Cancer Chemother Pharmacol (1981) 6:211–218

Cancer Chemotherapy and Pharmacology



# Quantitative Association Between the in vitro Human Tumor Stem Cell Assay and Clinical Response to Cancer Chemotherapy

Thomas E. Moon[1], Sydney E. Salmon[1,2], Carolyn S. White[1], H-S. George Chen[2], Frank L. Meyskens[1,2], Brian G. M. Durie[1,2], and David S. Alberts[1,2]

[1] Cancer Center and [2] Section of Hematology and Oncology, The University of Arizona, Health Sciences Center, Tucson, Arizona 85724, USA

***Summary.*** *Objective methods have been developed to quantitate results of the in vitro human tumor stem cell assay, the degree of the association between the in vitro assay and clinical response as well as the likelihood of response. Methods considered to quantitate in vitro assay data included first-order kinetics of percent survival with drug concentration, minimal percent of tumor colony-forming unit survival at low drug concentrations, and area under the in vitro percent survival-drug concentration curve. Based upon experimental data, the percent tumor colony survival and the area under the curve (i.e., in vitro sensitivity indices) were concluded to better account than other methods for the commonly observed nonlinear shape of the in vitro curves. The two methods also yielded equivalent quantitative descriptions of the in vitro data. A logistic regression model was used for explicit quantitation of the relationship between the in vitro sensitivity index and predicted probability of clinical response. Very high association was observed between the predicted in vivo and actual clinical response for the cytotoxic drugs considered. Incorporation of other pharmacologic and patient prognostic factors into the quantitative methods is discussed and shown to improve their effectiveness.*

## Introduction

The ability to accurately predict clinical response to cancer therapy for individual patients has not yet been achieved. This is, in part, due to the limited efficacy of currently available therapeutic agents and the heterogeneity between patients. The development of in vitro assays to predict in vivo (clinical) outcome has been the goal of many investigators. The recently developed in vitro soft-agar colony assay for human tumor stem cells [4, 8–11, 13, 16, 18, 21, 22] has the theoretical advantage over other assays of evaluating clonal growth of the cancer patient's own tumor. If a high degree of positive association exists between the in vitro human tumor stem cell assay and the in vivo outcome, then the in vitro assay could be effectively used to determine optimum patient cytotoxic treatment and predict the likelihood of its success.

Important to these efforts is the development of objective methods to quantitate results of the in vitro assay, evaluate the degree of in vitro-in vivo association, and predict in vivo outcome. The quantitative methods discussed below are based upon established statistical techniques and pharmacological principles which we have found useful in developing a quantitative assay system [1, 14, 17, 19].

### *Data Base*

One hundred fifty-six consecutive patients were included for analysis. Ninety patients had a diagnosis of ovarian cancer, while 25 myeloma and 41 melanoma patients were also included. The majority of patients had received extensive prior chemotherapy.

Original replicate tumor colony counts were obtained from control and drug-treated plates by the in vitro soft-agar colony assay. All assays were carried out in our laboratories and included tests of a wide variety of cytotoxic drugs including actinomycin D, adriamycin, AMSA, BCNU, bleomycin, *cis*-platinum, melphalan, methotrexate, vinblastine, vincristine, and vindesine. Tumor cells were exposed to drugs in nucleoside-free medium in suspension culture at 37° C for 1 h prior to plating in agar. Assay techniques have been detailed elsewhere [17, 18]. Other available information included in vivo outcome

*Reprint requests should be addressed to* T. E. Moon

0344-5704/81/0006/0211/$ 01.60

to drugs tested in vitro with clinically achievable concentrations. Efforts were made by the clinicians to administer drugs parenterally according to protocols that assured maximal or optimal clinical dosing schedules. In vivo tumor response was classified according to standard clinical criteria by the percent tumor reduction, as compared to the tumor size prior to treatment. Tumor size was defined for ovarian and melanoma patients as the sum of the maximal perpendicular products of all measurable tumor lesions. Cell mass was used to quantitate tumor size for myeloma patients [6]. Patients were defined as sensitive in vivo if they achieved at least 50% tumor reduction when treated by the agent tested in vitro. Patients achieving less than 50% tumor reduction were defined as resistant in vivo.

