Preclinical Screening and Evaluation of Agents for the Chemotherapy of Cancer: A Review*

ABRAHAM GOLDIN, JOHN M. VENDITTI, AND NATHAN MANTEL

(Laboratory of Chemical Pharmacology, and Biometry Branch, National Cancer Institute, Bethesda, Md.)

The current extensive programs on antitumor chemotherapeutic screening and drug evaluation are conducted mainly in rodents with transplantable tumors (7, 11, 13, 14, 19, 24, 26, 43, 45, 46, 48, 56, 57, 60–65, 67, 72, 75, 78, 84–91, 94, 99). Additional programs are being conducted with spontaneous and carcinogen-induced tumors (12, 48, 69, 79, 80, 96) and with tissue culture (8, 17, 18, 21, 22, 47, 59, 88, 95), microbiological (1, 5, 6, 16, 20, 21, 23, 50–53, 55, 77, 82, 88, 92), and biochemical (9, 13, 49, 66, 71, 82) systems, and systems involving development and differentiation (3, 58, 81), etc. Numbers of compounds are passing the screening and evaluation tests and are being introduced into preclinical and clinical pharmacology and into clinical trial.

There is considerable interest in the question of how well the screening and evaluation tests may predict clinical usefulness (25, 73, 75, 83). Will activity in specific animal tumor systems correlate well with activity in specific types of human tumor systems? Can the animal systems not only detect antitumor activity, but also rate drugs with respect to such activity? Will the rating of the drugs in the animal systems correlate with the comparative therapeutic evaluation of antitumor effectiveness in man (102)?

Basic to any attempt to determine correlation of animal testing and chemotherapeutic effectiveness in humans is the validity of the animal screen or evaluation test itself. What information does the animal screen or test provide? Is it actually evaluating activity in the animal system? Is it appropriately ordering the compounds in terms of relative effectiveness? Are the criteria of measurement of therapeutic effect appropriate? Is the assay system paying sufficient attention to the host-tumor-drug relationship? This basic question is prompted by the work of E. K. Marshall (68), who pointed out that any evaluation of agents in infection chemotherapy should take into account the triad of parasite, host, and drug. The current review was undertaken to determine what information the common animal screening and evaluation procedures may provide. The parameters of drug action examined are those employed in common practice. In the analysis no attempt is made to be exhaustive, but rather to review the types of common screening and evaluation procedures and their meaning. It is hoped that this may contribute to the re-examination of ways and means of improving animal and related screens, so that any attempt at correlation with results in humans may be more meaningful.

TUMOR INHIBITION ASSAYS

In the study of the antitumor activity of drugs, one may determine the effect of the drug on the tumor without any regard to its effect on the host. This procedure conceivably could be useful, if the antitumor agents were nontoxic for the host. In such case, the evaluation of drug effectiveness could be limited to the determination of the amount of drug required to produce a desired degree of inhibition or cure of the tumor. Or the evaluation could be based on the time or number of injections required to produce a specified antitumor effect, or the rapidity of occurrence of a refractory state, or the degree of activity for a spectrum of tumors, etc. Assay systems could, in the above cases, be limited primarily to simple dose-response studies with respect to the tumor, in the main ignoring effects of the drugs on the host. Although the drugs might differ in physiological disposition, including transport, excretion, or metabolic alteration, etc., host toxicity would be removed as a factor in the assay. The methodology would be similar to that employed in the in vitro tissue culture and bacterial systems, and the extent of correlation of results with these systems could be determined.

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From the point of view of therapeutic applicability, assay systems of drug effectiveness based on antitumor effect alone pertain to too simple a situation, since they presuppose that antitumor agents are nontoxic for the host. All of the known antitumor agents are toxic for the host as well as for the tumor, and the toxicity for the host severely limits their usefulness.

If the tumor system employed for assay is extremely sensitive to drug treatment this could, in effect, remove host-toxicity as a factor. In such case, since relatively nontoxic doses would exert antitumor effect, it might still be possible to limit the assay to evaluation of antitumor action of the drug. Such a system may be provided by using a tumor system empirically found to be sensitive, or selection could be practiced for sensitivity. A tumor system could also be made more sensitive by diminishing the size of the inoculum (41, 44) or by initiating treatment at an early time following tumor implantation (37, 35, 40, 78, 98). Such a system could then be employed to compare the relative effectiveness of drugs in inhibiting tumor growth (57, 78, 98). In some instances, it may be desirable to employ a relatively sensitive tumor system for determining marginal antitumor effects of drugs. This may have a practical value—for example, in screening for drugs to be employed as surgical adjuvants.

However, care must be exercised in the utilization of such assay systems. If two drugs completely inhibit early tumor growth at nontoxic doses, which is the more effective drug? It could, presumably, be the one, for example, which accomplishes complete inhibition at a lower dose. However, this might indeed be misleading if neither drug were capable of inhibiting tumor growth in mice with more advanced tumor.

