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Letter to the Editor

Role of Murine Tumor Models in Cancer Treatment Research[1]

Daniel S. Martin, M. Earl Balis, Bernard Fisher, Emil Frei, Emil J Freireich, Gloria H. Heppner, James F. Holland, Janet A. Houghton, Peter J. Houghton, Randall K. Johnson, Arnold Mittelman, Youcef Rustum, Robert C. Sawyer, Franz A. Schmid, Robert L. Stolfi, and Charles W. Young

Catholic Medical Center, Woodhaven, New York 11421 [D. S. M., R. C. S., R. L. S.]; Memorial Sloan-Kettering Cancer Center, New York, New York 10021 [D. S. M. M. E. B., F. A. S., C. W. Y.]; University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15261 [B. F.]; Sidney Farber Cancer Center, Boston, Massachusetts 02115 [E. F.]; M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77030 [E. J F.]; Michigan Cancer Foundation, Detroit, Michigan 48201 [G. H. H.]; Mt. Sinai Medical School, New York, New York 10029 [J. F. H.]; St. Jude Children's Research Hospital, Memphis, Tennessee 38101 [J. A. H., P. J. H.]; Smith, Kline and French Laboratories, Philadelphia, Pennsylvania 19101 [R. K. J.]; and Roswell Park Memorial Institute, Buffalo, New York 14263 [A. M., Y. R.]

Abstract

Two major factors have contributed to a widely held disenchantment with murine tumor models for drug screening in cancer research: (*a*) the higher costs of these models in comparison to studies performed with tumor cells *in vitro*; and (*b*) the perception that these models have failed to demonstrate satisfactory correlation of chemosensitivity with analogous human tumor types; *i.e.*, murine tumors generally have proved to be sensitive to many more agents than are found to be active in the clinic. The perceived failure of the murine models is discussed with particular reference to the difference in criteria used for evaluating drug sensitivity in murine tumor models *versus* clinical trials, and we conclude that the perception about murine models is not tenable in light of present information. The very important role of murine tumor models in optimizing dosage and administration schedules and, most importantly, in the development of a new drug to its most useful potential in combination chemotherapy is discussed. The value of this *in vivo* methodology is stressed.

Introduction

Most major progress in medicine, including advances in cancer treatment, has involved animal research, and it is therefore reasonable to expect that future progress in the treatment of disease will also depend upon animal experimentation. However, the increased cost of buying and maintaining laboratory animals has forced a substantial decrease in the number of animals used in research in recent years. [The National Academy of Sciences reported a 40% drop between 1968 and 1978 (1).] This trend has become increasingly evident in the cancer field, and there is now a discernible decrease in the number of research programs using *in vivo* murine model systems.

Individual cancer investigators and institutions such as the National Cancer Institute are increasingly using *in vitro* models. A major reason for this shift from *in vivo* to *in vitro* studies is cost. Despite the general requirement that National Cancer Institute reviews of research grants should separate budgetary considerations from evaluation of scientific merit, criticisms may be voiced about the greater expense of *in vivo* tumor models and emphasis may be placed on less costly studies *in vitro*. The National Cancer Institute has markedly reduced its contract-supported research programs; currently, only a single contract exists for evaluation of *in vivo* preclinical studies in combination chemotherapy. The economic basis of many decisions about animal tumor systems derives in large part from the low productivity of tumor-bearing mice for primary screening. The shift away from *in vivo* studies has led to what we perceive to be improper and dangerous neglect of the field. However, tumor-bearing mice are indispensable for experimental therapeutics involving pharmacokinetics, immunological mechanisms, biochemical pharmacology, and combination chemotherapy. In the absence of ongoing investigations in these fields, relevant new studies will be unavailable or will be worked out in humans at much greater risk and cost. Without the reasonable expectation of support, young investigators initially interested in *in vivo* experimental therapeutics may turn elsewhere. For example, despite the fact that both preclinical (2–6) and clinical (7, 8) studies have long established that combination chemotherapy is more effective therapeutically than drugs used individually, the unfortunate result of the above events is that few modern studies of polychemotherapy have been conducted or are planned in preclinical *in vivo* systems (9).