*Quantification of the in vitro Assay*

In vitro human tumor stem cell assay data are commonly plotted as shown in Fig. 1, as the percent survival of tumor colony-forming units (TCFU) on the vertical axis versus drug concentration on the horizontal axis. Percent survival is calculated by dividing the mean number of surviving TCFU in the treated sample by the mean number in the concurrent untreated control sample and multiplying by 100. To obtain an overview of such data, plots are prepared with the aid of a linear percent survival scale. Each series of line segments represents a separate patient's data for TCFU survival 7–14 days after initial exposure to selected drug concentrations. Two or more clinically achievable concentrations are selected, with three replications at each concentration. The standard error of each percent survival is also shown in Fig. 1; this was calculated by the method of propagation of error [12]. The choice of a linear scale for percent survival, as discussed below, was made because of the frequent appearance of marked clonal heterogeneity (as indicated by a plateau in the curve). Also, more than 100 colonies/dish are infrequently observed with current assay procedures, which prohibits the estimation of in vitro survival below 1%.

In vitro survival-concentration curves can be quantified in a variety of ways. Because only two to four in vitro concentrations plus controls are frequently evaluated for each patient, the ability to develop and validate a complex quantitative description that estimates the total shape of the patient's in vitro survival curve is limited. One approach would be to plot in vitro survival data on a semilog scale. Based upon first-order kinetic methods, estimates of the shoulder and slope ($D_0^{-1}$) of an assumed negative exponential shaped survival curve would then be obtained. While this approach is widely used for tumor cell lines and may be applicable for acute leukemia, in vitro human stem cell assay plots of experimental data for many tumors frequently do not follow such a simple pattern. The appearance of a plateau or marked decrease in the slope of an in vitro survival curve, as seen from curves B and C, indicate more complex shapes than a constant rate of decrease in survival. Thus, the use of a semilog scale to plot the in vitro assay data and quantify the in vitro survival curve by a slope ($D_0^{-1}$) distorts the actual appearance and interpretation of the data.

Other relatively simple approaches that have been considered include use of the percent survival of TCFU and the area under the linear in vitro survival-concentration curve. While the percent survival has been used frequently to quantify in vitro cell lethality by other investigators, the area under the curve has only recently been used [14, 17]. The area under the curve, however, has been extensively used in pharmacokinetics, and also furnishes a computationally simple approach to quantification of the in vitro human tumor stem cell assay. It is defined as the area under the percent survival curve between 0 and

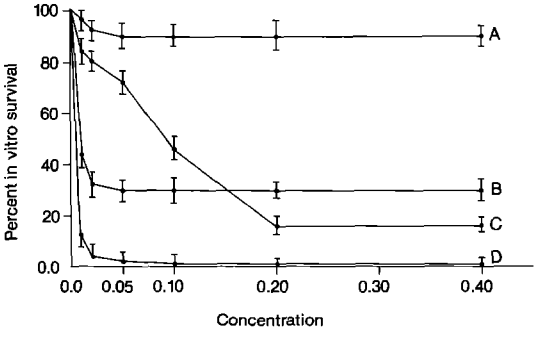


**Fig. 1.** Percent in vitro survival versus drug concentration

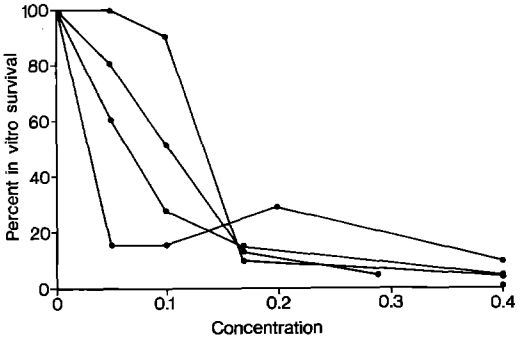


**Fig. 2.** In vitro survival for treated patients

an upper cut-off drug concentration limit. It can also be calculated by using the trapezoidal method, which adds the areas of the triangles and rectangles that can be placed entirely under the in vitro curve. Both the percent survival and area under the curve require that an upper concentration be defined.

Pharmacological parameters and mechanisms of action of the cytotoxic drugs used varied considerably; this is a point of which we are cognizant and has been discussed elsewhere [1].

With this in perspective, the somewhat simplifying procedures discussed below have nonetheless provided insight into selection of in vitro concentrations and exposure boundaries.

The upper concentration used in this paper was defined by clinically achievable concentration-time products and specified, for the drugs studied, to be 0.1 μg/ml. In most in vitro studies conducted with standard cytotoxic drugs, where pharmacokinetic data were available, the 0.1 μg/ml upper cut-off was a concentration corresponding to less than 10% of the maximally achievable concentration-time product [1]. The observation that TCFUs show decreased survival for drug concentrations that are less than 10% of clinically achievable levels is analogous to reports for prediction from in vitro assay for drugs effective against bacteria. In fact, for clinical correlations with in vitro bacterial cultures, the best in vivo results are observed when the effective in vitro concentration is less than 1% of the pharmacologically achievable dosage.

