Cancer Prognosis

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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 prognosis.
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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

Adachi, M., T. Taki, et al. (1996). "Correlation of KAI1/CD82 gene expression with good prognosis in patients with non-small cell lung cancer." Cancer Res **56**(8): 1751-5.

As part of our evaluation of members of the transmembrane 4 super-family as possible prognostic predictors, we performed a retrospective study on the expression of the recently identified KAI1 gene by tumors of the lung. This gene, which is identical to CD82, suppresses tumor metastasis of prostate cancer, and its decreased expression may be involved in malignant progression. We used reverse transcription-PCR to analyze tumor tissues from 151 lung cancer patients; 74 tumors were stage I, 17 were stage II, and 60 were stage III. Our results indicate that while 35 patients had tumors in which the KAI1/CD82 gene was conserved (positive), 116 patients had tumors with reduced gene expression (negative). The overall survival rate of patients with KAI1/CD82-positive tumors was significantly higher than that of patients with KAI1/CD82-negative tumors (77.4% versus 38.5%; P=0.002). Furthermore, the overall survival rate of patients with KAI1/CD82-positive adenocarcinoma was also much higher than that of individuals whose adenocarcinoma had reduced KAI1/CD82 expression (73.4% versus 27.1%;P=0.009). Multivariate analysis with the Cox regression model indicated that KAII/CD82 positivity correlated best with the overall survival rate, except for lymph node status. Our data suggest that high KAII/CD82 gene expression by tumors of the lung may be associated with a good prognosis. These findings complement our earlier studies on MRP-1/CD9, another member of the transmembrane 4 superfamily, whose reduced expression in non-small cell lung cancer appears to be a factor of poor prognosis. This set of observations suggests that assessment of the expression status of KAI1/CD82 and MRP-1/CD9 by tumors may provide prognostic information on the clinical behavior of lung cancer.

Ahnen, D. J. (1992). "Abnormal DNA content as a biomarker of large bowel cancer risk and prognosis." J Cell Biochem Suppl **16G**: 143-50.

Aneuploid cell populations can be defined as those that contain an abnormal number of chromosomes or an abnormal amount of DNA. Aneuploidy can be reliably detected by flow cytometric analysis of DNA content. This technique not only identifies aneuploid cell populations but can also quantify the percent of cells in various phases of the cell cycle, thus giving an indication of the proliferative activity of a tissue. Aneuploidy occurs in approximately 60% of established colorectal cancers, and many studies have demonstrated that patients with aneuploid tumors have a poorer prognosis than patients with diploid colon cancers. Some studies have suggested that the proliferative rate of tumors, as assessed by the percent of cells in S phase, also has prognostic significance. Until recently, aneuploidy was thought to occur only in malignant tissues, but it has been clearly shown that aneuploid cell populations can be identified in benign adenomatous polyps as well as in non-neoplastic-appearing mucosa of patients with chronic ulcerative colitis and Barrett's esophagus. In chronic ulcerative colitis, aneuploidy occurs more frequently in patients with dysplasia or cancer than in those with no evidence of neoplasia.

Similarly, dysplastic and malignant biopsies are more commonly aneuploid than non-neoplastic biopsies. Patients who have undergone colectomy for cancer or dysplasia in the setting of chronic ulcerative colitis frequently have multiple areas of aneuploidy throughout the remainder of their colon. Whether aneuploidy can be useful as a marker of cancer risk in patients with chronic ulcerative colitis deserves further investigation.

Anbazhagan, R., R. D. Gelber, et al. (1991). "Association of c-erbB-2 expression and S-phase fraction in the prognosis of node positive breast cancer." Ann Oncol **2**(1): 47-53.

An immunohistochemical study was performed on 211 primary breast carcinomas for c-erbB-2 expression. All patients had involvement of axillary lymph nodes and all were randomised onto one of the Ludwig Breast Cancer Trials I-IV between July 1978 and August 1981. c-erbB-2 overexpression significantly correlated with high S-phase fraction, four or more positive axillary nodes involved, estrogen receptor negative primaries, progesterone receptor negative primaries, high grade tumours and DNA aneuploidy. With a nine year median follow-up c-erbB-2 positive tumours had worse disease-free survival (p = 0.0002) and overall survival (p less than 0.0001). Multivariate analyses using proportional hazard regression models demonstrated that c-erbB-2 positivity continued to predict a poor outcome even when accounting for the effects of other prognostic factors.

Andrulis, I. L., S. B. Bull, et al. (1998). "neu/erbB-2 amplification identifies a poor-prognosis group of women with node-negative breast cancer. Toronto Breast Cancer Study Group." J Clin Oncol **16**(4): 1340-9.

PURPOSE: It remains a challenge to predict which women with axillary node-negative (ANN) breast cancer at greatest risk of relapse may benefit most from adjuvant therapy. Increases in neu/erbB-2 have been implicated in breast cancer prognosis. Although overexpression has been investigated extensively, this study represents the first prospective assessment of the prognostic value of neu/erbB-2 DNA amplification in a cohort of women with newly diagnosed ANN. METHODS: A consecutive series of women was monitored for recurrence (median follow-up duration, 36 months) and tumors from 580 individuals were analyzed for amplification. The association of amplification with risk of recurrence was examined in survival analyses with traditional and histologic markers as prognostic factors. RESULTS: Neu/erbB-2 was amplified in 20% of cases. We found an increased risk of disease recurrence when neu/erbB-2 was amplified > or = twofold that persisted with adjustment for other prognostic factors (relative risk, 2.36; P = .002). We found some evidence that amplification was more important in patients who received chemotherapy compared with untreated patients. CONCLUSION: neu/erbB-2 amplification is an independent prognostic factor for risk of recurrence in ANN breast cancer. Women with tumors without neu/erbB-2 amplification have a good prognosis; aggressive therapy in this group is therefore difficult to justify. On the other hand, even with adjuvant chemotherapeutic treatment, women whose tumors exhibit neu/erbB-2 amplification have an increased risk of recurrence. We encourage a randomized trial to compare more aggressive adjuvant chemotherapy versus standard chemotherapy for ANN women whose tumors exhibit neu/erbB-2 amplification.

