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## *Introduction*

Great strides have been made using chemotherapy to treat patients with cancer; great distances remain to be covered. The use of methotrexate to treat patients with choriocarcinoma demonstrated the important principle, in humans, that systemic chemotherapy could be used to destroy the neoplasm yet spare the patient (1). With the development of new chemotherapeutic agents, new strategies for their administration and combination, and more effective approaches to supportive care, it has become possible to cure patients with acute lymphoblastic leukemia (ALL),[1] Hodgkin's disease, diffuse histiocytic lymphoma, Burkitt's lymphoma, testicular cancer, Wilms' tumor, embryonal rhabdomyosarcoma, and Ewing's sarcoma (2, 3). Chemotherapy can also be used to achieve significant tumor regression and improve survival in patients with breast, gastric, prostatic, ovarian, and small cell lung cancers as well as for those with acute and chronic myelogenous leukemia and soft tissue sarcomas (2, 3).

## *Established strategies and principles*

Further experience provided discomforting evidence that in patients with ALL, the blood–brain barrier made the central nervous system a unique sanctuary where leukemic or other tumor cells could escape the effects of systemic chemotherapy. The ultimate result was central nervous system relapse in some patients who otherwise seemed to be in systemic remission (7). The development of multimodality therapy using cranial irradiation combined with intrathecal and systemic chemotherapy was therefore important in the treatment and prophylaxis of meningeal leukemia and in improving the long-term remission and cure rate of ALL (8, 9). This multimodality approach of combining chemotherapy in varying strategic sequences with surgery, radiation therapy, hormonal therapy, and more recently with immunotherapy, has increased the rate of long-term remissions and of apparent cures in several diseases. For example, many children with Wilms' tumor can be cured with a multimodality approach that combines surgical excision of the tumor, radiation therapy, and systemic chemotherapy with actinomycin D and vincristine (10).

Another approach to multimodality therapy is the use of adjuvant chemotherapy to treat undetectable and unrecognizable

tumor size so that the tumor can be dealt with more efficiently

by surgery and/or radiation therapy. Recent studies in patients with head and neck cancer show that *cis*-Platinum and fluorouracil, when administered simultaneously with radiation therapy, can be used in this fashion to reduce tumor size and permit more conservative, less disfiguring surgery and improved long-term, disease-free intervals (18).

### *Strategies for combination chemotherapy*

A major advance in cancer therapy, which contributed to all of the successes outlined above, occurred with development of the concept of combination chemotherapy—the use of multiple agents to produce additive or synergistic antitumor effects without compounding toxicity (19, 20). Successful combination chemotherapy regimens have been developed on the basis of empiric considerations, which take into account principles of pharmacology, biochemistry, and cell cycle and tumor kinetics. One of the most successful strategies for combination chemotherapy has been to combine agents that have different mechanisms of action and different toxicities. The first successful application of this approach was the treatment of acute lymphoblastic leukemia with the VAMP regimen, that is, vincristine, a mitotic tubule inhibitor with neurotoxicity; amethopterin, a folic acid antagonist with mucous membrane toxicity; mercaptopurine, an inhibitor of de novo purine nucleotide synthesis with bone marrow toxicity; and prednisone, a lympholytic corticosteroid whose use is complicated by aspects of Cushing's syndrome. Each of these agents used alone induced remissions in a small fraction of patients with ALL, whereas the VAMP combination produced apparently complete remissions in 80–90% of patients (4). Subsequent examples of successful combination chemotherapy regimens include mechlorethamine, oncovin, prednisone, and procarbazine (MOPP), and the noncross-resistant adriamycin, bleomycin, vinblastine, and dacarbazine (ABVD) for Hodgkin's disease (21, 22), cyclophosphamide, adriamycin, and fluorouracil (CAF) for breast cancer (23), fluorouracil, adriamycin, and mitomycin (FAM) for gastric cancer (24), cyclophosphamide, hydroxydaunomycin, oncovin, and prednisone (CHOP) for histiocytic lymphoma (25), and platinum, vinblastine, and bleomycin (PVB) for testicular cancer (26). An important principle that was learned in the development of these combinations was that agents that showed some activity against a particular tumor when given alone, usually contributed to a higher response rate when given in combination (27). Conversely, drugs that were not active as single agents rarely improved the response rate when given as part of a combination.

In the cell kinetic approach to combination chemotherapy, agents are combined at different time intervals to kill cells as they pass through different phases of the cell cycle (28, 29). Sometimes the first agent is given to synchronize cell division such that a greater percentage of tumor cells are in a particularly sensitive phase of the cell cycle at the time that the second agent is administered. Depending on their scheduling, cell cycle agents can produce antagonistic or synergistic antitumor effects (28, 29). While the efficacy of this approach has been clearly demonstrated in model systems (28, 29), it has not been extensively applied in humans. One successful example of its application, however, is the use of cytosine arabinoside and 6-thioguanine at 12-h intervals for the treatment of acute myelocytic leukemia (30, 31).