Since the known antitumor agents are limited in their usefulness because of their toxicity for the host, an assay system in which drug toxicity for the host is limiting may more closely reflect the clinical situation. A standard procedure employed in screening programs has been to measure the extent of inhibition of growth of the local transplanted tumor after a specified number of treatments. Usually the animal is sacrificed and the tumor is enucleated and weighed. Alternatively, the local tumor may be measured in one or more dimensions. Comparison is then made with the size or weight of the tumor for untreated control mice. The per cent inhibition of tumor growth is thus determined and must usually exceed a specified figure in order for a drug to pass a primary screen. The ratio \( t/c \) (fractional growth of treated tumor as compared with control) is commonly employed, where \( t \) is the average weight or size of treated tumors and \( c \) is the control average. In order to take into account nonspecific toxicity for the host it is usually required that \( t/c \) must not only be less than a specified fraction, but that this must be achieved with a limited animal weight loss and/or a limited host mortality response. The above type of procedure has been employed in primary screening programs to determine whether drugs are of sufficient interest for further study (7, 12, 19, 24, 26, 61–65, 72, 84, 85, 87, 89, 90, 94).

By such a test system, the compounds may be ordered with respect to the relative degree of effectiveness; i.e., comparisons may be made of \( t/c \) at a maximum tolerated dose.

Where both host effect and tumor effect are to be taken into consideration, the question arises as to the choice of parameters for each. Toxicity for the host is usually measured in terms of drug lethality, or as body weight loss. The measure of antitumor effect may be based on tumor size or weight, or on some other characteristic of the tumor, such as protein content, etc. Cytoxic effects or biochemical effects may be measured. The growth curve of the tumor may provide a useful measure of antitumor effect. Survival time of all mice or, alternatively, only of mice not succumbing to drug toxicity, have been used as measures of drug effectiveness. Where drug effects are extensive, or the tumor challenge weak, the percentage "cures" elicited may provide an index of drug effect.

Survival time assays

Whereas routine screening procedures most commonly employ tumor size or tumor weight as the basis for evaluating antitumor effect, there are some notable exceptions. For example, in chemotherapeutic screening with the L1210 tumor, the Cancer Chemotherapy National Service Center employs an index of effect based on the prolongation in survival time of tumor-bearing mice (61–65). Survival time as a measure of chemotherapeutic effect has been used by various investigators in laboratory research with the tumors (11, 13, 14, 24, 48, 56, 57, 67, 69, 78, 80, 86, 96). Since increasing survival time, rather than reducing tumor size, more properly reflects the purpose of treatment, such extension would, in many circumstances, more appropriately describe the therapeutic efficacy of treatment. Accordingly, in this laboratory, assay systems have been developed in which alternative chemotherapies are compared on the basis of the survival time of the tumors (27, 31, 40, 43–46, 99). Since, for each drug on a specified treatment schedule, survival time varies with dose level, an appropriate measure for
a drug on a schedule is the maximum survival time it elicits. At dosages beyond some optimal level, toxic effects for the host outweigh antitumor effects so that further increases in dosage lead to reductions rather than extensions in survival time. Use of the maximum survival time elicited with a drug on a schedule as the measure of therapeutic effect leads to certain modifications in laboratory procedure. To ensure bracketing the optimal dose level, a relatively wide dosage range should be employed. Also, the interval between successive doses must be small enough so that the observed optimal dose and maximal survival time may be taken as near approximations to their unknown true values. In employing this methodology, described in detail in other publications (40, 43–46, 99), it has been the practice of the authors to use the median survival time of a group of mice as the summary measure of survival time. Individual survival times may, nevertheless, be reported (45, 46, 99), protection thus being afforded against instances where the median survival time may be misleading. Chart 1 provides an illustration of the use of this procedure for determining the relative effectiveness of several antileukemic agents.

TUMOR SIZE VS. SURVIVAL TIME

If extension in the survival time of tumor-bearing animals is the motivation of experimental chemotherapy, justification for using inhibition in tumor size as a criterion, aside from perhaps its simpler ascertainment, would be dependent upon the existence of an underlying correlation between the two measures of effectiveness. The question, then, is whether or how tumor inhibition can be used to select agents capable of achieving important increases in survival time.

There are two aspects to this question. First, where an agent can, in fact, elicit important increases in survival time, or possibly even cures, how likely is this agent to be detected in a tumor-inhibition assay? Second, to what extent may agents, capable of eliciting only limited, nonspecific increases in survival time, give rise to reductions in tumor size? These aspects will be discussed below.

Where the use of tumor inhibition is premised on its being an easily ascertainable basis for selecting effective agents, the question of how much more efficient tumor-inhibition assays may be also arises. Goldin et al. (44) have suggested that,
under certain circumstances, survival time assays can be performed with efficient use of time and animals. In such case, tumor-inhibition assays may be unnecessary.

Although the emphasis in survival time assays is on the longevity of treated animals, it is not intended that in such assays toxic manifestations should be overlooked. One would obviously be dubious about accepting as an effective agent one which, while increasing survival time, resulted in permanent injury to a vital organ.