Araujo, S. E., W. M. Bernardo, et al. (2007). "DNA ploidy status and prognosis in colorectal cancer: a meta-analysis of published data." Dis Colon Rectum **50**(11): 1800-10.

PURPOSE: In colorectal cancer, the negative effect of aneuploidy has been a controversy for more than 20 years. Studies to determine a survival-deoxyribonucleic acid content relationship have conflicting results. A systematic literature search followed by a meta-analysis of published studies addressing prognostic effect of aneuploidy for patients who underwent surgical treatment of colon and rectal cancer was conducted. METHODS: The main outcome measure was the five-year overall mortality rate after surgical resection. For the selected studies, we estimated this outcome for three subsets of patients through separate meta-analyses: 1) for all patients with colorectal cancer; 2) only between patients with Stage II colon cancer; and 3) only for studies in which follow-up losses were declared. The presence of publication bias was assessed with a funnel plot for asymmetry. RESULTS: A total of 5,478 patients with colorectal cancer were represented in 32 studies (Group 1), we estimated a reduction in the five-year overall mortality from 43.2 percent for aneuploid tumors to 29.2 percent for diploid tumors (combined relative risk = 1.44; 95 percent confidence interval = 1.34-1.55; P < 0.001). In addition, 357 patients with Stage II colon cancer (Group 2) extracted from three studies had an absolute reduction of 14.3 percent in five-year overall mortality favoring diploid tumors (combined relative risk = 1.93; 95 percent confidence interval = 1.29-2.89; P = 0.001). Lastly, of 14 studies in which follow-up losses were declared (Group 3), 2,221 patients were represented and a 15.7 percent mortality reduction was measured favoring patients with diploid tumors (combined relative risk = 1.44; 95

percent confidence interval = 1.3-1.61; P < 0.001). CONCLUSIONS: Patients who undergo an aneuploid colorectal cancer surgical resection have a higher risk of death after five years. This finding may ultimately impact survival of patients with node-negative colon cancer through adjuvant therapy.

Bartolucci, R., J. Wei, et al. (2009). "XPG mRNA expression levels modulate prognosis in resected non-small-cell lung cancer in conjunction with BRCA1 and ERCC1 expression." Clin Lung Cancer **10**(1): 47-52.

Background: Molecular markers can help identify patients with early-stage non-small-cell lung cancer (NSCLC) with a high risk of relapse. Excision repair cross-complementing 1 (ERCC1), Xeroderma pigmentosum group G (XPG), and breast cancer 1 (BRCA1) are involved in DNA damage repair, whereas ribonucleotide reductase M1 (RRM1) is implicated in DNA synthesis. Expression levels of these molecules might therefore have a prognostic role in lung cancer. Patients and Methods: We examined ERCC1, RRM1, XPG, and BRCA1 mRNA levels by real-time quantitative polymerase chain reaction in 54 patients with stage IB-IIB resected NSCLC. A strong correlation was observed between the 4 genes. Results: For patients with low BRCA1, regardless of XPG mRNA expression levels, disease-free survival (DFS) was not reached. For patients with intermediate/high BRCA1 and high XPG, DFS was 50.7 months. However, for patients with intermediate/high BRCA1 and low/intermediate XPG, DFS decreased to 16.3 months (P = .002). Similar differences were observed in overall survival, with median survival not reached for patients with low BRCA1, regardless of XPG levels, or for patients with intermediate/high BRCA1 and high XPG. Conversely, for patients with intermediate/high BRCA1 levels and low/intermediate XPG levels, median survival dropped to 25.5 months (P = .007). Conclusion: BRCA1 and XPG were identified as independent prognostic factors for both median survival and DFS. High BRCA1 mRNA expression confers poor prognosis in early NSCLC, and the combination of high BRCA1 and low XPG expression still further increases the risk of shorter survival. These findings can help optimize the customization of adjuvant chemotherapy.

Barton, C. A., N. F. Hacker, et al. (2008). "DNA methylation changes in ovarian cancer: implications for early diagnosis, prognosis and treatment." Gynecol Oncol **109**(1): 129-39.

OBJECTIVE: To review epigenetic changes identified in ovarian cancer, focusing on their potential as clinical markers for detection, monitoring of disease progression and as markers of therapeutic response. METHODS: A comprehensive review of English language scientific literature on the topics of methylation and ovarian cancer was conducted. RESULTS: Genome-wide demethylation of normally methylated and silenced chromosomal regions, and hypermethylation and silencing of genes including tumor suppressors are common features of cancer cells. Epigenetic alterations, including CpG island DNA methylation, occur in ovarian cancer and the identification of specific genes that are altered by epigenetic events is an area of intense research. Aberrant DNA methylation in ovarian cancer is observed in early cancer development, can be detected in DNA circulating in the blood and hence provides the promise of a non-invasive cancer detection test. In addition, identification of ovarian cancer-specific epigenetic changes has promise in molecular classification and disease stratification. CONCLUSIONS: The detection of cancer-specific DNA methylation changes heralds an exciting new era in cancer diagnosis as well as evaluation of prognosis and therapeutic responsiveness and warrants further investigation.

