety of drugs and concepts. Based on the observation that continuous infusion of bleomycin for 48 hr produced an increase in LI in the nodules of patients with melanoma (27) (presumably as a result of S-G_2 arrest and subsequent release), Costanzi designed a regimen (28) in which 17 patients with disseminated carcinoma of the head and neck were treated with bleomycin as in i.v. infusion at 7.5 units/m^2/24 hr for 48 hr. Methotrexate (30 mg/m^2) and hydroxyurea (2000 mg/m^2) were administered in a single dose after a 24-hr rest period to allow the synchronized cohort to traverse G_2, M, and G_1 and reach the G_1-S interface. Tumor response was seen in 10 out of 17 (59%), not better than what one might achieve with methotrexate alone. Costanzi then adopted a 96-hr bleomycin infusion (to allow for S-G_2 trapping of more cells) and changed the rest period to 48 hr and the executor to methotrexate, 250 mg/m^2 with citrovorum factor rescue. Early results are promising, but no kinetic data are available.

Samuels (29) has compared a program in which bleomycin was given on a standard twice weekly schedule, with intermittent high dose vinblastine (VB-1), to one in which bleomycin was given as a 96-hr continuous infusion, followed by vinblastine. Patients with metastatic embryonal carcinoma of the testis appeared to

fare better on VB-3, while those with teratocarcinomas responded better to VB-1. Unique toxicity (jaundice and hemolysis) was observed with bleomycin as a continuous infusion.

The observation that vincristine reliably produces stathmokinetic arrest (i.e. in G_2 and M) in human tissues 6 to 24 hr after injection, coupled with the apparent cell-kill specificity for cells in G_2 and M of bleomycin, led Livingston et al to design a two-drug combination in which the former was given 6 hr earlier (30). An encouraging response rate in this pilot study was seen in patients with lung carcinoma (4 of 15) and led to its inclusion in more complex, combination regimens designed primarily for patients with lung tumors (31, 32). Unfortunately, kinetic data were never obtained to support this hypothesis and the most recent trial involving this combination indicates that vincristine and "staggered" bleomycin adds nothing to the antitumor efficacy of a simpler, three-drug regimen (Southwest Oncology Group, unpublished data). However, encouraging results have been obtained with a similar staggered schedule in carcinoma of the cervix (33); in a study of adriamycin, vincristine, and bleomycin in testicular cancer, (34); and in Einhorn's recent study of vinblastine, bleomycin, and platinum in testicular cancer (35). It is conceivable that this combination may produce an increase in the therapeutic index of bleomycin, given (*a*) some degree of inherent efficacy for bleomycin against the tumor cells involved, and (*b*) a sufficiently large growth fraction that arrest of cells in G_2 and M by vincristine becomes meaningful, in terms of subsequent mitotic cell kill of a synchronized cohort. The lack of a suitable experimental model has prevented testing of the concept to date at a basic level.

Only one published clinical study has looked at ara-C in solid tumors as a potential synchronizing agent (36). In this study, a push dose of ara-C (200 mg/m^2) was followed by methotrexate 24 hr later, in a variety of patients who were already refractory to first-line chemotherapy. No striking evidence of synergy was seen, possibly because the empiric schedule was too imprecise or simply wrong in its timing. This study again points up the need for serial *measurements* of kinetic effects if meaningful clinical studies, seeking to demonstrate efficacy of kinetic manipulations, are to be conducted.

A number of investigators have explored "kinetically oriented" approaches in which cell-cycle nonspecific agent was followed by a cell-cycle specific one, with varying results. Burke & Owens (37) employed in acute leukemia a sequence of cyclophosphamide followed by vincristine at 24 hr and ara-C, as a 48 hr i.v. infusion begun 12 hr after vincristine. No consistent effect of cyclophosphamide was seen on LI prior to vincristine and ara-C, but it could be argued that the cyclophosphamide dose was too low and the time interval too short. Salmon recently reviewed (38) attempts to capitalize on the apparent increase in growth fraction (39), manifested as an increase in LI, after therapy with an alkylating agent in myeloma: "Despite this apparent expansion of the growth fraction, most patients do not appear to have further tumor regression induced with cycle active agents such as ara-C, azathioprine, hydroxyurea, or methotrexate, although optimal schedules and doses may not have been utilized." The other clinical study which offers laboratory support for an increase in growth fraction after alkylator therapy recently was reported by Mauer

