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*Guest editorial* *

# Drug resistance in oncology: from concepts to applications

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**Summary.** The complex problem of drug resistance is discussed with respect to host toxicity, to tumor characteristics (kinetic resistance, heterogeneity of cell subpopulations, hypoxia, mutation and gene amplification), and to the medication itself (pharmacokinetic and pharmacodynamic resistance: cell membrane, intracellular metabolism, intracellular target). After detailing each type of resistance, the possibilities of fighting against drug resistance are explored (dealing with host toxicity, tumor characteristics and drugs – intensifying therapy, multiple drug therapy, biochemical modulation, particular modalities of drug administration). Finally, perspectives of research and development of new drugs are summarized.

**Key words:** Oncopharmacology – Resistance to chemotherapy – PgP – GST – Topoisomerases

## Introduction

Paul Ehrlich coined the term "chemotherapy" almost half a century ago. Since the first use of alkylating agents in hematological malignancies, the role of chemotherapy in the treatment of cancers has been ever increasing (Calvert 1989). The original idea of a therapy that seeks out and destroys cancerous cells throughout the body has evolved into a more sophisticated treatment that tries to balance antineoplastic activity with host toxicity. The existence of varyious tumor sensitivities is another concept that has emerged since the development of early chemotherapy regimens and has led to the concept of resistance to chemotherapy; a term that will first be defined.

According to Goldin, chemotherapy resistance is "the failure to achieve or maintain a therapeutic response" (Goldin 1989). De Vita adds precision in his definition: "specific drug resistance is traditionally studied in vitro, or by using transplantable rodent tumors, where variables can be carefully controlled. Clinical studies are, however, difficult to control, and what we know as "the problem of resistance" is in reality a mixture of variables that affect the outcome in different ways. At the clinical level, resistance is measured solely by what happens to measurable tumor masses in patients with advanced disease, they either fail to respond, or grow during treatment. If they respond, we measure whether or not the response is durable enough to be classified as a cure" (De Vita 1990). The laboratory studies, with the use of experimental models, allow us to increase our knowledge of drug resistance, with the identification of molecular mechanisms.

With these theoretical considerations in mind, how do we classify tumors according to their probability of response to chemotherapy? Hodgkin's disease, choriocarcinoma, testicular malignancies, hematological malignancies, most pediatric cancers, and embryonic tumors usually respond fairly well to chemotherapy. In contrast, renal tumors, gastrointestinal tumors, and melanomas are usually resistant, while breast, endometrium, cervix, prostate, and head and neck tumors have an intermediate sensitivity to chemotherapy.

## The different types of resistance

Keeping in mind the difference between short-term drug sensitivity, and long-term curability, certain authors stress the difference between primary or intrinsic resistance (tumors that do not respond to chemotherapy at the first attempt) and secondary resistance, i.e. acquired during repeated cycles of treatment (tumors that respond initially and then relapse). Others refer to time, distinguishing temporary from permanent resistance.

We will outline and illustrate the three components of resistance: host, tumor and drug. A single mechanism can vary in intensity and may not be entirely responsible for resistance. Indeed, there are many factors responsible for resis-

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tance to chemotherapy, and most have been studied in vitro. We will not be detailing host/tumor interactions in this paper though they are the subject of much research today.

*Resistance related to host toxicity*

It is known that increasing the dose of a given drug increase the pharmacological effect of the substance and is limited by the major toxic side-effects.

The therapeutic index must be determined by calculating the optimal dose, i.e. the maximal pharmacological activity with acceptable toxicity. With chemotherapeutic agents, this index is often quite narrow. This is illustrated in the laboratory by an animal with a tumor treated by a given chemotherapeutic agent with potential toxicity and administered in a standard fashion (Goldin 1989). A very low total dose will cause the animal to die rapidly from the overwhelming tumor burden. Similarly, the animal will die, but more slowly, from persistent tumor if the dose is insufficient. We would not call this drug resistance. Of course, death ensues rapidly from acute toxicity if the dose is too high, as it does if the dose is only moderately elevated, in this case death also ensues from toxicity, but less rapidly. It is the cumulative toxicity, rather than drug resistance that is responsible for the host's death since the host cannot recuperate between cycles. Again, the optimal regimen is one that is within the therapeutic window and combines maximal drug activity with a good drug tolerance.

There are many different types of drug toxicities in man, including target organ, degree of toxicity, time of onset and whether or not the are reversible (Armand et al. 1986). This aspect of chemotherapy treatment, though not discussed in this paper, is crucial during therapy as it explains why some effective agents are not used because of high toxicities, and why treatment must occasionally be interrupted because of poor tolerance by the patient. As a result, the activity is diminished and, if the interval between cycles is prolonged, tumor cells can arise that were never exposed to the chemotherapy.

*Resistance related to tumor characteristics*

One must realize that the tumor has adaptive and dynamic characteristics that allow it to modify itself in time. In order to explain the behaviour of neoplastic cells, we will first summarize the fundamentals of cell kinetics.

*Kinetic resistance.* The classic model of a given cell population proposes that there is an equilibrium between cycling cells and resting cells. Cell loss results from the relative proportion of each cell population. The cell cycle is mostly dependent on the G1 phase, and anticancer drug regimens vary depending on which phase of the cell cycle they affect.

