Resistance in Malignant Tumors: Can Resistance Assays Optimize Cytostatic Chemotherapy?

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Key Words

Malignant tumors • Chemotherapy resistance • Resistance assays

Abstract

The frequent resistance of malignant tumors to chemotherapy documents the fact that numerous patients are uselessly traumatized by highly toxic drugs. Investigations into this resistance have shown that many different mechanisms exist which can abolish the antitumor action of chemotherapeutics. So far, no safe method has been found to counteract this resistance. To date, the only way to avoid harm by an ineffective chemotherapeutic is to refrain from its use when resistance to it is detected. The present survey reports on the available assays that diagnose resistance reliably; sensitivity tests are excluded as being unreliable. Resistance assays are still not fully recognized in hospitals, despite the fact that they are able to optimize chemotherapy by eliminating treatment that is ineffective and merely harmful. Since the number of chemotherapeutics will increase enormously in the future, pharmacological cancer treatment cannot dispense with resistance assays.

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Introduction

Resistance, i.e. the failure of a disease to respond to drug therapy, occurs relatively frequently in medicine. Particularly serious is the resistance of malignant tumors to treatment with chemotherapeutics. Whereas a curative therapy is frequently rendered possible by operative removal of a tumor in its early stages, in advanced stages chemotherapy is the only option for treatment.

The poor efficacy of the therapy has essentially two causes: firstly, the very complex biological properties of malignant tumors, and secondly the use of drugs whose action is largely untargeted.

The great variability of growth potentials of malignant tumors has long been known [1]. The mechanisms leading to uncontrolled growth, however, are not well understood. Histological structural analysis by the pathologist can determine the malignancy, but not the individual growth potential of the tumor. The present treatment strategies of oncology, using standard therapies for histologically homogeneous tumors, however, ignore individual biological tumor reactions. The large number of failures indicates that this concept needs to be optimized.

Furthermore, only limited results can be achieved by the currently available drug arsenal. The mechanism of action of established chemotherapeutics (cytostatics) is not tumor specific; it consists in principle in the destruction of tumor cells by reason of their growing faster than normal cells.

Since the cells of vital organs, for instance those of the gastrointestinal tract or the hematopoietic system, also grow fast, and may therefore also be destroyed, chemotherapy often causes severe side effects [2–4]. Nontargeted chemotherapeutics are afflicted with a further serious handicap, that is, the risk of inducing resistance. Diagnosing resistance is therefore of great importance. Not only are unsuccessful chemotherapy treatments very costly, but patients suffering from a life-threatening disease are further harmed by the perilous side effects of the drugs.

In the extremely voluminous oncological literature, unambiguous data on the extent of tumor resistance are sparse, although the resistance dilemma is familiar to oncologists. Some figures can be found in publications for specialists: thus nearly 50% of all cancer patients suffer from malignancies that are intrinsically resistant to chemotherapy. A large proportion of the remaining half of patients develop drug resistance during treatment [5].

Resistance Mechanisms

Great efforts have been made to clarify the development of tumor resistance. In vitro investigations have shown that numerous mechanisms are demonstrable in resistant tumor cells, and that no one mechanism alone accounts for resistance to a cytostatic drug. Thus, in cases of lung cancer a great number of different resistance-related proteins were found, such as P-glycoprotein, glutathione-dependent enzymes, topoisomerase II, metallothioneins, O-6-alkylguanine-DNA alkyltransferase, thymidylate synthase, dihydrofolate reductase and heat shock proteins. But the presence of all these resistance proteins alone cannot explain the drug-resistant phenotype. Further factors, such as cell cycle-related proteins, angiogenic factors, protooncogenes and tumor suppressor genes also contribute to the phenotype of the resistance to drugs used against lung cancer [6].

Similar resistance mechanisms can be found in the case of numerous other cancers, in different quantitative compositions, however [7]. The varying resistance patterns measured indicate that each tumor has its own unique resistance factor profile. Thus, a close relationship exists between the extent of resistance and the number of resistance mechanisms detected [7].

