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# Combination Cancer Therapy: Presidential Address

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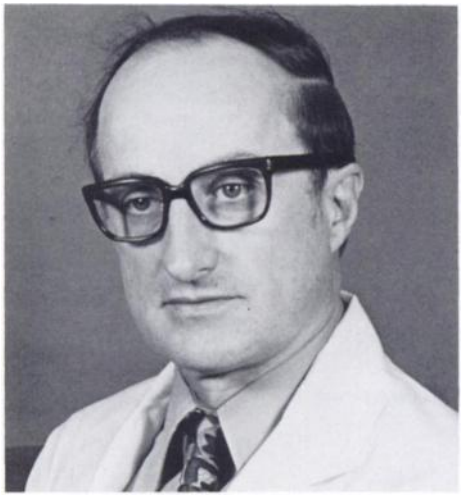

First I want to thank you for the privilege and honor of serving as your President during this past year. It has been a turbulent and an important year for cancer research and accelerated support is in process. I would like to take this opportunity to present some of the conceptual and practical advances that have been made in cancer chemotherapy in the past 15 to 20 years and to outline future projections.

In 1955 I arrived at the National Cancer Institute. The need for the development of systemic treatment for cancer, the encouragement from my superiors, and the opportunity to observe the very dramatic effect that could be achieved occasionally in the leukemias through treatment developed by Dr. S. Farber and Dr. J. H. Burchenal confirmed my interest in medical oncology and chemotherapy.

I have been extraordinarily fortunate in the superiors, associates, and trainees with whom I have been associated. I would like to mention three specifically. When I arrived at the National Cancer Institute, it was Dr. Gordon Zubrod who provided the friendliness, stimulation, guidance, and opportunity to work, and he has continued to do so. Five months after my arrival, a gentleman appeared in my office and announced that his name was Emil Freireich. I allowed that, aside from being somewhat long, this was indeed a very excellent name and that anyone with a name like that had to be good. I offered him a job on the spot, which was easily one of the smartest things I ever did; we have been working, playing, and fighting together ever since. Last but not least I wanted to introduce my wife Elizabeth. Unfortunately, she could not attend because of the urgency of her work on behalf of attaining peace in Indo-China.

Seventeen years ago the chemotherapist was isolated from his fellow clinicians in other disciplines relating to cancer mainly because he had relatively little to offer. He was also isolated from the bridging and from the basic scientists, partially because the field was in its very early stages of development but largely because most scientists, clinical and otherwise, were sceptical concerning the future of cancer chemotherapy.

A schematic diagram of drug treatment categories of disseminated cancer is presented in Chart 1. Our goal is to rid the patient of his neoplastic cells. Using rather crude techniques, such as Chalkly counts, to determine the number of neoplastic cells at autopsy, we found that patients with disseminated neoplastic disease have approximately one trillion or $10^{12}$ cells (8). Similar values have been obtained via more sophisticated techniques in myeloma by dividing the total paraprotein production by the amount of paraprotein produced by individual cells (23). The initial category is called remission induction wherein the goal is to produce complete remission, that is, to eliminate all clinical evidence of the disease. This necessitates at least a 99% reduction in neoplastic cells or, to use a colloquialism, a 2-log or greater reduction in the number of neoplastic cells. Once this is achieved, treatment during remission is initiated. For relatively homogeneous populations of tumor cells, a 1st-order kinetic effect of treatment obtains. This means that it takes just as much treatment to reduce the number of neoplastic cells from a million to a thousand (less than 1 mg of tissue) as it does to reduce the number of neoplastic cells from $10^{12}$ to $10^{9}$ (1 kg of tumor). Thus, the production of complete remission represents only the destruction of the top of the "exponential iceberg," and continued treatment in remission is necessary. As the number of cells are reduced there is cytokinetic and more direct experimental evidence that the slope of the curve with treatment may in fact become more steep, mainly because a larger proportion of the cells enter cycle. There is also experimental evidence that negotiating the final hurdle from almost complete eradication of neoplastic cells to complete eradication may necessitate an altered approach to

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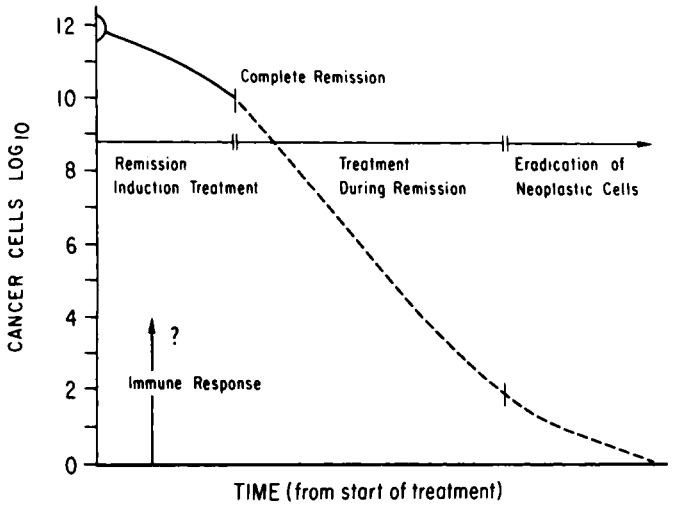


Chart 1. Temporal categories of treatment of cancer.

treatment because of selected $G_0$ cells, drug-resistant cells, pharmacological barriers, and other factors.

We get a variable amount of help from the host immune response which sets the threshold at higher than zero cells. As will be discussed later, it is important to interfere with that immune response as little as possible and indeed to improve it wherever possible.

There is only one human disease wherein the subclinical number of neoplastic cells can be measured with precision; this is choriocarcinoma (17, 22) (Chart 2). This is a semilogarithmic plot of chorionic gonadotropin titers as affected by treatment with methotrexate. The curves are generally consistent with those in Chart 1. During the initial phases of treatment there is, in general, a delay in the rate of decrease, followed by a more rapid reduction, a reverse Gompertzian curve, if you will, and finally a decreased effectiveness of treatment as the patient's titer approaches and falls within the normal range.

Table 1 presents data for childhood acute lymphocytic leukemia as of 1961 and is mainly of historical interest. These observations had major influence on the direction of chemotherapeutic research. With individual or single agents, complete remissions ranging from 21 to 57% were achieved. Combinations of 2 agents produced an additive or synergistic effect and indeed, with today's use of prednisone and vincristine, fully 90% of children with acute lymphocytic leukemia will enter complete remission (8). Contemplation of these data led to 3 major conceptual and practical advances.

The first was that agents with qualitatively different toxicity and different mechanisms of action could usually be combined at full doses with resultant additive or synergistic antitumor effect. This obtains for prednisone and vincristine and for prednisone and 6-mercaptopurine. In contrast, agents with similar dose-limiting toxicity, such as the myelosuppression for methotrexate and 6-mercaptopurine, can be combined safely only by reducing the doses. Because of this a lesser therapeutic effect occurs. In fact, the clinical basis for much if not most of the progress in combination chemotherapy has resulted from this rationale.

The 2nd observation was that appropriate combinations of agents, for this disease at least, might be capable of destroying

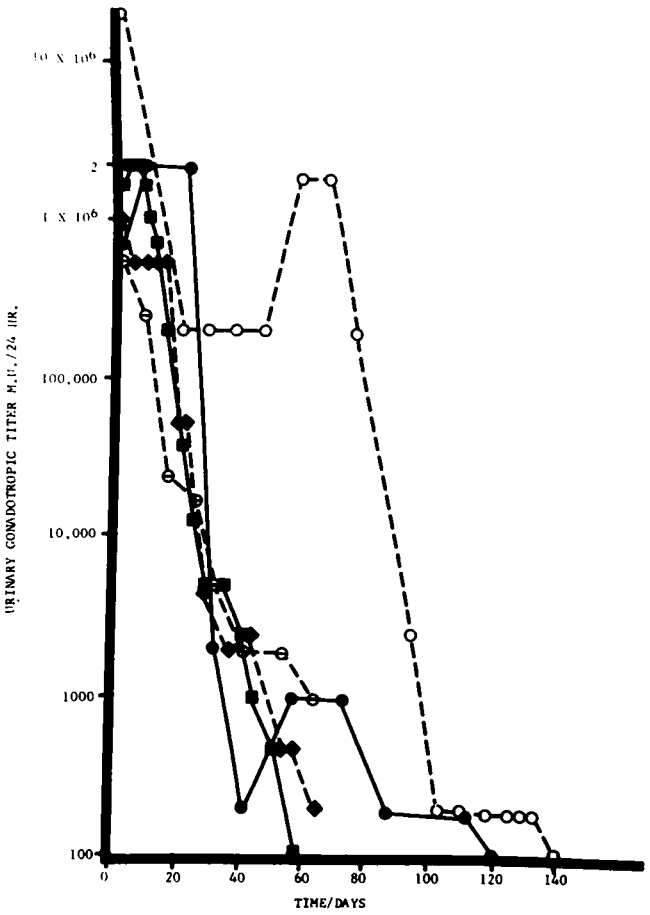


Chart 2. Hormone titers in 5 women with metastatic choriocarcinoma treated with methotrexate. Data are from the papers of Hertz *et al.* (17) and Ross *et al.* (22). *M.U.*, mouse units.