Assays to determine effectiveness of agents.—It is perhaps naïve to expect too high a correlation between the tumor-inhibitory effects of agents and their potential for extending survival time. Measurement of inhibition of tumor growth does not permit accurate extrapolation of survival time. Factors which may influence the relationship of inhibition of tumor growth to survival time include the drug dosage, schedule of therapy, and the nutritional status of the host. The relationship may vary in accordance with the time at which the observations are made. Agents effective in increasing survival time may achieve such prolongation in a number of ways. For an effective agent with a true tumoricidal effect a correlation might be readily obtained between tumor inhibition and survival time, provided the chemical agent is relatively nontoxic for the host. The correlation would be less readily demonstrable if the agent is toxic for the host. Alternatively, an agent may behave as a carcinostat, perhaps even with stasis not being evident until after the time selected for sacrifice of animals in a tumor-inhibition assay. While carcinostasis might lead to increased longevity, it would not necessarily produce effects detectable in a tumor-inhibition assay. There may be agents capable only of inhibiting invasion and metastasis of the neoplastic process without affecting the primary tumor; here the infiltrating tumor would appear to be sensitive and the local tumor resistant. In such instance, an agent could be overlooked readily in a tumor-inhibition assay. Conversely, there may be agents which inhibit the primary tumor but fail to retard metastatic infiltration. Increase in survival time of mice with leukemia L1210 has been observed to occur with either progressive growth of local tumor at the subcutaneous site of inoculation of leukemic cells or a decrease in size or actual disappearance of the local tumor (51). If agents producing the kinds of effects described above were encountered, restriction of measurement to observation of local tumor size should be particularly hazardous as a measure of therapeutic effectiveness.

When one considers results obtained with a range of dose levels, some difficulties can be visualized. For an effective drug, as the dose is increased, the rate of tumor growth may be diminished and the survival time increased. It is clear, however, that, owing to toxicity for the host, the very highest dose levels may give the highest degree of tumor inhibition, yet show the shortest survival times. This difficulty can be met in part by requiring that the dosage level employed be one that yields little lethality (or toxicity), at least as of the time of sacrifice. This may only yield fresh complications, due to differences among agents in the time at which they cause death or other toxic manifestations. With some agents, animals which have received lethal treatment may still be alive and in apparently good condition as of the scheduled date of sacrifice. Tumor inhibition would here be ascertained at what is, in fact, a nontolerated dose.

Further difficulties arise when one considers the possibility of cumulative toxic effects for the host. Where cumulative effects are minimal, one may continue to treat successfully animals which have not yet shown important tumor inhibition. Conversely, the greater inhibitory effect for another agent may be accompanied by such cumulative toxic effects for the host as to make impossible further successful treatment.

It is important to emphasize that, in the employment of survival time assay systems, one is guarded against the above types of situations. A variety of relationships encountered between tumor inhibition results and survival time extension is illustrated in Charts 2a, b, 3a, b.

Non-specific effects of treatment.—Non-specific drug toxicity for the host, including reduced caloric intake and body weight loss, may alter tumor growth and survival time. There need be no precise correlation between the effect of non-specific toxicity on tumor size and survival time. Inhibition of tumor growth by caloric restriction may or may not result in an increase in survival time, or it may, if the restriction is too extensive, actually result in a decrease in survival time. With increasing weight loss there may be progressive inhibition of tumor growth, but survival time may increase with moderate weight loss and decrease with excessive weight loss. Various investigators (2, 4, 28, 54, 76, 80, 93, 101) have produced tumor inhibition by caloric restriction. In one series of experiments it was shown that the degree of tumor inhibition of Sarcoma 180 elicited with a variety of agents corresponded to that obtained with levels of caloric restriction giving the same weight loss (28).
Charts 2a and 2b.—Examples of experimental antitumor activity employing Carcinoma 755. These charts permit following the progressive antitumor effects of treatment and show also its therapeutic efficacy as evidenced by a prolongation in survival time. Each curve shows the average tumor diameter, as measured by palpation, of a group of treated mice on specific days following tumor implantation. The curves are discontinued on the day of median survival of the group.

For the experiment represented by Chart 2a, daily treatment with graded dosages of compound was initiated 4 days following tumor inoculation and was continued until death of the animals. It can be seen that both 6-mercaptopurine (6-MP) and Cytoxan were effective at the optimal dosages of 8.1 and 6.2 mg/kg daily, respectively, in increasing the median survival from 24 days for untreated controls (C) to 50 days. With Cytoxan this increase in survival time was accompanied by only a moderate tumor-inhibitory effect, contrasting with the marked inhibition due to 6-MP. Had tumor inhibition at some early sacrifice date been the only criterion for evaluating an agent, standards by which 6-MP would have been accepted could well have led to the rejection of Cytoxan, an equally effective drug by survival time standards. It may be noted, especially for the Cytoxan data, that doses beyond the optimal, while showing increased tumor inhibition, lead to decreased survival times.

For the experiment represented by Chart 2b, daily drug treatment was initiated on day 4 and continued for only 5 days. With this curtailed treatment the optimal daily dosage was greatly increased, to 65 mg/kg for 6-MP and to 108 mg/kg for Cytoxan. Again it is evident that the efficacy of 6-MP (greater this time than that of Cytoxan) is accompanied by marked tumor-inhibitory effects, these being so great that tumors are not evident for an extended period following the discontinuance of treatment. Overdosing with 6-MP to a level of 180 mg/kg daily resulted in a decrease in survival time.

5-Bis(1-aziridinyl)-3,6-bis(2-methoxyethoxy)-p-benzquinone (A-139) which elicited clear tumor inhibition at a daily dosage of 5 mg/kg did not produce any important extension in survival time.

In other experiments with Carcinoma 755, not shown here, treatment with 6-MP or with Cytoxan was not initiated until as late as day 13, at which time mice already had clear palpable tumors. Drug treatment at this time, which was effective in extending survival time, caused the tumors to regress, though ultimately there was regrowth.
is not to say that the antitumor effect of the agents was necessarily mediated through a food restriction mechanism. However, it does suggest that the effects observed were nonspecific, reflecting only damage occurring to the host.