Chemotherapy resistance is not only to be regarded as a defense against cytostatic drugs, but rather seems to be a more common process with the pathophysiological function of protecting the organism from harm by toxic

substances. Thus resistance mechanisms are not restricted to cancer cells, but are also present in normal cells.

The finding is of interest that lung carcinoma cells in smokers are more frequently resistant and show higher levels of resistance factors than do corresponding tumors in nonsmokers [8, 9]. There are indications that detoxifying systems may share common regulatory elements.

So far, no clinically applicable method for the demonstration of tumor resistance, based on the knowledge of resistance mechanisms, has been realized. Little attention has, however, hitherto been paid to the development of relevant tests. Major efforts have now begun to be made in this important field of pharmacological cancer therapy [10].

Research on resistance continues to be intensive, but apart from more knowledge of the complexity of the mechanisms and their interactions, no direct, clinically applicable progress has been made in the last few years [5, 11–13].

Attempts to Reverse Drug Resistance

The detection of resistance mechanisms has aroused interest in developing strategies for resistance reversal. Tsuruo et al. [14], in the 1980s, were the first to report that the calcium blocker verapamil could reverse resistance. In the following years, a wide range of substances, of natural and synthetic pharmacological classes, were tested for their ability to reverse resistance in cell lines. Some modulators could be found in the following classes: calcium channel blockers, calmodulin antagonists, cyclosporins, quinolines and antiestrogens [7]. Monoclonal antibodies against resistance proteins also showed resistance-reducing actions.

The in vitro effects, however, could not be satisfactorily reproduced in in vivo experiments. Despite some promising results in hematological malignancy, results obtained in the treatment of solid tumors by these modulators have been rather disappointing so far. An explanation for this failure could be the multifactorial resistance of cancer cells; that is, in the case of the blockage of one resistance factor, others will replace and compensate for the function, in order to restore the former defense position.

Caution has also been advised as concerns the reversal of resistance by modulators which inhibit enzymes. As is known, such enzymes are also present in normal tissues such as those of the liver, kidney and intestine, where they fulfill vital functions. Therefore, enhanced toxic effects in these tissues by cytostatics must be expected.

The development of further strategies for resistance modulation, such as treatment with immunotoxins, bi-specific antibodies, antisense oligodeoxynucleotides, ribozymes and albumin-conjugated drugs has failed to produce better results [7]. The hitherto unsuccessful attempts to reverse resistance to chemotherapy treatment demonstrate the fact that cancer cells are able to utilize excessive interchangeable pathways to overcome the cytotoxic effects of chemotherapy.

Today, the strategy practiced for reducing resistance in cancer treatment consists in the use of combination chemotherapy, its various components attacking different targets. It is assumed that broad-acting chemotherapy is able to minimize resistance in most cases, making the search for individual resistance unnecessary.

The composition of combination regimens is, however, made difficult by a number of elements of uncertainty [15]. Complicated calculations based on results found in experiments on cultured cancer cell lines are mainly used. Results of animal experiments that would provide further information are usually available only in small numbers, for reasons of cost.

Since so many assumptions are necessary in order to compose regimens, it is difficult to be sure when an optimum drug combination has been found. Only screening in patients, which is very hazardous [16], is able to prove whether the calculated combination regimen has any therapeutic value.

As the possibilities of combination are great – in fact the number of potential combinations seems virtually unlimited – and as clinical studies to demonstrate effectiveness are very expensive, few combinations have, thanks to improvements compared with others, achieved routine clinical use. There can be no proof that a regimen that finds its way into clinical practice is the best.

The combination regimens meanwhile established as standard therapies are prescribed blind, i.e. taking no account of individual tumor resistance. In spite of the hopeful expectation triggered by sophisticated theoretical concepts, the failure rate in combination chemotherapy, especially with solid malignant tumors, shows that no drastic reduction is achieved in cases of resistance.

The clinical use of an early diagnosis of resistance is of eminent importance. Only knowledge of the resistance status of a tumor permits an accurate choice of medication and avoids harmful therapeutic intervention.

