PROGNOSTIC SIGNIFICANCE OF P-GLYCOPROTEIN IN MULTIDRUG RESISTANCE AND THE EFFECT OF ITS INHIBITION ON RESPONSE TO CHEMOTHERAPY IN BREAST CANCER PATIENTS

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Contents

Introduction

Cancer is an important factor in the global burden of disease. The estimated number of new cases each year is expected to rise to reach 15 million by 2025, with 60% of those cases occurring in developing countries. Breast cancer is the most common cancer in women in the Eastern Mediterranean Region and the leading cause of cancer mortality worldwide. There is geographic variation, with the standardized age incidence rate being lower in developing than industrialized countries.[1]

In Africa, Breast Cancer has overtaken cervical cancer as the most common malignancy affecting women and the incidence rates appear to be rising. These increases in incidence are due to changes in the demography, socio-economic parameters, epidemiologic risk factors, better reporting and awareness of the disease.[2] While mortality rates are declining in the developed world as a result of early diagnosis, screening, and improved cancer treatment programs, the reverse is true in the developing world as well as in eastern and central Europe.[3]

Breast cancer and its treatment constitute a great physical, psychosocial and economic challenge in resource-limited societies as found in Africa. The hallmarks of the disease in Africa are patients presenting at advanced stage, lack of adequate mammography screening programs, prevalence of younger pre-menopausal patients, and high morbidity and mortality rats.[4]

Among the Egyptian National Cancer Institute series out of 10,556 patients, during the year 2001, breast cancer was the most common cancer among women, representing 18.9% of total cancer cases (35.1% in women and 2.2% in men) with an age-adjusted rate of 49.6 per 100,000 population.[5] The Alexandria Cancer Registry 2000-2007 showed that out of 19,467 female cancer cases registered 8,386 (22%) were breast cancer. [6]

The high incidence and mortality rates of breast cancer, as well as the high cost of treatment and limited resources available, require that it should continue to be a focus of attention for public health authorities and policy-makers.[7] The costs and benefits of fighting breast cancer, including the positive impact that early detection and screening can have, need to be carefully weighed against other competing health needs. Although early detection, precise surgery with wide margins and adjuvant therapy has improved the results, relapse is frequent, with fatal outcome after diagnosis of metastatic disease. To date, no tool has been available to monitor the effect of adjuvant treatment apart from statistical analyses of the frequency of relapses.[8]

Prognostic factors in breast cancer

Prognostic factors should be used to provide an estimate of risk of recurrence in women with early-stage breast cancer. A useful prognostic factor has the following characteristics: it has significant and independent predictive value that has been validated by clinical testing, its determination must be feasible, reproducible, and widely available, with quality control and it must be readily interpretable by the clinician and have

therapeutic implications. Tumor diameter, lymph node status (LNMs) and histological grade are the most important prognostic factors in breast cancer. Besides these morphologic parameters numerous biological markers have been determined, but their importance as prognostic factors is still a matter of debate.[9]

- **Regional nodal involvement**

The extent of regional nodal involvement is the most powerful prognostic factor for predicting probability of recurrence. For simplicity, however, most clinical trials stratify patients based on four nodal groups that are based on National Surgical Adjuvant Breast and Bowel Project (NSABP) data: negative nodes, 1-3 positive nodes, 4-9 positive nodes, and 10 or more positive nodes. The 5-year survival for patients with node-negative disease is 82.8% compared with 73% for 1- 3 positive nodes, 45.7% for 4-12 positive nodes, and 28.4% for ≥13 positive nodes .[10] When present, axillary nodal involvement appears to obscure the prognostic significance of other factors. Immunohistochemical demonstration of otherwise occult micrometastatic nodal involvement has a prognostic significance, but this procedure at the moment is still investigational. Involvement of internal mammary nodes is also prognostically relevant, but this analysis is usually not performed because their involvement is rare in case of negative axillary involvement and mostly limited to inner-quadrant tumors. [11]