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Misperceptions Regarding the Value of Murine Tumor Models as Screening Tools for Potential New Anticancer Agents

Many clinical investigators believe that murine tumor models are not relevant to the human cancer problem because claims of anticancer activity for drugs in experimental tumor systems often were not verified when these drugs were used in human cancer patients (10, 11). Although there are a number of reasons for the poor correlation between animal tumor model determinations of drug activity and clinical efficacy, the most important is the use of different criteria for measuring activity in the two settings. Mere inhibition of solid tumor growth is usually used as the criterion for activity in the preclinical setting, whereas 50% or greater tumor regression, requiring the killing of 2 or more logs of the clonogenic tumor cells, is the acceptable criterion for activity in clinical trials. When different end points are used to evaluate data from chemotherapy protocols, disparate results are obtained (12). When different end points are used in animal models and in the clinic, the lack of positive correlation between drug response of human cancer and animal models of human cancer should be no surprise. After all, even total lack of tumor growth during chemotherapy (which conventionally would be considered very significant in experimental systems) is merely tumor stabilization as a clinical parameter and is considered relatively insignificant clinically. Clearly there has been a lack of effective communication and understanding regarding the significance of the difference between these two criteria for determining anticancer activity.

Laboratory oncologists are ambivalent regarding the chemotherapeutic predictive value of *in vivo* murine tumor models. On the one hand, they note that the models clearly have not been totally unsuccessfully: "Pre-clinical models continue to contribute to an increasing human cure rate" (13). Thus, the models are credited for the discovery of some 40 clinically active drugs that have radically altered the clinical approach to cancer therapy (14–16). On the other hand, laboratory oncolo-

gists often seem dissatisfied with the tumor models: "The greatest deficiency is the lack of objectively demonstrated positive correlation between the response of any specific experimental tumor and any specific human tumor" (14). And some laboratory oncologists have faulted themselves for using inhibition of tumor growth as an end point for activity in light of the different evaluation criteria used clinically, stating that this decision was "a serious error in judgment in collecting and interpreting chemotherapy data from experimental trials with animal tumors" (17).

Unless the same end point for measuring therapeutic activity in the laboratory and the clinic is used and a lack of correlation of chemotherapeutic activity is thereby demonstrated, murine tumor models should not be rejected as inherently false analogues of human cancer on the basis of the presently reported poor correlations (11). Rather, the methodologies need reordering first. It may be that the design, interpretation, and extrapolation of the laboratory chemotherapeutic data to the clinic need more revision than the tumor models.

Furthermore, the pressures to provide agents for clinical evaluation have resulted in the acceptance, as clinical candidates, of numerous drugs with minimal activity in only 1 or 2 animal tumor models. It is not surprising that such agents (bruceantin, triciribine phosphate, spirogermanium, and anguidine, to name a few recent examples) have failed in clinical trials. Agents with the greatest clinical utility, such as cyclophosphamide, doxorubicin, and *cis*-Diamminedichloroplatinum (II), have a high degree of activity in a broad spectrum of animal tumor models. The predictive value of animal tumor models could be readily improved simply by imposing more rigorous criteria for the selection of clinical candidates. If there is to be more selectivity, fewer new agents may be available for evaluation in humans in a given time.

Importance to a Cancer Drug Evaluation Program of the Realization That There Can Be No "Magic Bullet" Anticancer Agent

Cancer-associated genetic instability causes biochemical cellular heterogeneity with a variable cellular overlap among all types of mammalian neoplasms; this in turn leads to a variable chemotherapeutic correlation (18–20). The variability of chemotherapeutic response, which conforms to clinical experience, is a natural consequence of the somatic mutation model of cancer and is precisely what should be expected (21). The assumption that there should be a common pattern of cellular heterogeneity for identical histological types of cancer arising from the same organ system is not warranted. Heterogeneity cannot be expected to be duplicated from neoplasm to neoplasm.