Bazan, V., L. Bruno, et al. (2006). "Molecular detection of TP53, Ki-Ras and p16INK4A promoter methylation in plasma of patients with colorectal cancer and its association with prognosis. Results of a 3-year GOIM (Gruppo Oncologico dell'Italia Meridionale) prospective study." Ann Oncol **17 Suppl 7**: vii84-90.

BACKGROUND: Despite the improvement in detection and surgical therapy in the last years, the outcome of patients affected by colorectal carcinoma (CRC) remains limited by metastatic relapse. The aim of this study was to investigate the presence of free tumor DNA in the plasma of CRC patients in order to understand its possible prognostic role. PATIENTS AND METHODS: Ki-Ras, TP53 mutations and p16(INK4A) methylation status were prospectively evaluated in tumor tissues and plasma of 66 CRC patients. RESULTS: In 50 of the 66 primitive tumor cases (76%) at least one significant alteration was identified in Ki-Ras and/or TP53 and/or p16(INK4A) genes. Eighteen of the 50 patients presented the same alteration both in the plasma and in the tumor tissue. At univariate analysis, Ki-Ras mutations proved to be significantly related to quicker relapse (P <0.01), whereas only a trend towards statistical significance (P = 0.083) was observed for the TP53 mutations CONCLUSIONS: Detection of Ki-Ras and TP53 mutation in plasma should be significantly related to disease recurrence. These data suggest that patients with a high risk of recurrence can be identified by

means of the analysis of tumor-derived plasma DNA with the use of fairly non-invasive techniques.

Benatti, P., R. Gafa, et al. (2005). "Microsatellite instability and colorectal cancer prognosis." Clin Cancer Res **11**(23): 8332-40.

PURPOSE: Many studies have evaluated the role of high levels of microsatellite instability (MSI) as a prognostic marker and predictor of the response to chemotherapy in colorectal cancer (CRC); however, the results are not conclusive. The aim of this study was to analyze the prognostic significance of high levels of MSI (MSI-H) in CRC patients in relation to fluorouracil-based chemotherapy. EXPERIMENTAL DESIGN: In three different institutions, 1,263 patients with CRC were tested for the presence of MSI, and CRC-specific survival was then analyzed in relation to MSI status, chemotherapy, and other clinical and pathologic variables. RESULTS: Two hundred and fifty-six tumors were MSI-H (20.3%): these were more frequently at a less advanced stage, right-sided, poorly differentiated, with mucinous phenotype, and expansive growth pattern than microsatellite stable carcinomas. Univariate and multivariate analyses of 5-year-specific survival revealed stage, tumor location, grade of differentiation, MSI, gender, and age as significant prognostic factors. The prognostic advantage of MSI tumors was particularly evident in stages II and III in which chemotherapy did not significantly affect the survival of MSI-H patients. Finally, we analyzed survival in MSI-H patients in relation to the presence of mismatch repair gene mutations. MSI-H patients with hereditary non-polyposis colorectal cancer showed a better prognosis as compared with sporadic MSI-H; however, in multivariate analysis, this difference disappeared. CONCLUSIONS: The type of genomic instability could influence the prognosis of CRC, in particular in stages II and III. Fluorouracil-based chemotherapy does not seem to improve survival among MSI-H patients. The survival benefit for patients with hereditary non-polyposis colorectal cancer is mainly determined by younger age and less advanced stage as compared with sporadic MSI-H counterpart.

Bertucci, F., R. Houlgatte, et al. (2002). "Prognosis of breast cancer and gene expression profiling using DNA arrays." Ann N Y Acad Sci **975**: 217-31.

Breast cancer is a complex genetic disease characterized by the accumulation of multiple molecular alterations. The resulting clinical heterogeneity makes current therapeutic strategies-based on clinicopathlogical factors-less than perfectly adapted to each patient. Today, DNA arrays, by allowing the simultaneous and quantitative analysis of the mRNA expression levels of thousands of genes in a single assay, provide novel tools to tackle this complexity. Potential applications are multiple in the cancer field and the first research results are promising. Using home-made DNA arrays in an approach easily compatible with academic research-nylon support and radioactive detection-we identified a predictor set of 23 genes whose expression patterns differentiated two groups of breast cancer patients with different survival after adjuvant chemotherapy. We then validated and further extended these results in a larger, independent and homogeneous series of poor prognosis primary breast cancers treated with adjuvant anthracyclin-based chemotherapy. We confirmed the prognostic classification provided by the 23-gene set predictor. We then improved the predictor set and refined the classification by sorting the tumors into three classes with significantly different long-term survival. These results show the potential of the technology with an accessible approach for academic research teams. We also showed that nylon DNA arrays with radioactive detection are associated with excellent sensitivity, an advantage in clinical situations where the amount of available material is limited.

Borresen, A. L., T. I. Andersen, et al. (1995). "TP53 mutations and breast cancer prognosis: particularly poor survival rates for cases with mutations in the zinc-binding domains." Genes Chromosomes Cancer **14**(1): 71-5.

Acquired mutations in TP53 as well as immunohistochemically detectable protein expression have been implicated as prognostic factors for breast cancer. We have evaluated the relationship between mutations detected in 119 breast tumours and various clinicohistopathological indices, stratifying the mutations according to the functional domains as defined by the recent elucidation of the crystal structure of the protein. Patients with missense mutations located in regions encoding parts of the protein involved in zinc-binding had significantly decreased disease-free and overall survival relative to patients whose tumours had mutations in other domains. These results indicate that these biochemically defined domains also have biological relevance in terms of breast cancer disease course, and suggest that some mutations in TP53, more than others, can contribute to the development of clinically more aggressive and perhaps treatment resistant breast tumours. When confirmed, this will be of potential importance in predicting the clinical behaviour of breast cancer and its responsiveness to therapy.