The percent survival and area under the curve depend, to differing degrees, on the actual shape of the survival curve, the in vitro drug concentrations, and the upper cut-off used. A survival curve that has a very small surviving fraction for a low concentration and then plateaus could yield the same surviving fraction at a larger cut-off concentration than another curve that does not reflect such a marked reduction in survival at a lower concentration. For example, Fig. 2 shows the actual in vitro survival curves for four different patients. If 0.4 μg/ml were chosen as the upper cut-off, then all four patients would have nearly the same percent survival but would have differing areas under their curves. However, the choice of 0.1 μg/ml would yield differing values for both percent survival and area under the curves. While the above example suggests that the area under the curve should more accurately reflect the true, but unknown, shape of the survival curve, the calculated Pearson correlation coefficient of percent survival and area under the curve was 0.91 for the 156 patients considered. Thus, the actual use of a limited number of the drug concentrations in each patient's in vitro assay results in the two methods yielding essentially equivalent in vitro quantitative indexes. Both methods will be evaluated with respect to their association with in vivo studies.

### *Association Between in vivo and in vitro Studies*

The appearance of the in vitro survival-concentration curves as shown in Fig. 1 suggests an association between the in vitro sensitivity index (i.e., percent survival or area under the curve) and the in vivo tumor response. If the in vitro survival curve rapidly decreases at low concentrations, as seen in curve D, a low percent of in vitro survival is obtained at low drug concentrations, (i.e., in vitro sensitivity). Assuming a high correlation between the concentration-time product for the in vitro study and that required in vivo occurs, this would lead to the prediction of high in vivo effectiveness (i.e., the patient would be predicted to be clinically responsive to the treatment). Conversely, a minimal decrease in the survival curve, as shown by curve A, corresponds to a high percent survival for any concentration utilized in the in vitro assay and would lead to the prediction of in vivo resistance. An intermediate reduction or a plateauing in the in vitro survival curve, as seen in curves B and C, indicates a reduction in survival, followed possibly by minimal additional change in survival beyond a certain drug concentration. Conceptually, this suggests the presence of subpopulations of TCFU that are kinetically or biochemically resistant to the agent tested. The presence of in vitro survival curves exemplified by curves B and C underscores the necessity to develop objective methods to quantitate the in vitro survival assay and the corresponding in vitro-in vivo association.

An additional factor which may prove important is biochemical heterogeneity at metastases. Should different metastases prove to have different drug sensitivity in vivo, multiple biopsies may prove necessary for adequate in vitro testing.

For patients that received multidrug in vivo treatment, the in vitro percent survival and area under the curve were separately calculated for each drug. The drug with the smallest value was used to quantify in vitro sensitivity. Because of the high correlation between percent survival and area under the curve, in all patients, the drug that yielded the smallest percent survival also yielded the smallest area under the curve. Once the in vitro and corresponding in vivo studies were individually quantified for each patient, a line plot was drawn illustrating the association.

The top panel of Fig. 3 shows the percent survival scale divided into mutually disjoint regions. Also

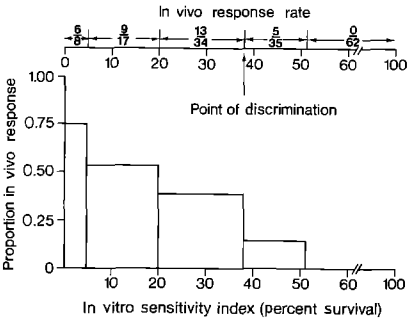


**Fig. 3.** In vitro sensitivity index and in vivo outcome

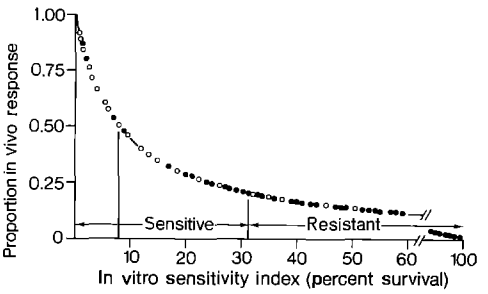


**Fig. 4.** Logistic regression model relating in vitro sensitivity index and in vivo outcome. (○) In vivo response; (●) No in vivo response