Toxic effects for the host are generally evident from the over-all weight loss which follows. Where the nonspecific effect on a tumor is due to a toxic effect not reflected in over-all host weight loss, the effect may mistakenly be taken as real. Alternatively, real antitumor effects which happen to be accompanied by weight loss can be mistaken as nonspecific. When caloric restriction is employed as the nonspecific agent, tumor-inhibitory effects extensive enough to meet most screening requirements can be achieved. However, these effects will, in general, be accompanied by substantial weight loss.

The relationship of tumor inhibition and survival time of the animals may be dependent upon the extent of underfeeding. The relationship may change with the time at which the measurements of tumor size are taken. The schedule of underfeeding, including the time at which underfeeding is terminated, may also influence the relationship, etc.

Alteration of diet may also alter tumor growth-survival time relationships, etc. With feeding of

![Chart 3a](image)

**CHARTS 3a AND 3b.**—Examples of experimental antitumor activity employing Sarcoma 37. For explanation of how results are shown in these charts see legend for Charts 2a and 2b, first paragraph.

For the experiment represented by Chart 3a, daily treatment with graded dosages of compound were initiated 3 days following tumor inoculation and were continued until death of the animals. No increase in survival time was elicited with either nitrogen mustard or hydrocortisone. Overdosages with these compounds which reduced survival time did not generally produce tumor-inhibitory effects. At an optimal daily dose of 120 mg/kg, N-methyl formamide yielded a median survival time of 35 days, compared with 17.5 days for untreated controls (C). This was not accompanied by important tumor inhibition. The overdose of 333 mg/kg daily yielded much more extensive tumor inhibition, but survival time was reduced.

For the experiment summarized in Chart 3b, daily treatment with 8-azaguanine, 6-thioguanine, or 4-aminopyrazolo(3,4-d)pyrimidine (4-APP) was initiated on day 3 and continued for only 5 days. None of the three compounds was effective in increasing survival time, and only one, 6-thioguanine, gave evidence of tumor inhibition. For the other two compounds, antitumor effects were not clearly evident even at overdosages.

In Charts 2a, 2b, 3a, and 3b, a variety of relationships between drug effects may be seen. Drug efficacy in extending survival time may be accompanied by a greater or less degree of tumor inhibition. Drug toxicity for the host may or may not be accompanied by tumor-inhibitory effects.

![Chart 3b](image)
desiccated tumor, the Walker tumor continued to grow, and survival time was increased (70).

The age and weight of the mice may also alter tumor growth-survival time relationships (39, 36).

In general, where survival time is the end-point employed, the problem of nonspecific effects is likely to be less critical. Work in this laboratory has shown that, while caloric restriction may produce important reductions in tumor size, the increases in survival time to which it gives rise may tend to be less dramatic (54).

In view of the drastic effects that diminished food intake may have on the growth of transplantable tumors, and the possibility of underfeeding actually increasing survival time, the importance of characterizing experimental tumor systems with respect to the influence of underfeeding cannot be overemphasized. If the experimental tumor system is sensitive to underfeeding, and/or weight loss, what would it behoove us to screen thousands of drugs, only to select as positive those drugs that depress appetite! Further testing would be required, in such cases, to determine to what extent diminished food intake could account for the observed inhibition of tumor growth. Experiments involving paired feeding or isocaloric controls would be required, and they are time-consuming.

It may be profitable in setting up chemotherapeutic assay systems to establish regression lines for the tumors employed, relating degree of underfeeding to antitumor effect or degree of weight loss to antitumor effect. The antitumor effect could be measured as tumor weight, survival time, etc. The extent of correspondence of the drug-induced antitumor effect to the regression lines may then be determined. A close correspondence would be indicative of nonspecific antitumor action of the drug.

The complication of nonspecific toxicity in evaluation of the antitumor effectiveness of a drug may frequently be overcome in large measure by appropriate adjustment of the assay procedure. If it is found that the test system employed is too sensitive to nonspecific calorie restriction or host toxicity, this may sometimes be remedied by increasing the inoculum level or withholding therapy until the tumor is more advanced. Thus, food restriction may inhibit local tumor growth and increase survival time of mice inoculated with leukemia L1210 when the restriction is initiated within several days following leukemic inoculation. However, when the disease is sufficiently advanced, caloric restriction produces minimal inhibition of the local tumor growth and does not increase survival time (54). This obviates the need for further testing of compounds (with isocaloric controls, etc.) that are found active against advanced leukemia L1210. It removes the need to be concerned about any possible failure of correlation of effect of drug-imposed caloric restriction on tumor growth and survival time in attempts to interpret specific drug effects. Charts 4 and 5 illustrate some of the points made in the foregoing, relative to the effects of food restriction.

THE THERAPEUTIC INDEX

A concept which has been useful in pharmacological research and which has been applied in ex-
Experimental cancer chemotherapy is that of the "therapeutic index." This index is one which takes account of the fact that the action of an agent has a dual nature. There are effects both on the disease process for which the agent is being applied and on the diseased host. When appropriately employed, the therapeutic index is a parameter, valuable in characterizing the action of a therapeutic agent. In general, the therapeutic index may be used to rate antitumor agents with respect to the margin of safety in their use. The higher the therapeutic index, the greater the margin of safety in the achievement of a specified antitumor effect.