The doubling time of a given cell population, which depends to a lesser degree on the duration of the cell cycle, is inversely proportional to the fractions of proliferating cells and cellular losses. These differ considerably among different types of cancers and have a significant impact on chemotherapy regimens (Tubiana and Malaise 1973; Tannock 1989). For example, chemotherapy is more effective on embryonic tumors characterized by short doubling times, 90% of cycling cells and only 6% of surviving tumor cells, than on adenocarcinomas with a very long doubling time, only 6% of cycling cells and a cellular loss of 71%. In other words, according to kinetic resistance, tumors that are chemosensitive are those that have rapid doubling times and are poorly differentiated. Chemotherapy given for such tumors is associated with toxicities to normal tissues composed of cells with rapid doubling times such as bone marrow and gastrointestinal mucosa.

Skipper (Skipper et al. 1950) first proposed an experimental model of the chemotherapy response using the L1210 leukemia model in mice; further work by Goldie and Colman (see later) completed this model. Skipper considered that host defense mechanisms were not involved and established the following three rules.

First: the host can be destroyed by the proliferation of a single cell, so every single cell must be destroyed: "total cell kill".

Second: for a constant tumor cell doubling time, host survival is inversely proportional to the initial number of inoculated or surviving cells and depends directly on the cytotoxic effect of the drug.

Third: cellular loss depends on first-order kinetics; chemotherapy eradicates a constant proportion rather than a constant number of tumor cells: this is the notion of "fractional cell kill".

Unfortunately, there are several reasons why Skipper's experimental model rules do not apply to clinical situations in man. First, the murine L1210 leukemia is a tumor that grows rapidly (doubling time = 12 h) and has a very large proliferating fraction, which is not the case in many human tumors. Secondly, the drug dose required to kill the last remaining tumor cell would be much too toxic for animal and man, except in the case of very small tumors. Thirdly, monoclonal tumors, such as chronic myelogenous leukemia, are an exception rather than the rule, and most human tumors are heterogeneous, often containing distinct cell subpopulations within the primary tumor.

*Heterogeneity of cell subpopulations.* Interactive cell subpopulations differ mainly in their karyotype, their growth kinetics, their morphology, their immunological characteristics, the products they express, their ability to metastasize, and, most importantly, in their genetic instability and their sensitivity to chemotherapeutic agents (Heppner 1984; Price 1990).

It is accepted today that the degree of cellular heterogeneity within a tumor is an important predictor of curability. A given tumor consists of several defined coexisting dynamic clones that have a greater rate of proliferation than of cell loss. The tumor grows according to a complex process, and kinetics are most frequently similar to the Gompertzian model. This is an exponential growth with constant cell division and cell loss followed by a phase characterized by an increased doubling time. Retsky has proposed other tumor growth models that add the notion of a growth plateau to the Gompertzian model (Retsky et al. 1987). These models seem to correspond better to the clinical situation where tumor growth is less regular, with progression, stabilization phases,

and occasional spontaneous partial regressions. Regardless of the different models used, in practice, the natural course of malignant tumors is the proliferation of the tumor mass and metastatic dissemination.

*Hypoxia.* As a tumor increases in volume, the center of the mass usually contains more and more hypoxic foci in which tumor cells have a prolonged doubling time or a blocked cycle. Much experimental work has been devoted to the understanding of tumor vascularization, pH, oxygen and nutrient distribution in different zones of murine tumors. Although less work has been done in human tumors, it is accepted that there are two types of hypoxia (Vaupel et al. 1989): first, areas of central tumor necrosis due to chronic hypoxia because of poor oxygen diffusion across tissues, and, second, acute hypoxia because of temporary suddenly decreased blood flow from tumor-feeding vessels (Brown 1979, 1990). This distinction is important when choosing among various chemotherapeutic regimens.

The resistance to chemotherapy increases in the hypoxic centers of tumors to which drugs have little access. This explains why patients with smaller tumors, who are potentially curable by chemotherapy, have a better prognosis than those with larger tumors. There are exceptions however; for example, with colon cancer and melanoma, which are usually resistant to chemotherapy, the size of the primary tumor has no effect on response to chemotherapy. Tumor size is, in fact, only one of many factors to take into account when considering a failure to respond to chemotherapy, and intrinsic resistance is often responsible for tumor resistance. Indeed, if there is a response to chemotherapy, this initial response usually manifests itself whatever the size of the primary tumor.

*Mutation and gene amplification.* Another important facet of resistance is the notion of the natural evolution of a tumor to defend itself by adapting to its environment. Poupon considers that during the course of its evolution, the tumor passes through an "obstacle course", and develops in time several defense mechanisms against the host or therapeutics (Poupon 1989). This is a result of genomic instability, which generates mutations giving cells survival advantages in particular with respect to chemotherapy treatment. These cells have acquired characteristics that can protect them from aggression, and can survive even if the total number of cells is decreased as a consequence of the initial chemotherapy. Tumor progression is often related to increased cellular heterogeneity, hence, the notion of "intrinsic" resistance, which becomes an important concept in cancer treatment. This explains why a clinically evident tumor results from the selection of many mutant-resistant tumor cells that appear during the long preclinical phase from the diverse cell subpopulations. As the tumor grows, the number of mutations increases and so does the resistance to chemotherapy. The end result is the frequent transformation of chemosensitive tumors into partially or fully resistant tumors during treatment.

The concept of spontaneous resistance is now well established and one must look back at work on antibiotic resistance to find the origins of this discovery. Luria and Delbrück in 1943 used the "fluctuation test" to prove the genetic and aleatory origins of bacterial resistance to the T1 bacteriophage (Luria and Delbrück 1943). The great fluctuation of the number of parallel colonies of *Escherichia coli* obtained after subculturing implied that there were spontaneous mutants that appeared before exposure to the bacteriophage. If they had appeared as a result of T1 infection, there should have been the same number of surviving colonies in all the petri dishes. There were few colonies if the first resistant strain appeared late, and many colonies if the resistant strains appeared early on. The number of resistant bacteria also depended on the size of the initial inoculum.

