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Summary of Informal Discussion on the Management of Drug-refractory Malignancy and Toxicity

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Dr. Holland stated that we have in fact tested the use of some of the drugs mentioned by Dr. Bruce in repetitive schedules at varying intervals. These have been tested in step-wise fashion, and very often we have met the limitation of the growth fraction of normal cells, specifically in the gastrointestinal tract and in the marrow. Without some innovations it seemed to Dr. Holland that the fine points, mentioned by Dr. Bruce as steps of strategy, will shortly lead to toxicity as a limiting feature before getting to use drugs now available in ways that are unique or different from the methods in which they have already been tested. He questioned the premise that one needs extracorporeal test systems. Each animal in the experimental system described by Dr. Bruce that has been sacrificed to show the number of residual thymic malignant stem cells could have served as his own test system by quantification of the time it took for his demise. In essence, if that surmise is correct, all patients serve as their own assay systems. In that sense, despite the intrepid clinical approaches that so far have been made, the outcome has been nearly uniformly disappointing in patients with a large number of cancers. On the other hand, there are unique circumstances. Dr. Holland believes that chemotherapeutic "cures" have occurred in rare individuals with a variety of solid tumors ordinarily considered too refractory. Probably we have understressed or underestimated the role of the host in the triad which Dr. Goldin earlier described. There is room for an empiric, but perhaps individualized, approach with respect to the host's participation in destruction of the residual small number of tumor cells that may remain after chemotherapy.

In replying to Dr. Holland, Dr. Bruce stated that it is not possible to estimate the effect of treatment on the survival of malignant cells from changes in tumor mass or the survival of animals. As examples, he quoted experiments in which treatment caused very little change in tumor mass, but bioassays for the numbers of surviving malignant cells, capable of regenerating the tumors, showed that the treatments had reduced the numbers of these cells by two or three decades (4, 5). Bruce has tested the clinical approach in the treatment of spontaneous lymphomas in AKR mice. The treatment of these mice with a single dose of cyclophosphamide caused lymph nodes to shrink temporarily but had little effect on the survival of the mice. When the numbers of malignant cells surviving in the thymus of these mice were assayed, they were found to be reduced through four to five decades within 24 hours after treatment; they then regrew rapidly to reach a plateau at the pretreatment level. Death occurred at varying intervals after this plateau was reached. In this experiment, the large fraction of malignant cells killed by the drug was not reflected by a prolongation of survival. If the response of the host were to contribute to the control of surviving malignant cells, this would be recognized by a change in the rate of regrowth of the tumor.

In replying to Dr. Zubrod, Dr. Bruce indicated that he did not mean to imply that his approach was unique. The main point of the paper was to show that the investigators listed in his table are using a slightly different approach to "curing" animal tumors than has been used clinically with patients. With respect to determining the numbers of tumor cells in resistant phases of the cell cycle (i.e, G_1, G_2, and G_0), Dr. Bruce felt that it did not much matter why these cells were resistant to chemotherapy. The numbers of tumor stem cells which survive the maximally tolerated dose of therapy represent the fraction of the tumor which are resistant, and the problem is to find some way of dealing with these cells. The empirical approach is to test different combinations of drugs for synergism, different dosage schedules, etc., and Dr. Bruce felt that this approach will be more productive than the approach of finding out why the cells are resistant.

With respect to Dr. Karrer's comments, Dr. Bruce agreed that first-generation transplants are useful tumors to study, but spontaneous animal tumors are more difficult to cure and are closer to the clinical problems encountered in patients. The uptake of the drug into the tumor is an important problem, but this is just another way of looking at the anoxia problem dealt with by radiation biologists. The survival of malignant cells located some distance from the blood supply is reflected in the plateau of the dosage-survival curve. Partly killed cells fall into the same category, for if they are capable of recovering into viable tumor cells, they will regenerate and kill the animal, and they will be recognized in the surviving fraction. If these cells are not capable of recovering, they will die and will no longer be a problem. Assaying tumors spontaneously, by determining the number of metastases following treatment, is treacherous because we do not know what controls the rate at which metastases occur.

Several discussants (notably Drs. Zubrod, Skipper, and Mendelsohn) objected to Dr. Bruce's use of the word "empirical," for they felt that his work did not reflect this type of approach. Bruce, however, stuck to his guns and reemphasized the fact that if one has good assays for malignant tumor cells (tumor stem cells) and the important normal stem cells so that the effects of changing drug combinations and schedules can be determined directly, the

best approach to improving cancer chemotherapy will be empirical. With these tools it should be possible to find a way of eliminating the resistant cell population, and if this is possible, it doesn't matter why some cells are more resistant to therapy than others.

During the discussion, Dr. Bruce stated that the spleen assay was designed to detect cells which have an extensive proliferative capacity. Cells which lose the capacity to undergo 17 to 20 doublings, as a result of differentiation or of damage following therapy, do not form macroscopic colonies and would not be detected by this assay. However, we are not interested in these cells, for they have lost their lethal potential. Dr. Bruce also was not concerned about malignant cells which may be pushed into G_0 by therapy, for a cell that stays in G_0 is not a threat.