Chemotherapy based on tumor kinetics is useful in some patients who harbor large tumor masses in which only a small proportion of the cells appear to be proliferating. The first chemotherapeutic agent is selected to kill a high proportion of tumor cells and stimulate the remaining tumor cells to enter a proliferative phase. A second, phase-specific agent is then administered to kill proliferating cells. This approach has been successfully used to combine adriamycin and cytosine arabinoside in the treatment of acute myelogenous leukemia (32).

Biochemical strategies for combination chemotherapy include the use of multiple agents to provide sequential blockade of a single pathway, concurrent inhibition of alternate or complementary pathways, and the use of one drug to enhance the activity or block the degradation of a second agent. For example, phosphono-*N*-acetyl-L-aspartic acid (PALA), a potent inhibitor of aspartate transcarbamylase, has been used to inhibit de novo pyrimidine synthesis and to expand the cellular pool of phosphoribosyl pyrophosphate in order to increase the activation of fluorouracil to fluorouridine monophosphate (33). Similarly, thymidine administration has been used to potentiate the activity of fluorouracil, presumably by increasing its incorporation into RNA (34). These and other agents have been combined to produce synergistic results in experimental systems, but they have not shown clinically useful results. Thus, it was reported in a recent study that the effect of fluorouracil alone in patients with colon cancer could not be significantly potentiated by combined treatment with thymidine, with phosphono-*N*-acetyl-L-aspartic acid, with levamisole, or with the combination of methyl chloroethyl cyclohexyl nitrosourea, vincristine, and streptozotocin (35).

The failure to achieve synergy in clinical studies may result from the relative lack of effectiveness of some of these modulators as single agents. Although these drugs are now used as biochemical modulators, it still seems prudent to adhere to the clinical observation that agents must show activity when given alone in order to contribute to the synergistic efficacy of a combination chemotherapy regimen (27). It is also possible that the agents whose biochemistry has been modulated have reached a plateau in their effects and cannot have their activity significantly enhanced in the target tumors. The lack of success with these regimens may also be associated with failure to achieve the desired biochemical endpoints. This is due, in part, to difficulties in measuring tumor metabolites. Consequently, drugs are frequently scheduled on an arbitrary basis without regard to their pharmacokinetics or to the extent of their metabolic effects. Thus, the future of biochemical modulation as an approach to chemotherapy may well depend on the development of more effective means of detecting and monitoring the metabolites in question. Perhaps, new techniques such as positron emission tomography, nuclear magnetic resonance, and stereotactic biopsy combined with microchemical analysis will provide us with the required monitoring tools.

### *Strategies for high dose chemotherapy*

A very important basis for success with chemotherapy was elucidation of the principle that chemotherapeutic agents act by first order kinetics, that is, there is a linear relation between the dose of the chemotherapeutic agent and the fraction of cells killed (36, 37). Thus, a given dose of a chemotherapeutic agent will kill a constant fraction of a cell population. Thus, if a tumor is sensitive to a particular agent, higher doses will eradicate a larger fraction of the tumor cell population. This principle also indicates that it should be easier to eradicate a tumor when it is present in low rather than high cell number. Higher doses may also circumvent the problem of tumor cell heterogeneity in which different cells in the population have variable levels of sensitivity or resistance to particular agents (38). High dose therapy can

also be expected to be more toxic to normal tissues and several strategies have been developed to maximize its benefit while sparing normal tissues. One approach to achieving high dose therapy is to provide the highest concentration of chemotherapeutic agent to the tumor-bearing region (39). Thus, hepatic artery infusion, limb perfusion, and intraperitoneal chemotherapy have been used to provide high local concentrations of chemotherapeutic agents. These approaches produce some degree of control of regional disease without severe systemic toxicity (39–42).

Another important strategy for the ultimate development of curative chemotherapy, which derives from the principle of first order kinetics in tumor cell kill, is the use of high dose systemic chemotherapy in combination with autologous bone marrow infusion to rescue the patient from the myelotoxic effects of the chemotherapy (43, 44). In this approach, the patient's bone marrow is harvested and stored while the patient is treated with high dose chemotherapy to produce maximal tumor reduction. The major toxic effect of this approach is ablation of the regenerative capacity of the bone marrow. When the systemic level of chemotherapeutic agent is reduced below a toxic level, the stored bone marrow is reinfused, which rescues the patient from toxic myelosuppression or the ablative effect of the chemotherapy. Using single agents and single courses of therapy, this approach has already been shown to produce marked tumor regression as well as some long-term remissions and apparent cures (45, 46). Some patients with otherwise refractory lymphomas, treated by this approach, have achieved complete remissions and survivals in excess of two years (46). As research continues on the efficacy and toxicities of high dose single agents, more regimens will be designed using high doses of agents in new combinations. These are likely to produce increased success rates similar to those achieved by the application of combination chemotherapy principles at more usual pharmacological doses.