Principles of the appearance of spontaneous resistance and the notion of "critical size", which means that there is a zero probability of not finding any resistant bacteria, were adapted to oncology in 1952 by Law who was studying murine leukemic cells and folic acid analogues (Law 1952). It was not until 1979 that Law's studies of somatic mutations found clinical applications through the works of Goldie and Coldman (Goldie and Coldman 1979; Goldie et al. 1982). The mathematical model of these authors is based on the following postulate: the number of resistant cancerous cells is a function of the frequency of spontaneous mutations and the delay between the appearance of the first resistant cell and the time at which treatment begins. The greater the size of the tumor the greater the number of resistant cells. This model helped inspire the development of multiple-drug regimens and neoadjuvant chemotherapy.

As with gene mutations, resistance to chemotherapy can also be related to the overexpression of normal genes of a drug target (Calvo et al. 1989); the origins of this gene amplification, preexistent or induced, are still under investigation. In the case of methotrexate, two types of cytogenetic anomalies are encountered in vivo and in vitro in the resistant cells. The number of copies of the dihydrofolate reductase gene can be so high as to produce elongated chromosome bands. These homogeneously staining regions are stable since they persist in the absence of selection pressure from the given agent (Biedler and Spengler 1971; Trent et al. 1984). There are also smaller extrachromosomal elements dubbed "double minutes", which confer an unstable resistance (Kaufman et al. 1979; Curt et al. 1983).

### *Resistance related to the drug*

Four choices are made in the prescription of a chemotherapeutic regimen: the drug(s), the dose, the route of administration and the duration of the treatment. The correct formulation of the drug depends on the pharmaco-toxicological, biopharmaceutical and physicochemical characteristics of the active agents, the characteristics of the excipient(s), the stability of the product, possible drug/drug interactions, and the bioavailability of the active agent(s). Strict quality control of all these phases, including dispensing, administration and monitoring, is critical, in order to exclude a resistance to chemotherapy caused by a careless pharmaceutical stage. Thus, the compliance of the treatment, a sine qua non condition of the objective measurement of therapeutic activity, may be guaranteed.

The study of the pharmacological resistance may be divided into its pharmacokinetic and pharmacodynamic components.

*Pharmacokinetic resistance*

Some primary or secondary tumors may be difficult to treat because of their anatomical location: central nervous system tumors, for example, are protected by the blood/brain barrier and thus remain out of reach of many agents (Skipper et al. 1961). However, this safe haven is not inviolate and, under certain conditions, the barrier can be transgressed; the physicochemical characteristics of the drug may determine whether it crosses the blood/brain barrier and this explains why nitrosoureas, which possess such properties, may be used in the treatment of intracerebral tumors.

Large individual variations in the pharmacokinetics of chemotherapy agents for a given compound and a given administration route are known, but to this day remain mainly unpredictable (Rowland and Tozer 1989; Benet et al. 1990). Contributing to these variations are genetic predispositions, age, sex and weight of patients, renal and hepatic functions, concurrent medications and previous treatments. There are also temporal variations in the pharmacodynamics of the medication i.e. "chronopharmacokinetics" (Cazin et al. 1991). As a result of this multifactorial variability, a standard treatment can be very effective in one patient, very toxic for another patient, and ineffective in yet another patient, falsely leading the clinician to believe that there is a drug resistance.

A common view is to consider that the cytotoxic effect of a drug is a function of the product of its concentration $c$ and exposure time $t$ at this concentration. As a result, we can estimate that, as long as $c \times t$ is constant, there should be a similar cytotoxic effect regardless of the mode of administration of the drug. However, these parameters are strongly linked. An agent that is dependent on a phase of the cycle and is administered as a bolus will not have time to be effective. One could conclude that there is resistance to chemotherapy, though if this same agent were administered as a continuous infusion, it would be clinically effective since the level would be adequate; vincristine, phase-M-dependent, with a long half-life of elimination, allows a bolus administration while a continuous infusion is a better route of injection for 5-fluorouracil, phase-S-dependent, with a short half-life of elimination. On the other hand, for a cycle-dependent agent, the administration of choice would be a bolus injection, which is easier to perform; in this case, modalities of administration are unimportant because $c \times t$ is the determinant of the efficacy.

*Pharmacodynamic resistance*

There are three important elements: the cell membrane, intracellular metabolism, and intracellular targets.

*Cell membrane.* Singer and Nicolson's dynamic fluid mosaic model of the cell membrane is the best description available today (Singer and Nicolson 1972). The cellular membrane is considered as a fluid phospholipidic bilayer (with an internal hydrophobic portion and an external hydrophilic portion) with inserted proteins. Gennis added the notion of functional channels, which helps explain movements across the cell membrane (Gennis 1989). If influx and efflux of drugs across the cell membrane are responsible for intracellular concentration, then the mechanism of transport of these agents into the cell may be of crucial importance.

As opposed to passive diffusion, which is a result of the existence of a concentration gradient, the properties of active carrier-mediated membrane transport mechanisms are the following: specificity, saturability, and competitive inhibition. Facilitated diffusion does not require any metabolic energy whereas active transport does. Should a defect in transport occur, such as a decrease in the membrane level of carrier or its affinity for the drug, then we can expect a decrease in the influx of a given drug. This has been shown to be the case for resistance to methotrexate, to cytosine arabinoside, to nitrogen mustard and to melphalan (Ohnoshi et al. 1982).