shown for each of the regions is the ratio of the number of in vivo responses to the total number of patients tested in vivo. A distinct pattern was observed, which suggested that patients with an in vitro sensitive index of less than 38% survival frequently obtained an in vivo response, while patients with a greater than 38% survival consistently did not achieve a response. A similar association was observed where area under the in vitro survival curve was used as the sensitivity index. The distinct pattern observed for these patients strongly suggested that a small value for the sensitivity index does correlate with subsequent response in vivo and a large value correlated with resistance in vivo. Our first approach was to calculate a point of discrimination that could be used to classify each patient's sensitivity index into two disjoint prognostic regions [2]. Patients whose sensitivity index was less than this point of discrimination would be predicted to have a response in vivo, while patients whose sensitivity index was greater than the point would be predicted to have resistance in vivo. The point of discrimination was calculated to be 38 when the in vitro sensitivity index was defined by the surviving fraction at 0.1 μg/ml in vitro drug concentration. When the sensitivity index was defined by the area under the in vitro survival curve between 0–0.1 μg/ml concentration, five was determined to be the point of discrimination.

Such a simple classification procedure does not fully consider the in vitro-in vivo relationship suggested by Fig. 3. A gradient of response in vivo can be noted ranging from 75% for a sensitivity index of less than 5 units to 0% response in vivo for a sensitivity index greater than 51 units. The same information is shown on the bottom panel of Fig. 3, plotted on a two-dimensional graph with proportion of response in vivo along the vertical axis and in vitro sensitivity index or percent survival along the horizontal axis. A similar-shaped curve is obtained when area under the in vitro survival curve is used as the sensitivity index.

To obtain an explicit relationship between in vitro sensitivity index and corresponding response in vivo, a logistic regression model was used [5]. A logistic model is a commonly used method of explicit quantification of such relationships. In addition to laboratory bioassay studies where the dose levels are defined prior to treatment, the logistic model is widely used to relate clinical or laboratory prognostic factors with subsequent clinical response. With the use of the logistic model to predict clinical response, the values of prognostic factors are commonly observed and not defined prior to treatment [5].

The combined in vitro-in vivo patient data shown in Fig. 3 as a training set, and the slope and intercept of a logistic regression model were statistically estimated; they were significantly different than zero ($P < 0.05$). The curve representing the model is shown in Fig. 4. Also shown are the actual data points indicating the observed in vitro sensitivity index and corresponding in vivo outcome for each patient.

One can utilize the resulting explicit quantitative relationship to predict patient response in vivo, prior to treatment, based upon their observed sensitivity index (i.e., percent survival) on the in vitro survival curve. For example, a patient who is tested in vitro and determined to have an 8% survival at 0.1 μg/ml would be predicted to have a 0.50 probability of attaining a response in vivo if treated with the same drug in vivo in clinically achievable doses. In contrast, a patient with a 40% survival would have a predicted probability of less than 0.20 of obtaining a clinical response. The statistical variation associated with the predicted probability of response is discussed below.

To furnish the clinician with a somewhat simpler method of predicting patients' response in vivo to agents tested in vitro, the sensitivity index scale was separated into three disjoint regions (Fig. 4). The regions, identified with high, intermediate, or low in vivo sensitivity, enable the clinician to identify

treatments that may have very different predicted probabilities of furnishing a patient with a response in vivo. The sensitivity regions were defined as corresponding with predicted probabilities of response in vivo and the overall in vivo response rate ($33/156 = 0.21$). The resistant (or low-sensitivity) region was defined as the portion of the in vitro sensitivity index scale that corresponded with less than a 0.20 predicted probability of response in vivo. The region that corresponds with a predicted probability of response of less than 0.50 but greater than 0.20 was defined as the intermediate region. The highly sensitive region corresponds to a predicted probability of greater than 0.50

The sensitivity boundaries for all patients combined are shown in Table 1. The boundaries were computed using the percent survival and separately the area under the survival-drug concentration curve as the quantitative in vitro sensitivity index. The standard errors of estimated sensitivity boundaries are also shown in Table 1, and were calculated according to the method of propagation of error [6]. Sensitivity boundaries and associated standard errors were also calculated for the patients with the same tumor histology. The difference between the estimated resistance boundary for the myeloma patients is suggestively different than the boundary for ovarian and melanoma patients (i.e., 20.7 vs 37.9, $0.05 < P < 0.10$). The interpretation of this difference has not been clarified, but may be due partly to in vitro assay growth conditions.