- **Tumor size**

In cases without axillary involvement, pathologic tumor size is the best predictor of outcome. Five-year recurrence rate ranges from approximately 10% for patients with tumors of less than 1 cm in diameter to 30-50% of patients with tumors of 5 or more cm in diameter. [9] However, this might not be the case for slow-growing very large tumors of certain subtypes (i.e. mucinous, papillary, tubular, medullary or adenocystic) that might not have high proliferative capacity. At the moment it is not known if invasive tumors of 1 cm or less in diameter detected by screening mammography might be considered of similar aggressiveness as those detected by physical examination.[9]

- **Nuclear grade**

This is a well-documented factor. When determined by experienced pathologists, it discriminates favorable and unfavorable prognostic groups. High nuclear grade is associated with a higher rate of recurrence. Nuclear grade is not currently part of the routine pathologic review of breast cancer specimens. The pathology community should adopt a uniform grading system and routinely use this discriminate. Several well-characterized histological subtypes impart a favorable prognosis, although they are a distinct minority of all breast cancer cases. These subtypes include tubular, colloid (mucinous), and papillary types .[10]

- **Hormone receptors(HR)**

Some tumours, notably carcinoma of the breast and prostate, are often responsive to hormones, a property which has become exploited through endocrine surgery and more

recently medically through drugs which influence hormonal levels or inhibit their effects on tumour cells.[12]

Steroid hormones bind with high specificity and affinity to intracellular receptors. These steroid receptors belong to a 'superfamily' of proteins whose function is to control the transportation of a repertoire of other cellular genes. Steroid receptors such as estrogen receptor (ER) and progesterone receptor (PR) are located in the cell nucleus. Hormone is believed to diffuse into or be transported to the nucleus where a steroid–receptor complex is formed with receptor dimerization. Some of the genes regulated by steroid receptors are involved in controlling cell growth and it is currently believed that these effects are the most relevant to oestrogen receptor influences on the behaviour and treatment of breast cancer. [12]

Approximately 30% of the patients with breast cancer will respond to endocrine therapy. However, by assay of oestrogen receptor status, using radioligand binding assay on tumour cytosol samples, a response is seen in 50–60% of patients with ER positive tumours compared with a response of less than 10% in patients with ER negative tumours . Prediction of response can be refined further by combining ER and PR assay; ER PR positive tumours carry a 78% response rate whilst patients with ER PR negative tumours respond in less than 10% of cases. [13]

- **Molecular markers**

Apart from the hormone receptors referred to above an extensive range of novel variables have been proposed as putative prognostic factors. Most are associated, experimentally at least, with mechanisms of differentiation, invasion, and metastasis or growth rate of neoplasms. They include expression of epithelial mucins including growth factor receptors (e.g. human epidermal growth factor receptor2 HER-2) , the tumor suppresser gene p53 ,tumour DNA ploidy and S-phase fraction ,proliferation markers, ,proteinases, for example Cathepsin D and adhesion molecules such as E-cadherin . Tumour angiogenesis has also received much attention as a possible prognostic or even predictive factor. [13]

However, although most of these factors can be shown to have some prognostic significance in univariate analysis none has so far been proven to be relevant universally in multivariate analysis. At the present time, perhaps paradoxically, the variables which have been shown consistently to be of independent significance are the routine histopathological factors described above. No doubt further objective biological factors will emerge but until then it is the traditional factors that should be used for patient management. [13]

- **Estimating individual risk**

Currently available prognostic factors are associated with a broad range of risk of recurrence in node negative breast cancer patients. There are extremes of high and low risk where it is possible to make recommendations about adjuvant systemic therapy. For example, outside of clinical trials, it is reasonable not to treat patients with tumors less than or equal to 1 cm in diameter because their chance of recurrence is less than 10 percent at

