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Biopharmaceutical applications of the tumor response assay

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The TCA-100 Tumor Chemosensitivity Assay first reported in 1990 has been further developed into the TRA Tumor Response Assay for both biopharmaceutical and clinical applications. Changes to the original cell culture and ATP bioluminescence test system provide a simpler, faster, and less expensive test system that is better suited for biopharmaceutical applications, genomic studies, and sequential testing of clinical specimens. These changes include culture microplates containing dried test drugs and controls, and counting microplates containing dried ATP extraction reagent, luciferin/luciferase counting reagent, and ATP standards. A principal rationale for modifying the established test system is to perform DNA microarray studies that will be discussed. Results will be presented demonstrating application of the TRA system for peptide biopharmaceutical agents, monoclonal antibodies, new chemotherapeutic drugs, and sequential testing of clinical specimens using standard chemotherapeutic drugs.

The use of the MTT assay for chemosensitivity testing

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Despite substantial advances in the biological and molecular characterisation of cancer, drug resistance remains a major problem. Identification of resistance in individual patients allows customisation of therapy giving improved prognosis. We have found the short-term MTT assay to be a simple, reproducible chemosensitivity technique, suitable for use throughout the time course of disease. We now have 12 years experience of using this method in a variety of tumour types, both maematological and solid tumours. Tumour cells are isolated from bone marrow, malignant effusions or solid biopsies and subjected to drug exposure for 48–96 hours. Cell survival is measured by re-incubation in MTT for 4 hours. We have found a significant correlation of *in vitro* results with *in vivo* outcome for AML and for ovarian cancer (both $p < 0.0001$) with an assay sensitivity of 98% for AML and 81% for ovarian cancer. Furthermore, the 5 year survival of ovarian cancer patients treated with a drug found sensitive *in vitro* is significantly higher than that for patients treated with a drug found resistant *in vitro* ($p = 0.033$). We have correlated assay results with drug resistance markers. For example, expression of the newly described half transporter BCRP correlates with daunorubicin resistance ($p < 0.05$). The MTT assay is also suitable for screening for modulation of drug resistance. We have found the DNA polymerase inhibitor aphidicolin markedly increases *in vitro* sensitivity to the platinums in ovarian cancer and cytosine arabinoside in AML in the majority of patients. The greatest effect was seen for patients deemed resistant *in vitro* to these agents. We have also identified novel drug combinations which demonstrate significant synergism using this methodology. In conclusion, we have found the MTT assay to be a simple, repeatable, adaptable technique which produces accurate information to help the clinician select treatment for individual cases.

Chemosensitivity testing of human tumors using Si-sensor-chips

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The chemotherapy is, on its own or in combination with other treatments, a very effective anticancer therapy. Introduced in the middle of the last century, chemotherapy today still faces the problem of determining which specific agent or agents are able to yield the desired clinical therapeutical effect for a particular tumor and patient. Numerous tests *in vitro* were developed to detect chemosensitivity, chemoresistence and also for the screening of new drugs. Three groups of tests can be defined: 1.- cell viability tests, 2.- measurements of cell metabolism and 3.- clonogenic assays. Test time, tissue preparation and complexity of test performance and correlation with clinical progress of the disease are criteria used to judge how successful they are. The introduction of Si-sensor-chips, which are able to detect metabolic changes in living cells, has opened up new possibilities in this field. Basically two sensor principles or types can be considered: a) the light addressable potentiometer sensor (LAPS) and b) the multisensor array (MSA). Whereas LAPS measure one, MSA register online many parameters (for instance, impedance, pH, O2, temperature). The aim is to review this technology and to present recent applications using cells, tissue slices and biopsies.

Multiparametric sensor chips for chemosensitivity testing of sensitive and resistant tumor cells

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Even when chemotherapeutic regimes are developed for treating particular types of tumors, not every patient will benefit due to individual resistance patterns of tumors. Unnecessary chemotherapeutic treatment could be avoided and potentially more effective drugs could be selected if the sensitivity of the tumor for the drugs were known before the beginning of the treatment. To this end, many approaches have been employed to test for the sensitivity of the tumor to the drug *in vitro*. However, such assays are usually based on only one cellular/biochemical parameter, are performed only at selected time points, and therefore generally have little predictive value. The development of multiparametric micro-sensor chips overcomes such drawbacks. Cells and tissues grow directly on the sensors. A fluidic system mimics physiological conditions by providing nutrients (and drugs) and removing metabolic products. This setup allows for detection of changes in the immediate microenvironment of the cells. At least three parameters are presently amenable to sensor readings on the same chip: changes in the extracellular pH and pO_2, reflecting metabolic activities, and changes in impedance, reflecting morphological properties such as cell-cell and cell-matrix adhesion. Further advantages of this system include: long term culture (several days), continuous data acquisition, analysis before and after drug administration on the same specimen, and small amount of cellular specimen. Data obtained with drug sensitive and resistant cell lines as well as with cancer explants clearly demonstrate the utility of the microsensor-chip in showing the effects of antitumor drugs in changing the metabolism and morphology.

Development of new *in vitro* chemosensitivity testing using collagen gel droplet embedded culture and image analysis for clinical usefulness

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We have developed an *in vitro* chemosensitivity test using collagen gel droplet embedded culture and image analysis. This assay shows the following performances: 1) a high success rate for primary culture, 2) a small number of cells being required for the test, 3) easy quantification of the anticancer effects without contamination with fibroblasts, and 4) evaluating the anticancer effects under the conditions of clinically equivalent concentrations of drug. We have called this method the collagen gel droplet embedded culture drug sensitivity test namely CD-DST. The CD-DST was conducted with several types of solid cancer. The overall evaluable rate was more than 80% in case of lung, breast, gastric, colorectal and metastatic brain cancers. The clinical correlation to chemotherapy investigated the assay in 11 patients was 91%, with a 80% true positive and 100% true negative rate. We were going to introduce the clinical usefulness and possibility to chemosensitivity of CD-DST.

In vitro chemosensitivity testing of hematological cancer patients: detection of ornithine decarboxylase

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Ornithine decarboxylase (ODC), which catalyzes the formation of putrescine from ornithine, has been found to be a marker of proliferation. It is expressed early in the cell cycle, is present in all mammalian cells and has an extremely short half-life. A new method for the *in vitro* chemosensitivity testing of human lymphocytes has been developed. The test is based on the quantitative detection of ODC using an immunohistochemical method. Lymphocytes from cancer patients were treated with the selected drugs for 6–12 hours, followed by incubation with an ODC antibody and an FITC-linked second antibody. The *in vitro* chemosensitivity of lymphocytes from normal individuals were compared with lymphocytes from 50 hematological cancer patients. In drug-sensitive cells, the intensity of the marker declined in the presence of the drug, whereas resistance to the drug was established by the presence of the marker upon exposure to the drugs. A good correlation was found between the predicted chemosensitivity and the outcome of the treatment. Moreover, the test permitted the detection of multiple-drug resistant cells. It has been suggested that this approach could be used for the *in vitro* chemo-

sensitivity testing of hematological cancers and most likely also for other malignancies.

