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Perspectives and Commentaries

Test This Patient's Leukemic Stem Cells in a Non-clonogenic Chemosensitivity Assay, Treat According to Standard Protocols

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(A COMMENT ON: Maddox A-M, Johnston D A, Barlogie B, Haq M, Keating M J, Freireich E J. *In vitro* suppression of DNA synthesis by remission induction agent and its correlation with response in adult acute leukemia. *Eur J Cancer Clin Oncol* 1984, **20**, 507–514.)

MODERN chemotherapy, combining cytosine arabinoside (ara-C) and an anthracycline drug, regularly produces complete remission (CR) rates of 60–70% in acute non-lymphocytic leukemia (ANLL). However, three major problems persist: a proportion of patients (about 25%) is resistant to induction treatment, the majority of patients relapse within 2 yr and long-term survivors number only around 15% [1].

Since new active antileukemic agents are developed at a slow pace, improvement of today's results could be achieved by a better use of the available treatment modalities. For instance, investigators at the M.D. Anderson Hospital and Tumor Institute at Houston, TX, have developed a clinical model [2, 3] which, in their hands, discriminates a subset of patients unlikely to enter CR following ara-C/anthracycline therapy. However, even if such a predictive model proved to be valid on a large cohort of patients, it cannot provide information on specific leukemia cell drug sensitivity.

The expectation from an *in vitro* test is that it would allow selection of the individual treatment regimen for patients with acute leukemia. The O/E (observed/expected) CR rate of patients managed by '*in vitro*-prescribed' therapy would ideally approximate unity. The observed CR rate should increase if in the chemotherpy armamentarium the most active agents can be correctly identified and subsequently administered; if no such therapy appears appropriate, then expected CR would be more accurately assigned and patients could be allocated to alternate protocols (e.g. marrow transplantation).

In recent years *in vitro* chemosensitivity assays have mainly been directed at testing the survival potential of clonogenic leukemic cells in semi-solid media [4, 5] or estimating DNA synthesis by labeled precursor uptake following drug exposure [6, 7]. Both methods produce a quantitative measure for *in vitro* drug-induced cellular injury.

While it is not our intent to go into methodological details, one should keep in mind that tests based on the clonal expansion of the putative tumor stem cells rely on the addition of mitogenic factors (usually a leukocyte-conditioned medium). Typically, from 200,000 blasts, 100–200 clones will grow after 7 days in the incubator; this indicates that only 1–2 out of 2000 leukemic cells retain the ability to produce progeny. These figures do not acknowledge the even smaller fraction of cells that maintain a high proliferation status over time, i.e. regrow a second colony after disruption of the first [8]. This self-renewing cell is thought to represent the true leukemic stem cell, which must be eradicated if cure is to take place. Essentially, the test determines a drug's ability to reduce the blastogenic response of (relevant?)

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clonogenic cells. Obviously, this test does not yield results in specimens that fail to grow.

When [^{3}H]-thymidine uptake ([^{3}H]-TdR) is determined autoradiographically in blasts, one can find that 0–20% of leukemic cells (generally <15%) incorporate [^{3}H]-TdR during 1-hr pulse exposure. This makes evident that, on average, cells capable of [^{3}H]-TdR uptake comprise a much larger cell population than clonogenic cells (approximately 300 times larger). The bulk of these non-clonogenic cells is probably not of significance to tumor growth and the relevance of drug damage to them is uncertain.

The paper recently published in this journal by Maddox *et al.* from the M.D. Anderson Hospital and Tumor Institute at Houston presents a retrospective analysis of *in vitro* suppression of DNA synthesis by antileukemic drugs and its relation to clinical outcome in patients with acute leukemia.

This study well illustrates several problems encountered in evaluating the relevance of *in vitro* chemosensitivity testing: (1) the study involved patients with adult acute non-lymphoblastic and lymphoblastic leukemia, which are viewed today as a wide spectrum of diseases. Specifically, this group of diseases is not homogeneous when one considers survival, response to treatment, morphology, immunological markers, cytogenetics and, most critically in this instance, tumor cell kinetics; (2) patients were not treated homogeneously. Three regimens were used: all patients received ara-C, vincristine (Oncovin, O) and prednisone (P); in addition, 33 received adriamycin, 12 rubidazone and 8 AMSA due to predicted resistance to anthracyclines [2]. Thus, of 53 clinical trials, only 33 included adriamycin, which indicates that results from 9 of 42 *in vitro* trials with this drug did not have the matched *in vivo* equivalent; and (3) accurately defining the exact correlation of *in vitro*/*in vivo* single-agent effects is difficult when polychemotherapy is administered.

This paper also reports that >80% suppression of *in vitro* [^{3}H]-TdR uptake by ara-C at 0.004 μg/ml is significantly correlated with response to polychemotherapy in 80% of the patients. No such correlation is found for adriamycin. It is further described that patients failing to go into CR differ from those responding in that individually their measured dose–response curves for ara-C (and adriamycin) deviate from the constructed drug sensitivity curves of all responding patients (Mahalanobis' D^2 score). The need for such statistical analysis arises from the tremendous overlap of the *in vitro* results obtained from samples of responding and non-responding patients. A D^2 score below and above 10 for ara-C discriminates between responders and non-responders respectively. This score is reported to rank first in comparison to clinical and laboratory parameters (age, presence of metaphases, antecedent hematological disorder), predicting probability of response. Since selection of AMSA–OP treatment is based on these parameters, one is left with the impression that resistance to ara-C *in vitro* may be an indicator for resistance to anthracyclines *in vivo*! Most importantly, however, the authors themselves conclude that "the considerable overlap of values among patients did not allow it to be useful as a single parameter in helping to choose therapy". This tremendous overlap is not surprising when one considers that maintenance of a 'normal' hemogram and bone marrow for 30 days was used to separate responders from non-responders. While this definition of CR is used in most clinical trials, it clearly underscores the fact that in most so-called CRs leukemic blasts will rapidly regrow the bone marrow. Viewed from this perspective, the definition of CR should be reconsidered. Indeed, the major failure in treating acute leukemia is not the induction of a CR, but rather maintaining a CR over a prolonged period. One wonders if a test to determine the residual tumor of CR would not be more useful than predicting the likelihood of a 30-day CR.

Ideally, an *in vitro* assay should yield tumor cell drug sensitivity for individual antineoplastic agents. This should allow tailoring optimal therapy, the clinical implication being an increase in the number of expected CRs and possibly prolongation of survival. While this goal is reached by initially analyzing *in vitro* data on a retrospective basis, the ultimate proof of the validity of the test must be established by carefully performed prospective studies. Needless to say, this goal is not yet within reach.

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