How is resistance currently detected in routine clinical oncology? As hitherto, by the measurement of tumor mass, only following several weeks of experimental drug treatment, though nowadays with improved procedures

of imaging, such as sonography, computed tomography, and magnetic resonance imaging, but still with relatively sluggish parameters. Only following tumor progression, recognizable by an increase in tumor size, can resistance be diagnosed and can it be found which patients profit from the therapy and which do not. As the growth curve of tumors flattens with increasing size, according to Gompertz kinetics, the diagnosis of tumor progression with some reliability is often possible only after much delay.

Tumor mass measurement is, however, not the only technique available to obtain information on tumor progression and resistance. There are now laboratory methods available which are able to prove tumor resistance much earlier.

Tests to Demonstrate Intrinsic Drug Resistance

Some small groups of research workers have been involved for several decades in the development of resistance assays. As long ago as the 1950s, the first attempts were made to test the prospects of success in the therapy of cancer using cytostatic drugs in cell cultures. The clinical observation that patients with histologically identical tumors reacted very differently to the same cytostatic led at the time to the idea of testing tumors individually as to their behavior [17, 18]. The development of tests has until recently concentrated almost exclusively on the demonstration of resistance prior to medication. The principle of the tests consists in incubating tumor cells from fresh tumor tissue obtained during operations with various cytostatics, and registering reactions to the growth of the tumor cells. Widely differing lab techniques have been applied in recent decades, both in harvesting the tumor cells and in the evaluation of the effect on cell vitality [19–21]. The two test methods competing for the favor of researchers are related to the type of *in vitro* cell growth, namely clonogenic and nonclonogenic assays. In the former case, growth takes place in cell clusters, and in the latter in cell layers. The study endpoints measuring cell damage vary, relating either to the loss of cell membrane integrity, loss of mitochondrial Krebs cycle activity, loss of cellular ATP, or inhibition of the incorporation of thymidine in the cell DNA. Without going into more detail about the advantages and disadvantages of the individual tests, it can be stated that nonclonogenic cell tests have been applied for preference during the past decade.

Although most tests have been able, in different ways, to demonstrate a congruence between resistance *in vitro*

and effect in vivo [22], the clinical use of the tests as a pharmacological application criterion has gained little attention. One factor responsible for this is no doubt the wide variety of lab techniques applied, which have made it more difficult to arrive at a generally recognized test with standard conditions. As the pharmaceutical industry has shown little interest in resistance assays, the initiative for the introduction of standardized tests has remained with research institutions having limited financial means.

Two tests have meanwhile achieved commercial status, by which the great advantages of standardization of the procedures and improved comparability have been achieved. These are, firstly, the 'Extreme Drug Resistance Test' introduced by Kern and Weisenthal [23] which, through incubation of the cell cultures with high doses of cytostatics, determines the substances which allow cells to continue to grow. Resistance is determined from the unhindered incorporation of radioactive thymidine into cells. The second test is the 'ATP Tumor Chemosensitivity Assay' developed by Andreotti and co-workers [24] which, after administering several doses of a cytostatic, establishes a dose-response profile. The effects are obtained by measuring the intracellular ATP content. Apart from resistance, this test also provides, though with a low prediction value, data of sensitivity to cytostatics. Both tests register tumor resistances with an accuracy of around 90%.

For completeness' sake, the measurement of resistance by the transplantation of human tumor cells into immune-deficient mice should also be mentioned [25, 26]. Here too, the experiments, which began in the 1960s, have achieved a degree of perfection in the demonstration of the resistance of individual tumors to cytostatics. Good correlations between tumor resistances and clinical data have been reported. However, because of the high laboratory requirement, these tests are not suitable for routine clinical use. Some significance is attributed to them, however, for the development of anticancer drugs [27–29].

Tests to Demonstrate Acquired Drug Resistance

Acquired drug resistance, i.e. the development of resistance while the patient is undergoing chemotherapy, is a further problem in the pharmacotherapy of advanced cancer disease [5, 12]. The detection of this resistance at an early stage has remained very limited up to the present. Where tumor tissue is available, the cellular lab tests are also applicable in determining acquired resistance.