Another likely resistance mechanism is one that favors an increase in drug efflux from the cell. Multidrug resistance (mdr) has received much attention in the recent literature, especially for its potential importance in clinical resistance to chemotherapy (Pastan and Gottesman 1987; Fuqua et al. 1988; Robert et al. 1990). Biedler and Riehm first observed the phenomenon in New York in 1970 (Biedler and Riehm 1970). They described Chinese hamster lung cells exposed to actinomycin D that became resistant to this drug but also to other drugs they had never been exposed to before: mithramicin, vinblastine, vincristine, puromycin, daunomycin, demecolcin and mitomycin C. These preliminary results were explained 6 years later by Juliano and Ling from Toronto who discovered a "glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants" resistant to colchicine (Juliano and Ling 1976). They named this Pgp (permeability glycoprotein). It is also refered to as PG170 because of its 170-kDa molecular mass. Five important points concerning multidrug resistance (previously named pleiotropic resistance) can be drawn from the extensive work that followed Juliano and Ling's discovery (Gottesman and Pastan 1988; Rothenberg and Ling 1989).

1. Cancer cell lines that become resistant to a drug after incubation with increasing concentrations of this drug also become resistant to other drugs that have no similarities in structure or mechanism of action to the original. This cross-resistance particularly affects hydrophobic and heterocyclic compounds, natural products isolated from plant alkaloids and bacterial antibiotics or their hemisynthetic derivatives such as anthracyclines, *Vinca* alkaloids, colchicine, taxol, epipodo-phylotoxins and actinomycin D, but not all natural products are concerned (bleomycin). This phenomenon has never been observed with alkylating agents or antimetabolites. Along with the cross-resistance induced, increased sensitivity to other substances that act on the cell membrane has been observed, such as the effects of local anesthetics (procaine and lidocaine), and some steroid hormones (glucocorticoids and dehydrotestosterone). This phenomenon remains poorly understood (Bech-Hansen et al. 1976).

2. Numerous experimental arguments have proved that a cell that expresses the PgP-mdr phenotype will have diminished intracellular accumulation and concentration and increased ATP-dependent efflux of the anti-cancer agent.

3. The diminution of intracellular drug levels as well as the degree of resistance, both highly variable, are mostly related to the membrane concentration of PgP (Ling and Thompson 1973; Kartner et al. 1983). These observations

have led to a very attractive multidrug resistance theory, which still remains to be confirmed by in vivo studies. According to this concept, the P glycoprotein corresponds to an ATP-dependent pump located in the cell membrane, which evacuates intracellular drugs from the cell and protects it from toxic effects. Thus, a very brief presence of the drug in the cell would explain a multidrug-type resistance. Two mechanisms are possible: transmembrane efflux of the drug or, more likely, direct export from the cell membrane (Pastan and Gottesman 1991). The gene of the PgP was cloned by Chen et al. (1986), Gros et al. (1986b) and Gerlach et al. (1986). This trans-membrane glycoprotein has a 1276–1280 amino acid structure depending on the species (Chinese hamster, mouse, man) with one extracellular glycosylation site, one -$NH_2$ intracellular extremity and one -COOH intracellular extremity, two homologous independent chains, each with a cytoplasmic ATP-binding site, which suggests an internal duplication, and six transmembrane loci. Several monoclonal antibodies directed against the PgP molecule have been developed: the C219 (Kartner et al. 1985), MRK16 (Hamada and Tsuruo 1986), JSB1 (Scheper et al. 1988), Hyb612 and 241 (Meyers et al. 1989) and mAb 57 (Cenciarelli et al. 1991) are the most important. The existence of homogeneously staining regions and double minute chromosomes, characterized in many resistant cell lines, is cytogenetic evidence of gene overexpression (Riordan et al. 1985). In man, the P glycoprotein is coded for by the PgP *mdr* gene, which belongs to a family of genes involved in cellular exchanges and is highly conserved between species (Dhir et al. 1990). Three classes of genes have been identified in hamsters and in mice, two in man. The human *mdr2* gene is sometimes called *mdr3* some authors, whereas three distinct *mdr* genes exist in the mouse: *mdr1, mdr2, mdr3.* It is interesting to note that genes coding for proteins very similar to the PgP have been isolated in bacteria, for example, a hemolysin-transport protein (Gerlach et al. 1986; Gros et al. 1986a) and in parasites; this may explain the chloroquin resistance of *Plasmodium falciparum* (Foote et al. 1990). These observations tend to ascribe a greater role to PgP in terms of intracellular detoxification. Transfection experiments with the PgP *mdr1* gene have yielded resistant cells from cells that were originally sensitive (Gros et al. 1986b; Ueda et al. 1987).

