# Summary of Informal Discussion on the Effects of Chemotherapy on the Kinetics of Leukemic Cell Behavior

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Dr. Skipper, in his presentation, suggested that an obviously important goal of chemotherapy of leukemias is eradication of all (every single one) of the host's leukemic cells (regardless of anatomic distribution or metabolic heterogeneity) with dosages which will spare the host. If the leukemia cells carry a leukemia-inducing virus, it will also be necessary to inactivate residual virus permanently so that reinduction of *a* leukemic cell will not occur during the normal life span of the host. It was pointed out that a maximum tolerated *single dose* is often far superior to *chronic* daily maximum tolerated therapy (q.d. 1–15 days or q.d. 1–death) if eradication of all of an animal's leukemic cells is the goal.

The reason for this is now apparent for the model L1210 leukemia systems. The fractional reduction of a leukemic cell population *in vivo* (regardless of size) is directly proportional to the daily dosage level administered to the host; i.e., administration of $LD_{10}$ on day 1 only may reduce a host's leukemic cell population by 6 logs, thus "curing" about 90% of animals bearing $10^5$ leukemia cells, whereas a q.d. 1- to 15-day $LD_{10}$ may reduce the population by only 50% each day, thus "curing" none of the animals because the surviving leukemic cells (quadrupling in number each day) more than replace those killed each day ($10^6 \times 50\% = 5 \times 10^5 \times 4 = 2 \times 10^6$).

Dr. Friedkin pointed out that the implications of exponential growth of leukemic cells, although generally understood, are all too often forgotten; also that if 1 leukemia cell survives therapy—whether it be because of inadequate drug dosage, because of protection from drug in the brain, or because of biochemical alteration which confers drug resistance—the inexorable doubling process leads to a logarithmic increase that is eventually lethal to the host. The postulate made some years ago by Goldin and his associates that the number of leukemic cells in the host can influence the apparent effectiveness of drug treatment was cited by Drs. Skipper and Friedkin.

Dr. Friedkin was uncertain whether Dr. Skipper believes that the death of a leukemic patient can also be attributed to the continued proliferation of drug-sensitive cells because of inadequate treatment (to a considerable degree he does). Dr. Friedkin believes that the blood-brain barrier and drug resistance problem can be important elements of leukemic death in man (Dr. Skipper agrees completely, but thinks that the constant fractional survival of so-called sensitive leukemic cells often may predispose to the latter "obstacles to control of acute leukemias").

Dr. Friedkin posed the question: Does the chemotherapeutic action of amethopterin in a leukemic animal or man depend on the outright killing of a certain fraction of drug-sensitive cells or on the prolongation of the generation time of all drug-sensitive cells? (Dr. Skipper's experiments with the *in vivo* L1210 leukemia system carried out at Southern Research Institute, i.e., control and treated titrations from which the generation time of control and surviving treated leukemic cell populations may be estimated, indicate that high single doses of amethopterin usually do not significantly affect the generation time of the leukemic cells which survive such therapy. Studies with leukemic cells labeled with tritiated thymidine offer promise of a more unequivocal answer to Dr. Friedkin's pertinent question.)

Dr. Zubrod pointed out that, based on clinical combination chemotherapy trials and the kinetic information gained with model experimental leukemia systems, the National Cancer Institute and the Leukemia B Group have instituted studies designed to attempt eradication of the total leukemic cell population in man. Some of these studies have entailed the use of vincristine, amethopterin, 6-mercaptopurine, and prednisone (VAMP).

Dr. Frei remarked that about $10^9$ packed leukemia cells occupy 1 ml; therefore $10^{12}$ cells represent about 1 kg of leukemic cells. He estimated that the average 30-kg child with acute lymphatic leukemia (not in remission) should have of the order of 2–3 kg of leukemic cells or about $2–3 \times 10^{12}$ at the time treatment is initiated. Using this estimate, knowledge of the duration of unmaintained complete remission (median after prednisone 72 days, after vincristine 42), approximate information on the generation time of leukemic cells (2-6 days based on tritiated thymidine and hematology studies), and assuming log-phase kinetics, Dr. Frei calculated that the acute lymphatic leukemia cell population in patients may be reduced from more than $10^{12}$ to a median of $10^8$ in patients who achieve complete remission on prednisone and to $10^9$ in patients achieving complete remission on vincristine, or about 4 and 3 logs, respectively. The median duration of remission of patients receiving VAMP is of the order of 120 or 130 days (about $\frac{2}{3}$–$\frac{3}{4}$ of the patients in this study have relapsed). This is consistent with a generation time of perhaps 3–4 days and, if this model applies, with the fact that VAMP has driven the acute leukemic cell population down much below that achieved by prednisone or vincristine alone. Dr. Frei felt that such clinical approaches were moving in the right direction, but whether or not it will be possible to eradicate all of a patient's leukemic cells

with the agents now at hand (used in any conceivable fashion) remains to be seen.