Specifically, the therapeutic index is the ratio of the dosage level of an agent causing a defined degree of damage in the host to the dosage causing a defined effect on the disease process. Damage to the host may be in terms of lethality, weight loss, or other toxic effects of the agent, the defined degree of such effect ordinarily being at some minimal level. Relative to the disease process, the defined effect may be some specified reduction in population of the disease-causing entity below either its initial value or the final value for corresponding untreated controls; alternatively, the effect on the disease process may be in terms of an objectively measured degree of improvement in the host, or a certain percentage of cures, or a certain survival time increase. With experimental tumor systems, the therapeutic index may be taken as the ratio of a specified host toxicity to a specified antitumor effect (56, 57, 72, 84). For purposes of the current discussion, it may be defined as LD10/ED90, where the LD10 is the dosage lethal to 10 per cent of the test animals and the ED90 is the dosage yielding a 90 per cent reduction in tumor weight below untreated controls.

The medical advantages of a high therapeutic index in some situations can readily be seen. Suppose in a clinical situation the index were defined in terms of the ratio of dose just barely producing toxic manifestations in the patient to the dose giving some large percentage of cures. Where the ratio is high, a treatment dosage level can be selected somewhere between the effective dose and the tolerated dose so as to provide a wide margin of safety. There is enough latitude to ensure little chance of a patient's being either overtreated or ineffectively treated. An agent with a high index is thus ideal for routine medical use with only fairly general instructions to clinicians as to appropriate treatment levels. The lower margin of safety which would obtain when the therapeutic index is low could restrict the use of an agent to cases where conditions can be carefully controlled with respect to the characteristics both of the patient and the drug. It might be too optimistic to expect a clear-cut therapeutic advantage in man with a drug which has only a moderate increase in therapeutic index in an animal tumor system.

Return now to the therapeutic index defined

**CHART 5.**—Effects of food restriction on Carcinoma 755. Results are shown in the same way here for food restriction effects as they are for drug treatment effects in Charts 2a, 2b, 3a, and 3b. For explanation of what is shown, see legend for Charts 2a and 2b, first paragraph.

The top panel shows how food restriction can mimic the effects of a compound which, though inhibitory to the tumor, is ineffective in eliciting important survival time increases. Extreme food restriction, like overdosage with some drugs, leads to more pronounced tumor inhibition but survival time is reduced.

For the experiment of the bottom panel, the initiation of food restriction was delayed until day 10. In this experiment, food restriction does lead to an extension in survival time (untreated controls died sooner in this experiment than in the first). As with overdosages of an effective agent, the more extreme tumor inhibitions with still further food restriction caused earlier death of the animals. That food restriction can produce some degree of extension in survival time does suggest that agents to be judged effective should be required to yield still greater extensions when working with this experimental tumor system.

The phenomenon of food restriction being more effective when initiated late than early parallels effects observed with some chemical agents. If treatment with a tumor-inhibitory agent having cumulative toxicity for the host is initiated early, it will kill the host before the tumor dose; with delayed initiation of treatment, drug lethality occurs after the normal time of death from tumor.

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The phenomenon of food restriction being more effective when initiated late than early parallels effects observed with some chemical agents. If treatment with a tumor-inhibitory agent having cumulative toxicity for the host is initiated early, it will kill the host before the tumor dose; with delayed initiation of treatment, drug lethality occurs after the normal time of death from tumor.
above as the \( \text{LD}_{10}/\text{ED}_{90} \) in an experimental tumor system. Clearly, it is not sufficient to inhibit a tumor only 90 per cent; it may still be growing or may resume growth and destroy the host. Suppose one were willing to tolerate 10 per cent drug lethality for the host. Then, where the index is high, a large relative dosage increase over the \( \text{ED}_{90} \) can be effected without encountering undue lethality, while for a low index only a small increase can be tolerated. The increased effect on the tumor depends, however, not only on the dosage increase but also on the nature of the dosage-response curve for the tumor. Where there is parallelism of these curves for the two agents, ordinarily the larger dosage increase permitted with the high therapeutic index would result in greater antitumor effect. Such parallelism may not, however, exist. It is possible that the agent with the lower index may nevertheless display a so much steeper antitumor dosage response curve as to prove more efficacious at the tolerated level. The agent with the greater index may have so shallow a curve as to be little more effective against the tumor at the \( \text{LD}_{10} \) than at the \( \text{ED}_{90} \).

The therapeutic index may not be too discriminating if it is employed with weak or highly sensitive tumor systems. If the tumor system is extremely sensitive, any number of drugs having different therapeutic indices could effect complete inhibition of tumor growth. In such circumstances, the assay system would, in essence, determine the degree of safety with which any number of drugs may effect total inhibition of tumor growth.

A common practice in determining the therapeutic indexes of compounds is to obtain the host toxicity data in normal mice, with the tumor mice exclusively used to determine degree of antitumor inhibition. An additional element of error is involved in this procedure, since there may be a lack of correspondence of the dose-mortality curve for the normal host and the tumorous host. In general, the tumorous host may tolerate less of the drug. Additional studies would be required to compare drug toxicity in the normal and tumorous host. Even if the drugs are so inhibitory to tumor growth that the dose range for antitumor effect is much below the dose range for toxicity, the most efficient practice could be to determine the entire dose-response curves for tumor and host in the same population of tumorous animals. If it is considered that this dose range is too great, it might be useful in order to bring the antitumor and antitumor dose ranges closer, to alter the test system so that the antitumor effect is more difficult to obtain.