What procedures exist today to test the development of resistance under treatment with cytostatics? Small groups of researchers have long studied the use of tumor markers. These bio-markers are released into the bloodstream by tumors, and provide more or less reliable data on tumor growth [30]. However, there exist no reliable studies on tumor markers with regard to the occurrence of resistance, so that these parameters cannot yet be applied to determine individual resistances. It remains hard to understand why, in view of the great number of studies on chemotherapy, the role of tumor markers has not been systematically investigated at the same time in these studies.

A rapid and accurate measurement of resistance is, however, currently possible using a procedure which is clinically established, positron emission tomography (PET). Briefly summarized, this method is based on the intravenous injection of biological tracers, marked with short-living radioactive isotopes, which are taken up by the active tumor cells more rapidly than by normal body cells. PET permits the detection of very small active tumors, and a failure to respond to medication can thus in many cases be diagnosed shortly after commencing therapy. The sensitivity of this method is so high that, in contrast to computed tomography or magnetic resonance imaging, results can be obtained before morphological changes in the tumor take place [31].

A very important factor in the early detection of acquired resistance is knowledge of the time interval from the administering of cytostatics to the developing of resistance. There appear to be no studies on the measurement of such time intervals in tumor patients. There are, however, indications of a rapid development of resistance following the administering of cytostatics. Thus, it has been observed that toxic substances which damage the DNA are able within a very short time to induce the c-fos and c-jun genes, which in turn can set off a chain reaction by inducing various resistance proteins [7]. A further indication of a rapid development of resistance has been given by a recent experimental study on human mammary, ovarian, esophageal and colorectal tumors [32]. The results showed that drug-induced resistances had already occurred in histologically identical tumors a few days after treatment with chemotherapy. By use of the PET test, an early registration of resistance development seems to be possible.

PET research has made considerable advances in the last few years. In addition to the routine clinical application of the glucose analog ^{18}F 2-fluoro-2-deoxyglucose, which measures the energy metabolism of the cell, further cell functions can be tested in vivo. Thus, activities

of protein metabolism using marked amino acids, the rate of DNA synthesis using thymidine, and the composition of cell membrane using cholins can now be tested, i.e. functions which are also of importance for the cellular cytostatic effect. Currently, methods for measuring more specific cell functions are being investigated using PET, such as HER2/neu receptor activities [33]. The use of PET for individualized cancer treatment, as well as for the development of new anticancer drugs, is thought to be promising [34–36]. One weak point of PET, namely the lack of the exact anatomic position of the tumor activity measured, has recently been eliminated by the combination of the PET camera with computer tomography [37]. The cost of this testing method is, however, great. A benefit-risk analysis, comparing the high cost of the test with ineffective and furthermore harmful chemotherapy, should favor the test.

Pharmacogenetic Tests

A new field for the early detection of tumor resistances may be opened up by progress in pharmacogenetics [38]. The realization that individual reactions to drugs are influenced by genes could be of great importance for medical therapy as a whole. Since industrial interests are involved, great efforts are currently being undertaken to make a personalized application of drugs available in the chemotherapy of cancer as well. The principle of the tests consists in the fact that DNA of the tumors as biomarkers provides indications of the reaction of an individual organism to a given drug. As in the case of cell culture resistance assays, tissue samples of the tumor are necessary for analysis. The cell material no longer needs to be vital, i.e. fixed histological preparations can also be used.

The process of evaluation affords very high logistic demands, owing to the numerous and very complex alterations in the genes that affect the detection of the reactions resulting from the drug treatment. Only with recently invented technical equipment has it now become possible to analyze and calculate the great number of processes that take place in human genes.