**Table 1.** In vitro sensitivity regions

| Patients | N[a] | In vitro quantitative method[b] | Sensitivity[c] | |
|---|---|---|---|---|
| | | | High | Low[d] |
| Combined | 156 | PS | 7.7 ± 5.00[e] | 31.4 ± 3.94 |
| | | AUC | 3.4 ± 1.14 | 5.6 ± 0.73 |
| Ovarian | 90 | PS | 18.9 ± 7.13 | 37.9 ± 5.15 |
| | | AUC | 3.2 ± 1.48 | 6.3 ± 0.95 |
| Melanoma | 41 | PS | 4.9 ± 9.08 | 40.5 ± 6.52 |
| | | AUC | 3.3 ± 1.92 | 6.9 ± 1.23 |
| Myeloma | 25 | PS | 6.4 ± 6.98 | 20.7 ± 5.06 |
| | | AUC | 4.1 ± 1.27 | 5.3 ± 0.78 |

[a] N indicates the sample size
[b] PS and AUC relate to the percent survival and area under the survival-drug concentration curve methods to quantitate the results of the in vitro assay
[c] Intermediate sensitivity is defined as the region between high and low sensitivity
[d] Low sensitivity is commonly referred to as resistant. Sensitivity refers to either high or intermediate sensitivity
[e] Estimated mean ± standard error of the mean in vitro sensitivity index

*Selection of in vitro Cut-off Drug Concentration*

In the calculation of the in vitro sensitivity index (i.e., percent survival or area under the curve) and the estimation of the sensitivity boundaries, 0.1 μg/ml was used as the cut-off drug concentration. This value was chosen because it, or a concentration very close to it (generally 0.2 μg/ml), was consistently used for every patient's in vitro assay data considered. Also, preliminary experience suggested that the in vitro-in vivo association for the drugs tested was not increased by using concentrations much greater than 0.1–0.2 μg/ml (e.g., 1.0 μg/ml). Following these preliminary observations, increased information has been accumulated that permits a more objective choice of the cut-off drug concentrations.

For most drugs tested in vitro (for patients considered), 0.1 μg/ml for 1-h exposure corresponds to less than 10% of the clinically achievable concentration-time product or CXT [1]. However, for actinomycin D and the vinca alkaloids vinblastine, vincristine, and vindesine, 0.1 μg/ml corresponds to greater than 50% in vivo CXT. Figure 5 illustrates that a decreased in vitro-in vivo association is observed when an in vitro concentration corresponding to greater than 50% in vivo CXT is used.

The left panel of Fig. 5 shows that nine patients were tested in vitro (eight melanoma patients were tested with actinomycin D and one ovarian patient with vindesine). The results of Table 1 were used to classify all patients as in vitro sensitive (i.e., surviving fraction < 38), a concentration of 0.1 μg/ml being used. Only three (33%) were subsequently determined to have a response in vivo. However, these same patients also had an in vitro test with a concentration of less than 0.01 μg/ml for 1 h exposure (i.e., an in vitro concentration corresponding to less than 10% in vivo CXT). Four of the patients would have been classified as resistant in vitro and five patients as sensitive in vitro (from Table 1). The three patients who obtained a response in vivo would have been classified as sensitive in vitro at 0.01 μg/ml. Thus, seven of nine (78%) of the patients would have been correctly classified by the in vitro assay with an in vitro concentration corresponding to less than 10% in vivo CXT, in contrast to three of nine (33%) with the higher in vitro concentration of 0.1 μg/ml. It remains conceivable that higher in vitro concentrations may also be required to represent 10% of the achievable CXT in vivo for drugs given in multigram quantities in vivo. However, for those drugs administered commonly in milligram or microgram quantities, the current in vitro concentration limits appear suitable.

The right panel of Fig. 5 shows the in vitro-in vivo association for 31 ovarian cancer patients tested in vitro at 0.1 and 1.0 μg/ml for a 1-h exposure to adriamycin, *cis*-platinum, or bleomycin. All patients were classified as resistant in vitro at a concentration of 0.1 μg/ml, and were observed to be resistant in vivo. At an in vitro concentration of 1.0 μg/ml (i.e., corresponding to greater than 50% in vivo CXT for the drugs tested), eight patients (26%) had a surviving fraction less than 38% and would have been incorrectly classified by the in vitro assay (from Table 1). Thus, a decreased in vitro-in vivo association is again observed when an in vitro concentration corresponding to greater than 50% in vivo CXT is used.