Drs. E. Henderson and C. Heidelberger questioned this attitude. Dr. Henderson suggested that the late relapses of patients with acute lymphocytic leukemia (i.e., relapses after more than 300 days in remission without therapy) might be explained by the survival of latent leukemic cells, perhaps in G_0, which are stimulated to proliferate by an event occurring at varying intervals after complete remission status has been achieved. He asked Dr. Bruce whether the spleen colony assay could be relied on to detect such latent leukemic cells.

Dr. Bruce replied that resting hemopoietic stem cells (presumably in G_0) which are not in cell cycle when they are taken from a normal marrow (for they do not incorporate TdR-^{3}H) will form spleen colonies when they are injected into an irradiated host. He could not say whether the assay detects *all* of the G_0 cells, for there is no other independent assay to check this. Similarly, he could not say whether the spleen colony assay would detect all latent leukemic cells. However, in the treatment of mice with transplanted AKR lymphoma, he has found that dosage schedules which eliminate malignant cells capable of forming spleen colonies also result in apparent cures of the lymphoma. Also, in the treatment of the spontaneous lymphoma, long-term survivors are not obtained, and it has not been possible to eliminate lymphoma colony-forming cells from the thymus. Thus, in this instance, the results of the spleen colony assay for malignant cells has correlated well with the survival of the animals.

Dr. Goldin asked whether the malignant cells which survive very large doses of chemotherapeutic agents demonstrate resistance to the drug after transplantation. Dr. Bruce replied that this question has been investigated and that he was not able to demonstrate any change in the sensitivity of the surviving cells to cyclophosphamide.

Dr. Hellmann pointed out that another way to approach the problem of drug-resistant malignant cells is to develop new drugs. He presented data on the interesting new compound I.C.R.F. 159. Much of this material has since appeared in the literature (1, 2).

Dr. Rall commented on the increase in mitotic activity noted by Dr. Philips in mouse gastrointestinal epithelium 11 hours after a single dose of arabinosylcytosine (ara-C). Dr. Rall and his collaborators have found that two doses of ara-C separated by a 9-hour interval are much more toxic than if they are separated by 6, 12, 15, or 18 hours. This result agrees very nicely with the morphologic observations of Dr. Philips.

Dr. Elion commended Dr. LePage for bringing biochemistry back into the rationale of combination chemotherapy. However, she took issuance with his positive statement that the tumor inhibitory activity of thioguanine is due to its incorporation into DNA. She has found that 6-mercaptopurine (6-MP) is incorporated into DNA as thioguanine in a line of adenocarcinoma 755 that is resistant to 6-MP. Indeed, the incorporation as thioguanine is twice as great as in the sensitive line of these cells.

The explanation that Dr. LePage offered for the observation that the combination of thioguanine and ara-C did not produce an increased prolongation in survival time was that repeated doses were used, whereas the increased incorporation of thioguanine into DNA was obtained with a single dose of ara-C. However, the results could also be interpreted to mean that the incorporation of thioguanine has nothing to do with the mechanism of action. Dr. LePage replied that the amount of thioguanine which Dr. Elion observes to be incorporated into the DNA of adenocarcinoma 755 cells is below the threshold which he has found to correlate with response. Thioguanine has to be incorporated into DNA above the threshold of about one nucleoside per 1000 or the cells recover.

Dr. Garattini discussed the effects of tumors and other drugs on the host and the influence of these effects on drug toxicity. Certain tumors, such as the Walker carcinoma, cause a marked depletion of adipose tissue. Animals bearing an advanced Walker tumor will die if they are left overnight without food, whereas animals with other types of tumors can resist longer periods of fasting. If animals bearing advanced Walker tumors are treated with a drug which causes anorexia or vomiting, the animal will die as a result of not eating, rather than as a result of toxicity directed against a specific tissue. Certain tumors and drugs affect the microsomal enzymes of the liver, and this will effect the metabolism of other drugs.

In commenting on Dr. Perry's paper, Dr. Amiel recounted the experience of a pathogen-free isolation unit containing five beds which has been in use since 1965 at the Institut de Cancerologie et d'Immunogenetique in Villejuif, France. The use of this unit appears to have reduced the risk of infection in patients treated with intensive chemotherapy. Leukocytes, collected with the IBM continuous-flow separator, have been used to treat infections. It is of some interest that a few of these patients developed symptoms which closely resemble the secondary graft-versus-host syndrome.

Dr. Holland presented data on leukocyte mobilization in patients with acute and chronic leukemia. A preliminary report of these studies has been published in abstract form (3). Acute leukemia patients with a reduced ability to mobilize leukocytes have a marked increase in the frequency of infections and a higher mortality. He suggested that these high-risk patients should be selected for placement in the protected environments discussed by Dr. Perry.

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