Branca, M., C. Giorgi, et al. (2006). "Over-expression of topoisomerase IIalpha is related to the grade of cervical intraepithelial neoplasia (CIN) and high-risk

human papillomavirus (HPV), but does not predict prognosis in cervical cancer or HPV clearance after cone treatment." Int J Gynecol Pathol **25**(4): 383-92.

OBJECTIVE: One of the pathways leading to cervical cancer is a loss of normal cell cycle control. Topoisomerase IIalpha and IIbeta are important nuclear proteins controlling the G2/M checkpoint, and shown to be over-expressed in many human cancers. Their links to oncogenic human papillomavirus (HPV) types and their prognostic value in cervical cancer are practically unexplored. MATERIAL AND METHODS: As part of our HPV-PathogenISS study, a series of 150 squamous cell carcinomas (SCC) and 152 CIN lesions were examined using immunohistochemical (IHC) staining for topoisomerase IIalpha (topo IIalpha), and tested for HPV using PCR with three primer sets (MY09/11, GP5/GP6, SPF). Follow-up data were available from all SCC patients, and 67 CIN lesions had been monitored with serial PCR for HPV clearance/persistence after cone treatment. RESULTS: Topo IIalpha expression increased with increasing grade of CIN ($p = 0.0001$), with the most dramatic up-regulation upon progression from CIN2 to CIN3 and peaking in SCC (OR 16.23; 95%CI 7.89-33.38). Topo IIalpha up-regulation was also significantly associated with HR-HPV detection in univariate analysis (OR = 3.07; 95%CI 1.70-5.52), but was confounded by the histological grade (Mantel-Haenszel common OR = 1.622; 95%CI 0.782-3.365), and by entering both p16(INK4a) (9) and Survivin (33) in the multivariate regression model. Topo IIalpha did not predict clearance/persistence of HR-HPV after treatment of CIN, and it was not a prognostic factor in cervical cancer in either univariate or multivariate analysis. CONCLUSIONS: Over-expression of topo IIalpha is significantly associated with progression from CIN2 to CIN3, being a late marker of cell proliferation. Its close association with HR-HPV is plausibly explained by the fact that E7 oncoproteins of these HR-HPV (but not LR-HPV) block the normal pRb-mediated inhibition of topo IIalpha by degrading the wild-type Rb.

Brennan, D. J., S. L. O'Brien, et al. (2005). "Application of DNA microarray technology in determining breast cancer prognosis and therapeutic response." Expert Opin Biol Ther **5**(8): 1069-83.

There are > 1.15 million cases of breast cancer diagnosed worldwide annually, and it is the second leading cause of cancer death in the European Union. The optimum management of patients with breast cancer requires accurate prognostic and predictive factors. At present, only a small number of such factors are used clinically. DNA microarrays have the potential to measure the expression of tens of thousands of genes simultaneously. Recent preliminary findings suggest that DNA microarray-based gene expression profiling can provide powerful and independent prognostic information in patients with newly diagnosed breast cancer. As well as providing prognostic information, emerging results suggest that DNA microarrays can also be used for predicting response or resistance to treatment, especially to neoadjuvant chemotherapy. Prior to clinical application, these preliminary findings must be validated using large-scale prospective studies. This article reviews these advances and also examines the role of DNA microarrays in reducing the number of patients who receive inappropriate chemotherapy. The most recent data supporting the integration of various publicly available data sets is also reviewed in detail.

Brooks, S. A., A. J. Leathem, et al. (1993). "Markers of prognosis in breast cancer--the relationship between binding of the lectin HPA and histological grade, SPF, and ploidy." Breast Cancer Res Treat **25**(3): 247-56.

Abnormal cellular glycosylation as demonstrated by the binding of a lectin from Helix pomatia (HPA) to paraffin-embedded sections has been shown in several studies to be associated with aggressive biological behaviour and poor long-term patient prognosis in breast cancer. This study aims to address the possibility that expression of the HPA binding ligand may be of prognostic significance through an association with increased cellular proliferation (as measured by S-phase fraction and histological grade), anaplasia (reflected in histological grade), or ploidy (DNA index). In a 24 year retrospective study, paraffin-embedded sections of 366 primary breast cancers were stained for binding of HPA. All tumours were assessed for histological grade. Flow cytometry was performed on all cases for which sufficient tumour tissue was available (358/366 cases) and S-phase fraction (SPF) and ploidy calculated. Data regarding patient age at diagnosis, nodal status, and tumour size were also recorded. Life table analyses revealed survival advantage for HPA 'non stainers' in comparison to 'stainers' ($p < 0.001$); for patients with tumours of low grade vs. high grade ($p < 0.001$); and for those with tumours of low SPF vs. high SPF ($p < 0.001$). No survival advantage was shown for those with diploid vs. aneuploid tumours ($p = 0.17$). No association was apparent between HPA binding and grade, SPF, or ploidy (Chi squared values not significant). This was confirmed by multivariate analysis in which nodal status, tumour size, and SPF were independently predictive of survival. There was no confounding effect of grade, SPF, or ploidy upon the correlation between survival and HPA binding. HPA was, however, not independently predictive

owing to its strong association with nodal status. The results of this study suggest that the prognostic significance of altered glycosylation, as detected by HPA binding, is unlikely to be through an association with proliferative rate, degree of anaplasia, or cellular ploidy, but may rather be through a direct association with the presence of nodal metastases.