Neither a "magic bullet" curative agent nor an exact correspondence in chemotherapeutic sensitivity between histologically identical cancers is an expectation compatible with our biological knowledge of the heterogeneity of cancer. Heterogeneity indeed makes the cancer problem more difficult to solve, but cure nevertheless can be attained by combination chemotherapy. Some leukemias, lymphomas, and sarcomas, as well as some of the relatively uncommon carcinomas, are cured by combination chemotherapy (15, 16). Chemotherapeutic heterogeneity was the important original concept stimulating the promulgation of combination chemotherapy of cancer as opposed to single agent treatment (2, 4), and curative progress with combination chemotherapy continues (16).

With regard to chemotherapeutic heterogeneity, the cancer problem is more difficult than other medical problems. For example, in sensitivity testing for antibiotics in patients with bacterial infection, a positive test has a high correlation with clinical cure if the patient has normal immunological and leukocytic function. Due to heterogeneity of cancer cells, a single agent's positive tumor response rate in cancer patients can only rarely be expected to achieve cure, for some cancer cells will elude the single agent despite the appearance of a complete tumor regression, and tumor recurrence will eventuate (21). Further, neoplastic cell heterogeneity and past clinical experience make it most likely that the tumor response rate, for even a very good new single agent, will be in the range of only 30–50%. Although such findings, of course, would be very gratifying, the problem(s) of adding agents to seek cure would remain to be resolved.

Combination chemotherapy with the newly found agent may be evaluated clinically (without initial preclinical *in vivo* trials), but in general this has not proved to be an effective way to conduct this type of research. Enhanced selectivity of a drug combination *in vivo* is demonstrably dependent upon the variables of sequence, timing, schedule, dose, and dose ratio between agents. Given the infinite number of possible ways several drugs can be combined, only a minute fraction of these methods can actually be evaluated in patients. Clinical experience indicates that failure to obtain a positive result in the initial clinical trial usually results in a loss of clinical interest sufficient to preclude additional clinical investigation of a particular drug combination. It is therefore important to provide useful preclinical guidelines to aid the clinical investigator in the initial selection of a sequence, schedule, timing, and ratio of drugs in combination that will lead to positive results if such exist. Optimization can follow. It is precisely because the leap from preclinical models of whatever sort to the patient is so great that adjustment of many variables (*e.g.*, schedule, timing, dose, etc.) is necessary. Guidelines from *in vivo* murine model studies are useful. Lane (22) commented, "While many empirical combinations have been highly effective, many others have been no more effective or even less effective than single agents, due to antagonistic effects of drugs related to biochemical, pharmacological, pharmacokinetic, and cytokinetic mechanisms. This is a major argument for exploring various drug combinations and sequences in animals prior to clinical experimentation."

Xenografts of human tumors may represent, with considerable fidelity, metabolic characteristics of human malignant disease *in situ* (23, 24). As such they represent a first approximation to clinical cancer and may have value in selecting "histiotype-specific" agents when used as screening tools (25, 26). The value of such models awaits prospective trial. However, retrospective analysis indicates that xenografts parallel the qualitative and quantitative chemosensitivities of their specific histiotype. Thus, the use of a "small battery" of tumors of a particular histiotype representing the heterogeneity observed in a clinical setting may prove to be an effective preclinical screen (24, 25).

The human tumor xenograft models, when used appropriately (26), would appear to be of value in the screening program, but within the field of cancer research they should be regarded as additional model systems rather than alternatives to contemporary transplantable or spontaneous murine tumor models. Even if it were desirable to evaluate schedule, sequence, and drug combinations against xenografts representing each histiotype, this would present considerable logistical problems. Such large scale studies are best carried out in murine models, and then selected protocols should be evaluated against xenografts of the appropriate histiotype, to elucidate whether a particular combination demonstrates histiotype specificity.

The importance to a cancer drug evaluation program of facing the reality that there is no "magic bullet" lies in the recognition that the search for anticancer effectiveness does not end with the identification of a clinically active agent. It is only a good beginning. Additional experimental therapeutic studies in animals should follow the identification of a new agent to provide useful guidelines for more effective clinical trials.

What Are the Data Supporting the Notion That Findings in Murine Tumor Models Can Be Relevant to Human Cancer?