However, pharmacogenetic diagnostic is still in its infancy. Statements in this field must still be treated with caution, since misinterpretations of results are possible [39]. The microassays used for diagnosis are not yet standardized, which impedes comparisons of the lab-dependent results [40]. The first commercial pharmacogenetic tests, whose results are still of limited value, are already available. Thus a gene test approved by the American

FDA is able to assess the individual chances of treatment with the cytostatic irinotecan [41]. Other gene tests commercially available are the MammaPrint Microarray Test and the Oncotype DX test, which provide information on the danger of relapse in women operated on for breast cancer [42, 43]. These tests are, however, unable to give information on chemotherapy that may become necessary; they can at best help to avoid the use of an adjuvant chemotherapy in cases where a low risk is established.

Although already conceivable in theory, it seems unlikely that gene tests for a number of cancer drugs may soon be developed that provide predictions regarding the prospects of therapy. Also, some time will elapse before the existing gene tests are fully recognized, since so far no prospective studies on these tests exist [44].

How Valid Are Current Resistance Tests?

Following a critical report in the *New England Journal of Medicine* [45] on possible artifacts in a particular tumor cell test in the 1980s, the view became widespread among oncologists that all cell tests for the measurement of resistance are unreliable. In consequence, research activities, which were on the increase at the time, suffered an important setback [46]. Meanwhile, important progress has been made in lab techniques, and high correlations have been achieved between in vitro findings and patient reactions. The ATP-test described above shows a high rate of success, at approximately 90% positive predictions of resistance [22, 47]. Problems in testing tend to stem mainly from incorrectly submitted tumor material.

Although numerous studies document the value of the measurement of resistance based on primary cell cultures, the American Society for Clinical Oncology, a worldwide opinion leader, has retained its early rejective attitude towards these tests. In a statement dating from 2004, it arrives at the conclusion, based on a literature survey, that existing cell culture tests from fresh tumor tissue are still 'investigational', and should not be applied in individual cases. Since, however, a possible significance cannot be denied to in vitro tests, it states that their use can be justified only in clinical studies [48]. For this statement, of the many existing studies (over a thousand) only 12 were adduced, which fulfilled certain criteria. These were studies published between 1983 and 2003 and which had used various test methods (6 in all). There is no account of the individual lab techniques. It fails to

emerge from the publication whether resistance and sensitivity to cytostatics in the tests were assessed differently, although this is of fundamental importance. Whereas resistance behavior can be measured relatively reliably in isolated tumor cells, the sensitivity of the tumor to a cytostatic depends on numerous additional factors in the human organism. Thus, in the case of a positive resistance test, only ineffective chemotherapy can be prevented, but no conclusions can be drawn as to sensitivity. Finally, it is not surprising that in the evaluation of these inhomogeneous studies, no clear statement could be made on resistance-assay-assisted chemotherapy compared to chemotherapy with empirically administered cytostatics. The demand that prospective randomized studies be required for a recognition of resistance tests [48] can hardly be realized, for reasons of cost. The objection is raised elsewhere that no proof of successful treatment by randomized clinical studies was required for any other lab test, including estrogen receptor measurement [49].

A number of standard chemotherapies can be expected to be less frequently applied as a consequence of clinical use of tumor cell resistance tests. According to recent publications [12], a high degree of resistance is also found in new cytostatics that are used in standard combinations. Clinging to the idea that a histologically homogeneous tumor group can best be treated with standard chemotherapy appears to be still firmly rooted in the predominant opinion of oncologists. This is clearly expressed in the most recent mammoth work on cancer medicine, a recognized publication of the American Association for Cancer Research [2]. Although it describes in great detail almost all aspects of the wide field of cancer therapy, one finds no mention of resistance tests for an individual therapy of cancer patients.

Demonstrating acquired resistance involves considerably greater difficulties, as the resistance occurs during treatment. Tumor tissue is only available in exceptional cases, making *in vivo* tests necessary. Some methods already described are still at an investigational stage [50]. The PET-test, available only in highly specialized centers, is able to contribute considerably to the early detection of acquired resistance. A demand for validation by prospective randomized studies is not to be expected, since it is already clinically established.

It is currently open to question whether pharmacogenetic tests, which have yet to prove themselves in the demonstration of intrinsic resistance, also play a part in acquired resistance. The recent discovery of nucleic acids (DNA and RNA) in cell-free body fluids such as plasma,

serum and urine should open up new ways to promote research into individual disease monitoring, particularly in cancer [51].