The SPECIFICITY INDEX

While intended primarily as a measure of drug safety the therapeutic index may, under appropriate circumstances, be employed to compare the therapeutic potencies of various drugs. This is true when the tumor-dose response curves and also the host-dose response curves for the various agents are parallel. This may occur if the various agents being compared have related structures and thereby display similar activities. Care, however, should be taken in comparing therapeutic indices, particularly for unrelated structures. In general, when the agents do not have similar activities, the therapeutic index fails to provide an answer to the question, "For equivalent cost in toxicity or equivalent risk for the host, which drug is the more effective antitumor agent?"

An alternative measure, the specificity index, has been employed (27, 33-39) as a measure of therapeutic efficacy. In broadest terms, it is defined as the antitumor effect at a specified toxicity risk. The question is asked, "For equal host toxicity, which drug gives greater inhibition of tumor growth, or greater increase in survival time, or greater percentage cure, etc.?" Other parameters of host effect and tumor effect could be employed. The host effect could be body weight change, food intake, hematopoietic alteration, or a cytological or biochemical response, etc. The tumor effect could be change in cell number, level of enzyme, cytologic alteration including mitotic aberration, etc.

In principle, there is not only a specificity index for an agent but also a specificity curve. Suppose an agent has been administered to the tumorous host under conditions which permit ascertaining separate dose response curves for both tumor and host. A specificity curve can, in turn, be determined from these curves by relating the antitumor effect of drug treatment to the corresponding effect on the host. Drugs which have distinct response curves for tumor and host may yet have identical specificity curves.

Chart 6 illustrates this concept. For each of two drugs, \( A \) and \( B \), the panels of the figure illustrate the tumor response curve, the host response curve, and the specificity curve. In the illustration, drug \( A \), which has the lower response curve both with respect to the tumor and the host, has, nevertheless, the higher specificity curve. For simplicity, in the illustration, the response curves are shown as parallel straight lines; this is not a general requirement for obtaining or comparing specificity curves.

The methodology may be employed to rate drugs with respect to specificity, or to evaluate
regimens of therapy. This type of evaluation has been made with individual drugs (38, 39) and combinations of drugs (27, 32–34, 37, 39) used in treatment. The combinations of drugs have been employed in studies of synergism (27, 32, 39) and antagonism (27, 32–34, 37).

In the above types of assay the question may arise as to how to determine dose-response curves for host and for tumor in the same host. This has been accomplished in one type of experiment in which host mortality was used as the toxicity index and survival time of mice not succumbing to host toxicity as the tumor index, by designing the experimental test system to provide a temporal separation between the two parameters. In this system, animals succumbed to drug dose toxicity prior to the time that untreated controls succumbed to tumor.

**RELATIONSHIP BETWEEN THE THERAPEUTIC INDEX AND THE SPECIFICITY INDEX**

If the therapeutic specificity of an antitumor agent is defined in terms of its antineoplastic effect at a defined level of tolerance for the host (specificity index), it is seen that there is no necessary relationship between a drug’s therapeutic specificity and its therapeutic index. The therapeutic index has to do not so much with the efficacy of an agent, but rather with the care needed to be exercised in its employment. The importance of both the efficacy of an agent and the degree of safety in its use is evident. Since, in clinical cancer situations the utmost of care is exercised in the use of a drug, additional attention should be given to therapeutic specificity as an important basis for ranking candidate agents.

The concepts of the therapeutic index and therapeutic specificity have certain parallels in the assay procedures involving maximum survival time increases. As described above, the assay is directed toward ascertaining the maximum survival time which can be obtained with a drug on a particular schedule. This maximum can be thought of as corresponding to the drug’s therapeutic specificity. Use of dosage levels either above or below the optimal will result in shorter survival times. Assume there is some survival time which, though below the maximum, is nevertheless desirably high.

There should then be two dosage levels yielding this survival time; one will be above, the other below, the optimal dosage. The ratio of these two dosages, which corresponds to the therapeutic index, indicates how much latitude there is for varying the dosage without encountering undesirably low survival times. This index has been employed by Creech et al. (13) and can provide an important ancillary index to determination of the maximum survival time in the rating of chemotherapeutic agents.

The various concepts discussed in this section are illustrated graphically in Charts 7 and 8.

**THE TUMOR CHALLENGE**

Any experimental measure of the effect of an antineoplastic agent is necessarily specific to the circumstances surrounding its determination. Effectiveness will vary with the particular neoplasm.
Chart 7.—Schematic representation of various possible relationships between the antitumor specificity of an agent and its therapeutic index. Each of the four panels of the chart shows a hypothetical antitumor dose response curve and a hypothetical host toxicity curve for an agent. For simplicity, these are shown as straight lines. The vertical scaling is not in defined units and should be interpreted to be the appropriate scale for antitumor effect and host effect as the case may be. Antitumor effects and effects on the host are generally in non-comparable units making the concept of parallelism between their dose response curves inappropriate.

Panel A illustrates an agent with a high therapeutic index (the ratio of the dose yielding a designated level of toxicity to the dose yielding a designated antitumor effect is large) and a high specificity (there is a strong antitumor effect at a dosage level yielding a designated toxic effect for the host). In panel B it can be seen that the same high specificity can be obtained even when the therapeutic index is low, whereas panel C illustrates how the high therapeutic index can be coupled with a low specificity. Finally, panel D shows an association of low therapeutic index with low specificity.

