

## Cancer and Chemotherapy Literatures

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**Abstract:** Cancer is the cells that grow out of control. Cancer cells can also invade other tissues. Growing out of control and invading other tissues are what makes a cell a cancer cell. Involved in more than 100 diseases, the cancer can cause serious illness and death. Normally, the cells become cancer cells because of DNA damage. This material is a literature collection of the researches on the cancer and the chemotherapy.

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### 1. Introduction

Cancer is the general name for a group of more than 100 diseases. Although there are many kinds of cancer, all cancers start because abnormal cells grow out of control. Untreated cancers can cause serious illness and death. The body is made up of trillions of living cells. Normal body cells grow, divide, and die in an orderly fashion. During the early years of a person's life, normal cells divide faster to allow the person to grow. After the person becomes an adult, most cells divide only to replace worn-out or dying cells or to repair injuries.

### Literatures

Akatsu, Y., Y. Saikawa, et al. (2007). "Predictive value of GADD153, p21 and c-Jun for chemotherapy response in gastric cancer." *Cancer Sci* **98**(5): 707-15.

We sought to determine whether changes in the expression of early response genes (GADD153, p21 and c-Jun) are indicators of chemotherapy response in gastric cancer. Three human gastric cancer cell lines were exposed to 5-fluorouracil or cisplatin in vitro. Xenografts of TMK-1 cells in nude mice were also treated with 5-fluorouracil or cisplatin in vivo. For each of these treatments, we tested for a correlation between early gene expression levels and inhibition ratios derived at a later time. A 5-fluorouracil derivative, S-1, and cisplatin were administered to 12 patients with advanced gastric cancer for 3 weeks. Gene expression levels were measured using biopsy specimens obtained by endoscopy soon after initiation of chemotherapy. There was a significant correlation between expression levels of these genes at 24 h and inhibition ratios at 72 h in vitro. Cut-off values determined from receiver-operating characteristic curves were 1.3 for GADD153, 1.8 for p21 and 2.1 for c-Jun. There was also a significant correlation between gene expression levels at 2 days and inhibition ratios at 21 days in vivo. Cut-off values were 1.8 for GADD153, 1.9 for

p21 and 2.2 for c-Jun. Levels of early response gene expression in patients showing progressive disease were significantly lower than those in patients with partial response. Changes in the expression of the three early response genes soon after drug administration could improve predictions of the final outcome of chemotherapy in gastric cancer.

Alberti, C. (2009). "Cytoskeleton structure and dynamic behaviour: quick excursus from basic molecular mechanisms to some implications in cancer chemotherapy." *Eur Rev Med Pharmacol Sci* **13**(1): 13-21.

Novel nanoscale microscopic technologies are driving dramatic advances in the knowledge of cytoskeleton structure and dynamics. Cytoskeleton, that is organized into microtubules, actin meshwork and intermediate filaments, besides providing cells with important mechanical properties, allows, within the cell, not only the molecule cargo transport, but also the charged particle/biophoton transmission, so that the cell signaling might be considered as consisting of both molecule/chemically- and charged particle/physically-addressed systems. Molecular motors that drive molecule cargo translocation along the cytoskeletal highway, either through endocytic or secretory-exocytic mechanisms, include kinesin and cytoplasmic dynein, traveling on microtubule, and myosin family members, traveling along actin meshwork. The membrane-bound organelles and protein complexes are sorted with high specificity to their various destinations. In the field of highly structured cell signaling machinery, the endocytosis appears to play an important role with following specific changes in gene expression. In the opposite direction, the exocytosis involves many intracellular steps toward the vesicle fusion with the plasma membrane. Insights into cytoskeleton structure and dynamics are providing important progress in identifying proper targets for cancer therapy. Taxane

and Vinca alkaloids, by stabilizing the polymerized microtubules, are able to suppress their dynamic behaviour with subsequent cell death. Epithelones, by acting in same way, are emerging as a new class of anticancer drugs, moreover their toxicity resulting unaffected also towards taxane-resistant cancer cells. Even the alkylating agent nitrogen mustard exerts some cytotoxic effects at the level of the microtubule, whereas azaspiracid-1 induces cytoskeletal actin disorganization without affecting microtubule architecture. Regarding the influences of extracellular mechanical forces on changes in cell adhesion gene expression, the iatrogenic pressure-induced tumor cell implantation within surgical wounds may be prevented by perioperative administration of microtubule/actin inhibitors. Even though, among the different cancer therapy strategies, the chemotherapy could appear to be conceptually outclassed because of its low cancer cell-selectivity in comparison with novel molecular mechanism-based agents. However "combo-strategies", that combine the chemotherapeutic high killing potential with new molecule targeted agents, may be an effective curative measure. Some anticytoskeleton agents are under evaluation for their applications in tumor chemotherapy; benomyl, griseofulvin, sulfonamides, that are used as antimycotic and antimicrobial drugs, appear to have a powerful antitumor potential by targeting microtubule assembly dynamics, together with exhibiting, in comparison with taxane and Vinca alkaloids, a more limited toxicity. An exciting challenge for the next future will be to properly define the cytoskeleton structure and dynamic behaviour to design more effective drugs for cancer chemotherapy.

Als, A. B., L. Dyrskjot, et al. (2007). "Emmprin and survivin predict response and survival following cisplatin-containing chemotherapy in patients with advanced bladder cancer." *Clin Cancer Res* **13**(15 Pt 1): 4407-14.

Cisplatin-containing chemotherapy is the standard of care for patients with locally advanced and metastatic transitional cell carcinoma of the urothelium. The response rate is approximately 50% and tumor-derived molecular prognostic markers are desirable for improved estimation of response and survival. **EXPERIMENTAL DESIGN:** Affymetrix GeneChip expression profiling was carried out using tumor material from 30 patients. A set of genes with an expression highly correlated to survival time after chemotherapy was identified. Two genes were selected for validation by immunohistochemistry in an independent material of 124 patients receiving cisplatin-containing therapy. Fifty-five differentially expressed genes correlated significantly to survival time. Two of the protein products (emmprin and

survivin) were validated using immunohistochemistry. Multivariate analysis identified emmprin expression (hazard ratio, 2.23;  $P < 0.0001$ ) and survivin expression (hazard ratio, 2.46;  $P < 0.0001$ ) as independent prognostic markers for poor outcome, together with the presence of visceral metastases (hazard ratio, 2.62;  $P < 0.0001$ ). In the clinical good prognostic group of patients without visceral metastases, both markers showed significant discriminating power as supplemental risk factors ( $P < 0.0001$ ). Within this group of patients, the subgroups of patients with no positive, one positive, or two positive immunohistochemistry scores (emmprin and survivin) had estimated 5-year survival rates of 44.0%, 21.1%, and 0%, respectively. Response to chemotherapy could also be predicted with an odds ratio of 4.41 (95% confidence interval, 1.91-10.1) and 2.48 (95% confidence interval, 1.1-5.5) for emmprin and survivin, respectively. **CONCLUSIONS:** Emmprin and survivin proteins were identified as strong independent prognostic factors for response and survival after cisplatin-containing chemotherapy in patients with advanced bladder cancer.

Ansari, M. and M. Krajcinovic (2007). "Pharmacogenomics in cancer treatment defining genetic bases for inter-individual differences in responses to chemotherapy." *Curr Opin Pediatr* **19**(1): 15-22.

Pharmacogenomics is evolving rapidly due to the expansion of genomics and proteomics, the emerging technologies, knowledge of the molecular basis of neoplasms and of drug pathways. This article will give an update on the genetic basis of variable therapeutic responses to anticancer agents in children. **RECENT FINDINGS:** The majority of recent findings concern the pharmacogenetics of key components of acute lymphoblastic leukemia treatment, 6-mercaptopurine and methotrexate. This is not surprising given that leukemia is the most common cancer affecting children, accounting for 25-35% of childhood malignancies worldwide with acute lymphoblastic leukemia comprising 80% of leukemia cases. In certain patients treatment fails due to drug resistance, rendering acute lymphoblastic leukemia the leading cause of cancer-related death in children. Most of the studies use a candidate gene approach adding a new body of evidence to existing knowledge. Recent findings relating to other childhood tumors and the potential to optimize treatment of these malignancies are briefly discussed. Interindividual differences in drug responses are an important cause of resistance to treatment and adverse drug reactions. Pharmacogenetics tends to identify the genetic basis of these suboptimal responses allowing traditional

treatment to be complemented by genotype-based drug dose adjustment.

Azzoli, C. G., B. J. Park, et al. (2008). "Molecularly tailored adjuvant chemotherapy for resected non-small cell lung cancer: a time for excitement and equipoise." *J Thorac Oncol* **3**(1): 84-93.

In patients with previously-untreated, completely-resected pathologic stage II-III non-small cell lung cancer, 4 months of postoperative cisplatin-based chemotherapy reduces the risk of death by approximately 20%. To date, the only prospectively validated prognostic and predictive factor which can be used to guide clinical practice is pathologic stage. Higher stage patients have a worse prognosis, but derive more benefit from adjuvant chemotherapy. Numerous molecular markers are being developed with the potential to help decide which patients to treat with adjuvant chemotherapy, and which drugs to use. This paper will review the molecular markers which are having immediate impact on treatment decisions in routine practice, and which merit further study in the next generation of adjuvant chemotherapy trials.

Bellone, M., A. Mondino, et al. (2008). "Vascular targeting, chemotherapy and active immunotherapy: teaming up to attack cancer." *Trends Immunol* **29**(5): 235-41.

Chemotherapy has been combined with therapeutic tumor-specific vaccination in an attempt to simultaneously debulk tumors, increase the effector lymphocyte:tumor cell ratio, and favor immune-mediated tumor rejection. However, chemotherapy is often inadequate because of insufficient and uneven drug penetration into tumors, and because it might also cause, in some instances, undesirable side effects and immunosuppression. Here, we suggest a combined approach based on targeted alteration of the endothelial barrier function with vascular disrupting agents, such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), before chemotherapy and tumor-specific vaccination. This approach has the potential to empower chemoimmunotherapeutic strategies by improving cytotoxic drug penetration into tumors while exploiting the proinflammatory and immunostimulating activities of TNF- $\alpha$  and active immunotherapy.

Bertheau, P., M. Espie, et al. (2008). "TP53 status and response to chemotherapy in breast cancer." *Pathobiology* **75**(2): 132-9.

Despite its central role in the control of apoptosis, senescence and cell cycle arrest, the tumor suppressor protein p53 remains an enigma for its possible role in predicting response to chemotherapy

in cancer patients. Many studies remained inconclusive, others showed a better response for tumors with normal p53, and some recent studies showed adverse effects of normal p53 for response to treatment. p53 is not only a powerful pro-apoptotic factor in response to drug-induced DNA damages but also a potential inducer of cell cycle arrest, protecting tumor cells from further cytotoxic damages. Our review describes the classical as well as the more recent concepts. In order to draw definite conclusions, future works should use more reliable methods to assess the TP53 status and should address more homogeneous tumor subpopulations treated with homogeneous chemotherapy regimens.

Bhat, K. M. and V. Setaluri (2007). "Microtubule-associated proteins as targets in cancer chemotherapy." *Clin Cancer Res* **13**(10): 2849-54.

Natural and synthetic compounds that disrupt microtubule dynamics are among the most successful and widely used cancer chemotherapeutic agents. However, lack of reliable markers that predict sensitivity of cancers to these agents and development of resistance remain vexing issues. There is accumulating evidence that a family of cellular proteins that are associated with and alter the dynamics of microtubules can determine sensitivity of cancer cells to microtubule-targeting agents and play a role in tumor cell resistance to these agents. This growing family of microtubule-associated proteins (MAP) includes products of oncogenes, tumor suppressors, and apoptosis regulators, suggesting that alteration of microtubule dynamics may be one of the critical events in tumorigenesis and tumor progression. The objective of this review is to integrate the knowledge on these seemingly unrelated proteins that share a common function and examine their relevance to microtubule-targeting therapies and highlight MAPs-tubulin-drug interactions as a novel avenue for new drug discovery. Based on the available evidence, we propose that rational microtubule-targeting cancer therapeutic approaches should ideally include proteomic profiling of tumor MAPs before administration of microtubule-stabilizing/destabilizing agents preferentially in combination with agents that modulate the expression of relevant MAPs.

Bocci, G., A. Falcone, et al. (2008). "Antiangiogenic and anticolorrectal cancer effects of metronomic irinotecan chemotherapy alone and in combination with semaxinib." *Br J Cancer* **98**(10): 1619-29.

Metronomic chemotherapy refers to the administration of chemotherapy at low, nontoxic doses on a frequent schedule with no prolonged breaks. The aim of the study is to rationally develop a CPT-11 metronomic regimen in preclinical settings of

colon cancer. In vitro cell proliferation, apoptosis and thrombospondin-1/vascular endothelial growth factor (TSP-1/VEGF) expression analyses were performed on endothelial (HUVEC, HMVEC-d) and colorectal cancer (HT-29, SW620) cells exposed for 144 h to metronomic concentrations of SN-38, the active metabolite of CPT-11. HT-29 human colorectal cancer xenograft model was used, and tumour growth, microvessel density and VEGF/TSP-1 quantification was performed in tumours. In vitro and in vivo combination studies with the tyrosine inhibitor semaxinib were also performed. SN-38 preferentially inhibited endothelial cell proliferation alone and interacted synergistically with semaxinib; it induced apoptosis and increased the expression and secretion of TSP-1. Metronomic CPT-11 alone and combined with semaxinib significantly inhibits tumour growth in the absence of toxicity, which was accompanied by decreases in microvessel density and increases in TSP-1 gene expression in tumour tissues. In vitro results show the antiangiogenic properties of low-concentration SN-38, suggesting a key role of TSP-1 in this effect. In vivo, the CPT-11 metronomic schedule is effective against tumour and microvessel growth without toxic effect on mice.

Bonnefoi, H., A. Potti, et al. (2007). "Validation of gene signatures that predict the response of breast cancer to neoadjuvant chemotherapy: a substudy of the EORTC 10994/BIG 00-01 clinical trial." *Lancet Oncol* **8**(12): 1071-8.

We have previously described gene-expression signatures that predict growth inhibitory and cytotoxic effects of common chemotherapeutic drugs in vitro. The aim of this study was to confirm the validity of these gene-expression signatures in a large series of patients with oestrogen-receptor-negative breast tumours who were treated in a phase III neoadjuvant clinical trial. This trial compares a non-taxane regimen (fluorouracil, epirubicin, and cyclophosphamide [FEC] for six cycles) with a taxane regimen (docetaxel for three cycles followed by epirubicin plus docetaxel [TET] for three cycles) in women with oestrogen-receptor-negative breast cancer. The primary endpoint of the study is the difference in progression-free survival based on TP53 status and will be reported later. Predicting response with gene signatures was a planned secondary endpoint of the trial and is reported here. Pathological complete response, defined as complete disappearance of the tumour with no more than a few scattered tumour cells detected by the pathologist in the resection specimen, was used to assess chemosensitivity. RNA was prepared from sections of frozen biopsies taken at diagnosis and hybridised to Affymetrix X3P microarrays. In-vitro single-agent

drug sensitivity signatures were combined to obtain FEC and TET regimen-specific signatures. This study is registered on the clinical trials site of the US National Cancer Institute website <http://www.clinicaltrials.gov/ct/show/NCT00017095>. FINDINGS: Of 212 patients with oestrogen-receptor-negative tumours assessed, 87 patients were excluded. 125 oestrogen-receptor-negative tumours (55 that showed pathological complete responses) were tested: 66 in the FEC group (28 that showed pathological complete responses) and 59 in the TET group (27 that showed pathological complete responses). The regimen-specific signatures significantly predicted pathological complete response in patients treated with the appropriate regimen ( $p < 0.0001$ ). The FEC predictor had a sensitivity of 96% (27 of 28 patients [95% CI 82-99]), specificity of 66% (25 of 38 patients [50-79]), positive predictive value (PPV) of 68% (27 of 40 patients [52-80]), and negative predictive value (NPV) of 96% (25 of 26 patients [81-99]). The TET predictor had a sensitivity of 93% (25 of 27 patients [77-98]), specificity 69% (22 of 32 patients [51-82]), PPV of 71% (25 of 35 patients [55-84]), and NPV of 92% (22 of 24 patients [74-98]). Analysis of tumour size, grade, nodal status, age, and regimen-specific signatures showed that the genomic signatures were the only independent variables predicting pathological complete response at  $p < 0.01$ . Selection of patients with these signatures would increase the proportion of patients with pathological complete responses from 44% to around 70% in the patients studied here. We have validated the use of regimen-specific drug sensitivity signatures in the context of a multicentre randomised trial. The high NPV of both signatures may allow early selection of patients with breast cancer who should be considered for trials with new drugs.

Booton, R., T. Ward, et al. (2007). "ERCC1 mRNA expression is not associated with response and survival after platinum-based chemotherapy regimens in advanced non-small cell lung cancer." *J Thorac Oncol* **2**(10): 902-6.

Platinum-based therapy is pivotal to the treatment of advanced non-small cell lung Cancer (NSCLC). Excision repair cross-complementation group 1 (ERCC1) is a key component of the platinum-DNA repair machinery responsible for nucleotide excision repair. We sought to determine the influence of ERCC1 mRNA expression in advanced NSCLC on chemotherapy response, toxicity, and survival after platinum-based chemotherapy. Patients randomized to a phase III trial of platinum-based chemotherapy were eligible for inclusion. Formalin-fixed paraffin-embedded tumor biopsies were retrieved for mRNA extraction and purification before quantitative real-

time polymerase chain reaction analysis using Taqman technology. Expression data were correlated with treatment response, toxicity, and overall survival. Sixty-six patients were enrolled. No statistically significant relationship existed between ERCC1 mRNA expression and response to chemotherapy ( $p = 0.794$ ) or hematological toxicity. No statistically significant difference in median survival was demonstrated according to ERCC1 expression (high expression, 415 days, 95% confidence interval [95%CI]: 197-633 days; low expression, 327 days [95%CI: 211-433 days];  $p = 0.801$ ). High ERCC1 mRNA expression was associated with a hazard ratio for death of 0.96 (95% CI 0.919-1.004;  $p = 0.08$ ). In contrast to recent publications, ERCC1 mRNA expression in our study did not favor a prognostically better outcome after platinum-based chemotherapy in advanced NSCLC. We explore potential reasons for this, including the need for cautious interpretation of mRNA expression data from archival materials and highlight the need for additional translational research linking gene expression with a promising ERCC1 polymorphism.

Brabender, J., D. Vallbohmer, et al. (2008). "ERCC1 RNA expression in peripheral blood predicts minor histopathological response to neoadjuvant radio-chemotherapy in patients with locally advanced cancer of the esophagus." *J Gastrointest Surg* **12**(11): 1815-21.

The aim of this study was to determine the gene is spelled excision repair cross-complementing gene 1 (ERCC1) RNA-expression in peripheral blood as a non-invasive molecular predictor of response to neoadjuvant radio-chemotherapy in patients with locally advanced cancer of the esophagus. Only patients with locally advanced cancer of the esophagus with a major histopathological response to neoadjuvant radio-chemotherapy benefit from this treatment. No non-invasive molecular marker exists that can reliably predict response to neoadjuvant therapy in this disease. To improve the treatment of patients with cancer of the esophagus, molecular predictors of response are desperately needed. Blood samples were drawn from 29 patients with esophageal cancer prior to neoadjuvant radio-chemotherapy. After extraction of cellular tumor-RNA from blood samples, quantitative expression analysis of ERCC1 was done by real-time reverse transcription polymerase chain reaction. Nineteen (65.5%) patients showed a minor and ten (34.5%) a major histopathological response to neoadjuvant therapy. ERCC1 expression in blood of patients was detectable in 82.8%. The median ERCC1 expression was 0.62 (minimum 0.00, maximum 2.48) in minor responders and 0.24 (minimum 0.00, maximum 0.45) in major responders ( $p = 0.004$ ). No

significant associations were detectable between ERCC1 levels and patients' clinical variables. Relative ERCC1 levels above 0.452 were not associated with major histopathological response (sensitivity, 68.4; specificity, 100%), and 13 of 19 patients with minor response could be unequivocally identified. Minor responders to the applied therapy show a significant higher ERCC1 expression level in their blood compared to major responders. ERCC1 appears to be a highly specific non-invasive predictor of response to neoadjuvant therapy in esophageal cancer.

Breen, L., M. Heenan, et al. (2007). "Investigation of the role of p53 in chemotherapy resistance of lung cancer cell lines." *Anticancer Res* **27**(3A): 1361-4.

p53 is a tumour suppressor gene, which is mutated in more than half of all tumours. Most chemotherapeutic drugs cause DNA damage, which is sensed by p53; the cell can then try to repair the damage or induce cell suicide. If the p53 machinery is defective, effective chemotherapy is made more difficult. Wild-type p53 was transfected into lung cancer cell lines with different p53 status. The transfected cells were tested for changes in sensitivity to a range of chemotherapeutic agents. We observed only modest changes in the sensitivity to the chemotherapeutic agents adriamycin, taxol and carboplatin in the transfected cells lines. p53 protein was detected in a transfected clone of the cell line H1299, whose parent cells are p53 null. However, the protein did not accumulate after DNA damage, suggesting that this cell line utilises alternative pathways for responding to stress, and no longer has a functional p53 pathway. The results suggest that introduction of wild-type p53 alone is not sufficient to substantially alter the sensitivity of a cell line to a given chemotherapeutic agent.

Broet, P., S. Camilleri-Broet, et al. (2009). "Prediction of clinical outcome in multiple lung cancer cohorts by integrative genomics: implications for chemotherapy selection." *Cancer Res* **69**(3): 1055-62.

The role of adjuvant chemotherapy in patients with stage IB non-small-cell lung cancer (NSCLC) is controversial. Identifying patient subgroups with the greatest risk of relapse and, consequently, most likely to benefit from adjuvant treatment thus remains an important clinical challenge. Here, we hypothesized that recurrent patterns of genomic amplifications and deletions in lung tumors could be integrated with gene expression information to establish a robust predictor of clinical outcome in stage IB NSCLC. Using high-resolution microarrays, we generated tandem DNA copy number and gene expression profiles for 85 stage IB lung adenocarcinomas/large cell carcinomas. We identified

specific copy number alterations linked to relapse-free survival and selected genes within these regions exhibiting copy number-driven expression to construct a novel integrated signature (IS) capable of predicting clinical outcome in this series ( $P = 0.02$ ). Importantly, the IS also significantly predicted clinical outcome in two other independent stage I NSCLC cohorts ( $P = 0.003$  and  $P = 0.025$ ), showing its robustness. In contrast, a more conventional molecular predictor based solely on gene expression, while capable of predicting outcome in the initial series, failed to significantly predict outcome in the two independent data sets. Our results suggest that recurrent copy number alterations, when combined with gene expression information, can be successfully used to create robust predictors of clinical outcome in early-stage NSCLC. The utility of the IS in identifying early-stage NSCLC patients as candidates for adjuvant treatment should be further evaluated in a clinical trial.

Cai, M., G. B. Wang, et al. (2009). "Enhanced chemotherapy sensitivity of human colon cancer cells to 5-fluorouracil by siRNA recombinant expression vector targeting survivin gene." *Chin Med Sci J* **24**(2): 97-101.

To investigate the effects of small interfering RNA (siRNA) recombinant expression vector targeting survivin gene on chemotherapy sensitivity of human colon cancer cells to 5-fluorouracil, siRNA recombinant expression vector targeting survivin gene was constructed and transfected into human colon cancer cell lines LOVO. After 48 hours of transfection, cells were harvested for analysis of survivin mRNA and protein expressions using RT-PCR and Western blot. In addition, after human colon cancer cell lines were treated with Survivin siRNA and/or 5-fluorouracil, MTT assay and flow cytometry were used to analyze cell proliferation and apoptosis. Restriction endonuclease analysis confirmed that siRNA recombinant expression vector targeting survivin gene was successfully constructed. Inhibitory ratios of survivin mRNA and protein expressions by Survivin siRNA were 36.33% and 44.65%, respectively. Survivin siRNA combined with 5-fluorouracil significantly increased the cell proliferation inhibitory ratio and apoptosis ratio compared with 5-fluorouracil treating alone ( $P < 0.05$ ). The siRNA recombinant expression vector targeting survivin gene can inhibit the expression of survivin gene, and enhance chemotherapy sensitivity of human colon cancer cells to 5-fluorouracil.

Camps, C., R. Sirera, et al. (2007). "Gene expression and polymorphisms of DNA repair enzymes: cancer

susceptibility and response to chemotherapy." *Clin Lung Cancer* **8**(6): 369-75.

Platinum compounds play a central role in cancer chemotherapy. Although treatment is limited by side effects, they continue to have widespread application. One of the main aims of clinical or translational research in cancer is the search for genetic factors that could foresee treatment outcomes, in biologic activity and toxic effects. This genetic analysis might allow selection of patients who will have the greatest benefit from chemotherapy. Furthermore, a better knowledge of the underlying molecular profile of the host and the tumor will facilitate screening for lung cancer susceptibility and tailoring of chemotherapy in individual patients, choosing those most likely to respond, adjusting doses more precisely in order to reduce less adverse effects, and establishing safety profiles based on individual genetic analyses. Herein, we discuss current knowledge regarding gene expression and polymorphisms of DNA repair enzymes in regard to cancer susceptibility and response to chemotherapy.

Cappuzzo, F., G. Finocchiaro, et al. (2008). "EGFR FISH assay predicts for response to cetuximab in chemotherapy refractory colorectal cancer patients." *Ann Oncol* **19**(4): 717-23.

Standardized conditions to distinguish subpopulations of colorectal cancer (CRC) patients more and less sensitive to cetuximab therapy remain undefined. We retrospectively analyzed epidermal growth factor receptor (EGFR) copy number by fluorescence in situ hybridization (FISH) in paraffin-embedded tumor blocks from 85 chemorefractory CRC patients treated with cetuximab. Results were analyzed according to different score systems previously reported in colorectal and lung cancers. The primary end point of the study was identification of the EGFR FISH score that best associates with response rate (RR). Using receiver operating characteristic (ROC) analysis, the cut-off that best discriminated responders versus nonresponders to cetuximab was a mean of 2.92 EGFR gene copies per cell. This model showed sensitivity of 58.6% [95% confidence interval (CI) = 47.1-70.1] and specificity of 93.3% (95% CI = 80.6-100). EGFR FISH-positive patients ( $N = 43$ , 50.6%) had significantly higher RR ( $P = 0.0001$ ) and significantly longer time to disease progression ( $P = 0.02$ ) than EGFR FISH negative ( $N = 42$ , 49.4%). Other scoring systems resulted less accurate in discriminating patients with the highest likelihood of response to cetuximab therapy. CONCLUSIONS: CRC patients with high EGFR gene copy number have an increased likelihood to respond to cetuximab therapy. Prospective clinical trials with a

careful standardization of assay conditions and pattern interpretation are urgently needed.

Cappuzzo, F., C. Ligorio, et al. (2007). "EGFR and HER2 gene copy number and response to first-line chemotherapy in patients with advanced non-small cell lung cancer (NSCLC)." *J Thorac Oncol* **2**(5): 423-9.

A critical point in designing clinical trials comparing chemotherapy with epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) in patients with non-small cell lung cancer (NSCLC) is the expected benefit with standard chemotherapy in presence of biological features indicative of TKI sensitivity. The aim of this study was to assess whether EGFR and HER2 gene copy number and Akt activation are associated with response to first-line chemotherapy. Tumor samples from 190 patients with NSCLC were analyzed. EGFR and HER2 gene copy number were evaluated by fluorescence in situ hybridization in 185 and 184 cases, respectively. Akt activation was assessed by immunohistochemistry (n = 176). Additional biomarkers included EGFR DNA sequencing (n = 65), and EGFR immunohistochemistry (n = 185). Response rate was not associated with EGFR, HER2, and P-Akt status, irrespective of the method used for biomarker assessment. Among patients with EGFR gene mutations, response to chemotherapy was observed only in individuals with exon 19 deletion (response rate: 46.6% versus 0%, p = 0.02). Among the 190 patients analyzed, 123 received a treatment with a TKI as second- or third-line therapy. When assessed by fluorescence in situ hybridization or DNA sequencing, EGFR-positive patients seemed to be more sensitive to TKIs than to chemotherapy in terms of response rate and time to progression, whereas in EGFR-negative patients, response rate and time to progression favored chemotherapy. This study suggested that EGFR expression and gene copy number, HER2 gene copy number, and P-Akt expression are not associated with response to first-line chemotherapy in NSCLC. Prospective phase III trials should compare standard chemotherapy with a TKI in selected NSCLC.

Chang, C. H., K. Y. Chen, et al. (2008). "The safety and efficacy of gefitinib versus platinum-based doublets chemotherapy as the first-line treatment for advanced non-small-cell lung cancer patients in East Asia: a meta-analysis." *Lung Cancer* **62**(2): 242-52.

To evaluate the risk/benefit profiles of gefitinib in comparison with platinum-based doublets chemotherapy as a first-line treatment for chemo-naïve patients with advanced non-small-cell lung cancer in East Asia. We searched MEDLINE, EMBASE,

Cochrane Library, and ClinicalTrials.gov to identify randomized and non-randomized phase II or III clinical trials of gefitinib or chemotherapy treatment in East Asian patients published before 4/30/2007. Two reviewers independently applied selection criteria, performed quality assessment, and extracted data. Treatment arms with gefitinib 250mg/day and platinum-based doublets chemotherapy irrespective of dosage and schedule were combined to calculate the pooled estimates for efficacy and safety outcomes of interest. We identified 7 gefitinib and 41 platinum-based doublets chemotherapy trials with nearly 3000 enrolled patients for planned comparison. The pooled response rate (95% confidence interval) to gefitinib for unselected chemo-naïve population was 31% (23-38%), not substantially different from 34% (31-38%) reported by platinum-based doublets chemotherapy trials. Patients with certain characteristics were more likely to benefit from gefitinib treatment, with pooled response rates as high as 75% (60-90%) for patients with epidermal growth factor receptor (EGFR) exon 18-21 mutations; 56% (38-74%) for never smokers; 55% (41-69%) for female; and 43% (30-57%) for adenocarcinoma or bronchioalveolar carcinoma. Severe hematological adverse events related to gefitinib treatment were not observed in any of the included trials. However, the risks of severe liver and lung injury related to gefitinib treatment were both approximately 6%, significantly higher than 1% and 0.2% reported by platinum-based doublets chemotherapy trials. Our data suggest that one third of chemo-naïve NSCLC patients in East Asia would respond to oral gefitinib monotherapy while 6% would develop severe liver and lung injury. Although patients with EGFR gene mutations, female gender, non-smokers, or adenocarcinoma were more likely to respond to gefitinib, further study with valid comparison groups are needed to identify the optimal treatment strategy in these subpopulations.

Checinska, A., B. S. Hoogeland, et al. (2007). "Role of XIAP in inhibiting cisplatin-induced caspase activation in non-small cell lung cancer cells: a small molecule Smac mimic sensitizes for chemotherapy-induced apoptosis by enhancing caspase-3 activation." *Exp Cell Res* **313**(6): 1215-24.

X-linked IAP (XIAP) suppresses apoptosis by binding to initiator caspase-9 and effector caspases-3 and -7. Smac/DIABLO that is released from mitochondria during apoptosis can relieve its inhibitory activity. Here we investigated the role of XIAP in the previously found obstruction of chemotherapy-induced caspase-9 activation in non-small cell lung cancer (NSCLC) cells. Endogenously expressed XIAP bound active forms of both caspase-9 and caspase-3. However, downregulation of XIAP

using shRNA or disruption of XIAP/caspase-9 interaction using a small molecule Smac mimic were unable to significantly induce caspase-9 activity, indicating that despite a strong binding potential of XIAP to caspase-9 it is not a major determinant in blocking caspase-9 in NSCLC cells. Although unable to revert caspase-9 blockage, the Smac mimic was able to enhance cisplatin-induced apoptosis, which was accompanied by increased caspase-3 activity. Additionally, a more detailed analysis of caspase activation in response to cisplatin indicated a reverse order of activation, whereby caspase-3 cleaved caspase-9 yielding an inactive form. Our findings indicate that the use of small molecule Smac mimic, when combined with an apoptotic trigger, may have therapeutic potential for the treatment of NSCLC.

Chen, A. M., M. Zhang, et al. (2009). "Co-delivery of doxorubicin and Bcl-2 siRNA by mesoporous silica nanoparticles enhances the efficacy of chemotherapy in multidrug-resistant cancer cells." *Small* 5(23): 2673-7.

Chen, J., F. Xie, et al. (2009). "ERCC5 promoter polymorphisms at -763 and +25 predict the response to oxaliplatin-based chemotherapy in patients with advanced colorectal cancer." *Cancer Biol Ther* 8(14): 1424-30.

This study aimed to investigate whether single nucleotide polymorphisms (SNPs) in the promoter of the excision repair cross complementation group 5 (ERCC5) gene influences response to oxaliplatin-based chemotherapy. Eighty-three patients with cytologically or histologically confirmed advanced colorectal cancer (CRC), at least one measurable lesion and underwent oxaliplatin-based chemotherapy were studied. To this end, six polymorphisms (-1415C>T, -763A>G, -413C>T, +25A>G, +202C>T, +372C>T) in the ERCC5 promoter were selected for investigation. Genomic DNA was obtained from peripheral blood cells, and polymerase chain reaction-ligation detection reaction was used to analyze these SNPs. The chi(2) test or Fisher's exact test was then used to investigate the association between polymorphisms and chemotherapy response. Our results showed that the response rate among patients with the -763GG genotype (72.7%) was significantly higher than that of other genotypes (22.2% for AA genotype,  $p = 0.008$  and 37.2% for AG genotype,  $p = 0.046$  respectively). In addition, the response rate among patients with the +25AA genotype (75%) was significantly higher than that of other genotypes (24.1% for GG genotype,  $p = 0.004$  and 35.7% for AG genotype,  $p = 0.022$  respectively). Patients with the -763A/+25G haplotype had a higher risk of non-response to oxaliplatin

chemotherapy compared to those carrying the -763G/+25A haplotype (OR 2.672, 95% CI 1.353-5.278,  $p = 0.004$ ). However, no genetic variation was observed at site -413, and no significant association was found between the -1415C>T, +202C>T or +372C>T polymorphisms and chemotherapy response. Therefore, these data suggest that ERCC5 promoter polymorphisms at -763 and +25 may be important predictors of response to oxaliplatin chemotherapy.

Chen, Z. K., L. W. Lin, et al. (2009). "Interstitial chemotherapy with ricin-loaded thermosensitive hydrogel in pancreatic cancer xenograft." *Hepatobiliary Pancreat Dis Int* 8(4): 418-23.