Carey, F. A., E. Gray, et al. (1996). "A comparison of flow and image DNA cytometry in prediction of patient prognosis in surgically resected small cell lung cancer." Anal Cell Pathol **12**(3): 137-43.

We have previously reported that flow cytometric tumor DNA content may be of prognostic significance in surgically resected small cell lung cancer (SCLC). We are particularly interested in determining prospective parameters for better selection of 'good prognosis' patients to proceed to surgery. Since flow cytometric measurements are poorly, if at all, applicable to endoscopic biopsy and cytology specimens we compared an image cytometric system to flow cytometry and clinical parameters in an extended series of surgically resected SCLC. Clinical follow-up was obtained on 75 patients having surgical resection for SCLC in the years 1981-92. Paraffin blocks were prepared for cytometry in standard fashion. Flow DNA histograms were characterised as diploid/tetraploid (n = 45) or DNA aneuploid (n = 27). DNA histograms obtained by image analysis were divided into type I (peridiploid n = 43) or type II (non-diploid with a minority of cells in peridiploid region; n = 31); 5c exceeding rate, 2c deviation index and malignancy grade were also computed. Overall two year survival was 27/75 patients (36%). Stage of disease was confirmed as a predictor of outcome with only 3/17 (18%) N2 patients surviving for 2 years as compared to 24/52 (46%) patients with N0/N1 disease. Image cytometric histogram classification just reached statistical significance with type I histograms indicating a better prognosis (20/43 survivors (47%) versus 7/31 (23%) patients with type II profiles, $P < 0.05$). Flow cytometry, 5cER, 2cD1 and malignancy grade were not useful in predicting prognosis. The results do not indicate a significant role for cytometry in SCLC.

Chang, Y. S., L. Wang, et al. (2002). "Correlation between insulin-like growth factor-binding protein-3 promoter methylation and prognosis of patients with stage I non-small cell lung cancer." Clin Cancer Res **8**(12): 3669-75.

PURPOSE: The activities of insulin-like growth factors (IGFs) in regulating cell proliferation,differentiation, and apoptosis are modulated by a family of high-affinity specific IGF-binding proteins (IGFBPs), especially IGFBP-3, the most abundant IGFBP in circulation. Hypermethylation of the promoter represses the expression of the IGFBP-3 gene. The purpose of this study was to determine whether the methylation status of IGFBP-3 promoter influences the prognosis of non-small cell lung cancer (NSCLC). EXPERIMENTAL DESIGN: Eighty-three patients with pathological stage I NSCLC who had undergone curative surgery were investigated for promoter hypermethylation of IGFBP-3 by methylation-specific PCR. Statistical analyses, all two-sided, were performed to determine the prognostic effect of methylation status of the IGFBP-3 promoter on various clinical parameters. IGFBP-3 was the only molecular parameter tested on these tissues in this study. RESULTS: Hypermethylation of the IGFBP-3 promoter was found in 51 (61.5%) of the 83 tumors. The clinicopathological factors, such as age, histological type, histological grade, gender, and smoking status, of corresponding patients, did not exhibit statistically significant association with the methylation status of IGFBP-3 promoter. However, patients with a hypermethylated IGFBP-3 promoter had a significantly lower 5-year disease-specific, disease-free, and overall survival rate than did those without a methylated IGFBP-3 promoter (53.1% versus 86.1%, $P = 0.006$; 36.5% versus 76.2%, $P = 0.007$; and 38.9% versus 64.0%, $P = 0.022$, respectively). Moreover, multivariate analysis indicated that hypermethylation of the IGFBP-3 promoter was the only independent predictor for disease-free and disease-specific survival among the clinical and histological parameters tested. CONCLUSIONS: Hypermethylation of the IGFBP-3 promoter, as measured by methylation-specific PCR, is a frequent phenomenon and strongly associated with poor prognosis among patients with stage I NSCLC.

Chapman, J. A., H. L. Lickley, et al. (2006). "Ascertaining prognosis for breast cancer in node-negative patients with innovative survival analysis." Breast J **12**(1): 37-47.

Clinical decisions to administer adjuvant systemic therapy to women with early breast cancer require knowledge about baseline prognosis, which is only assessable in the absence of such adjuvant treatment, which most patients currently do receive. The Cox model is the standard tool for assessing the effect of prognostic factors; however, there may be substantive differences in the estimated prognosis obtained by the Cox model rather than a log-normal model. For more than 50 years, clinical breast cancer data for cohorts of patients have supported the choice of a log-normal model. The prognostic impact of model type is examined here for a cohort of breast cancer patients, only 7% of whom received adjuvant

systemic therapy. We quantitated prognosis utilizing Kaplan-Meier, Cox, and log-normal survival analyses for 415 consecutive T1-T3, M0, histologically node-negative patients who were operated on for primary breast cancer at Women's College Hospital between 1977 and 1986. Recurrence outside the breast for disease-free interval (DFI) and breast cancer death for disease-specific survival (DSS) were the events of interest. The patient follow-up for these investigations was 96% complete: a median 8 years for those surviving. Factors used in these investigations were age, weight, tumor size, histology, tumor grade, nuclear grade, lymphovascular invasion, estrogen receptor (ER), progesterone receptor (PR), combined ER/PR receptor, overexpression of neu oncoprotein, DNA ploidy, S-phase, and adjuvant therapy. In our study we found evidence against the Cox assumption of proportional hazards, which is not an assumption for the log-normal approach. We identified patients with greater than 96% and others with less than 40% DSS at 10 years. The difference in prognosis determined by using the Cox versus the log-normal model ranged for DFI from 1.2% to 8.1%, and for DSS from 0.4% to 6.2%; interestingly, the difference was more substantial for patients with a high risk of recurrence or death from breast cancer. Estimated prognoses may differ substantially by survival analysis model type, by amounts that might affect patient management, and we think that the log-normal model has a major advantage over the Cox model for survival analysis.

