METHODS IN CANCER RESEARCH

Edited by

HARRIS BUSCH

VOLUME X

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Preface

The advancing frontier of cancer research has posed many important problems involved in diagnosis, therapy, and evaluation of causal factors in human tumors. In the continuing evolution of important methods dealing with human and experimental tumors, few are more important than the evaluation of chemotherapy. Some of the critical parameters are discussed in Chapter I. In addition, critical factors involved in immune response to tumors are dealt with in Chapter II. Enzyme cytochemistry is discussed in Chapter III, and some of the problems in dealing with α-fetoproteins are presented in Chapter IV. A variety of endocrine tumors are of both clinical and laboratory importance. These topics are reviewed in detail in a series of chapters that deal with tumors of the brain, thyroid, mammary gland, pituitary, and prostate.

The Editor is greatly indebted to the many excellent colleagues who have participated in the development of the ten volumes of this series of *Methods in Cancer Research*. It is their hope and mine that these volumes will continue to offer a platform of current methodology from which important advances in all aspects of oncology can continue to evolve.

HARRIS BUSCH

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CHEMOTHERAPY

CHAPTER I

CLINICAL PARAMETERS OF COMBINATION CHEMOTHERAPY*

JOSEPH H. BURCHENAL

I. Introduction

The parameters for the clinical evaluation of combination chemotherapy are much the same as the evaluation of combination chemotherapy in mouse leukemia. In the latter, it is generally agreed that, for a combination to have real value, it must produce a greater increase in survival time than the maximum tolerated dose of either drug alone, and it should also hopefully be able to produce a higher percentage of long-term survivors or cures than either drug alone at any dose.

In discussing combination chemotherapy, one assumption that is often made is that two drugs with clinical activity against a given tumor, but with diverse limiting toxicities, are generally more likely to be useful in combination therapy than those with similar toxicities.

Another important classification of drugs, in addition to their toxicities, is their effect on proliferating and nonproliferating cells, since in any given stage of the development of a tumor, there will be some cells in the active cell cycle and some cells resting in a prolonged G_1 or G_0 phase. Since some agents act only in the phase of DNA synthesis and are thus completely cell-cycle dependent, the growth fraction of a tumor assumes great importance in determing which type of drug will be useful. Thus, there might be advantages in combining drugs to kill both resting and proliferating tumor cells. In Burkitt's tumor, where the cell generation time is short and almost 100% of the cells are in cycle (Cooper *et al.*, 1966; Ziegler *et al.*, 1972), agents that are cell-cycle dependent would be more likely to be useful. A similar situation probably obtains in acute lymphoblastic leukemia in early remission, where

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maintenance therapy with cycle-active drugs is very effective. On the other hand, in some of the slower growing tumors, such as carcinoma of the colon and carcinoma of the breast, it is probable that combinations of non-cycle-dependent drugs would be more effective, at least until the tumor mass had markedly decreased, at which time the addition of a cell cycle-specific agent might kill those previously dormant cells that had been recruited to cycle activity. This type of theoretical recruitment leading eventually to total cell kill was shown in idealized form by Schabel (1969) and is reproduced in Fig. 1.

One must always bear in mind, however, that the essential normal cells, such as bone marrow and intestinal epithelium, are also made up of a mixture