Pancreatic cancer is one of the most aggressive malignancies, and has a poor prognosis. Despite efforts made in multiple fields, there has been little success in improving the disease-free survival rate of patients. This study was undertaken to investigate the effectiveness and feasibility of using intra-tumoral injection of ricin-loaded thermosensitive hydrogel for treatment of pancreatic cancer xenografts, attempting to develop a new treatment for human pancreatic cancer. BALB/c-(nu/nu) nude mice were inoculated subcutaneously in the right flank with the human pancreatic cancer cells, SW1990. Fourteen days after inoculation, 32 mice, bearing tumors of volume 1.5-2.0 cm<sup>3</sup>, were randomly assigned to one of four groups, and given an intra-tumoral injection of: (1) saline; (2) 23% w/w thermosensitive hydrogel alone; (3) ricin, 10 microg/kg; or (4) 10 microg/kg ricin loaded in thermosensitive hydrogel. On day 14 after administration, the tumors were excised to calculate the inhibition rate of tumor growth and perform histopathological examination. Tumor cell apoptosis was detected by flow cytometry, and RT-PCR was performed to evaluate the mRNA expression levels of Bcl2 and Bax. Intra-tumoral injection of ricin-loaded thermosensitive hydrogel resulted in remarkable control of tumor growth. The tumor became necrotic by day 14 after administration. The histological results clearly confirmed that the tumor cells were lysed. The percentage of apoptotic cells detected by flow cytometry was higher in the ricin hydrogel group than in the other groups. Semi-quantitative RT-PCR revealed that the mRNA expression level of Bcl2 was down-regulated whereas Bax was upregulated. CONCLUSIONS: Intra-tumoral injection of ricin-loaded thermosensitive hydrogel may provide an effective approach for interstitial chemotherapy in pancreatic cancer. Inducing apoptosis by downregulating Bcl2 expression and upregulating Bax expression may be a key molecular mechanism.

Chintamani, B. P. Jha, et al. (2007). "The expression of mismatched repair genes and their correlation with clinicopathological parameters and response to neo-adjuvant chemotherapy in breast cancer." *Int Semin Surg Oncol* 4: 5.

The DNA mismatch repair (MMR) pathway is an important post-replicative repair process. It is involved in the maintenance of genomic stability and MMR genes have therefore been named the proofreaders of replicating DNA. These genes repair the replicative errors of DNA and are thus imperative for genomic stability. The MMR genes have been found to be involved in promoting cytotoxicity, apoptosis, p53 phosphorylation and cell cycle arrest following exposure to exogenous DNA damaging agents. Loss of MMR function prevents the correction of replicative errors leading to instability of the genome, and can be detected by polymorphisms in micro satellites (1-6 nucleotide repeat sequences scattered in whole of the genome). This phenomenon, known as micro satellite instability (MSI), is a hallmark of MMR dysfunction and can be used as a marker of MMR dysfunction in colorectal and other malignancies. An alternative method for detection of MMR dysfunction is to test the expression of protein products of the MMR genes by immunohistochemistry (IHC), as mutations in these genes lead to reduced or absent expression of their gene products. Correlation between loss of MMR function and clinical, histopathological, behavioral parameters of the tumor and its response to chemotherapy in breast cancers may be of value in predicting tumor behavior and response to neoadjuvant chemotherapy (NACT). Neoadjuvant chemotherapy is an integral part of multimodal therapy for locally advanced breast cancer and predicting response may help in tailoring regimens in patients for optimum response. MATERIALS: After approval by the IRB (Institutional Review Board) and ethical committee of the hospital, 31 cases of locally advanced breast carcinoma (LABC) were studied to assess the correlation between MMR dysfunction, clinicopathological parameters and objective clinical response to neoadjuvant chemotherapy using immunohistochemistry. The immunohistochemical analysis for four MMR protein products--MLH1, MSH2, MSH6 and PMS2 was done in the pre NACT trucut biopsy specimen and after three cycles of NACT with C AF (cyclophosphamide, adriamycin, 5-fluorouracil) regimen, in the modified radical mastectomy specimen. There was no significant correlation observed between expression of MMR proteins and age, family history, tumor size or histological type. However there was a statistically significant negative correlation between MLH1, MSH2 expression and histological grade. There was

also a negative correlation observed between PMS2 expression after neo-adjuvant chemotherapy and clinical response. Cases with high post NACT expression of PMS2 were poor responders to chemotherapy. MSH6 was the most frequently altered MMR gene, with a negativity rate of 48% and the patients with high expression responded poorly to NACT. The study highlights the possible role of MMR expression in predicting aggressive tumor behavior (histological grade) and response to neoadjuvant chemotherapy in patients with LABC.

Climent, J., P. Dimitrow, et al. (2007). "Deletion of chromosome 11q predicts response to anthracycline-based chemotherapy in early breast cancer." *Cancer Res* 67(2): 818-26.

Despite the recent consensus on the eligibility of adjuvant systemic therapy in patients with lymph node-negative breast cancer (NNBC) based on clinicopathologic criteria, specific biological markers are needed to predict sensitivity to the different available therapeutic options. We examined the feasibility of developing a genomic predictor of chemotherapy response and recurrence risk in 185 patients with NNBC using assembled arrays containing 2,460 bacterial artificial chromosome clones for scanning the genome for DNA copy number changes. After surgery, 90 patients received anthracycline-based chemotherapy, whereas 95 did not. Tamoxifen was administered to patients with hormone receptor-positive tumors. The association of genomic and clinicopathologic data and outcome was computed using Cox proportional hazard models and multiple testing adjustment procedures. Analysis of NNBC genomes revealed a common genomic signature. Specific DNA copy number aberrations were associated with hormonal receptor status, but not with other clinicopathologic variables. In patients treated with chemotherapy, none of the genomic changes were significantly correlated with recurrence. In patients not receiving chemotherapy, deletion of eight bacterial artificial chromosome clones clustered to chromosome 11q was independently associated with relapse (disease-free survival at 10 years $\pm$ SE, 40% $\pm$ 14% versus 86% $\pm$ 6%;  $P < 0.0001$ ). The 54 patients with deletion of 11q (29%) did not present more aggressive clinicopathologic features than those without 11q loss. The adverse influence of 11q deletion on clinical outcome was confirmed in an independent validation series of 88 patients with NNBC. Our data suggests that patients with NNBC with the 11q deletion might benefit from anthracycline-based chemotherapy despite other clinical, pathologic, or genetic features. However, these initial findings should be evaluated in randomized clinical trials.

Coley, H. M. (2008). "Mechanisms and strategies to overcome chemotherapy resistance in metastatic breast cancer." *Cancer Treat Rev* 34(4): 378-90.

Resistance to chemotherapeutic agents is a significant issue in the management of patients with breast cancer. Anthracyclines, although first used over 30 years ago, are still part of the standard chemotherapy for this disease. Subsequently, the taxanes heralded a new era in chemotherapy and have been used extensively in the treatment of metastatic breast cancer. Unfortunately, along with other constituents of combination chemotherapy for metastatic breast cancer such as cyclophosphamide, these agents become increasingly ineffective in progressive disease and tumours are then deemed to be drug resistant - frequently multidrug resistant. A number of processes have been identified that can underlie clinical drug resistance, and these largely stem from in vitro laboratory-based studies in human cancer cell lines. A large proportion of these studies have focused on multidrug resistance associated with resistance to natural product anticancer agents due to the presence of putative drug transporter proteins such as P-glycoprotein, MRP1, and BCRP. Other studies have highlighted mechanisms whereby breast cancer cells show resistance to chemotherapeutic agents by altered regulation of DNA repair processes, with many other factors influencing drug detoxification processes and altering drug targets. New developmental agents with improved specificity for tumour cells, such as trastuzumab, and those with low susceptibility to common tumour-resistance mechanisms, such as ixabepilone, have provided new hope for effective treatment of breast cancer. Ixabepilone is the first in a new class of neoplastics, the epothilones. With these developments in therapy, and the technology of gene expression profiling, the future holds more promise for the development of more effective treatment for metastatic breast cancer.

Cortesi, L., A. Barchetti, et al. (2009). "Identification of protein clusters predictive of response to chemotherapy in breast cancer patients." *J Proteome Res* 8(11): 4916-33.

An attempt for the identification of potential biomarkers predictive of response to chemotherapy (CHT) in breast cancer patients has been performed by the use of two-dimensional electrophoresis and mass spectrometry analysis. Since growth and progression of tumor cells depend also on stromal factors in the microenvironment, we choose to investigate the proteins secreted in Tumor Interstitial Fluid (TIF) and in Normal Interstitial Fluids (NIF). One-hundred and twenty-two proteins have been analyzed and a comparison was also made between the proteomic

profile of responders versus nonresponders to CHT. At baseline, proteins isolated in TIF and NIF of all the 28 patients show significant differences in expression. Two clusters of proteins, differentially expressed in TIF with respect to NIF were found. Most significant is the decreased expression in TIF of CRYAB. In the protein metabolism group, also FIBB was found decreased. Some proteins involved in energy pathways were overexpressed (PGAM-1, ALDO A, PGK1, G3Pcn), while some other were down-regulated (CAH2, G3Pdx, PRDX6, TPIS). The same trend was observed for signal transduction proteins, with 14-3-3-Z overexpressed, and ANXA2 and PEBP 1 down-regulated. Moreover, an analysis has been conducted comparing protein expression in interstitial fluids of responders and nonresponders, irrespective of TIF or NIF source. This analysis lead us to identify two clusters of proteins with a modified expression, which might be predictive of response to CHT. In responders, an increase in expression of LDHA, G3Pdx, PGK1sx (energy pathways), VIME (cell growth and maintenance) and 14-3-3-Z (signal transduction), coupled with a decreased expression of TPIS, CAH 2, G3Pdx, PGK 1dx (energy pathways), TBB5 (cell growth and maintenance), LDHB and FIBB (protein metabolism), was found. We observed that CHT modifies the expression of these cluster proteins since, after treatment, their expression in TIF of responder is generally decreased. Patients not responding to CHT show an unchanged expression pattern in TIF, with the exception of protein 14-3-3-Z, which is overexpressed, and a decreased expression in NIF of several cluster proteins. In conclusion, the identification of protein clusters associated with response to CHT might be important for predicting the efficacy of a specific antineoplastic drug and for the development of less empiric strategies in choosing the therapy to be prescribed to the single patient.

Cosse, J. P. and C. Michiels (2008). "Tumour hypoxia affects the responsiveness of cancer cells to chemotherapy and promotes cancer progression." *Anticancer Agents Med Chem* 8(7): 790-7.

A solid tumour forms an organ-like structure that is comprised of cancer cells as well as stroma cells (fibroblasts, inflammatory cells) that are embedded in an extracellular matrix and are nourished by vascular network. However, tumoral microenvironment is heterogeneous due to the abnormal vasculature network and high proliferation rate of cancer cells. Because of these features, some regions are starved from oxygen, a phenomenon called hypoxia. Transient hypoxia is associated with inadequate blood flow while chronic hypoxia is the consequence of the increased oxygen diffusion distance due to tumour expansion. Both types of

hypoxia are correlated with poor outcome for patients. Moreover, hypoxia also enhances chemoresistance of cancer cells. Firstly, the delivery of drugs in hypoxic area and cellular uptake of it are affected by hypoxia or associated acidity. Secondly, some chemotherapeutic drugs require oxygen to generate free radicals that contribute to cytotoxicity. Last, hypoxia induces cellular adaptations that compromise the effectiveness of chemotherapy. In response to nutrient deprivation due to hypoxia, the rate of proliferation of cancer cells decreases but chemotherapeutic drugs are more effective against proliferating cells. On the other hand, hypoxia induces adaptation by post-translational and transcriptional changes that promote cell survival and resistance to chemotherapy. Through these changes, hypoxia promotes angiogenesis, shift to glycolytic metabolism, expression of ABC transporters, cell survival by inducing the expression of genes encoding growth factors and the modulation of apoptotic process. The aim of this review is to provide a description of known hypoxia-induced mechanisms of chemoresistance at a cellular level.

Crino, L., F. Cappuzzo, et al. (2008). "Gefitinib versus vinorelbine in chemotherapy-naïve elderly patients with advanced non-small-cell lung cancer (INVITE): a randomized, phase II study." *J Clin Oncol* **26**(26): 4253-60.

This phase II, open-label, parallel-group study compared gefitinib with vinorelbine in chemotherapy-naïve elderly patients with advanced non-small-cell lung cancer (NSCLC). Chemotherapy-naïve patients (age  $\geq 70$  years) were randomly assigned to gefitinib (250 mg/d orally) or vinorelbine (30 mg/m<sup>2</sup>) infusion on days 1 and 8 of a 21-day cycle. The primary end point was progression-free survival (PFS). Secondary end points were overall survival (OS), objective response rate (ORR), quality of life (QOL), pulmonary symptom improvement (PSI), and tolerability. Exploratory end points included epidermal growth factor receptor (EGFR) gene copy number by fluorescent in situ hybridization (FISH). Patients were randomly assigned to gefitinib (n = 97) or to vinorelbine (n = 99). Hazard ratios (HR; gefitinib v vinorelbine) were 1.19 (95% CI, 0.85 to 1.65) for PFS and 0.98 (95% CI, 0.66 to 1.47) for OS. ORR and disease control rates were 3.1% (95% CI, 0.6 to 8.8) and 43.3% (for gefitinib) and 5.1% (95% CI, 1.7 to 11.4) and 53.5% (for vinorelbine), respectively. Overall QOL improvement and PSI rates were 24.3% and 36.6% (for gefitinib) and 10.9% and 31.0% (for vinorelbine), respectively. In the 54 patients who were EGFR FISH-positive, HRs were 3.13 (95% CI, 1.45 to 6.76) for PFS and 2.88 (95% CI, 1.21 to 6.83) for OS. There were fewer treatment-

related grade 3 to 5 adverse events with gefitinib (12.8%) than with vinorelbine (41.7%). There was no statistical difference between gefitinib and vinorelbine in efficacy in chemotherapy-naïve, unselected elderly patients with advanced NSCLC, but there was better tolerability with gefitinib. Individuals who were EGFR FISH-positive benefited more from vinorelbine than from gefitinib; this unexpected finding requires further study.

Custodio, A. B., J. L. Gonzalez-Larriba, et al. (2009). "Prognostic and predictive markers of benefit from adjuvant chemotherapy in early-stage non-small cell lung cancer." *J Thorac Oncol* **4**(7): 891-910.

Lung cancer-predominantly non-small cell lung cancer (NSCLC)-is the leading cause of death from cancer in most industrialized countries. Patients with early-stage NSCLC are at substantial risk for recurrence and death even after potentially curative surgery. Multiple large randomized trials have demonstrated that adjuvant chemotherapy using modern cisplatin-based regimens can significantly improve 5-year survival in carefully selected patients with NSCLC. The current staging system is inadequate for predicting the outcome of treatment and the prognosis in an individual patient. Molecular markers may provide additional information about the likelihood of relapse beyond that obtained from pathologic staging. They may also have value in determining which patients will benefit from adjuvant platinum-based chemotherapy. This is a review focused on approaches and specific markers under study, including gene expression profiles, DNA repair pathways, class III beta-tubulin expression, abnormalities in the k-ras oncogene and p53 tumor suppressor gene, and DNA methylation markers. Additional studies will be required to determine whether these markers are useful in selecting patients for adjuvant platinum-based chemotherapy.

Dai, Z., A. C. Papp, et al. (2008). "Genotyping panel for assessing response to cancer chemotherapy." *BMC Med Genomics* **1**: 24.

Variants in numerous genes are thought to affect the success or failure of cancer chemotherapy. Interindividual variability can result from genes involved in drug metabolism and transport, drug targets (receptors, enzymes, etc), and proteins relevant to cell survival (e.g., cell cycle, DNA repair, and apoptosis). The purpose of the current study is to establish a flexible, cost-effective, high-throughput genotyping platform for candidate genes involved in chemoresistance and -sensitivity, and treatment outcomes. We have adopted SNPlex for genotyping 432 single nucleotide polymorphisms (SNPs) in 160 candidate genes implicated in response to anticancer

chemotherapy. The genotyping panels were applied to 39 patients with chronic lymphocytic leukemia undergoing flavopiridol chemotherapy, and 90 patients with colorectal cancer. 408 SNPs (94%) produced successful genotyping results. Additional genotyping methods were established for polymorphisms undetectable by SNPlex, including multiplexed SNaPshot for CYP2D6 SNPs, and PCR amplification with fluorescently labeled primers for the UGT1A1 promoter (TA)<sub>n</sub>TAA repeat polymorphism. This genotyping panel is useful for supporting clinical anticancer drug trials to identify polymorphisms that contribute to interindividual variability in drug response. Availability of population genetic data across multiple studies has the potential to yield genetic biomarkers for optimizing anticancer therapy.

Dai, Z., A. C. Papp, et al. (2008). "Genotyping panel for assessing response to cancer chemotherapy." *BMC Med Genomics* 1: 24.

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d'Amato, T. A., R. J. Landreneau, et al. (2007). "Chemotherapy resistance and oncogene expression in non-small cell lung cancer." *J Thorac Cardiovasc Surg* 133(2): 352-63.

**OBJECTIVES:** Empiric chemotherapy for patients with non-small cell lung cancer who have undergone resection is recommended without knowledge of the tumor's specific biologic characteristics, and many patients may not benefit. In vitro chemotherapy resistance is associated with clinical unresponsiveness in some tumors, and in lung cancer, chemotherapy resistance is prevalent. Multiple-agent chemotherapy resistance and association of chemotherapy resistance with molecular markers are described. Chemotherapy resistance to doublets--carboplatin and paclitaxel, cisplatin and navelbine, cisplatin and docetaxel, and cisplatin and gemcitabine--was analyzed in 4571 non-small cell lung cancer tumors with the extreme drug resistance assay. Chemotherapy resistance is defined as follows: extreme drug resistance, 1 SD above the median chemotherapy resistance; intermediate drug resistance, between the median and extreme drug resistances; and low drug resistance, 1 SD below the median. Chemotherapy resistance was compared with DNA ploidy measured by flow cytometry, and markers p53 and epithelial growth factor receptor were assayed by immunohistochemistry. Tumors with extreme or intermediate drug resistance were noted in 30% to carboplatin-paclitaxel, in 24% to cisplatin-avelbine, in 42% to cisplatin-gemcitabine, and in 27% to cisplatin-docetaxel. Extreme or intermediate drug resistance to at least one drug occurred in 74% to carboplatin-paclitaxel, in 68% to cisplatin-avelbine, in 88% to cisplatin-gemcitabine, and in 68% to cisplatin-docetaxel. More intermediate plus extreme chemotherapy resistances occurred in aneuploid tumors to etoposide (53% vs 36%,  $P = .0002$ ) and topotecan (48% vs 36%,  $P = .0094$ ), with less intermediate or extreme chemotherapy resistance to gemcitabine (88% vs 81%,  $P = .0345$ ). p53-Positive tumors had more intermediate or extreme resistance to etoposide (57% vs 44%,  $P = .0009$ ) and doxorubicin (73% vs. 58%,  $P = .0324$ ) and less intermediate or extreme resistance to cisplatin (44% vs 54%,  $P = .0125$ ), to carboplatin (47% vs 57%,  $P = .0129$ ), to taxol (47% vs 57%,  $P = .0056$ ), and to gemcitabine (78% vs 87%,  $P = .0108$ ). Fewer epithelial growth factor receptor-positive tumors were extremely drug resistant to cisplatin (13% vs 26%,  $P = .0074$ ) and carboplatin (13% v. 30%,  $P = .0008$ ).

**CONCLUSIONS:** Multi-drug chemotherapy resistance in non-small cell lung cancer tumor cultures is common, and associations between molecular markers and in vitro chemotherapy resistance are noted. Clinical validation through integration of such testing into clinical trials seems warranted.

Danesi, R., G. Pasqualetti, et al. (2009). "Pharmacogenomics in non-small-cell lung cancer chemotherapy." *Adv Drug Deliv Rev* **61**(5): 408-17.

The disappointing results in long-term survival of patients who have a resectable non-small cell lung cancer (NSCLC) may reflect the lack of knowledge on the way by which molecular abnormalities in neoplastic cells affect responsiveness to adjuvant therapy. This issue deserves intensive investigation to select methodological approaches for a new generation of chemotherapeutic strategies. Remarkable advances in the understanding of NSCLC biology have been made, including the discovery of critical mutations in oncogenes (i.e. K-Ras and c-myc), as well as the loss of tumor-suppressor genes, such as TP53, p16(INK4) or Rb. Other studies demonstrated the role of mutations or deregulation of the expression of several molecular determinants involved in cell cycle control such as epidermal growth factor receptor (EGFR). All these characteristics, as well as alterations in gene products directly related to drug activity, might contribute to the aggressive behaviour of NSCLC. The future challenge of chemotherapy of NSCLC relies on the identification of molecular markers that are predictive of drug sensitivity and are helpful in the selection of chemotherapeutic agents best suited to the individual patient. Other intriguing issues will be the identification of the optimal drug sequence in combination regimens and the pharmacogenetics of severe toxicities. Moreover, due to the developments of novel technologies to decipher genetic alterations involved in tumor progression, new agents are gaining momentum, including inhibitors of intracellular signal transduction, and a large body of research, using prospective clinical trials, should be devoted to this area.

Davidovich, S., O. Ben-Izhak, et al. (2008). "Over-expression of Skp2 is associated with resistance to preoperative doxorubicin-based chemotherapy in primary breast cancer." *Breast Cancer Res* **10**(4): R63.

**INTRODUCTION:** Preoperative chemotherapy is often used in patients with locally advanced breast cancer. However, commonly used clinical and pathological parameters are poor predictors of response to this type of therapy. Recent studies have suggested that altered regulation of the cell cycle in cancer may be involved in resistance to chemotherapy. Over-expression of the ubiquitin ligase Skp2 results in loss of the cell cycle inhibitor p27Kip1 and is associated with poor prognosis in early breast cancer. The purpose of the present study was to examine the role of these proteins as predictors of clinical outcome and response to chemotherapy in locally advanced breast cancer. The expression levels

of Skp2 and p27Kip1 were determined by immunohistochemistry both before and after preoperative chemotherapy in 40 patients with locally advanced breast cancer. All patients were treated with cyclophosphamide/doxorubicin (adriamycin)/5-fluorouracil (CAF) and some patients received additional treatment with docetaxel. Expression data were compared with patients' clinical and pathological features, clinical outcome, and response to chemotherapy. Skp2 expression before preoperative chemotherapy was inversely related to p27Kip1 levels, tumor grade, and expression of estrogen and progesterone receptors. Both Skp2 and p27Kip1 were found to be accurate prognostic markers for disease-free and overall survival. High preoperative expression of Skp2 was associated with resistance to CAF therapy in 94% of patients ( $P < 0.0001$ ) but not with resistance to docetaxel. Skp2 expression may be a useful marker for predicting response to doxorubicin-based preoperative chemotherapy and clinical outcome in patients with locally advanced breast cancer.

de Haas, E. C., N. Zwart, et al. (2008). "Variation in bleomycin hydrolase gene is associated with reduced survival after chemotherapy for testicular germ cell cancer." *J Clin Oncol* **26**(11): 1817-23.

Response to chemotherapy may be determined by gene polymorphisms involved in metabolism of cytotoxic drugs. A plausible candidate is the gene for bleomycin hydrolase (BLMH), an enzyme that inactivates bleomycin, an essential component of chemotherapy regimens for disseminated testicular germ-cell cancer (TC). We investigated whether the single nucleotide polymorphism (SNP) A1450G of the BLMH gene (rs1050565) is associated with survival. PATIENTS AND Data were collected on survival and BLMH genotype of 304 patients with TC treated with bleomycin-containing chemotherapy at the University Medical Center Groningen, the Netherlands, between 1977 and 2003. Survival according to genotype was analyzed using Kaplan-Meier curves with log-rank testing and Cox regression analysis with adjustment for confounders. BLMH gene SNP A1450G has a significant effect on TC-related survival (log-rank  $P = .001$ ). The homozygous variant (G/G) genotype ( $n = 31$ ) is associated with decreased TC related survival compared with the heterozygous variant (A/G;  $n = 133$ ) and the wild-type (A/A;  $n = 140$ ). With Cox regression the G/G genotype proves to be an unfavorable prognostic factor, in addition to the commonly used International Germ Cell Consensus Classification prognosis group, with a hazard ratio of 4.97 (95% CI, 2.17 to 11.39) for TC-related death. Furthermore, the G/G genotype shows a higher

prevalence of early relapses. The homozygous variant G/G of BLMH gene SNP A1450G is associated with reduced survival and higher prevalence of early relapses in TC patients treated with bleomycin-containing chemotherapy. This association is hypothesis generating and may eventually be of value for risk classification and selection for alternative treatment strategies in patients with disseminated TC.

Delbes, G., D. Chan, et al. (2009). "Impact of the chemotherapy cocktail used to treat testicular cancer on the gene expression profile of germ cells from male Brown-Norway rats." *Biol Reprod* **80**(2): 320-7.

Advances in treatment for testicular cancer that include the coadministration of bleomycin, etoposide, and cisplatin (BEP) have brought the cure rate to higher than 90%. The goal of this study was to elucidate the impact of BEP treatment on gene expression in male germ cells. Brown-Norway rats were treated for 9 wk with vehicle (0x) or BEP at doses equivalent to 0.3x and 0.6x the human dose. At the end of treatment, spermatogenesis was affected, showing altered histology and a decreased sperm count; spermatozoa had a higher number of DNA breaks. After 9 wk of treatment, round spermatids were isolated, and RNA was extracted and probed on Rat230-2.0 Affymetrix arrays. Of the 31 099 probe sets present on the array, 59% were expressed in control round spermatids. BEP treatment significantly altered the expression of 221 probe sets, with at least a 1.5-fold change compared with controls; 80% were upregulated. We observed a dose-dependent increase in the expression of oxidative stress response genes and no change in the expression of genes involved in DNA repair. BEP upregulated genes were implicated in pathways related to Jun and Junb protooncogenes. Increased mRNA levels of Jun and Junb were confirmed by quantitative RT-PCR; furthermore, JUN protein was increased in elongating spermatids. Thus, BEP exposure triggers an oxidative stress response in round spermatids and induces many pathways that may lead to the survival of damaged cells and production of abnormal sperm.

Dylla, S. J., L. Beviglia, et al. (2008). "Colorectal cancer stem cells are enriched in xenogeneic tumors following chemotherapy." *PLoS One* **3**(6): e2428.

Patients generally die of cancer after the failure of current therapies to eliminate residual disease. A subpopulation of tumor cells, termed cancer stem cells (CSC), appears uniquely able to fuel the growth of phenotypically and histologically diverse tumors. It has been proposed, therefore, that failure to effectively treat cancer may in part be due to preferential resistance of these CSC to chemotherapeutic agents. The subpopulation of human

colorectal tumor cells with an ESA(+)CD44(+) phenotype are uniquely responsible for tumorigenesis and have the capacity to generate heterogeneous tumors in a xenograft setting (i.e. CoCSC). We hypothesized that if non-tumorigenic cells are more susceptible to chemotherapeutic agents, then residual tumors might be expected to contain a higher frequency of CoCSC. **METHODS AND FINDINGS:** Xenogeneic tumors initiated with CoCSC were allowed to reach approximately 400 mm<sup>3</sup>, at which point mice were randomized and chemotherapeutic regimens involving cyclophosphamide or Irinotecan were initiated. Data from individual tumor phenotypic analysis and serial transplants performed in limiting dilution show that residual tumors are enriched for cells with the CoCSC phenotype and have increased tumorigenic cell frequency. Moreover, the inherent ability of residual CoCSC to generate tumors appears preserved. Aldehyde dehydrogenase 1 gene expression and enzymatic activity are elevated in CoCSC and using an in vitro culture system that maintains CoCSC as demonstrated by serial transplants and lentiviral marking of single cell-derived clones, we further show that ALDH1 enzymatic activity is a major mediator of resistance to cyclophosphamide: a classical chemotherapeutic agent. **CONCLUSIONS:** CoCSC are enriched in colon tumors following chemotherapy and remain capable of rapidly regenerating tumors from which they originated. By focusing on the biology of CoCSC, major resistance mechanisms to specific chemotherapeutic agents can be attributed to specific genes, thereby suggesting avenues for improving cancer therapy.

Dziedziszko, R., B. Holm, et al. (2007). "Epidermal growth factor receptor gene copy number and protein level are not associated with outcome of non-small-cell lung cancer patients treated with chemotherapy." *Ann Oncol* **18**(3): 447-52.

Survival benefit of non-small-cell lung cancer (NSCLC) patients treated with epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors is predicted by high EGFR gene copy number and by strong EGFR protein expression. Clinical relevance of these features in patients treated with chemotherapy has not been reported. **PATIENTS AND METHODS:** This study included 82 NSCLC patients treated with chemotherapy. There were 45% of females, 6% of never smokers and 45% of patients diagnosed with adenocarcinoma. EGFR gene copy number was evaluated by fluorescence in situ hybridization and EGFR protein level by immunohistochemistry. High EGFR gene copy number and protein level were found in 33% and 71% of patients, respectively. Both markers were significantly associated (P = 0.01). For objective response and disease control, there was no

difference between patients defined as negative or positive for both EGFR gene copy number ( $P = 0.39$  and  $P = 1.00$ , respectively) and for EGFR protein ( $P = 1.00$  and  $P = 0.80$ , respectively). There were no differences in progression-free and overall survival according to EGFR gene copy number ( $P = 0.76$  and  $P = 0.82$ , respectively) and protein level ( $P = 0.67$  and  $P = 0.62$ , respectively). In chemotherapy-treated NSCLC patients, EGFR gene copy number was positively associated with protein level but none of the features were predictive for either treatment response or survival.

Ebi, N., H. Semba, et al. (2008). "A phase II trial of gefitinib monotherapy in chemotherapy-naive patients of 75 years or older with advanced non-small cell lung cancer." *J Thorac Oncol* 3(10): 1166-71.

Gefitinib has shown modest activity in patients with recurrent non-small cell lung cancer (NSCLC) after platinum-based chemotherapy. However, the activity of gefitinib as first-line chemotherapy remains unclear, especially unknown in elderly patients. A multicenter phase II trial was conducted to evaluate the efficacy and tolerability of gefitinib for elderly patients with chemotherapy-naive NSCLC. Elderly chemotherapy-naive patients with advanced NSCLC, ECOG PS of 0-2, and adequate organ functions received 250 mg/day of gefitinib. The primary objective of this study was to determine the objective response rate (RR). Secondary endpoints were tolerability, disease-related symptom using lung cancer subscale (LCS) in FACT-L, progression free survival (PFS) and overall survival (OS). We investigated mutation status of the epidermal growth factor receptor (EGFR) gene in cases with available tumor samples. Fifty patients were enrolled, of whom 49 were eligible. Median age (range) was 80 (75-90) years. Thirty-two patients were female (65%) and 40 patients had adenocarcinoma (82%). The objective RR was 25% (CI 95%, 13-39). Median survival time was 10 months (CI 95%, 7-20) and 1-year survival rate was 50%. The most frequent adverse events were skin disorders (76%). Fifteen patients (30%) experienced toxicities  $\geq$  grade 3. There were four patients with possible interstitial lung disease including two treatment-related deaths. Symptom improvement rate using LCS was 49% at 4 weeks of gefitinib therapy. Tumor samples from 17 patients were analyzed for EGFR mutation status. EGFR mutations were detected in tumor tissues from 7 patients, of which 5 had partial responses (71%). CONCLUSIONS: Gefitinib monotherapy is effective and relatively well tolerated in chemotherapy-naive elderly patients with advanced NSCLC. Gefitinib has potential as a first-line therapeutic option in elderly patients with advanced NSCLC.

Edelman, M. J., D. Watson, et al. (2008). "Eicosanoid modulation in advanced lung cancer: cyclooxygenase-2 expression is a positive predictive factor for celecoxib + chemotherapy--Cancer and Leukemia Group B Trial 30203." *J Clin Oncol* 26(6): 848-55.

Increased expression of eicosanoids in cancer has been associated with adverse prognosis. We performed a randomized phase II trial to test the hypothesis that inhibitors of two eicosanoid pathways (cyclooxygenase-2 [COX-2], celecoxib and 5-lipoxygenase [5-LOX], zileuton) added to chemotherapy would improve outcome in advanced non-small-cell lung cancer (NSCLC). PATIENTS AND Patients with advanced NSCLC, a performance status of 0 to 2, and no prior therapy were eligible. All patients received carboplatin area under the curve (AUC) 5.5 mg/mL x min day 1 + gemcitabine (1,000 mg/m<sup>2</sup>) days 1 and 8. Patients were randomly assigned to: (a) zileuton 600 mg PO qid, (b) celecoxib 400 mg PO bid, or (c) celecoxib and zileuton at the same doses. Immunohistochemical staining for COX-2 and 5-LOX was performed without knowledge of outcomes. One hundred forty patients were entered and 134 were eligible and treated. There was no survival difference between the arms. COX-2 expression was a negative prognostic marker for overall survival (OS; hazard ratio [HR] = 2.51,  $P = .019$  for index  $\geq 4$ ; HR = 4.16,  $P = .005$  for index = 9) for patients not receiving celecoxib. Patients with increased COX-2 expression (index  $\geq 4$ ), receiving celecoxib had better survival than did COX-2-expressing patients not receiving drug (HR = .342,  $P = .005$  for OS; HR = .294,  $P = .002$  for failure-free survival). Multivariate analysis confirmed the interaction of COX-2 and celecoxib on survival. 5-LOX expression was neither prognostic nor predictive. This study failed to demonstrate the value of dual eicosanoid inhibition or benefit from either agent alone in addition to chemotherapy. However, a prospectively defined subset analysis suggests an advantage for celecoxib and chemotherapy for patients with moderate to high COX-2 expression.

Farmer, P., H. Bonnefoi, et al. (2009). "A stroma-related gene signature predicts resistance to neoadjuvant chemotherapy in breast cancer." *Nat Med* 15(1): 68-74.

To better understand the relationship between tumor-host interactions and the efficacy of chemotherapy, we have developed an analytical approach to quantify several biological processes observed in gene expression data sets. We tested the approach on tumor biopsies from individuals with estrogen receptor-negative breast cancer treated with chemotherapy. We report that increased stromal gene

expression predicts resistance to preoperative chemotherapy with 5-fluorouracil, epirubicin and cyclophosphamide (FEC) in subjects in the EORTC 10994/BIG 00-01 trial. The predictive value of the stromal signature was successfully validated in two independent cohorts of subjects who received chemotherapy but not in an untreated control group, indicating that the signature is predictive rather than prognostic. The genes in the signature are expressed in reactive stroma, according to reanalysis of data from microdissected breast tumor samples. These findings identify a previously undescribed resistance mechanism to FEC treatment and suggest that antistromal agents may offer new ways to overcome resistance to chemotherapy.

Felip, E., F. Rojo, et al. (2008). "A phase II pharmacodynamic study of erlotinib in patients with advanced non-small cell lung cancer previously treated with platinum-based chemotherapy." *Clin Cancer Res* **14**(12): 3867-74.