10 years. With increasing tumor diameter, other prognostic factors should be weighed in the decision to use adjuvant treatment. A major goal is the development of risk profile systems with sufficient accuracy and reproducibility to estimate prognosis in the individual patient.[9]

Adjuvant Chemotherapy role in breast cancer

Adjuvant chemotherapy has been defined as the administration of antitumor drugs to kill or inhibit clinically undetectable micrometastasis after primary surgery.[14] Such an approach is prudent, as adjuvant systemic chemotherapy with or without hormonal therapy has been demonstrated to improve survival in both node-negative and node-positive disease. Adjuvant chemotherapy may increase 10-year survival by 7–11% in premenopausal women with early stage disease and by 2–3% in women aged over 50.[15]

The hypothesis that adjuvant systemic treatment would reduce the risk for recurrence and improve the chances of survival in women with primary breast cancer was formulated in the late 1960s to early 1970s. Since then, significant advances have been made through the conduct of a large number of prospective, randomized clinical trials analyzing different strategies, chemotherapeutic regimens, and durations of treatment; however, only a few of these trials had sufficient statistical power. Therefore, in 1985, a meta-analysis of adjuvant clinical trials was undertaken by the Early Breast Cancer Trialists' Collaborative Group (EBCTCG) in an attempt to provide reliable estimations of relative and absolute average treatment benefits. Since then it has been updated every 5 years.[16]

The EBCTCG meta-analysis demonstrated that adjuvant chemotherapy significantly reduces the risk for relapse and death in operable breast cancer patients regardless of age, the involvement of axillary nodes, hormone receptors status (HR), and menopausal status, although the absolute advantage is proportional to the risk for relapse and decreases with increasing age. [17] The resources of chemotherapeutic agents available for the treatment of breast cancer have expanded greatly over the past several decades, and complex regimens are nearly universal today. [18]

- **Therapeutic Regimens used in the treatment of primary breast cancer**

The administration of polychemotherapy (two or more agents) is superior to the administration of single agents. Three to six courses of treatment (3-6 months) appear to provide optimal benefit, with the administration of additional courses adding to toxicity without substantially improving overall outcome. Popular regimes include:

- Cyclophosphamide, methotrexate and fluorouracil (CMF).[19]
- Cyclophosphamide, doxorubicin (Adriamycin), and fluorouracil (CAF). [19]
- Combined anthracycline–taxane regimens. [19]

- **CMF regimen**

The CMF regimen proposed by Bonadonna[20] has been widely tested, and its long-term benefits in improving Disease free survival(DFS)and overall survival (OS) were confirmed after 30 years of follow-up. This regimen is still used in the adjuvant setting, particularly in Node-negative women and in cases of anthracycline contraindications.[20]

CMF was the mainstay of adjuvant chemotherapy for many years and the Oxford overview analysis of 47 trials, the majority of which used CMF-based regimens, clearly established the role of adjuvant chemotherapy in significantly reducing the risk of recurrence and death.[16] These results were significant irrespective of lymph node status, ER status and tamoxifen use, but the degree of benefit was influenced by age and menopausal status. For all women under the age of 50 years, chemotherapy significantly improved the absolute 10-year survival by >10% for those with node-positive disease and by 6% for those with node-negative disease . When results were analysed by age in decades, there was a strong trend towards the younger the age, the greater the benefit. No significant benefit was demonstrated for patients over the age of 70 years, but the number of patients involved was small.[21]

- **Anthracycline-Based Regimens**

A decade of clinical research was necessary to firmly establish the superiority of anthracycline-based regimens over classic CMF or CMF-like regimens. Randomized adjuvant trials have generated conflicting results.[22] This can be explained by the number of patients enrolled and their selection, the different anthracycline-based and CMF-like regimens, and different doses of drugs in similar regimens. The EBCTCG meta-analysis shows that the benefits of adjuvant anthracyclines are persistent for 15 years, with a 26% reduction in breast cancer death in young patients.[16]