Chart 8.—Schematic representation of various possible relationships between the therapeutic efficacy of an agent and the width of its effective dose range. These relationships are intended to parallel those between antitumor specificity and therapeutic index as shown in Chart 7. Each of the four panels of the chart shows a hypothetical survival time dose-response curve. Characteristically, the survival time of treated tumor-bearing animals increases with dosage up to a point; increased dosage beyond some optimal level results in reductions rather than increases in survival time. The therapeutic efficacy of an agent is defined as the survival time elicited by its optimal dosage. Arbitrarily here, the width of the effective dose range is defined as the ratio of the highest dose yielding a twofold or better increase in survival time over untreated controls to the lowest dose doing so.

It can be seen from the various panels that agents with high efficacy (panels A and B) may be effective over either a wide or narrow effective dose range; also agents having a wide effective dose range (panels A and C) may or may not be especially efficacious. These parallel the possible situations existing between the antitumor specificity of a drug and its therapeutic index as shown in Chart 7.
employed and its source, and with the particular host employed and its age, sex, or weight. Varying the route of tumor inoculation or drug treatment may result in changed measure of effect (45, 100). Altered schedules of drug therapy may increase or diminish effectiveness (35, 37, 40, 97). Changes in definition also can change one's measure of effectiveness. Defining the effective antitumor dose as the one yielding 50 per cent tumor weight loss in 5 days will give different measures of the therapeutic index than defining it as the dose yielding 90 per cent inhibition in 7 days. Alternative measures can flow from defining the effective dose in terms of effect on tumor diameter or on survival time; similar changes can occur from changes in definition of the tolerated dose, as, for example, the dose yielding not more than 20 per cent weight loss or the dose yielding not more than 10 per cent lethality.

Changes in measure of drug effectiveness such as the last, resulting from altered definitions, are only of academic interest. Others may be of important theoretical interest. It may be worth while to learn why a particular drug is effective with a certain host, or more effective when the host is older, etc. Other causes of altered drug effectiveness may be of important practical interest. If variation in method of use of an agent, as by altered schedules of therapy, can make the drug appear to be more or less effective, how most effectively to use the method of use of an agent, as by altered schedules

The work of this laboratory has been characterized by the use of increasing levels of tumor challenge as more effective agents have been identified (44). Thus, when it became apparent that complete inhibition of mouse leukemia L1210 could readily be achieved with early drug treatment, provided the tumor inoculum was barely large enough to ensure 100 per cent takes, the challenge was made more difficult. The inoculum was raised to one about 100 times that giving 100 per cent takes in the tumor titration. Effectiveness in such a system now became the criterion for rating agents.

An increasing measure of success against this massive inoculum in turn led to the employment of still more extensive tumor challenges. It was recognized that, while appropriate therapy did lead to "cure," even though the inoculum employed was massive, nevertheless, treatment had been initiated while the tumor was still not yet palpable. This did not approximate very well the clinical situation. The further increase in the tumor challenge made at this point was one in which test animals were not subjected to drug therapy until tumors were palpable at the site of inoculation and the disease was systemic.

With this challenge amethopterin was more effective than the various known antileukemic agents. It produced several weeks' extension in survival time, even though treatment was withheld until only 2 or 3 days before death would otherwise have occurred. This provided a yardstick for comparing the effectiveness of still other agents. Finally, use of the assay procedure employing delayed initiation of treatment led to the finding that certain halogenated analogs of folic acid could be used routinely to obtain a high percentage of cures. No little part in the cure of experimental leukemia L1210, which only a few years ago was thought to be among the more virulent of the laboratory neoplasms, was played by the use of a criterion of efficacy which emphasized the therapeutic results achieved rather than the dose level employed. On an equimolar basis, amethopterin exerts greater antileukemic effect than 3',5'-dichloroamethopterin. However, the halogenated analog is considerably less toxic for the host. This permitted the use of relatively massive doses of the halogenated analog, resulting in extensive antileukemic effect unaccompanied by host toxicity (31, 42, 99).

It may be noted that use of a lesser tumor challenge may, under certain circumstances, provide a reasonable basis for rating candidate agents. When the challenge is limited, one can limit the extent to which the test compound is used. Thus, when
drugs were tested in this laboratory against the more massive tumor inoculum, but with treatment given early, such treatment was given on only a single or on only a limited number of days. As indicated above, this device permitted a separation of the lethal effects and the antitumor effects of such treatment. The agents could then be rated on the basis of the specificity indices (38).

The various systems employed in this laboratory have been useful not only for rating candidate agents, but also for elucidating the effects of experimental modifications. Successive kinds of assay performed in this laboratory employing progressively more severe challenges are illustrated in Charts 9–13.

**SUMMARY AND CONCLUSIONS**

Various aspects of the philosophy and conduct of antitumor drug screening and evaluation programs are reviewed and illustrated in the present report.

The yield of screening and evaluation programs is influenced by the experimental procedures employed, the end-points observed in measuring drug effect, and the rating procedure by which drug effectiveness is evaluated.

The value of tumor inhibition, per se, as a measure of effectiveness is limited, even when the restriction is made that such inhibition be not accompanied by untoward toxic effects for the host. In concept, there are a variety of modes of action by which an agent may provide an effective therapy without its being reflected in a high degree of tumor inhibition at some specified time. There is no necessary correlation between tumor size and survival time. Examples have been given where inhibition of tumor growth was accompanied by either an increase, or no increase, or a decrease in survival time.