Apoptosis examined by TUNEL and TEM is correlated with chemosensitivity or chemoresistance in human solid tumours

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Chemotherapy should not be administered to cancer patients under a block-buster policy. We believe that each cancer type is like a fingerprint for the individual cancer patient and that treatment should be tailored to each cancer patient's needs. Thus, we examine *in vitro* chemosensitivity of tumour specimens from cancer patients taken from biopsy or surgical excision. Tumour cells are isolated by the collagenase method and they are incubated for 24 hours with 22 drugs which include antimetabolites, topoisomerase inhibitors, alkylating agents and plant alkaloids.In case there is reported chemoresistance, we try to identify by PCR, Western blot and immunochemistry overexpression, mutations and deletions in aminoacid sequences of oncogenes and tumour suppressor genes. Then, we interfere with oligonucleotides or antibodies to downregulate oncogene expression, and, we replace inactivated tumour suppressor genes. These gene corrections will allow induction of apoptosis after chemotherapy. The chemosensitivity test is performed as follows:

Apoptosis is determined in tumour cells of small specimens of various solid tumours after incubation with anticancer agents in culture medium for 24 hours by terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick labeling (TUNEL) and transmission electron microscopy. We check the percentage of TUNEL-positive tumour cells (TUNEL positivity) and formation of apoptotic bodies which indicate an irreversible D2 stage. We are interested to find the four most active drugs which produce the highest TUNEL positivity and highest percentage of apoptotic bodies termed as high level apoptosis (HLA) with $>50\%$ of apoptotic tumour cells. If we get low level apoptosis (LLA) or no detected apoptosis, we proceed with identification of genetic alterations which are correlated with inhibition of apoptosis (p53, bcl-2, HER-2, Apaf1, Ras etc). Concluding, chemosensitivity was correlated with apoptotic percentage of tumour cells.

Towards *in vitro* predicting of *in vivo* response of human tumor cells with a fast chemosensitivity assay

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The chemosensitivity assay Chemo*Select*® has its roots in pharmaceutical drug screening and is a sensor-chip based diagnostic test which permits the functional and continuous real-time measureent of induced tumor cell cytotoxicity following the administration of chemotherapeutic drugs. We used this test to study the applicability of this assay for tumor cells based on the analysis of tumor cell lines and tumor specimens. In this preliminary study, this test was studied in predicting chemoresistance and chemosensitivity in cell lines and tumor specimens for which the result was already predetermined by the properties of the cell line or the tumor specimen used in the experiment. The applicability in a clinical setting was studied by confirming the trends on selected drug sensitivity and drug resistance with an interim analysis of an ongoing clinical study in selected patients with breast cancer undergoing neoadjuvant chemotherapy. The minimum detection limit of cells and biologic cell responses, an important variable determining the applicability of the test in routine clinical use, was also assessed.

Chemoluminescence detection of ATP in heterologous cell culture after cytostatic treatment

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Introduction: A modified testing system for the evaluation of cytotoxicity effects of cytostatics in heterologous tumor single cell cultures was established.

Method: The tumor tissue is digested into single cells using a collagenase-DNAse cocktail at 37°C for maximally 60 min. Non-digested tissue is then treated with Pancreatin at 37°C for short time. Small tissue pieces are then passaged through a 100 μm cell mesh for further dissociation to single cells. 20.000 cells/well are cultivated with RPMI in clear-bottom ELISA plates in the presence or without cytostatics. Cytostatics are diluted in a linear degression. Viability, morphologic and sterile controls are continuously

performed during culture. Effects of cytostatics are measured using a ATP detection system basing on a chemoluminescent ELISA by Roche Diagnostics. Optical densities of ATP measured in cell cultures are correlated with the viability of cells.

Results: In this test we measure the effects of all cytostatics commercially available in heterologous cell cultures of all metastatic tumor types. We test fine needle biopsies, solid tumor tissue and cell material derived from ascites and pleural punctures. Cellular ATP produced in the presence of cytostatics is detected in comparison to ATP secretion of non-treated, and of not lysed cells and in comparison to a ATP standard curve. Cell culture is performed for three days to avoid production of cytotoxic stimulators during culture. ATP is measured with a chemoluminescence -detecting TECAN XFluor4 program. Correlation of ATP with the viability of cells is calculated with a non-commercial program developed by M. Mappes. Cells not needed for testing can be stored or used for immunohistochemical detection of specific tumor markers.

Discussion: We plan to reproduce our test system in a study.

Application of *in vitro* ATP testing for development of gastrointestinal cancer chemotherapy

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In vitro methods can prioritize the choice of novel empirical therapy for testing as well as discover novel treatments for individual patients. Testing a panel of disease and treatment history specific tumors allows ordering of candidate drug pairs based on the frequency with which they produce synergism supplemented by additional criteria defined and used in an algorithm-patent pending. In the case of tumors with conventional high rates of resistance to conventional therapy such as pancreatic cancer the frequency of synergism is not great enough to predict success for the conventional drug pairs now in testing, pairs such as gemcitabine (G) FU/FA and with either cisplatin or oxaliplatin. Similarly G and either taxanes (T) or irinotecan (Ir) in spite of their single agent activity seem unlikely or only marginal prospects for success in spite of some rate of *in vitro* synergism. Even the three drug combinations in current testing GFP and GF LOHP appear destined for marginal if any success based on the algorithm. The three potential interactions do not occur with adequate frequency, GP > FP > GF. Adding a 4th drug, Irinotecan, increases the number of drug pairs with potential interaction from three to six interactive pairs. Taxanes represent another candidate drug found promising *in vitro* for three and four drug combinations. The Irinotecan regimens appear active in all GI cancers and the Taxanes in all UGI cancers tested *in vitro*. Clinically several laboratory derived regimens have been used by the author in broad fashion with promising preliminary results against cholangio ca, stomach and pancreatic cancer. *In vitro* findings also predicted the clinically observed activity of the IrP combination and predict further improvement adding G and FU/FA producing the G-FLIP regimen, ASCO and SOMPS 2001. As predicted by *in vitro* findings AACR1998 and 1999 clinically 80 mg/M2 can reactivate GFP for pancreatic patients already failing the three drugs. This produced > 50% response and stable disease and 10 added mos. median survival after first clinical failure vs. the expected 9–18% benefit and 4mos added survival. Results in gastric cancer may be even better after failure of FU/FA P. As primary therapy G-FLIP produces 13/15 response and stable disease in high risk patients, half the patients were too sick for conventional protocol therapy. Taxanes which are also active at the 10 or 20% level depending on the choice of *in vitro* criteria (perhaps stable disease included in the latter 20% predictive criteria), *In vitro* preliminary information predicts T can produce third treatment response when used in combination and this has been confirmed clinically using I-T-P ± Xeloda or G-T ± Xeloda ± P, even after failure of G-FLIP, prior failure of all the drugs except T, an other example of recruitment, as the *in vitro* test predicted.