4. The fourth important point is the relevance of this phenomenon in normal and malignant tissues and the possibility that it may predict the response to chemotherapy (Ma and Bell 1989; Goldstein et al. 1991). Until recently, it was thought that only the PgP *mdr1* gene, located on chromosome 7 and transcribed by a 4.5-kilobase mRNA existed in man. The *mdr2* (or *3*) gene expression is now known to exist and has been found in some type-B lymphocytic leukemias (Herweijer et al. 1990). Data collected from the literature show that PgP and/or *mdr1* mRNA have been identified at high levels in normal adrenal tissue, pancreatic tissue, and tissues responsible for elimination of exogenous and endogenous toxins, such as liver, kidney, and the small and large intestine. This supports PgP's role in detoxification. It is also found in CNS blood vessels, and may partly help to explain the blood/brain barrier. According to the numerous methods available to determine gene expression and/or to detect the protein, contrasting results were obtained by different authors; however, the major clinical correlates seems to be the following (Goldstein et al. 1991). Previously untreated tumors can be classified into four groups. First, one finds, among previously untreated cancers, increased levels of PgP in tumors from organs normally having overexpression of the *mdr1* gene, such as phenochromocytomas, hepatomas, colon cancer, and renal, adrenal and pancreatic cancers. Increased expression is also found during blast crisis in chronic myelocytic leukemias and in carcinoid tumors. This might explain the intrinsic resistance to chemotherapy usually found in these tumors. Secondly, tumors such as neuroblastomas, non-Hodgkin lymphomas, and acute lymphocytic and non-lymphocytic leukemias of adulthood sometimes have a high level of expression. Thirdly, there are tumors such as bladder, breast and non-small-cell lung cancers that have a low signal and, finally, those that have little or no expression, and are usually very sensitive to chemotherapy such as small-cell lung cancers, chronic myeloid leukemia in chronic phase, ovarian cancer, thymoma, thyroid cancer, Wilms tumor, prostate cancer and sarcoma. In this group there are also tumors that do not express the *mdr* gene and are not chemosensitive, such as melanoma, mesothelioma and esophagus, stomach and head and neck cancer. In these cases, a (or several) mechanism(s) other than PgP *mdr* gene expression must probably exist. Another interesting group of tumors comprises the previously treated tumors that have increased *mdr* expression after a recurrence, such as non-Hodgkin lymphomas, lymphocytic leukemias in adults and children, blast crisis of chronic myeloid leukemias, acute lymphoblastic leukemia, acute myeloid leukemia (Musto et al. 1991), adult T-cell leukemia (Kuwazuru et al. 1990), pheochromocytomas, neuroblastomas and ovarian and breast cancers. Gene amplification in these cases has not yet been reported in vivo.

5. The last point is the partial or total reversal of the *mdr1* phenotype and this will be discussed later.

To summarize the body of work done on PgP-mediated drug resistance we can say, from the few clinical series, that PgP seems to play a role in intrinsic or acquired resistance but is not "the last frontier" (Kellen 1991); other mechanisms must concurrently exist and should be sought after.

*Intracellular metabolism.* Once a drug enters the cell, it can be transformed into active moities, metabolized into inactive compounds, or it can be subjected to the cell detoxification mechanisms.

a) The first mechanism is a reduction in the intracellular activation of the drug. For example, methotrexate is mostly transformed into polyglutamic derivatives. These derivatives tend to remain in the intracellular compartment, unlike methotrexate, hence a decrease in polyglutamation can be responsible for drug resistance to methotrexate (Fabre et al. 1984). A similar mechanism confers resistance to cytosine arabinoside (AraC) by decreasing the activity of deoxycytidine kinase, which catalyzes the conversion of AraC into AraCTP, the active component (Tattersall et al. 1974). There is a similar decrease of uridine cytidine kinase for 5-azacytidine and a decrease of hypoxanthine–guanine phosphoribosyl transferase for both 6-mercaptopurine and 6-thioguanine. In the case of cyclophosphamide, a prodrug that must be activated in the liver to 4-hydroxycyclophosphamide to become cytotoxic, a decrease in liver enzymes, notably *P*-450, can confer resistance to this drug. Resistance to 5-fluorouracil develops from

a decrease of activity of key metabolic enzymes such as uridine kinase, uridine phosphorylase and orotic acid phosphoribosyltransferase.

b) Another mechanism thought to be involved in resistance is an increased metabolic inactivation of drugs. This is seen, especially with AraC, as an increase in the activity of cytidine deaminase, which converts AraC to AraU, but also with cyclophosphamide and aldehyde dehydrogenase, purine analoges and membrane alkaline phosphatase, bleomycin and bleomycin hydrolase.

c) The third possibility for metabolic resistance is an increase in the rate of intracellular detoxification. Some authors have implicated increased intracellular levels of metallothioneins, protein sulfhydryls, in platinum-derived resistance (Endressen et al. 1984; Kelley et al. 1988). Another compound of interest is glutathione, which is a non-protein sulfhydryl compound with many in vivo metabolic functions. Notably, it is conjugated with electrophilic substrates, such as free radicals, and contributes to cellular detoxification of many molecules, including anticancer drugs and carcinogens, by increasing their hydrosolubility. The catalysts for these reactions are mainly glutathione peroxidase and a family of isoenzymes known as glutathione *S*-transferases. The best studied in man are the cytosolic isoenzymes $\alpha$ (basic), $\mu$ (neutral) and especially $\Pi$ (acid). An increase in glutathione peroxidase and *S*-transferase, along with an increase of glutathione levels, has been described in several cell lines, especially in the case of isoenzyme $\alpha$ for nitrogen mustard, of isoenzyme $\mu$ for nitrosureas, of isoenzyme $\Pi$ for cisplatin and doxorubicin.