Dr. Frei stated that some 15 patients over the last 7 or 8 years at the National Cancer Institute have died in complete bone marrow remission and asked Dr. Thomas to comment on his histologic examination of tissues from these patients. Dr. Thomas recalled that in 10 of the 15 it was possible to find small foci of leukemic cells: some in the liver, kidney, testes, bowel, lung, and (in 1 patient) the arachnoid.

Dr. Wintrobe asked about the significance of these cells. How much could be concluded by microscopic examination? What were they doing biochemically, immunologically, or by any other type of test? Dr. Thomas felt that he could not determine whether these cells were actively proliferating or were inactive residual leukemic cells. Dr. Wintrobe asked if one could be certain that they were leukemic cells, and not lymphocytic infiltrates of a benign nature. Dr. Thomas stated that one could not be certain on this point but that his judgment that they were leukemic cells was based on the distribution of the cells in the tissues and their histologic appearance. He believed that they could have been detected on blind diagnosis. A complete description of this case material will be published soon.[1]

Dr. Wintrobe stated that he had some reservations regarding histologic evidence on small numbers of residual "viable" leukemic cells.

(Somewhat later during the discussion the transcriber wrote, "At this point several of the doctors had a discussion, all at once, concerning the result.")

Dr. Goldin presented data showing that cytoxan, over a series of single doses administered 3 days following leukemic cell inoculation, produced a rather high percentage of failures to take. Of a series of drugs, BCNU and cytoxan were the most effective in eradication of L1210 leukemia cells *in vivo*. Methotrexate, when given in a maximum single dose, did not provide "cures" but did increase host life span. This agent was more effective in increasing host life span when dosages were spaced by 4 days. A combination of amethopterin and citrovorum factor (alternate days) allowed a rather high dose of amethopterin and reduced the effective inoculum from 100,000 leukemic cells to approximately 25,000 cells. Good responses of advanced L1210 leukemia were obtained by administration of a single dose of BCNU followed by methotrexate (every 4th day).

[1] Nies, B., Bodey, G. P., Thomas, L. B., Brecher, G., and Freireich, E. J. The Persistence of Extramedullary Leukemic Infiltrates during Bone Marrow Remission of Acute Leukemia. To be published.

Dr. Goldin felt that with the emphasis on short-term, high-level therapy one should not discount the potential of extensive (long-term) treatment. In the model devised by Friedkin and Goldin, a generation time of 1 day was assumed; thus if there were 50% survival or an equivalent delay in generation time the leukemic cell number would remain static under daily treatment, and if greater than 50% of the population were killed each day the population curve would move downward during a long course of chronic therapy. A situation such as this appears to result from continuous treatment of L1210 leukemia with dichloroamethopterin.

Dr. Louis stated that if an optimal dose of cytoxan is exceeded in acute leukemia the response rate goes down. He has noted similar results with chemotherapy of lymphosarcoma. Dr. Louis suggested that the clinical evidence indicates that one must consider the host's reaction to the dose level in determining the drug activity.

Dr. Kaplan, in support of the concept of exponential killing of cancer cells, pointed out that many quantitative data have been obtained with radiation. It has been established that the killing of tumor cells and normal cells (in culture) by radiation also follows exponential kinetics, usually with an initial shoulder. Puck and others have examined very carefully the question whether the predicted tumoricidal dose would reduce an estimated tumor cell population down to very low levels such as mentioned by Dr. Skipper. The predictions are, in fact, in agreement with clinical experience. The $LD_{10}$ for squamous carcinoma *in vitro* is about 300 r, and a single dose of 3000 r (10 $LD_{10}$'s for the culture system) is a pretty good, curative dose level in clinical experience. Dr. Kaplan emphasized the necessity of considering differential kill (neoplastic *versus* normal cells) when attempting to decide on the optimal chemotherapeutic dosage schedule.

Dr. Kaplan further mentioned the current notion that in certain instances radiation may block mitotic progress in cells, "incompletely" killing cells, and that the cells which escape such blockage are in partial synchrony. He wondered whether cells escaping similar blocks induced by chemotherapy might not be unusually sensitive to a second dosage strategically timed in relation to generation time, i.e., the most sensitive stage of the mitotic cycle.

Dr. Kaplan also referred to cases that have long, relapse-free time which do not seem to fit a model of exponential growth. Work by Dr. Mortimer Mendelson of Philadelphia and others suggests that a fraction of many tumor cell populations is in a condition which he has termed "$G_0$." This seems to precede the $G_1$ state. Such cells are dormant in a certain sense until certain conditions reactivate their mitotic activity.

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