Table 1

*Combination chemotherapy in acute lymphatic leukemia of childhood*

Data are from Leukemia Chemotherapy Group B.

| Agent(s) | No. of patients | Complete remission | | Reference |
|---|---|---|---|---|
| | | No. | % | |
| Mtx[a] | 48 | 10 | 21 | 11 |
| 6-MP | 43 | 12 | 27 | 11 |
| VCR | 81 | 38 | 47 | 27 |
| Pred. | 72 | 41 | 57 | 1 |
| Mtx + 6-MP | 39 | 17 | 45 (42)[b] | 11 |
| Pred. + 6-MP | 154 | 127 | 82 (69) | 26 |
| Pred. + VCR | 63 | 53 | 84 (77) | 28 |

[a] Mtx, methotrexate; 6-MP, 6-mercaptopurine; VCR, vincristine; Pred., prednisone.

[b] Numbers in parentheses, complete remission rate (CRR) calculated assuming independent drug action:

$$CRR_{A+B} = CRR_A + CRR_B\,[(100 - CRR_A)/100]$$

$10^{12}$ leukemia cells and effecting cellular cure. Thus, if each agent producing complete remission effected a 3-log reduction, that is a $10^{-3}$ effect, and 4 such agents could be combined, a $10^{-12}$ effect was theoretically achievable. With these data and concepts in mind, the 1st treatment program, which had as its primary goal the cellular cure of childhood acute lymphocytic

leukemia, was initiated by Freireich *et al.* in 1963 (11) (Table 2). This program involved five 10-day courses of the 4-drug combination referred to as VAMP. This included vincristine, methotrexate, 6-mercaptopurine, and prednisone. The duration of remission following the end of treatment, that is, the duration of unmaintained remission, was used as the parameter of response. It was clear that such treatment provided significantly longer durations of unmaintained remission than single agents. If one assumed a 4-day generation time for the leukemic cells and cellular homogeneity, a very substantial reduction in neoplastic cells was achieved (8). However, these same 4 drugs, with a somewhat modified dose and schedule, the POMP program, given for a total of 14 months as compared to 6 months, resulted in a much longer duration of unmaintained remission (15). This could not be explained on the basis of simple cytokinetic models and focused attention on the need for studies of the cytokinetic, cellular, pharmacological, and other leukemic cell heterogeneity. It also indicated that the longer the duration of treatment, at least up to 14 months, the longer is the duration of unmaintained remission. This has resulted in more prolonged treatment during remission, and the problem of the optimal duration of treatment for patients in complete remission for most neoplastic diseases remains a difficult one. Finally, it has focused attention on the need for altered approaches to chemotherapy and perhaps immunotherapy for the eradication of minimal residual disease.

A 3rd derivative of the fact that 90% of children with acute lymphocytic leukemia could be placed in complete remission was that one could now approach quantitatively the problem of treatment during remission (Chart 3). In a series of studies by Holland, Pinkel, and others, extrapolated from Phase I clinical investigations and experimental therapeutic studies conducted by Skipper, Goldin, Schabel, Bruce, and others, different dose schedules, combinations of agents, the cyclic use of agents, reinduction therapy, and other techniques have progressively prolonged the duration of remission and survival. Each of the curves in Chart 3 represents a study by the Acute Leukemia Group B or St. Jude Hospital and the year the study was started. The number of patients in the study is given in parentheses. Actual 5-year survivals of 20 to 30% have occurred in studies started in 1966 and a realistic 5-year survival estimate for some of the more recent studies is 50% (1, 18). Burchenal, in a retrospective study of 5-year survivors, has found that the risk of recurrent disease and death following the 5th year decreases with time and that 50% of 5-year survivors are alive and in remission at the 11th year. These patients have a minimal risk of subsequent relapse. Making relatively risk-free assumptions, one can multiply the 50% by 50% and find that 25% of patients whose treatment is initiated today will have long-term tumor-free survival. Moreover, there is every reason to believe that the rapid progress that has been made will continue.

These principles derived from studies of acute leukemia have been applied to a number of other neoplastic diseases with notable success in some, such as Hodgkin's disease (Table 3). As in acute leukemia there are a number of agents that individually are effective, but the complete remission rate is only 10 to 20% (20, 21). This was improved with the combination of 3 agents (21) and particularly with the 4-drug combination developed by DeVita *et al.* (5) known as the MOPP program. This combination involves mustargen, vincristine, procarbazine, and prednisone, all of which are active in Hodgkin's disease and only 2 of which have

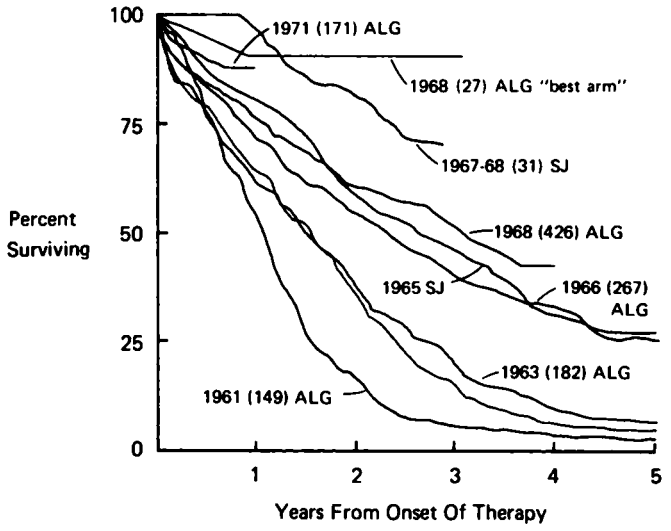


**Chart 3. Acute lymphocytic leukemia in children improving survival. The data are from the Acute Leukemia Group B (*ALG*) and St. Jude Hospital (*SJ*).**

Table 2

*Duration of unmaintained remission in childhood acute lymphocytic leukemia*

| Remission induction treatment | Early remission treatment | Total duration of treatment (mo.) | Duration of unmaintained remission (days) |
|---|---|---|---|
| V[a] | None | 1.5 | 40 |
| P | None | 1.5 | 60 |
| VAMP (VP + Mtx + MP) | | 6 | 140 |
| POMP (VP + Mtx + MP) | | 14 | 240 |
| VP | Mtx | 2.5 | 110 |
| VP | Mtx → MP → Cyt → BCNU | 3.5 | 160 |
| VP | Mtx ⇌ VP | 8.0 | 315 |

[a] V, vincristine; P, prednisone; Mtx, methotrexate; MP, 6-mercaptopurine; Cyt, Cytoxan; BCNU, 1,2-bis(chloroethyl)nitrosourea.

Table 3

*Remission induction in Hodgkin's disease*

All patients had Stage III or IV disease and 76% had Stage IVB disease.

| | No. of patients | Complete remission (%) |
|---|---|---|
| Single agents | | |
| Pred.[a] | | <10 |
| Nitrogen mustard | | <10 |
| Cyclo | 389 | 11 |
| VCR | 23 | 13 |
| VLB | 88 | 20 |
| Procarb. | 48 | 18 |
| Combination chemotherapy | | |
| COP (Cyclo, VCR, Pred.) | 107 | 36 |
| MOPP ($HN_2$, VCR, Procarb., Pred.) | | |
| National Cancer Institute | 43 | 81 |
| Southwest Group | 110 | 72 |

[a] Pred., prednisone; Cyclo, cyclophosphamide; VCR, vincristine; VLB, vinblastine; Procarb., procarbazine.

overlapping dose-limiting toxicity. This combination, in patients with disseminated, largely Stage IVB disease, has produced complete remission rates approaching 80%, a highly significant change from single agents. Chart 4 indicates the importance of achieving a complete remission initially in patients with Hodgkin's disease with respect to the ultimate parameter of response, that is, survival.[2] Of patients who achieve complete remission, 80 to 100% remain alive at over 3 years with slight differences depending upon treatment during remission. For patients who achieved only a partial remission and for patients who did not respond to initial treatment, survival was markedly shortened. This observation concerning Hodgkin's disease can be generalized, almost without exception, for cancer chemotherapy; the production of a complete remission initially markedly improves prognosis. Even more dramatic has been the effect of MOPP treatment on the duration of remission of patients with Hodgkin's disease. Following single-agent treatment and induction of complete remission, the median duration of remission following treatment, *i.e.*, unmaintained remission, is only 40 days. In the initial National Cancer Institute study of MOPP, 10-day courses were given at monthly intervals for 6 courses, following which no further treatment was given. The minimum follow-up time from end of treatment is 5 years, and the median duration of unmaintained remission, or the relapse-free interval, approaches 4 years and has leveled off at approximately 45% from the 4th to the 6th year (Chart 5).

The Southwest Group investigated the importance of the duration of MOPP treatment on the duration of remission and survival. The schematic diagram for this study is presented in Chart 5. Patients were given 6 courses of MOPP treatment at monthly intervals. At this point patients in complete remission were randomly allocated to (*a*) no treatment and (*b*) 9 courses of MOPP at bimonthly intervals. Thus, this latter group of patients would receive a total of 24 months of treatment. This duration was selected on the basis of the rate of decrease in volume of Hodgkin's tumor with MOPP treatment and extrapolating down on a semilogarithmic plot. If one assumed cellular homogeneity, such extrapolation indicated that 12 to 18 months of treatment would be required to produce neoplastic cell eradication. While the follow-up has not been as long, it is important to emphasize that, for the unmaintained patients the curve is comparable to the National Cancer Institute curve. Furthermore, for the maintained patients at 3 years, 68% are in complete remission as compared to 45% for the unmaintained patients ($p = 0.04$).[2]

These very excellent results in childhood leukemia and disseminated Hodgkin's disease have brought into focus the question of whether such treatment is curative in a proportion of patients. The usual definition of cure is that, following treatment and over a decade or 2, there is a cohort of patients whose relative survival is comparable to that of aged matched controls. While this is an acceptable definition, it is a totally impractical one for evaluating the progress of therapy, since it would take a minimum of 10 to 20 years to determine cure rates. Accordingly, the relapse-free interval following radiotherapy of Hodgkin's disease has been analyzed (19). Radiotherapy is curative in a large proportion of patients with localized diseases.