Chavez-Uribe, E., J. Cameselle-Teijeiro, et al. (2007). "Hypoploidy defines patients with poor prognosis in breast cancer." Oncol Rep **17**(5): 1109-14.

The clinicopathological features currently used in breast cancer prognosis often fail to characterize the clinical heterogeneity of the disease accurately. Our study is aimed to investigate the predictive value of DNA flow cytometry in breast cancer. Previously untreated breast carcinoma samples (584) were snap frozen for flow-cytometry. Tumors were classified into three DNA index (DI) categories: i) tumors showing a DI =0.96-1.15 (diploid and near-diploid); ii) tumors with a DI >or=1.16 (hyperdiploid, tetraploid, multiploid and/or those with more than one diploid population); and iii) tumors with a DI <or=0.95 (hypoploid). The 5- and 10-year cumulative survival rates +/- SE for Group I (n=191) were 98+/-1% and 98+/-1%. For Group II (n=361) these rates were 77+/-2% at 5 years and 63+/-5% at 10 years. In Group III (n=32) the rate at 5 years was 23+/-8%, with no patients alive at 10 years (p<0.0001). In univariate analysis, tumor size, node status, grade, karyometry, S-phase fraction, MIB-1 index, and estrogen receptors retained prognostic significance; in multivariate analysis, only DI <or=0.95 (hypoploid) was retained as an independent prognostic factor for overall survival. Our data strongly support that DNA hypoploid has a strong, independent prognostic value for predicting the short-term clinical outcome of breast carcinoma patients.

Chiang, J. W., B. Y. Karlan, et al. (2006). "BRCA1 promoter methylation predicts adverse ovarian cancer prognosis." Gynecol Oncol **101**(3): 403-10.

OBJECTIVE: To compare the clinical outcome of ovarian cancer patients whose tumors contain BRCA1 genes silenced by promoter hypermethylation to patients with germline BRCA1 mutations and to patients with wild-type BRCA genes. METHODS: Ovarian cancers from a hospital-based tumor bank were characterized as having a BRCA1 mutation; or a methylated BRCA1, BRCA1 pseudogene or MLH1 promotor; or a wild-type BRCA gene. Survival of patients with methylated BRCA1 promoters (N = 11) was compared to that of patients with wild-type BRCA genes (N = 30) and BRCA1 mutations (N = 22). A methylator phenotype was defined to include tumors with hypermethylation of BRCA1, hMLH1 and/or dBRCA1 pseudogene promoters (N = 23). RESULTS: All cohorts had comparable clinical factors except for age at diagnosis. Median age of methylated BRCA1 and wild-type BRCA patients was older than BRCA1 mutation carriers (60 and 63 versus 48 years; P = 0.04). The median disease-free interval was significantly shorter for patients with a methylated BRCA1 promoter (9.8 months) than for BRCA1 mutation carriers (39.5 months; P = 0.04). Median overall survival was also significantly shorter for patients with a methylated BRCA1 promoter (35.6 months) than BRCA1 mutation carriers (78.6 months; P = 0.02). The combined methylator phenotype cohort had significantly shorter survival (36.1 months) compared to wild-type BRCA patients (63.3 months; P = 0.02). CONCLUSION: These data suggest that methylation of the BRCA1 promoter is associated with poor patient outcome. BRCA1 may be part of a global panel of methylated genes associated with aggressive disease.

Choma, D., J. P. Daures, et al. (2001). "Aneuploidy and prognosis of non-small-cell lung cancer: a meta-analysis of published data." Br J Cancer **85**(1): 14-22.

In lung cancer, DNA content abnormalities have been described as a heterogeneous spectrum of impaired tumour cell DNA histogram patterns. They are merged into the common term of aneuploidy and probably reflect a high genotypic instability. In non-small-cell lung cancer, the negative effect of aneuploidy has been a subject of controversy

inasmuch as studies aimed at determining the survival-DNA content relationship have reported conflicting results. We made a meta-analysis of published studies aimed at determining the prognostic effect of aneuploidy in surgically resected non-small-cell lung cancer. 35 trials have been identified in the literature. A comprehensive collection of data has been constructed taking into account the following parameters: quality of specimen, DNA content assessment method, aneuploidy definition, histology and stage grouping, quality of surgical resection and demographic characteristics of the analysed population. Among the 4033 assessable patients, 2626 suffered from non-small-cell lung cancer with aneuploid DNA content (overall frequency of aneuploidy: 0.65; 95% CI: (0.64-0.67)). The DerSimonian and Laird method was used to estimate the size effects and the Peto and Yusuf method was used in order to generate the odds ratios (OR) of reduction in risk of death for patients affected by a nearly diploid (non-aneuploid) non-small-cell lung cancer. Survivals following surgical resection, from 1 to 5 years, were chosen as the end-points of our meta-analysis. Patients suffering from a nearly diploid tumour benefited from a significant reduction in risk of death at 1, 2, 3 and 4 years with respective OR: 0.51, 0.51, 0.45 and 0.67 (P< 10(-4)for each end-point). 5 years after resection, the reduction of death was of lesser magnitude: OR: 0.87 (P = 0.08). The test for overall statistical heterogeneity was conventionally significant (P< 0.01) for all 5 end-points, however. None of the recorded characteristics of the studies could explain this phenomenon precluding a subset analysis. Therefore, the DerSimonian and Laird method was applied inasmuch as this method allows a correction for heterogeneity. This method demonstrated an increase in survival at 1, 2, 3, 4 and 5 years for patients with diploid tumours with respective size effects of 0.11, 0.15, 0.20, 0.20 and 0.21 (value taking into account the correction for heterogeneity;P< 10(-4)for each end-point). Patients who benefit from a surgical resection for non-small-cell lung cancer with aneuploid DNA content prove to have a higher risk of death. This negative prognostic factor decreases the probability of survival by 11% at one year, a negative effect deteriorating up to 21% at 5 years following surgery.