Future Prospects for Resistance Assays

The search for new anticancer drugs for targeted use has never been more intensive than at present. Great efforts are being made to develop new therapy strategies with antibodies against overexpressed growth factors. In view of the heterogeneity of cancer properties, with cellular defense against drugs used, problems of resistance are also bound to predominate in this new field.

The problems in cancer therapy to be expected in the future are discussed in detail in a recently published survey [52]. According to this, a flood of new anticancer drugs must be expected in the next few years. The number of 500 substances tested in studies in 2005 is estimated to rise to 5,000 by 2010. Meanwhile, the cost of cancer therapy will increase considerably. The authors assume that, despite many new drugs, whose development will swallow up huge sums, curative progress will remain small. New technologies are required, to find out which patients profit from a given therapy and which do not. Of interest is the conclusion that pharmaceutical firms will not necessarily make a contribution to the development of such technologies, as this would fragment the market for cytostatics. A personalized cancer therapy must also render it possible to include tumor cases that would be better off without drug cancer therapy [52].

In conclusion, it may be recognized that, in view of the heterogeneity of the biological properties of malignant solid tumors, an individual, personalized cancer therapy is indispensable. An individualized chemotherapy is already possible at present, by measuring tumor resistances before medication by means of laboratory cell culture tests. This requires tumor tissue in vital condition obtained during operative removal of the tumor. Thus, the decision to measure resistance must be made prior to operation. The PET test, however, can be carried out *in vivo* without extirpation of the tumor. With resistance tests, chemotherapy can be optimized by avoiding the use of individually ineffective drugs. Since standard therapies only harm patients in cases of tumor resistance, the 'trial-and-error' strategy of the blind administering of highly toxic cytostatics is indefensible. An important step forward to an individualized cancer therapy can be expected from the further development of easily performed and reliable tumor resistance tests.