To examine potential markers of clinical benefit and the effects of erlotinib on the epidermal growth factor receptor (EGFR) signaling pathway in advanced non-small cell lung cancer patients refractory to platinum-based chemotherapy. **EXPERIMENTAL DESIGN:** Patients were given erlotinib (150 mg/d). Tumor biopsies were done immediately before treatment and in a subgroup of patients after 6 weeks' treatment. Of 73 evaluable patients, 7 (10%) had partial response and 28 (38%) had stable disease. In 53 patients with baseline tumor samples, no relationship was observed between pretreatment levels of EGFR, phosphorylated (p)-EGFR, p-AKT, p-mitogen-activated protein kinase (MAPK), or p27 and clinical benefit (i.e., response, or stable disease  $\geq$ 12 weeks). Tumors from 15 of 57 patients had high EGFR gene copy number, assessed using fluorescence in situ hybridization (FISH positive), 10 of whom had clinical benefit, compared with 5 of 42 FISH-negative patients. FISH-positive patients had longer median progression-free [137 versus 43 days,  $P = 0.002$ ; hazard ratio (HR), 0.37] and overall (226 versus 106 days,  $P = 0.267$ ; HR, 0.70) survival than FISH-negative patients. In paired biopsy samples from 14 patients, p-EGFR ( $P = 0.002$ ), p-MAPK ( $P = 0.001$ ), and Ki-67 ( $P = 0.025$ ) levels were significantly reduced after 6 weeks' treatment. Apoptosis was significantly increased in patients with clinical benefit ( $P = 0.029$ ), and may be a marker of clinical benefit. In this study, EGFR FISH-positive status was associated with improved outcome after erlotinib therapy. Erlotinib led to reduced levels of p-EGFR, p-MAPK, and Ki-67, and stimulated apoptosis in tumor samples from patients with clinical benefit.

Felip, E. and R. Rosell (2007). "Testing for excision repair cross-complementing 1 in patients with non-small-cell lung cancer for chemotherapy response." *Expert Rev Mol Diagn* **7**(3): 261-8.

Non-small-cell lung cancer (NSCLC) accounts for 80% of all cases of lung cancer, which is the leading cause of cancer mortality. Most patients with NSCLC are diagnosed in the advanced stages, with the majority of patients presenting with Stage III or IV disease. Despite the introduction of more effective chemotherapeutic agents, it appears that a survival plateau has been reached. Thus, new treatment strategies are clearly needed. One such approach is the study of genes influencing drug activity, which offer the possibility of tailoring therapy according to the specific genetic profile of individual patients. Approximately 90% of lung cancer mortality among men and 80% among women is attributable to smoking. Cigarette smoking has been found to induce DNA damage. Lower DNA-repair capacities have been associated with a higher risk of lung cancer. Once cancer has been diagnosed, defective DNA-repair capacities can confer a favorable cytotoxic effect. The nucleotide excision repair system plays a major role in repairing a variety of distorting lesions, notably platinum-induced DNA adducts. The present review focuses on the excision repair cross-complementing (ERCC)1 which is the lead enzyme in the nucleotide excision repair process. Several groups have investigated the influence of ERCC1 on resistance to chemotherapy. Overall, the data suggest that ERCC1 is a marker for resistance to cisplatin. At present, molecular markers, such as ERCC1, represent a potential parameter to help guide clinical treatment decisions. Prospective pharmacogenomic studies are therefore a research priority in NSCLC.

Feng, J., X. Sun, et al. (2009). "XPA A23G polymorphism is associated with the elevated response to platinum-based chemotherapy in advanced non-small cell lung cancer." *Acta Biochim Biophys Sin (Shanghai)* **41**(5): 429-35.

DNA repair capacity (DRC) is correlated with sensitivity of cancer cells toward platinum-based chemotherapy. We hypothesize that genetic polymorphisms in DNA repair gene XPA (xeroderma pigmentosum group A) and XPG (xeroderma pigmentosum group G) (ERCC5, excision repair cross-complementation group 5), which result in inter-individual differences in DNA repair efficiency, may predict clinical response to platinum agents in advanced non-small cell lung cancer (NSCLC) patients. In this study, we find that the A right curved arrow G change of XPA A23G polymorphism significantly increased response to platinum-based

chemotherapy. Polymorphism in XPG His46His was associated with a decreased treatment response, but was not statistically significant.

Feng, J. F., J. Z. Wu, et al. (2009). "Polymorphisms of the ribonucleotide reductase M1 gene and sensitivity to platin-based chemotherapy in non-small cell lung cancer." *Lung Cancer* **66**(3): 344-9.

Ribonucleotide reductase catalyzes the rate limiting step of deoxyribonucleotide formation, a crucially important step in DNA synthesis and repair. The regulatory subunit M1 of ribonucleotide reductase (RRM1) is the necessary part of the RR function and controls substrate specificity and global on/off enzyme activity. Despite recent research progress, the role of RRM1 in lung cancer sensitivity to chemotherapeutics remains to be elucidated. This study was to investigate the relationship between polymorphisms of the RRM1 gene and sensitivity to platinum-based chemotherapy in non-small cell lung cancer (NSCLC). Genomic DNA samples from 214 NSCLC patients treated with platinum-based chemotherapy were used to determine the RRM1 promoter allelotypes. The RR37CC-RR524TT was the most frequent allelotype (38.50%), followed by RR37AC-RR524CT (26.76%) and RR37CC-RR524CT (14.95%). The average response rate for chemotherapy was 44.4%. The response rates to the treatment regimens in the RR37CC-RR524TT, RR37AC-RR524CT and RR37CC-RR524CT allelotypes were 43.9%, 52.6%, and 51.6%, respectively. The response rates to therapy among patients with RRM1 (-)524 allelotypes were significantly different ( $p=0.046$ ), whereas that among patients with RRM1 (-)37 allelotypes were not significant. Further analysis showed that the response rate in the patients with RR524CT allelotype (52.3%) was the highest, compared with that with RR37CC-RR524TT allelotype (43.9%,  $p=0.28$ ), or the Others (RR524CC and RR37AC-RR524TT, 30.2%,  $p=0.02$ ). Our results suggest that the RR524CT allelotype may be associated with an increased sensitivity to platinum-based chemotherapy in NSCLC. Further research on determining RR524CT as a clinical marker for predicting response to platinum-based therapy in NSCLC patients is warranted.

Filipits, M., V. Haddad, et al. (2007). "Multidrug resistance proteins do not predict benefit of adjuvant chemotherapy in patients with completely resected non-small cell lung cancer: International Adjuvant Lung Cancer Trial Biologic Program." *Clin Cancer Res* **13**(13): 3892-8.

The purpose of our study was to determine whether multidrug resistance proteins (MRP) are of prognostic and/or predictive value in patients who were enrolled into the International Adjuvant Lung

Cancer Trial (IALT). EXPERIMENTAL DESIGN: Expression of MRP1 and MRP2 was immunohistochemically assessed in tumor specimens obtained from 782 IALT patients. Prognostic and predictive analyses were based on Cox models adjusted for clinical and pathologic variables. MRP1 expression was considered positive in 364 (47%) patients and MRP2 expression in 313 (40%) patients. MRP2-positive patients had a significantly shorter overall survival than MRP2-negative patients in the total patient population [adjusted hazard ratio for death, 1.37; 95% confidence interval (95% CI), 1.09-1.72;  $P = 0.007$ ]. There was no significant association between MRP1 expression and overall survival. Neither MRP1 nor MRP2 predicted response to adjuvant cisplatin-based chemotherapy. CONCLUSIONS: MRP2 expression is an independent prognostic factor in patients with completely resected non-small cell lung cancer but neither MRP1 nor MRP2 was of predictive value in patients enrolled into the IALT.

Filipits, M., R. Pirker, et al. (2007). "Cell cycle regulators and outcome of adjuvant cisplatin-based chemotherapy in completely resected non-small-cell lung cancer: the International Adjuvant Lung Cancer Trial Biologic Program." *J Clin Oncol* **25**(19): 2735-40.

The International Adjuvant Lung Cancer Trial (IALT) demonstrated that adjuvant cisplatin-based chemotherapy improves the survival of patients with completely resected non-small-cell lung cancer (NSCLC). The purpose of our study was to determine whether cell cycle regulators are of prognostic and/or predictive value in patients who were enrolled onto the IALT. PATIENTS AND Expression of p27Kip1, p16INK4A, cyclin D1, cyclin D3, cyclin E, and Ki-67 was immunohistochemically assessed in tumor specimens obtained from 778 IALT patients. Prognostic and predictive analyses were based on Cox models adjusted for clinical and pathologic parameters. There was a relationship between p27Kip1 status and benefit of cisplatin-based chemotherapy (test for interaction,  $P = .02$ ). Among patients with p27Kip1-negative tumors, cisplatin-based chemotherapy resulted in longer overall survival compared with controls (adjusted hazard ratio [HR] for death = 0.66; 95% CI, 0.50 to 0.88;  $P = .006$ ). In patients with p27Kip1-positive tumors, overall survival was not different between patients treated with cisplatin-based chemotherapy and controls (adjusted HR for death = 1.09; 95% CI, 0.82 to 1.45;  $P = .54$ ). The other cell cycle regulators and Ki-67 did not predict benefit of adjuvant cisplatin-based chemotherapy. None of these biomarkers was significantly associated with overall survival of the

patients in the total study population. NSCLC patients with p27Kip1-negative tumors benefit from adjuvant cisplatin-based chemotherapy after complete tumor resection. Before establishing p27Kip1 as a routine marker for selection of patients for adjuvant chemotherapy, the predictive value of p27Kip1 has to be confirmed in patients from other trials.

Fillmore, C. M. and C. Kuperwasser (2008). "Human breast cancer cell lines contain stem-like cells that self-renew, give rise to phenotypically diverse progeny and survive chemotherapy." Breast Cancer Res **10**(2): R25.

**INTRODUCTION:** The phenotypic and functional differences between cells that initiate human breast tumors (cancer stem cells) and those that comprise the tumor bulk are difficult to study using only primary tumor tissue. We embarked on this study hypothesizing that breast cancer cell lines would contain analogous hierarchical differentiation programs to those found in primary breast tumors. Eight human breast cell lines (human mammary epithelial cells, and MCF10A, MCF7, SUM149, SUM159, SUM1315 and MDA.MB.231 cells) were analyzed using flow cytometry for CD44, CD24, and epithelial-specific antigen (ESA) expression. Limiting dilution orthotopic injections were used to evaluate tumor initiation, while serial colony-forming unit, reconstitution and tumorsphere assays were performed to assess self-renewal and differentiation. Pulse-chase bromodeoxyuridine (5-bromo-2-deoxyuridine [BrdU]) labeling was used to examine cell cycle and label-retention of cancer stem cells. Cells were treated with paclitaxel and 5-fluorouracil to test selective resistance to chemotherapy, and gene expression profile after chemotherapy were examined. The percentage of CD44+/CD24- cells within cell lines does not correlate with tumorigenicity, but as few as 100 cells can form tumors when sorted for CD44+/CD24-/low/ESA+. Furthermore, CD44+/CD24-/ESA+ cells can self-renew, reconstitute the parental cell line, retain BrdU label, and preferentially survive chemotherapy. These data validate the use of cancer cell lines as models for the development and testing of novel therapeutics aimed at eradicating cancer stem cells.

Francipane, M. G., V. Eterno, et al. (2009). "Suppressor of cytokine signaling 3 sensitizes anaplastic thyroid cancer to standard chemotherapy." Cancer Res **69**(15): 6141-8.

We previously showed that cancer cells from papillary, follicular, and anaplastic thyroid carcinomas produce interleukin-4 and interleukin-10, which counteract the cytotoxic activity of conventional chemotherapy through the up-regulation of

antiapoptotic molecules. Here, we identify Janus kinase/signal transducers and activators of transcription (STAT) and phosphatidylinositol 3-kinase (PI3K)/AKT as the down-stream pathways through which these cytokines confer resistance to cell death in thyroid cancer. We found that the absence of suppressors of cytokine signaling (SOCS) molecules allows the propagation of the survival signaling. Exogenous expression of SOCS1, SOCS3, and SOCS5 in the highly aggressive anaplastic thyroid cancer cells reduces or abolishes STAT3 and 6 phosphorylation and PI3K/Akt pathway activation resulting in alteration in the balance of proapoptotic and antiapoptotic molecules and sensitization to chemotherapeutic drugs in vitro. Likewise, exogenous expression of SOCS3 significantly reduces tumor growth and potentially enhances the efficacy of chemotherapy in vivo. Our results indicate that SOCS3 regulation of cytokines-prosurvival programs might represent a new strategy to overcome the resistance to chemotherapy-induced cell death of thyroid cancer.

Fujimoto-Ouchi, K., F. Sekiguchi, et al. (2007). "Antitumor activity of trastuzumab in combination with chemotherapy in human gastric cancer xenograft models." Cancer Chemother Pharmacol **59**(6): 795-805.

To clarify the antitumor activity of trastuzumab and its potential as an effective treatment for gastric cancer patients. Levels of HER2 expression in tumor tissues of gastric cancer cell lines were examined using immunohistochemistry (IHC), fluorescence in situ hybridization (FISH), and mRNA quantification. Efficacy of trastuzumab was examined as a single agent or in combination with chemotherapeutic agents widely used clinically for gastric cancers in HER2-overexpressing human gastric cancer xenograft models. Two of nine human gastric cancer xenograft models, NCI-N87 and 4-1ST, showed overexpression of HER2 mRNA and protein by IHC (HercepTest) and HER2 gene amplification by FISH (Pathvysion). HER2 protein showed potent staining in peripheral membranes, similar to the staining pattern of breast cancer. FISH scores were also comparable to those of breast cancer models. Trastuzumab as a single agent inhibited the tumor growth in both of the HER2-overexpressing models but not in the HER2-negative models, GXF97 and MKN-45. In any combination with capecitabine, cisplatin, irinotecan, docetaxel, or paclitaxel, trastuzumab showed more potent antitumor activity than the anticancer agents alone. A three-drug combination of capecitabine, cisplatin, and trastuzumab showed remarkable tumor growth inhibition. In NCI-N87 in vitro, trastuzumab showed

direct antiproliferative activity according to cell count or crystal violet dying, and showed indirect antitumor activity such as antibody-dependent cellular cytotoxicity. The antitumor activity of trastuzumab observed in human gastric cancer models warrants consideration of its use in clinical treatment regimens for human gastric cancer as a single agent or a combination drug with various chemotherapeutic agents.

Gan, L., S. Chen, et al. (2009). "Inhibition of the androgen receptor as a novel mechanism of taxol chemotherapy in prostate cancer." *Cancer Res* **69**(21): 8386-94.

Taxol chemotherapy is one of the few therapeutic options for men with castration-resistant prostate cancer (CRPC). However, the working mechanisms for Taxol are not fully understood. Here, we showed that treatment of 22Rv1, a PTEN-positive CRPC cell line, with paclitaxel and its semisynthetic analogue docetaxel decreases expression of the androgen receptor (AR)-activated genes prostate-specific antigen (PSA) and Nkx3.1 but increases expression of the AR repression gene maspin, suggesting that Taxol treatment inhibits AR activity. This was further supported by the observation that the activity of AR luciferase reporter genes was inhibited by paclitaxel. In contrast, paclitaxel treatment failed to inhibit AR activity in the PTEN-null CRPC cell line C4-2. However, pretreatment of C4-2 cells with the phosphoinositide 3-kinase inhibitor LY294002 restored paclitaxel inhibition of the AR. Treatment of 22Rv1 xenografts in mice with docetaxel induced mitotic arrest and a decrease in PSA expression in tumor cells adjacent to vascular vessels. We further showed that paclitaxel induces nuclear accumulation of FOXO1, a known AR suppressive nuclear factor, and increases the association of FOXO1 with AR proteins in the nucleus. FOXO1 knockdown with small interfering RNA attenuated the inhibitory effect of paclitaxel on AR transcriptional activity, expression of PSA and Nkx3.1, and cell survival. These data reveal a previously uncharacterized, FOXO1-mediated, AR-inhibitory effect of Taxol in CRPC cells that may play an important role in Taxol-mediated inhibition of CRPC growth.

Gautschi, O., P. C. Mack, et al. (2008). "Pharmacogenomic approaches to individualizing chemotherapy for non-small-cell lung cancer: current status and new directions." *Clin Lung Cancer* **9 Suppl 3**: S129-38.

At present, selection of chemotherapy regimens for individual patients remains largely an empirical process. Nevertheless, developing pharmacogenomic approaches for selection of

chemotherapy through predictive biomarkers now appears feasible because of improved understanding of underlying molecular mechanisms. Although diverse terminology has been applied to such pharmacogenomic approaches (individualizing, customizing, or personalizing therapy), all rely on commonly shared principles for assessing tumor- or host-related factors. Herein, we summarize emerging data regarding pharmacogenomic approaches to treatment selection for non-small-cell lung cancer, focusing primarily on biomarkers relevant to 2 important chemotherapeutic drug classes: platinum compounds and antimicrotubule agents. The results of pilot studies and the first randomized prospective trial testing this concept are described, including limitations in the clinical setting of advanced-stage disease. Methodologic and technical aspects of pharmacogenomic approaches are elucidated, recommendations for clinical application are provided, and new directions in this field are projected.

Giaccone, G., R. B. Iacona, et al. (2009). "Epidermal growth factor receptor expression analysis in chemotherapy-naive patients with advanced non-small-cell lung cancer treated with gefitinib or placebo in combination with platinum-based chemotherapy." *J Cancer Res Clin Oncol* **135**(3): 467-76.

Two large, randomized, placebo-controlled trials (IRESSA NSCLC Trial Assessing Combination Therapy; INTACT 1 and 2) in non-small-cell lung cancer (NSCLC) failed to show survival benefit for gefitinib (IRESSA) in combination with first-line platinum-based chemotherapy. Epidermal growth factor receptor (EGFR) staining was assessed retrospectively in relation to survival response to gefitinib in combination with chemotherapy. Tumor biopsies obtained prior to start of therapy were assessed by immunohistochemistry for EGFR using the Dako EGFR pharmDx assay (Dako, Denmark). Analyses were stratified by trial and performed independently for patients randomized to placebo and gefitinib as well as for both treatment groups combined. A restricted backwards elimination Cox regression analysis was conducted to identify independent EGFR factors that were statistically significant ( $P < 0.10$ ), and these were also tested for treatment interaction to assess if they served as predictive factors. Analyses found two statistically significant EGFR-based prognostic factors representing growth pattern and percent membrane staining in patients treated with gefitinib ( $P = 0.0023$ ), placebo ( $P = 0.0128$ ), and both combined ( $P < 0.0001$ ). The prognostic effect was independent of other known prognostic factors. There was no predictive effect of either the growth pattern or membrane staining variable. CONCLUSIONS: While

some previous studies indicate that higher EGFR expression correlates with poor survival, our analyses provide statistically significant evidence that the combination of EGFR expression and growth pattern is a strong prognostic indicator for improved survival within this setting. The effects of membrane staining and growth pattern are still significant when adjusting for mutation.

Goldmann, T., D. Dromann, et al. (2008). "CXCR7 transcription in human non-small cell lung cancer and tumor-free lung tissues; possible regulation upon chemotherapy." *Virchows Arch* **452**(3): 347-8.

Gomez, L. A., A. de Las Pozas, et al. (2007). "Increased expression of cyclin B1 sensitizes prostate cancer cells to apoptosis induced by chemotherapy." *Mol Cancer Ther* **6**(5): 1534-43.

Chemotherapeutic drugs ideally should take advantage of the differences between transformed and normal cells and induce apoptosis only in cancer cells. One such difference may be the overexpression of cyclin B1 protein in cancer cells, which is required for the proper progression through mitosis. Previously, we showed that treatment of human prostate cancer cells with 2-methoxyestradiol (2-ME) or docetaxel results in an accumulation of cyclin B1 protein and an increase in cyclin B1 kinase activity, followed by induction of apoptotic cell death. Inhibition of cyclin B1 kinase lowers apoptosis induced by 2-ME and docetaxel. In this study, we established a positive correlation between cyclin B1 protein and apoptosis induced by chemotherapy in prostate cancer cells. There is minimal cyclin B1 and induction of apoptosis by chemotherapy in nontransformed cells. LNCaP and PC-3 prostate cancer cells stably overexpressing cyclin B1 are more sensitive to apoptosis induced by chemotherapy. LNCaP cells expressing cyclin B1 small interfering RNA to lower cyclin B1 protein or dominant negative cyclin-dependent kinase 1 to inhibit cyclin B1 kinase show a decrease in apoptosis. Increased sensitivity to apoptosis by overexpression of cyclin B1 may be due to lower Bcl-2, higher p53, and decreased neuroendocrine differentiation. We suggest that a cancer-specific mechanism whereby 2-ME and docetaxel may exert anti-prostate cancer activity is the deregulated activation of cyclin B1 kinase, leading to the induction of apoptotic cell death. Our results also suggest that higher levels of cyclin B1 in prostate cancer cells may be a good prognostic marker for chemotherapy.

Goss, G., D. Ferry, et al. (2009). "Randomized phase II study of gefitinib compared with placebo in chemotherapy-naive patients with advanced non-

small-cell lung cancer and poor performance status." *J Clin Oncol* **27**(13): 2253-60.

To compare gefitinib with placebo in chemotherapy naive patients with advanced non-small-cell lung cancer (NSCLC) and poor performance status. PATIENTS AND NSCLC patients (chemotherapy naive, WHO performance status 2 or 3; unfit for chemotherapy; stage IIIB/IV) were randomly assigned to gefitinib (250 mg/d) plus best supportive care (BSC; n = 100) or placebo plus BSC (n = 101). The primary end point was progression-free survival (PFS). Secondary end points included overall survival (OS), objective response rate (ORR), quality of life (QOL), pulmonary symptom improvement (PSI), and safety. Correlation of gefitinib efficacy with EGFR gene copy number (fluorescent in situ hybridization [FISH]) was explored. Hazard ratios (HRs; gefitinib:placebo) were 0.82 (95% CI, 0.60 to 1.12; P = .217) for PFS and 0.84 (95% CI, 0.62 to 1.15; P = .272) for OS. As expected for this patient population, OS for both arms was poor, at about 3 months. ORRs were 6.0% (gefitinib) and 1.0% (placebo). QOL and PSI rates were 21.1% and 28.3% (gefitinib) and 20.0% and 28.3% (placebo), respectively. In EGFR FISH-positive patients (n = 32), HRs were 0.29 (95% CI, 0.11 to 0.73) for PFS and 0.44 (95% CI, 0.17 to 1.12) for OS. No unexpected adverse events occurred. There was no statistically significant difference in PFS, OS, and ORRs after treatment with gefitinib or placebo, in the overall population; improvements in QOL and symptoms were similar in both groups. Tolerability profile of gefitinib was consistent with previous studies. PFS was statistically significantly improved for gefitinib-treated patients with EGFR FISH-positive tumors.

Graessmann, M., B. Berg, et al. (2007). "Chemotherapy resistance of mouse WAP-SVT/t breast cancer cells is mediated by osteopontin, inhibiting apoptosis downstream of caspase-3." *Oncogene* **26**(20): 2840-50.

Impairment of the complex regulatory network of cell death and survival is frequently the reason for therapy resistance of breast cancer cells and a major cause of tumor progression. We established two independent cell lines from a fast growing mouse breast tumor (WAP-SVT/t transgenic animal). Cells from one line (ME-A cells) are sensitive to apoptotic stimuli such as growth factor depletion or treatment with antitumor agents (e.g. doxorubicin). Cells from the second line (ME-C cells), which carry a missense mutation at the p53 codon 242, are very insensitive to apoptotic stimuli. Co-cultivation experiments revealed that the ME-C cells mediate cell death resistance to the ME-A cells. Microarray and Western blot analysis

showed that osteopontin (OPN) is selectively overexpressed by the ME-C cells. This glycoprotein is the most abundant protein secreted by the ME-C cells and we obtained strong indications that OPN is the main antiapoptotic factor. However, the OPN containing ME-C cell medium does not alter the expression level of pro- or antiapoptotic genes or known inhibitors of apoptosis (IAPs). Its signaling involves mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) kinase (MEK)1/2 as the kinase inhibitor PD98059 restores apoptosis but not the Akt inhibitor. In the ME-A cells, mitochondrial cytochrome c release occurs with and without external apoptotic stimuli. OPN containing ME-C cell medium does not prevent the mitochondrial cytochrome c release and caspase-9 processing. In serum starved ME-A cells, the OPN containing ME-C cell medium prevents caspase-3 activation. However, in doxorubicin-treated cells, although apoptosis is blocked, it does not inhibit caspase-3. This indicates that the ME-A cells distinguish between the initial apoptotic stimuli and that the cells possess a further uncharacterized control element acting downstream from caspase-3.

Gray, J., G. Simon, et al. (2007). "Molecular predictors of chemotherapy response in non-small-cell lung cancer." *Expert Rev Anticancer Ther* 7(4): 545-9.

Lung cancer is the number one cause of cancer-related mortality. In order to improve the outcome of patients, advances in the understanding of cancer biology and the development of therapeutic modalities that target key proliferation and survival mechanisms are needed. In vitro data have demonstrated that the genes RRM1 and ERCC1 are important components of these mechanisms. Recently, how these genes affect lung cancer therapy has been explored in the clinical setting with the goal of finding customized treatment algorithms to optimize efficacy, improve outcomes and minimize toxicity.

Guo, X., N. Ma, et al. (2008). "Increased p38-MAPK is responsible for chemotherapy resistance in human gastric cancer cells." *BMC Cancer* 8: 375.

Chemoresistance is one of the main obstacles to successful cancer therapy and is frequently associated with Multidrug resistance (MDR). Many different mechanisms have been suggested to explain the development of an MDR phenotype in cancer cells. One of the most studied mechanisms is the overexpression of P-glycoprotein (P-gp), which is a product of the MDR1 gene. Tumor cells often acquire the drug-resistance phenotype due to upregulation of the MDR1 gene. Overexpression of MDR1 gene has often been reported in primary gastric adenocarcinoma. This study investigated the role of

p38-MAPK signal pathway in vincristine-resistant SGC7901/VCR cells. P-gp and MDR1 RNA were detected by Western blot analysis and RT-PCR amplification. Mitogen-activated protein kinases and function of P-gp were demonstrated by Western blot and FACS Aria cytometer analysis. Ap-1 activity and cell apoptosis were detected by Dual-Luciferase Reporter Assay and annexin V-PI dual staining. The vincristine-resistant SGC7901/VCR cells with increased expression of the multidrug-resistance 1 (MDR1) gene were resistant to P-gp-related drug and P-gp-unrelated drugs. Constitutive increases of phosphorylated p38-MAPK and AP-1 activities were also found in the drug-resistant cells. Inhibition of p38-MAPK by SB202190 reduced activator protein-1 (AP-1) activity and MDR1 expression levels and increased the sensitivity of SGC7901/VCR cells to chemotherapy. Activation of the p38-MAPK pathway might be responsible for the modulation of P-glycoprotein-mediated and P-glycoprotein-unmediated multidrug resistance in the SGC7901/VCR cell line.

Guo, X. L., N. N. Ma, et al. (2009). "Up-regulation of hTERT expression by low-dose cisplatin contributes to chemotherapy resistance in human hepatocellular cancer cells." *Oncol Rep* 22(3): 549-56.

The telomerase is specifically activated in most malignant tumors but is usually inactive in normal somatic cells. It has been reported that telomerase has an anti-apoptotic role and up-regulation of telomerase helps cancer cells to be resistant to chemotherapeutic agent-induced cell death. The effect of cisplatin on telomerase activity is complex, and the exact mechanism remains largely unknown. In this study, we found that cisplatin activated telomerase activity and human telomerase reverse transcriptase (hTERT) expression in SMMC7721 human hepatocellular carcinoma cell line. Low-dose cisplatin up-regulated hTERT and NF-kappaB p65 expression and increased telomerase and NF-kappaB activity. Inhibition of NF-kappaB attenuated the hTERT expression and telomerase activity exposed to cisplatin, suggesting that NF-kappaB is responsible for the cisplatin-induced activation of the hTERT. Furthermore, preincubation of low-dose cisplatin which induced high expression of hTERT help hepatocellular carcinoma SMMC7721 cells survive under the high concentration of anti-cancer drugs. Inhibition of hTERT increased sensitivity of SMMC7721 cells to chemotherapy. Taken together, these results suggested that up-regulation of hTERT expression by low-dose cisplatin is NF-kappaB-dependent and contributes to chemotherapy resistance in human hepatocellular cancer cells.

Hamm, J. T., J. W. Wilson, et al. (2008). "Gemcitabine/epirubicin/paclitaxel as neoadjuvant chemotherapy in locally advanced breast cancer: a phase II trial of the NSABP Foundation Research Group." *Clin Breast Cancer* **8**(3): 257-63.

This phase II protocol of neoadjuvant chemotherapy with gemcitabine/epirubicin/paclitaxel (GET) was designed to determine the pathologic complete response (pCR) rate in the breast, clinical response rate, disease-free survival, and overall survival at 2 years as well as toxicity in patients with locally advanced breast cancer. This trial also evaluated the feasibility of tissue collection for gene-expression profiling. PATIENTS AND Seventy-six women with stage IIB, IIIA, and IIIB breast cancer were entered into this trial. Patients received a maximum of 6 cycles of neoadjuvant GET chemotherapy every 21 days (gemcitabine 1000 mg/m<sup>2</sup> intravenously [i.v.] on days 1 and 4, epirubicin 90 mg/m<sup>2</sup> i.v. bolus on day 1, and paclitaxel 175 mg/m<sup>2</sup> i.v. on day 1). After chemotherapy, patients underwent surgery and were assessed for pathologic response. The pCR rate among the 74 patients evaluable for efficacy was 23% (95% CI, 14%-34.2%). Adverse events among the 76 patients evaluable for toxicity included anemia requiring transfusion (14.5%), infection with grade 3/4 neutropenia (10.5%), febrile neutropenia (7.9%), and platelet transfusion (6.6%). Infectious complications occurred in 24 patients (31.6%), of whom 18.4% were in the setting of neutropenia. High-quality RNA and successful probe synthesis were obtained from all pretreatment core biopsy specimens that contained tumor cells (n=66; 88%). Neoadjuvant GET chemotherapy is an active regimen but with substantial toxicity. Tissue collection for gene-expression profiling is feasible in a multi-institutional setting.

Henriette Tanja, L., H. J. Guchelaar, et al. (2009). "Pharmacogenetics in chemotherapy of colorectal cancer." *Best Pract Res Clin Gastroenterol* **23**(2): 257-73.

Although in recent years, chemotherapeutic options for colorectal carcinoma have expanded, overall response rates are still too low, with high rates of toxicity. Pharmacogenetics aim at predicting both treatment response and adverse effects in individual patients. This review describes the current knowledge of pharmacogenetic markers in the systemic treatment of colorectal cancer. UGT1A1\*28 leads to reduced conjugation of SN-38, the active metabolite of irinotecan, resulting in an increased rate of adverse effects, especially neutropenia. To a lesser extent, increased 5-FU toxicity is predicted by DPYD\*2A. A variable number of tandem repeats polymorphism in

the thymidylate synthase enhancer region, in combination with a single nucleotide polymorphism C>G, may predict poorer response to 5-FU. Efficacy of oxaliplatin is influenced by polymorphisms in components of DNA repair systems, such as ERCC1 and XRCC1. Polymorphic changes in the endothelial growth factor receptor probably predict cetuximab efficacy. Furthermore, the antibody-dependent cell-mediated cytotoxic effect of cetuximab may be reduced by polymorphisms in the immunoglobulin G fragment C receptors. Bevacizumab efficacy is suspected to be influenced by polymorphisms in the VEGF gene and the hypoxia inducible factor 1alpha gene. Although the interpretation of pharmacogenetic studies is complicated, results imply a promising way of pretreatment prediction of chemotherapy efficacy and toxicity.

Hirsch, F. R., R. S. Herbst, et al. (2008). "Increased EGFR gene copy number detected by fluorescent in situ hybridization predicts outcome in non-small-cell lung cancer patients treated with cetuximab and chemotherapy." *J Clin Oncol* **26**(20): 3351-7.

Epidermal growth factor receptor (EGFR) gene copy number detected by fluorescent in situ hybridization (FISH) has proven to be useful for selection of non-small-cell lung cancer (NSCLC) patients for treatment with EGFR tyrosine kinase inhibitors. Here, we evaluate EGFR FISH as a predictive marker in NSCLC patients receiving the EGFR monoclonal antibody inhibitor cetuximab plus chemotherapy. PATIENTS AND Two hundred twenty-nine chemotherapy-naïve patients with advanced-stage NSCLC were enrolled onto a phase II selection trial evaluating sequential or concurrent chemotherapy (paclitaxel plus carboplatin) with cetuximab. EGFR FISH was assessable in 76 patients with available tumor tissue and classified as positive (four or more gene copies per cell in  $\geq$  40% of the cells or gene amplification) in 59.2%. Response (complete response/partial response) was numerically higher in FISH-positive (45%) versus FISH-negative (26%) patients (P = .14), whereas disease control rate (complete response/partial response plus stable disease) was statistically superior (81% v 55%, respectively; P = .02). Patients with FISH-positive tumors had a median progression-free survival time of 6 months compared with 3 months for FISH-negative patients (P = .0008). Median survival time was 15 months for the FISH-positive group compared with 7 months for patients who were FISH negative. (P = .04). Furthermore, survival favored FISH-positive patients receiving concurrent therapy. These results are the first to suggest that EGFR FISH is a predictive factor for selection of NSCLC patients for cetuximab

plus chemotherapy. Prospective validation of these findings is warranted.

Holm, N. T., F. Abreo, et al. (2009). "Elevated chemokine receptor CXCR4 expression in primary tumors following neoadjuvant chemotherapy predicts poor outcomes for patients with locally advanced breast cancer (LABC)." *Breast Cancer Res Treat* **113**(2): 293-9.

Purpose Patients with locally advanced breast cancer (LABC) have a poor outcome. A molecular predictor to identify at-risk patients is sorely needed. CXCR4 is a chemokine receptor that has been linked to breast cancer invasion and metastasis. We postulate that in patients with LABC, CXCR4 overexpression levels in cancer specimens following neoadjuvant chemotherapy predict cancer outcome. Experimental design 54 patients with LABC were prospectively accrued and analyzed. All had neoadjuvant chemotherapy and definitive surgical therapy. Study homogeneity was maintained by standardized treatment, surveillance, and compliance protocols. A 1 cm<sup>3</sup> cancer from the surgical specimens of each patient was retrieved for analysis. CXCR4 levels were detected using Western blots, and results were quantified against 1 mug of protein from HeLa cells. CXCR4 expression was defined as low (<6.6-fold) or high (> or =6.6-fold). Primary endpoints were cancer recurrence and death. Statistical analysis performed included independent samples t-test, chi-square test, Spearman Rank analysis, Kaplan-Meier survival analysis, log-rank test, and Cox proportional hazard model. Results With a median follow-up of 30 months, patients with high CXCR4 overexpression (> or =6.6-fold) had a significantly higher incidence of recurrence (P = 0.0006) and cancer death (P = 0.0128) than those with low CXCR4 overexpression (<6.6-fold). The relative risks for recurrence and death in the high CXCR4 group were 27.3-fold (95% CI: 6.2-120.8; P = 0.001) and 4.8-fold (95% CI: 1.5-15.0; P = 0.0076) higher, respectively than those in the low CXCR4 group. Conclusion High CXCR4 overexpression in specimens from LABC patients receiving neoadjuvant chemotherapy was predictive of cancer outcome.