In Table 4, the risk of relapse after radiotherapy is analyzed as a function of the time intervals following treatment (9). Thus, for case accrual dating back to 1932, the risk of subsequent relapse in Peter's series immediately following radiotherapy was 67%, but even at 4 years 32% of patients were destined subsequently to relapse. With improved staging systems and with our ability to identify disease in previously "silent" areas, such as the retroperitoneum, these figures have changed substantially. Thus, for series, wherein case accrual started between 1953 and 1957, the risk of relapse decreases with time. While the numbers are not large, the data strongly suggest that, if a patient remains relapse free 4 years after treatment, he has a less than 10% chance of relapsing subsequently or, conversely, a 90% chance of being cured (9). Thus, the fact that chemotherapy has produced relapse-free intervals at 4 years in over 40% of patients is extremely promising. While there are theoretical reasons for believing that the relapse-free curve following chemotherapy could be qualitatively dissimilar to that following radiotherapy, such has not been the case to date. Thus, observing the relapse-free interval following treatment for a period of several years may provide reasonably precise estimates of the cure rate.

The radiotherapists have derived considerable information concerning the effectiveness of radiotherapy and the nature of the spread of Hodgkin's disease by observing the relapse pattern. In Chart 6, the anatomical distribution of relapse following MOPP chemotherapy in the Southwest Group study is presented. The figure at the left in each box represents the pretreatment distribution of major sites of involvement and the figure at the right shows the sites where relapse first appeared. All of these patients entered complete remission and these 15 relapses occurred from 9 to 48 months later. There is an excellent correlation between the site of initial relapse and the site of major, pre-MOPP treatment, involvement. Indeed, this correlation was exact in 9 and good in 6 of the 15 patients;[2] it clearly indicates that, in contrast to radiotherapy

[2] E. Frei, III, J. K. Luce, J. F. Gamble, C. A. Coltman, Jr., J. Costanzi, R. W. Talley, R. W. Monto, H. E. Wilson, J. S. Hewlett, A. Haut, F. C. Delaney, and E. A. Gehan. Combination Chemotherapy (MOPP) in the Remission Induction and Maintenance of Advanced Hodgkin's Disease, submitted for publication.

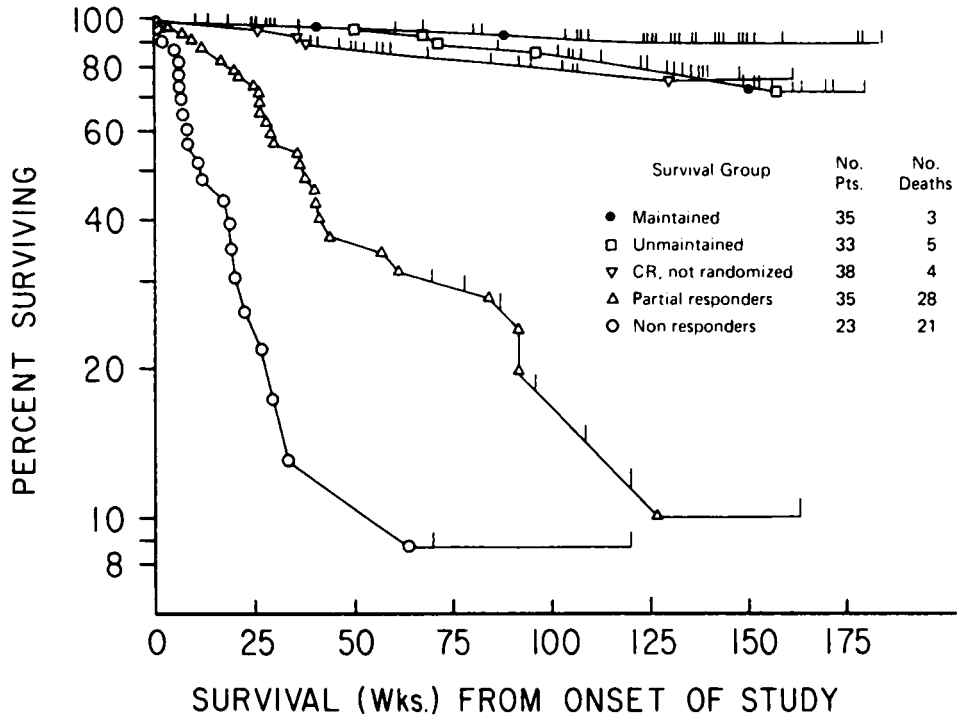


Chart 4. Effect of remission and maintenance treatment on survival. *CR*, complete remission; *Pts.*, patients.

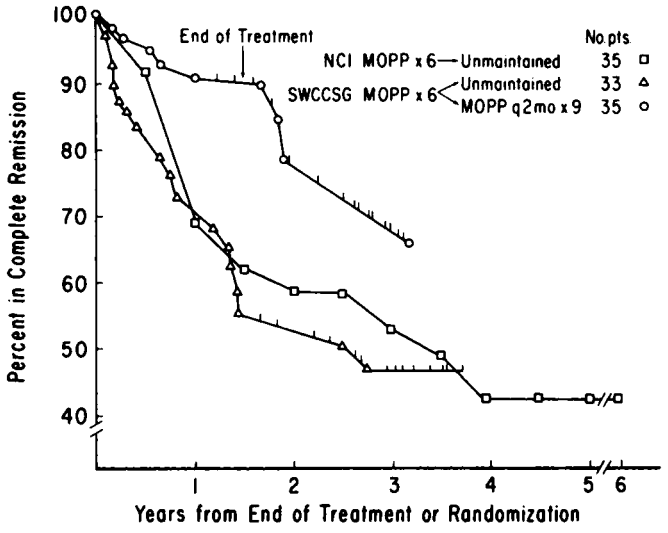


**Chart 5. Effect of the duration of MOPP therapy on the remission of Hodgkin's disease (all Stage III and IV disease; 76% Stage IVB). NCI, National Cancer Institute; *SWCCSG*, Southwest Group; *q 2 mo*, every 2 months.**

where relapses occur in untreated sites, the relapses following combination chemotherapy occur with a high degree of predictability at sites of major pretreatment involvement. Considering the 1st-order kinetic effects of chemotherapy and the vascular, cytokinetic, pharmacological, and other adverse features of large tumors with respect to response to chemotherapy, this correlation is not surprising. It has a major therapeutic implication which is currently under study. Since radiotherapy is capable of sterilizing local Hodgkin's lesions, a major quantitative study has been undertaken by Coltman and the Southwest Group, wherein radiotherapy is delivered to sites of major pretreatment involvement in those patients who have entered complete remission on MOPP therapy.

The marked effectiveness for radiotherapy in patients with localized Hodgkin's disease, and the increasing effectiveness of chemotherapy for patients at the other end of the spectrum, that is, patients with disseminated symptomatic disease, has brought the disciplines of radiotherapy and chemotherapy together. The results of this collaboration are indicated in Chart 7.

In this randomized study Kaplan and Rosenberg compared total nodal radiotherapy to total nodal radiotherapy followed by 6 courses of MOPP chemotherapy in patients with intermediate stages of Hodgkin's disease. This included patients with Stages IIB, IIIA, and IIIB disease. The percentage of relapse-free patients for the 2 treatment programs are plotted from the end of treatment. Even with limited follow-up, the combination, that is the addition of MOPP chemotherapy to total nodal radiotherapy, is significantly superior to radiotherapy alone (4). Thus, it is probable that a 2nd disease has been identified (the 1st one was Wilm's tumor) wherein chemotherapy added to radiotherapy and/or surgery improves the cure rate.

The scientific basis for the construction of therapeutic trials is becoming increasingly broad and relevant. I would like to illustrate a few areas of investigation in this area relating to cytokinetics.

Vincristine arrests cells during metaphase. Thus, following a single dose of vincristine the mitotic index of human tumors increases sharply with the peak at 6 to 12 hr (Chart 8) (10).

*In vitro* cytokinetic studies of the antitumor antibiotic bleomycin are presented in Chart 9. In Chinese hamster cells synchronized in various stages of the mitotic cycle, it was

Table 4
*Risk of relapse after radiotherapy in Hodgkin's disease*

| Author | Stages | Accrual time | No. of patients | Proportion of subsequent relapses at following time interval from treatment (yr) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 0 | 1 | 2 | 3 | 4 | (*)[a] | 5 | 6 | 7 |
| Peters | I and II | 1932–1960 | 365 | 0.67 | 0.54 | 0.42 | 0.36 | 0.32 | (164) | 0.26 | 0.20 | 0.14 |
| Musshoff | All | 1953– | 332 | 0.51 | 0.25 | 0.19 | 0.12 | 0.08 | (200) | 0.04 | 0.04 | 0.03 |
| Kaplan | I and II | 1956–1968 | 145 | 0.50 | 0.36 | 0.22 | 0.08 | 0.05 | (25) | 0 | 0 | 0 |
| Kligerman | I and II | 1957– | 78 | 0.42 | 0.34 | 0.21 | 0.12 | 0.08 | (23) | 0.06 | 0.06 | 0.06 |

[a] (*), no. of patients at risk at the 4th year.