The chemosensitivity profile of retinoblastoma

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Introduction: Retinoblastoma is a rare malignant tumour of the developing retina with an incidence of 1 in 20 000 live births in all human races. Chemotherapy is used in retinoblastoma as adjuvant therapy to prevent the growth of metastases and to treat metastatic disease once this has become clinically apparent. Current regimens are based on empirical drug combinations and few clinical trials have been

conducted due to the rarity of this tumour. Chemosensitivity testing offers a way of testing a large number of agents against tumours. The ATP-based chemosensitivity assay (ATP-TCA) has already helped to design new regimens for melanoma, breast and ovarian cancer.

Methods: Primary retinoblastoma tumour material was obtained from ten eyes, seven of which contained sufficient viable cells for ATP-TCA.

Results: The results show very high sensitivity to single agents, particularly cisplatin, doxorubicin and vinca alkaloids. Of the anti-metabolites tested, 5-FU is relatively disappointing (though still active), and gemcitabine shows considerable activity consistent with a cytotoxic effect. The shape of the inhibition curves is interesting. There is a plateau effect with the topoisomerase inhibitors and vinblastine, which is not present with the cisplatin. One tumour was much more resistant than the others tested particularly to vinblastine, but also to the topoisomerase inhibitors which fail to achieve complete kill at any concentration tested, consistent with a multidrug resistance phenotype. Of the combinations (VAC and VEC), the VAC regimen looks marginally more active in the more resistant of the two cases tested to date.

Conclusions: These data confirm that retinoblastoma is a rapidly growing malignancy that is very susceptible to cytotoxic drugs of all types. Chemosensitivity testing provides a practical method of testing new regimens before clinical trials in retinoblastoma patients.

Chemosensitivity testing in malignant melanoma

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The prognosis of patients with metastatic melanoma remains poor. The most effective single agents in stage IV patients produce response rates between 10–15% and the expectation for 5-year survival is less than 10%. More aggressive treatments with chemotherapy combinations yield approximately 40% responses but have no survival advantage over treatment with single agents. Dacarbazine remains the reference standard treatment for stage IV melanoma. We have examined the heterogeneity of chemosensitivity and chemoresistance in metastatic melanoma patients using an *in vivo* ATP-based chemosensitivity assay (ATP-TCA). A total number of 196 tests with 9 different single cytotoxic agents and with 7 different combinations of cytotoxic agents was performed. The chemosensitivity was analysed using an arbitrary sensitivity index. We found a considerable heterogeneity of chemosensitivity and chemoresistance. The highest cytotoxic activity was observed by the following cytotoxic drug combinations: treosulfane + gemcitabine, gemcitabine + cisplatin and paclitaxel + doxorubicine. Our data indicate that the ATP-TCA can be used to select patients who might benefit from specific chemotherapeutic combinations. Based on the results we have enrolled a German multicenter trial of a ATP-TCA-directed chemotherapy in metastatic melanoma.

In vitro drug resistance in childhood brain tumors and retinoblastomas

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Determination of *in vitro* drug resistance has shown clinical relevance in childhood leukemia. We therefore adapted the MTT assay to measure *in vitro* drug resistance in different types of childhood solid tumors and correlated the results with cell biologic features. Fresh samples of 95 tumors (23 central primitive neuro-ectodermal tumor [cPNET], 13 ependymoma, 15 pilocytic astrocytoma and 44 retinoblastoma) were included. The procedure to obtain a tumor cell suspension was standardized with a filter method, enzymatic handling and ammonium shock in case of high erythrocyte count. After determination of contaminating leukocytes by CD45 and endothelial cells by CD34 phenotyping, purification by magnetic beads was performed if the percentage of tumor cells was below 80. The MTT assay was used to determine *in vitro* resistance to ten drugs. Additionally S-phase, DNA ploidy, differentiation markers (GFAP, NSE, NF and synaptophysin) and proliferation markers (PCNA and MIB-1) were determined by immunohistochemistry. The viability of the cell suspensions was adequate in cPNET, ependymoma and pilocytic astrocytoma. The enzymatic handling had to be omitted in retinoblastomas. Purity of the samples was not sufficient in pilocytic astrocytomas, due to high numbers of leukocytes and endothelial cells. The use of immunomagnetic beads resulted in too much cell loss, so pilocytic astrocytomas could not be tested in cell suspensions. There was a significant cross-resistance between structurally related drugs, such as platinum analogues and anthracyclines. Ependymoma

was the most resistant tumor type, retinoblastoma was most sensitive to carboplatin and cisplatin. There was an increased relative resistance (RR) to most drugs as compared to childhood ALL, being most profound for cytarabine (RR >450 in all tumor types) and vincristin (RR >700 in ependymoma). Neuronal differentiation (NSE) was correlated with increased drug sensitivity in retinoblastoma. In cPNET high S-phase and higher MIB-1 expression was correlated with ifosfamide resistance. In conclusion, adaptation of the MTT assay was adequate in cPNET, ependymoma and retinoblastoma. Contamination of pilocytic astrocytoma with normal cells demands a different drug resistance assay. Ependymoma is the most drug resistant tumor. Proliferation seems to correlate with *in vitro* drug resistance.

The correlation of the topoisomerase-II alpha and HER-2/neu status towards the *ex vivo* chemosensitivity in breast cancer