The EBCTCG overview also showed a small but significant benefit in relapse-free survival and OS for anthracyclines compared with the more traditional CMF regimens (68.4% versus 64.1% for OS). Since this overview, other supportive studies have confirmed the benefit of anthracyclines-based therapy. [21]Anthracyclines, as integral components of most regimens, are central to the accepted treatment standards. they are important factors in optimizing adjuvant and neoadjuvant treatment and are indicated for adjuvant therapy regardless of the extent of nodal involvement , hormone receptor status, or human epidermal growth factor receptor 2 (HER-2) expression level of the tumor.[18]

- **Taxanes**

The potential importance of the taxanes as adjuvant therapy is emphasised by the large number of major trials currently running. Four important trials assessing the addition of a taxane to anthracycline chemotherapy in patients with node positive disease have been reported. [21]

In the Cancer and Leukemia Group B (CALGB 9344) trial, 3121 women who had received four cycles of Adriamycin and Cyclophosphamide (AC) regimen at different dose levels were randomized to receive four further courses of paclitaxel or no further

chemotherapy. The addition of paclitaxel resulted in a small but statistically significant improvement in DFS and OS.[23]

In a similarly designed trial, the National Surgical Adjuvant Breast and Bowel Project (NSABP B-28) randomised 3000 women to four courses of AC chemotherapy followed by four courses of paclitaxel versus four courses of AC chemotherapy alone .Sixty-six per cent of the patients were ER-positive and received concurrent tamoxifen, including all women aged >50 years regardless of ER status. These results showed a significant absolute 4% improvement in the DFS in the paclitaxel arm but no difference in OS . [24]

These two trials are open to the criticism that efficacy differences could be explained by differences in treatment duration rather than the addition of paclitaxel. [21]

The third trial, Breast Cancer International Research Group (BCIRG), involved docetaxel, used concurrently with an anthracycline rather than sequentially .The study included 1491 women, who were randomised to receive six cycles of standard FAC versus six cycles of TAC (docetaxel, adriamycin, cyclophosphamide). The second interim results after a median follow-up of 55 months and 399 events showed a significant improvement in DFS (75% versus 68%) and OS (87% versus 81%) at 5 years for the TAC group. The rate of febrile neutropenia was 24.7% in the TAC arm (despite prophylactic use of oral ciprofloxacin) compared with 2.5% in the FAC arm. [25]

In the fourth trial, a French group compared six cycles of fluoronacil, epirubicin , cyclophosphamide (FEC) regimen using epirubicin 100 mg/m^2 versus three cycles of FEC followed by three courses of docetaxel 100 mg/m2 for women with node-positive cancer . and first results were presented at San Antonio 2004, but have not yet been published.These showed a significant improvement in DFS in favour of the switch to docetaxel (5-year DFS 78.3% versus 73.2%). Curiously, the benefit was significant in women over the age of 50 years, but not for those under 50 years. The trial also showed a small but significant OS advantage (90.7% versus 86.7% 5-year OS).

Therefore, all four taxane trials that have so far produced data have shown a significant DFS gain, and three of the four have shown an OS gain. There is now good evidence to support the use of taxanes in patients with node-positive breast cancer. [21]

- **Anthracyclines**

The anthracyclines are derivatives of rhodomycin B, a red-pigmented polyketide antibiotic, isolated in the 1950s from Gram-positive *Streptomyces* present in an Indian soil sample. After the discovery of the antitumor activity and chemistry of rhodomycin B, Farmitalia initiated a program to find new anticancer compounds produced by novel strains of microbes isolated from soil. In 1957, a colony of *Streptomyces* producing a red pigment was grown from a soil sample taken at Castel del Monte near the city of Andria in southeastern Italy. This microbe produced a substance named daunorubicin after a pre-Roman tribe in southeastern Italy; Di Marco demonstrated antitumor activity in 1963. At nearly the same time this compound was isolated by French researchers at Rhône Poulenc, who named it rubidomycin. Later on, it became clear that rubidomycin and daunomycin

were identical and daunorubicin became the only name for this compound. In 1969, Arcamone and his co-workers succeeded in isolating and purifying doxorubicin (14-hydroxydaunomycin) from *Streptomyces peucetius* variety *caesius*, a mutant of the original *Streptomyces* strain found near the Adriatic Sea. This is the reason why doxorubicin was named Adriamycin.[26]