Horne, H. N., P. S. Lee, et al. (2009). "Inactivation of the MAL gene in breast cancer is a common event that predicts benefit from adjuvant chemotherapy." *Mol Cancer Res* **7**(2): 199-209.

Dysregulation of MAL (myelin and lymphocyte protein) has been implicated in several malignancies including esophageal, ovarian, and cervical cancers. The MAL protein functions in apical transport in polarized epithelial cells; therefore, its disruption may lead to loss of organized polarity

characteristic of most solid malignancies. Bisulfite sequencing of the MAL promoter CpG island revealed hypermethylation in breast cancer cell lines and 69% of primary tumors analyzed compared with normal breast epithelial cells. Differential methylation between normal and cancer DNA was confined to the proximal promoter region. In a subset of breast cancer cell lines including T47D and MCF7 cells, promoter methylation correlated with transcriptional silencing that was reversible with the methylation inhibitor 5-aza-2'-deoxycytidine. In addition, expression of MAL reduced motility and resulted in a redistribution of lipid raft components in MCF10A cells. MAL protein expression measured by immunohistochemistry revealed no significant correlation with clinicopathologic features. However, in patients who did not receive adjuvant chemotherapy, reduced MAL expression was a significant predictive factor for disease-free survival. These data implicate MAL as a commonly altered gene in breast cancer with implications for response to chemotherapy.

Hotta, K., K. Kiura, et al. (2007). "Clinical significance of epidermal growth factor receptor gene mutations on treatment outcome after first-line cytotoxic chemotherapy in Japanese patients with non-small cell lung cancer." *J Thorac Oncol* **2**(7): 632-7.

INTRODUCTION: The relationship between the EGFR gene mutation status and clinical outcome has not fully been assessed in patients with non-small cell lung cancer (NSCLC) who received cytotoxic agents. The aim of this study was to clarify its association. We also examined whether this association could be affected by previous gefitinib treatment. Patients with advanced or postoperative recurrent NSCLC who received both cytotoxic chemotherapy and gefitinib monotherapy in their treatment course were included in this study. An EGFR mutation was determined in exons 19 and 21 by direct sequencing. Of 194 Japanese patients with advanced or relapsed NSCLC assessable for mutation analysis, 60 received both cytotoxic chemotherapy and gefitinib monotherapy through their treatment courses. EGFR mutations significantly affected progression-free survival (PFS) in the first-line cytotoxic chemotherapy regimens in the multivariate analysis (hazard ratio for PFS = 0.422; p = 0.0422). In contrast, in 28 (47%) of the 60 patients who also received cytotoxic chemotherapy after the relapse to gefitinib monotherapy, there were no differences in PFS stratified by EGFR mutation status. The sensitivity to gefitinib was, however, correlated with EGFR mutation status, and its sensitivity was retained even in the second-line treatment setting in patients with EGFR mutations. CONCLUSIONS: EGFR mutations were therefore significantly associated with

a better PFS in the first-line cytotoxic chemotherapy regimens. However, its association was not observed in the cytotoxic regimens administered after the relapse to gefitinib monotherapy, whereas gefitinib sensitivity was associated with an EGFR mutation even in the second-line or later treatment settings.

Hsu, F. Y., Y. Zhao, et al. (2007). "Downregulation of NPM-ALK by siRNA causes anaplastic large cell lymphoma cell growth inhibition and augments the anti cancer effects of chemotherapy in vitro." *Cancer Invest* **25**(4): 240-8.

The fusion protein, nucleophosmin-anaplastic lymphoma kinase (NPM-ALK), results from the chromosome translocation t(2;5)(p23;q25) and is present in 50-70 percent of anaplastic large-cell lymphomas (ALCLs). NPM-ALK is a constitutively activated kinase that transforms cells through stimulating several mitogenic signaling pathways. To examine if the NPM-ALK is a potential therapeutic target in ALCL, we used siRNA to specifically downregulate the expression of the NPM-ALK in ALCL cell lines. In this report, we demonstrated viability loss in t(2;5)-positive ALCL cell lines, SUDHL-1 and Karpas 299 cells, but not in lymphoma cell lines without the chromosome translocation, Jurkat and Granta 519 cells. Further study demonstrated that the downregulation of NPM-ALK resulted in decreased cell proliferation and increased cell apoptosis. When used in combination with chemotherapeutic agents, such as doxorubicin, the inhibition of the NPM-ALK augments the chemosensitivity of the tumor cells. These results revealed the importance of continuous expression of NPM-ALK in maintaining the growth of ALCL cells. Our data also suggested that the repression of the fusion gene might be a potential novel therapeutic strategy for NPM-ALK positive ALCLs.

Huang, Y. (2007). "Pharmacogenetics/genomics of membrane transporters in cancer chemotherapy." *Cancer Metastasis Rev* **26**(1): 183-201.

Inter-individual variability in drug response and the emergence of adverse drug reactions are main causes of treatment failure in cancer therapy. Recently, membrane transporters have been recognized as an important determinant of drug disposition, thereby affecting chemosensitivity and -resistance. Genetic factors contribute to inter-individual variability in drug transport and targeting. Therefore, pharmacogenetic studies of membrane transporters can lead to new approaches for optimizing cancer therapy. This review discusses genetic variations in efflux transporters of the ATP-binding cassette (ABC) family such as ABCB1 (MDR1, P-glycoprotein), ABCC1 (MRP1), ABCC2

(MRP2) and ABCG2 (BCRP), and uptake transporters of the solute carrier (SLC) family such as SLC19A1 (RFC1) and SLCO1B1 (SLC21A6), and their relevance to cancer chemotherapy. Furthermore, a pharmacogenomic approach is outlined, which using correlations between the growth inhibitory potency of anticancer drugs and transporter gene expression in multiple human cancer cell lines, has shown promise for determining the relevant transporters for any given drugs and predicting anticancer drug response.

Iacopetta, B., K. Kawakami, et al. (2008). "Predicting clinical outcome of 5-fluorouracil-based chemotherapy for colon cancer patients: is the CpG island methylator phenotype the 5-fluorouracil-responsive subgroup?" *Int J Clin Oncol* **13**(6): 498-503.

The CpG island methylator phenotype (CIMP+) of colorectal cancer (CRC) occurs predominantly in the proximal colon and is characterized by frequent hypermethylation of gene promoter regions. In this review, we present evidence suggesting CIMP+ represents the subgroup of colon cancers that are responsive to 5-fluorouracil (5-FU)-based treatments. CIMP+ has been associated with survival benefit from 5-FU in a clinical study of CRC, with additional evidence coming from studies on gastric cancer and tumor cell lines. Elevated concentrations of 5-10-methylene tetrahydrofolate (CH(2)FH(4)) occur in CIMP+ tumors and are probably due to low expression levels for gamma-glutamyl hydrolase (GGH). Clinical and in vitro work has previously shown that high CH(2)FH(4) and low GGH expression levels correlate with good response to 5-FU. Methylation-induced silencing of dihydropyrimidine dehydrogenase, the rate-limiting enzyme in 5-FU degradation, may also provide a link between CIMP+ and good response to 5-FU. The CIMP+-related phenotype referred to as microsatellite instability (MSI+) has been widely investigated as a predictive marker of response to 5-FU, with contradictory results. The interpretation of these studies is likely to be confounded by the fact that some MSI+ tumors occur in the background of CIMP+, but a significant proportion of others do not. Further studies on tumors from randomized clinical trials are required to confirm the value of CIMP+ and associated molecular features for the prediction of clinical outcome to 5-FU-based chemotherapy.

Ibanez de Caceres, I. and P. Cairns (2007). "Methylated DNA sequences for early cancer detection, molecular classification and chemotherapy response prediction." *Clin Transl Oncol* **9**(7): 429-37.

Molecular studies of many types of cancer have revealed that clinically evident tumours carry

multiple genetic and epigenetic abnormalities, including DNA sequence alterations, chromosome copy number changes and aberrant promoter hypermethylation. Together, these aberrant changes result in the activation of oncogenes and inactivation of tumour-suppressor genes (TSG). In many cases these abnormalities can be found in premalignant lesions and even in histological normal adjacent cells. Many tumour types are difficult to detect early and are frequently resistant to available chemotherapy and radiotherapy. Therefore, the early detection, chemoprevention and the design of new therapeutic strategies based on the increased understanding of cancer molecular changes are one of the great challenges nowadays. Insertions of a methyl group at the fifth carbon of cytosines within the dinucleotide 5'-CpG-3' is the best studied epigenetic mechanism. DNA methylation acts together with others mechanisms like histone modification, chromatin remodelling and microRNAs to mould the DNA structure according to the functional state required. The aberrant methylation of the CpG islands located at the promoter region of specific genes is a common and early event involved in cancer development. Thus, hypermethylated DNA sequences from tumours are one of the most promising markers for early detection screenings as well as tumour classification and chemotherapy response in many types of cancer.

Ishikawa, T. and H. Nakagawa (2009). "Human ABC transporter ABCG2 in cancer chemotherapy and pharmacogenomics." *J Exp Ther Oncol* **8**(1): 5-24.

The ability of cancer cells to acquire resistance to multiple anticancer agents, termed multidrug resistance, is often mediated by overexpression of ATP-binding cassette (ABC) transporters that remove drugs out of the cell against a concentration gradient. ABCG2, or breast cancer resistance protein (BCRP), is an ABC transporter that has been the subject of intense study since its discovery a decade ago. While ABCG2 overexpression has been demonstrated in cancer cells after in vitro drug treatment, endogenous ABCG2 expression in certain cancers is considered as a reflection of the differentiated phenotype of the cell of origin and likely contributes to intrinsic drug resistance. Notably, ABCG2 is often expressed in stem cell populations, where it plays a critical role in cellular protection. ABCG2 exhibits a broad range of substrate specificity. New technologies of high-speed screening and quantitative structure-activity-relationship (QSAR) analysis have been developed to analyze the interactions of drugs with ABCG2. As ABCG2 reportedly transports porphyrins, its contribution to photodynamic therapy of human cancer is also implicated. Protein expression levels of

ABCG2 in cancer cells are regulated by both transcriptional activation and protein degradation. The ABCG2 protein undergoes endosomal and/or ubiquitin-mediated proteasomal degradations. Furthermore, genetic polymorphisms in the ABCG2 gene are important factors in cancer chemotherapy to circumvent adverse effects and/or to enhance the efficacy of anticancer drugs. The present review article addresses recent advances in molecular pharmacology and pharmacogenomics of ABCG2 and provides novel ideas to improve cancer chemotherapy.

Jackman, D. M., B. Y. Yeap, et al. (2007). "Phase II clinical trial of chemotherapy-naïve patients > or = 70 years of age treated with erlotinib for advanced non-small-cell lung cancer." *J Clin Oncol* **25**(7): 760-6.

This is a phase II, multicenter, open-label study of chemotherapy-naïve patients with non-small-cell lung cancer (NSCLC) and age > or = 70 years who were treated with erlotinib and evaluated to determine the median, 1-year, and 2-year survival. The secondary end points include radiographic response rate, time to progression (TTP), toxicity, and symptom improvement. PATIENTS AND Eligible patients with NSCLC were treated with erlotinib 150 mg/d until disease progression or significant toxicity. Tumor response was assessed every 8 weeks by computed tomography scan using Response Evaluation Criteria in Solid Tumors. Tumor samples were analyzed for the presence of somatic mutations in EGFR and KRAS. Eighty eligible patients initiated erlotinib therapy between March 2003 and May 2005. There were eight partial responses (10%), and an additional 33 patients (41%) had stable disease for 2 months or longer. The median TTP was 3.5 months (95% CI, 2.0 to 5.5 months). The median survival time was 10.9 months (95% CI, 7.8 to 14.6 months). The 1- and 2- year survival rates were 46% and 19%, respectively. The most common toxicities were acneiform rash (79%) and diarrhea (69%). Four patients developed interstitial lung disease of grade 3 or higher, with one treatment-related death. EGFR mutations were detected in nine of 43 patients studied. The presence of an EGFR mutation was strongly correlated with disease control, prolonged TTP, and survival. Erlotinib monotherapy is active and relatively well tolerated in chemotherapy-naïve elderly patients with advanced NSCLC. Erlotinib merits consideration for further investigation as a first-line therapeutic option in elderly patients.

Jin, Y. T., X. X. Ying, et al. (2008). "aPKC inhibitors might be the sensitizer of chemotherapy and adoptive immunotherapy in the treatment of hASIPa-overexpressed breast cancer." *Oncol Res* **17**(2): 59-68.

The atypical protein kinase C (aPKC) plays an important role in cell growth through the interaction with its substrates, including human ASIP (hASIP), which contains an aPKC phosphorylation site encoded by exon 17b of the gene. hASIP is expressed as numerous alternative splicing isoforms in the cells. Our results showed that hASIPa, an exon 17b-containing isoform of hASIP, is overexpressed in human breast cancer (HBC) MDA-MB-231, SK-BR-3, and MCF-7 cell lines and HBC specimens. The anticancer effects of 5-FU chemotherapy or adoptive immunotherapy and the synergic action of aPKC inhibitor against hASIPa-overexpressed HBC cells were tested. The results indicated that HBC MDA-MB-231 and SK-BR-3 cells were sensitive to 5-FU treatment *in vitro*. The combined treatment of aPKC inhibitor and 5-FU raised the anticancer activities against hASIPa-overexpressed HBC cells. The coculture of cytokine-induced killer (CIK) cells and autologous dendritic cells (DCs) with or without Her-2 peptide GP2 pulse created two new populations of effective immune-active T-cell populations called DC-modulated and cytokine-induced killer (DCIK) cells and peptide-DC-modulated and cytokine-induced killer (DCIK-P) cells. The DCIK cells showed cytotoxic activities on MDA-MB-231, SK-BR-3, and MCF-7 cells in MHC unrestricted manner. The DCIK-P cells possessed extra-enhanced cytotoxic activities against HLA-A2+/Her-2+ MDA-MB-231 cells in MHC restricted manner, but not for HLA-A2+/-/Her-2+ SK-BR-3 cells and HLA-A2+/Her-2+/- MCF-7 cells. The data suggested specific cytotoxic T-lymphocyte (CTL) activity of DCIK-P cells on MDA-MB-231 cells. The combined treatment of aPKC inhibitor with DCIK/DCIK-P cells further raised the anticancer activities against hASIPa-overexpressed HBC cells. The results demonstrated that the hASIPa/aPKC signaling pathway functions as an important regulator in the growth of HBC cells and aPKC inhibitor treatment showed the synergic activities on 5-FU or DCIK/DCIK-P cells adoptive immunotherapy against hASIPa-overexpressed HBC cells.

Johnson, J. B., S. John, et al. (2009). "Pretreatment with alternate day modified fast will permit higher dose and frequency of cancer chemotherapy and better cure rates." *Med Hypotheses* 72(4): 381-2.

It is established that calorie restriction (CR) increases the resistance of cells to various stressors such as oxidative damage, excitotoxins, mercury and acetaminophen. Alternate day feeding (ADF) may confer greater stress resistance than daily CR of 30% or 40%. A recent study in three strains of mouse showed that a fast of 48 or 60 h prevented toxic effects due to administration of doses 2-4 times the

maximum human dose of etoposide, a chemotherapy agent which acts through increased oxidative stress. In addition, mice inoculated with neuroblastoma survived longer when pretreated with fasting, then given high dose etoposide, as well as not exhibiting toxicity. This increased survival was construed as evidence of differential stress resistance between normal and cancer cells, the cancer cells being only partially protected by the pretreatment fast. In clinical practice, increased differential stress resistance could lead to the use of much higher doses of chemotherapy agents, and in the absence of toxicity, make it possible to repeat the treatment to kill residual cancer cells. Humans are unlikely to comply with a total fast of longer than 24 or 48 h, which may be insufficient to activate the same gene expression process. Based on published data we estimate that an optimal time period for development of stress resistance is 2-3 weeks when alternate day feeding is employed. Our previously published experience suggests that 2-3 weeks of alternate day modified fast in which subjects eat *ad libitum* one day and <20% of one's estimated caloric requirement the next will confer a similar stress resistance. Compliance with this diet is high and greater maintenance of body weight is feasible. We hypothesize that a pretreatment of 2-3 weeks with the alternate day modified fast will improve outcomes in cancer chemotherapy, decreasing morbidity and raising cure rates.

Kaliberova, L. N., D. L. Della Manna, et al. (2008). "Molecular chemotherapy of pancreatic cancer using novel mutant bacterial cytosine deaminase gene." *Mol Cancer Ther* 7(9): 2845-54.

The combination of molecular chemotherapy with radiation therapy has the potential to become a powerful approach for treatment of pancreatic cancer. We have developed an adenoviral vector (AdbCD-D314A) encoding a mutant bacterial cytosine deaminase (bCD) gene, which converts the prodrug 5-fluorocytosine (5-FC) into the active drug 5-fluorouracil. The aim of this study was to investigate AdbCD-D314A/5-FC-mediated cytotoxicity *in vitro* and therapeutic efficacy *in vivo* alone and in combination with radiation against human pancreatic cancer cells and xenografts. AdbCD-D314A/5-FC-mediated cytotoxicity alone and in combination with radiation was analyzed using crystal violet inclusion and clonogenic survival assays. CD enzyme activity was determined by measuring conversion of [3H]5-FC to [3H]5-fluorouracil after adenoviral infection of pancreatic cancer cells *in vitro* and pancreatic tumor xenografts by TLC. S.c. pancreatic tumor xenografts were used to evaluate the therapeutic efficacy of AdbCD-D314A/5-FC molecular chemotherapy in combination with radiation therapy. AdbCD-D314A

infection resulted in increased 5-FC-mediated pancreatic cancer cell killing that correlated with significantly enhanced CD enzyme activity compared with AdbCDwt encoding wild-type of bCD. Animal studies showed significant inhibition of growth of human pancreatic tumors treated with AdbCD-D314A/5-FC in comparison with AdbCDwt/5-FC. Also, a significantly greater inhibition of growth of Panc2.03 and MIA PaCA-2 tumor xenografts was produced by the combination of AdbCD-D314A/5-FC with radiation compared with either agent alone. The results indicate that the combination of AdbCD-D314A/5-FC molecular chemotherapy with radiation therapy significantly enhanced cytotoxicity of pancreatic cancer cells in vitro and increased therapeutic efficacy against human pancreatic tumor xenografts.

Kamada, M., A. So, et al. (2007). "Hsp27 knockdown using nucleotide-based therapies inhibit tumor growth and enhance chemotherapy in human bladder cancer cells." *Mol Cancer Ther* 6(1): 299-308.

Heat shock protein 27 (Hsp27) is a cytoprotective chaperone that is phosphoactivated during cell stress that prevents aggregation and/or regulate activity and degradation of certain client proteins. Recent evidence suggests that Hsp27 may be involved in tumor progression and the development of treatment resistance in various tumors, including bladder cancer. The purpose of this study was to examine, both in vitro and in vivo, the effects of overexpression of Hsp27 and, correspondingly, the down-regulation of Hsp27 using small interfering (si) RNA and OGX-427, a second-generation antisense oligonucleotide targeting Hsp27. Hsp27 overexpression increased UMUC-3 cell growth and resistance to paclitaxel. Both OGX-427 and Hsp27 siRNA decreased Hsp27 protein and mRNA levels by >90% in a dose- and sequence-specific manner in human bladder cancer UMUC-3 cells. OGX-427 or Hsp27 siRNA treatment induced apoptosis and enhanced sensitivity to paclitaxel in UMUC-3 cells. In vivo, OGX-427 significantly inhibited tumor growth in mice, enhanced sensitivity to paclitaxel, and induced significantly higher levels of apoptosis compared with xenografts treated with control oligonucleotides. Collectively, these findings suggest that Hsp27 knockdown with OGX-427 and combined therapy with paclitaxel could be a novel strategy to inhibit the progression of bladder cancer.

Kandioler, D., G. Stamatis, et al. (2008). "Growing clinical evidence for the interaction of the p53 genotype and response to induction chemotherapy in advanced non-small cell lung cancer." *J Thorac Cardiovasc Surg* 135(5): 1036-41.

The objective of this study is to establish clinical evidence that the p53 genotype can serve as a predictive marker for response to cisplatin-based induction therapy. Patients with advanced non-small cell lung cancer who had received neoadjuvant chemotherapy in the context of a prospective phase II trial were analyzed for the p53 genotype of their tumors. Response to induction therapy was then correlated to the p53 genotype as assessed by complete direct DNA sequencing. Patients had received 3 cycles of cisplatin and etoposide, and 1 cycle of simultaneous radiochemotherapy. All 3 treatment components mediate their cytotoxic effect through induction of apoptosis, which is suggested to require an intact p53 gene. In addition, the results from a previously published hypothesis-finding study are updated to demonstrate the consistency of clinical results and summarize currently available clinical evidence. In the phase II trial, 35 patients underwent resection after induction chemotherapy, allowing a pathohistologic response assessment. The presence of a mutant p53 genotype was highly indicative of resistance to induction chemotherapy ( $P < .002$ ). The sensitivity of a mutant p53 genotype to identify nonresponders was 94% (71.3-99.9 confidence interval). A normal p53 gene was significantly associated with radical resection ( $P < .004$ ) and survival advantage ( $P = .02$ ). This is the second clinical evaluation demonstrating a significant relation between p53 genotype and response to induction therapy in non-small cell lung cancer. We conclude that the p53 genotype should be evaluated as a predictive marker for response to induction therapy in prospective randomized protocols.

Keam, B., S. A. Im, et al. (2008). "Modified FOLFOX-6 chemotherapy in advanced gastric cancer: Results of phase II study and comprehensive analysis of polymorphisms as a predictive and prognostic marker." *BMC Cancer* 8: 148.

The objective of this study was to evaluate the efficacy and toxicity of infusional 5-fluorouracil (5-FU), folinic acid and oxaliplatin (modified FOLFOX-6) in patients with advanced gastric cancer (AGC), as first-line palliative combination chemotherapy. We also analyzed the predictive or prognostic value of germline polymorphisms of candidate genes associated with 5-FU and oxaliplatin. Seventy-three patients were administered a 2 hour infusion of oxaliplatin (100 mg/m<sup>2</sup>) and folinic acid (100 mg/m<sup>2</sup>) followed by a 46 hour continuous infusion of 5-FU (2,400 mg/m<sup>2</sup>). Genomic DNA from the patients' peripheral blood mononuclear cells was extracted. Ten polymorphisms within five genes were investigated including TS, GSTP, ERCC, XPD and XRCC. The overall response rate (RR) was 43.8%.

Median time to progression (TTP) and overall survival (OS) were 6.0 months and 12.6 months, respectively. Toxicities were generally tolerable and manageable. The RR was significantly higher in patients with a 6-bp deletion homozygote (-6 bp/-6 bp) in TS-3'UTR (55.0% vs. 30.3% in +6 bp/+6 bp or +6 bp/-6 bp,  $p = 0.034$ ), and C/A or A/A in XPD156 (52.0% vs. 26.1% in C/C,  $p = 0.038$ ). The -6 bp/-6 bp in TS-3'UTR was significantly associated with a prolonged TTP and OS. In a multivariate analysis, the 6-bp deletion in TS-3'UTR was identified as an independent prognostic marker of TTP (hazard ratio = 0.561,  $p = 0.032$ ). Modified FOLFOX-6 chemotherapy appears to be active and well tolerated as first line chemotherapy in AGC patients. The 6-bp deletion in TS-3'UTR might be a candidate to select patients who are likely to benefit from 5-FU based modified FOLFOX-6 in future large scale trial.

Khan, A. and H. Lu (2008). "Inhibition of MDM2-p53 feedback loop by various small molecules for potential cancer chemotherapy." *Cancer Biol Ther* 7(6): 853-5.

Kim, H. T., J. E. Lee, et al. (2008). "Effect of BRCA1 haplotype on survival of non-small-cell lung cancer patients treated with platinum-based chemotherapy." *J Clin Oncol* 26(36): 5972-9.

To determine whether germ-line variations in BRCA1 affect outcome in non-small-cell lung cancer (NSCLC) patients treated with platinum combination chemotherapy. PATIENTS AND We evaluated the associations of four tagging BRCA1 polymorphisms and their haplotypes with treatment outcome in 300 NSCLC patients at stages IIIA (16%), IIIB (31%), and IV (53%). The median age was 63 years (range, 28 to 89 years). Histologically, 139 (46.3%) of the patients had squamous cell carcinomas and 137 (45.7%) had adenocarcinomas. Patient median survival time (MST) was 13.0 months. We observed no significant association between any of the tagging polymorphisms [S1613G, IVS13-1893 (A>C), IVS12-1207 (C>T), and IVS12+112 (C>A)] and overall survival. Of the five haplotypes evaluated (AACC, AACA, GCTC, GATC, and AATC), the survival of patients with two copies of the AACC (wild-type) haplotype was significantly shorter than that of patients with zero to one copies (MST, 8.47 v 14.57 months; log-rank  $P = .0066$ ), even after adjustment for body weight loss, performance status, stage, second-line treatment, and radiation therapy (hazard ratio = 2.097; 95% CI, 1.339 to 3.284). The survival of patients with squamous cell carcinoma and two copies was significantly shorter than that of other patients with squamous cell carcinoma (MST, 6.8 v 15.3 months; log-rank  $P = 3.6 \times 10^{-5}$ ), whereas

differences in survival between the two adenocarcinoma groups was not significant (log-rank  $P = .677$ ). These findings suggest that the AACC haplotype of the BRCA1 gene is an important prognostic marker in NSCLC patients treated with platinum combination chemotherapy.

Koike Folgueira, M. A., H. Brentani, et al. (2009). "Gene expression profile of residual breast cancer after doxorubicin and cyclophosphamide neoadjuvant chemotherapy." *Oncol Rep* 22(4): 805-13.

In breast cancer patients, primary chemotherapy is associated with the same survival benefits as adjuvant chemotherapy. Residual tumors represent a clinical challenge, as they may be resistant to additional cycles of the same drugs. Our aim was to identify differential transcripts expressed in residual tumors, after neoadjuvant chemotherapy, that might be related with tumor resistance. Hence, 16 patients with paired tumor samples, collected before and after treatment (4 cycles doxorubicin/cyclophosphamide, AC) had their gene expression evaluated on cDNA microarray slides containing 4,608 genes. Three hundred and eighty-nine genes were differentially expressed (paired Student's t-test,  $pFDR < 0.01$ ) between pre- and post-chemotherapy samples and among the regulated functions were the JNK cascade and cell death. Unsupervised hierarchical clustering identified one branch comprising exclusively, eight pre-chemotherapy samples and another branch, including the former correspondent eight post-chemotherapy samples and other 16 paired pre/post-chemotherapy samples. No differences in clinical and tumor parameters could explain this clustering. Another group of 11 patients with paired samples had expression of selected genes determined by real-time RT-PCR and CTGF and DUSP1 were confirmed more expressed in post- as compared to pre-chemotherapy samples. After neoadjuvant chemotherapy some residual samples may retain their molecular signature while others present significant changes in their gene expression, probably induced by the treatment. CTGF and DUSP1 overexpression in residual samples may be a reflection of resistance to further administration of AC regimen.

Kolasa, I. K., A. Rembiszewska, et al. (2009). "PIK3CA amplification associates with resistance to chemotherapy in ovarian cancer patients." *Cancer Biol Ther* 8(1): 21-6.

PI3K/AKT signalling pathway controls important cellular processes such as the cell proliferation and apoptosis. PIK3CA gene encoding a catalytic subunit of the PI3K is mutated and/or amplified in various neoplasms, including ovarian cancer. We aimed to evaluate PIK3CA alterations and

their clinical importance in ovarian cancer patients. Molecular analysis was performed on 117 ovarian carcinomas with the use of qPCR, SSCP and sequencing. In a group of 98 patients with complete clinical data, 62 patients were treated with standard taxane-platinum regimens and 36 patients with platinum-cyclophosphamide regimens. A multivariate analysis was performed by the Cox's and logistic regression models. PIK3CA mutations occurred in 5/117 (4.3%) carcinomas, exclusively in the endometrioid and clear cell types ( $p = 0.0002$ ); they were also associated with low FIGO stage ( $p = 0.0003$ ), low tumor grade ( $p = 0.045$ ) and early patient's age at diagnosis ( $p = 0.0005$ ). The PIK3CA amplification (predominantly a low-level) was found in 28/117 (24%) ovarian carcinomas. It was more frequent in TP53 mutant tumors ( $p = 0.012$ ) and tended to associate with high pAKT expression ( $p = 0.061$ ). The PIK3CA amplification strongly diminished odds of complete remission (OR = 0.25,  $p = 0.033$ ) and platinum sensitive response (PS, OR = 0.12,  $p = 0.004$ ) in the taxane-platinum treated patients. The odds of PS were also much lower in all patients with the PIK3CA amplification evaluated together, regardless of the treatment applied (OR = 0.18,  $p = 0.001$ ). Our results suggest that PIK3CA amplification may be a marker predicting ovarian cancer response to chemotherapy.

Koopman, M., S. Venderbosch, et al. (2009). "Predictive and prognostic markers for the outcome of chemotherapy in advanced colorectal cancer, a retrospective analysis of the phase III randomised CAIRO study." *Eur J Cancer* **45**(11): 1999-2006.

We have tested several biomarkers [dihydropyrimidine dehydrogenase (DPD), orotate phosphoribosyl transferase (OPRT), thymidine phosphorylase (TP), thymidylate synthase (TS) and excision cross-complementing gene (ERCC1)] for their prognostic and predictive value in relation to the outcome of chemotherapy in tumour tissues of 556 advanced colorectal cancer (ACC) patients who were randomised between sequential treatment and combination treatment in the CApecitabine, IRinotecan, Oxaliplatin (CAIRO) study. DPD expression showed a statistically significant predictive value for combination treatment with capecitabine plus irinotecan with low versus high values resulting in an improved median progression-free survival (PFS) and median overall survival (OS) of 8.9 (95% confidence interval (CI) 8.3-9.9) versus 7.2 months (95% CI 6.5-8.1,  $p=0.006$ ), and 21.5 months (95% CI 17.9-26.5) versus 16.9 months (95% CI 13.0-19.1,  $p=0.04$ ), respectively. In the overall patient population a high OPRT expression in stromal cells was a favourable prognostic parameter for OS, with 21.5

months (95% CI 17.9-27.3) versus 17.2 months (95% CI 15.1-18.6,  $p=0.036$ ), respectively. A similar effect was observed for PFS. In a multivariate analysis that included known prognostic factors these results remained significant and also showed that a high OPRT expression in tumour cells was an unfavourable prognostic parameter for PFS and OS. In conclusion, in this largest study on capecitabine with or without irinotecan to date we found a predictive value of DPD expression. Our results on the prognostic value of OPRT expression warrant further studies on the role of stromal cells in the outcome of treatments. The divergent results of ours and previous studies underscore the complexity of these biomarkers and currently prevent the routine use of these markers in daily clinical practice.

Kuo, M. T. (2009). "Redox regulation of multidrug resistance in cancer chemotherapy: molecular mechanisms and therapeutic opportunities." *Antioxid Redox Signal* **11**(1): 99-133.

The development of multidrug resistance to cancer chemotherapy is a major obstacle to the effective treatment of human malignancies. It has been established that membrane proteins, notably multidrug resistance (MDR), multidrug resistance protein (MRP), and breast cancer resistance protein (BCRP) of the ATP binding cassette (ABC) transporter family encoding efflux pumps, play important roles in the development of multidrug resistance. Overexpression of these transporters has been observed frequently in many types of human malignancies and correlated with poor responses to chemotherapeutic agents. Evidence has accumulated showing that redox signals are activated in response to drug treatments that affect the expression and activity of these transporters by multiple mechanisms, including (a) conformational changes in the transporters, (b) regulation of the biosynthesis cofactors required for the transporter's function, (c) regulation of the expression of transporters at transcriptional, posttranscriptional, and epigenetic levels, and (d) amplification of the copy number of genes encoding these transporters. This review describes various specific factors and their relevant signaling pathways that are involved in the regulation. Finally, the roles of redox signaling in the maintenance and evolution of cancer stem cells and their implications in the development of intrinsic and acquired multidrug resistance in cancer chemotherapy are discussed.

Lassmann, S., L. Tang, et al. (2007). "Predictive molecular markers for colorectal cancer patients with resected liver metastasis and adjuvant chemotherapy." *Gastroenterology* **133**(6): 1831-9.

**BACKGROUND & AIMS:** The aims of the study were to evaluate the predictive value of 8 candidate molecular markers for colorectal cancer (CRC) patients receiving hepatic arterial infusion (floxuridine [FUDR] and dexamethasone) and systemic irinotecan (CPT11) post resection of liver metastasis. RNA was extracted from microdissected tumor cells of fixed and embedded specimens of resected liver metastases (94 cases) and analyzed by quantitative reverse-transcription polymerase chain reaction (RT-PCR) for thymidine phosphorylase, dihydropyrimidine dehydrogenase, thymidylate synthase, uridine phosphorylase, uridine/cytidine (monophospho)kinase, Bcl-2 related protein, Cyclin-D1, and Survivin expression. Uni- and multivariate statistical analyses and an explorative hierarchical clustering analysis of quantitative RT-PCR data were performed for overall survival and recurrent disease. After adjustment for multiple clinicopathologic parameters, none of the markers were significantly associated with overall survival (except, marginally, Cyclin-D1;  $P = .06$ ) or extrahepatic recurrence. However, high Survivin ( $P = .03$ ) and Cyclin-D1 ( $P = .05$ ) levels were predictive for hepatic recurrence. Hierarchical cluster analysis identified 7 of 94 patients associated with lower hepatic recurrence ( $P < .001$ ). This patient group was characterized by low Cyclin-D1 and Survivin messenger RNA levels, both genes also clustering together. **CONCLUSIONS:** Cyclin-D1 and Survivin messenger RNA analyzed by standardized, quantitative RT-PCR are predictive markers for CRC patients receiving hepatic arterial infusion (FUDR/dexamethasone) and systemic CPT11 post resection of liver metastasis. Moreover, our exploratory hierarchical cluster analysis of quantitative RT-PCR data supports its potential as an application to define clinically relevant patient subgroups.

Lee, J., Y. H. Im, et al. (2008). "Evaluation of ER and Ki-67 proliferation index as prognostic factors for survival following neoadjuvant chemotherapy with doxorubicin/docetaxel for locally advanced breast cancer." *Cancer Chemother Pharmacol* **61**(4): 569-77.