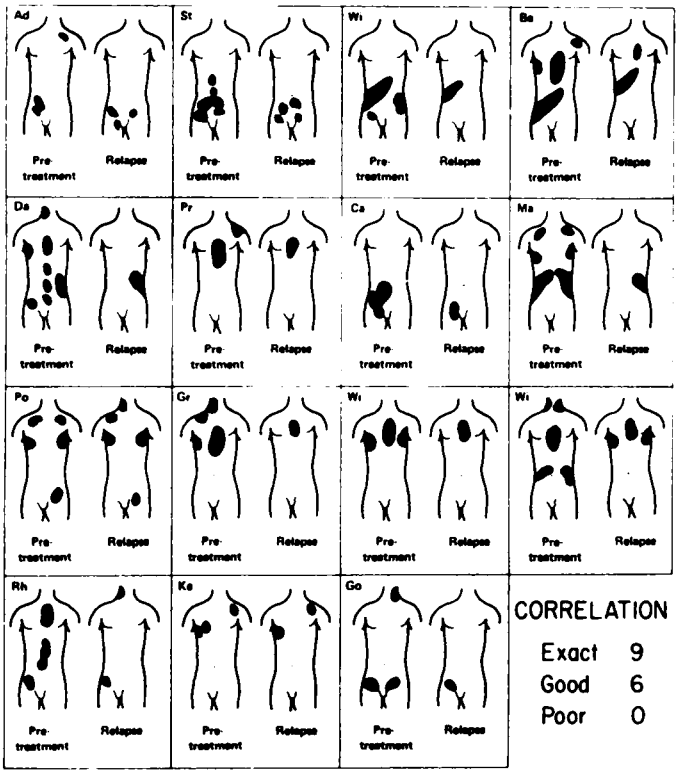


Chart 6. MOPP in Hodgkin's disease. Correlation between treatment and relapse sites of involvement.

found that bleomycin compromised the viability of the cells much more markedly when it was present during the process of mitosis (2). This suggested that, if one produced mitotic arrest with vincristine and if bleomycin were delivered at the appropriate interval following vincristine, a much greater destruction of cells would result. This was demonstrated *in vivo* in the Lewis lung system where vincristine followed by bleomycin was found to be synergistic (F. Schabel, Jr., and H. E. Skipper, unpublished observations). In a clinical study in patients with metastatic lung cancer, a program involving vincristine followed in 6 hr by bleomycin twice weekly has been developed (Table 5). These preliminary results are being extended.

Similarly, 2 agents that are commonly used in combination in man are vincristine and ara-C.[3] In general, little or no attention is paid to the intimate time relationship of the dose schedule. That such is important is indicated in Chart 10 wherein vinblastine, which is a metaphase arrester similar to vincristine, and ara-C, a DNA synthesis inhibitor, are examined in the L1210 mouse leukemia system (27). The antileukemic effect is presented in the ordinate. Vinblastine and ara-C were given at fixed doses every other day alone or in close time sequence. The effect of the agents used alone is indicated. When the 2 drugs are given simultaneously there is an actual reduction in the antileukemic effect. When ara-C is given 16 hr after vinblastine, the maximum effect is achieved. In cytokinetic studies it was demonstrated that vinblastine results in maximum synchrony in the S period by 16 hr. This is the period most sensitive to ara-C. Clearly, such information is important in the development of dose schedules for combination chemotherapy in man.

One of the most important problems in chemotherapy has been the selection of new agents and treatment programs for clinical trial. While the transplanted tumor systems, particularly the L1210 mouse leukemia, are effective and useful, they are dissimilar to their clinical counterpart in a variety of ways. This may compromise the correlation between the experimental and clinical systems and thus reduce the predictability value. While a great deal of work has occurred in this area, I would like to emphasize particularly the spontaneous AKR mouse leukemia system shown in Table 6 (24, 25). Like the human disease, acute myelogenous leukemia, it is spontaneous in contrast to the transplanted systems. It is diagnosed by palpation of the spleen and like human leukemia is widely disseminated at the time of diagnosis. Cytokinetically, it has a long leukemia cell-doubling time which corresponds to the clinical disease. This is related, in considerable part, to the fact that the growth fraction in both systems is relatively low in contrast to the L1210. The recovery time for the normal bone marrow in the mouse is 3 to 4 days following cell cycle-specific agent administration in contrast to man in whom it is 9 to 15 days. After a series of studies, Schabel and Skipper have observed that the best treatment programs with cell cycle-specific agents such as ara-C for both the L1210 and the AKR consists of 24- to 48-hr courses of continuous treatment followed by interruption between courses sufficient to allow for complete bone marrow recovery (25). Translated cytokinetically to man, this would represent roughly 5-day courses of continuous ara-C every 14 days. In noncomparative studies such interrupted courses of treatment are superior to various types of daily treatment, for both complete remission induction and maintenance and for survival (Table 7) (Ref. 7; J. Bickers and E. J Freireich, manuscript in preparation). The spontaneous AKR system has also been used to evaluate combination chemotherapy (Chart

[3] The abbreviation used is: ara-C, 1-β-D-arabinofuranosylcytosine.

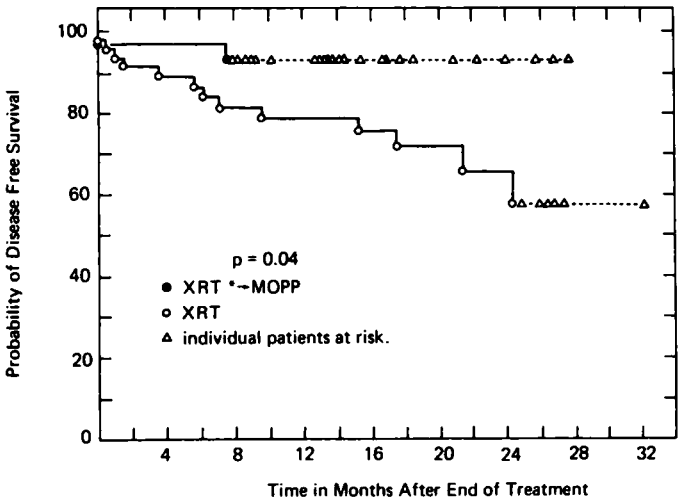


Chart 7. Effect of combined radiotherapy and chemotherapy on Stages IIB, IIIA, and IIIB of Hodgkin's disease. *XRT*, total nodal radiotherapy.

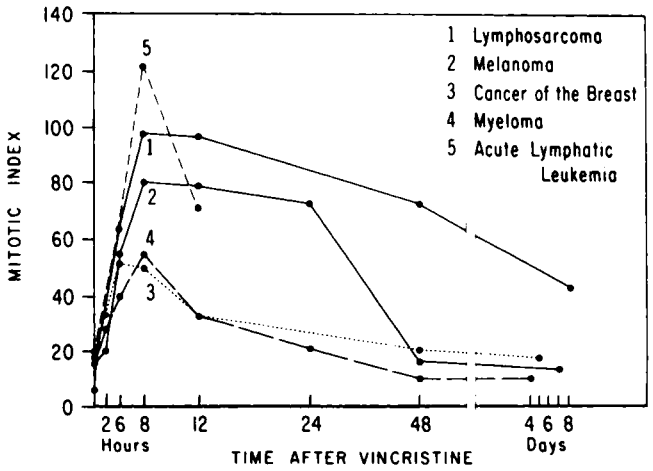


Chart 8. The effect of vincristine on the mitotic index in the bone marrow. *Mitotic index*, no. of mitoses/1000 cells.

11). The best combination with appropriate attention to schedule has been cyclophosphamide combined with ara-C and also with vincristine and prednisone. The survival curve of untreated (control) mice with spontaneous AKR leukemia is described. For combined treatment the survival curve levels off at about the 50% survival level and, by bioassay, no tumor cells could be demonstrated in the thymus in 50% of these animals following treatment (H. E. Skipper and F. J. Schabel, Jr., unpublished observations). After 4 weeks, mortality from leukemia again appears, probably because of background reinduction by Gross virus. That cellular cure in all probability can be achieved by chemotherapy in this spontaneous tumor system is of signal importance. These animals have 100 million leukemic cells at the time of treatment, and it is reliably estimated that a greater than 1,000-fold destruction of normal stem cells would be fatal. Thus, this chemotherapy was capable of delivering a 100,000-fold selectivity against the neoplastic cells. When this combination was subjected to a randomized study by Bodey and the Southwest Group and compared directly to ara-C, it was highly effective and was significantly superior in terms of the complete remission rate, the duration of complete remission, and survival (Table 8). Additional studies by Goldin and Schabel indicate that the spontaneous AKR leukemia generally responds to agents that are effective in the leukemias and lymphomas in man. Thus, the development of this system represents major progress in the critical area of selecting new agents and new treatment programs for clinical trial.

As already indicated, there is evidence, both in experimental and in clinical tumor systems, that intensive short-term courses of treatment are superior to continuous treatment. In man this would appear to be particularly true in Burkitt's lymphoma, choriocarcinoma, myeloma, Hodgkin's disease, and adult acute leukemia. Perhaps one of the most compelling arguments in favor of intermittent treatment relates to chemical immunosuppression. As our treatment becomes more effective in terms of remission induction and as we move to more long-term treatment, the relative effects of various treatment programs on suppression of immune response will become increasingly important.