(40). Cyclophosphamide was administered to children with disseminated neuroblastoma, at a dose of 150 mg/m^2/day for seven days. The patients received adriamycin 25 mg/m^2 on day 8. Among 14 patients whose tumor cells could be selectively studied from bone marrow aspirates, 10 had an increase in LI after the cyclophosphamide, and all responded with a decrease in LI and complete remission after adriamycin. Furthermore, if the LI and MI decreased immediately after the 7 days of cyclophosphamide, the clinical response to the sequential regimen was poor.

Other studies with encouraging clinical results, but no kinetic measurements, have been carried out in oat-cell carcinoma of the lung (41) and non-oat-cell lung tumors (42) with the sequential combination of cyclophosphamide and methotrexate. However, equally encouraging results have been obtained in these tumor categories using simultaneous combinations of drugs without any kinetic rationale.

Klein's work with vincristine in Ehrlich ascites tumor led him to do clinical trials with two doses of vincristine, followed at various, empirically chosen intervals by cyclophosphamide (7). He treated 55 patients, of whom 39 had lymphomas, 7 had oat cell lung carcinoma, 7 had acute leukemia, and 2 had unknown primary sites. He observed a 60% complete and 90% complete plus partial response rate; although these are good results, they are not extraordinary, particularly since all but two of the complete remissions were in leukemia or lymphoma. Pouillart et al (43, 44) have also treated patients with lymphoma, lung carcinoma, and other malignancies with vincristine given twice, followed by other executor compounds, with good results but not kinetic data. As Pouillart himself has emphasized (45), some of the heightened effectiveness of the combinations may be due to pharmacodynamic interactions, rather than kinetic effects.

Early work by Rentschler et al (46) has shown encouraging results in solid tumors with sequential asparaginase and methotrexate in a regimen similar to that of Capizzi in acute leukemia. Further studies in this area, including measurement of tumor cell LI when possible, are in progress.

CORRELATION OF KINETIC PARAMETERS AND CLINICAL RESPONSE

Another use of cell kinetic data, which may prove more fruitful than the design of "kinetic" clinical protocols, lies in the possible predictive relationship of these parameters (especially LI) to response and survival.

Pretreatment LI: Response and Survival

Hart et al (47) reported that a direct relationship exists between height of the pretreatment LI and the likelihood of complete response for patients with acute leukemia, studying a population composed largely of adults with AML. Hart further found a direct relationship between LI and tumor burden, as estimated by "absolute leukemic infiltrate" (percentage of blasts X percentage of cellularity of bone marrow X 100). Thus, although patients with a high percentage of LI had a greater chance of responding to (primarily cycle-active) chemotherapy, they also tended to

have *shorter* remission durations, possibly related to a greater residual "subclinical" burden of leukemia and/or inherently a more aggressive growth of residual disease. But high LI and leukemic infiltrate also correlated with longer survival, because these patients tended to be more easily reinduced and very few had early deaths. Hillen (48), Vogler (49), and Burke (37) have reported a similar correlation between pretreatment LI and response, as shown in Table 1. It is noteworthy that these regimens relied primarily on cytosine arabinoside or methotrexate, both S-phase specific agents, to induce remission.

When the "backbone" of induction therapy is ara-C plus thioguanine given simultaneously on a twice-daily schedule, the relationship of pretreatment LI to response may disappear: Vogler found no difference in LI between 45 responders and 27 nonresponders on such a regimen (50), nor did Raich (51) or Arlin (52). This suggests that thioguanine, designed as an antimetabolite but known to be incorporated into the DNA of "non-S" cells (53), may play a role in the induction of response among patients with a low LI, while ara-C is most important for those with a higher LI. However, Crowther also reported no correlation between pretreatment LI and response (54) in adult patients with AML who received a combination of daunorubicin and ara-C; moreover, in these 58 patients a *positive* correlation was reported for LI with length of remission, as well as with survival among those who achieved remission. In children with ALL, Mauer (55) has observed no relationship between pretreatment LI and response; here, of course, a major component of remission induction is the cell-cycle nonspecific agent, prednisone.