The clinical development of daunorubicin started in 1964 for the treatment of acute leukemias, and doxorubicin in 1968, and this drug was broadly evaluated in patients with leukemia, lymphoma and most solid tumors. The first clinical experiments with doxorubicin were performed in Milano by Bonadonna which showed remarkable antitumor activity that were later confirmed by studies in the USA. Only 6 years later, in 1974, doxorubicin was approved by the US Food and Drug Administration (FDA). At the end of the 1970s the two anthracyclines dauno- and doxorubicin were the most efficacious anticancer drugs with an enormous impact on the development of anticancer therapy with cytotoxic drugs and medical oncology which grew up to an independent medical discipline within internal medicine.[26]

- **Chemistry**

Structurally, all anthracyclines share a common four-ringed 7,8,9,10-tetrahydrotetracene-5,12-quinone structure and usually require glycosylation at specific sites for biological activity. The anthracyclines are a subgroup of the aromatic polyketides that form one of the largest families of naturally occurring bioactive compounds comprising 5,000 members, of which 2,000 belong to the anthracycline-type family. Mathematical approaches that consider the detailed basis of structural diversity of these compounds suggest that more than 10,000 theoretical anthracycline-analogs structures could be possible. The general structure
of anthracyclines is depicted in Figure 1 that illustrates the partial planar
structure of the tetracyclic ring system (ring B,C,D) which represents the chromophore (anthracyclines are red compounds) and includes the quinone structure.[27]

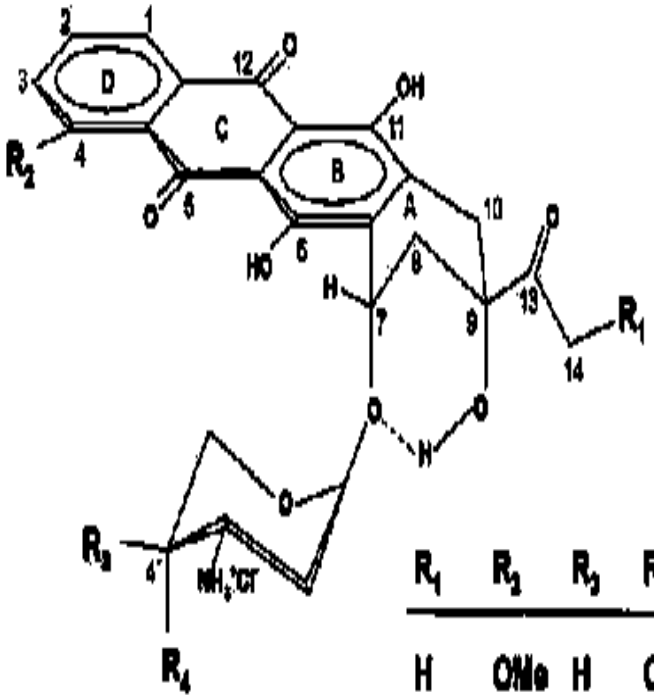
The 7 and 9 position in ring A are important because the daunosamine sugar moiety is linked glycosidically at the 7-position and at the 9-position a sidechain with a ketone group is tethered. The name anthracycline was created in the late 1950s based on the presence of an anthraquinone chromophore and the polycyclic ring system in the chemical structure (Fig 1), which is similar to that of tetracyclines.[27]