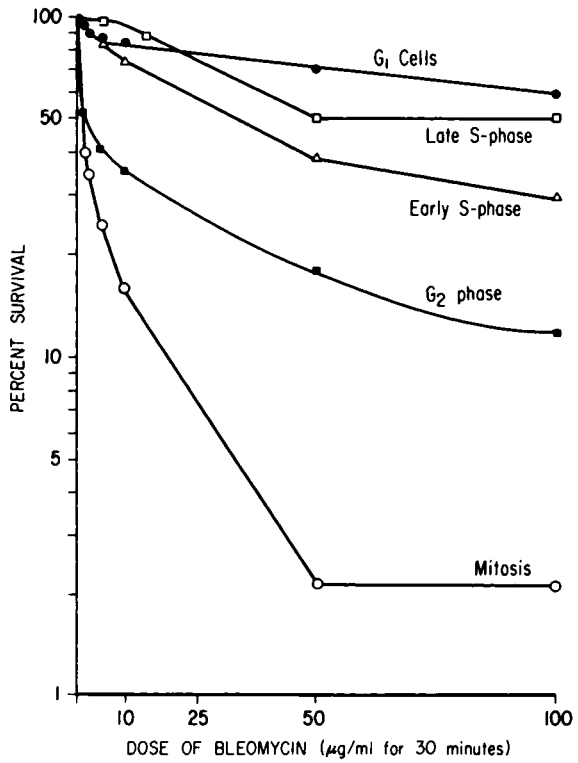


Chart 9. Effect of bleomycin on survival of mammalian cells synchronized in various stages of the cell cycle.

Table 5

*VCR[a]-Bleo sequence for lung cancer*

| | No. of cases | Partial response | |
|---|---|---|---|
| | | No. | % |
| Bleo | 77 | 2 | 3 |
| VCR → Bleo[b] | 13 | 4 | 31 |

[a] VCR, vincristine; Bleo, bleomycin.
[b] VCR followed in 6 hr by Bleo twice a week.

Hersh has studied immunosuppression in patients receiving 5-day courses of intensive chemotherapy (Chart 12). The immunological parameters evaluated included macrophage entry into experimental inflammatory sites (the skin window), response to primary antigenic stimulation, and lymphocyte blastogenesis to phytohemagglutinin (3). There is a marked decrease in all of these parameters while the patient is on treatment. However, within 2 to 3 days following such treatment there is complete or nearly complete recovery in immunological response and indeed, in many patients, immunological "overshoot" occurs during the recovery period. Thus, when such 5-day courses of treatment are given every 2 to 4 weeks, the patient's immunological apparatus is normal most of the time. This contrasts to what is seen in continuous treatment wherein a lesser initial degree of immunological depression occurs but is sustained and tends to be progressive. Indeed, there is evidence that intermittent chemotherapy has a greater effect on circulating antibody response as compared to continuous treatment, which has a relatively greater effect on cellular immunity. These data are presented in Charts 13 and 14 wherein kidney transplant patients receiving continuous daily chemotherapy are compared to patients with cancer receiving intermittent chemotherapy. In Chart 13 the humoral antibody response to keyhole limpet hemocyanin is substantially impaired as compared to controls in both groups of patients, but more so in those receiving intermittent chemotherapy. In contrast (Chart 14) lymphocyte blastogenesic response to keyhole limpet hemocyanin, a measure of cellular immunity, is markedly impaired in patients receiving continuous chemotherapy as compared to those receiving intermittent chemotherapy. While the many differences between the 2 groups of patients is recognized, the discordance in the cellular and humoral immune response as a function of the schedule of chemotherapy is of note. Since cellular, *i.e.*, T-cell, immunity is the major tumor-restraining facet of immune response and since humoral immunity may be "enhancing or blocking," intermittent chemotherapy not only provides a lesser overall immunosuppression but tends to preserve cellular immunity.

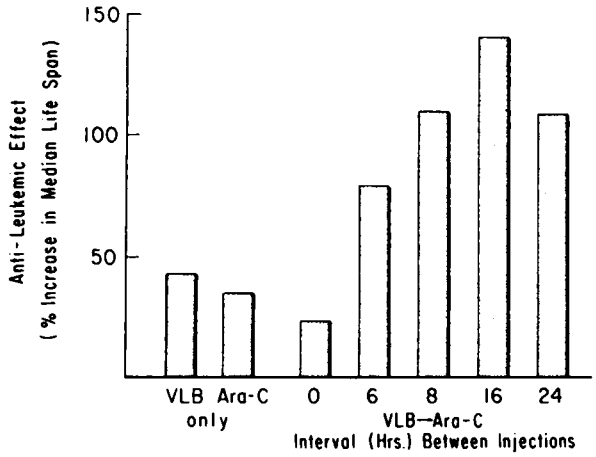


Chart 10. Schedule of vinblastine (*VLB*) and ara-C combination in L1210 cells. Vinblastine (1 mg/kg) and ara-C (20 mg/kg) given every 2 days.

There is ample evidence in experimental systems that the preservation and indeed the augmentation of immunity and particularly cellular immunity is important to the antitumor effect of chemotherapy. There is also compelling evidence in man that response to chemotherapy is highly correlated with a patient's immune response prior to treatment. This is illustrated in Table 9 in which the response of adults with acute leukemia to established skin test antigens and the blastogenic response to phytohemagglutinin prior to treatment and at the time of recovery following the 1st course of treatment was evaluated (16). For these patients who had a remission as a result of chemotherapy, there was a significantly superior cellular immunity as compared to those patients who did not respond; this difference was particularly true after recovery from the 1st course of chemical immunosuppression. Thus, those patients destined to have a remission (major

Table 7

*Effect of dose schedule on ara-C in acute myelogenous leukemia*

Separate studies.

| Schedule | No. of patients | Complete remission (%) | Duration Remission median mo. | Survival 50% | Survival 25% |
|---|---|---|---|---|---|
| Daily | 98 | 8–25 | 7 | 2.5 | 8 |
| 5-day infusions every 2–3 wk | 85 | 38 | 12 | 2 | 14 |

Table 6

*Spontaneous AKR mouse leukemia model*

| Tumor | Origin | Status at time of initial treatment | Doubling time (days) | $T_C$ (days) | $T_S$ (hr) | Growth fraction | Normal marrow recovery (days)[a] | Best schedule[b] for cell cycle-specific agent |
|---|---|---|---|---|---|---|---|---|
| L1210 | Transplanted | Variable | 0.5 | 0.5 | 0.9 | 1 | 3 | 24+-hr courses every 4 days |
| Spontaneous AKR | "Spontaneous" Gross virus tolerant | Disseminated $10^8$ cells | >5 | 1.0 | 11 | 0.5 | 3–4 | 24+-hr courses every 4 days |
| Acute myelogenous leukemia | "Spontaneous" | Disseminated $10^{12}$ cells | >5 | 2–4 | 20 | 0.2–0.4 | 9–15 | 5-day courses every 14 days |

[a] After suppression with cell cycle-specific agents.

[b] Best schedule for cell cycle-specific agent consisted of courses of treatment for at least twice the $T_C$ with treatment-free internal between courses to allow for normal marrow recovery.

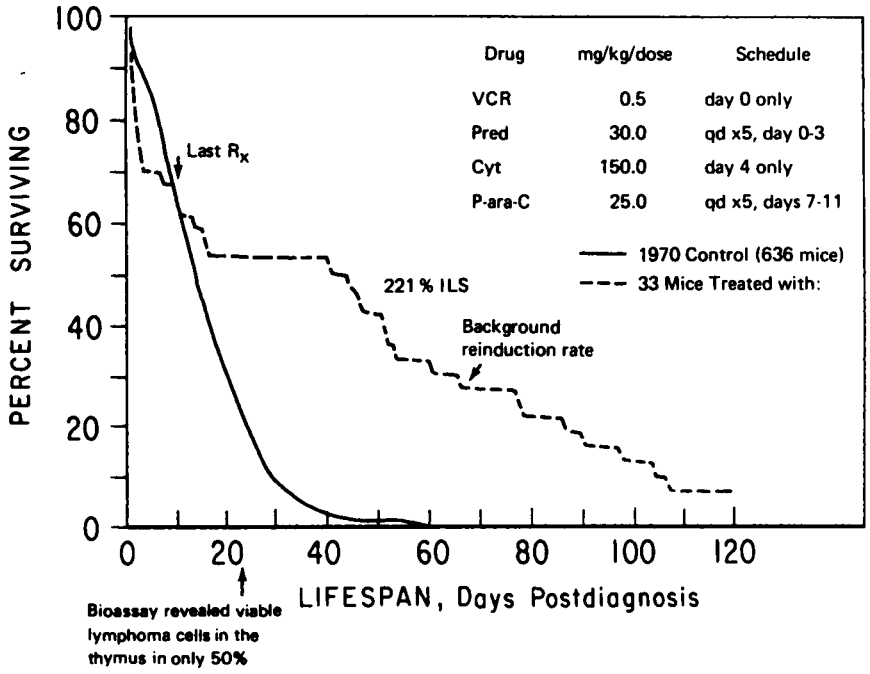


Chart 11. Bifunction survival of AKR lymphoma after "curative" chemotherapy. *ILS*, increased life-span.