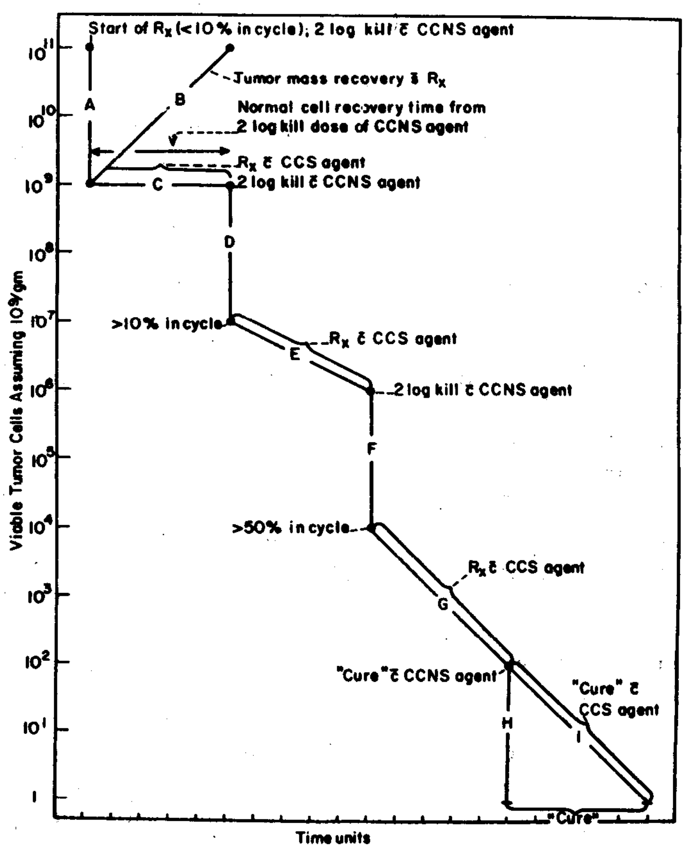

FIG. 1. Idealized approach to curative therapy of advanced tumors using a cell cycle-nonspecific (CCNS) agent (e.g., alkylating agent) followed by a cell cycle-specific (CCS) (e.g., antimetabolite) in repeated courses. (From Schabel, 1969.)

of proliferating and resting cells. For this reason, it is important to search for intermittent intensive schedules that will inflict the maximum damage on the tumor cells while allowing the vital normal cells to recover before the second course is begun. A typical schedule with arabinosyl cytosine (ara-C), which has an extremely short half-life, in mouse leukemia calls for administration of the drug in relatively large doses (15 mg/kg) every 3 hours for eight doses, then allowing a 72-hour rest period for the normal cells to recover. Such intensive intermittent treatments with ara-C repeated every 4 days for four separate courses allows complete destruction of the transplanted leukemic cell population while doing very little or no cumulative damage to the normal bone marrow or intestinal epithelium of the host (Skipper *et al.*, 1967). Similar schedules seem to be optimal with hydroxyurea, guanazole, and 5-hydroxypicolinaldehyde thiosemicarbazone (Skipper *et al.*, 1970), and long ago Goldin *et al.* (1956) showed that methotrexate had a better therapeutic index in relatively early leukemia when given in large doses every fourth day instead of daily. The latter has been confirmed in the clinic in short intensive courses used successfully in the treatment of uterine choriocarcinoma (Li *et al.*, 1956) and Burkitt's tumor (Oettgen *et al.*, 1963).

II. Clinical Results

With these ideas in mind, an attempt will be made to classify the various agents which have shown clinical activity according to their primary limiting toxicity, their cell-cycle specificity, and their areas of clinical activity (Table I). This grouping of drugs into marrow-depressant and non-marrow-depressant toxicities would then suggest that one or more non-marrow-depressant drugs might well combine beneficially with a marrow-depressant drug with no serious additive toxicity and yet with summation of therapeutic effects on the tumor. If, in addition, at least one drug of the combination is active against resting cells (in G_1 or G_0) while another affects cells in DNA synthesis, the combination might be expected to be even more effective. Indeed, this has been found to be true since the combination of prednisone and mercaptopurine induced a considerably higher percentage of remissions in acute lymphoblastic leukemia than either member of the combination alone (Frei *et al.*, 1965).

Similarly, the studies of Pratt *et al.* (1972) have shown that vincristine and Cytoxan together, with or without the addition of actinomycin D, as adjuvant therapy for Ewing's tumor after intensive local irradiation to the primary lesion are giving results superior to those previously obtained with either drug alone. They reported 5 of 15 patients as showing no evidence of disease (NED) at 21–91 months, and another 5 NED at 4–19 months. There have been only five recurrences in this series of 15 patients.