Clark, A. J., R. Barnetson, et al. (2004). "Prognosis in DNA mismatch repair deficient colorectal cancer: are all MSI tumours equivalent?" Fam Cancer **3**(2): 85-91.

Microsatellite instability (MSI) in colorectal tumours is the hallmark of defective DNA mismatch repair (MMR) and high level MSI can be detected in up to 15% of incident colorectal cancers. MSI in sporadic colorectal tumours is primarily due to epigenetic silencing of MLH1 while MSI is almost universal in tumours from HNPCC family members due to germline MMR gene mutation with loss or mutational inactivation of the second copy as a somatic event. There is evidence that tumour MSI is associated with a better outcome than the generality of large bowel malignancy. However, although MSI occurs in both sporadic colorectal cancer and in tumours arising in patients with germline MMR gene mutations, cancer survival should not be considered to be equivalent for these two groups with MSI tumours simply because both exhibit similarities in molecular phenotype. Here, we review the evidence on prognosis in patients with sporadic MSI tumours compared to those who have inherited a germline DNA MMR repair gene defect. In addition, we explore whether there are variables that afford opportunity to distinguish three groups on the basis of MSI status, namely: sporadic MSI tumours; MSI tumours in carriers of germline MMR gene defects; microsatellite stable (MSS) tumours. Differences in prognosis between these three groups is important because it underpins the rationale for surveillance and early identification of tumours in MMR gene carriers, as well as refining understanding of the influence of MSI on cancer progression. Furthermore, we discuss the effect of MSI on the effectiveness of chemotherapy regimens.

Creighton, C. J., A. Casa, et al. (2008). "Insulin-like growth factor-I activates gene transcription programs strongly associated with poor breast cancer prognosis." J Clin Oncol **26**(25): 4078-85.

PURPOSE: Substantial evidence implicates insulin-like growth factor-I (IGF-I) signaling in the development and progression of breast cancer. To more clearly elucidate the role of IGF in human breast cancer, we identified and then examined gene expression patterns of IGF-I-treated breast cancer cells. METHODS: MCF-7 cells were stimulated with IGF-I for 3 or 24 hours and were profiled for greater than 22,000 RNA transcripts. We defined an IGF-I signature pattern of more than 800 genes that were up- or downregulated at both time points. The gene signature was examined in clinical breast tumors and in experimental models that represented other oncogenic pathways. The signature was correlated with clinical and pathologic variables and with patient outcome. RESULTS: IGF-I caused temporal changes in gene expression that were strongly associated with cell proliferation, metabolism, and DNA repair. Genes with early and sustained regulation by IGF-I were highly enriched for transcriptional targets of the estrogen receptor (ER), Ras/extracellular signal-related kinase 1/2, and phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin pathways.

In three large, independent data sets of profiled human breast tumors, the IGF-I signature was manifested in the majority of ER-negative breast tumors and in a subset (approximately 25%) of ER-positive breast tumors. Patients who had tumors that manifested the IGF-I signature (including patients who did not receive adjuvant therapy) had a shorter time to a poor outcome event. The IGF gene signature was highly correlated with numerous poor prognostic factors and was one of the strongest indicators of disease outcome. CONCLUSION: Transcriptional targets of IGF-I represent pathways of increased aggressiveness and possibly hormone independence in clinical breast cancers.

Davis, J. R., S. Aristizabal, et al. (1989). "DNA ploidy, grade, and stage in prognosis of uterine cervical cancer." Gynecol Oncol **32**(1): 4-7.

A retrospective study of 56 cases of uterine cervical squamous carcinoma evaluated DNA content, histological grade, and clinical stage as indicators of prognosis. Minimum survivor follow-up was 24 months. Following standard radiation therapy, there were 40 cures and 16 treatment failures. DNA content was measured by flow cytometry of pretreatment biopsies removed from paraffin. There were 18 diploid cases and 38 aneuploid (67.9%). Aneuploid cases included 6 with very high G2-M peaks (greater than or equal to 15% of the cell sample). DNA ploidy correlation with prognosis was not statistically significant. However, both grading by a multiple parameter method (P less than 0.0133) and staging (P less than 0.0064) were significant prognostic factors. Higher grade and stage correlated with treatment failure.

de Sa, V. K., F. C. Canavez, et al. (2009). "Isoforms of hyaluronidases can be a predictor of a prostate cancer of good prognosis." Urol Oncol **27**(4): 377-81.

INTRODUCTION: Hyaluronidases (HAases) are enzymes related to cancer progression. Isoforms of HAases have been described as products of alternative splicing responsible for differences in enzyme activity. The heterogeneity of HAase expression has been identified in tumors and could be related to the differences in their biological behavior. METHODS: Thirty-seven patients subjected to radical prostatectomy for prostate cancer were divided into 2 groups for the analyses: Low (< or =6-18) and high (> or =7-19) Gleason score and tumor behavior; recurrence 15 and nonrecurrence 22, mean follow-up 52.6 months. CONCLUSION: A profile of HAase related to low Gleason score and non-tumor recurrence was characterized by expression of HYAL3-v1, HYAL1-v3, and HYAL3-v2. More studies should be made in order to confirm with larger series.