The four major anthracyclines in clinical use differ in the residuals R1 to R4. The smallest difference is found between doxo- (DOX) and epirubicin (EPI) which differ only in the C-4 position of the OH-group: in the case of DOX the hydroxy group has an axial orientation in case of EPI an equatorial orientation. This orientation renders EPI a good substrate for human D-glucuranyl transferases, and EPI is therefore conjugated in vivo at the daunosamine sugar moity with glucuronic acid, which is not a metabolite known for DOX.[27]

- **Mechanism of action**

Despite extensive clinical utilization, the mechanisms of action of anthracyclines in cancer cells remain a matter of controversy. In a seminal commentary the following mechanisms were considered (Figure 2):

1) intercalation into DNA, leading to inhibited synthesis of macromolecules.
2) generation of free radicals, leading to DNA damage or lipid peroxidation.
3) DNA binding and alkylation.
4) DNA cross-linking.
5) interference with DNA unwinding or DNA strand separation and helicase activity.
6) direct membrane effects.
7) initiation of DNA damage via inhibition of topoisomerase II.
8) induction of apoptosis in response to topoisomerase II inhibition. [28]



R_1	R_2	R_3	R_4	Anthracycline
H	OMe	H	OH	Daunorubicin
OH	OMe	H	OH	Doxorubicin
OH	OMe	OH	H	Epirubicin
H	H	H	OH	Idarubicin

Figure 1: Chemical structure of the anthracyclines daunorubicin (DNR), doxorubicin (DOX), epirubicin (EPI) and idarubicin (IDA).

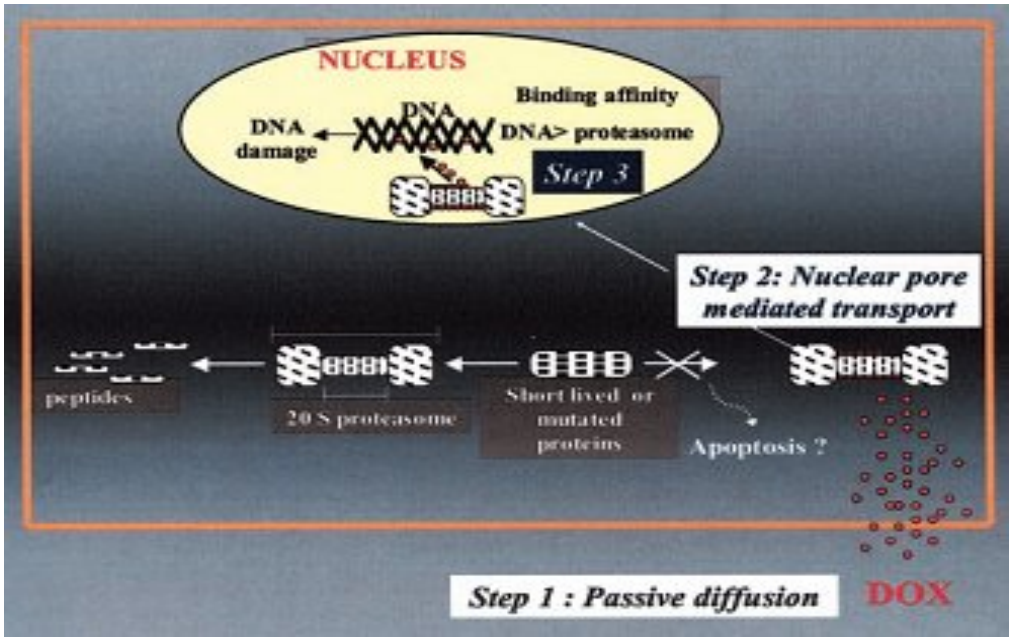


Figure 2: The proposed mechanisms of action of anthracyclines in cancer cells.