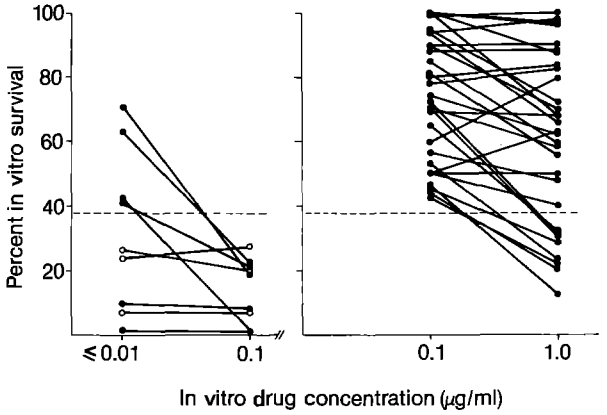


**Fig. 5.** Effect of in vitro drug concentration on the prediction of in vivo outcome. (○) In vivo Response; (●) No in vivo response

### *Evaluation of the Logistic Model to Quantify the Association Between in vitro and in vivo Studies*

The excellent fit of the training set by the corresponding logistic regression model, as illustrated in Fig. 4, is partly due to the retrospective comparison of the data. An effective way to validate the model is based upon independent patients, not considered in the training set, whose in vitro sensitivity index is used to correlate prospectively with the in vivo response subsequently observed. While all available patients' in vitro-in vivo correlations were used in the training set, the prospective evaluation of the model must await the collection of additional information.

**Table 2.** Association between in vitro and in vivo studies

| Patients | N[a] | Association[b] | | | | Accuracy[c] | | | $P$[e] |
|---|---|---|---|---|---|---|---|---|---|
| | | SS | SR | RS | RR | P (S) | P (S \| S) | P (R \| R) | |
| Combined | 156 | | | | | | | | > 0.60 |
| PS | | 23 | 24 | 10 | 99 | 0.21 | 0.49 | 0.91 | |
| AUC | | 24 | 26 | 9 | 97 | 0.21 (0.03)[d] | 0.48 (0.07) | 0.92 (0.03) | |
| Ovarian | 90 | | | | | | | | > 0.50 |
| PS | | 14 | 10 | 1 | 65 | 0.17 | 0.58 | 0.98 | |
| AUC | | 13 | 13 | 2 | 62 | 0.17 (0.04) | 0.50 (0.08) | 0.97 (0.03) | |
| Melanoma | 41 | | | | | | | | > 0.30 |
| PS | | 11 | 15 | 1 | 14 | 0.29 | 0.42 | 0.93 | |
| AUC | | 11 | 13 | 1 | 16 | 0.29 (0.07) | 0.46 (0.10) | 0.94 (0.07) | |
| Myeloma | 25 | | | | | | | | > 0.40 |
| PS | | 6 | 2 | 0 | 17 | 0.24 | 0.75 | 1.00 | |
| AUC | | 5 | 3 | 1 | 16 | 0.24 (0.09) | 0.63 (0.17) | 0.94 (0.06) | |

[a] N indicates the sample size

[b] SS, number of patients classified as sensitive in vitro an in vivo; SR, number of patients classified as sensitive in vitro and resistant in vivo; RS, number of patients classified as resistant in vitro and sensitive in vivo; RR, number of patients classified as resistant in vitro and in vivo

[c] P (S), observed in vivo response rate for the patients considered; P (S | S), rate of in vivo response among those patients who where in vitro sensitive, according to the logistic regression model [i.e., SS/(SS+SR)]; P (R | R), rate of in vivo resistance among those patients who where in vitro resistant [i.e., RR/(RR+RS)]

[d] The numbers in parantheses indicate the standard error of the estimated response rates

[e] $P$, significance level of the chi-squared goodness-of-fit test for the logistic regression model

The fit of the calculated logistic model to the training set is shown in Table 2. For all patients, combined and separately by tumor histology, the in vitro sensitivity index is partitioned into two distinct regions (i.e., sensitive and resistant, as described above) and various summary and test statistics calculated. Both percent survival and area under the in vitro curve were used to quantify the in vitro assay data. In vitro resistance has been previously defined as the low-sensitivity region on the sensitivity index scale and in vivo resistance as a less than 50% reduction in pretreatment tumor size. Focusing on the combined group of patients, with similar results obtained for the subgroups, the chi-squared goodness-of-fit test described by Tsiatis [20] yielded a $P$-value $> 0.60$, indicating a good fit of the logistic regression model to the training set. It was noted (Table 2) that the accuracy is comparable whether the percent survival or area under the in vitro curve is used. Thus, the percent survival and the area under the in vitro survival curve are not only highly correlated but also are equally associated with in vivo studies.

### *Identification of Patient Responsiveness to Chemotherapy*

One of the uses of the human tumor stem cell assay is the selection of therapy to be given to a particular patient. While some patients (e.g., those with no prior therapy) may be sensitive in vitro to a number of chemotherapeutic drugs, Table 3 suggests that the assay yields more information than simply whether a patient will or will not be a 'responder' in vivo to chemotherapy of any type.