The aim of the study was to identify reliable predictive biological markers for treatment outcome following neoadjuvant adriamycin/docetaxel (AT) chemotherapy in locally advanced breast cancer patients. **MATERIALS AND METHODS:** This study was a phase II study on AT neoadjuvant chemotherapy in locally advanced breast cancer patients. Patients received 50 mg/m<sup>2</sup> of doxorubicin intravenously (IV) over 15 min followed by docetaxel 75 mg/m<sup>2</sup> infused over 1 h, repeated every 3 weeks for three cycles. Surgery was performed within 3-4 weeks following the last cycle of chemotherapy. We analyzed the pre-treatment

and post-treatment expression levels of ER, PgR, HER-2, Ki-67 proliferation index, and p53 and examined the correlation between the markers and clinical parameters with treatment response, overall survival and relapse-free survival following neoadjuvant treatment. From July 2001 to September 2004, 61 patients were enrolled. The meaningful parameters adversely influencing survival were post-treatment ER(-) status ( $P = 0.013$ ) and post-treatment Ki-67 index above 1.0% ( $P = 0.013$ ). At the multivariate level, the post-treatment Ki-67 proliferation index  $\leq 1.0$  was the only meaningful prognostic factor for better survival ( $P = 0.033$ ). Notably, tumors with Ki-67 index  $\leq 1.0$  were more likely to express ER with statistical significance ( $P = 0.002$ ). Tumors with ER(+) and Ki-67 index  $\leq 1.0$  showed the highest survival rate, followed by ER(+) and Ki-67 index  $> 1.0\%$ , ER(-) and Ki-67  $\leq 1.0\%$ , and ER(-) and Ki-67  $> 1.0\%$  with the worst survival ( $P = 0.033$ ). Collectively, post-treatment ER status and Ki-67 proliferation index were prognostic of overall survival following neoadjuvant AT chemotherapy.

Lee, T. B., J. H. Park, et al. (2008). "Epigenetic mechanisms involved in differential MDR1 mRNA expression between gastric and colon cancer cell lines and rationales for clinical chemotherapy." *BMC Gastroenterol* **8**: 33.

The membrane transporters such as P-glycoprotein (Pgp), the MDR1 gene product, are one of causes of treatment failure in cancer patients. In this study, the epigenetic mechanisms involved in differential MDR1 mRNA expression were compared between 10 gastric and 9 colon cancer cell lines. The MDR1 mRNA levels were determined using PCR and real-time PCR assays after reverse transcription. Cytotoxicity was performed using the MTT assay. Methylation status was explored by quantification PCR-based methylation and bisulfite DNA sequencing analyses. The MDR1 mRNA levels obtained by 35 cycles of RT-PCR in gastric cancer cells were just comparable to those obtained by 22 cycles of RT-PCR in colon cancer cells. Real-time RT-PCR analysis revealed that MDR1 mRNA was not detected in the 10 gastric cancer cell lines but variable MDR1 mRNA levels in 7 of 9 colon cancer cell lines except the SNU-C5 and HT-29 cells. MTT assay showed that Pgp inhibitors such as cyclosporine A, verapamil and PSC833 sensitized Colo320HSR (colon, highest MDR1 expression) but not SNU-668 (gastric, highest) and SNU-C5 (gastric, no expression) to paclitaxel. Quantification PCR-based methylation analysis revealed that 90% of gastric cancer cells, and 33% of colon cancer cells were methylated, which were completely matched with the results obtained by

bisulfite DNA sequencing analysis. 5-aza-2'-deoxycytidine (5AC, a DNA methyltransferase inhibitor) increased the MDR1 mRNA levels in 60% of gastric cells, and in 11% of colon cancer cells. Trichostatin A (TSA, histone deacetylase inhibitor) increased the MDR1 mRNA levels in 70% of gastric cancer cells and 55% of colon cancer cells. The combined treatment of 5AC with TSA increased the MDR1 mRNA levels additively in 20% of gastric cancer cells, but synergistically in 40% of gastric and 11% of colon cancer cells. These results indicate that the MDR1 mRNA levels in gastric cancer cells are significantly lower than those in colon cancer cells, which is at least in part due to different epigenetic regulations such as DNA methylation and/or histone deacetylation. These results can provide a better understanding of the efficacy of combined chemotherapy as well as their oral bioavailability.

L'Esperance, S., M. Bachvarova, et al. (2008). "Global gene expression analysis of early response to chemotherapy treatment in ovarian cancer spheroids." *BMC Genomics* 9: 99.

Chemotherapy (CT) resistance in ovarian cancer (OC) is broad and encompasses diverse unrelated drugs, suggesting more than one mechanism of resistance. To better understand the molecular mechanisms controlling the immediate response of OC cells to CT exposure, we have performed gene expression profiling in spheroid cultures derived from six OC cell lines (OVCAR3, SKOV3, TOV-112, TOV-21, OV-90 and TOV-155), following treatment with 10,0 microM cisplatin, 2,5 microM paclitaxel or 5,0 microM topotecan for 72 hours. Exposure of OC spheroids to these CT drugs resulted in differential expression of genes associated with cell growth and proliferation, cellular assembly and organization, cell death, cell cycle control and cell signaling. Genes, functionally involved in DNA repair, DNA replication and cell cycle arrest were mostly overexpressed, while genes implicated in metabolism (especially lipid metabolism), signal transduction, immune and inflammatory response, transport, transcription regulation and protein biosynthesis, were commonly suppressed following all treatments. Cisplatin and topotecan treatments triggered similar alterations in gene and pathway expression patterns, while paclitaxel action was mainly associated with induction of genes and pathways linked to cellular assembly and organization (including numerous tubulin genes), cell death and protein synthesis. The microarray data were further confirmed by pathway and network analyses. Most alterations in gene expression were directly related to mechanisms of the cytotoxic actions in OC spheroids. However, the induction of genes linked to mechanisms of DNA replication and repair in

cisplatin- and topotecan-treated OC spheroids could be associated with immediate adaptive response to treatment. Similarly, overexpression of different tubulin genes upon exposure to paclitaxel could represent an early compensatory effect to this drug action. Finally, multicellular growth conditions that are known to alter gene expression (including cell adhesion and cytoskeleton organization), could substantially contribute in reducing the initial effectiveness of CT drugs in OC spheroids. Results described in this study underscore the potential of the microarray technology for unraveling the complex mechanisms of CT drugs actions in OC spheroids and early cellular response to treatment.

Li, C., A. Gemma, et al. (2007). "In vitro simulation study of individualized chemotherapy in lung cancer." *J Nippon Med Sch* 74(3): 217-22.

The primary aim of this in vitro simulation study was to evaluate the utility of gene expression profile analysis in predicting the effect of varying drug combinations for the treatment of lung cancer. Using 10 human cancer cell lines, we focused our gene expression analysis on a cohort of candidate sensitivity-prediction factors, previously reported using cDNA filter arrays, with a view to predicting the ability of a set of anti-cancer drugs commonly used to treat lung cancer, namely cisplatin, 5-fluorouracil (5FU), SN38, docetaxel, gemcitabine, and vinorelbine. Altered expression of genes for glutathione-S-transferase-pi, uridine phosphorylase, O-6-methylguanine-DNA methyltransferase, and multidrug resistance 1 was identified in lung cancer cell lines. Drug sensitivity testing, in the form of methylthiotetrazol analysis, was performed using these six anti-cancer drugs against the panel of 10 lung cancer cell lines. We compared the predicted chemosensitivity based on the gene expression pattern of 19 well-known sensitivity-related genes with the cytotoxic activity of each of these anti-cancer drugs. Molecular profiling data predicted resistance to CDDP in LK-2 cells, 5FU in LK-2, PC7, A549, NCI-N231, Lu135 cells, irinotecan in PC9 cells, and VNR in PC7 cells. However, the prediction efficacy (number of predicted inactive drugs by gene expression analysis/number of inactive drugs by methylthiotetrazol assay) was 21.6% (8 of 37). No false-positive findings in relation to sensitivity-related genes were obtained on the basis of this molecular analysis. Thus, prediction of sensitivity to lung cancer by molecular analysis appears possible. With elucidation of additional drug sensitivity factors, selection of appropriate anticancer drugs by gene expression profiling may make it possible to increase the response rate in lung cancer chemotherapy.

Li, J., Z. N. Li, et al. (2009). "Expression of MRP1, BCRP, LRP, and ERCC1 in advanced non-small-cell lung cancer: correlation with response to chemotherapy and survival." *Clin Lung Cancer* **10**(6): 414-21.

We investigated the prognostic value of the expression of multidrug resistance protein-1 (MRP1), breast cancer resistance protein (BCRP), lung resistance-related protein (LRP), and excision repair cross-complementing group-1 (ERCC1) in patients with advanced non-small-cell lung cancer (NSCLC) treated with cisplatin-based chemotherapy. PATIENTS AND Semiquantitative reverse-transcriptase polymerase chain reaction (RT-PCR) was used for detecting the expression of MRP1, BCRP, LRP, and ERCC1 mRNA in 66 transbronchial biopsy (TBB) samples from untreated patients with advanced NSCLC. All of the patients received cisplatin-based chemotherapy. Response to chemotherapy, progression-free survival (PFS), and overall survival (OS) were compared in relation to expression of each gene and clinicopathologic factors. Results showed that tumor stage ( $P = .028$ ) and the expression of MRP1 ( $P = .046$ ) and LRP ( $P = .012$ ) correlated with response to chemotherapy. Poor performance status (PS;  $P = .016$ ), advanced tumor stage ( $P = .004$ ), and the high expression of MRP1 ( $P = .012$ ) and LRP ( $P = .002$ ) predicted poorer PFS. Performance status ( $P = .009$ ); tumor stage ( $P = .003$ ); and the expression of MRP1 ( $P = .017$ ), LRP ( $P = .005$ ), and ERCC1 ( $P = .002$ ) were predictive for OS. In a Cox proportional hazards multivariable analysis, PS ( $P = .042$ ), tumor stage ( $P = .007$ ), and the expression of LRP ( $P = .011$ ) and ERCC1 ( $P = .026$ ) were identified as independent prognostic factors for OS. Our data suggested that determination of MRP1, LRP, and ERCC1 mRNA expression using RT-PCR in TBB samples might be helpful in predicting outcome of patients with advanced NSCLC treated with cisplatin-based chemotherapy and in optimizing therapeutic strategy based on the expression of these genes.

Li, X., J. Yu, et al. (2008). "Chemical conjugation of muramyl dipeptide and paclitaxel to explore the combination of immunotherapy and chemotherapy for cancer." *Glycoconj J* **25**(5): 415-25.

Paclitaxel (Taxol) conjugated to muramyl dipeptide (MDP) is described. Biological testing showed that the conjugation of MDP at 2'-O-paclitaxel (2'-O-MTC-01) not only has antitumor activity, but also have immunoenhancement capacity. Compared with paclitaxel or MDP alone or with a mixture of paclitaxel + MDP, 2'-O-MTC-01 significantly increases the production and expression of TNF-alpha and IL-12 from mouse peritoneal macrophages, which

demonstrates a synergism of MDP and paclitaxel in one conjugated molecule.

Li, X. Q., J. Li, et al. (2009). "Expression of MRP1, BCRP, LRP and ERCC1 as prognostic factors in non-small cell lung cancer patients receiving postoperative cisplatin-based chemotherapy." *Int J Biol Markers* **24**(4): 230-7.

The development of resistance to chemotherapy is one of the major obstacles in the treatment of non-small cell lung cancer (NSCLC). The purpose of this study was to investigate the prognostic value of multidrug resistance protein 1 (MRP1), breast cancer resistance protein (BCRP), lung resistance-related protein (LRP), and excision repair cross-complementing 1 (ERCC1) in NSCLC patients receiving cisplatin-based adjuvant chemotherapy (cisplatin plus vinorelbine or gemcitabine) after tumor resection. We used semiquantitative reverse-transcription polymerase chain reaction to detect the expression of MRP1, BCRP, LRP and ERCC1 mRNA in surgical resection specimens of 60 patients with stage IB through IIIA NSCLC. The expression level of each gene was analyzed in relation to clinicopathological factors, tumor-free survival (TFS), and overall survival. The results showed that stage IIIA ( $p=0.011$ ), N1 and N2 status ( $p=0.008$ ), high expression of MRP1 ( $p=0.034$ ) and LRP ( $p=0.018$ ) were associated with shorter TFS. Stage IIIA ( $p=0.0105$ ), N1 and N2 status ( $p=0.009$ ), high expression of MRP1 ( $p=0.021$ ) and ERCC1 ( $p=0.012$ ) were related to a shorter overall survival. Cox multivariate analyses revealed that early stage ( $p=0.013$  and  $p=0.024$ ), negative lymph node status ( $p=0.006$  and  $p=0.011$ ), and low MRP1 expression ( $p=0.022$  and  $p=0.035$ ) were independent predictors of favorable TFS and overall survival, respectively. Additionally, ERCC1 ( $p=0.019$ ) was an independent predictor of favorable overall survival.

Liang, H., H. J. Zhan, et al. (2007). "Change in expression of apoptosis genes after hyperthermia, chemotherapy and radiotherapy in human colon cancer transplanted into nude mice." *World J Gastroenterol* **13**(32): 4365-71.

AIM: To investigate the change in expression of p53, Bcl-2, and Bax genes in human colon cancer cells transplanted into nude mice after hyperthermia, chemotherapy, radiotherapy, thermochemotherapy, thermoradiotherapy and thermochemoradiotherapy. Human colon cancer cell line (HT29) was transplanted into the hind limbs of nude mice. Under laboratory simulated conditions of hyperthermia (43 centigrade, 60 min), the actual radiation doses and doses of mitomycin C (MMC) were calculated in reference to the clinical radiotherapy for human rectal cancer and

chemotherapy prescription for colon cancer. The mice were divided into 6 groups according to the treatment approaches: hyperthermia, chemotherapy, radiotherapy, thermochemotherapy, thermoradiotherapy, and thermochemoradiotherapy. The mice were sacrificed at different time points and the tumor tissue was taken for further procedures. The morphologic changes in membrane, cytoplasm and nuclei of tumor cells of p53, Bcl-2, and Bax after treatment, were observed by immunohistochemistry staining. All of the six treatment modalities down-regulated the expression of p53, Bcl-2 and up-regulated the expression of Bax at different levels. The combined therapy of hyperthermia, with chemotherapy, and/or irradiation showed a greater effect on down-regulating the expression of p53 (0.208 +/- 0.009 vs 0.155 +/- 0.0115,  $P < 0.01$ ) and Bcl-2 (0.086 +/- 0.010 vs 0.026 +/- 0.0170,  $P < 0.01$ ) and up-regulating Bax expression (0.091 +/- 0.0013 vs 0.207 +/- 0.027,  $P < 0.01$ ) compared with any single therapy. Hyperthermia enhances the effect of radio- and chemotherapy on tumors by changing the expression of apoptosis genes, such as p53, Bcl-2 and Bax.

Liao, D. J., A. Thakur, et al. (2007). "Perspectives on c-Myc, Cyclin D1, and their interaction in cancer formation, progression, and response to chemotherapy." *Crit Rev Oncog* **13**(2): 93-158.

C-myc is an oncogene that functions both in the stimulation of cell proliferation and in and apoptosis. C-myc elicits its oncogenic activity by causing immortalization, and to a lesser extent the transformation of cells, in addition to several other mechanisms. C-myc may also enhance or reduce the sensitivity of cancer cells to chemotherapy, but how this dual function is controlled is largely unclear. Cyclin D1 (D1) is another oncogene that drives cell cycle progression; it acts as a growth factor sensor to integrate extracellular signals with the cell cycle machinery, though it may also promote apoptosis. C-Myc collaborates with TGF $\alpha$ , epidermal growth factor receptor, Ras, PI3K/Akt, and NF- $\kappa$ B. in part via coordination in regulation of D1 expression, because D1 is a common downstream effector of these growth pathways. Coordination of c-Myc with D1 or its upstream activators not only accelerates tumor formation, but also may drive tumor progression to a more aggressive phenotype. Because c-Myc may effect immortalization while D1 or its upstream activators elicit transformation, targeting c-myc and D1 may be a good strategy for cancer prevention. Moreover, since D1 imposes chemoresistance on cancer cells, targeting D1 may also be a good strategy for cancer chemotherapy, whereas practitioners

should be cautious to downregulate c-myc for chemotherapy, since c-Myc may elicit apoptosis.

Lin, C. S., L. S. Wang, et al. (2008). "Low copy number and low oxidative damage of mitochondrial DNA are associated with tumor progression in lung cancer tissues after neoadjuvant chemotherapy." *Interact Cardiovasc Thorac Surg* **7**(6): 954-8.

The decrease in the copy number of mitochondrial DNA (mtDNA) in cancer tissues might be associated with a decrease in oxidative mtDNA damage to achieve cancer immortalization and progression. Lung cancer specimens were collected from 29 patients with stage III non-small cell lung cancer (NSCLC) after neoadjuvant chemotherapy followed by surgical resection. The relative mtDNA copy number and the oxidative mtDNA damage (formation of 8-OHdG in mtDNA) of each cancer tissue were measured by quantitative real-time PCR. Seven female and 22 male lung cancer patients, with a mean age of 63.5 years were evaluated. Tumors of five patients became progressive, 13 stable, and 11 partially responsive after preoperative chemotherapy. Low mtDNA copy number ( $P=0.089$ ) and low degree of oxidative mtDNA damage ( $P=0.036$ ) were found to associate with tumor progression. Moreover, mtDNA copy number was significantly related to the degree of oxidative mtDNA damage ( $P=0.031$ ). The mtDNA copy number and oxidative mtDNA damage were lower in advanced NSCLC after chemotherapy. This finding suggests that a decrease in the content of mtDNA may result in a decrease of mitochondrial density in cancer cells, which leads to a decrease of endogenous ROS production and reduction of ROS-triggered DNA damage to achieve immortalization.

Lin, K., D. Ye, et al. (2008). "Protein expression levels of excision repair cross-complementation group 1 and xeroderma pigmentosum D correlate with response to platinum-based chemotherapy in the patients with advanced epithelial ovarian cancer." *Int J Gynecol Cancer* **18**(5): 1007-12.

This study was undertaken to examine whether there is an association between excision repair cross-complementation group 1 (ERCC1) and xeroderma pigmentosum D (XPD) protein expression levels and response to platinum-based chemotherapy in epithelial ovarian cancer (EOC). The study cohort consisted of 91 consecutive patients suffering from stage III or IV disease of primary EOC from 1999 to 2004 at the Women's Hospital, School of Medicine, Zhejiang University. There were 36 sensitive cases of serous ovarian cancer, 27 resistant cases of serous ovarian cancer, 15 cases of clear cell cancer, and 13 cases with serous ovarian cancer receiving neoadjuvant chemotherapy. The ovarian tissue

microsections were stained by standard immunohistochemical techniques to show ERCC1 and XPD protein expression levels. In resistance group of serous ovarian cancer, ERCC1 and XPD protein expression levels were significantly higher than those of sensitivity group, and after receiving neoadjuvant chemotherapy, they showed 23% and 32% higher than before. Meanwhile, their levels of clear cell cancer group were significantly higher than serous ovarian cancer group's. Upregulation of ERCC1 and XPD protein expression was associated with resistance process to platinum-based chemotherapy in advanced EOC. This study provided evidence that differences of nucleotide excision repair-related genes expression may have an effect on the observed differences in clinical behavior of EOC.

Liu, H., T. Zhang, et al. (2008). "Predictive value of MMP-7 expression for response to chemotherapy and survival in patients with non-small cell lung cancer." *Cancer Sci* **99**(11): 2185-92.

Fundamental studies have suggested that matrix metalloproteinases-7 (MMP-7) expression is associated with chemoresistance and constitutes a prognostic factor in several solid tumors. The present study assessed the prognostic and predictive value of MMP-7 in tumors of patients with advanced non-small cell lung cancer (NSCLC) treated with platinum-based chemotherapy. In total, 159 patients with stage III and IV NSCLC were retrospectively enrolled. Immunohistochemistry was performed to evaluate the expression of MMP-7, apoptosis-related proteins Bcl-2, Bax, Fas and FasL and the Ki-67 proliferation marker. The TUNEL (terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling) method was performed to investigate tumor apoptosis. Ninety carcinomas (56.6%) were identified as high expression of MMP-7. Overexpression of MMP-7 was more frequent in adenocarcinomas than in squamous cell carcinomas ( $P = 0.032$ ). The expression of MMP-7 was positively related with Ki-67 index and Bcl-2, but not apoptosis index. MMP-7 status was correlated inversely with response to chemotherapy in overall patients (response rates, 20.0% and 35.8%, for patients with high-MMP-7 and low-MMP-7 tumors, respectively,  $P = 0.036$ ), especially in adenocarcinoma ( $P = 0.021$ ), but not in patients with squamous cell carcinomas ( $P = 0.373$ ). The overall survival was significantly lower in NSCLC patients with high MMP-7 than in those with low MMP-7 ( $P < 0.001$ ). A Cox regression analyses also demonstrated MMP-7 status to be a significant prognostic factor (hazard ratio, 5.49  $P = 0.001$ ). These findings suggest that the expression level of MMP-7 in tumor cells is predictive of response to chemotherapy and outcome in patients

with advanced NSCLC receiving platinum-based chemotherapy.

Loignon, M., W. Miao, et al. (2009). "Cul3 overexpression depletes Nrf2 in breast cancer and is associated with sensitivity to carcinogens, to oxidative stress, and to chemotherapy." *Mol Cancer Ther* **8**(8): 2432-40.

Nrf2 is the key transcription factor for cytoprotective gene programs. Nrf2 is normally maintained at very low concentrations by proteasomal degradation, through its interaction with the adapter protein Keap1 and the Cul3 E3 ligase. Increased Nrf2 concentration resulting from loss of function Keap1 mutations has been described in chemoresistant non-small cell lung cancer. Previous studies in breast cancer showed low levels of some Nrf2-regulated detoxification genes, but the mechanism has not been systematically examined. We found that half of the breast cancer cell lines examined have decreased concentration of Nrf2 compared with normal mammary epithelial cell lines, associated with variable but detectable levels in Keap1 levels, and consistently increased Cul3 mRNA and protein. Immunohistochemistry showed that 7 of 10 breast cancer specimens examined also have low Nrf2 levels and increased Cul3. Keap1 protein levels are variable. We found no C23Y mutation in Keap1 of any of the cell lines. Using siRNA, we silenced Cul3 in MCF-7 breast cancer cells, and microarray analysis reveals the induction of GCL, NQO1, AKR1C1, UGDH, and TXN by at least 2-fold. The Nrf2-regulated ABCC1 drug transporter was also found to be increased. These Cul3-silenced MCF7 cells are highly resistant to oxidative stress induced by H<sub>2</sub>O<sub>2</sub> to the carcinogen benzo(a)pyrene, and to both Doxorubicin and Paclitaxel. This high Cul3/low Nrf2 signature may be key to cellular sensitivity to both chemical carcinogenic stimuli as well as to cytotoxicity of commonly used chemotherapeutic drugs in established breast cancers.

Lu, J., J. S. Kovach, et al. (2009). "Inhibition of serine/threonine phosphatase PP2A enhances cancer chemotherapy by blocking DNA damage induced defense mechanisms." *Proc Natl Acad Sci U S A* **106**(28): 11697-702.

A variety of mechanisms maintain the integrity of the genome in the face of cell stress. Cancer cell response to chemotherapeutic and radiation-induced DNA damage is mediated by multiple defense mechanisms including polo-like kinase 1 (Plk-1), protein kinase B (Akt-1), and/or p53 pathways leading to either apoptosis or cell cycle arrest. Subsequently, a subpopulation of arrested viable cancer cells may remain and recur despite

aggressive and repetitive therapy. Here, we show that modulation (activation of Akt-1 and Plk-1 and repression of p53) of these pathways simultaneously results in paradoxical enhancement of the effectiveness of cytotoxic chemotherapy. We demonstrate that a small molecule inhibitor, LB-1.2, of protein phosphatase 2A (PP2A) activates Plk-1 and Akt-1 and decreases p53 abundance in tumor cells. Combined with temozolomide (TMZ; a DNA-methylating chemotherapeutic drug), LB-1.2 causes complete regression of glioblastoma multiforme (GBM) xenografts without recurrence in 50% of animals (up to 28 weeks) and complete inhibition of growth of neuroblastoma (NB) xenografts. Treatment with either drug alone results in only short-term inhibition/regression with all xenografts resuming rapid growth. Combined with another widely used anticancer drug, Doxorubicin (DOX, a DNA intercalating agent), LB-1.2 also causes marked GBM xenograft regression, whereas DOX alone only slows growth. Inhibition of PP2A by LB-1.2 blocks cell-cycle arrest and increases progression of cell cycle in the presence of TMZ or DOX. Pharmacologic inhibition of PP2A may be a general method for enhancing the effectiveness of cancer treatments that damage DNA or disrupt components of cell replication.

Luo, J. (2009). "Glycogen synthase kinase 3beta (GSK3beta) in tumorigenesis and cancer chemotherapy." *Cancer Lett* **273**(2): 194-200.

Glycogen synthase kinase 3beta (GSK3beta), a multifunctional serine/threonine kinase found in all eukaryotes, had been initially identified as a key regulator of insulin-dependent glycogen synthesis. It is now known that GSK3beta functions in diverse cellular processes including proliferation, differentiation, motility and survival. Aberrant regulation of GSK3beta has been implicated in a range of human pathologies including non-insulin-dependent diabetes mellitus, cardiovascular disease, some neurodegenerative diseases, and bipolar disorder. As a consequence, the therapeutic potential of GSK3beta inhibitors has become an important area of investigation. However, GSK3beta also participates in neoplastic transformation and tumor development. The role of GSK3beta in tumorigenesis and cancer progression remains controversial; it may function as a "tumor suppressor" for certain types of tumors, but promotes growth and development for some others. GSK3beta also mediates drug sensitivity/resistance in cancer chemotherapy. Therefore, although GSK3beta is an attractive therapeutic target for a number of human diseases, its potential impact on tumorigenesis and cancer chemotherapy needs to be carefully evaluated. This mini-review discusses the role of

GSK3beta in tumorigenesis/cancer progression as well as its modulation of cancer chemotherapy.

Makizumi, R., W. L. Yang, et al. (2008). "Alteration of Drug Sensitivity in Human Colon Cancer Cells after Exposure to Heat: Implications for Liver Metastasis Therapy using RFA and Chemotherapy." *Int J Clin Exp Med* **1**(2): 117-29.

Radiofrequency ablation (RFA) is gaining popularity for treating colorectal liver metastases by inducing image guided tumor hyperthermia. In order to reduce tumor recurrence, adjuvant therapies have been administered post-RFA. We hypothesized that tumor cells escaping RFA cytotoxicity by being in the sublethal zones of tumor might develop differential behavior toward cytotoxic drugs. Here, we used cultured human colorectal cancer cells to evaluate the interaction between heat treatment and chemotherapeutic agents. Human colon cancer cell lines HT29 and HCT116 were subjected to temperatures of 42 degrees to 50 degrees C for 15 min, in combination with 5-fluorouracil, oxaliplatin, or irinotecan at different sequences. Cytotoxicity was determined by MTT assay. The cell cycle progression was analyzed by flow cytometry with propidium iodide staining. The expression of several genes associated with drug sensitivity was quantitated by real-time RT-PCR before and after heat treatment. Either heat treatment at 45 degrees C by simultaneous or pre-treatment with three different chemotherapeutic agents didn't affect the cytotoxicity of the combined treatment to HT29 and HCT116 cells, except for irinotecan treatment in HCT116 cells. However, when pre-exposure to 45 degrees C, HCT116 cells, but not HT29 cells, developed resistance to these three drugs. In an analysis of cell cycle profile after the drug followed heat treatment, a longer delay in cell cycle progression in HCT116 cells was observed in comparison to HT29 cells. Furthermore, HCT116 and HT29 cells exhibited different expression profiles of several drug-related genes in response to heat treatment at 45 degrees C. An observation of a differential response to the drug and heat treatment sequences between two human colon cancer cell lines suggests that tumor heterogeneity and selection of chemotherapeutic agents need to be under consideration in the clinical setting.

Meslin, F., R. Conforti, et al. (2007). "Efficacy of adjuvant chemotherapy according to Prion protein expression in patients with estrogen receptor-negative breast cancer." *Ann Oncol* **18**(11): 1793-8.

Prion protein (PrPc) has been previously reported to be associated with resistance to proapoptotic stimuli. We evaluated whether the expression of PrPc was associated with the resistance

to adjuvant chemotherapy in patients with estrogen receptor (ER) -negative breast cancer. PATIENTS AND The expression of PrPc by primary tumors was assessed by immunohistochemistry in a series of 756 patients included in two randomized trials that compared anthracycline-based chemotherapy to no chemotherapy. The PrPc expression was correlated with ER expression and the benefit of adjuvant chemotherapy was assessed according to PrPc expression in patients with ER-negative tumors. Immunostaining analysis showed that PrPc was mainly expressed by myoepithelial cells in normal breast tissue. Tissue microarray analysis from 756 breast tumors showed that PrPc was associated with ER-negative breast cancer subsets ( $P < 0.001$ ). Adjuvant chemotherapy was not associated with a significant risk reduction for death in patients with ER-negative/PrPc-positive disease [adjusted hazard ratio (HR) for death = 0.98, 95% confidence interval (CI) 0.45-2.1,  $P = 0.95$ ], while it decreased the risk for death (HR = 0.39, 95% CI 0.2-0.74,  $P = 0.004$ ) in patients with ER-negative/PrPc-negative tumors. These data indicate that ER-negative/PrPc-negative phenotype is associated with a high sensitivity to adjuvant chemotherapy.

Mitani, Y., N. Oue, et al. (2007). "Reg IV is a serum biomarker for gastric cancer patients and predicts response to 5-fluorouracil-based chemotherapy." *Oncogene* **26**(30): 4383-93.

Regenerating gene family, member 4 (Reg IV), a secreted protein, is overexpressed in several cancers, including gastric cancer (GC). In the present study, we measured Reg IV levels in sera from patients with GC by enzyme-linked immunosorbent assay. We also examined the effect of forced Reg IV expression on the apoptotic susceptibility to 5-fluorouracil (5-FU). Forced expression of Reg IV inhibited 5-FU-induced apoptosis. Induction of Bcl-2 and dihydropyrimidine dehydrogenase was involved in inhibition of apoptosis. Among 36 GC patients treated with a combination chemotherapy of low-dose 5-FU and cisplatin, all 14 Reg IV-positive patients showed no change or disease progression. The serum Reg IV concentration was similar between healthy individuals (mean $\pm$ s.e., 0.52 $\pm$ 0.05 ng/ml) and patients with chronic-active gastritis (0.36 $\pm$ 0.09 ng/ml). However, the serum Reg IV concentration in presurgical GC patients was significantly elevated (1.96 $\pm$ 0.17 ng/ml), even at stage I. The diagnostic sensitivity of serum Reg IV (36.1%) was superior to that of serum carcinoembryonic antigen (11.5%) or carbohydrate antigen 19-9 (13.1%). These results indicate that expression of Reg IV is a marker for prediction of resistance to 5-FU-based chemotherapy

in patients with GC. Serum Reg IV represents a novel biomarker for GC.

Morgensztern, D. and R. Govindan (2008). "Adjuvant chemotherapy for lung cancer: cisplatin doublets only?" *J Natl Compr Canc Netw* **6**(3): 277-84.

Lung cancer is the leading cause of cancer-related mortality worldwide. Despite adequate resection, more than half of patients die of recurrent disease, usually at distant sites. Adjuvant systemic chemotherapy is mainly used to eradicate micrometastatic disease. Since the seminal 1995 meta-analysis from earlier studies showed a trend toward improved survival with the use of cisplatin-based adjuvant chemotherapy, several randomized prospective adjuvant trials have addressed this question and eventually established the role for platinum-based adjuvant chemotherapy in patients with stage II or IIIA non-small cell lung cancer who have undergone complete resection. The role of adjuvant chemotherapy in patients with stage I disease remains controversial. Although no clinical or molecular predictors of recurrent disease after surgical resection are reliable, encouraging preliminary data on gene expression studies suggest that identifying, and perhaps treating, only patients at high risk for relapse might be possible in the near future. Furthermore, molecular predictors of resistance may guide the selection of chemotherapy in this setting.

Moriyama-Gonda, N., H. Shiina, et al. (2008). "Rationale and clinical implication of combined chemotherapy with cisplatin and oestrogen in prostate cancer: primary evidence based on methylation analysis of oestrogen receptor-alpha." *BJU Int* **101**(4): 485-91.

To determine whether oestrogen enhances platinum sensitivity, and if promoter CpG methylation of the oestrogen receptor-alpha (ER-alpha) gene determines the potential of cisplatin-induced apoptosis in prostate cancer, as the high-mobility group 1 (HMG1) preferentially binds to cisplatin-modified DNA and is up-regulated after oestrogen treatment in breast cancer cell line MCF-7. MATERIALS AND The study comprised prostate cancer cell lines (LNCaP and PC-3), 156 pathologically confirmed 156 radical prostatectomy samples and eight hormone-refractory prostate cancer (HRPC) samples (from needle biopsy). Expression of HMG1 in cell lines was analysed by Western blotting or differential reverse-transcription-polymerase chain reaction (PCR). The methylation status of ER-alpha was analysed by methylation-specific PCR using bisulphite DNA as a template or bisulphite DNA sequencing. In LNCaP cells, treatment with oestrogen increased HMG1 expression and co-treatment with cisplatin and

oestrogen reduced cell viability by accelerating apoptosis, compared with cisplatin alone. However, in PC-3, oestrogen did not up-regulate HMG1 or accelerate the cisplatin-induced apoptosis. Although ER-beta was expressed in both LNCaP and PC-3, ER-alpha was expressed only in LNCaP. Bisulphite DNA sequencing of the ER-alpha promoter showed partial methylation in LNCaP but complete methylation in PC-3. ER-alpha AS transfection diminished the cisplatin-induced apoptosis in oestrogen-treated LNCaP cells. In clinical samples there was ER-alpha hypermethylation in 40% of prostate cancers this correlated with Gleason score (GS, 31% for GS < 7, 50% for GS = 7 and 56% for GS > 7). In addition, five of eight HRPc samples showed ER-alpha hypermethylation. These findings suggest that HMG1 induction as an enhancer of platinum sensitivity is mediated through interaction between oestrogen and ER-alpha. As CpG hypermethylation of the ER-alpha promoter is a frequent event in aggressive prostate cancer, negative conversion of ER-alpha methylation is essential to achieve the most beneficial effect when combined chemotherapy of cisplatin with oestrogen is used in patients with prostate cancer.

Mozaffari, F., C. Lindemalm, et al. (2009). "Systemic immune effects of adjuvant chemotherapy with 5-fluorouracil, epirubicin and cyclophosphamide and/or radiotherapy in breast cancer: a longitudinal study." *Cancer Immunol Immunother* **58**(1): 111-20.

Immunotherapy is being increasingly utilized for adjuvant treatment for breast cancer (BC). We have previously described immune functions during primary therapy for BC. The present study describes immune recovery patterns during long-term, unmaintained follow-up after completion of adjuvant therapy. A group of patients with primary BC had been treated with adjuvant radio-chemotherapy (RT + CT) 5-fluorouracil, epirubicin and cyclophosphamide (FEC) (n = 21) and another group with radiotherapy (RT) (n = 20) alone. Immunological testing of NK and T-cell functions was performed initially at the end of adjuvant treatment and repeated after 2, 6 and 12 months. NK cell cytotoxicity was significantly higher (P < 0.05) at all time-points in patients than in age-matched controls and did not differ between the two treatments groups during one year observation. In contrast, lower numbers of CD4 T-cells and lower expression of CD28 on T-cells was observed particularly in RT + CT patients and did not normalize during the observation period. The numbers of T(reg) cells (CD4(+)/CD25(high)) were low in the RT + CT group during follow-up, as well as expression of TCR $\alpha$ , Zap70, p56(lck), P59(fyn) and PI3 k in CD4(+) cells. In contrast, expression of intracellular cytokines (IFN-gamma, IL-2, IL-4) in CD4 and CD8

T cells were significantly higher in RT + CT patients than in the RT group and the difference increased during follow-up. In conclusion, NK-cell cytotoxicity increased during unmaintained long-term follow-up whereas CD4 and regulatory T cells as well as signal transduction molecules remained low following adjuvant radio-chemotherapy.