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Overexpression of the HER-2/neu oncogene, which encodes a transmembrane receptor tyrosine kinase, has been shown to be associated with poor prognosis in breast cancer. Recent studies indicate that HER-2/neu may also be involved in determining the chemosensitivity of human breast cancer. The HER-2/neu gene is located on chromosome 17q21 near the locus encoding for topoisomerase II alpha (topo II alpha), and topo II alpha alterations in tumors amplifying HER-2/neu have been frequently reported. Topo II alpha is associated with active cell proliferation and is a target for chemotherapeutic agents. The predictive value of HER-2/neu which may be partly depend on alterations of the cellular topo II alpha content is still a subject of controversy. Since individual sensitivity profiles are difficult to obtain clinically, this study was initiated to compare the HER-2/neu and topo II alpha status of native breast carcinomas with the results of the *ex vivo* ATP-based tumor chemosensitivity assay (ATP-TCA) Native tumor tissue of 77 patients diagnosed with breast cancer (67 primaries, 10 relapses) were screened for their HER-2/neu and topo II alpha expression by immunohistochemistry (ICH). The ATP-TCA was performed simultaneously using corresponding tumor specimens. The following agents and their combinations were tested: epirubicin (EPI), doxorubicin (DOX), mitoxantrone (MX), paclitaxel (PCT), docetaxel (DCT), EPI+4-OOH-Cyclophosphamide (EC), 4-OOH-Cyclophosphamide/Methotrexate/5-Fluorouracil (CMF). Out of 67 primaries, 21% showed HER-2/neu overexpression, 7 out of these 14 were positive for topo II alpha. In the group of 10 relapses, 4 were stained as HER-2/neu positive, 2 out of these 4 tumors showed also topo II alpha overexpression. Regarding the results for anthracyclines, HER/2-neu negative tumors (59/77) were slightly more chemosensitive than HER/2-neu positive tumors. Considering the HER/2-status in correlation to the topo II alpha, topo II alpha positive tumors tended to be more chemosensitive than the topo II alpha negative ones. A similar effect could be shown with the taxanes related to a positive topo II alpha status. The combination of taxanes and anthracyclines resembled to the situation with the taxanes and anthracyclines for both single agents and EC. Our data suggest that topoisomerase II alpha status , which correlates with HER-2/neu expression, may be a major determinant of chemosensitivity in HER-2/neu overexpressing breast cancers.

Differential growth inhibition of sodium salicylate in colorectal cancers using an individualized histoculture system

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There is strong epidemiological evidence that non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin have chemopreventive effect on colorectal cancer. Anti-neoplastic effect of aspirin and its metabolites has also been observed in a few cell lines. We therefore undertook a study to explore the chemotherapeutical potential of sodium salicylate, an aspirin metabolite by using a three-dimensional histoculture system. Fresh surgical specimens of 24 colorectal cancer patients were histocultured for 4 days and then treated with sodium salicylate from 1 to 10 mM for 24 h or 48 h. The proliferation of the tissues was measured by 48 h cumulative Bromodeoxyuridine (BrdU) incorporation into replicating DNA. Apoptosis was evaluated by morphology and presence of cleaved 89 KD fragment of poly (ADP-ribose) polymerase. Twenty tumors were successfully cultured. The mean BrdU labeling index (LI) of the control tissues was $41 \pm 14\%$. No dose-relevant induction of apoptosis was observed. However, the LI in 16

out of 20 tumors (80%) decreased with dose, suggesting that sodium salicylate has antiproliferative effect on colorectal cancer. Further analysis showed that 12 of these 16 cancers (60%) showed dose effect relationship with sodium salicylate within the physiological relevant concentrations (IC_{50}: 1.4–4.1 mM). The different sensitivity, in term of cell proliferation, to sodium salicylate reflects inter-tumor heterogeneity, emphasizing superiority of the individualized histoculture system. Chemosensitivity to sodium salicylate was significantly correlated to age and tumor site, reflecting possibly underlying genetic differences and/or environmental exposure. In addition, initial finding with 4 specimen showed that extending the exposure time from 24 to 48 h decrease the IC_{50} values ranging from 20% to 80% suggesting the possibility of achieve similar inhibitory effect with a lower dose over an extended time.

Effect of C-erbB-2 and topoisomerase II-alpha on Chemosensitivity of native ovarian carcinomas *ex vivo*

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C-erbB-2 overexpression is likely to be associated with high proliferative activity and poor prognosis of epithelial ovarian cancer (EOC). It also may modulate chemosensitivity. The c-erbB-2 oncogene is located closely to the topoisomeraseII alpha (topoII alpha) gene which determines chemosensitivity towards topoII alpha inhibitors. In our study, 79 EOCs were screened for c-erbB-2 and topoII-alpha by immunohistochemistry, as well as for chemosensitivity assessed by the *ex vivo* ATP tumor chemosensitivity assay (ATP-TCA). 10% of EOCs (8/79) were c-erbB-2+, 24% (19/79) were topoII alpha+. The ATP-TCA used anthracenes (ANT; doxorubicin or mitoxantrone), paclitaxel (PCT), cisplatin (CDDP) and 4-OOH-cyclophosphamide (4-HC). Population-based cumulative dose-response curves for both IC50 and IC90 were calculated for each drug, the area under the curves (AUC) were used for quantitative comparison of chemosensitivity with high values indicating high sensitivity. AUC differences of 15% were regarded as significant. C-erbB-2- EOCs showed a higher chemosensitivity than c-erbB-2+ EOCs towards ANT, 4-HC (IC50,IC90) and PCT (IC90). Regarding CDDP (IC50,IC90) and PCT (IC50) the c-erbB-2+ were more chemosensitive than the c-erbB-2- tumors. TopoII alpha+ tumors tended to be more chemosensitive towards ANT, CDDP (IC50) and PCT (IC50,IC90) than the topoII alpha- tumors. The activity of 4-HC was only marginally affected by both proteins. In conclusion, the predictive value of c-erbB-2 towards cellular chemosensitivity is limited. C-erbB-2 should be regarded more likely as part of a complex functional network determining the phenotype of an individual EOC.

Multidrug resistance mediated by ATP-dependent export pumps

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Overexpression of some ATP-dependent export pumps in the plasma membrane of tumor cells may lead to resistance of malignant tumors to chemotherapy. Enhanced activity of export pumps represents one of several mechanisms leading to multidrug resistance. Transfection of genes encoding ATP-binding cassette (ABC) transporters has been shown to confer multidrug resistance as demonstrated for MDR1 P-glycoprotein, MRP1, MRP2, MRP3, MRP4, MRP5, and MXR (BCRP). The expression pattern of these genes varies in different tumors and in normal tissues. Furthermore, localization of these export pumps differs in polarized cells. Highly selective inhibitors have been developed for MDR1 P-glycoprotein, such as LY335979, GG918, and others. These compounds may clarify the role of MDR1-P-glycoprotein in clinical multidrug resistance. Inhibitors for ATP-dependent transporters of the MRP family are under development.

The ATP-dependent export pumps of the multidrug resistance protein (MRP) family have been characterized with respect to their substrate specificity and their kinetic properties. MRP1 and MRP2 mediate the ATP-dependent transport of anionic conjugates and the co-transport of reduced glutathione with chemotherapeutic agents such as Vinca alkaloids and anthracyclines. MRP4 and MRP5 mediate the resistance to nucleoside analogs because of the ATP-dependent export of nucleoside phosphates, as exemplified by the MRP5-mediated transport of guanosine 3', 5'-cyclic monophosphate.

Drug resistance in melanoma—anything new?