Since high dose chemotherapy with autologous marrow rescue is experimental and very rigorous, it is reserved by many physicians for use as salvage therapy. The severity of the therapy can be effectively managed, however, by experienced teams that are knowledgeable in the use of blood components, antibiotics, and other aspects of supportive care. The principle that tumor cells are killed by first order kinetics also indicates that high dose chemotherapy should be most effective as an early regimen when tumor cell mass is small and more likely to be eradicated by exposure to the high doses. This aggressive approach should now be evaluated in the adjuvant setting for some tumors with very high recurrence rates. Thus, high dose chemotherapy should be evaluated to determine if it will prevent recurrences in patients with malignant melanoma who, after surgical resection of the tumor primary and positive regional lymph nodes, appear to be free of disease, but who almost certainly harbor undetectable tumor cells. High dose chemotherapy should also be investigated as an early regimen for treatment of recurrent breast cancer. Strong support for this approach comes from the recent demonstration that an aggressive, initial approach using high dose combination chemotherapy for small cell lung cancer increases the number of patients who achieve complete remissions. Some of the patients went on to disease-free survivals in excess of four years (47).

### *New strategies from molecular biology and biotechnology*

The discovery of oncogenes and the elucidation of their function in the regulation of growth should certainly provide us with new targets such as tyrosine–protein kinases and guanosine 5′-triphosphate (GTP) binding proteins for attack by cancer chemotherapeutic agents (48–50). Surely, these targets will have susceptible counterparts involved in normal cell function, and it will be the task of the molecular biologist and clinical chemotherapists to develop strategies to exploit the differences in the oncogene activities in normal and neoplastic cells. While immunotherapy is not the subject of this review, monoclonal antibodies specific for tumor surface antigens should be useful to target chemotherapeutic agents. Thus, cytotoxic drugs could be selectively delivered to a tumor by conjugation directly to a tumor-specific antibody, or such antibodies could be incorporated into the surface of drug-loaded liposomes.

### *New strategies for modulating DNA repair pathways*

While DNA is the principal target of most alkylating agents, their antitumor effects can be drastically affected by cellular levels of glutathione and other nonprotein thiols. Glutathione functions as an intracellular reductant that is important for protection against oxidative damage initiated by radiation or chemotherapeutic agents (51, 52). It also forms thioether complexes and inactivates many chemotherapeutic agents (51, 52). Buthionine sulfoximine inhibits gamma glutamylcysteine synthesis, which results in the depletion of cellular glutathione levels and increased sensitivity to such chemotherapeutic agents as melphalan, bleomycin, mechlorethamine, and nitrosoureas (51–55). The increased sensitivity to chemotherapeutic agents produced by glutathione depletion can be used as a strategy for designing new approaches to combination chemotherapy and overcoming drug resistance (54, 55). Agents affecting thiol biochemistry are also being investigated for their ability to sensitize or protect tissues during radiation exposure (51).

Since the mechanism of action of many chemotherapeutic agents involves the production of DNA damage, elucidation of the type of damage and the DNA repair pathways involved in their restitution should provide new opportunities for biochemical modulation. For example, the first step in the pathway for 1,3-bis-(2 chloroethyl)1-nitrosourea (BCNU) to produce DNA cross-links involves the formation of an adduct at the $O^6$ position of a guanine residue in DNA (56, 57). The enzyme $O^6$-alkylguanine–DNA alkyltransferase rapidly and efficiently repairs $O^6$-alkylguanine adducts (58, 59). The enzyme functions in a stoichiometric fashion that covalently and irreversibly transfers the alkyl moiety from the nucleic acid base to the enzyme protein, which results in self-inactivation and leaves an intact guanine residue in the DNA (59, 60). Cells with high levels of alkyl transferase remain resistant to the cytotoxic effects of nitrosoureas until a sufficient number of $O^6$-alkylguanine adducts are formed to inactivate and deplete the enzyme protein. In contrast, cells deficient in alkyltransferase are significantly more sensitive to nitrosoureas compared with proficient cells (59, 61, 62). Thus, a new strategy for combination chemotherapy is to convert alkyltransferase-proficient cells to -deficient cells, rendering them more sensitive to nitrosoureas. This can be accomplished with the modified nucleoside, $O^6$-methylguanine, which is taken up by cells and acts as a methyl donor to irreversibly inactivate the alkyltransferase (63). $O^6$-Methylguanine has been shown in tissue culture to sensitize tumor cells to the toxic effects of alkylating agents (64). This approach will serve as another new strategy for developing clinical trials.