Muller, P. J., H. Dally, et al. (2009). "Polymorphisms in ABCG2, ABCC3 and CNT1 genes and their possible impact on chemotherapy outcome of lung cancer patients." *Int J Cancer* **124**(7): 1669-74.

The prognosis of lung cancer patients treated with chemotherapy is poor, motivating the search for predictive factors. Single nucleotide polymorphisms (SNPs) in membrane transporter genes could influence the pharmacokinetics of cytostatic drugs and therefore affect treatment outcome. We examined 6 SNPs with known or suspected phenotypic effect: ABCG2 G34A, C421A; ABCC3 C-211T, G3890A, C3942T and CNT1 G565A. For 349 Caucasian patients with primary lung cancer [161 small cell lung cancer (SCLC), 187 nonsmall cell lung cancer (NSCLC) and 1 mixed] receiving first-line chemotherapy 3 different endpoints were analyzed: response after the 2nd cycle (R), progression-free survival (PFS) and overall survival (OS). The prognostic value of the SNPs was analyzed using multivariable logistic regression, calculating odds ratios (ORs) when comparing genotype frequencies in responders and nonresponders after the 2nd cycle. Hazard ratios (HRs) for PFS and for OS were calculated using Cox regression methods. In all lung cancer patients, none of the investigated polymorphisms modified response statistically significant. The only significant result in the histological subpopulations was in SCLC patients carrying the ABCC3 -211T allele who showed significantly worsened PFS (HR: 1.79; 95% confidence interval (CI) 1.13-2.82). In an exploratory subgroup analysis significantly worse OS was seen for carriers of the ABCG2 421A-allele treated with platinum-based drugs (HR: 1.60; 95% CI 1.04-2.47; n = 256). In conclusion, this study prioritizes ABCC3 C-211T and ABCG2 C421A as candidate transporter SNPs to be further investigated as possible predictors of the clinical outcome of chemotherapy in lung cancer patients.

Nakanishi, T. (2007). "Drug transporters as targets for cancer chemotherapy." *Cancer Genomics Proteomics* **4**(3): 241-54.

Transporter proteins play an important role in taking up nutrients into and effluxing xenobiotics out of cells to sustain cell survival. Transporters that affect drug absorption, distribution and excretion are the so-called drug transporters. In the last decade, a

number of studies revealed interactions between drug transporters and clinically important anticancer agents. Utilizing the knowledge of transporter functions offers us the possibility of delivering a drug to the target tissues, avoiding distribution to other tissues and improving oral bioavailability. Many transporters have been reported to be differentially up-regulated in cancer cells compared to normal tissues, suggesting that the differential expression of transporters in cancer cells may provide good targets for enhancing drug delivery as well as diagnostic markers for cancer therapy. This review will focus on the role of drug transporters in the adaptation and growth of tumors and in their potential usefulness as therapeutic targets in cancer.

Noma, B., T. Sasaki, et al. (2008). "Expression of multidrug resistance-associated protein 2 is involved in chemotherapy resistance in human pancreatic cancer." *Int J Oncol* **33**(6): 1187-94.

Despite the recent introduction of the new anticancer agents gemcitabine (GEM) and TS-1, as well as combination regimens such as GEM plus cisplatin (CDDP), pancreatic cancer treatment remains relatively ineffective. Both intrinsic and acquired resistance to chemotherapy are major roadblocks to the successful treatment of pancreatic cancer patients. The aims of this study were to examine the expression of multidrug resistance-associated proteins (MRPs) MRP1, MRP2 and MRP3 and to evaluate the correlation between MRP2 expression and CDDP resistance in human pancreatic cancer. Five human pancreatic cancer cell lines and several surgically resected pancreatic cancer tissues were subjected to reverse-transcriptase (RT)-PCR, real-time PCR and immunohistochemical analysis. While MRP1 and MRP2 mRNA was expressed in all cell lines, MRP3 mRNA was only detected in two cell lines. In resected pancreatic cancer tissues, only MRP2 mRNA was expressed and it was overexpressed compared with normal pancreatic tissues. MRP2 protein expression was observed in 77.5% (31/40) of cancer tissues, primarily in the cytoplasm of cancer cells, but was not observed in normal pancreatic tissue. Two CDDP-resistant pancreatic cancer cell line SUIT-2 variants, SUIT-2-CD3 and SUIT-2-CD4, were established by continuously administering 10 nM CDDP to SUIT-2 cell lines for 3 and 4 months, respectively. Incubation of these cells with CDDP in the presence of anti-MRP2 antibody or the MRP2 inhibitor MK-571 in a growth inhibition assay demonstrated that the CDDP-resistant variants were more resistant to CDDP than the parent cell line and this resistance was diminished by either anti-MRP2 antibody or MK-571. Moreover, RT-PCR and real-time PCR revealed that while induction of MRP2 mRNA expression was increased

in CDDP-resistant compared with parent cells, MRP1 and MRP3 expression remained unchanged. These observations suggest that MRP2 may correlate to intrinsic and acquired resistance for CDDP in human pancreatic cancer.

Okoshi, K., S. Nagayama, et al. (2009). "A case report of pathologically complete response of a huge rectal cancer after systemic chemotherapy with mFOLFOX6." *Jpn J Clin Oncol* **39**(8): 528-33.

A 54-year-old man was referred to our hospital because of a huge, unresectable rectal cancer occupying his entire pelvic space with a solitary liver metastasis. He had undergone a laparotomy for surgical resection, but ended up with a sigmoid colostomy due to possible invasion into the urinary bladder and pelvic wall. At the completion of seven cycles of FOLFOX regimen, radiographic examination revealed remarkable reduction of the primary rectal tumor and regional lymph nodes, and also a complete response (CR) of the liver metastasis. The tumor was extirpated without any macroscopic residues by a low anterior resection of the rectum, along with a partial resection of the urinary bladder and seminal vesicles. Since pathological and immunohistochemical examinations showed no viable cancer cells in any parts of the resected specimens, the lesion was regarded as a pathologically CR. Analysis for single-nucleotide polymorphisms in the genes involved in nucleotide excision repair, excision repair cross-complementing group 1 and xeroderma pigmentosum group D, showed a genotypic pattern sensitive to oxaliplatin. To our knowledge, this is a rare case of an initially unresectable primary rectal cancer, which was down-staged to a pathologically CR by FOLFOX chemotherapy instead of chemoradiotherapy.

Olaussen, K. A., G. Mountzios, et al. (2007). "ERCC1 as a risk stratifier in platinum-based chemotherapy for nonsmall-cell lung cancer." *Curr Opin Pulm Med* **13**(4): 284-9.

**PURPOSE OF REVIEW:** Cisplatin-based chemotherapy remains the treatment of choice in advanced nonsmall-cell lung cancer. The development of predictive biomarkers able to identify lung-cancer patients who are most likely to benefit from cisplatin-based chemotherapy would be a powerful tool. Many reports have explored the role of ERCC1 expression in the repair mechanism of cisplatin-induced DNA adducts in cancer cells. **RECENT FINDINGS:** Using immunohistochemistry in resected tumors, the International Adjuvant Lung Cancer Trial showed that high ERCC1 protein expression was associated with improved survival in patients who did not receive chemotherapy. In contrast, the benefit of adjuvant

cisplatin-based chemotherapy was more profound in patients with low ERCC1 expression. Other investigators studying mRNA expression in tumor biopsies from patients treated with cisplatin and gemcitabine showed that patients with low ERCC1 mRNA expression have a longer median survival compared to those with high expression. SUMMARY: High ERCC1 expression is predictive of resistance to platinum-based therapy. Thus, there is solid evidence to support ERCC1 as a useful marker of clinical resistance to platinum-based chemotherapy in the adjuvant setting of nonsmall-cell lung cancer. Meanwhile, optimization of methodology and standardization of technical procedures seem necessary before larger prospective studies can address the same question.

Orlando, L., B. Del Curto, et al. (2008). "Topoisomerase IIalpha gene status and prediction of pathological complete remission after anthracycline-based neoadjuvant chemotherapy in endocrine non-responsive Her2/neu-positive breast cancer." *Breast* 17(5): 506-11.

Topoisomerase IIalpha (Topo II) is a potential marker of responsiveness to anthracycline-based therapy. We analyzed the role of Topo II gene status in the prediction of pathological complete remission (pCR) after primary anthracycline-based chemotherapy in non-endocrine responsive breast cancers overexpressing Her2/neu. Twenty-three patients, with T2-T4, ER and PgR absent, overexpressing Her2/neu breast cancers treated with anthracycline-based chemotherapy were evaluated. Topo II gene status was assessed by FISH in pre-treatment tumor specimens and the results were correlated to pathological and clinical responses. Overall, six patients had a pCR (26%). Topo II was amplified in 5 (22%) of the tumors. In all patients with Topo II amplification, Her2/neu gene amplification was also detected. Among patients without amplification, one had polysomia of chromosome (Cr) 17 and four patients had deletion of the Topo II gene. A higher probability of pCR was observed when Topo II amplification and Cr 17 polysomy were present: pCR was reported in 3 of 5 amplified tumors (60%), in the polysomic tumor (amplified plus polysomic 67%) and in only 2 out of 13 tumors without alteration of Topo II status (15%). If we compare the frequency of pCR in tumors with amplification or polysomy versus the frequency of tumors with not amplification (deletion or no modification), a significant difference was detected ( $p=0.02$ ). One progressive disease (PD) was reported in one tumor with Topo II deletion (1/4, 25%) and one in tumor without any modification of Topo II gene status (1/13, 8%). CONCLUSIONS: In patients with endocrine unresponsive and Her2

overexpressing tumors, Topo II amplification or the presence of chromosome 17 polysomy correlate with a significantly high probability of achieving pCR after neoadjuvant, anthracycline-based chemotherapy. Further prospective studies in order to more clearly define the predictive role of Topo II status in this subgroup of patients are warranted.

Ota, S., G. Ishii, et al. (2009). "Immunohistochemical expression of BCRP and ERCC1 in biopsy specimen predicts survival in advanced non-small-cell lung cancer treated with cisplatin-based chemotherapy." *Lung Cancer* 64(1): 98-104.

The aim of this study was to determine the prognostic value of expression of ATP binding cassette (ABC) transporter proteins and DNA repair gene proteins by immunohistochemically staining tumor biopsy specimens from patients with advanced non-small-cell lung cancer (NSCLC) being treated with platinum-based chemotherapy. EXPERIMENTAL DESIGN: Expression of ABC transporter proteins, including BCRP (breast cancer resistance protein) and MRP2 (multidrug resistance proteins 2), and the DNA-repair-related proteins, ERCC1 (excision repair cross-complementation group 1) and BRCA1 (breast cancer type 1 susceptibility protein) was assessed immunohistochemically in 156 tumor samples from untreated stage IV NSCLC patients. All of the patients had received platinum-based chemotherapy. Response to chemotherapy, progression-free survival (PFS), and overall survival were compared in relation to expression of each of the proteins and to clinicopathological factors. High ERCC1 expression was associated with short survival (237 days vs. 453 days, log-rank  $P = 0.03$ ), but not with response to chemotherapy or PFS. And high BCRP expression was associated with short survival (214 days vs. 412 days, log-rank  $P = 0.02$ ) but not with response to chemotherapy or PFS. Multivariate analysis confirmed that negativity for the expression of BCRP tends to be an independent variable related to overall survival ( $P = 0.06$ ). CONCLUSIONS: This study examined ERCC1 and BCRP expression in biopsy specimens as candidates for predictors of the survival of patients with advanced NSCLC treated with platinum-based chemotherapy.

Pan, J. H., J. X. Han, et al. (2009). "MDR1 single nucleotide polymorphism G2677T/A and haplotype are correlated with response to docetaxel-cisplatin chemotherapy in patients with non-small-cell lung cancer." *Respiration* 78(1): 49-55.

The multidrug resistance gene 1 (MDR1) encodes P-glycoprotein (P-gp), which plays an important role in mediating multidrug resistance to chemotherapeutic agents. MDR1 gene polymorphisms

may have an impact on the expression and function of P-gp, thereby influencing the response to chemotherapy. OBJECTIVES: To investigate whether the MDR1 2677 and 3435 genotypes are associated with the sensitivity of non-small-cell lung cancer (NSCLC) to docetaxel. In this study we investigated the potential association of MDR1 2677G>T at exon 21, 3435C>T at exon 26 and their haplotypes with chemotherapy response of 54 Han Chinese patients with NSCLC. The patients were treated with docetaxel-cisplatin. The 2677 GG genotype was associated with a significantly better response to chemotherapy compared with the combined 2677 GT and TT genotype ( $p = 0.035$ ). The 3435 CC genotype was also associated with a better response to chemotherapy compared with the combined 3435 CT and TT genotypes although the difference was not statistically significant ( $p = 0.123$ ). Moreover, patients harboring the 2677G-3435C haplotype had a statistically significant better response to chemotherapy compared with those with the other haplotypes combined ( $p = 0.015$ ). Our findings suggest that the MDR1 2677G>T/A polymorphism and the 2677G-3435C haplotype are predictors of treatment response to docetaxel-cisplatin chemotherapy in NSCLC patients.

Pan, J. H., J. X. Han, et al. (2007). "CYP450 polymorphisms predict clinic outcomes to vinorelbine-based chemotherapy in patients with non-small-cell lung cancer." *Acta Oncol* **46**(3): 361-6.

CYP2C19\*2(G681A), CYP2C19\*3(G636A), CYP2D6\*4(C188T), CYP2D6\*2(C2938T, G4268C), CYP3A1\*3- G44A and CYP3A5\*3(A22893G) are the most common polymorphisms detected among Chinese that may influence the efficacy of vinorelbine-based therapies to treat non-small-cell lung cancer (NSCLC). We detected the genotypes of these polymorphisms by PCR-RFLP in 59 patients with NSCLC and assessed their responses to vinorelbine. CYP2D6\*4(C188T), CYP3A1\*3 (G -44 A) and CYP3A5\*3 were found to be associated with response to vinorelbine. For the 2D6\*4 polymorphism, the 18 of 32 (56.25%) patients with homozygous (C/C) responded to this therapy, while 6 of 27 (22.22%) of those heterozygous (C/T) at this site responded. ( $\chi^2=5.68$ ,  $p < 0.05$ ) For the 3A1\*1/\*3 polymorphism, 12 of 42 (28.57%) patients with homozygous (A/A) responded, while 12 of 17 (70.59%) with heterozygous (A/G) and homozygous (G/G) responded ( $\chi^2=7.19$ ,  $p < 0.01$ ). CYP3A5\*3 polymorphism has a result corresponding to 3A1\*3 polymorphism. Other polymorphisms were not associated with response to vinorelbine. No significant difference in toxicity and survival was observed according to SNP genotype.

Pan, J. H., J. X. Han, et al. (2008). "MDR1 single nucleotide polymorphisms predict response to vinorelbine-based chemotherapy in patients with non-small cell lung cancer." *Respiration* **75**(4): 380-5.

The polymorphisms of genes participate in metabolism and transport, and therefore may have an impact on the response to vinorelbine. OBJECTIVES: To investigate whether genotypes of CYP3A5, MDR1 and cyclooxygenase-2 (COX-2) are associated with the response to vinorelbine in non-small cell lung cancers (NSCLC). We determined the genotypes of CYP3A5(\*3), MDR1 (2677G-->T at exon 21 and 3435C-->T at exon 26 and their haplotypes) and COX-2 (-1195G-->A) polymorphisms by PCR-RFLP and chemotherapy response in 69 Chinese Han patients with NSCLC who received a combination chemotherapy of vinorelbine-cisplatin (VC). The chi(2) test was used to investigate potential associations between genotypes and response to chemotherapy. Odds ratios and 95% confidence intervals were calculated. The 3435 CC genotype was associated with a significantly better chemotherapy response compared with the combined 3435 CT and TT genotypes ( $p = 0.025$ ). The 2677 GG genotype was also associated with a better chemotherapy response compared with the combined 2677 GT and TT genotype, although it was not statistically significant. Moreover, we analyzed the haplotypes of MDR1 3435-2677: patients harboring the 2677G-3435C haplotype had a statistically significantly better response to chemotherapy compared with those with the other haplotypes combined ( $p = 0.015$ ). CYP3A5\*3 is not likely to correlate with sensitivity to vinorelbine in NSCLC. COX-2 (-1195G) is likely to result in a better response to vinorelbine (nonsignificant). CONCLUSIONS: Our findings suggest that MDR1 2677G-->T/A and 3435C-->T polymorphisms can be used to predict treatment response to VC chemotherapy in NSCLC patients.

Park, S., C. Shimizu, et al. (2006). "Gene expression profiling of ATP-binding cassette (ABC) transporters as a predictor of the pathologic response to neoadjuvant chemotherapy in breast cancer patients." *Breast Cancer Res Treat* **99**(1): 9-17.

Drug resistance is a major obstacle to the successful chemotherapy. Several ATP-binding cassette (ABC) transporters including ABCB1, ABCC1 and ABCG2 have been known to be important mediators of chemoresistance. Using oligonucleotide microarrays (HG-U133 Plus 2.0; Affymetrix), we analyzed the ABC transporter gene expression profiles in breast cancer patients who underwent sequential weekly paclitaxel/FEC (5-fluorouracil, epirubicin and cyclophosphamide)

neoadjuvant chemotherapy. We compared the ABC transporter expression profile between two classes of pretreatment tumor samples divided by the patients' pathological response to neoadjuvant chemotherapy (residual disease [RD] versus pathologic complete response [pCR]) ABCB3, ABCC7 and ABCF2 showed significantly high expression in the pCR. Several ABC transporters including ABCC5, ABCA12, ABCA1 ABCC13, ABCB6 and ABCC11 showed significantly increased expression in the RD ( $p < 0.05$ ). We evaluated the feasibility of developing a multigene predictor model of pathologic response to neoadjuvant chemotherapy using gene expression profiles of ABC transporters. The prediction error was evaluated by leave-one-out cross-validation (LOOCV). A multigene predictor model with the ABC transporters differentially expressed between the two classes ( $p < 0.003$ ) showed an average 92.8% of predictive accuracy (95% CI, 88.0-97.4%) with a 93.2% (95% CI, 85.2-100%) positive predictive value for pCR, a 93.6% (95% CI, 87.8-99.4%) negative predictive value, a sensitivity of 88.1% (95% CI, 76.8-99.4%), and a specificity of 95.9% (91.1% CI, 87.8-100%). Our results suggest that several ABC transporters in human breast cancer cells may affect the clinical response to neoadjuvant chemotherapy, and transcriptional profiling of these genes may be useful to predict the pathologic response to sequential weekly paclitaxel/FEC in breast cancer patients.

Perez-Plasencia, C. and A. Duenas-Gonzalez (2006). "Can the state of cancer chemotherapy resistance be reverted by epigenetic therapy?" *Mol Cancer* 5: 27.

Transcriptome analysis shows that the chemotherapy innate resistance state of tumors is characterized by: poorly dividing tumor cells; an increased DNA repair; an increased drug efflux potential by ABC-transporters; and a dysfunctional ECM. Because chemotherapy resistance involves multiple genes, epigenetic-mediated changes could be the main force responsible of this phenotype. Our hypothesis deals with the potential role of epigenetic therapy for affecting the chemotherapy resistant phenotype of malignant tumors. PRESENTATION OF THE HYPOTHESIS: Recent studies reveal the involvement of DNA methylation and histone modifications in the reprogramming of the genome of mammalian cells in cancer. In this sense, it can be hypothesized that epigenetic reprogramming can participate in the establishment of an epigenetic mark associated with the chemotherapy resistant phenotype. If this were correct, then it could be expected that agents targeting DNA methylation and histone deacetylation would by reverting the epigenetic mark induce a global expression profile that mirror the observed in untreated resistant cells. TESTING THE

HYPOTHESIS: It is proposed to perform a detailed analysis using all the available databases where the gene expression of primary tumors was analyzed and data correlated with the therapeutic outcome to determine whether a transcriptome profiling of "resistance" is observed. Assuming an epigenetic programming determines at some level the intrinsic resistant phenotype, then a similar pattern of gene expression dictated by an epigenetic mark should also be found in cell acquiring drug resistance. If these expectations are met, then it should be further investigated at the genomic level whether these phenotypes are associated to certain patterns of DNA methylation and chromatin modification. Once confirmed the existence of an epigenetic mark associated to either the intrinsic or acquired chemotherapy resistant phenotype, then a causal association should be investigated. These preclinical findings should also be tested in a clinical setting. IMPLICATIONS OF THE HYPOTHESIS: Our hypothesis on the ability of epigenetic therapy to revert the epigenetic changes leading to a transcriptome profile that defines the resistant state will eventually be a more rational and effective way to treat malignant tumors.

Piccart-Gebhart, M. J. and C. Sotiriou (2007). "Adjuvant chemotherapy--yes or no? Prognostic markers in early breast cancer." *Ann Oncol* 18 Suppl 12: xii2-7.

The question of whether to offer adjuvant chemotherapy to patients with early-stage breast cancer continues to challenge clinicians on a daily basis. Many patients with node-negative or low-risk breast cancer could be spared the trauma of receiving chemotherapy, but more reliable prognostic markers are still needed to aid our therapy decision making. Gene expression profiling is a welcome product of the 'omic' era and several multi-gene expression panels ('signatures') have now been developed that show promise in predicting which breast cancer patients are likely to develop metastatic disease if adjuvant chemotherapy is not administered. The value of gene expression profiling as a prognostic tool in clinical practice is currently being appraised more fully in two large, prospective, randomised studies--TAILORx and MINDACT. These studies should provide level I evidence of the prognostic power of gene expression profiling and will hopefully allow us to one day quantify risk of progression in the individual patient and tailor treatment accordingly. Genetic profiling of circulating tumour cells and micrometastases should further enhance our understanding of breast cancer biology and allow us to personalise therapy based on functional maps of critical tumour pathways. Close

collaboration between clinicians and scientists will be essential to achieve this goal.

Porter, P. L., W. E. Barlow, et al. (2006). "p27(Kip1) and cyclin E expression and breast cancer survival after treatment with adjuvant chemotherapy." *J Natl Cancer Inst* **98**(23): 1723-31.

Abnormal expression of the cell cycle regulatory proteins p27(Kip1) (p27) and cyclin E may be associated with breast cancer survival and relapse. We studied these markers in a clinical trial setting with patients with breast cancer treated by a uniform drug regimen so that treatment was not associated with variability in outcome. We used tissue microarrays to evaluate the expression of p27 and cyclin E proteins by immunohistochemistry in tumor tissue from 2123 (68%) of 3122 patients with moderate-risk primary breast cancer who were enrolled in Southwest Oncology Group-Intergroup Trial S9313, in which patients were assigned to receive doxorubicin and cyclophosphamide administered concurrently (n = 1595) or sequentially (n = 1527). Disease-free and overall survival were equivalent in the two arms. Expression of the proteins was rated on a scale of 1-7, and the median value was used as the cut point. Log-rank tests and Cox regression analyses were used to assess associations with survival. Overall survival was defined as time to death from all causes; disease-free survival was defined as time to recurrence or death. All P values were from two-sided statistical tests. Lower p27 expression was associated with worse overall survival (unadjusted hazard ratio [HR] = 1.50, 95% confidence interval [CI] = 1.21 to 1.86) and disease-free survival (unadjusted HR = 1.31, 95% CI = 1.10 to 1.57) than higher p27 expression. Among hormone receptor-positive patients, lower p27 expression was associated with worse overall survival (HR = 1.42, 95% CI = 1.05 to 1.94) and worse disease-free survival (HR = 1.27, 95% CI = 0.99 to 1.63) than higher p27 expression after adjustment for treatment, menopausal status, tumor size, and number of positive lymph nodes. Among these patients, 5-year overall survival associated with higher p27 expression (0.91, 95% CI = 0.89 to 0.93) was similar to that associated with lower p27 expression (0.85, 95% CI = 0.82 to 0.87). No association between p27 expression and survival was found in hormone receptor-negative patients. Cyclin E expression was not statistically significantly associated with overall survival (HR = 1.12, 95% CI = 0.91 to 1.38) or disease-free survival (HR = 1.09, 95% CI = 0.92 to 1.29). CONCLUSIONS: Low p27 expression appears to be associated with poor prognosis, especially among patients with steroid receptor-positive tumors.

Potti, A. and A. K. Ganti (2006). "Adjuvant chemotherapy for early-stage non-small cell lung cancer: the past, the present and the future." *Expert Opin Biol Ther* **6**(7): 709-16.

Complete resection is mandatory in order to achieve a cure in patients with early-stage non-small cell lung cancer (NSCLC). However, despite complete resection, a substantial proportion of patients have disease recurrence, with distant metastases being the primary sites of failure. Recent trials have conclusively demonstrated the benefit of platinum-based adjuvant therapy in patients with resected stage IB and II NSCLC. The role of adjuvant chemotherapy in resected stage III NSCLC is less clear, with trials showing conflicting results. The role of targeted agents in this setting is being investigated. Gene expression profiling studies should help direct chemotherapy to those who would actually benefit from it, thereby saving others from unnecessary toxicity.

Pritchard, K. I., L. E. Shepherd, et al. (2006). "HER2 and responsiveness of breast cancer to adjuvant chemotherapy." *N Engl J Med* **354**(20): 2103-11.

Amplification of the human epidermal growth factor receptor type 2 (HER2, also called HER2/neu) gene and overexpression of its product in breast-cancer cells may be associated with responsiveness to anthracycline-containing chemotherapy regimens. In the randomized, controlled Mammary.5 trial, we studied 639 formalin-fixed paraffin-embedded specimens obtained from 710 premenopausal women with node-positive breast cancer who had received either cyclophosphamide, epirubicin, and fluorouracil (CEF) or cyclophosphamide, methotrexate, and fluorouracil (CMF) as adjuvant chemotherapy. HER2 amplification or overexpression was evaluated with the use of fluorescence in situ hybridization, immunohistochemical analysis, and polymerase-chain-reaction analysis. Amplification of HER2 was associated with a poor prognosis regardless of the type of treatment. In patients whose tumors showed amplification of HER2, CEF was superior to CMF when assessed on the basis of relapse-free survival (hazard ratio, 0.52; 95 percent confidence interval, 0.34 to 0.80; P=0.003) and overall survival (hazard ratio, 0.65; 95 percent confidence interval, 0.42 to 1.02; P=0.06). For women whose tumors lacked amplification of HER2, CEF did not improve relapse-free survival (hazard ratio for relapse, 0.91; 95 percent confidence interval, 0.71 to 1.18; P=0.49) or overall survival (hazard ratio for death, 1.06; 95 percent confidence interval, 0.83 to 1.44; P=0.68). The adjusted hazard ratio for the interaction between treatment and HER2 amplification was 1.96 for

relapse-free survival (95 percent confidence interval, 1.15 to 3.36;  $P=0.01$ ) and 2.04 for overall survival (95 percent confidence interval, 1.14 to 3.65;  $P=0.02$ ). CONCLUSIONS: Amplification of HER2 in breast-cancer cells is associated with clinical responsiveness to anthracycline-containing chemotherapy. (cancer.gov number, NCI-V90-0027.).

Quinn, J. E., J. E. Carser, et al. (2009). "BRCA1 and implications for response to chemotherapy in ovarian cancer." *Gynecol Oncol* **113**(1): 134-42.

OBJECTIVES: Treatment of epithelial ovarian cancer (EOC) remains a challenge, despite advances in surgery and chemotherapy. Hereditary ovarian cancer is primarily due to germline mutations in the BRCA1 tumour suppressor gene. In addition, sporadic EOC tumours display significant loss of BRCA1 function due to epigenetic inactivation of the BRCA1 gene. This article reviews the preclinical and clinical evidence to support a role for BRCA1 as a potential predictive biomarker of response to both platinum and taxane based chemotherapy in EOC. We conducted a Medline and Pubmed search for reports between 1990 and 2008 using the search terms: BRCA1 and hereditary ovarian cancer, BRCA1 and sporadic ovarian cancer, ovarian cancer and chemotherapy, ovarian cancer and taxanes, ovarian cancer and platinum, ovarian cancer and clinical response, BRCA1 and DNA damage, BRCA1 and DNA repair, BRCA1 and mitotic checkpoint. If reports identified by these criteria referred to other papers not in the initial search, then these were also reviewed if relevant to BRCA1 and ovarian cancer. The BRCA1 pathway plays a significant role in the development of both hereditary and sporadic EOC. Evidence suggests that BRCA1 is a potential biomarker of response to platinum chemotherapy in EOC with BRCA1 deficiency predicting for enhanced response. In contrast, initial evidence suggests that loss of BRCA1 function results in reduced response to antimicrotubule-based chemotherapy. The ability of BRCA1 to differentially modulate response to these agents involves loss of BRCA1 mediated DNA repair and mitotic checkpoint control, respectively. CONCLUSIONS: Standard first line treatment of EOC consists of a combination of platinum and taxane chemotherapy, however clinically useful biomarkers for predicting response to these agents have yet to be established. BRCA1 may prove useful as a biomarker in EOC for assigning chemotherapy treatments based on the presence or absence of BRCA1 function.

Quintela-Fandino, M., J. M. Lopez, et al. (2006). "Breast cancer-specific mRNA transcripts presence in peripheral blood after adjuvant chemotherapy predicts poor survival among high-risk breast cancer patients

treated with high-dose chemotherapy with peripheral blood stem cell support." *J Clin Oncol* **24**(22): 3611-8.

To study the prognostic significance of the presence of breast cancer-specific mRNA transcripts in peripheral blood (PB), defined by serial analysis of gene expression, in high-risk breast cancer (HRBC) patients undergoing high-dose chemotherapy after receiving adjuvant chemotherapy. From 1994 to 2000, 84 HRBC patients (median age, 44 years; > 10 nodes; 74%) received adjuvant chemotherapy (fluorouracil, epirubicin, and cyclophosphamide for six cycles [83%] or doxorubicin and cyclophosphamide followed by paclitaxel) before undergoing one course of cyclophosphamide plus thiotepa plus carboplatin (STAMP V). Radiotherapy or hormone therapy was administered whenever indicated. Aliquots of apheresis-mononuclear blood cells were frozen from each patient. mRNA was isolated using an automatic nucleic acid extractor based on the magnetic beads technology; reverse transcription was performed using random hexamers. Cytokeratin 19, HER-2, P1B, PS2, and EGP2 transcripts were quantified to B-glucuronidase by real-time polymerase chain reaction (RT-PCR) using a linear DNA probe marked with a quencher and reporter fluorophores used in RT-PCR. Presence of PB micrometastases, estrogen receptor and progesterone receptor status, tumor size, age, tumor grade, number of nodes affected, and treatment with paclitaxel were included in the statistical analysis. Median follow-up was 68.3 months (range, 6 months to 103 months). Forty-seven relapses (56%) and 35 deaths (41.7%) were registered. Both tumor size and presence of micrometastases reached statistical significance according to the Cox multivariate model. Relapse hazard ratio (HR) for those patients with PB micrometastases was 269% ( $P = .006$ ); death HR, 300% ( $P = .011$ ). Time relapse was 53 months longer for patients without micrometastases: 31.3 v 84.2 months ( $P = .021$ ). PB micrometastases presence after adjuvant chemotherapy predicts both relapse and death more powerful than classical factors in HRBC patients undergoing high-dose chemotherapy. Micrometastases search using a gene panel appears to be a more accurate procedure than classical approaches involving only one or two genes.

Raguz, S., R. A. Randle, et al. (2008). "Production of P-glycoprotein from the MDR1 upstream promoter is insufficient to affect the response to first-line chemotherapy in advanced breast cancer." *Int J Cancer* **122**(5): 1058-67.

Multidrug resistance, the phenomenon by which cells treated with a drug become resistant to the cytotoxic effect of a variety of other structurally and functionally unrelated drugs, is often associated with

the expression of P-glycoprotein, an efflux membrane pump coded by the MDR1 (ABCB1) gene. Transcription from MDR1 can start at 2 promoters: a well-characterized downstream promoter and an as yet uncharacterized upstream promoter (USP). We have previously determined that the USP is activated in some drug-resistant cell lines, in primary breast tumors and in metastatic epithelial cells isolated from the lymph nodes of breast cancer patients. In this study, we report the cloning and characterization of the MDR1 USP and studied its association with chemotherapy response in breast cancer patients. Deletion analysis indicated that a nearby endogenous retroviral long terminal repeat is not responsible for promoter activation, and that the region within the first 400 nucleotides upstream from the transcription start point contained all the elements necessary for promoter activity in drug-resistant cells. We identified an element recognized by the transcription factor NF-IL6 (activated upon interleukin-6 exposure) which is necessary for promoter activity in drug-resistant cells and plays a role in the activation of the promoter in response to interleukin-6 in breast cancer MCF-7 cells. Although transcripts from this promoter are associated with translating polyribosomes, their low abundance makes the amount of synthesized P-glycoprotein insufficient to affect the response to first-line chemotherapy in patients with advanced breast cancer.

Raki, M., M. Sarkioja, et al. (2008). "Oncolytic adenovirus Ad5/3-delta24 and chemotherapy for treatment of orthotopic ovarian cancer." *Gynecol Oncol* **108**(1): 166-72.

Oncolytic adenoviruses capable of replication selectively in tumor cells are an appealing approach for the treatment of neoplastic diseases refractory to conventional therapies. The aim of this study was to evaluate the effect of dose and scheduling of a tropism-modified, adenovirus serotype 3 receptor-targeted, Rb/p16 pathway-selective replication-competent adenovirus, Ad5/3-delta24, against human ovarian adenocarcinoma. As oncolytic viruses and chemotherapy can have synergistic interactions, the antitumor efficacy of Ad5/3-delta24 was also studied in combination with epirubicin and gemcitabine, common second-line treatment options for platinum-resistant ovarian cancer. Orthotopic murine models of peritoneally disseminated ovarian cancer were utilized to compare survival of mice treated with either a single viral dose or weekly delivery. The lowest effective dose of intraperitoneal Ad5/3-delta24 was determined. Combinations of Ad5/3-delta24 and gemcitabine or epirubicin were studied in vitro as well as in vivo. Treatment outcome after administration of a single dose of Ad5/3-delta24 was as effective as

delivery of several weekly doses. Our results also demonstrate that a single intraperitoneal injection of 100 viral particles significantly increased the survival of mice compared to untreated animals. Further, combining Ad5/3-delta24 with either gemcitabine or epirubicin resulted in greater therapeutic benefit than either agent alone. These preclinical data suggest that Ad5/3-delta24 represents a promising treatment strategy for advanced ovarian cancer as a single agent or in combination with chemotherapy.

Redondo, M., T. Tellez, et al. (2007). "Anticlusterin treatment of breast cancer cells increases the sensitivities of chemotherapy and tamoxifen and counteracts the inhibitory action of dexamethasone on chemotherapy-induced cytotoxicity." *Breast Cancer Res* **9**(6): R86.