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Malignant melanoma is a highly drug-resistant tumor and commonly used anticancer agents do not alter the clinical course of the disease. Various mechanisms have been described as mediating drug-resistance in various types of cancer, but the cellular mechanisms conferring chemoresistance in malignant melanoma are only poorly understood. Therefore we have human melanoma cells, MeWo, exposed to increasing amounts of various cytostatic drugs (CDDP, Fotemustine, Vindesine and etoposide) to establish a reproducible cell system for studying drug resistance. Membrane transport proteins such as MDR-1 were shown not to of major importance in melanoma cells. Furthermore enzyme activation and DNA repair were demonstrated to mediate a part of the resistance pattern. Recent publications demonstrated that chemotherapeutic agents exert their cytotoxicity by inducing apoptosis leading to the attractive concept that acquired drug-resistance may be the result of modulated apoptotic processes . We have assessed the effect of etoposide and cisplatin on the apoptotic pathways of the drug-sensitive human melanoma cell line MeWo as well as its etoposide- and cisplatin-resistant sublines ($MeWo_{Eto01}$; $MeWo_{Eto1}$ and $MeWo_{Cis01}$; $MeWo_{Cis1}$). Etoposide and cisplatin induced apoptosis in drug-sensitive MeWo cells as indicated by dose-dependent 1. cytochrome c release, 2. caspase-activation, 3. DNA-fragmentation and 4. cleavage of poly(ADP-ribose)polymerase (PARP). In contrast, while low etoposide-resistant cells ($MeWo_{Eto01}$) demonstrated reduced but detectable apoptotic activities, highly etoposide-resistant cells ($MeWo_{Eto1}$) did not exhibit any of the apoptotic events observed in etoposide-induced cell death. However, highly cisplatin-resistant cells ($MeWo_{Cis1}$) demonstrated a reduced caspase-9 activity and cytochrome c release but the extent of effector caspase activation as well as DNA-fragmentation was comparable to that of sensitive MeWo cells at equitoxic concentrations. In addition, PARP-cleavage was strongly reduced in highly cisplatin-resistant sublines. Taken together, sensitive and drug-resistant MeWo cells utilized different apoptotic pathways upon drug exposure in a drug-dependent fashion and apoptosis deficiency was highly associated with the drug-resistant phenotype.

Antineoplastic agents induce mdr1-gene expression in ovarian cancer cell lines

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The clinical observation of the multidrug resistance (MDR) phenotype is often associated with overexpression of the *mdr1*-gene, in particular with respect to ovarian cancer. However, until now the *mdr1*-inducing potential of commonly used antineoplastics has been only incompletely explored.

We performed short-term cultures of 7 established ovarian cancer cell lines exposed to either blank medium or each cisplatin, doxorubicin or paclitaxel, respectively, at concentrations related to the clinically achievable plasma peak concentration. A highly specific quantitative real time RT-PCR was used to detect the *Mdr1*-transcripts. *Mdr1*-mRNA contents were calibrated in relation to co-amplified GAPDH-mRNA.

Mdr1-mRNA was detectable in each cell line. In 13 assays (62%) the specific anticancer drug being tested induced *mdr1*-transcription with a considerable variation. No decrease in *mdr1*-mRNA concentration was observed.

Our data indicate that *mdr1*-induction by antineoplastics is one of the reasons for failure of ovarian cancer therapy but may vary individually.

Modulation of irinotecan cytotoxicity by lesions in p53 gene and MMR system

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Background: Colon carcinomas (CRC) which are refractory to 5-fluorouracil (5-FU) treatment respond in 30% to treatment with irinotecan (CPT-11). The lesions occurring in the mismatch repair (MMR) system and in the *p53* gene were shown previously to modulate the effects of 5-FU. Mutations in *p53* gene or in genes coding for MMR proteins are frequently occurring in colon cancer; about 70% of CRC cases have been described as p53-defective tumors, while about 15% of CRC is characterized by microsatellite instability (MSI), due to a mutation or inactivation of a MMR protein.

Objective: To study the effects of lesions in *p53* gene and in the MMR system on the cytotoxicity of CPT-11 in colon carcinoma cells.

Methods: The effect of the MMR system and the *p53* gene on the sensitivity to CPT-11 were compared using HCT116 cell lines with the genotypes $p53^{wt}$, MMR^-; $p53^{wt}$, MMR^+; $p53^{-/-}$, MMR^-. Sensitivity to CPT-11 was assayed by clonogenic assay. Cell cycle analysis was performed by flow cytometry. DNA fragmentation was analysed by Cell Death Detection ELISA Kit (Boehringer Mannheim). The expression of the following proteins was analysed by Western blot: p53, p21, BAX, Bcl-X_L, cdc2, cyclin B1, 14-3-3 σ. The activity of the cdc2-cyclin B1 complex was detected by histone H1 phosphorylation assay.

Results: After CPT-11 treatment, the two $p53^{wt}$ cell lines mantained a long-lasting G_2/M arrest, while the $p53^{-/-}$ cell line was only transiently arrested. The prolonged G_2/M arrest was concomitant with a sustained overexpression of p53 protein, p21 protein and the inhibition of the kinase activity of the cdc2-cyclin B1 complex. On the other hand, the $p53^{-/-}$ cell line underwent after CPT-11 treatment a massive apoptosis, while the two $p53^{wt}$ cell lines showed only little induction of cell death. The induction of apoptosis in the $p53^{-/-}$ cell line was concomitant with decrease of the Bcl-X_L expression and, consequently, with an increase of BAX/Bcl-X_L ratio. This suggested a p53-independent role of Bcl-X_L in CPT-11-induced apoptosis. The sensitivity of the three cell lines, as judged by clonogenic assay, was identical.

Conclusions: The data presented here show that CPT-11 exerts two types of effects on colon carcinoma cells, depending mainly on their p53 status. CPT-11 induces a prolonged G_2/M arrest in $p53^{wt}$ cell lines, while it preferentially induces apoptosis in $p53^{-/-}$ cells. The overall result of these two different effects if measured in clonogenic assay is the same. These data offer new insight into the dependence of sensitivity of colon carcinoma cells to CPT-11 on genetic lesions and suggest possible ways of a rational improvement of CPT-11 therapy.