*Intracellular target.* There are several different types of resistance at the cellular target level.

a) The first is the absence of target: this phenomenon remains incompletely understood. The existence of subsidiary metabolic routes, more or less significant according to the tissues to spare or synthesize dTMP, leads to a highly variable sensitivity of tumors to 5-fluorouracil.

b) The second of these mechanisms of resistance to be described was the mutation of tubulin, which is the target of vincristine (Cabral et al. 1980). Another increases the pool of intracellular nucleotides, like dCTP, which competes with AraCTP and is related to resistance to cytosine arabinoside.

c) Thirdly, there exists the quantitative or qualitative modification of target enzymes. Unknown in normal cells, this defense mechanism involving overexpression of normal genes is seen in the thymidilate synthetase gene for 5-fluorouracil, the ribonucleotide reductase gene for hydroxyurea, the aspartate transcarbamylase gene for N-phosphonoacetyl-L-aspartate (PALA), and the glutathione *S*-transferase genes for the nitrosureas. This is also the case for the *mdr1* gene of multiple drug resistance, as previously mentioned, and for methotrexate resistance via its target enzyme, dihydrofolate reductase (DHFR). Methotrexate and its polyglutamic derivatives are powerful DHFR inhibitors. DHFR is the catalyst for the transformation of dihydrofolate to tetrahydrofolate, which is required to synthesize purine nucleotides and thymidylate, indispensible in cellular metabolism (Albrecht and Biedler 1984). One of the defense mechanisms of resistant cells is to multiply, sometimes by a factor of $10^2$–$10^3$, the unique DHFR gene (Alt et al. 1978). This bypasses the temporary metabolic block caused by methotrexate. An increase in intracellular DHFR or a decreased affinity of this enzyme for methotrexate may thus lead to resistance. Likewise, resistance to 5-fluorouracil by increased activity of thymidilate synthetase and of hydroxyurea by ribonucleotide reductase has been observed.

d) Other mechanisms of resistance are related to DNA topoisomerases (Topo). Resistance to camptothecin, because of a mutation of DNA Topo I, has been described. Another mechanism of resistance is related to inhibitors of DNA Topo II. This enzyme is necessary in order to maintain DNA stability. The drug's effectiveness (formation of stabilized enzyme/DNA cleavable complexes) is proportional to the amount of DNA Topo II located in the cell's nucleus (Liu 1990). Beck et al. (1987) first described a particular type of resistance in a cell line resistant to teniposide, then to etoposide and to anthracyclines but remaining sensitive to *Vinca* alkaloids. This type of resistance, with no expression of PgP and no decrease in intracellular drug concentrations was called "atypical multidrug resistance" or at-mdr, as opposed to "classical mdr". This type of resistance is always associated with a mutation of DNA Topo II, and is also called "altered topoisomerase resistance".

e) The last resistance mechanism to be described is related to activation of the DNA repair mechanisms of the cell. Antineoplastic drugs either produce lethal DNA damage or damage that can be repaired by the cell's complex set of repair enzymes: ligases, insertases, and alkyltransferases for direct repair; glycosylases, endonucleases, ligases, exonucleases, and topoisomerases, polymerases in cases of excision repair (Epstein 1990). This increased enzyme activity is seen with most alkylating agents and anthracyclines (Bungo et al. 1990). There is an increase in the activity of $O^6$-alkylguanyltransferase, which specifically repairs damage done by nitrosureas: this suicide enzyme transfers alkyl groups from the $O^6$ of a DNA guanine onto its cysteine residues, becomes irreversibly inactive and repairs the DNA (D'incalci et al. 1988).

## Strategies to beat resistance: realities and perspectives

### *Dealing with host toxicity*

In brief, recent innovations in pharmacology and surgical and biological techniques have allowed patients to tolerate aggressive treatment better. New anti-(5-hydroxytryptamine 3) medications can help prevent severe nausea and vomiting induced by many chemotherapeutic agents. Autologous bone marrow transplantations, administration of hematopoetic growth factors, and transfection of the *mdr1* gene to normal target cells, such as bone marrow cells and gastrointestinal mucosa, are techniques that are making previously fatal toxicities now acceptable (Storb 1989; Grunberg 1990; Marty et al. 1990; Mc Lachlin et al. 1990).

### *Tumor characteristics*

According to Heppner, "recognition of tumor heterogeneity is essential. Tumor societies are highly adapted for survival.

Having recognized their complexity, we must now learn to annihilate tumor societies" (Heppner 1984). Indeed, understanding the behavior, the structure, and the characteristics of tumor cell subpopulations and their interdependence within a given tumor is of crucial importance and challenges the development of highly efficient and specific therapies. At the present time, we are unable to modify the frequent of mutations, intrinsic properties of the tumor, but we are capable of initiating early treatment before tumors reach their "critical mass", before the appearance of mutations, hence the rationale of neoadjuvant chemotherapy. There is also work being done on the inactivation of gene amplification by experimental elaboration of false substrates or antisense codons. Another facet of research is an attempt to oxygenate poorly oxygenated tumors (Sartorelli 1988). Bioreductive agents, such as nitroimidazoles and mitomycin C and their derivatives, can be used for a limited time in acute hypoxic tumors, or for much longer periods in the case of chronic hypoxia (Coleman 1989). There are also new prodrugs that can be specifically activated by a hypoxic state (Connors 1989).

*Drugs*

Much can still be done to improve the drugs currently available, especially with pharmacokinetic modulation or new information concerning tumor biology and host characteristics such as biological rhythms (Hrushesky 1985).