INTRODUCTION: Overexpression of the apoptosis-related protein clusterin is associated with breast cancer development and tumor progression. We describe the use of clusterin-specific antisense oligonucleotides and antibodies to sensitize breast carcinoma cells to anticancer drugs routinely used in breast cancer therapy. MCF-7 and MDA-MB-231 cells were treated with the oligonucleotide or antibody, chemotherapeutic agents (doxorubicin or paclitaxel), tamoxifen, or with combinations of these. Treatments that include antisense clusterin oligonucleotide or antibody to clusterin have been shown to reduce the number of viable cells more effectively than treatment with the drugs alone. We also demonstrate that dexamethasone pretreatment of breast cancer cell lines inhibits chemotherapy-induced cytotoxicity and is associated with the transcriptional induction of clusterin. However, anticlusterin treatment increases chemotherapy-induced cytotoxicity, even in the presence of glucocorticoids, suggesting a possible role for these proteins in glucocorticoid-mediated survival. These data suggest that combined treatment with antibodies to clusterin or antisense clusterin oligodeoxynucleotides and paclitaxel, doxorubicin, or tamoxifen could be a novel and attractive strategy to inhibit the progression of breast carcinoma by regulation of the clusterin function. Moreover, glucocorticoid activation in breast cancer cells regulates survival signaling by the direct transactivation of genes like clusterin which encode proteins that decrease susceptibility to apoptosis. Given the widespread clinical administration of dexamethasone before chemotherapy, understanding glucocorticoid-induced survival mechanisms is essential for achieving optimal therapeutic responses.

Rejiba, S., S. Wack, et al. (2007). "K-ras oncogene silencing strategy reduces tumor growth and enhances

gemcitabine chemotherapy efficacy for pancreatic cancer treatment." *Cancer Sci* **98**(7): 1128-36.

Pancreatic adenocarcinoma remains a fatal disease characterized by rapid tumor progression, high metastatic potential and profound chemoresistance. Gemcitabine is the current standard chemotherapy for advanced pancreatic cancer, but it is still far from optimal and novel therapeutic strategies are needed urgently. Mutations in the k-ras gene have been found in more than 90% of pancreatic cancers and are believed to play a key role in this malignancy. Thus, the goal of this study was to investigate the impact of k-ras oncogene silencing on pancreatic tumor growth. Additionally, we examined whether combining k-ras small interfering RNA (siRNA) with gemcitabine has therapeutic potential for pancreatic cancer. The treatment of tumor cell cultures with the corresponding k-ras siRNA resulted in a significant inhibition of k-ras endogenous expression and cell proliferation. In vivo, tumor xenografts were significantly reduced with k-ras siRNA(GAT) delivered by electroporation. Moreover, combined treatment with pSsik-ras(GAT) plus gemcitabine resulted in strong growth inhibition of orthotopic pancreatic tumors. Survival rate was significantly prolonged and the mean tumor volume was dramatically reduced in mice receiving the combined treatment compared with single agents. Collectively, these findings show that targeting mutant k-ras through specific siRNA might be effective for k-ras oncogene silencing and tumor growth inhibition. The improvement of gemcitabine-based chemotherapy suggests that this strategy might be used therapeutically against human pancreatic cancer to potentiate the effects of conventional therapy.

Rody, A., T. Karn, et al. (2006). "Gene expression profiles of breast cancer obtained from core cut biopsies before neoadjuvant docetaxel, adriamycin, and cyclophosphamide chemotherapy correlate with routine prognostic markers and could be used to identify predictive signatures." *Zentralbl Gynakol* **128**(2): 76-81.

Neoadjuvant administration of chemotherapy provides a unique opportunity to monitor response to treatment in breast cancer and assesses response exactly. Global gene expression profiling by microarrays has been used as a valuable tool for the identification of prognostic and predictive marker genes. Even though this technology is now wide spread and relatively standardized, there are only few data available which compare established parameters with expression values to determine reliability of this method. Therefore we analyzed gene expression data of pretreatment biopsies of breast cancer patients and compared them with the results of the

immunohistochemical receptor expression for ER/ PR and Her-2, as well as FISH testing for HER-2 amplification. We analyzed the change of expression of these markers before and after neoadjuvant chemotherapy. Furthermore we evaluated the predictive significance of prognostic gene signatures as described by Sorlie, van't Veer and Ahr for response to neoadjuvant chemotherapy. Pretherapeutic core biopsies were obtained from 70 patients undergoing neoadjuvant TAC chemotherapy within the GEPARTRIO-trial. Samples were characterized according to standard pathology including ER, PR and HER2 IHC and amount of cancer cells. Only biopsies with more than 80 % tumor cells were considered for further examination. RNA was isolated and expression profiling performed using Affymetrix Hg U133 Arrays (22 500 genes). GeneData's Expressionist software was used for bioinformatic analyses. More than two thirds of the biopsies yielded sufficient amounts (> 5 microg) of RNA for expression profiling and high quality data were obtained for 50 samples. Unsupervised clustering broadly revealed a correlation with hormone receptor status. When ER-alpha, PR and HER2 as analyzed by immunohistochemistry were compared to the corresponding mRNA data from gene chips more than 90 % concordance was observed. We could observe a switch of receptor expression for ER, PR or HER-2 from positive to negative and vice versa in 16/35 cases (45.7 %) and 5/22 cases (22.7 %) respectively. The prognostic marker sets of Sorlie, van't Veer and Ahr could not discriminate responders from non-responders in our patient group. CONCLUSIONS: Our results demonstrate that reliable expression profiles can be achieved by using limited amounts of tissue obtained during neoadjuvant chemotherapy. Microarray data capture conventional prognostic markers but might contain additional informative gene sets correlated with treatment outcome. Prognostic marker sets are not suitable to predict tumor response in the neoadjuvant setting, suggesting the necessity of class prediction methods to identify marker sets predictive for the type of therapy used.

Rogers, D., K. D. Nylander, et al. (2008). "Molecular predictors of human nervous system cancer responsiveness to enediyne chemotherapy." *Cancer Chemother Pharmacol* **62**(4): 699-706.

To identify and mathematically model molecular predictors of response to the enediyne chemotherapeutic agent, neocarzinostatin, in nervous system cancer cell lines. Human neuroblastoma, breast cancer, glioma, and medulloblastoma cell lines were maintained in culture. Content of caspase-3 and Bcl-2, respectively, was determined relative to actin content for each cell line by Western blotting and optical

densitometry. For each cell line, sensitivity to neocarzinostatin was determined. Brain tumor cell lines were stably transfected with human Bcl-2 cDNA cloned into the pcDNA3 plasmid vector. In human tumor cell lines of different tissue origins, sensitivity to neocarzinostatin is proportional to the product of the relative contents of Bcl-2 and caspase-3 ( $r(2) = 0.9$ ;  $P < 0.01$ ). Neuroblastoma and brain tumor cell lines are particularly sensitive to neocarzinostatin; the sensitivity of brain tumor lines to neocarzinostatin is enhanced by transfection with an expression construct for Bcl-2 and is proportional in transfected cells to the product of the relative contents of Bcl-2 and caspase-3 ( $r(2) = 0.7$ ). These studies underscore the potential of molecular profiling in identifying effective chemotherapeutic paradigms for cancer in general and tumors of the nervous system in particular.

Rogers, K. M., M. Thomas, et al. (2007). "Cellular FLICE-inhibitory protein regulates chemotherapy-induced apoptosis in breast cancer cells." *Mol Cancer Ther* 6(5): 1544-51.

Combination treatment regimens that include topoisomerase-II-targeted drugs, such as doxorubicin, are widely used in the treatment of breast cancer. Previously, we showed that IFN-gamma and doxorubicin cotreatment synergistically induced apoptosis in MDA435 breast cancer cells in a signal transducer and activator of transcription 1-dependent manner. In this study, we found that this synergy was caspase-8 dependent. In addition, we found that IFN-gamma down-regulated the expression of the caspase-8 inhibitor cellular FLICE-inhibitory protein (c-FLIP). Furthermore, IFN-gamma down-regulated c-FLIP in a manner that was dependent on the transcription factors signal transducer and activator of transcription 1 and IFN regulatory factor-1. However, IFN-gamma had no effect on c-FLIP mRNA levels, indicating that c-FLIP was down-regulated at a posttranscriptional level following IFN-gamma treatment. Characterization of the functional significance of c-FLIP modulation by small interfering RNA gene silencing and stable overexpression studies revealed it to be a key regulator of IFN-gamma- and doxorubicin-induced apoptosis in MDA435 cells. Analysis of a panel of breast cancer cell lines indicated that c-FLIP was an important general determinant of doxorubicin- and IFN-gamma-induced apoptosis in breast cancer cells. Furthermore, c-FLIP gene silencing sensitized MDA435 cells to other chemotherapies, including etoposide, mitoxantrone, and SN-38. These results suggest that c-FLIP plays a pivotal role in modulating drug-induced apoptosis in breast cancer cells.

Rom, J., G. von Minckwitz, et al. (2009). "Phase I study of apoptosis gene modulation with oblimersen

within preoperative chemotherapy in patients with primary breast cancer." *Ann Oncol* 20(11): 1829-35.

Expression of the Bcl-2 protein confers resistance to chemotherapy-mediated apoptotic signals in patients with breast cancer. We investigated effects of Bcl-2 down-regulation by the Bcl-2 antisense oligodeoxynucleotide oblimersen in breast tumor biopsies. Oblimersen targets Bcl-2 messenger RNA (mRNA), down-regulates Bcl-2 protein translation and enhances antitumor effects of subtherapeutic chemotherapy doses. Within a phase I trial, we administered escalating doses of oblimersen (3, 5 or 7 mg/kg/day) as continuous infusion on days 1-7 in combination with standard-dose docetaxel (Taxotere), Adriamycin and cyclophosphamide (TAC) on day 5 as preoperative chemotherapy in 28 patients with T2-4 tumors. Effects of oblimersen were evaluated in tumor biopsies and peripheral blood mononuclear cells (PBMCs) 4 days after start of oblimersen and before TAC treatment by quantitative microfluidic real-time PCR. Read-outs consisted in measurement of Bcl-2 mRNA modulations and of 18 putative predictive markers. Two of 13 patients showed a diminution of Bcl-2 transcripts after 4 days of treatment with oblimersen 5 mg/kg/day. PBMCs could not be evaluated as a surrogate tissue because no qualified RNA could be isolated. Nevertheless, we demonstrated feasibility to process clinical samples and to obtain good quality RNA from tumor biopsies and indicated the potential of oblimersen to lower Bcl-2 mRNA in breast cancer.

Rosell, R., F. Cecere, et al. (2006). "Predicting the outcome of chemotherapy for lung cancer." *Curr Opin Pharmacol* 6(4): 323-31.

Lung cancer is a worldwide problem. At the time of diagnosis, 50% of patients have advanced incurable disease. Different chemotherapy combinations--with or without targeted therapies--yield similar results despite the continuous efforts of clinicians. However, molecular biological studies have already shed a great deal of light on the existence of multiple genetic aberrations that can be useful for customizing treatment. mRNA transcripts involved in DNA repair pathways, such as ERCC1 and BRCA1, confer selective resistance to cisplatin or taxanes, whereas thioredoxin confers a broad spectrum of chemoresistance. Polymorphisms in DNA repair genes and methylation of checkpoint genes in circulating serum DNA could become important predictive markers of survival in certain cisplatin-based regimens. Epidermal growth factor receptor tyrosine kinase mutations are the crux of targeted therapies, whereas epithelial-mesenchymal transitions and HER3 mRNA levels are promising ancillary markers for

treatment with epidermal growth factor receptor tyrosine kinase inhibitors.

Rosell, R., C. Manegold, et al. (2008). "Can we customize chemotherapy? Individualizing cytotoxic regimens in advanced non-small-cell lung cancer." *Clin Lung Cancer* **9 Suppl 2**: S76-82.

Metastatic non-small-cell lung cancer remains a fatal disease with a median survival of < 1 year. A critical challenge is to develop predictive markers for customizing platinum-based treatment. The first studies focused on the excision repair cross-complementing 1 (ERCC1) gene in this difficult task. Several layers of evidence indicate that ERCC1 mRNA expression could be a predictive marker for cisplatin alone or in combination with certain drugs such as etoposide, gemcitabine, and 5-fluorouracil but not in combination with antimicrotubule drugs. Several retrospective studies demonstrated an impressive survival advantage for gemcitabine plus cisplatin but not for other combinations in tumors with low ERCC1 expression. A customized phase III ERCC1-based trial met the primary endpoint of improvement in response but not in survival, leading us to hypothesize that docetaxel might not be the most appropriate partner for cisplatin in the presence of low ERCC1 levels or for gemcitabine in the presence of high ERCC1 levels. A phase II study demonstrated the feasibility of combining carboplatin, gemcitabine, docetaxel, and vinorelbine according to ERCC1 and ribonucleotide reductase subunit M1 expression levels. These findings highlight the importance of continual learning, and decision-making strategies for customizing treatment should reflect the limitations of our knowledge.

Rottenberg, S., A. O. Nygren, et al. (2007). "Selective induction of chemotherapy resistance of mammary tumors in a conditional mouse model for hereditary breast cancer." *Proc Natl Acad Sci U S A* **104**(29): 12117-22.

We have studied in vivo responses of "spontaneous" Brca1- and p53-deficient mammary tumors arising in conditional mouse mutants to treatment with doxorubicin, docetaxel, or cisplatin. Like human tumors, the response of individual mouse tumors varies, but eventually they all become resistant to the maximum tolerable dose of doxorubicin or docetaxel. The tumors also respond well to cisplatin but do not become resistant, even after multiple treatments in which tumors appear to regrow from a small fraction of surviving cells. Classical biochemical resistance mechanisms, such as up-regulated drug transporters, appear to be responsible for doxorubicin resistance, rather than alterations in drug-damage effector pathways. Our results underline

the promise of these mouse tumors for the study of tumor-initiating cells and of drug therapy of human cancer.

Saad, A. F., W. D. Meacham, et al. (2007). "The functional effects of acid ceramidase overexpression in prostate cancer progression and resistance to chemotherapy." *Cancer Biol Ther* **6**(9): 1455-60.

Among the many processes regulating cell death, ceramide signaling is a vital component. We previously determined that acid ceramidase (AC) is upregulated in 60% of primary prostate cancer (PCa) tissues, suggesting that AC may play a role in tumor development. In order to determine the significance of AC elevation, stable clones of DU145 cells with AC overexpression (AC-EGFP) were generated. Compared to controls (EGFP), AC-EGFP cells exhibited enhanced cell proliferation and migration. Subcutaneous injection of AC-EGFP cells into Nu/Nu mice resulted in larger tumor volumes compared to EGFP controls. Moreover, using the MTS viability assay, AC-EGFP cells were more resistant to cell death induced by doxorubicin, cisplatin, etoposide, gemcitabine or C6-ceramide. Conversely, knock down of AC using siRNA, sensitized AC-EGFP cells to these drugs. In addition, mass spectroscopic analysis of sphingolipids indicated that long chain ceramide levels were decreased in AC-EGFP cells treated with either doxorubicin or etoposide. In conclusion, this study implicates AC as a critical regulator of PCa progression by affecting not only tumor cell proliferation and migration but also responses to drug therapy, suggesting AC as a potential therapeutic target in advanced PCa.

Safra, T. (2009). "Hereditary ovarian cancer: biology, response to chemotherapy and prognosis." *Womens Health (Lond Engl)* **5**(5): 543-53.

Recent evidence has indicated that the prognosis of women with epithelial ovarian cancer who are BRCA-mutation carriers may be better than for noncarriers. Part of the explanation is a higher sensitivity to platinum and other chemotherapies, as was demonstrated in in vitro studies, as well as a possible different biology. BRCA genes are important in double-strand DNA break repair and in other important processes of the cell cycle. Mutation or reduced activity of BRCA genes leads to a higher vulnerability to DNA damage (caused by chemotherapy and radiotherapy) compared with malignant tumors of noncarriers. New targeted drugs, such as poly (ADP-ribose) polymerase-1 and -2 inhibitors, are currently under investigation, as are new biomarkers that will hopefully lead the way to better treatment and longer survival. Testing for the BRCA mutation should be carried out and used as a

guide for therapy in most patients with epithelial ovarian cancer.

Saito, K., K. Khan, et al. (2009). "The predictive and therapeutic value of thymidine phosphorylase and dihydropyrimidine dehydrogenase in capecitabine (Xeloda)-based chemotherapy for head and neck cancer." *Laryngoscope* **119**(1): 82-8.

**OBJECTIVES:** To evaluate whether two molecular biomarkers, thymidine phosphorylase (TP) and dihydropyrimidine dehydrogenase (DPD), could be clinically useful in predicting and improving the chemotherapeutic outcome of the oral fluoropyrimidine capecitabine (5'-DFUR or Xeloda), in the treatment of human head and neck squamous cell carcinoma (HNSCC). **EXPERIMENTAL DESIGN:** Quantitative reverse-transcriptase polymerase chain reaction was used to determine the TP and DPD expression levels in different HNSCC cell lines. The TP to DPD ratio was calculated and compared to the relative chemosensitivity between cell lines after treatment with 5'-DFUR. The effect of TP transgene expression to alter the TP to DPD ratio and hence optimize the therapeutic outcome of capecitabine treatment was further evaluated in a murine model of human HNSCC using immunohistochemistry to detect TP and DPD expression in vivo. No correlation was detected between sensitivity to 5'-DFUR and the relative expression levels of TP or DPD in the multiple HNSCC cell lines tested. However, significant correlation was observed between the TP to DPD ratio versus drug resistance of the HNSCC cells ( $r = -0.914$ ,  $p = 0.0281$ ). In addition, we demonstrate that transgene expression of TP significantly enhanced the tumoricidal effect of capecitabine in HNSCC tumors with otherwise low endogenous TP to DPD ratios. This antitumor effect was observed up to 30 days after treatment. **CONCLUSIONS:** The results of this study suggest that HNSCC patients who would most benefit from capecitabine-based chemotherapy could be identified by examining the TP to DPD ratio of their tumors. Furthermore, we demonstrate the potential role of TP gene therapy in TP to DPD ratio manipulation to optimize the tumoricidal effect of capecitabine.

Sato, K., Y. Kitajima, et al. (2007). "CPT-11 (SN-38) chemotherapy may be selectively applicable to biliary tract cancer with low hMLH1 expression." *Anticancer Res* **27**(2): 865-72.

Biliary tract cancer is of high malignancy with a poor 5-year survival. However, established chemotherapeutic regimens have not yet been established. Previously, we have reported that hMLH1, a mismatch repair (MMR) gene was

frequently (57%) found to be lacking in surgically resected biliary tract carcinomas and the patients lacking the expression of hMLH1 revealed a poorer prognosis than those patients who possessed it. The MMR gene has been considered to be associated with sensitivity to various chemotherapeutic agents that act on DNA. A loss of MMR expression has been reported to increase sensitivity to topoisomerase inhibitors such as etoposide (ETP) or camptothecins (CPT). In the present study, whether or not hMLH1 deficiency resulted in a higher sensitivity to irinotecan (CPT-11) active form (SN-38) was investigated using a short interfering (Si)RNA system. A quantitative reverse transcription-polymerase chain reaction (RT-PCR) was conducted to measure the levels of hMLH1 expression in seven cancer cell lines, and this was compared with the drug sensitivity (IC<sub>50</sub>) to SN-38. The hMLH1 expression was correlated with the IC<sub>50</sub> for SN-38, although the relationship was not statistically significant ( $R = 0.717$ ,  $p = 0.0715$ ). SiRNA double strand RNA (dsRNA) was transiently transfected into KMG-C (gallbladder cancer) cells. hMLH1 mRNA expression was repressed by hMLH1 dsRNA in a dose-dependent manner in comparison to the control dsRNA. The cell growth of the hMLH1 dsRNA transfected group was decreased by approximately 50% by SN-38 exposure. Flow cytometry was also carried out to examine the effect of the SN-38 treatment on the cell cycle. Following hMLH1 dsRNA transfection, the subG1 fraction was increased in comparison with the control in a dose-dependent manner. In conclusion, a low expression of hMLH1 in biliary tract cancer may aid in predicting its responsiveness to CPT-11 (SN38).

Schipper, M. L., M. L. Goris, et al. (2007). "Evaluation of herpes simplex virus 1 thymidine kinase-mediated trapping of (131)I FIAU and prodrug activation of ganciclovir as a synergistic cancer radio/chemotherapy." *Mol Imaging Biol* **9**(3): 110-6.

Evaluation of selective killing of Herpes Simplex Virus 1 thymidine kinase (HSV1-tk) expressing tumors by radiolabeled (131)I-fialuridine (FIAU), and of synergy between (131)I-FIAU and Ganciclovir (GCV). **PROCEDURES:** HSV1-tk-expressing cell lines and parental cell lines were exposed to (131)I-FIAU alone, GCV alone, or combinations. Activity and concentration were varied widely, concurrent and sequential administrations tested, and dose rate effects were studied. HSV1-tk-expressing cells accumulated up to 15.7-fold more (131)I-FIAU, were growth inhibited by 2  $\mu\text{Ci/ml}$ , or 5  $\mu\text{Ci/ml}$  (131)I-FIAU, and were inhibited by two log orders lower concentrations of GCV than parental cells. However, no synergy or additive effect was observed. Dose rate variations, or sequential

treatment, did not alter outcome. Radioisotope therapy of HSV1-tk-expressing tumor cells with (131)I-FIAU is reported for the first time. Lack of synergy between (131)I-FIAU and GCV does not warrant further investigation of combination treatment with the two agents.

Segura-Pacheco, B., E. Perez-Cardenas, et al. (2006). "Global DNA hypermethylation-associated cancer chemotherapy resistance and its reversion with the demethylating agent hydralazine." *J Transl Med* 4: 32.

The development of resistance to cytotoxic chemotherapy continues to be a major obstacle for successful anticancer therapy. It has been shown that cells exposed to toxic concentrations of commonly used cancer chemotherapy agents develop DNA hypermethylation. Hence, demethylating agents could play a role in overcoming drug resistance. MCF-7 cells were rendered adriamycin-resistant by weekly treatment with adriamycin. Wild-type and the resulting MCF-7/Adr cells were analyzed for global DNA methylation. DNA methyltransferase activity and DNA methyltransferase (dnmt) gene expression were also determined. MCF-7/Adr cells were then subjected to antisense targeting of dnmt1, -3a, and -b genes and to treatment with the DNA methylation inhibitor hydralazine to investigate whether DNA demethylation restores sensitivity to adriamycin. MCF-7/Adr cells exhibited the multi-drug resistant phenotype as demonstrated by adriamycin resistance, *mdr1* gene over-expression, decreased intracellular accumulation of adriamycin, and cross-resistance to paclitaxel. The *mdr* phenotype was accompanied by global DNA hypermethylation, over-expression of *dnmt* genes, and increased DNA methyltransferase activity as compared with wild-type MCF-7 cells. DNA demethylation through antisense targeting of *dnmts* or hydralazine restored adriamycin sensitivity of MCF-7/Adr cells to a greater extent than verapamil, a known inhibitor of *mdr* protein, suggesting that DNA demethylation interferes with the epigenetic reprogramming that participates in the drug-resistant phenotype. We provide evidence that DNA hypermethylation is at least partly responsible for development of the multidrug-resistant phenotype in the MCF-7/Adr model and that hydralazine, a known DNA demethylating agent, can revert the resistant phenotype.

Shanmugam, R., V. Jayaprakasan, et al. (2006). "Restoring chemotherapy and hormone therapy sensitivity by parthenolide in a xenograft hormone refractory prostate cancer model." *Prostate* 66(14): 1498-511.

Nuclear Factor kappa B (NFkappaB) is a eukaryotic transcription factor that is constitutively

active in human cancers and can be inhibited by the naturally occurring sesquiterpene lactone, parthenolide (P). The *in vitro* effects of P were assessed using the androgen independent cell line, CWR22Rv1, and human umbilical endothelial cells (HUVECs). The *in vivo* activity of P as a single agent and its ability to augment the efficacy of docetaxel and the anti-androgen, bicalutamide, were determined using the CWR22Rv1 xenograft model. Parthenolide at low micromolar concentration inhibited proliferation of CWR22Rv1 and HUVEC cells, promoted apoptosis and abrogated NFkappaB-DNA binding. Parthenolide downregulated anti-apoptotic genes under NFkappaB control, TRAF 1 and 2, and promoted sustained activation of c-jun-NH2 kinase (JNK). Parthenolide also augmented the *in vivo* efficacy of docetaxel and restored sensitivity to anti-androgen therapy. These studies demonstrate parthenolide's anti-tumor and anti-angiogenic activity, and its potential to augment the efficacy of chemotherapy and hormonal therapy.

Shibata, T., A. Kokubu, et al. (2008). "Genetic alteration of Keap1 confers constitutive Nrf2 activation and resistance to chemotherapy in gallbladder cancer." *Gastroenterology* 135(4): 1358-1368, 1368 e1-4.

**BACKGROUND & AIMS:** Biliary tract cancer (BTC) is a highly malignant tumor, and identification of effective therapeutic targets to improve prognosis is urgently required. Oncogenic activation of survival genes is important for cancer cells to overcome oxidative stresses induced by their microenvironments that include chronic inflammation or exposure to anticancer drugs. We attempted to examine whether deregulation of Nrf2, a master transcriptional factor of various cytoprotective genes against oxidative stress, plays a role in the carcinogenesis of BTC. We screened genetic alteration of Keap1, a negative regulator of Nrf2, in BTC including tumors originated from gallbladder and extra- and intrahepatic bile ducts. Functional analysis of cancer-related mutant Keap1 in Nrf2 repression and the association between Nrf2 activation and resistance to 5-fluorouracil (5-FU) were investigated. Recurrent (in 1/11 cell lines and 6/53 primary tumors) Keap1 gene alterations were observed in BTC and were especially frequent (4/13, 30.7%) in gallbladder cancer (GBC). These alterations led to a considerable loss of Nrf2 repression activity, caused constitutive activation of Nrf2, and promoted cell proliferation. Down-regulation of Nrf2 activity by either Keap1 complementation or Nrf2 short interference RNA increased sensitivity to 5-FU in Keap1-altered BTC cells. **CONCLUSIONS:** Keap1 mutation occurs frequently in GBC. Aberrant Nrf2 activation provoked

by Keap1 alteration is one of the molecular mechanisms for chemotherapeutic resistance in GBC and will be a novel therapeutic target as an enhancer of sensitivity to 5-FU-based regimens.

Shibata, T., A. Kokubu, et al. (2009). "Oncogenic mutation of PIK3CA in small cell lung carcinoma: a potential therapeutic target pathway for chemotherapy-resistant lung cancer." *Cancer Lett* **283**(2): 203-11.

Lung cancer is one of the most prevalent cancers worldwide. This study focused on small cell lung cancer (SCLC), which has a poor clinical prognosis, and attempted to elucidate potential therapeutic molecular targets. A target-specific mutational search revealed mutation of the PIK3CA gene in three of 13 SCLC cell lines and two of 15 primary SCLCs. By introducing these mutant PIK3CA cDNAs, we established artificial "PIK3CA-addicted" cells and found that Tricribine, a small-molecule inhibitor of AKT signaling that is located downstream from PIK3CA, significantly inhibited the growth and colony formation activity of these cells. Using cancer cell lines, we further showed that PIK3CA-mutated SCLC cells are more sensitive to Tricribine than PIK3CA wild-type cells. Additionally, we found that a cisplatin-resistant subclone of PIK3CA-mutant SCLC cells was equally sensitive to Tricribine. This study for the first time uncovered PIK3CA alterations in SCLC, and our findings suggest that anti-AKT molecular therapy could be effective for a subgroup of SCLC, which shows activation of specific genes, such as PIK3CA mutation, and that genetic stratification of SCLC according to the activation status of individual therapeutic target pathways could be clinically beneficial, especially for chemotherapy-resistant/relapsing tumors.

Simon, G. R. (2008). "Individualizing chemotherapy for non-small cell lung cancer (NSCLC) in the adjuvant and advanced setting: current status and future directions." *Curr Treat Options Oncol* **9**(4-6): 300-12.

Treatments based on molecular determinants are likely to be increasingly explored in the future. Future challenges will include ensuring that molecular determinants are identified as novel chemotherapeutic agents are being developed, enabling rationale therapeutics. Clinical trials will then enrich patients with the molecular determinants, potentially increasing response rates and survival in a specific group of patients who express this molecular determinant. It is hoped that this patient-specific 'individualized cocktails' will substantially improve response rates and survival.

Singh, A., S. Boldin-Adamsky, et al. (2008). "RNAi-mediated silencing of nuclear factor erythroid-2-related factor 2 gene expression in non-small cell lung cancer inhibits tumor growth and increases efficacy of chemotherapy." *Cancer Res* **68**(19): 7975-84.

Nuclear factor erythroid-2-related factor 2 (Nrf2) is a redox-sensitive transcription factor that regulates the expression of electrophile and xenobiotic detoxification enzymes and efflux proteins, which confer cytoprotection against oxidative stress and apoptosis in normal cells. Loss of function mutations in the Nrf2 inhibitor, Kelch-like ECH-associated protein (Keap1), results in constitutive activation of Nrf2 function in non-small cell lung cancer. In this study, we show that constitutive activation of Nrf2 in lung cancer cells promotes tumorigenicity and contributes to chemoresistance by up-regulation of glutathione, thioredoxin, and the drug efflux pathways involved in detoxification of electrophiles and broad spectrum of drugs. RNAi-mediated reduction of Nrf2 expression in lung cancer cells induces generation of reactive oxygen species, suppresses tumor growth, and results in increased sensitivity to chemotherapeutic drug-induced cell death in vitro and in vivo. Inhibiting Nrf2 expression using naked siRNA duplexes in combination with carboplatin significantly inhibits tumor growth in a subcutaneous model of lung cancer. Thus, targeting Nrf2 activity in lung cancers, particularly those with Keap1 mutations, could be a promising strategy to inhibit tumor growth and circumvent chemoresistance.

Sohn, J. W., S. Y. Lee, et al. (2006). "MDR1 polymorphisms predict the response to etoposide-cisplatin combination chemotherapy in small cell lung cancer." *Jpn J Clin Oncol* **36**(3): 137-41.

The MDR1 gene encodes P-glycoprotein (PGP), which plays an important role in mediating multidrug resistance to chemotherapeutic agents. Polymorphisms in the MDR1 gene may have an impact on the expression and function of PGP, thereby influencing the response to chemotherapy. We investigated the potential association of MDR1 polymorphisms (2677G>T at exon 21 and 3435C>T at exon 26) and their haplotypes with chemotherapy response in 54 small cell lung cancer (SCLC) patients who received a combination chemotherapy of etoposide-cisplatin. The 3435 CC genotype was associated with a significantly better chemotherapy response compared with the combined 3435 CT and TT genotype ( $P = 0.025$ ). The 2677 GG genotype was also associated with a better chemotherapy response compared with the combined 2677 GT and TT genotype, although it was not statistically significant. Consistent with the results of genotyping analyses, patients harboring the 2677G-3435C haplotype had a

statistically significant better response to chemotherapy compared with those with the other haplotypes combined ( $P = 0.015$ ). CONCLUSIONS: Our findings suggest that the MDR1 2677G>T and 3435C>T polymorphisms can be used for predicting treatment response to etoposide-cisplatin chemotherapy in SCLC patients.

Steffensen, K. D., M. Waldstrom, et al. (2008). "Prediction of response to chemotherapy by ERCC1 immunohistochemistry and ERCC1 polymorphism in ovarian cancer." *Int J Gynecol Cancer* **18**(4): 702-10.

The response of tumor cells to platinum-based chemotherapy involves DNA repair mechanisms. Excision repair cross-complementation group 1 (*ercc1*) is one of the leading genes involved in DNA repair, and several studies have linked *ercc1* to platinum resistance in cell lines and in human cancers. A common single nucleotide polymorphism (SNP) of *ercc1* at codon 118 has been proposed to impair *ercc1* translation and reduce ERCC1 protein expression and consequently influence the response to platinum-based chemotherapy. The primary aim of the present study was to evaluate ERCC1 expression and *ercc1* codon 118 polymorphism in epithelial ovarian cancer (EOC) and their possible predictive value in patients treated with platinum-based chemotherapy. Formalin-fixed, paraffin-embedded tissue sections from 159 patients with advanced EOC were used for immunohistochemistry. *Ercc1* codon 118 SNP genotyping was performed by real-time polymerase chain reaction. ERCC1 protein overexpression was found in 37.7% of the tumors. The CA-125 response rate was 94.5% (52/55) in patients with ERCC1-negative tumors compared to 80% (36/45) in patients with ERCC1-positive tumors ( $P = 0.026$ ,  $\chi^2$ ). The T/T genotype (44%) signalized a better response to chemotherapy than C/C (15%) + C/T (41%) variants ( $P = 0.045$ , trend test). Patients with ERCC1-negative tumors appear to have significantly better response to platinum-based chemotherapy compared to patients with ERCC1-positive tumors, but the differences in response rates did not translate into differences in survival. In addition, the TT genotype seems to be favorable toward better response to platinum-based chemotherapy.

Sun, S. H., M. Zheng, et al. (2008). "A small molecule that disrupts Mdm2-p53 binding activates p53, induces apoptosis and sensitizes lung cancer cells to chemotherapy." *Cancer Biol Ther* **7**(6): 845-52.

Mdm2 binds to p53 and promotes its degradation. A class of small molecule Mdm2 inhibitors (MI) was recently discovered that binds to Mdm2 and disrupts Mdm2-p53 binding. The efficacy of MI-43 was tested against two pairs of human lung

cancer lines differing in p53 status: adenocarcinoma A549 (p53 wild-type, wt) and H522 (p53-null) and non-small cell lung carcinoma H460 (p53wt) and H1299 (p53-null). MI-43 induced the accumulation of p53 and its downstream target genes, Mdm2, p21, Noxa and Puma only in wt p53-containing cells, indicating that disruption of Mdm2-p53 binding increases p53, which is transcriptionally active. MI-43 preferentially inhibited the growth of wt p53-containing cells in a p53 dependent manner, but was much less effective in p53-null cells. Mechanistically, MI-43 induced G(1) or G(2) arrest at low concentration as result of p21 induction, and apoptosis at high concentration due to Puma/Noxa induction. Importantly, MI-43 is much less toxic to normal fetal lung fibroblast, MRC5 cells. Finally, when used in combination, MI-43 sensitized chemo-resistant A549 cells to etoposide-induced apoptosis. Thus, MI-43 or its analogues could be further developed as a novel class of anticancer drug for lung cancer cells harboring wt p53 as a single agent or in combination with chemo-drugs.

Sunaga, N., Y. Tomizawa, et al. (2007). "Phase II prospective study of the efficacy of gefitinib for the treatment of stage III/IV non-small cell lung cancer with EGFR mutations, irrespective of previous chemotherapy." *Lung Cancer* **56**(3): 383-9.