Table 3 demonstrates the relationship between the number of in vitro drugs tested and the likelihood of identifying at least one drug that is sensitive in vitro. As the number of drugs tested in vitro increases, the percentage of patients sensitive in vitro to at least one drug also increases. The data for the combined group of patients, and similarly for each tumor histology-specific subgroup, indicated that over 78% of all patients whose tumors are successfully grown in vitro were sensitive to at least one of four or more drugs tested in vitro. When eight or more different drugs were tested in vitro, the current information indicates than an even higher percentage of patients were sensitive to at least one of the drugs. Thus, the value of the human tumor stem cell assay is not in the identification of patients with or without a responsive tumor, but in the identification in vitro of specific drugs and the prediction of the effectiveness in vivo.

**Table 3.** Identification of drugs sensitive in vitro related to the number of drugs tested

| Number of drugs tested in vitro | Number of patients sensitive[a] | Number of patients resistant[b] | Total number of patients tested |
|---|---|---|---|
| ≤ 3 | 13 (0.42) | 18 | 31 |
| 4–7 | 82 (0.77) | 24 | 106 |
| 7+ | 19 (1.00) | 0 | 19 |
| Total | 114 (0.73) | 42 | 156 |

[a] Sensitive means sensitive in vitro to at least one of the drugs tested

[b] Resistant means resistant to all drugs tested in vitro

### *Improvement of Methodology*

The existence of an intermediate in vitro sensitivity region indicates that for a fraction of patients the human stem cell assay system does not currently furnish sufficient information to satisfactorily predict in vivo outcome. This suggests that for certain drugs the current in vitro exposure system may not be adequate, or that there may be other factors related to in vivo response in addition to efficacy of cytotoxic agents. With respect to in vitro drug exposure, the 1-h time period was selected because this encompasses the major period of in vivo pharmacologic exposure for most standard drugs, many of which are cell cycle- or cell cycle–stage-nonspecific and are given by intermittent injections (i.e., weekly or monthly). Several drugs appear to be schedule-dependent, however, some having a long metabolic half-life, or are in advertently given clinically by continuous or multiple daily dosing schedules. Relatively brief in vitro exposures to these agents could potentially lead to underestimation of their in vivo efficacy, as kinetic resistance could be mistaken for biochemical resistance. However, kinetic resistance becomes irrelevant with prolonged drug exposure. Such drugs might be better assessed with prolonged in vitro exposure (i.e., greater than 1 h). Examples include bleomycin and cytosine arabinoside, both of which yield the best clinical results when administered frequently (e.g., every 8 h SC or by continuous infusion [3]. Recent observations in our laboratories have led to more detailed evaluation of such drugs, to assure that they are assessed adequately in the in vitro assay. Also, the development of improved culture media for testing of antimetabolites is another area for further study.

The identification of patient characteristics that are associated with clinical response and the development of quantitative prognostic factor models are

active areas of clinical research. Commonly observed patient characteristics, such as prior therapy, histology, stage, and others, can readily be incorporated into a logistic regression model (such as discussed above) in an explicit manner. Such factors are important in high risk of relapse or in rapidly fatal neoplasms. For example, in acute myeloid leukemia it is not only important to select an effective treatment (as might be predicted from in vitro sensitivity), but the patient's age, infection status, degree of thrombocytopenia, and availability of supportive care [15] are also important in such a predictive equation. The significant difference between myeloma patients and patients with melanoma or ovarian cancer (Table 1) indicates a substantial difference in the interpretation of the in vitro sensitivity index for different tumor histologies. A myeloma patient must demonstrate a greater degree of in vitro sensitivity than a corresponding ovarian carcinoma or melanoma patient to have the same predicted probability of response in vivo. While this may be a reflection of the ability of the human stem cell assay system to clone different histologies successfully, an important point is that there is a marked difference between the histology-specific patient groups. This difference can be quantitatively incorporated into the logistic regression model and more effectively reflect the experimental data. While tumor repopulation rates in vivo may also influence the completeness of tumor regression, we currently do not measure this parameter routinely, although measurements of the $^3$H-thymadine labeling index provides some approximation of this factor [7].

### *Concluding Remarks*

To allow effective comparison of results between different investigators, the need for objective and versatile quantitative methods will increase. While various methods can be utilized simply to quantify and evaluate the relationship between in vitro and in vivo studies, consideration of the experimental data has influenced the selection of the quantitative methods that we currently utilize. As the human stem cell assay system is influenced by developing knowledge of tumor stem cell biology and colony-counting techniques, the currently utilized quantitative methods will undoubtedly evolve. Quantitative predictive techniques, however, will remain no less important for many years to come. Subsequent experimental data may indicate in which histologic types and which cancer therapies the in vitro human stem cell system is most appropriate for the individualization of cancer patient treatment.