*Optimization of currently available drugs*

*Intensifying therapy.* Because dose/response curves vary among patients and tumors, intensification of therapy has been suggested. Saturation phenomena apart, a dose greater than that usually delivered in standard practice should deliver a greater amount of drug at the target level and should have a greater cytotoxic effect (both desirable and undesirable), and Hryniuk defined the expression "dose intensity" as the quantity of drug delivered per unit of time, regardless of the modalities of administration (Hryniuk 1987, 1989). An increased dose can help eradicate sensitive cells, can increase drug levels in poorly vascularized tissues, can expose less sensitive subpopulations to higher doses, and can expose cells to effective doses for longer periods of time. The dose/response curve can now be further explored. Under close hematological monitoring, doses up three to ten times the standard dose can be delivered, until the second toxicity is reached. This is particularly true for cyclophosmamide and cisplatin, but should currently be reserved for patients with tumors that have proven sensitive and no failures of other target organ systems. This concept of intensifying therapy is also applicable to combined drug therapy.

*Multiple drug therapy.* Multiple drug therapy is based on Law's and Goldie's hypotheses that if the frequency of mutation of a cancerous cell vis-à-vis a first drug is $10^{-5}$, and $10^{-7}$ vis-à-vis a second drug, then the probability of double resistance to these two simultaneously administered drugs would be $10^{-12}$ (Goldin 1989; Goldie et al. 1982). Despite these theoretical considerations, multiple drug chemotherapy has been adopted as the treatment of choice except in a few rare cases (methotrexate for choriocarcinomas, and cyclophosmamide for Burkitt's lymphoma). When combining several chemotherapeutic agents in a protocol, several rules apply (De Vita 1991). Drugs should have partial effectiveness individually and be administered according to specific time and dose protocols in order to improve their clinical effectiveness. The interval between two cycles must be short enough to prevent tumor regrowth between cycles, yet allow time for the patient to recover from the side-effects. In general, drugs with cross-resistance should not be combined, nor should drugs with metabolic competition or similar side-effects. In contrast, drugs with different mechanisms of action are good candidates for combination, when they are used in sequence or simultaneously, their cytotoxic effects being additive. Interesting strategies are, for example, to begin with a drug that synchronizes resistant cells in a given cell phase and then to add a drug that eradicates these cells by acting specifically on the next cell phase. Such ideas, however, do not translate very well in vivo, notably because of the heterogeneity of tumor cell subpopulations.

*Biochemical modulation.* Some drugs can be given at very high doses because of a biochemical modulation that is included in the treatment. For example: methotrexate is given at a high dose because it is followed by a folinic acid ($N^5$-formyltetrahydrofolic acid) rescue after the cytotoxic period. This schedule is used for the treatment of osteosarcomas (Rosen and Nirenberg 1986). Another example is the combination of 5-fluorouracil (5-FUra) and folinic acid. The active 5-FUra form, 5-FdUMP, kills cells by depriving cells of dTMP. This inhibition of the thymidylate synthetase occurs via the covalent ternary complex between 5-FdUMP-thymidylate synthetase and $N^5$, $N^{10}$-methylenetetrahydrofolate. A long-term inhibition of thymidylate synthetase and a sufficient amount of $N^5$, $N^{10}$-methylenetetrahydrofolate are both necessary for cytotoxic activity of 5-FUra. Administration of folinic acid, especially its active form "*l*", leads to an increase in the pool of this cofactor and of its polyglutamates. This association has been successfuly used to treat colorectal cancers (Erlichmann et al. 1988). Other trials have used modulation by deoxythymidine, which helps transform 5-FUra into 5-FdUrd and deoxyinosine, source of deoxyribose phosphate (Rustum 1990). Scanlon et al. (1986), in the case of 5-FUra/cisplatin association, hypothesize that cisplatin diminishes cellular absorption of methionine and, in response, induces its increased biosynthesis as well as the increased biosynthesis of reduced folates, hence potentializing the action of 5-FUra. Others have shown that tetrahydrouridine (which inhibits cytidine deaminase) is capable of reversing aracitine resistance due to the increase in the activity of this enzyme.

Another mechanism is resistance due to an increased detoxification secondary to increased storage of glutathione. Buthionine sulfoximine can reverse cyclophosphamide, melphalan, and nitrosurea resistance in vitro by inhibiting glutamylcysteine synthetase, thereby decreasing intracellular glutathione (Ozols et al. 1987). This is also the case with ethacrynic acid, which inhibits glutathione *S*-transferase.

Also, aphidicolin, which inhibits DNA polymerases $\alpha$ and $\beta$, can suppress the repair of DNA in vitro and partially

restore activity of melphalan and cisplatin in initially resistant tumors. Buthionine sulfoximine and aphidicolin have also been combined, with promising results (Lai et al. 1989). Lastly, streptozotocin can reverse resistance to nitrosureas because of an increase in the activity of $O^6$-alklguanyltransferase.