Table 8
*ara-C vs. COAP[a] in adult acute leukemia*
Randomized comparative study.

| Treatment[a] | No. of patients | Complete remission | Duration of remission median (mo.) | Survival 50% | Survival 25% |
|---|---|---|---|---|---|
| ara-C | 57 | 23% | 9 | 2 | 13 |
| COAP[b] | 64 | 50% | 14 | 8 | 25 |
| | | $p < 0.01$ | $p = 0.05$ | $p < 0.01$ | |

[a] COAP, *C*ytoxan, *O*ncovin (vincristine), *a*ra-C, and *P*rednisone.
[b] Both treatment programs given as 5-day courses every 2 to 3 weeks.

response) recovered completely whereas recovery was faulty or lacking in the patients destined not to respond to chemotherapy. In summary, proper attention to the scheduling of chemotherapeutic agents, with emphasis on intensive short-term courses of intermittent therapy, tends to preserve immune response and, particularly, cellular immune response. As active immunotherapeutic programs are combined with chemotherapy, the active immunization can be administered at the time of immunological recovery or "overshoot." Finally, there is evidence that esoteric infections such as fungus, viral, and *Pneumocystis* infections are less frequent in patients maintained on long-term intermittent chemotherapy than in those on continuous chemotherapy.

The discussion up to this point has dealt with hematological cancers. I would like to turn now to selected solid tumors. Some of the cytokinetic, pharmacological, vascular, and other features of solid tumors as compared to the leukemias that might adversely affect response have been emphasized. I would like to indicate another difference which I don't believe has been sufficiently recognized (Table 10). In the acute

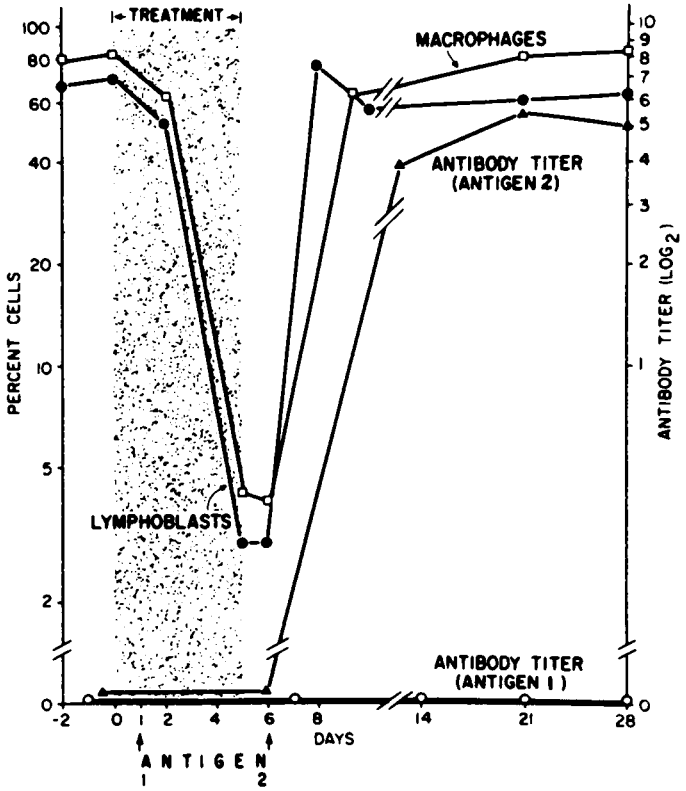


Chart 12. Effect of chemotherapy on immune response.

leukemias, while cytogenetic abnormalities occur in the leukemic cells in about 50% of patients, these usually represent a single clone, are pseudodiploid or near diploid, and, most important, usually remain stable throughout the disease (14). For these patients, responses are frequent and, with appropriate treatment, prolonged. Similarly, in the early

phase of chronic myelogenous leukemia where aneuploidy or pseudodiploidy is slight, response to treatment is excellent. In the blast phase, however, aneuploidy, which may be unstable, is common and response to treatment is poor (26). While

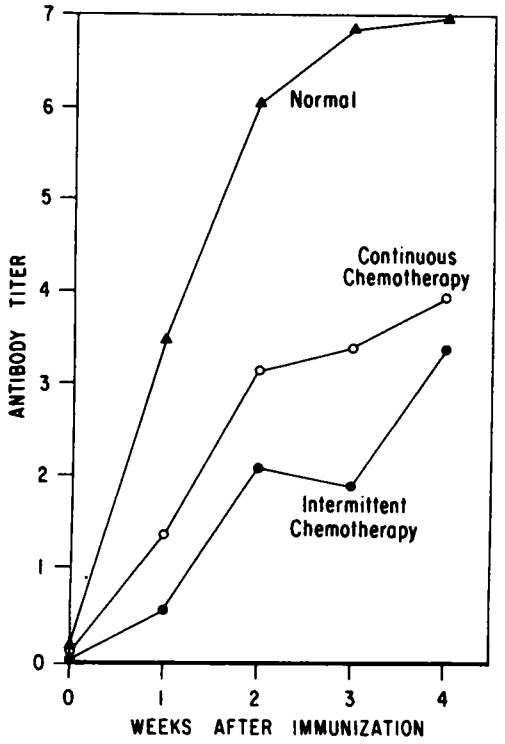


Chart 13. Total antibody response of various groups to keyhole limpet hemocyanin.

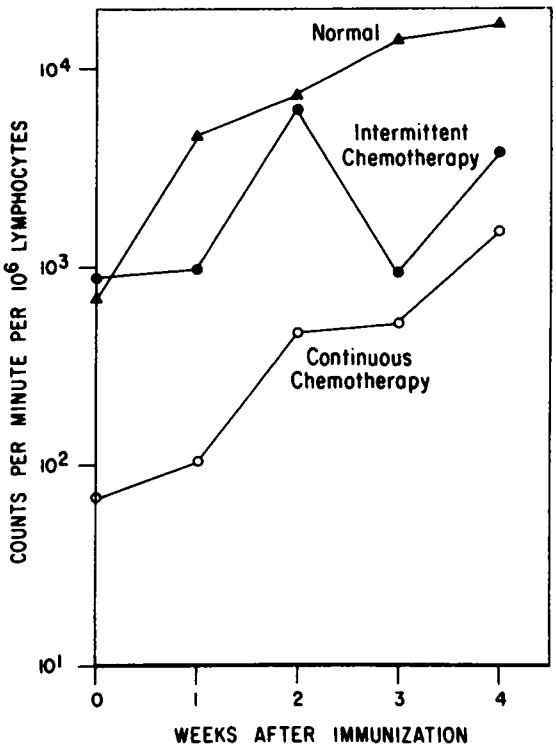


Chart 14. Lymphocyte response of various groups to keyhole limpet hemocyanin.

Table 9

*Effect of various types of chemotherapy on the delayed hypersensitivity response to keyhole limpet hemocyanin*

| | Keyhole limpet hemocyanin skin test response | | |
|---|---|---|---|
| Group | Positive/tested | % positive | Diameter of positive reactions |
| Continuous therapy | 2/16 | 12.5 | 7.5 |
| Intermittent therapy | 16/20 | 80.0 | 8.5 |
| Control | 13/14 | 92.8 | 9.5 |

metastatic solid tumors in general have been studied insufficiently, the existing data suggest that they are substantially different from the leukemias. Thus for metastatic melanoma, marked aneuploidy with hyperdiploid up through hypertetraploid lines are common, stem cell lines are multiple, and clonal evolution (*i.e.*, instability) is very common (28). This heterogeneity should be theoretically ideal for the development or selection of drug resistance.

The effects of this cytogenetic heterogeneity of human melanoma metastases on chemotherapy are indicated in Charts 15 and 16. Four clones of melanoma cells were isolated from the same metastasis, and their responsiveness *in vitro* to ara-C and to 1,2-bis(chloroethyl)nitrosourea was determined. For ara-C, under the conditions of the experiment, the clones varied in responsiveness from a 0 to 50% reduction in the survival fraction (Chart 15). A similar or even greater variation was seen for 1,2-bis(chloroethyl)nitrosourea (Chart 16; S. Barranco and R. Humphrey, unpublished observations). In view of this it is not surprising that ara-C is not particularly effective in the treatment of metastatic melanoma. Thirteen patients with metastatic melanoma to the skin were treated with ara-C and are ranked in order of response (Table 11). The pretreatment tumor size is expressed as 100%. There was only 1 really good response (Patient V. M.) which lasted for 7 months. Two marginal partial responses occurred which lasted only 2 months. With the marked variability in chemotherapeutic response of clones within a given metastasis, one would expect the rapid selection of the resistant clones upon treatment with ara-C. ara-C is activated by the enzyme deoxycytidine kinase (K) to the nucleotide, at which level it inhibits DNA polymerase and is substantially inactivated by deoxycytidine (or ara-C) deaminase (D). It was postulated that, for a given tumor, the ratio of these 2 enzymes should determine the activation and the tissue susceptibility to ara-C. This proved to be the case. The K/D ratio was much higher in responding patients (Table 11) ($p = 0.04$) (13). These results have 2 implications. The first is that, with modern techniques, it should become increasingly possible to determine in advance *in vivo* susceptibility to chemotherapeutic agents. The isolation of different clones from a given tumor may further assist in this goal. Most particularly, this heterogeneity constitutes a strong argument for a multifaceted approach to metastatic disease including combination chemotherapy and immunotherapy.