References

- Woodruff M: Cellular Variation and Adaption in Cancer, Biological Basis and Therapeutic Consequences. Oxford, Oxford University Press, 1990.
- Kufe DW, Bast RC, Hait WN, Hong WK, Pollack RE, Weichselbaum RR, Holand JF, Frei E: Cancer Medicine. London, BC Decker, 2006.
- Chang AE, Ganz PA, Hayes, DF, Kinsella TJ, Pass HI, Schiller JH, Stone RM, Strecher VJ: Oncology: An Evidence-Based Approach. New York, Springer, 2006.
- Berger DP, Engelhardt R, Mertelsmann R: Das Rote Buch, Hämatologie und Internistische Onkologie. Landsberg/Lech, Ecomed Medizin, 2006.
- Pinedo HM, Giaccone G: Drug Resistance in the Treatment of Cancer. Cambridge, Cambridge University Press, 2006.
- Volm M, Mattern J: Resistance mechanisms and their regulation in lung cancer. *Crit Rev Oncogen* 1996;7:227–244.
- Volm M: Multidrug resistance and its reversal. *Anticancer Res* 1998;18:2905–2918.
- Volm M, Mattern J, Koomägi R: Expression of lung resistance protein (LRP) in non-small cell lung carcinomas of smokers and non-smokers and its predictive value for doxorubicin resistance. *Anti-Cancer Drugs* 1997;8:931–936.
- Volm M, Mattern J, Samsel B: Overexpression of P-glycoprotein and glutathione S-transferase- π in resistant non-small cell lung carcinomas of smokers. *Br J Cancer* 1991;64:700–704.
- Volm M, Koomagi R, Efferth T: Prediction of drug sensitivity and resistance of cancer by protein expression profiling. *Cancer Genomics Proteomics* 2004;1:157–166.
- Andersson B, Murray D: Clinically Relevant Resistance in Cancer Chemotherapy. Boston, Kluwer Academic Publishers, 2002.
- Teicher BA: Cancer Drug Resistance. Totowa, Humana Press, 2006.
- Moscow J, Schneider E, Sikic BI, Morrow CS, Cowan KH: Drug resistance and its clinical circumvention; in Kufe DW, Bast RC, Hait WN, Hong WK, Pollack RE, Weichselbaum RR, Holland JF, Frei E (eds): Cancer Medicine. London, BC Decker, 2006, pp 630–647.
- Tsuruo T, Iida H, Tsukagoshi S, Sakurai Y: Overcoming of vincristine resistance in P 388 leukemia in vivo and in vitro through enhanced cytotoxicity of vincristine and vinblastine by verapamil. *Cancer Res* 1981;41:1967–1972.
- Decker S, Sausville EA: Preclinical modeling of combination treatments: fantasy or requirement? *Ann New York Acad Sci* 2005;1059:61–69.
- Weisenthal LM: Antineoplastic drug screening belongs in the laboratory, not in the clinic. *J Natl Cancer Inst* 1992;84:1288–1290.
- Dawson M, Dryden WF: Tissue culture in the study of the effects of drugs. *J Pharm Sci* 1967;56:545–561.
- Tanneberger S: Gewebekultur und Krebschemotherapie. *Arch Geschwulstforsch* 1968;31:387–400.
- Dendy PP, Hill BT: Human Tumour Drug Sensitivity Testing in vitro: Techniques and Clinical Applications. New York, Academic Press, 1983.
- Masters JRW: Human Cancer in Primary Culture, a Handbook. Dordrecht, Kluwer Academic Publishers, 1991.
- Dickson RB, Lippman ME: Drug und Hormonal Resistance in Breast Cancer Cellular and Molecular Mechanisms. New York, Ellis Horwood, 1995.
- Reinhold U, Tilgen W: Chemosensitivity Testing in Oncology. Berlin, Springer, 2003.
- Kern DH, Weisenthal ML: Highly specific prediction of antineoplastic drug resistance with an in vitro assay utilizing suprapharmacologic drug exposures. *J Nat Cancer Inst* 1990;82:582–588.
- Andreotti PE, Cree IA, Kurbacher CM, Hartmann DM, Linder D, Harel G, Gleibermann I, Caruso PA, Ricks SH, Untch M, Satori C, Bruckner HW: Chemosensitivity testing of human tumors using a microplate adenosine triphosphate luminescence assay: clinical correlation for cisplatin resistance of ovarian cancer. *Cancer Res* 1995;55:5276–5282.
- Mattern J, Bak M, Hahn EW, Volm M: Human tumor xenografts as model for drug testing. *Cancer Metastasis Rev* 1988;7:263–284.
- Reynolds CP, Sun B, DeClerck YA, Moats RA: Assessing growth and response to therapy in murine tumor models; in Blumenthal RD (ed): Chemosensitivity. In vivo Models, Imaging and Molecular Regulators. Totowa, Humana Press, 2005, vol 2, pp 335–350.
- Winograd B, Boven E, Lobberzoo MW, Pinedo HM: Human tumor xenografts in the nude mouse and their value as test models in anticancer drug development. *In Vivo* 1987;1:1–13.
- Fiebig HH, Maier A, Burger AM: Clonogenic assay with established tumour xenografts: correlation of in vitro to in vivo activity as a basis for anticancer drug discovery. *Eur J Cancer* 2004;40:802–820.
- Teicher BA: Tumor models for efficacy determination. *Mol Cancer Ther* 2006;5:2435–2443.
- Diamandis EP, Fritsche HA, Lilja H, Chan DW, Schwartz MK: Tumor Markers, Physiology, Pathobiology, Technology and Clinical Applications. Washington, AACCC Press, 2002.
- Jerusalem G, Belhocine TZ: Metabolic monitoring of chemosensitivity with ^{18}F FDG PET; in Blumenthal RD (ed): Chemosensitivity. In vivo Models, Imaging, and Molecular Regulators. Totowa, Humana Press, 2005, vol 2, pp 417–440.
- Di Nicolantonio F, Mercer SJ, Knight LA, Gabriel FG, Whitehouse PA, Sharma S, Fernando A, Glaysher S, DiPalma S, Johnson P, Somers SS, Toh S, Higgins B, Lamont A, Gulliford T, Hurren J, Yiangou C, Cree IA: Cancer cell adaptation to chemotherapy. *BMC Cancer* 2005;5:78.
- Jager PL, de Korte MA, Lub-de Hooge MN, van Waarde A, Koopmans KP, Perik PJ, de Vries EG: Molecular imaging: what can be used today. *Cancer Imaging* 2005;5:S27–S32.
- Jain KK: Personalised medicine for cancer: from drug development into clinical practice. *Expert Opin Pharmacother* 2005;6:1463–1476.
- Czernin J, Weber WA, Herschman HR: Molecular imaging in the development of cancer therapeutics. *Ann Rev Med* 2006;57:99–118.
- Kenny LM, Aboagye EO, Price PM: Positron emission tomography imaging of cell proliferation in oncology. *Clin Oncol* 2004;16:176–185.
- Conti PS: The future of radioimmunotherapy: a PET perspective. *Semin Oncol* 2005;32:(suppl 1):S63–S67.
- Efferth T, Volm M: Pharmacogenetics for individualized chemotherapy. *Pharmacol Ther* 2005;107:155–176.
- Dupuy A, Simon RM: Critical review of published microarray studies for cancer outcome and guidelines on statistical analysis and reporting. *J Natl Cancer Inst* 2007;99:147–157.
- Kawasaki ES: The end of the microarray Tower of Babel: will universal standards lead the way? *J Biomol Tech* 2006;17:200–206.
- Hasegawa Y, Ando Y, Shimokata K: Screening for adverse reactions to irinotecan treatment using Invader UGT1A1 molecular assay. *Expert Rev Mol Diagn* 2006;6:527–533.
- Glas AM, Floore A, Delahaye LJ, Witteveen AT, Pover RC, Bakx N, Lahti-Domenici JS, Bruinsma TJ, Warmoes MO, Bernards R, Wessels LF, Van't Veer LJ: Converting a breast cancer microarray signature into a high-throughput diagnostic test. *BMC Genomics* 2006;7:278.
- Kaklamani V: A genetic signature can predict prognosis and response to therapy in breast cancer: Oncotype DX. *Expert Rev Mol Diagn* 2006;6:803–809.
- Kaklamani V, Gradishar WJ: Gene expression in breast cancer. *Curr Treat Options Oncol* 2006;7:123–128.