The extrapolation of concepts and biological principles regarding tumor response to therapy from the murine species to humans is documented in the literature. Much of the conceptual framework and general principles of clinical cancer chemotherapy evolved from work on the murine leukemia L1210 (6). Research on murine solid tumors provided an understanding of the basis for the need for clinical combination chemotherapy (2, 4, 5). A clinically important concept from chemotherapy studies in animal tumor models is that the total body burden of tumor cells may affect the therapeutic outcome (9, 27). The clinical therapeutic advantage of combining the modalities of surgery and chemotherapy (adjuvant chemotherapy) evolved from murine tumor model studies (28). The conceptual framework that heterogeneity was a central clinical problem in cancer therapy evolved largely from animal studies (18). The importance of dose as a critical factor in clinical cancer chemotherapy was first established in experimental *in vivo* tumor systems (29). A comparison of the quantitative toxicity of anticancer agents in mice and humans determined that the maximum tolerated dose per m^2 of body surface area (mg/m^2) was approximately the same (30). An important clinical concept from chemotherapy studies in animals is that the schedule of administration may markedly affect the therapeutic outcome (31).

In contrast to the broad conceptual advances that have been made through the use of murine tumor models, their utility as models to provide specific guidelines for drug treatment scheduling in cancer patients has produced uncertain correlations with clinical results. Analysis of these data reveals that judgment and knowledge must be used to effect successful transfer of the details of the animal data to the clinical situation.

Sometimes the model may be viewed as a means to produce a "cookbook" type prescription ("recipe") of a drug treatment schedule. A schedule found to be optimally effective in the model is assumed to be directly transferable to clinical scheduling. The classical example of this use is the optimal schedule for treatment of residual L1210 leukemia with methotrexate (32), confirmed to be effective for humans with leukemia as a maintenance therapy in the absence of manifest disease (33).

There are also clinical studies that do not correlate with successful preclinical findings in murine animal tumor models. However, when these reports are closely examined, the laboratory treatment schedule ("recipe") has often not been properly followed in the clinic. As Saunders (34) has noted, "It has been a fact of scientific life in the United States that with respect to cancer, fundamental biochemical research and applied clinical research have not generally been carried out in a coordinated way." For example, preclinical studies stressed that 6-methylmercaptopurine ribonucleoside could enhance the therapeutic activity of 6-mercaptopurine only if the two drugs were given in an appropriate sequence and time interval (35, 36). Despite these preclinical caveats, clinical trial was performed with the concomitant administration of the two drugs, and it is not surprising that the negative clinical results did not agree with the positive preclinical findings (37).

Successful application of a "recipe" from a "cookbook" requires that all of the details of the recipe are closely followed and that there is a close correlation between the situation in the clinic and the model (38). Much more often, however, there are clear quantitative differences (*i.e.*, tumor mass, cell proliferation rate, a markedly differing degree of chemotherapeutic response between two agents that are to be evaluated in combination, etc.) between the model and the clinical situation that warrant reconsideration of the "recipe" approach. One cannot translate what one sees in specific animal tumors to specific human cancers in a recipe-like manner without taking into consideration the marked quantitative differences. Skipper and Schabel (39) have noted, "None ... (of the animal models for solid tumors) ... will save the experimental and clinical investigator the pain of having to learn to "translate" in both directions. Translation means the changes in regimen required to achieve a similar response rate in a second tumor system when a given response to some therapeutic procedure is observed in one tumor system ...." Note that the call is to "translate," not to transpose (*i.e.*, not the "recipe" approach), the laboratory findings to clinical trial. An example of the need to "translate" has been reported recently (40).

All of the factors of experimental design, including proper controls, sequence, dosage, ratios, and the proper time interval between administration of drugs, are among the important factors that require attention. Dosages and exposure times may be worked out in the laboratory, but unless the details are properly translated into the clinic with the aid of detailed pharmacological studies, the successful preclinical results may not be achieved in the complex clinical situation. If these details are not properly followed, the murine tumor model cannot be faulted for a failure to predict a successful clinical trial.

It is likely that clinical disappointment with many combination chemotherapy trials will be minimized if appropriate *in vivo* test steps are interposed between active single agent and combination chemotherapy clinical trials. We believe that compounds identified as being clinically active should be brought back to the laboratory for reevaluation in preclinical *in vivo* investigations to discover or to optimize schedules, sequencing, time intervals, and other details of combination chemotherapy.

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