Lack of correlation between p53 status and *ex vivo* chemosensitivity of clinical ovarian carcinomas

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While loss of p53 function has been associated with worse prognosis in epithelial ovarian cancer (EOC) its predictive value towards increased chemoresistance still remains debatable (Kurbacher et. al., Proc. AACR 1998). In breast cancer, point mutations in the functional domains of the p53 gene (exons 5-9) rather than occurrence of mutated p53 protein per se has been demonstrated to confer to increased doxorubicin resistance (Aas et al, Nature Med. 1996). We have analyzed 54 clinical tumor samples derived from patients with advanced primary (n = 31) or recurrent EOC (n = 23) for both mutated p53 by routine immunohistochemistry (IHC) and for p53 point mutations in exons 5-9 by PCR and sequencing of the genomic DNA. *Ex vivo* chemosensitivity testing was performed in the same tumors using the ATP-based tumor chemosensitivity assay (ATP-TCA; Andreotti et al., Cancer Res. 1995) using a panel of 9 single agents such as cisplatin (DDP), 4-OOH-cyclophosphamide (4-HC), treosulfan (TREO), doxorubicin (DOX), mitoxantrone (MX), etoposide (VP-16), paclitaxel (PCT), cytosine arabinoside (ara-C), and gemcitabine (dFdC) and 8 combinations. 18 samples were found to be p53+ by IHC (33%). A total of 11 p53 point mutations (9 missense, 1 nonsense, 1 silent) were found in either 4 primary and recurrent EOC (15%), three of them regarded to be wild type ($p53^{WT}$) by IHC. Only one recurrent tumor carried more than one point mutation. *Ex vivo* chemosensitivity against all regimens tested did not differ significantly between $p53^{WT}$ and $p53^+$ tumors classified by IHC. Moreover, the group of tumors with p53 point mutations generally did not exhibit higher overall drug resistance. Controversially, higher *ex vivo* sensitivity could be detected for MX, MX + PCT, and DDP + dFdC. However, broad pleiotropic *ex vivo* chemoresistance could be demonstrated in one particular EOC carrying 4 point mutations all located in exon 5 of the p53 gene. We therefore conclude that neither mutated p53 status identified by ICH nor single point mutations in exons 5-9 of the p53 gene are reliable predictors of chemoresistance in EOC.

Role of novel criteria and objectives for development of algorithms designed to facilitate both disease specific drug development and individual sensitivity testing

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Systematic testing of panels of disease and treatment history specific de novo tumors directly obtained from patients and free of common laboratory selection artifacts discovered evidence that a series of current operating assumptions applied to *in vitro* drug testing may often be false or suboptimum. Based on this

evidence there have been several changes in the methods of data generation and processing leading to new operational algorithms for prioritizing regimens for drug development and selection of treatments for individuals. (i)-selection of the dose response range and analysis of the curve place new emphasis on findings at low drug concentrations and often reject conventional decisions based on tests of extreme drug resistance concentrations. (ii) Drugs clinically eligible for testing now include conventionally untested clinically failed drugs and analogues rejected untested for the specific disease. (iii) Lower levels of inhibition are now sometimes considered predictive of clinical utility in the context of specific diseases, added drugs, frequency of drug administration and utility of disease stabilization. (iv) Single drug activity and especially maximum inhibition at high concentrations is now given lower priority than synergism demonstrable at low concentrations or ability of a drug to recruit otherwise inactive standard drugs. (New clinical objectives are now achievable, given equal efficacy, including safety, patient specific safety, reserving a salvage treatment option and major economy). (vi) The definition of the panel and significant level of activity have been redefined in a disease and drug (combination) specific manner for the algorithm. (vii) Drug synergism is geometrically maximized in practice when combinations use three or more drugs and represents an alternative to current strategies designed to maximize one or at most two drugs by dose intensification. When drug synergism replaces single drug activity in the drug selection algorithm–patent pending–the opportunities, safety and cost of treatment benefit improve markedly; more than 20% compared to empirical and extreme drug resistance based selection methods. This appears true for all six diseases tested to date-cancers of the breast, skin (melanoma), ovary, stomach, and pancreas–the level of complexity in selection explains past and current failures of EDR methods and lends itself to sophisticated computer supported algorithms.

Maximizing chemoresistance testing data for the clinican

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Selection of an optimal therapeutic regimen for women with recurrent ovarian cancer requires a case by case analysis of several clinical factors to arrive at each individual's treatment plan. While it is not yet prudent to choose therapy based solely on an *in vitro* assay, given the fact that all second line agents have similar response rates and the paucity of prospective randomized phase III trials of second line therapy, it is rational to include *in vitro* test results as part of the selection process. Review of the data on *in vitro* assays indicates that patients treated with agents classified to be resistant *in vitro* have inferior outcomes, supporting the notion that *in vitro* tests can assist the physician in the elimination of resistant drugs that are toxic with no benefit. On the other hand, the intuitively obvious counterpart that treatment with low resistance agents will improve outcomes requires confirmation in a randomized phase III study. This fact does not, however, proscribe the use of *in vitro* tests for individuals outside of the trial setting in order to eliminate drugs found to be inactive. Given that recurrent ovarian cancer is rarely curable, that response rates of 10 to 30 percent are reported for all second-line therapies, and that response times are generally less than 6 months, it is intuitively obvious that *in vitro* assays may be better at identifying resistant drugs than sensitive drugs. Two recent phase II studies from our laboratory compared median progression free survival for newly diagnosed ovarian cancer patients treated with platinum compounds to which their tumors demonstrated either extreme drug resistant (EDR) or low drug resistance (LDR) in the assay:

Investigator	Tumor type	(*N*)	EDR	LDR	*P*-value
Holloway	ovarian	79	6.0 m	24 m	0.011
Fruehauf	ovarian	31	4.0 m	24 m	0.006

These results demonstrated inferior survival for patients treated with drugs that were in the EDR category and support the use of *in vitro* test data when choosing therapy for a specific patient with ovarian cancer. *In vitro* drug resistance assays may be of particular use in the recurrent setting.

Chemosensitivity testing as an aid to anti-cancer drug and regimen development

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The ATP-based chemosensitivity assay has proved particularly useful for the evaluation of new anti-

cancer agents and combinations. The majority of our publications in this area have concentrated on topoisomerase inhibitors. Comparison of mitoxantrone with doxorubicin convinced us that these two agents were not completely cross-resistant and led to the design of the mitoxantrone + paclitaxel regimen which is now in clinical practice. Re-assessment of treosulfan in uveal melanoma led to the design of a new regimen combining this alkylating agent with gemcitabine, again with rapid introduction of this combination to clinical practice. The assay has recently been used to examine the concentration-activity curve to determine which tumours might benefit from liposomal preparations capable of delivering 4–16× the standard dose without cardiotoxicity. Assay-directed use of Caelyx is producing encouraging results and we are now examining this drug in combination with others. We recently showed that XR5000, a combined inhibitor of topoisomerase I and II was effective against melanoma as well as ovarian cancer, but at concentrations which were unlikely to be achieved in patients. These data confirm our suggestion that use of the assay could reduce the time to introduction of new anti-cancer drugs and the cost of this process.