The extent to which drug treatment increases the survival time of tumor-bearing animals is suggested as a measure of drug effectiveness which gives weight to both the antitumor effects of treatment and its toxic effects for the host, and which should be appropriate whatever the mode of therapeutic action. The survival time measurement re-
This chart illustrates how the procedure exemplified in Chart 10 was employed to ascertain the susceptibility of L1210 to treatment with amaminopterin when administered as a single dose at various times following tumor implantation. The leukemia was found to be more susceptible on day 3 than on either day 1 or day 6. When treatment was delayed until day 9, no important increases in survival time were noted and there was no evidence of progressive increase in survival time with increasing levels of amaminopterin.

**TREATMENT OF ADVANCED LEUKEMIA (L1210)**

**CHART 13.**—Illustration of especially efficacious antileukemic (L1210) compounds detected using the maximum survival time procedure. The chart is from Goldin et al. (31).

In this experiment, amethopterin (MTX) shows its usual high capacity (cf. Chart 12) for extending the survival time of mice bearing advanced leukemia L1210. Its dihalogenated forms, 3',5'-dichloroamethopterin (DCM) and 3'-bromo-5'-chloroamethopterin (BCM), however, show antileukemic activity of a nature not previously observed. These agents elicit rather extreme increases in median survival time and also yield substantial numbers of 160-day survivors (29) (100-day survivors are shown in parentheses in the figure). With survivors of advanced leukemia established as the reference effect, it may become desirable to alter assay procedures to permit detection of agents having the capacity to cure advanced L1210 rather than only to increase survival time.

It may be noted that the effective dose levels of the halogenated derivatives are many times that of amethopterin. The increased antileukemic efficacy stems primarily from a sharply reduced toxicity, on a molar basis, for the host, without as extensive a reduction in antileukemic effect. This permits the safe employment of high dose levels with resulting increased therapeutic effects.

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The text continues with further details and results from experiments involving different treatments and their effects on leukemia survival times.
fects the net effect of the drug on the host-tumor relationship. In general, a drug which is capable of eliciting important increases in survival time is likely to inhibit tumor growth. However, a therapeutic effect may be achieved, even when a drug has little or no direct effect on a tumor, i.e., (a) in this laboratory, increase in survival time of mice with leukemia L1210 has been observed with either progressive growth of the local tumor at the site of inoculation of the leukemic cells, or a decrease in size or disappearance of the local tumor; (b) the drug may have a salutary effect on the host and thereby prolong life. There are other situations in which measurement of local tumor size alone would not adequately describe the therapeutic value of a drug. (a) The drug effect on the tumor may require the mediation of the host. It may, for example, be necessary for the host to alter the drug to an active form. Such effects, and the toxic limitation of the drug for the host, could be masked if measurement is limited to local tumor growth. (b) Where a tumor metastasizes, measurement of local tumor growth would fail to take into account the infiltration of the disease into vital organs, etc. (c) If tumor size or weight is measured on a specified day and animal weight change is taken into account, this may still not accurately reflect therapeutic response as adjudged by survival time. For example, the tumor-inhibitory effect or toxicity for the host could occur subsequent to the time of measurement, etc.

Thus, a screening system or evaluation program limited to measurement of local tumor growth may miss compounds which produce therapeutic effects without extensive inhibition of tumor growth. In addition, such a screen may give too many false positives. Screening systems employing survival time as the major response with measurement of local tumor growth response as an ancillary index of effect would appear to be highly desirable.

Both the tumor response and the survival time of the tumorous animals should be characterized with respect to the influence of nonspecific effects. Regression lines may be established relating host response, tumor response, and survival time response to underfeeding and weight loss of the host. If the system is too sensitive to nonspecific toxicity, steps may be taken to provide a more difficult challenge. This may be accomplished by increasing the inoculum level, delaying treatment until the tumor is well established, etc.

Survival time measurement lends itself well to precise quantitative evaluation of drug effectiveness. It may be employed in assay systems for the determination of the specificity of action of drugs. Assay systems with survival time used as the primary criterion of drug effectiveness permit the ordering of compounds in terms of their maximum effectiveness, or in terms of their effectiveness at a given cost in toxicity for the host. The therapeutic index, as a measure of the safety with which a drug may be employed, provides an important index of the action of the drug. Taken in conjunction with the assay of specificity of action it provides a broad characterization of the action of the drug. Preferably, the therapeutic index employed should incorporate survival time as well as tumor size as criteria of drug response. Such assay systems are applicable also to study of combinations of drugs in synergism and antagonism and to determination of the influence of schedules of therapy. The assay system may be employed in pharmacological and biochemical studies involving inhibition analysis.

The assay systems should remain flexible. The tumor challenge should be increased as more effective drugs are found. Whereas moderate increases in survival time may at first be the requirement for drug effectiveness, extensive survival time and cure with advanced systemic disease is the ultimate objective. The assay systems with transplantable tumor systems may, in turn, be extended to carcinogen-induced and spontaneous tumor systems.

The development of wholly quantitative assay procedures for the determination, rating, and study of drug effectiveness, and the use of screening procedures related to these assay procedures should provide a firm foundation for study of the relationship of the influence of drugs in animal tumor systems and the clinic.

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Abraham Goldin, John M. Venditti and Nathan Mantel


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