TABLE I
CLASSIFICATION OF CHEMOTHERAPEUTIC AGENTS ACCORDING TO CELL CYCLE SPECIFICITY AND PRIMARY TOXICITY

Compound	Primary toxicity and special features	Cell cycle dependence	Refs.[a]	Clinical areas of activity
Actinomycin D	Bone marrow Skin	Yes, DNA synthesis	(1)	Carcinoma Sarcoma
Adriamycin	Bone marrow Mucosal ulcerations	DNA synthesis	(2,3)	Acute leukemia Lymphoma Carcinoma Sarcoma
Ara-C[b]	Bone marrow	Yes, DNA synthesis	(4,5)	Acute leukemia
Asparaginase	Allergic Hepatic CNS	Yes, DNA, RNA, and protein synthesis		Acute leukemia
Azacytidine	Bone marrow	None, RNA synthesis	(6)	Acute leukemia
Bleomycin	Skin (dermatitis) Pulmonary fibrosis	?		Lymphoma Carcinoma
Cis-platinum diamminodichloride	Bone marrow Renal	?		? Lymphoma ? Carcinoma
Cytoxan[c]	Bone marrow Platelet sparing Oral activity	Yes, but not particularly DNA synthesis	(1)	Acute and chronic leukemia Lymphoma Carcinoma
Daunomycin	Bone marrow	DNA synthesis	(2,3)	Acute leukemia Lymphoma
DTIC[d]	Bone marrow	No	(7)	Melanoma
5-Fluorouracil	Bone marrow Gastrointestinal tract (G-I)	Yes	(1)	Carcinoma
5-HP[e]	Bone marrow G-I	Yes, DNA synthesis	(5)	? Acute leukemia
Guanazole	Bone marrow	Yes, DNA synthesis	(5)	Acute leukemia
HN_2	Bone marrow	None		Chronic leukemia Lymphoma Carcinoma
Hydroxyurea	Bone marrow Oral activity	Yes, DNA synthesis	(5)	Chronic leukemia

Methotrexate	Megaloblastosis G-I Oral Activity	Yes, DNA, RNA and protein synthesis	(1)	Acute leukemia Lymphoma Carcinoma
Mitomycin C	Bone marrow	?		Chronic leukemia Carcinoma
Nitrosoureas				
BCNU[f]	Bone marrow	None	(8)	Acute leukemia
CCNU[g]	Cross blood–brain barrier	?	(1)	Lymphoma
Methyl-CCNU	Oral activity			Carcinoma
Phenylalanine mustard	Bone marrow	None		Chronic leukemia, lymphoma, carcinoma
Prednisone	Hypertension Hyperglycemia Immunosuppression	No		Acute and chronic leukemia Lymphoma
Procarbazine	Bone marrow Oral activity	?		Lymphoma
6-Mercaptopurine	Bone marrow Oral activity	Yes, DNA and RNA synthesis		Acute and chronic leukemia leukemia
Streptonigrin	Bone marrow	?		Carcinoma Lymphoma
Streptozotocin	Pancreatic endocrine toxicity	?		Carcinoma
Thioguanine	Bone marrow	Yes, DNA synthesis		Acute leukemia
Vinblastine	Bone marrow	Acts on mitosis		Lymphoma
Vincristine	Neurotoxicity	Mitosis		Acute leukemia, lymphoma
VM-26[h]	Bone marrow	Acts on mitosis		Lymphoma
VP-16[i]	Bone marrow	Acts on mitosis		? Lymphoma

[a]Key to references: (1) Bruce *et al.* (1967). (2) Kim and Kim (1972). (3) Razek (1971). (4) Skipper *et al.* (1967). (5) Skipper *et al.* (1970). (6) Burchenal *et al.* (1972a). (7) Hoffman *et al.* (1968). (8) Burchenal *et al.* (1972b).

[b]Ara-C; 1-β-D-arabinofuranosyl cytosine.

[c]Cytoxan; Mead Johnson brand name for cyclophosphamide.

[d]DTIC; 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide.

[e]5-HP; 5-hydroxypicolinaldehyde thiosemicarbazone.

[f]BCNU; 1,3-bis(2-chloroethyl)-1-nitrosourea.

[g]CCNU; 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea.

[h]VM-26; *N*-demethylepipodophyllotoxin thenylidine glucoside.

[i]VP-16; *N*-demethylepipodothyllotoxin ethylidine glucoside