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Treosulfan in the treatment of metastatic melanoma: from chemosensitivity testing to clinical trials

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The therapy of metastatic malignant melanoma is limited by poor responses and short overall survival. Thus, it remains an important issue to identify and test potential new drugs in this disease. To examine the effects of the bifunctional alkylating cytostatic treosulfan an *in vitro* microplate ATP bioluminescence assay (ATP-TCA) was used. Five highly chemoresistant melanoma cell lines and melanoma cells freshly isolated from metastases surgically resected from stage IV melanoma patients (n = 10) were incubated with treosulfan. Three cell lines and 4/8 tested tumor cells isolated from melanoma metastases showed tumor growth inhibition after incubation with treosulfan. Therefore, 14 patients with rapidly progressing stage IV malignant melanoma that were pretreated with at least one standard chemotherapy regimen received treosulfan. In this population of patients with highly refractory advanced melanoma 1 complete remission (7.1%), 2 partial remissions (14.3%) and 3 stable disease (21.4%) were observed. Median survival for all patients measured from the beginning of treosulfan treatment was 9 months. Based on these data a multicenter Phase II trial was initiated. A total of 31 patients with stage IV melanoma were included and treated second-line with 8 g/m2 i.v. treosulfan. From this group 26 patients were evaluable. No objective remission (CR, PR) was observed, 5/26 patients (19%) had stable disease and 21 patients had progressive disease. Median overall survival was 6.5 months (95% CI: 3.1–10 months). Toxicity of treosulfan was moderate. Further clinical trials testing treosulfan first line or in combination regimens (e.g. gemcitabine) are warranted.

Chemosensitivity testing and test-directed chemotherapy in pancreatic cancer

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Human pancreatic cancer is a devastating disease with poor prognosis. In many cases it is diagnosed at stages in which a complete resection is not possible. However, even after complete resection most tumors recur. Therefore, several chemotherapeutic strategies have been developed, however, with little impact on the clinical outcome. Because one of the hallmarks of human pancreatic cancer is its general resistance to chemotherapeutic agents, it seems important to develop strategies to individualize chemotherapy and to render cells more sensible to chemotherapeutic agents.

Here we describe our method of *in vitro* chemosensitivity testing using the human tumor colony-forming assay for pancreatic cancer in comparison with colorectal cancer and how our *in vitro* results influence chemotherapy. We further demonstrate new developments of mRNA-quantitation of chemoresistance target enzymes based on real-time PCR which may help in the future to individualize chemotherapy of pancreatic cancer. Finally, we present the results of our studies about cyclin D1. Inhibition of cyclin D1 by cyclin D1 antisense mRNA expression was associated with growth inhibition and an increase in chemosensitivity to fluoropyrimidines and platinum compounds, suggesting that chemotherapy in combination with

strategies to increase chemosensitivity may be a reasonable combination for the treatment of advance human pancreatic cancer in the future.

In comparison to other gastrointestinal cancers human pancreatic cancer is relatively chemoresistant. Additionally, material for chemosensitivity testing is difficult to obtain and to analyse. Therefore, future investigations should aim at the development of more sensitive methods to analyse individual chemosensitivity and of strategies which increase its chemosensitivity.

Clinical relevance of drug resistance in childhood leukemia

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Cellular drug resistance is an important determinant of the response to chemotherapy, and its precise measurement may have clinical relevance. Potential applications are: prognostic factor for risk-group stratification, tailored chemotherapy for subgroups or individual patients with a specific cellular drug resistance profile, determination of cross-resistance patterns, study of resistance modulation or circumvention, and screening of novel compounds. We mainly use the colorimetric 4-days MTT assay, which has a success-rate of about 80% for fresh ALL and AML samples. For methotrexate (MTX) a different assay must be used, and we therefore adapted the thymidylate synthase inhibition assay (TSIA). We and others have reported the independent prognostic significance of *in vitro* drug resistance data in childhood ALL. Now, an ongoing clinical trial of the German COALL group (head, G.E. Janka-Schaub) in collaboration with us and colleagues in Rotterdam (head, R. Pieters) shows the feasibility of risk-group stratification based on MTT (if necessary DiSC) assay results in children with ALL. In ALL, we also demonstrated the relevance of TSIA results, by showing an increased resistance to MTX of T-cell and relapsed ALL samples as compared to common/pre-B ALL. This resistance could be overcome by continuous long-term exposure to MTX in T-cell ALL, but not in relapsed ALL. Also, TEL-AML1 positive ALL samples were more sensitive to l-asparaginase, while DNA hyperdiploid ALL cases were more sensitive to a large number of drugs, which may help explain the good prognosis of these cytogenetic subgroups. Similarly, AML samples from children with Down syndrome were remarkably *in vitro* sensitive to ''AML-like'' and ''ALL-like'' drugs, except to glucocorticoids. The latter type of drugs actually induced proliferation in about 25% of AML samples (but in even 13 out of 24 FAB M5 samples). AML samples with chromosome 5 and 7 abnormalities were more resistant to cytarabine, etoposide and daunorubicin. *In vitro* cytarabine resistance in AML could be decreased by aphidicolin and fludarabine. AML samples in general as compared to ALL samples were more resistant to most drugs, but equally sensitive to cytarabine and thiopurines, and more sensitive to 2-chloro-deoxyadenosine. In conclusion, cellular drug resistance testing provides clinically relevant information. The data are more and more being used in clinical trials in leukemia.

ATP chemosensitivity testing in ovarian cancer: early clinical trials

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The ATP-based tumor chemosensitivity assay (ATP-TCA) provides both a satisfying laboratory methodology and good correlation of assay results and the clinical outcome of patients with ovarian carcinoma (Andreotti *et al.*, 1995; Konecny *et al.*, 2000). We have initiated two prospective clinical trials to determine the value of the ATP-TCA to direct chemotherapy (Ctx) for heavily pretreated patients with recurrent ovarian cancer (ROC). The first trial was based on a preclinical study in 42 native ROC specimens suggesting that 83% of tumors tested may be sensitive to a combination of mitoxantrone (MX) and paclitaxel (PCT), NT. Subsequently, 35 ROC patients (31 with platinum-refractory ROC), in whom no direct tumor testing was possible, received a total of 39 NT therapies either at a q1w or aq3w schedule. The overall response rate (ORR) was 67% (14 CR, 12 PR) resulting in a median progression-free survival (PFS) of 40 weeks and a median overall survival (OS) of 89 weeks. In the second trial, 59 patients–31 with platinum-sensitive (PSROC) and 28 with platinum-resistant ROC (PRROC)–who failed 1–5 prior Ctx were recruited to be treated with ATP-TCA-directed Ctx. All patients were assayed against single agents and combinations over a 2 *log* dose range, ATP-TCA always confirmed clinical platinum-resistance. Novel combinations as indicated by ATP-TCA were used in 46 patients. During 316 cycles assessable for toxicity, myelosuppression did not differ significantly between the various regimens: G3 leucopenia in

⩽13%, G3 anemia in ⩽10%, and G3–4 thrombocytopenia in ⩽12% of cycles with one Ctx-related death (1.7%). Organ toxicity was infrequent did not excedd G2. Three patients with PSROC were not evaluable for response. Novel regimens (NT, platinum + cytarabine or gemcitabine) produced 31of 39 remissions with an ORR of 66% (evaluable patients: 70%): PSROC, CR 6, PR 11; PRROC, CR 14, PR 8. Median PFS was 45 wks: PSROC, 40 wks; PRROC, 50 wks. Median OS was 90 wks: PSROC, 97 wks; PRROC, 87 wks. Neither RR nor survival (OS and PFS) was signifiantly affected by clinial platinum resistance. These results show that the ATP-TCA is a valuable means to select Ctx regimens with promising activity for further clinical use. Moreover, Ctx for ROC individually directed by the ATP-TCA is clinically feasible, well tolerated, and produced an exceptionally high RR resulting in a promising PFS and OS. A European phase III trial is on the way to confirm the particularly good results of ATP-TCA-directed Ctx in PRROC achieved in the second study reported.