### *New strategies focused on the mechanism of cell death*

We are developing a strategy for combination chemotherapy focused on enhancing the mechanisms by which chemothera-

peutic agents kill cells. This, of course, requires a knowledge of the biochemistry of cell death—an area in which there is surprisingly little information. For example, we know that radiation causes DNA strand breaks (65), methotrexate inhibits dihydrofolate reductase (66), cytosine arabinoside inhibits DNA polymerase (67), vincristine binds microtubules (68), and *cis*-Platinum cross-links DNA (69). We also know that steroid cytotoxicity requires binding to a steroid receptor (70), and VP-16 toxicity correlates with formation of protein cross-linked–DNA strand breaks, presumably involving topoisomerase II (71). But how do any of these agents cause cell death? Why should inhibition of DNA polymerase cause cell death when many resting, intermitotic cells get along quite well with low or absent levels of this enzyme (72)? Why should DNA strand breaks cause cell death when the red blood cell functions quite well without any DNA? The answer most frequently provided for these mechanisms of cell death is "unbalanced cell growth." Still, the biochemical pathways of unbalanced cell growth have not been defined.

Studies in our laboratory indicate that one mechanism of cell death is mediated by activation of the chromatin-bound enzyme poly(ADP-ribose) polymerase and the consequent depletion of cellular energy metabolites (73, 74). This enzyme is activated by DNA strand breaks to cleave NAD at the glycosylic bond between the nicotinamide and adenosine diphosphoribose moieties (75–77). The latter moieties are joined by the same enzyme into linear or branched chain polymers of ADP-ribose (75). Poly(ADP-ribose) polymerase activity is proportional to the number and duration of DNA strand breaks (76, 77), and we have shown that alkylating agent-induced DNA damage can sufficiently activate the enzyme to deplete cellular NAD pools in 30–60 min (73). Consumption of NAD pools results in loss of ability to synthesize ATP, with consequent depletion of ATP and loss of all energy-dependent functions. The result is inability to phosphorylate and use glucose, loss of the ability to conduct DNA, RNA, or protein synthesis, and an inability to maintain membrane integrity (73, 74, 78). The consequence is cell death. This mechanism may represent a final common pathway of cell death that is initiated by many diverse agents. Activation of poly(ADP-ribose) polymerase and depletion of NAD pools has recently been shown to account for the T cell toxicity occurring in adenosine deaminase deficiency, and contributes to the cytotoxic effects of deoxycoformycin (79). This pathway also accounts for some of the cytotoxicity induced by active oxygen species that damages nuclear DNA (80). A consequence of methotrexate treatment is decreased thymidine synthesis (81). As a result, deoxyuridine is incorporated into DNA, and is subsequently recognized and excised by uracil-*N*-glycosylase and an apyrimidinic endonuclease, bringing about DNA strand breaks (82). Thus, while the primary site of methotrexate activity is inhibition of dihydrofolate reductase (66), its mechanism of cell killing may well be the induction of DNA strand breaks, activation of poly(ADP-ribose) polymerase, and consequent depletion of energy metabolites. Further studies are clearly needed to define the final pathway of cell death exerted by many other chemotherapeutic agents.

The importance of defining a mechanism of cell death is that it provides a new focus for combination chemotherapy. For example, in the pathway outlined above, the use of agents to inhibit synthesis of NAD or other metabolites should serve to enhance chemotherapeutic efficacy. We have recently shown that one of the metabolic effects of tiazofurin is to inhibit NAD synthesis, and this compound significantly potentiates the cytotoxic effects of BCNU (83). Another agent that interferes with NAD synthesis, 6-aminonicotinamide (84, 85), can be combined with tiazofurin and BCNU to mediate even further synergistic antitumor effects in tissue culture and in vivo. This modulation of a biochemical pathway of cell death may provide an important new strategic approach to cancer chemotherapy. An interesting aspect of this mechanism of cell death is that depletion of ATP can be monitored in vivo using phosphorous nuclear magnetic resonance (86). It may, therefore, become possible to directly monitor tumor metabolite concentrations to determine optimal dose and scheduling for combination chemotherapy agents. Other common pathways leading to cell death must also exist and need to be explored.

Great strides have been made using chemotherapy to treat patients with cancer; great distances remain to be covered. New strategies and new agents will surely provide us with seven-league boots to span the distance.

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