- **Doxorubicin Pharmacokinetics**

Pharmacokinetic studies, determined in patients with various types of tumors undergoing either single or multi-agent therapy have shown that doxorubicin follows a multiphasic disposition after intravenous injection.[29]

Distribution: The initial distribution half-life is approximately 5 minutes suggests rapid tissue uptake of doxorubicin, while its slow elimination from tissues is reflected by a terminal half-life of 20 to 48 hours. Binding of doxorubicin and its major metabolite, doxorubicinol, to plasma proteins is about 74 to 76% and is independent of plasma concentration of doxorubicin.[30]

Metabolism: Doxorubicin is metabolized by the liver, with reduction and hydrolysis of the ring substituents. An alcohol form is an active metabolite whereas the aglycone is inactive. Most of the drug and its metabolites are excreated in bile and about one-sixth is excreted in urine.[30]

Excretion: the disappearance curve for doxorubicin is multiphasic with elimination half-lives of 3 hours and about 24-30 hours.[29]

Multi Drug Resistance(MDR)

Chemotherapy failure is a major problem in the management of patients with breast cancer. In general, breast cancer is considered to be one of the more chemosensitive solid tumors, and major response rates (40%) in patients with metastatic disease have been reported. However, complete remissions of the initially responsive tumors do not occur and develop resistance to multiple anticancer agents of different structure and function.[31]

The main reason for the limited efficacy of chemotherapy is multidrug resistance (MDR), defined as cellular resistance to multiple, structurally and functionally divergent drugs. The resistance of tumors occurs not only to a single cytotoxic drug used, but also occurs as a cross-resistance to a whole range of drugs with different structures and cellular targets. [32] Once MDR appears, using high doses of drugs to overcome resistance is ineffective, toxic effects appear and resistance is further stimulated. MDR severely limits the effectiveness of chemotherapy in a variety of common malignancies and is responsible for the overall poor efficacy of cancer chemotherapy.[33]

Studies show that the molecular mechanisms of MDR are numerous and operating at different steps of the cytotoxic action of the drug that include (Figure 3):

1- MDR cells sometimes exhibit altered kinetics of cellular drug uptake linked to changes in membrane permeability (e.g. expression of transporter proteins as Pglycoprotein (P-gp); Multidrug-resistance protein family members (MRPs) breast cancer resistant protein BCRP). [34]
2- Increased drug detoxification linked to over expression of enzymes such as glutathione-S-transferase or elevated intracellular glutathione concentrations. [34]
3- Changes in drug effectiveness are also documented, e.g., increased DNA damage repair via alterations in O-6-methylguanine DNA methyl transferase or changes in topoisomerase II activity. [34]
4- Defect in apoptotic pathways (eg, through overexpression of antiapoptotic proteins such as Bcl-2) are also efficient drug resistance mechanisms. [35]

- **Clinical importance of MDR**

From a clinical point of view, MDR-phenomena affect patients with a variety of blood cancers and solid tumors, including breast, ovarian, lung, and lower gastrointestinal tract cancers. Tumors usually consist of mixed populations of malignant cells, some of which are drug-sensitive while others are drug-resistant. Chemotherapy kills drug-sensitive cells, but leaves behind a proportion of drug-resistant cells. [36] Metastatic or recurrence might be due to the unrestricted growth of resistant cell clones within the original tumor, which replace those cells initially susceptible, or to the emergence of new mutant cell clones resistant to the treatment. [37]

The potential benefit of correlating the presence of MDR to cancer treatment is, however, large. As the rapid analysis of the MDR mechanism at the molecular level in tumor samples is entering the phase of clinical application, it will become possible to reconstruct a resistance profile for the predominant cell types in each tumor and to adjust the chemotherapy accordingly. This should at least make it possible to spare some patients an aggressive therapy that does not work. [38]

- **Importance of modulation of MDR**

Overcoming clinical drug resistance remains a challenge for the oncologist in the treatment of human malignancy, even in the current era of novel therapies. [39] Cancer defends itself actively using these mechanisms, and therefore their impairment is likely to