## References


1. Alberts DS, Chen H-SG, Salmon SE (1980) In vitro drug assay: Pharmacologic considerations. In: Salmon SE (ed) Cloning of human tumor stem cells, chap 16. Alan Liss, New York, pp 197–207
2. Armitage P (1971) Statistical methods in medical research. Wiley, New York, pp 375–384; 135–138
3. Baker LH, Opipari MI, wilson H, Bottomley R, Coltman CA (1978) Mitomycin C, vincristine, and bleomycin therapy for advanced cervical cancer. Obstet Gynecol 52: 146
4. Buick RN, Stanisic TH, Fry SE, Salmon SE, Trent JM, Krasovich R (1976) Development of an agar/methylcellulose clonogenic assay for progenitor cells in transitional cell carcinoma of the bladder. Cancer Res 39: 5051–5056
5. Cox DR (1970) Analysis of binary data. Methuen, London, pp 43–45
6. Durie BGM, Salmon SE (1975) Cellular kinetics in multiple myeloma. A new approach to staging and treatment. Arch Intern Med 135: 131–138
7. Durie BGM, Salmon SE, Moon TE (1980) Pretreatment tumor mass, cell kinetics and prognosis in multiple myeloma. Blood 55: 364–372
8. Hamburger AW, Salmon SE (1977a) Primary bioassay of human tumor stem cells. Science 197: 461–463
9. Hamburger AW, Salmon SE (1977b) Primary bioassay of human myeloma stem cells. J Clin Invest 60: 846–854
10. Hamburger AW, Salmon SE, Kim MB, Trent JM, Soehnlen BJ, Alberts DS, Schmidt HJ (1978) Direct cloning of human ovarian carcinoma cells in agar. Cancer Res 38: 3438–3443
11. Jones SE, Hamburger AW, Kim MB, Salmon SE (1979) The development of a bioassay for putative human lymphoma stem cells. Blood 53: 294–303
12. Kendall MG, Stuart A (1969)The advanced theory of statistics, 3rd edn, Part I, chap 10. Hafner, New York, p 6
13. Meyskens FL, Salmon SE (1979) Inhibition of human melanoma colony formation by retinoids. Cancer Res 40: 4055–4057
14. Moon TE (1980) Quantitative and statistical analysis of the association between in vitro and in vivo studies. In: Salmon SE (ed) Cloning of human tumor stem cells, chap 17. Alan Liss, New York, pp 209–221
15. Preisler HD (1980) Prediction of response to chemotherapy in acute myelocytic leukemia. Blood 56: 361–367
16. Salmon SE, Buick RN (1979) Preparation of permanent slides of intact soft agar colony cultures of hematopoietic and tumor stem cells. Cancer Res 39: 1133–1136
17. Salmon SE, Hamburger AW, Soehnlen BJ, Durie BGM, Alberts DS, Moon TE (1978) Quantitation of differential sensitivity of human tumor stem cells to anticancer drugs. N Engl J Med 298: 1321–1327
18. Salmon SE, Alberts DS, Meyskens FL, Durie BGM, Jones SE (1980a) Clinical correlations of in vitro drug sensitivity. In: Salmon SE, Soehnlen BJ, Young L, Chen H-SG, Moon TE (eds) Cloning of human tumor stem cells, chap 18. Alan Liss, New York, pp 223–245
19. Salmon SE, Alberts DS, Durie BGM, Meyskens FL, Soehnlen BJ, Chen H-SG, Moon TE (1980b) Clinical correlations of drug sensitivity in the human tumor stem cell assay. In: Mathé G, Muggia FM (eds) Recent results in cancer research, vol 74. Springer, Berlin Heidelberg New York, pp 300–305
20. Tsiatis A (19890) A note on a goodness-of-fit test for the logistic regression model. Biometrika 67: 250–251
21. Von Hoff DD, Johnson GE (1979) Secretion of tumor markers in the human tumor stem cell assay system (Abstract) Proc Am Assoc Cancer Res 20: 51
22. Von Hoff DD, Harris GJ, Johnson G, Glaubiger D (1980) Initial experience with the human tumor stem cell assay: Potential and Problems. In: Salmon SE (ed) Cloning of human tumor stem cells, chap 10. Alan Liss, New York, pp 113–124


Received February 4, 1981