Mutations in the epidermal growth factor receptor (EGFR) gene are associated with increased sensitivity of non-small cell lung cancer (NSCLC) to gefitinib, an EGFR tyrosine kinase inhibitor. The objective of this study was to prospectively evaluate the efficacy of gefitinib in patients with stage III/IV NSCLC whose tumors carried EGFR mutations, irrespective of previous chemotherapy. EXPERIMENTAL DESIGN: Genomic DNA was extracted from tumor specimens and EGFR mutations in exons 19 and 21 analyzed by direct sequencing. Patients with stage III/IV NSCLC whose tumors had the EGFR mutations received gefitinib (250 mg/day orally). Response, toxicity and survival data were assessed. RESULT: From November 2004-May 2006, 21 patients with EGFR mutations received gefitinib (median age: 59 years; 17 females; 19 non-smokers; all had adenocarcinomas). Two patients discontinued gefitinib and withdrew from the study 3 weeks after gefitinib initiation (interstitial pneumonitis, 1 patient; facial acne, 1 patient). Of 19 patients, 3 achieved complete response, 13 exhibited partial response and 3 had stable disease. Response and disease control rates were 76% (95% confidence interval [CI] 53-92) and 90% (95% CI 70-99), respectively. The most common adverse event was skin toxicity (67%); however, no grade 4 skin toxicities were seen. Ten patients relapsed and three died at a median follow-up period

of 12.6 months (range 5.6-23.8 months); median progression-free survival was 12.9 months. Analysis of tumor EGFR mutations in patients with NSCLC could be used to identify patients suitable for treatment with gefitinib to obtain optimum response and disease control rates.

Sussman, R. T., M. S. Ricci, et al. (2007). "Chemotherapy-resistant side-population of colon cancer cells has a higher sensitivity to TRAIL than the non-SP, a higher expression of c-Myc and TRAIL-receptor DR4." *Cancer Biol Ther* 6(9): 1490-5.

Cancer stem cells are resistant to chemotherapy and provide an important target for drug development. We found that, surprisingly, the dye-effluxing side population (SP) within SW480 human colon tumor cells, a population defined to possess stem cell characteristics, expresses a 10-fold higher level of pro-apoptotic TRAIL receptor DR4 as compared to non-SP cells. The TRAIL receptors are activated by the anti-tumor host immune system through the TRAIL ligand. SW480 SP-cells express similar levels of another TRAIL receptor (DR5), as non-SP cells. SP-cells from multiple tumorigenic human cell lines, which are most often resistant to chemotherapeutic agents such as etoposide, cisplatin and 5-FU, are more sensitive to TRAIL than non-SP cells. SP-cells express higher levels of c-Myc than non-SP cells which may explain their sensitivity to TRAIL. We have found c-Myc activates DR4 transcription through E-box DNA-response elements located in the DR4 promoter, thereby increasing the expression of cell-surface pro-apoptotic death receptors in TRAIL-resistant cell lines. TRAIL sensitivity of SP-cells may represent a safeguard against malignancy, and therefore, offers a therapeutic window and opportunity.

Taghizadeh, F., M. J. Tang, et al. (2007). "Synergism between vitamin D and secreted protein acidic and rich in cysteine-induced apoptosis and growth inhibition results in increased susceptibility of therapy-resistant colorectal cancer cells to chemotherapy." *Mol Cancer Ther* 6(1): 309-17.

Patients with advanced colorectal cancer continue to have poor outcomes because of therapy-refractory disease. We previously showed that secreted protein acidic and rich in cysteine (SPARC) gene and protein could function as a chemotherapy sensitizer by enhancing tumor regression in response to radiation and chemotherapy in tumor xenograft models of chemotherapy-resistant tumors. This function of SPARC was gleaned from a microarray analysis that also revealed down-regulation of the vitamin D receptor (VDR) in therapy-refractory colorectal cancer cells. This study examines the

potential synergistic effect of SPARC and vitamin D, which up-regulates VDR, in enhancing chemotherapy response in colorectal cancer. Using MIP101 colorectal cancer cell lines and SPARC-overexpressing MIP101 cells, we were able to show that, in the presence of SPARC, exposure to low doses of 1alpha,25-dihydroxyvitamin D(3) significantly reduces cell viability, enhances chemotherapy-induced apoptosis, and inhibits the growth of colorectal cancer cells. Moreover, in tumor xenograft mouse models, up-regulation of VDR was seen in tumors that had the greatest regression following treatment that combined SPARC with chemotherapy. Therefore, our findings reveal a synergistic effect between SPARC and low doses of 1alpha,25-dihydroxyvitamin D(3) that further augments the sensitivity of tumors to chemotherapy. This combination may prove to be a useful adjunct in the treatment of colorectal cancer, especially in those patients with therapy-refractory disease.

Takano, S., A. Togawa, et al. (2008). "Annexin II overexpression predicts rapid recurrence after surgery in pancreatic cancer patients undergoing gemcitabine-adjuvant chemotherapy." *Ann Surg Oncol* 15(11): 3157-68.

Gemcitabine has been shown to exhibit significant clinical activity against pancreatic cancer and has become a first-line chemotherapeutic for this disease in recent years. However, there are still many patients who do not respond to this treatment and it is expected to improve the clinical outcome if we can develop a method to predict the efficacy of gemcitabine before treatment. The purpose of this study was to determine novel factors that make pancreatic cancer resistant to gemcitabine. MATERIALS AND METHODS Using the high-resolution proteomic approach, agarose two-dimensional gel electrophoresis, we compared protein profiling of a gemcitabine-resistant pancreatic cancer cell line with its wild-type. We identified Annexin II as an up-regulated protein in the gemcitabine-resistant pancreatic cancer cell line. Immunohistochemistry demonstrated that Annexin II was mainly expressed at the cell surface of pancreatic cancer cells. Interestingly, Annexin II overexpression in cancer cells was significantly associated with rapid recurrence after gemcitabine adjuvant chemotherapy in postoperative patients ( $P = .0078$ ), and its staining was also an independent prognostic indicator of recurrence in pancreatic cancer patients who underwent adjuvant gemcitabine treatment after curative surgery on multivariate analysis ( $P = .0047$ ). In addition, inhibition of Annexin II expression by siRNA in pancreatic cancer cell lines increased the cytotoxic efficacy of gemcitabine. These results indicate that Annexin II overexpression may induce

gemcitabine resistance in pancreatic cancer resulting in rapid recurrence. CONCLUSIONS: Analysis of Annexin II expression in cancer tissues may predict the clinical outcome of gemcitabine treatment, leading to the development of a new method for tailor-made treatment for this disease.

Takara, K., T. Sakaeda, et al. (2006). "An update on overcoming MDR1-mediated multidrug resistance in cancer chemotherapy." *Curr Pharm Des* **12**(3): 273-86.

The intrinsic or acquired resistance to anticancer drugs remains one of the most significant factors impeding the progress of cancer chemotherapy. This phenomenon often involves simultaneous resistance to other anticancer drugs that differ in their chemical structure and mode of action and are not even used in chemotherapy. This phenotype has been called multidrug resistance (MDR). Although the cellular basis underlying MDR is not fully understood, several factors mediating therapy resistance in tumors have been proposed. One of the mechanisms leading to chemoresistance of tumor cells is the increased activity of transporter proteins. The best-characterized transporter protein is MDR1/P-glycoprotein, and a number of clinical investigations have suggested that its intrinsic or acquired overexpression resulted in a poor clinical outcome of chemotherapy. Various types of compounds and techniques for the reversal of MDR1/P-glycoprotein-mediated MDR have been developed, and efforts have concentrated on the inhibition of function and suppression of expression. This review summarizes the current state of knowledge of MDR1/P-glycoprotein and the modulation of MDR by targeting MDR1/P-glycoprotein.

Tang, R., Z. G. Zhu, et al. (2006). "The impact of hyperthermic chemotherapy on human gastric cancer cell lines: preliminary results." *Oncol Rep* **16**(3): 631-41.

In this preliminary study, we evaluated the impact of hyperthermia (HT) and hyperthermic chemotherapy (HTCT) on six human gastric cancer cell lines and explored the mechanisms of cell-killing effect under HTCT. Treatment conditions were categorized into 4 modes: i) normothermic control (NT), ii) HT, iii) normothermic chemotherapy (NTCT) and iv) HTCT. According to the data of MTT and LM observations, isolated HT only temporarily inhibited cell proliferation and had no cell-killing effect on gastric cancer cell lines employed in our study except for SNU-1. Combining with HT enhanced the cytotoxicity of CDDP in all gastric cell lines and the concentration inhibiting cell proliferation and inducing cell death of HTCT was much lower

than that of NTCT. There was a synergistic effect of HT and chemotherapy on inhibiting proliferation in each cell line in a certain range of CDDP concentration. The data of TEM and FCM proved that HTCT induced cell death with two modes - apoptosis and necrosis, and apoptosis was the major type. Microarray illustrated that, under HTCT, a total of 58 gene expressions were regulated according to the filtering criteria, including 10 extra genes with an expression change below the threshold or even unchanged when treated with either HT or CDDP alone. Five of these 10 genes were verified by QRT-PCR. These genes may include the target genes for the enhancing effect of HT on chemotherapy and their effects should be further validated by functional analysis.

Thomas, M., J. Clarhaut, et al. (2008). "Synthesis and biological evaluation of glucuronide prodrugs of the histone deacetylase inhibitor CI-994 for application in selective cancer chemotherapy." *Bioorg Med Chem* **16**(17): 8109-16.

Two glucuronide prodrugs of the histone deacetylase inhibitor CI-994 were synthesized. These compounds were found to be soluble in aqueous media and stable under physiological conditions. The carbamoyl derivatisation of CI-994 significantly decreased its toxicity towards NCI-H661 lung cancer cells. Prodrug incubation with beta-glucuronidase in the culture media led efficiently to the release of the parent drug and thereby restoring its ability to decrease cell proliferation, to inhibit HDAC and to induce E-Cadherin expression.

Tiezzi, D. G., J. M. De Andrade, et al. (2006). "Apoptosis induced by neoadjuvant chemotherapy in breast cancer." *Pathology* **38**(1): 21-7.

AIM: To evaluate the relationship between apoptosis induced by chemotherapy and clinical response in breast cancer. Apoptosis index (AI), mutant p53 and Bcl-2 protein expression were evaluated in 44 breast tumour samples from patients submitted to neoadjuvant chemotherapy. Objective response (OR) to primary chemotherapy was observed in 37 patients (84%) and no response (NR) in seven. AI was measured by the rate of apoptotic cells identified using morphological criteria. p53 and Bcl-2 protein expression were evaluated using an immunoperoxidase staining technique. The median AI change observed between pre-chemotherapy AI and post-chemotherapy AI was 0.84 in the OR group and 0.01 in the NR group, ( $\rho = 0.4$ ;  $p = 0.006$ ). There was no change in Bcl-2 protein expression following chemotherapy. In the OR group, p53 protein expression was positive in 41.6% of patients before and in 22.2% after chemotherapy (difference = 16.6%;

p = 0.03). No change was detected in the NR group. A positive correlation was found between the increase in AI and clinical response to neoadjuvant chemotherapy in locally advanced breast cancer.

Toiyama, Y., Y. Inoue, et al. (2008). "Alteration of the chemoresistant gene expression during chemotherapy for colon cancer: a molecular case report." *Oncol Rep* **19**(3): 755-9.

Chemotherapy remains the main choice of treatment for the management of unresectable or metastatic colorectal cancer. However, drug resistance is a major problem, limiting the effectiveness of the chemotherapies presently used to treat cancer. During treatment, drug resistance can also be acquired by tumors that are initially sensitive to chemotherapy. We present a case of metachronous splenic recurrence after a curative resection of appendical cancer with ovarian metastasis, although the patient had been treated with 5-FU/LV followed by mFOLFOX6 after surgery. Molecular analyses by RT-PCR also showed that the residual tumor after chemotherapy has cancer cells overexpressing 5-FU/1-OHP based chemotherapy-resistant genes. Therefore, it was suggested that a careful assessment of the disease status be undertaken during chemotherapy to ensure that the possibility of surgical resection, especially of the re-growth or partial response tumors, was not missed, since several genes, chemoresistant to the agents used, can be induced in residual tumors during chemotherapy.

Tsao, M. S., S. Aviel-Ronen, et al. (2007). "Prognostic and predictive importance of p53 and RAS for adjuvant chemotherapy in non small-cell lung cancer." *J Clin Oncol* **25**(33): 5240-7.

p53 and RAS are multifunctional proteins that are critical to cell cycle regulation, apoptosis, cell survival, gene transcription, response to stress, and DNA repair. We have evaluated the prognostic and predictive value of p53 gene/protein aberrations using tumor samples from JBR.10, a North American phase III intergroup trial that randomly assigned 482 patients with completely resected stage IB and II non-small-cell lung cancer (NSCLC) to receive four cycles of adjuvant cisplatin plus vinorelbine or observation alone. p53 protein expression was evaluated by immunohistochemistry. Mutations in exons 5 to 9 of the p53 gene were determined by denaturing high-performance liquid chromatography and confirmed by sequencing. RAS mutations were identified by allelic specific oligonucleotide hybridization. Of 253 patients, 132 (52%) were positive for p53 protein overexpression. Untreated p53-positive patients had significantly shorter overall survival than did patients with p53-negative tumors (hazard ratio [HR] = 1.89;

95% CI, 1.07 to 3.34; P = .03). However, these p53-positive patients also had a significantly greater survival benefit from adjuvant chemotherapy (HR = 0.54; P = .02) compared with patients with p53-negative tumors (HR = 1.40; P = .26; interaction P = .02). Mutations of p53 and RAS genes were found in 124 (31%) of 397 and 117 (26%) of 450 patients, respectively. Mutations in these genes were neither prognostic for survival nor predictive of a differential benefit from adjuvant chemotherapy. p53 protein overexpression is a significant prognostic marker of shortened survival, and also a significant predictive marker for a differentially greater benefit from adjuvant chemotherapy in completely resected NSCLC patients.

Ushijima, R., K. Takayama, et al. (2007). "Immunohistochemical expression of MRP2 and clinical resistance to platinum-based chemotherapy in small cell lung cancer." *Anticancer Res* **27**(6C): 4351-8.

Determining an effective predictor of clinical drug resistance in small cell lung cancer (SCLC) is considered to be important. In this study, the relationship between the expression of P-glycoprotein (P-gp), multidrug resistance-associated protein 1 (MRP1) and MRP2, which are the members of ATP-binding cassette superfamily transporter, and of the p53 tumor suppressor gene and the response to chemotherapy were analysed. The expression of P-gp, MRP1, MRP2, and p53 was determined by an immunohistochemical analysis of transbronchial biopsy (TBB) specimens from 61 SCLC patients. The relationship of such expression was also investigated regarding chemotherapy and clinicopathological factors. The response rate in the MRP2-negative group was significantly higher than that in the MRP2-positive group (88% versus 50%). The P-gp-negative group responded significantly better to chemotherapy than the P-gp-positive group, with a response rate of 81% versus 39%. No relationship could be found between the response to chemotherapy and immunostaining for MRP1 or p53. In 37 patients treated with platinum-based chemotherapy, the response rate of patients in the MRP2-negative group was significantly higher than that in the positive group (92% versus 50%). In a multiple logistic regression analysis, MRP2 as well as P-gp were shown to be statistically significant predictors of chemotherapy resistance. These results suggest that immunostaining of MRP2 for TBB specimens may help to predict clinical resistance to platinum agents. This is the first report which indicates that the immunohistochemical expression of MRP2 is positively related to a clinical resistance to platinum.

Van Schaeybroeck, S., J. Kyula, et al. (2006). "Chemotherapy-induced epidermal growth factor receptor activation determines response to combined gefitinib/chemotherapy treatment in non-small cell lung cancer cells." *Mol Cancer Ther* 5(5): 1154-65.

Activating epidermal growth factor receptor (EGFR) mutations have been linked with sensitivity to gefitinib and erlotinib; however, there are no established predictive markers for response to the combination of EGFR inhibitors with standard chemotherapy in non-small cell lung cancer (NSCLC) patients. In this study, we characterized a panel of human EGFR wild-type and mutant NSCLC cells for their sensitivity to gefitinib alone and in combination with cisplatin or Taxol. Cell viability was assessed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide and crystal violet cell viability assays. Cell cycle distribution was measured by flow cytometry. EGFR expression was measured by flow cytometry, real-time PCR, and Western blotting. EGFR/Her2/Akt and extracellular signal-regulated kinase 1/2 (Erk1/2) phosphorylation were measured by Western blotting. Two of nine EGFR wild type and one of two EGFR mutant NSCLC cells were sensitive to gefitinib, and this was associated with a decrease in phospho (p)-Akt and pErk1/2 following gefitinib exposure. There was no correlation between constitutive EGFR expression or activity and sensitivity to gefitinib nor was there a correlation between Her2/Akt and Erk1/2 activity and gefitinib sensitivity. However, in cells displaying a synergistic interaction between gefitinib and chemotherapy (cisplatin or Taxol), a dose-dependent increase in pEGFR was observed following chemotherapy exposure. In contrast, in cells where no change or a decrease in pEGFR following drug treatment was observed, we found an antagonistic or (at best) an additive interaction between the two compounds. Furthermore, the nature of this interaction was not dependent on the presence of a mutant EGFR. These novel findings suggest that modulation of EGFR activity following drug treatment determines response to gefitinib in combination with chemotherapy in NSCLC cells.

Wakelee, H. and L. Chhatwani (2008). "Adjuvant chemotherapy for resected non-small cell lung cancer." *Semin Thorac Cardiovasc Surg* 20(3): 198-203.

Surgery remains the mainstay of therapy for early stage non-small cell lung cancer (NSCLC), but even for stage IA, disease relapse rates remain as high as 30%. Patients with completely resected (R0) N1 disease have about a 50% chance of relapse. In the past 5 years, the benefit of adjuvant chemotherapy has finally been demonstrated for patients with lung

cancer. Improvements of 5% to 10% 5-year survival have been reported with cisplatin-based chemotherapy. Still, cure rates have significant room for improvement and ongoing trials with "targeted" agents such as those active against the vascular endothelial growth factor (VEGF), epidermal growth factor receptor (EGFR), and vaccine therapy will hopefully further increase the odds for patients with resected disease. Other studies looking at tumor gene and protein expression will lead us toward better identification of patients most likely to benefit from therapy.

Wang, Z., B. Xu, et al. (2008). "XRCC1 polymorphisms and severe toxicity in lung cancer patients treated with cisplatin-based chemotherapy in Chinese population." *Lung Cancer* 62(1): 99-104.

Cisplatin kills tumor cells through DNA cross linking. Alterations in the function of DNA repair genes may affect DNA repair proficiency and influence cancer patients' response to cisplatin. The predictability of DNA repair XRCC1 (X-ray repair cross-complementing group 1 protein) single nucleotide polymorphisms (SNPs) for cisplatin-based grades 3 and 4 chemotherapy-related toxicity in patients with newly diagnosed advanced lung cancer was evaluated. The genotypes of XRCC1 at the Arg194Trp, and Arg399Gln sites were determined by PCR-based restriction fragment length polymorphism (RFLP) methods. There was no statistically significant association between either the Arg194Trp or the Arg399Gln polymorphisms and hematologic grade 3 or 4 toxicity. However, carrying at least one variant XRCC1 Arg399Gln allele (399Arg/Gln or 399Gln/Gln) was associated with a significantly increased risk of overall grade 3 or 4 toxicity (odds ratio, 2.05; 95% confidence interval, 1.02-4.10; p=0.04); and grade 3 or 4 gastrointestinal toxicity (odds ratio, 2.53; 95% confidence interval, 1.06-6.03; p=0.03). Our results suggested that patients carrying at least one variant XRCC1 Arg399Gln allele have a 2.5-fold increased risk of grade 3 or 4 gastrointestinal toxicity when treated with first-line cisplatin-based chemotherapy.

Watanabe, Y. and F. Itoh (2006). "Epigenetics modulates the effect of chemotherapy on gastric cancer." *J Gastroenterol* 41(2): 180-1.

Weichselbaum, R. R., H. Ishwaran, et al. (2008). "An interferon-related gene signature for DNA damage resistance is a predictive marker for chemotherapy and radiation for breast cancer." *Proc Natl Acad Sci U S A* 105(47): 18490-5.

Individualization of cancer management requires prognostic markers and therapy-predictive

markers. Prognostic markers assess risk of disease progression independent of therapy, whereas therapy-predictive markers identify patients whose disease is sensitive or resistant to treatment. We show that an experimentally derived IFN-related DNA damage resistance signature (IRDS) is associated with resistance to chemotherapy and/or radiation across different cancer cell lines. The IRDS genes STAT1, ISG15, and IFIT1 all mediate experimental resistance. Clinical analyses reveal that IRDS(+) and IRDS(-) states exist among common human cancers. In breast cancer, a seven-gene-pair classifier predicts for efficacy of adjuvant chemotherapy and for local-regional control after radiation. By providing information on treatment sensitivity or resistance, the IRDS improves outcome prediction when combined with standard markers, risk groups, or other genomic classifiers.

Worrillow, L. J., A. G. Smith, et al. (2008). "Polymorphic MLH1 and risk of cancer after methylating chemotherapy for Hodgkin lymphoma." *J Med Genet* **45**(3): 142-6.

Methylating agents are effective chemotherapy agents for Hodgkin lymphoma, but are associated with the development of second primary cancers. Cytotoxicity of methylating agents is mediated primarily by the DNA mismatch repair (MMR) system. Loss of MLH1, a major component of DNA MMR, results in tolerance to the cytotoxic effects of methylating agents and persistence of mutagenised cells at high risk of malignant transformation. We hypothesised that a common substitution in the basal promoter of MLH1 (position -93, rs1800734) modifies the risk of cancer after methylating chemotherapy. 133 patients who developed cancer following chemotherapy and/or radiotherapy (n = 133), 420 patients diagnosed with de novo myeloid leukaemia, 242 patients diagnosed with primary Hodgkin lymphoma, and 1177 healthy controls were genotyped for the MLH1 -93 polymorphism by allelic discrimination polymerase chain reaction (PCR) and restriction fragment length polymorphism assay. Odds ratios and 95% confidence intervals for cancer risk by MLH1 -93 polymorphism status, and stratified by previous exposure to methylating chemotherapy, were calculated using unconditional logistic regression. Carrier frequency of the MLH1 -93 variant was higher in patients who developed therapy related acute myeloid leukaemia (t-AML) (75.0%, n = 12) or breast cancer (53.3%, n = 15) after methylating chemotherapy for Hodgkin lymphoma compared to patients without previous methylating exposure (t-AML, 30.4%, n = 69; breast cancer patients, 27.2%, n = 22). The MLH1 -93 variant allele was also over-represented in t-AML

cases when compared to de novo AML cases (36.9%, n = 420) and healthy controls (36.3%, n = 952), and was associated with a significantly increased risk of developing t-AML (odds ratio 5.31, 95% confidence interval 1.40 to 20.15), but only in patients previously treated with a methylating agent. CONCLUSIONS: These data support the hypothesis that the common polymorphism at position -93 in the core promoter of MLH1 defines a risk allele for the development of cancer after methylating chemotherapy for Hodgkin lymphoma. However, replication of this finding in larger studies is suggested.

Wu, X., J. Gu, et al. (2006). "Genetic variations in radiation and chemotherapy drug action pathways predict clinical outcomes in esophageal cancer." *J Clin Oncol* **24**(23): 3789-98.

Understanding how specific genetic variants modify drug action pathways may provide informative blueprints for individualized chemotherapy. We applied a pathway-based approach to examine the impact of a comprehensive panel of genetic polymorphisms on clinical outcomes in 210 esophageal cancer patients. In the Cox proportional hazards model, MTHFR Glu429Ala variant genotypes were associated with significantly improved survival (hazard ratio [HR] = 0.56; 95% CI, 0.35 to 0.89) in patients treated with fluorouracil (FU). The 3-year survival rates for patients with the variant genotypes and the wild genotypes were 65.26% and 46.43%, respectively. Joint analysis of five polymorphisms in three FU pathway genes showed a significant trend for reduced recurrence risk and longer recurrence-free survival as the number of adverse alleles decreased (P = .004). For patients receiving platinum drugs, the MDR1 C3435T variant allele was associated with significantly reduced recurrence risk (HR = 0.25; 95% CI, 0.10 to 0.64) and improved survival (HR = 0.44; 95% CI, 0.23 to 0.85). In nucleotide excision repair genes, there was a significant trend for a decreasing risk of death with a decreasing number of high-risk alleles (P for trend = .0008). In base excision repair genes, the variant alleles of XRCC1 Arg399Gln were significantly associated with the absence of pathologic complete response (odds ratio = 2.75; 95% CI, 1.14 to 6.12) and poor survival (HR = 1.92; 95% CI, 1.00 to 3.72). Several biologically plausible associations between individual single nucleotide polymorphisms and clinical outcomes were found. Our data also strongly suggest that combined pathway-based analysis may provide valuable prognostic markers of clinical outcomes.

Wu, X., C. Lu, et al. (2008). "Germline genetic variations in drug action pathways predict clinical outcomes in advanced lung cancer treated with

platinum-based chemotherapy." *Pharmacogenet Genomics* **18**(11): 955-65.

Genetic polymorphisms contribute to interindividual variation in drug response. However, a single polymorphism is likely to exhibit a modest effect. Therefore, we applied a pathway-based approach to evaluate the cumulative effect of multiple polymorphisms on clinical outcome of patients with non-small cell lung cancer. We genotyped 25 functional polymorphisms in 16 key genes involved in cisplatin metabolism and action and evaluated their associations with overall survival in 229 non-small cell lung cancer patients receiving first-line cisplatin-based chemotherapy. Several biologically plausible main effects were identified in individual analysis. More importantly, when six polymorphisms in nucleotide excision repair genes were analyzed jointly, a significant trend of reduced risk of death with decreasing number of putative unfavorable genotypes was observed (P for trend < 0.001 and log rank P < 0.001). Survival tree analysis revealed potential higher-order gene-gene interactions and categorized subgroups with dramatically different survival experiences, based on distinct genotype profiles. The median survival time was 78.5 months for terminal node 1 in the low-risk group, 15.1 months for terminal node 10 in the medium-risk group, and 6.7 months for terminal node 9 in the high-risk group (log rank P < 0.001). We also constructed a prediction hazard model. The area under the curve increased from 0.71 (using clinical variables only) to 0.84 (using clinical, epidemiological, and genetic variations from survival tree analysis). Our results highlight the clinical potential of taking a pathway-based approach and using survival tree analytic approach to identify subgroups of patients with distinctly differing outcomes.

Xu, J., J. Y. Zhou, et al. (2006). "Tumor necrosis factor-related apoptosis-inducing ligand is required for tumor necrosis factor alpha-mediated sensitization of human breast cancer cells to chemotherapy." *Cancer Res* **66**(20): 10092-9.

Tumor necrosis factor alpha (TNFalpha) induces apoptosis and sensitizes cancer cells to chemotherapy, but the mechanism underlying its sensitization is not fully understood. Here, we report that TNFalpha-mediated sensitization of cancer cells to chemotherapy involves activation of the TRAIL pathway. We show that the combined treatment of breast cancer cells with TNFalpha and Adriamycin significantly increases cell death compared with the treatment with either agent alone. The combined treatment activated both death receptor and mitochondrial apoptotic pathways, whereas Adriamycin alone activated only the mitochondrial

pathway, and TNFalpha failed to activate either. Furthermore, we show that TNFalpha induces TRAIL through a transcriptional mechanism. Using reporter gene assays in conjunction with chromatin immunoprecipitation assays, we show that TRAIL induction by TNFalpha is regulated via both nuclear factor-kappaB and Sp1 binding sites. Importantly, down-regulation of TRAIL by small interfering RNA silencing decreased TNFalpha-mediated Adriamycin-induced caspase activation and apoptosis, and thus enhanced breast cancer cell resistance to Adriamycin. Collectively, our results suggest that induction of TRAIL by TNFalpha is critical for sensitization of breast cancer cells to chemotherapy.

Yang, C. H., C. J. Yu, et al. (2008). "Specific EGFR mutations predict treatment outcome of stage IIIB/IV patients with chemotherapy-naive non-small-cell lung cancer receiving first-line gefitinib monotherapy." *J Clin Oncol* **26**(16): 2745-53.

To explore predictive factors for time to treatment failure (TTF) in chemotherapy-naive non-small-cell lung cancer (NSCLC) patients receiving gefitinib treatment. We designed a phase II study to test gefitinib antitumor efficacy in advanced-stage, chemotherapy-naive NSCLC patients. Patients were treated with gefitinib 250 mg/d. Tumor assessments were performed every 2 months. Responding or stable patients were treated until progression or unacceptable toxicity. All scans were reviewed independently. EGFR exons 18-21 sequence, K-ras exon 2 sequence, and MET gene copy numbers were examined in available samples. Clinical or molecular predictors of TTF were examined by multivariate analysis. One hundred six patients were enrolled. Ninety patients had tumor samples for biomarker tests. Overall response rate was 50.9% (95% CI, 41.4% to 60.4%). Median TTF was 5.5 months, and median overall survival (OS) was 22.4 months. The response rate and median TTF of the patients with exon 19 deletion (n = 20) were 95.0% and 8.9 months, for exon 21 L858R mutation (n = 23) were 73.9% and 9.1 month, and for other types of EGFR mutations (N = 12) were 16.7% and 2.3 months, respectively. In multivariate analysis, the presence of EGFR deletion exon 19 or L858R EGFR mutations in adenocarcinoma patients predicted longer TTF. High copy number of MET seemed to correlate with shorter TTF in patients with gefitinib-sensitive activating EGFR mutations. In this prospective study, EGFR exon 19 deletion or L858R mutations in adenocarcinoma were the best predictors for longer TTF in stage IIIB/IV chemotherapy-naive NSCLC patients receiving first-line gefitinib monotherapy.

Yang, S. X., S. M. Steinberg, et al. (2008). "Gene expression profile and angiogenic marker correlates with response to neoadjuvant bevacizumab followed by bevacizumab plus chemotherapy in breast cancer." *Clin Cancer Res* **14**(18): 5893-9.

To identify biomarkers and gene expression profile signatures to distinguish patients with partial response (PR) from those with stable disease (SD) and progressive disease (PD). EXPERIMENTAL DESIGN: Twenty patients with inflammatory breast cancer and one patient with locally advanced breast cancer received one cycle of bevacizumab followed by six cycles of bevacizumab plus docetaxel-doxorubicin before surgery. Baseline angiogenic/tumor markers were examined by immunohistochemistry and gene expression profiles were measured by Agilent Whole Human Genome arrays. Representative significant GO classes include spindle (11 genes;  $P = 0.001$ ), vascular endothelial growth factor receptor activity including PDGFR-beta (5 genes;  $P = 0.002$ ), and cell motility including CD31 (80 genes;  $P = 0.005$ ). Baseline CD31, PDGFR-beta, and GO classes for vascular endothelial growth factor receptor activity and mitosis were significantly associated with response to bevacizumab followed by bevacizumab plus chemotherapy.

Yoshioka, A., H. Miyata, et al. (2008). "The activation of Akt during preoperative chemotherapy for esophageal cancer correlates with poor prognosis." *Oncol Rep* **19**(5): 1099-107.

The activation of the PI3K/Akt/mTOR pathway plays an important role in tumorigenesis and resistance to anticancer drugs. The aim of this study was to elucidate the role of the Akt/mTOR pathway in chemoresistance and the prognosis of patients with esophageal squamous cell carcinoma (ESCC) who received preoperative chemotherapy. We evaluated p-Akt and p-mTOR expression by immunohistochemistry in the surgical specimens of 143 ESCC (51 patients with and 92 without preoperative chemotherapy). In 37 patients of the former group, paired tissue samples obtained before and after chemotherapy were examined immunohistochemically. The incidence of p-Akt expression was higher in ESCC with than without chemotherapy (51.0 vs. 25.0%,  $p=0.0018$ ). Although p-Akt expression was not associated with an advanced tumor stage, a comparison between before and after chemotherapy demonstrated an increased p-Akt expression during chemotherapy ( $p=0.0348$ ). The p-Akt expression did not correlate with survival in ESCC without chemotherapy, but was associated with poor prognosis in those with chemotherapy ( $p=0.0058$ ). In particular, an increased p-Akt expression during chemotherapy was associated with

poor survival ( $p=0.0022$ ). Notably, the p-mTOR expression did not correlate with p-Akt expression ( $p=0.1482$ ). The depth of the tumor invasion, clinical response and p-Akt expression correlated with the prognosis of 51 ESCC with chemotherapy. A multivariate analysis showed that p-Akt expression was the only independent predictor of poor prognosis in ESCC patients with chemotherapy. p-Akt expression increases after chemotherapy in ESCC and a high expression correlates with poor prognosis. Our results suggest that the activation of Akt is a potentially useful therapeutic target in ESCC patients treated with chemotherapy.

Zhang, C., A. Kolb, et al. (2006). "Corticosteroid co-treatment induces resistance to chemotherapy in surgical resections, xenografts and established cell lines of pancreatic cancer." *BMC Cancer* **6**: 61.

Chemotherapy for pancreatic carcinoma often has severe side effects that limit its efficacy. The glucocorticoid (GC) dexamethasone (DEX) is frequently used as co-treatment to prevent side effects of chemotherapy such as nausea, for palliative purposes and to treat allergic reactions. While the potent pro-apoptotic properties and the supportive effects of GCs to tumour therapy in lymphoid cells are well studied, the impact of GCs to cytotoxic treatment of pancreatic carcinoma is unknown. A prospective study of DEX-mediated resistance was performed using a pancreatic carcinoma xenografted to nude mice, 20 surgical resections and 10 established pancreatic carcinoma cell lines. Anti-apoptotic signaling in response to DEX was examined by Western blot analysis. In vitro, DEX inhibited drug-induced apoptosis and promoted the growth in all of 10 examined malignant cells. Ex vivo, DEX used in physiological concentrations significantly prevented the cytotoxic effect of gemcitabine and cisplatin in 18 of 20 freshly isolated cell lines from resected pancreatic tumours. No correlation with age, gender, histology, TNM and induction of therapy resistance by DEX co-treatment could be detected. In vivo, DEX totally prevented cytotoxicity of chemotherapy to pancreatic carcinoma cells xenografted to nude mice. Mechanistically, DEX upregulated pro-survival factors and anti-apoptotic genes in established pancreatic carcinoma cells. These data show that DEX induces therapy resistance in pancreatic carcinoma cells and raise the question whether GC-mediated protection of tumour cells from cancer therapy may be dangerous for patients.

Zhang, J. T. (2007). "Use of arrays to investigate the contribution of ATP-binding cassette transporters to drug resistance in cancer chemotherapy and prediction of chemosensitivity." *Cell Res* **17**(4): 311-23.

Multidrug resistance (MDR) is a major problem in cancer chemotherapy. One of the best known mechanisms of MDR is the elevated expression of ATP-binding cassette (ABC) transporters. While some members of human ABC transporters have been shown to cause drug resistance with elevated expression, it is not yet known whether the over-expression of other members could also contribute to drug resistance in many model cancer cell lines and clinics. The recent development of microarrays and quantitative PCR arrays for expression profiling analysis of ABC transporters has helped address these issues. In this article, various arrays with limited or full list of ABC transporter genes and their use in identifying ABC transporter genes in drug resistance and chemo-sensitivity prediction will be reviewed.

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