Among patients with solid tumors, data are now becoming available that suggest a relationship between pretreatment LI and response to a variety of cytotoxic drug regimens, at least for patients with breast cancer and melanoma. Sulkes et al (56) studied 56 patients with breast carcinoma and disseminated disease; among 28 with LI<8.3% prior to therapy, only 1 had > 50% tumor regression, while 12 out of 28 with LI > 8.3 responded. This difference was statistically significant, and held when the group was broken into smaller subgroups related to presence or absence of prior chemotherapy, and treatment with combinations versus single agents. Responders with a high LI also had significantly longer survival than low LI patients

Table 1 Acute leukemia: diagnosis and pretreatment kinetics versus complete remission rate

	Labeling index %				
	<9		>9		
Diagnosis	#CR/TOT.[a]	(%)	#CR/TOT.	(%)	P value
AML[b]	15/71	(21)	55/75	(73)	<0.001
ALL	3/8	(38)	18/23	(78)	0.09
All patients	18/79	(23)	73/98	(74)	<0.001

[a] #CR/TOT represents number of complete remissions/total.

[b] Results represent the pooled data of Hillen, Vogler, Hart, and Burke (37, 47-49).

(as a group) or nonresponders with a high LI. The "high" and "low" LI patients were found to be comparable with respect to age, menopausal status, and dominant metastatic site. In contrast, an earlier report by Kofman et al (57) showed no correlation between the uptake of C^{14}-formate by breast tumor tissues and their subsequent response to hormone therapy. These observations support the hypotheses that, in breast carcinoma, presently available cytotoxic chemotherapy preferentially affects proliferating cells, while hormonal manipulation, if effective, affects nonproliferating cells as well as (or better than) proliferating cells.

Thirlwell et al (58) recently reported that 8 patients with melanoma who responded to regimens containing DTIC had a median LI of 8.0, compared to 3.2 for 22 patients who were nonresponders. He also found, in an analysis of 59 patients with a variety of solid tumors (excluding melanoma and breast carcinoma), that the following relationship obtained between response rate to chemotherapy and median LI respectively: $>$ 40%, 15.8 (21 patients); 20–40%, 7.3 (15 patients); and $<$ 20%, 3.7 (23 patients).

Further suggestion of a relationship between growth fraction and response to cytotoxic chemotherapy comes from the clinical observation that lymphomas and oat-cell carcinoma of the lung, tumors with a high degree of drug responsiveness, are also among those with the highest LI in the untreated state (59). Skipper (60) has elegantly summarized the data that support a direct relationship between the growth fraction of experimental tumors and their responsiveness, especially to agents killing cells in S phase. Against this impressive volume of clinical and experimental data stands the observation of Wolberg et al (61) that pretreatment LI failed to correlate with clinical response to 5-FU in patients, most of whom had breast or colon carcinoma. Clearly, as emphasized by Hall (62), factors other than proliferative fraction are important in whether a tumor responds to chemotherapy. Yet the importance of this factor as an independent variable seems now sufficiently well established to suggest that measurements of pretreatment LI be used as an adjunct, where possible, in deciding at least whether S-phase specific agents are indicated. The combination of LI data and information regarding the presence of estrogen receptors (63) may be very helpful in making decisions about both "adjuvant" and "advanced" regimens for patients with breast carcinoma, with regard to the role of hormones versus "cytotoxic" agents.