Much work is also being done on the possibility of reversing the multidrug resistance phenotype. Some pharmacological agents can bind in vitro to the PgP, probably via a competitive action with the anticancer drug, and effectively block the efflux of the drug, increasing its intracellular level and thus partially or totally circumventing this type of multidrug resistance (Gottesman and Pastan 1989; Beck 1990; Ford and Hait 1990). Tsuruo et al. (1981) originally described such an activity with verapamil, a calcium channel blocker, and since then several other compounds have been shown to have the same properties: calmodulin inhibitors (phenothiazines), cephalosporins (cefoperazone), cyclosporins (A, C, G), amiodarone, reserpine, chloroquine, quinine, progesterone and tamoxifen. Because of the necessarily high plasma levels of these drugs, severe toxicities in clinical trials were noted, especially with verapamil (Pennock et al. 1991). However, the *R*-verapamil stereoisomer is a promising compound. Research in this area is focusing on a better understanding of the biochemical and molecular mechanisms involved in multidrug resistance. Studies are under way on the structure, function, specificity and regulation of PgP. Similar studies are focusing on the mechanisms that govern the binding of drug or modulator to the PgP, and on the identification of these binding sites. More work is necessary to develop analogs of reverting agents that were originally discovered empirically, by using experimental methods based on the use of resistant cell lines also by developing drugs with fewer side-effects and better pharmacokinetics. No doubt in a few years we will combine the administration of several modulators with an optimal therapeutic index (Hu et al. 1990). In the future, we might expect to develop a transport mechanism for a drug composed of biodegradable nanospheres, which would protect exposure to the PgP while entering the cell (Poupon et al. 1990). Other mechanisms include antibody inhibitors, monoclonal or chimeric (Hamada et al. 1989) anti-PgP coupled to toxins (Fitzgerald et al. 1987) or radioisotopes, ATPase inhibitors or antisense oligonucleotides inhibiting PgP synthesis. Studies are also under way to improve our understanding of atypical multidrug resistance, which occurs either simultaneously or without PgP *mdr*. Preliminary studies are under way using drugs that do not interact with PgP and are not substrates of DNA Topo II. Some investigators believe that DNA repair mechanisms could be defective in cells expressing atypical multidrug resistance (Beck 1990; Baguley et al. 1990).

*Particular modalities of drug administration.* Continuous-infusion pumps allow prolonged administration of drugs at low flow rates and can help overcome kinetic resistance due to slow cell division time. This is particularly useful in the treatment with 5-FUra and AraC, which seem to induce differentiation of leukemic cells.

For CNS tumors, to obtain an efficient $c \times t$ in tumor and protect normal tissues, intrathecal administration and Ommaya reservoirs can be used to treat meningeal lymphomas and leukemias with methotrexate and AraC.

Other routes, such as intrapleural or intraperitoneal, are used as well as portal administration of fluoropyrimidine derivatives in the treatment of colorectal cancers.

One of the goals of cancer chemotherapy is to hit the tumor cells selectively and spare healthy tissue. Ehrlich is responsible for the "magic bullet" theory and strategies have been devised to try to simulate such a situation, for example, by coupling drugs to macromolecules such as DNA, monoclonal antibodies or growth factors, monoclonal antibodies coupled with an enzyme able to activate a secondarily administrated prodrug, time-release mechanisms, liposomes, and hyperthermia. Despite great efforts, tumor-seeking and -destroying specificity is not yet optimal.

*Research and development of new drugs.* Antineoplastic drugs have been developed from the extraction of agents naturally occurring in animal or vegetable life (e.g. *Vinca* alkaloids), from synthesis or partial synthesis, from molecular biology techniques, or from serendipitous discoveries (cisplatin). The development of a new drug is a very tedious and long process with quite small returns.

New methods, based on in vitro and in vivo models, which are "more representative" of human cancers, today allow better mass screening programs (Phillips et al. 1990).

*Classical method.* The development of "classical" drugs, such as analogs of well-known parent drugs (using quantitative structure/activity relationships and computer-assisted conception), though not very original, is a time-tested method of improving the efficacy of an agent without significantly increasing its toxicity. These drugs are usually easier to administer. This type of drug is usually developed as a result of competition among pharmaceutical companies. The various derivatives of cisplatin and daunorubicin are such examples.

*New approaches.* The search for new types of antineoplastic mechanisms of action is a more interesting but much more costly and less predictable venture for drug companies. The past decade has been significant advances in the understanding of information processing among cells, cell differentiation, transformation, proliferation and mechanisms of metastasis (Workman 1990). Other than targeted drug-delivery systems, combinations of radiation and chemotherapy, and biological modifiers, there have been two major innovative strategies in cancer chemotherapy: the interruption of cellular signals and the inhibition of oncogene expression.

Chelating agents or complexants, and competitive antagonists can be used to interrupt proliferation cycles, by interacting with growth factors such as epidermal growth factor or transforming growth factor. There can also be direct action on growth factor receptors by monoclonal antibodies (Powis et al. 1990). Transduction signals can also be tampered with, for example, phosphorylation via second-messenger calcium and cAMP, as well as analogs of tyrosine kinase, inosine triphosphate or diacylglycerol (Fine et al. 1989).

Another approach is to block the expression of oncogenes using a very specific antisense nucleotide that highly specifically binds to the oncogene's mRNA (Deisseroth 1989).

## Conclusion

In this paper, we have tried to give an overview – though not an exhaustive one – of current concepts of drug resistance in oncology at the biological and clinical levels. Muggia and Norris (1990) set the following objectives: "restoring the sensibility present prior to the emergence of resistance, overcoming intrinsic drug resistance mechanisms and delaying the emergence of resistant populations". According to Young, "clinical drug resistance may be thought of in much the same way as one might view the multiple defenses displayed to protect a medieval castle from armed attack. Some of the ancient defense mechanisms, such as moats, stone wall and impenetrable terrain, were generally effective and non specific but generally inflexible. Others, such as arrows and boiling oil, were specific and flexible but only narrowly effective. Each tumor, like each medieval castle, probably utilizes a unique mixture of mechanisms to resist external attack" (Young 1989). Hence the challenges of today's research in pharmacology. May the pharmacologists of the next decades, with the use of always more refined protocols and individualized therapy, become more and more aggressive towards this castle.

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