In spite of the above limitations and problems, intense investigation of the chemotherapy of various solid tumors is underway and progress is being made. In the past, predictive or screening systems have emphasized transplanted leukemias and lymphomas. This has, at least in part, contributed to the fact

Table 10
*Cytogenetic abnormalities and response to chemotherapy*

| | Cytogenetic abnormalities | | | | |
|---|---|---|---|---|---|
| | Aneuploidy | | | | |
| | Frequency (%) | Severity | Stability | Stem lines | Response to chemotherapy |
| Acute leukemias | 50 | Slight | Stable | 1 | Frequent and prolonged |
| Chronic myelocytic leukemia | | | | | |
| Early phase | 80 | pH[1] | Stable | 1 | Frequent and prolonged |
| Late (blast) phase | 80+ | Slight | Unstable | Several | Poor |
| Metastatic solid tumors (melanoma) | 90+ | Major[a] | Unstable | Multiple | Poor |

[a] Hyperdiploid, triploid, tetraploid, and hypertetraploid cell lines are common.

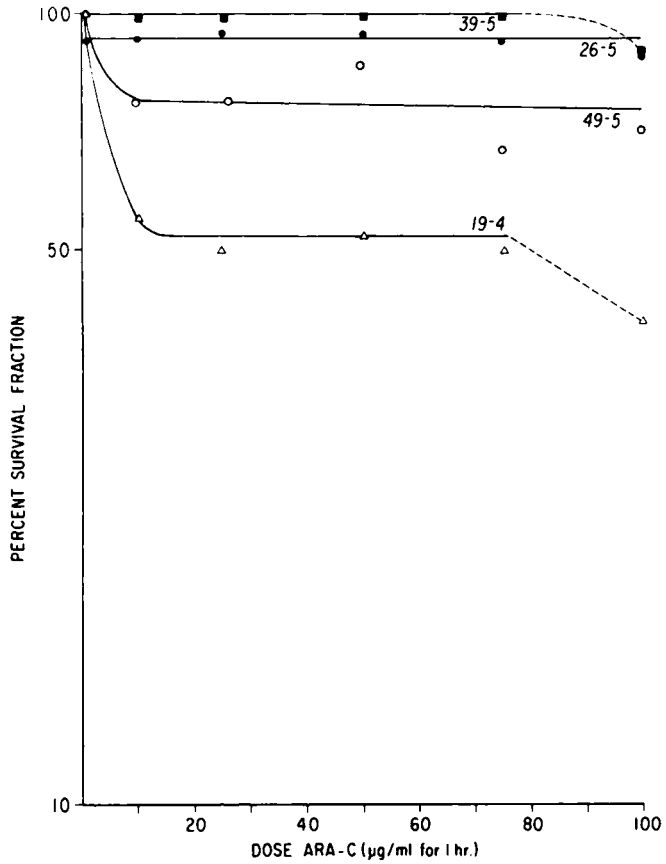


Chart 15. Effect of ara-C on 4 strains of human malignant melanoma growing asynchronously *in vitro*.

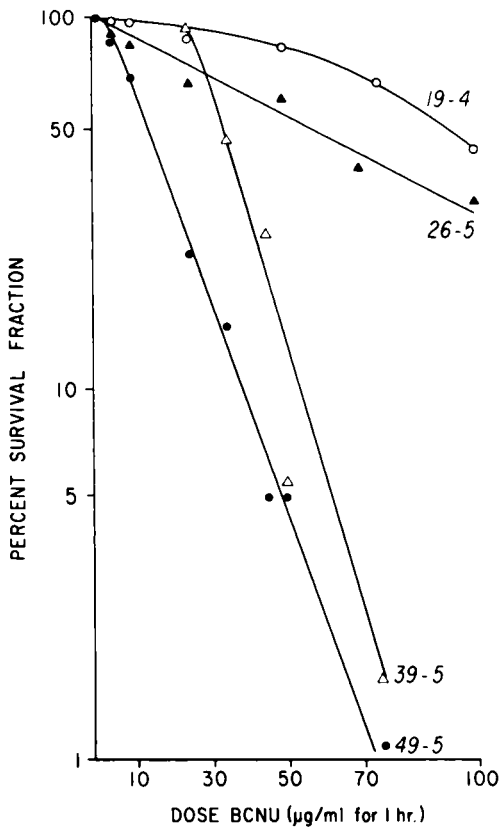


Chart 16. Effect of 1,2-bis(chloroethyl)nitrosourea (*BCNU*) on 4 strains of human malignant melanoma growing asynchronously *in vitro*.

that agents selected for clinical trial have tended to be most active in the hematological cancers. Increasing emphasis is being given to experimental solid tumor models such as those shown in Table 12. These data, largely from the Southern Research Institute, indicated the limited activity of agents that tend to be effective in the hematological cancers and that are, for the most part, cell cycle specific. However, some of the new agents such as bleomycin, the anthracyclines, the triazenoimidazoles, and particularly the nitrosoureas alone or in combination with cyclophosphamide have marked antitumor activity. These studies are currently being translated to man with positive results in some instances, as we shall see shortly. I would predict that major progress will soon be seen for breast cancer. There are at least 6 classes of chemotherapeutic agents capable of producing objective

Table 11

*Correlation of cellular pharmacology and response for ara-C in metastatic melanoma*

| Response | Patient | No. of patients | Reduction of tumor size (%)[a] | Enzyme assays (nmoles product/g tissue 1 hr) ara-C kinase (K) | ara-C deaminase (D) | (K/D) × 100 |
|---|---|---|---|---|---|---|
| Response | V. M. | 12 | 5 | 506 | 278 | 182 |
| | E. D. | 3 | 50 | 34 | 74 | 46 |
| | D. H. | 3 | 50 | 329 | 648 | 51 |
| Failure adequate trial | G. J. | 4 | 100 | 53 | 450 | 12 |
| | E. H. | 2 | 100 | 76 | 1240 | 6 |
| | W. M. | 2 | 120 | 11 | 122 | 9 |
| | J. T. | 3 | 150 | 72 | 1129 | 6 |
| | J. L. | 3 | 150 | 33 | 244 | 14 |
| | G. J. | 3 | 150 | 50 | 187 | 27 |
| | D. C. | 2 | 150 | 3 | 1059 | 0.3 |
| | L. M. | 1 | 150 | 0 | 402 | 0 |
| | G. S. | 1 | 150 | 232 | 419 | 55 |
| | L. F. | 3 | 200 | 0 | 296 | 0 |

[a] Pretreatment size, 100%.

Table 12

*Activity of various agents against a spectrum of solid tumors*

| Agent | Sarcoma 180 Tumor wt. T/C (%)[a] | Sarcoma 180 Cures | Ca 755 Tumor wt. T/C (%) | Ca 755 Cures | C3H (% ILS) | B16 melanoma (% ILS) | Lewis lung Early T/C (%) | Lewis lung Established rating |
|---|---|---|---|---|---|---|---|---|
| Methotrexate | 32 | 0/6 | 27 | 0/10 | 0 | 0 | 65 | – |
| 6-Mercaptopurine | 14 | 0/6 | 0 | 9/10 | 0 | 23 | 52 | – |
| 5-Fluorouracil | 3 | 3/6 | 13 | 0/10 | 9 | 0 | 51 | – |
| ara-C | 62 | 0/8 | 8 | 0/10 | 31 | ? | 87 | – |
| Hydroxyurea | 69 | 0/10 | 6 | 0/10 | 27 | | | |
| Vincristine | 46 | 0/10 | | | | | 63 | |
| Prednisone | | | | | | | | |
| Daunomycin | | | | | 0 | 18 | 56 | – |
| Actinomycin D | 13 | 0/7 | 0 | | 11 | 64 | 55 | |
| Bleomycin | | | | | | | 6 | |
| Nitrogen mustard (HN2) | 55 | 1/10 | 20 | 0/10 | | 22 | 65 | + |
| DL-Sarcolysin | 7 | 0/6 | 4 | 2/10 | 0 | 50 | 32 | |
| Cytoxan | 0 | 18/20 | 0 | 2/20 | 47 | 75–100 | 0 | – |
| 1,2-Bis(chloroethyl)nitrosourea | 10 | 0/6 | 13 | 1/10 | 10 | 47 | | – |
| 1-(2-Chloroethyl)-3-cyclohexyl-1-nitrosourea | | | | | 0 | | 0 | + |
| Methylcyclohexylurea | 0 | 18/20 | 5 | 0/10 | 16 | 75–100 | 0 | +++ |
| Methylcyclohexylurea + Cytoxan | 0 | 14/20 | 0 | 0/10 | 155 | >100 | | +++ |

[a] T/C, tumor size in treated animal/tumor size in control animal; ILS, increase in life-span of treated animals/control animals.

response. Several of these, such as the nitrosoureas and adriamycin, have been identified in the above screens only within the past several years. This situation resembles that for Hodgkin's disease 7 years ago and for childhood leukemia 11 years ago. The appropriate application of clinical investigation and basic science techniques and extrapolations, as well as the development of further new agents, will almost certainly result in improved control of metastatic breast disease. While there is conclusive evidence that combinations will increase the remission rate, the overall effect of this on the disease remains to be determined.

An example of recent progress in a tumor category that was almost totally unresponsive to chemotherapy just 3 years ago is indicated in Table 13. Soft tissue and bone sarcomas have been highly resistant to chemotherapy. This table represents data from the Southwest Group. Soft tissue and bone sarcomas have a 15% objective response rate to the triazenoimidazole and a 31% response rate to the new antibiotic, adriamycin. In experimental tumors this combination was found to be synergistic and to be tolerated in combination at full doses without an increase in dose-limiting toxicity. This was found also to be true for the combination in man with a resultant response rate of 41% and a median duration of response of over 5 months (12).