Changes in LI and Response

Sky-Peck (3) reported that a decrease in LI after a course of therapy correlated highly with the likelihood of subsequent clinical response to that therapy. Using a technique (64) that allowed for rapid autoradiography on representative samples of viable tumor cells, Murphy et al (65) confirmed this observation; in 63 patients with a variety of solid tumors and on numerous different chemotherapeutic regimens, clinical response was observed in 12 out of 16 patients with a significant decrease in LI after therapy, and in only 2 of 47 who failed to show such a decrease (59). Data from experimental tumors (66) also demonstrate that tumor regression after chemotherapy is accompanied by a decrease in LI; however, decreases of LI (though of lesser magnitude) have also been reported in tumors resistant to the agent em-

ployed (67, 68). Given that suppression of DNA synthesis in a tumor does not guarantee response, clinical and experimental data strongly suggest that it is a sine qua non for response to occur, at least with agents that preferentially kill proliferating cells. This appears to be true as well for radiation; in studies recently reported by Tubiana (69), among patients with head and neck tumors who underwent weekly LI determination during continuous radiation treatment, a $>$ 50% decrease in LI correlated with clinical radiosensitivity, and $<$ 50% decrease with nonresponse. Breitenecker et al (70) made a similar observation with radiation in carcinoma of the cervix.

As a clinically useful predictor of response, the study of LI pre- and post-therapy has two serious drawbacks: (*a*) the tumor most be accessible to repeated biopsy, and (*b*) the patient must be committed to a course of treatment before its effect can be evaluated. Several investigators have reported preliminary studies to test a more useful hypothesis (if correct): that drug therapy in vitro may predictably and *differentially* suppress DNA synthesis in sensitive cells. Cline (71) has summarized the conditions that should be met to test such a hypothesis: (*a*) the drug(s) must be in active form (or converted to it) in the in vitro system; (*b*) the drug(s) must be present in concentrations approximating those achieved in vivo; (*c*) the rate and other characteristics of DNA synthesis of the malignant cells in vivo and in vitro must be sufficiently similar so that drug effects under the two conditions are comparable; and (*d*) there must be sufficient time for drug action to become manifest. Zittoun et al used a test system (72) in which drugs were directly added to leukemic cells, at concentrations that produced a 50% decrease in 14-C-thymidine incorporation of control nonleukemic marrows after 2 hr; in vitro depression of labeled thymidine incorporation was more marked in those who responded to therapy than in the nonresponders. The mean decrease in 16 responders was 52% compared to 24% in 26 nonresponders (P $<$0.001). No difference was observed in depression of ^{3}H-uridine incorporation between responders and nonresponders. Lippmann et al (73) found that, when leukemic blasts from ALL patients were incubated 18 hr with dexamethasone directly added in vitro, ^{3}HTdR incorporation was significantly inhibited in glucocorticoid-sensitive, but not in glucocorticoid-resistant cells. Furthermore, the minimal concentration necessary to achieve this effect approximated that necessary to saturate steroid-binding protein receptor sites.

Problems associated with direct addition of drugs to in vitro systems include the following: (*a*) the drug may not be present in its active form in the in vitro system, and (*b*) the drug (or metabolite) concentration achievable in vivo may not be approximated under in vitro conditions, either through oversight or (commonly) lack of pharmacologic data. An attempt to circumvent these difficulties involves the use of "treated serum," defined as serum obtained from the patient shortly after drug administration, at a time when pharmacologic concentration of active metabolites may be nearly maximal. Such a technique has precedent in the use of various dilutions of host serum containing antibiotics to determine whether adequate bactericidal concentrations have been reached (74). The determination of antineoplastic activity of treated sera, for the purpose of determining duration of antitumor effect after a single dose, also has precedent in experimental systems (75).

Burns et al (76) measured ^{3}HTdR incorporation by scintillography of leukemic cells incubated with pretreatment (control) serum versus serum from the same patient obtained after 2 days of therapy with ara-C. The period of incubation with treated serum was 4 hr. All patients who had depression of thymidine uptake by 65% or more showed response, while depression by $< 50\%$ was not associated with clinical response.