- 45 Selby P, Buick RN, Tannock I: A critical appraisal of the human tumor stem cell assay. *New Engl J Med* 1983;308:129–134.
- 46 Weisenthal LM: Predictive assays for drug and radiation resistance; in Masters JRW (ed): *Human Cancer in Primary Culture*. Dordrecht, Kluwer Academic Publishers, 1991, pp 103–147.
- 47 Blumenthal RD: Chemosensitivity. In *vitro* Assays. Totowa, Humana Press, vol 1, 2005.
- 48 Schrag D, Garewal HS, Burstein HJ, Samson DJ, von Hoff DD, Sommerfield MR: American Society of Clinical Oncology Technology assessment: chemotherapy sensitivity and resistance assays. *J Clin Oncol* 2004;22:3631–3638.
- 49 Weisenthal LM: Fresh-tumor cell-culture assays for breast cancer; in Dickson RB, Lippmann ME (eds): *Drug and Hormonal Resistance in Breast Cancer, Cellular and Molecular Mechanisms*. New York, Ellis Horwood, 1995, pp 323–349.
- 50 Blumenthal RD: Chemosensitivity. In *vivo* Models, Imaging and Molecular Regulators. Totowa, Humana Press, vol 2, 2005.
- 51 Swaminathan R, Butt A, Gahan P: Circulating nucleic acids in plasma and serum IV. *Ann NY Acad Sci* 2006;1075:1–353.
- 52 Bosanquet N, Sikora K: *The Economics of Cancer Care*. Cambridge, Cambridge University Press, 2006.