It is apparent that a great deal of progress has been made and is continuing in the chemotherapy of cancer. However, new and more effective drugs are needed. Perhaps the rate of progress in chemotherapy is best illustrated by the increasing rate with which clinically effective antitumor agents are being discovered (Chart 17). The 23 clinically effective drugs are

Table 13
*Soft tissue and bone sarcomas*

| Treatment | No. of patients | Response (%) | No. of complete remissions | Duration of response median (mo.) |
|---|---|---|---|---|
| Diethyltriazenoimidazole carboximide[a] | 55 | 15 | 1 | |
| Adriamycin[a] | 108 | 31 | 1 | |
| Diethyltriazenoimidazole carboximide + adriamycin[b] | 100 | 41 | 5 | >5 |

[a] Collected series.
[b] Ref. 12.

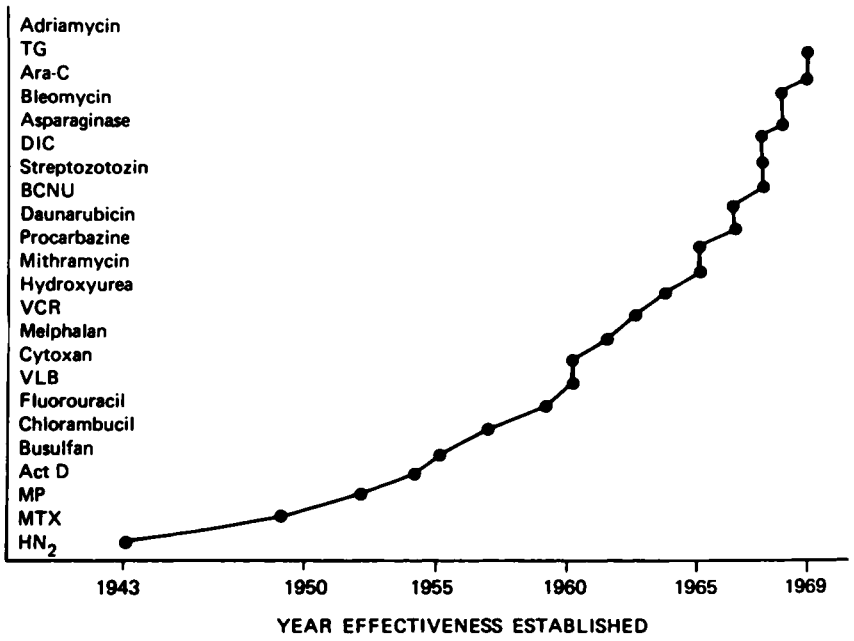


Chart 17. Rate of discovery of clinically effective antitumor agents exclusive of hormones. *TgG*, thioguanine; *DIC*, dimethyltriazenoimidazole carboxamide; *BCNU*, 1,2-bis(chloroethyl)nitrosourea; *VCR*, vincristine; *VLB*, vinblastine; *Act D*, actinomycin D; *MP*, 6-mercaptopurine; *MTX*, methotrexate.

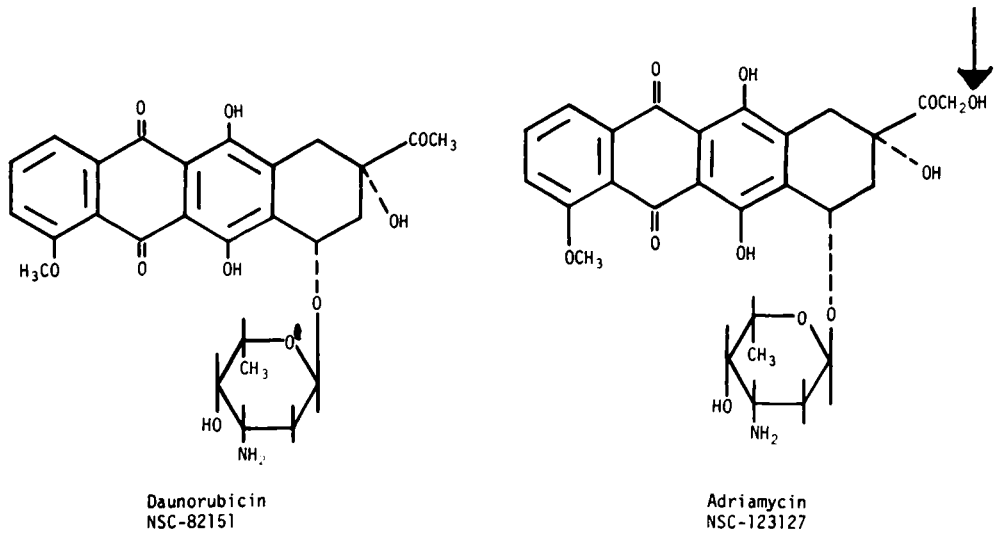


Chart 18. The anthracycline antibiotics.

Table 14
*Comparative antitumor effects of anthracycline antibiotics*

| | Daunorubicin | Adriamycin |
|---|---|---|
| Experimental antitumor activity | | |
| Ehrlich carcinoma | 2+ | 4+ |
| Spontaneous mammary carcinoma | 0–1+ | 3+ |
| L1210 | 0 | 1–2+ |
| Sarcoma 180 | 1+ | 2–3+ |
| Human antitumor activity | | |
| Lymphoma | 20 (87)[a] | 64 (44)[a] |
| Acute myelogenous leukemia | 37 (469) | 27 (51) |
| Acute lymphatic leukemia | 27 (388) | 43 (121) |
| Breast carcinoma | 12 (29) | 43 (23) |
| Sarcomas | 13 (46) | 31 (108) |
| Bladder carcinoma | | 37 (19) |

[a] % response (no. of patients). Collected series.

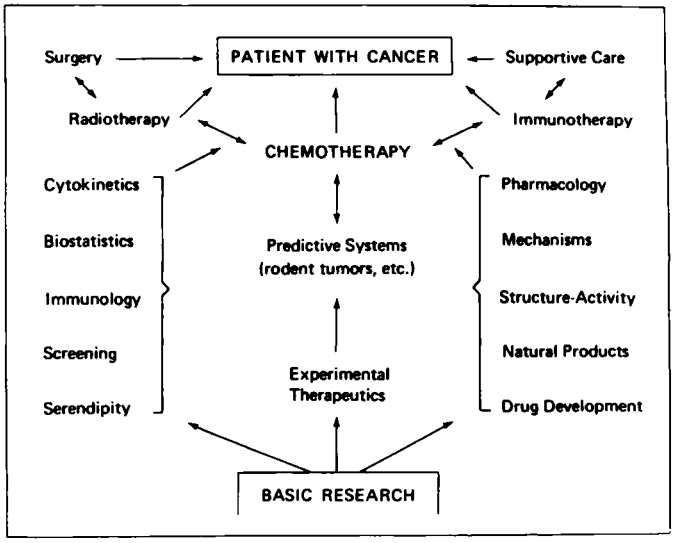


Chart 19. Chemotherapy. Relation to clinical and basic disciplines.

listed in ascending order on the Y-axis, and the year in which their clinical effectiveness was established is on the X-axis. While most of these represent new structures, a few congeners were selected only when they had unique clinical antitumor effects. The rate of progress increased during the 1950's and accelerated through the 1960's. I stopped the analysis after 1970 since the effectiveness of recently introduced agents is often controversial. However, there are currently 13 new agents in clinical trial. These data tend to underestimate the rate of progress. For example, while the folate antagonists were listed only once, that is, when Farber identified the effectiveness of aminopterin in acute leukemia, the curative value of methotrexate for choriocarcinoma was not discovered until more than 10 years later and certainly represents a separate and highly important advance. So it is with many of the drugs for which indications have been extended to other neoplastic diseases, to combinations, etc.

The revolutionary advances in chemistry and molecular biology have made it possible to exploit these observations with respect to new drugs with much greater rapidity and effectiveness. The anthracycline antibiotics illustrate this point (Chart 18).

Daunorubicin was the first anthracycline discovered and several years thereafter, adriamycin, which differs only in a hydroxyl group in position 14 was discovered (6). This slight difference in structure has resulted in a better therapeutic index in *in vivo* experimental systems including leukemias and solid tumors (Table 14). While the relative effects of the 2 anthracyclines in human leukemia has not been definitively determined, adriamycin is highly active in lymphomas, breast cancer, and sarcomas in man, in contrast to daunorubicin, and in preliminary studies has activity in other solid tumors as well (Table 14). The intercalation of the anthracyclines with DNA is under definitive study, and further modifications of the molecule based on the empirical, chemical; and biochemical rationale are underway.

To summarize I would like to refer to Chart 19. Chemotherapy is central here only because it was the subject of this presentation. It is the interaction of both the clinical disciplines and the basic disciplines that has been, and increasingly will be, responsible for progress in cancer control. In marked contrast to the picture 17 years ago the medical oncologist today is directly and extensively involved in the integration of diagnosis and patient care, particularly chemotherapy and supportive care, and increasingly in immunology and in immunotherapy. In order to apply the best of patient care the 5 clinical disciplines must appreciate each other's contributions, and their close interaction in the initial and long-term management of the patient must be implemented.

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