Pretherapeutic chemosensitivity testing and neoadjuvant chemotherapy in breast cancer

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The preoperative (neoadjuvant) therapy is an established concept in the treatment of breast cancer. This type of treatment is especially used in inflammatory breast cancer. There is an increasing role of preoperative chemotherapy in the treatment of operable breast cancer, with three major goals:

- Down staging and mobilisation of the primary tumor to increase the rate of breast conservation.
- Locoregional response in the breast and in the axillary lymph nodes correlate with disease-free and overall survival.
- The use of preoperative therapy as a surrogate marker to increase survival in innovative randomized protocols.

The choice of chemotherapeutic agents was historically decided by empirical data evaluation. In general, three to four cycles of AC, EC, FEC or FAC or CMF are used before operation.

The ATP-Chemosensitivity Assay (ATP-TCA) allows the pretherapeutic cytostatic drug testing in tissue samples from biopsy materials taken before chemotherapy. This would allow a more individualized therapy. Breast cancer tissue has been processed in the laboratory with the ATP-TCA and different and cytostatic combinations (CMF, FEC, Epirubicin/Taxol, Epirubicin/ Iphosphomide, Paclitaxel, Gemcitabine, Paclitaxel/ Vinorelbine, Paclitaxel/Carboplatin). Cellular ATP-measurement can be compared between treated and untreated controls and is calculated as growth inhibition for sensitivity in % of the tested samples on the IC50 and IC90 levels. IC50 and IC90 for FEC and CMF were similar for Epirubicin and Paclitaxel. The sequence or combination of Epirubicin and Paclitaxel is showing a very high response rate on the IC50 and especially on the IC90 level. Most of the tumor samples are responding to Paclitaxel/Epirubicin. With ATP-TCA preoperative chemotherapy could be used for therapy planning in the future. Early recognition of resistance mechanisms could have direct consequences in the preoperative therapy planning. Innovative concepts could be evaluated in shorter periods than it was with the adjuvant chemotherapy, avoiding long-term follow-up over at least 10 to 15 years until different drugs or combinations can be recognized as more effective than standard regimens.

Salvage therapy of patients with heavily pretreated breast and ovarian carcinoma: a clinical pilot trial based on results of an *ex vivo* study

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The ATP-based tumor chemosensitivity Assay (ATP-TCA) using native tumors provides a feasible and reliable means to preclinically screen for novel active chemotherapy (Ctx) regimens to be used in clinical oncology (Cree and Kurbacher, 1999). We here describe the *ex vivo* and clinical evaluation of the treosulfan (TREO) and gemcitabine (dFdC) combination (TG) to salvage heavily pretreated patients with metastatic breast (MBC) or recurrent ovarian cancer (ROC). A total 42 specimens derived from 39 ROC and 3 MBC patients were assayed against dFdC, TREO, and TG using ATP-TCA. Of all tumors tested, 79% were sensitive to TG whereas only 42% and 24% responded *ex vivo* to dFdC and TREO, respectively. A subsequent pilot trial was thus initiated including 25 patients with ROC (n = 13) or MBC (n = 12) who had failed 1-6 prior

Ctx regimens. All patients with ROC were platinum-refractory, 10/12 MBC patients relapsed after anthracycline pretreatment. Prior taxanes were given to either 10 patients with MBC and ROC. TG was administered q3w with 5 g/m^2 TREO on day 1 and 1250 mg/m^2 dFdC on days 1 and 8. During 93 courses eligible for toxicity, myelosuppression G3-4 was the predominant side effect with leukopenia in 33%, anemia in 14%, and thrombocytopenia in 8% of courses. Non-hematological toxicity was generally mild and occurred infrequently, G3 nausea/vomiting and arthralgia/myalgia was observed in either 1% of TG cycles. Due to unresolved myelosuppression, TG was given at a reduced dose intensity (DI) in 13 of 25 patients. 24 patients were evaluable for response with 4 CR (ROC: 3; MBC: 1); 8 PR (ROC: 4; MBC: 4), 4 SD, 8 PD, accounting for an objective response rate (RR) of 48% (50% in evaluable patients). The median progression-free survival (PFS) was 21 and the median overall survival (OS) was 110+ weeks (wks). Both RR and survival did not differ significantly between ROC and MBC. RR were similar in patients treated with 100% DI and those who received $<$100% DI. However PD was seen mostly in patients with reduced DI. Consecutively, patients receiving 100% DI had a significantly longer median PFS (27 vs 17.5 wks) and OS (110+ vs 74 wks). In conclusion, the ATP-TCA is an effective method to select active drug combinations for further clinical use. TG showed promising preclinical and clinical activity in heavily pretreated ROC and MBC. However, the regimen clearly need further improvements. DFdC at 250 mg/m^2 given over 6 h instead of 1250 mg/m^2 infused within 30 min may both reduce myelotoxicity and allow the maintenance of DI which was found to be the crucial to achieve long-term survival.

Chemosensitivity testing—present and future in Japan

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Radical surgery with extended lymph node dissection is the first and only curative treatment of gastrointestinal cancer. While the combined cancer chemotherapy have achieved 30$\sim$50% response rates, a controversy still remains in the significance of the adjuvant cancer chemotherapy after surgery. To breakthrough this limitation, we have introduced the chemosensitivity test in evaluating the appropriate adjuvant cancer chemotherapy for advanced gastrointestinal cancer. Our plural studies indicated that the chemosensitivity test would be useful in evaluating the appropriated adjuvant chemotherapy b increasing the survivals in the sensitive group. Recently, the molecular targets have been clarified for the conventionally available antitumor agents, e.g., thymidylate synthetase for 5-fluorouracil, ATP-binding cassette transporters for anthracyclines, glutathione-related detoxification for platins and topoisomerase I for CPT-11, which will be applied for clinical use in evaluating the appropriate cancer chemotherapy. The chemosensitivity test-guided adjuvant chemotherapy will result in the survival benefit for the patients with advanced gastrointestinal cancer, this test should be approved as ''social insurance'' for further wide clinical application.