It is probable that 4 hours of incubation is not, in many instances, a sufficient period of time for drug effect on DNA synthesis to become manifest. This is suggested by experimental tumor work with alkylating agents (67) which shows that maximal inhibition of DNA synthesis occurs 24–48 hr after their administration in vivo, and by the failure of systems using short-term exposure conditions to demonstrate consistent, good correlation between changes in DNA synthesis and response (77, 78). Using a 24-hr period of incubation with treated serum versus fresh and 24-hr control serum, Thirlwell et al (79) found the following: (*a*) the LI of tumor cells remained constant over 24 hr in 90% of controls, with no evidence of a nonspecific effect from serum itself; (*b*) treated sera did, in some cases, produce significant decreases in LI after 24 hr relative to controls; and (*c*) these decreases correlated with the subsequent observed clinical response. In 10 patients with solid tumors who had a significant decrease in vitro, who received the chemotherapy tested, 5 had partial responses and 3 improved; in 12 without a decrease, none had response, 1 improved, and 11 had progressive disease.

Other investigators have reported that depression of DNA synthesis in vitro by hormones correlates with the clinical response in patients with breast and endometrial cancer (80, 81). As with the use of cytotoxic drugs or treated serum, the results are encouraging but preliminary, and require confirmation.

FUTURE DIRECTIONS

Several areas involving measurement and manipulation of cellular proliferation have great potential for exploration. A leading possibility is to minimize cell division in the most drug-sensitive normal host tissues (usually bone marrow and gastrointestinal mucosa) in order to increase the therapeutic index of the administered drug against tumor tissue. Some ways in which this might be accomplished include: (*a*) intravenous hyperalimentation to reduce DNA synthesis in gastrointestinal mucosa (82, 83); (*b*) administration of an inhibitor of protein synthesis just prior to an S-phase specific agent (84); (*c*) administration of ara-C as a single injection, which reversibly and transiently suppresses bone marrow DNA synthesis, just prior to cytotoxic therapy with a short duration of action (e.g. nitrogen mustard, nitrosourea); and (*d*) administration of a large dose of glucocorticoid (to inhibit G_1-S transition) just prior to S-phase specific therapy. With all of these approaches, it will be critical to measure the effect on coexistent tumor cells as well; inhibition of their proliferation might negate any possible therapeutic advantage.

A second, corollary hypothesis involves maximization of bone marrow proliferation after chemotherapy, to speed recovery and allow for more intensive treatment of the tumor. This could involve (*a*) lithium administration (85), (*b*) *Corynebac-*

terium parvum (86) or other immunostimulants, (*c*) administration of stored autologous serum containing a high concentration of colony-forming units (e.g. as obtained after a previous course of myelosuppressive therapy), and (*d*) autologous marrow reinfusion (87).

Third, one may attempt hormonal or other "physiologic" stimulation of tumor growth to increase sensitivity to chemotherapy. Historically, this is illustrated by the administration of testosterone prior to radioactive phosphorus systemically, in patients with prostatic carcinoma. Experimental tumor work with organ cultures from rat mammary tumors (88) suggests that prolactin stimulates DNA synthesis in hormone-dependent but not in autonomous tissue. In addition, Kiang et al (89) have demonstrated stimulation of the DMBA-induced rat mammary tumor by low dose estrogen therapy. The provision of specific metabolites (e.g. asparagine) is another possible avenue of approach, in tumors whose dependency can be identified. Burke et al (90) studied sera containing a stimulator of leukemic and normal bone marrow cell DNA synthesis, the levels of which were maximal 9 days after administration of a massive dose (75 mg/kg) of cyclophosphamide, and suggested possible therapeutic usefulness of these growth regulators.

Young et al (91, 92) have shown that, in sensitive tumor systems, DNA synthesis is depressed in both tumor and normal tissues, but there is rapid recovery and overshoot, followed by a return to baseline values, in proliferation of the normal bone marrow and GI mucosa, with much slower recovery in DNA synthesis of the tumor. When resistance develops, this differential pattern of recovery is lost (68). This type of approach deserves application to more appropriate scheduling of chemotherapeutic agents in clinical trials, and may also indicate when relapse is imminent, in patients with serially accessible disease.

Finally, approaches toward *selective* kill of nonproliferating tumor cells can be explored and tested with appropriate measurements of kinetic parameters. They might include immunotherapy (to which S-phase cells appear most resistant) (93) or use of agents with selective toxicity toward hypoxic cells (94). In this setting one might expect to see an *increase* in the growth fraction of residual viable cells as an indicator of response.

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