Deshpande, N., I. Mitchell, et al. (1983). "Deoxyribonucleic acid (DNA) content of carcinomas and prognosis in human breast cancer." Int J Cancer **32**(6): 693-6.

DNA content was estimated in 705 breast carcinomas from patients with stage I or stage II disease undergoing mastectomy, to investigate whether it would predict the clinical course of the disease. The patients were then followed for up to 84 months during which time 166 of them developed recurrences. There were no statistically significant differences in the DNA content of carcinomas between stage I and stage II patients or between those with various numbers of involved nodes. The tumours from pre-menopausal patients as a group had significantly higher DNA content than those from post-menopausal cases. There was a gradual rise in DNA content with the malignancy grade of the carcinoma which showed significant differences between Grades I and III and between II and III. Life-table analyses showed that the measurement of DNA would not be an aid to prognosis. Furthermore, when the data were stratified according to menopausal status, stage, malignancy grade or numbers of lymph nodes involved, the results indicated that these estimation do not add significantly to the information obtained through established prognostic factors. Survival data were available on III patients with recurrent disease. There were no significant differences in tumour DNA content between short- and long-term survivors. It is concluded that these measurements seem unlikely to be a prognostic factor in human breast cancer.

Dworkin, A. M., T. H. Huang, et al. (2009). "Epigenetic alterations in the breast: Implications for breast cancer detection, prognosis and treatment." Semin Cancer Biol **19**(3): 165-71.

Epigenetic alterations of the genome such as DNA promoter methylation and chromatin remodeling play an important role in tumorigenesis. Recent findings indicate epigenetic modifications as key factors in breast carcinogenesis. These modifications are quite appealing as targets for preventative care and therapeutics because of their potential for reversal. Future medical care for breast cancer patients will likely depend upon a better understanding of the roles epigenetic modifications play in carcinogenesis. Here, we discuss the importance of epigenetics in breast cancer detection, prognosis, and therapy with an emphasis on mechanisms and epigenetic contributions to field cancerization effects.

Eder, A. M., X. Sui, et al. (2005). "Atypical PKCiota contributes to poor prognosis through loss of apical-basal polarity and cyclin E overexpression in ovarian cancer." Proc Natl Acad Sci U S A **102**(35): 12519-24.

We show that atypical PKCiota, which plays a critical role in the establishment and maintenance of epithelial cell polarity, is genomically amplified and overexpressed in serous epithelial ovarian cancers. Furthermore, PKCiota protein is markedly increased or mislocalized in all serous ovarian cancers. An increased PKCiota DNA copy number is associated with decreased progression-free survival in serous epithelial ovarian cancers. In a Drosophila in vivo epithelial tissue model, overexpression of persistently active atypical PKC results in defects in apical-basal polarity, increased Cyclin E protein expression, and increased proliferation. Similar to the Drosophila model, increased PKCiota proteins levels are associated with increased Cyclin E protein expression and proliferation in ovarian cancers. In nonserous ovarian cancers, increased PKCiota protein levels, particularly in the presence of Cyclin E, are associated with markedly decreased overall survival. These results implicate PKCiota as a potential oncogene in ovarian cancer regulating epithelial cell polarity and proliferation and suggest that PKCiota is a novel target for therapy.

Elledge, R. M., G. M. Clark, et al. (1994). "Tumor biologic factors and breast cancer prognosis among white, Hispanic, and black women in the United States." J Natl Cancer Inst **86**(9): 705-12.

BACKGROUND: In the United States, prognosis and survival after the diagnosis of breast cancer is poorer among black patients and, to a lesser extent, among Hispanic patients, compared with white patients. Patients who are black or Hispanic have been reported to present with higher stage or more advanced disease. Even after adjusting for stage, however, survival rates are lower for blacks but not for Hispanics. PURPOSE: Our purpose was to compare survival, age, tumor size, nodal status, estrogen-receptor (ER) and progesterone-receptor (PgR) status, histologic type, S-phase fraction, DNA ploidy status, HER-2/neu protein expression, and p53 protein status, along with systemic treatment, in a large group of white, black, and Hispanic U.S. women. METHODS: From 1970 to 1991, breast tumor specimens were submitted to The University of Texas Health Science Center from 31 contributing hospitals throughout the United States for ER and PgR assay. A total of 4885 white, 1016 black, and 777 Hispanic women were eligible for this study. Median follow-up was 57 months. RESULTS: Overall, white women were significantly more likely to be older and to have smaller tumors, have less lymph node involvement, have tumors with positive ER and PgR status, and have a lower S-phase fraction compared with Hispanic or black women. There were no clinically important differences in DNA ploidy, histologic type, HER-2/neu, and p53 expression among the three groups. Considering all stages, white women had the best overall survival (date of diagnosis to date of death) at 5 years--75% +/- 1% (means +/- SE), with a median survival of 166 months, but Hispanic women had an intermediate survival--70% +/- 2% (median survival, 156 months), and black women had the worst survival--65% +/- 2% (median survival, 117 months) ($P < .0001$). For node-negative patients, there was no significant difference in disease-free survival (date of diagnosis to date of first recurrence) or overall survival, although blacks tended to have a worse prognosis. For node-positive or locally advanced disease and for metastatic disease, blacks had significantly ($P < .0001$) worse disease-free and overall survival than did white or Hispanic women. Differences in the use of systemic therapy did not explain these outcomes. CONCLUSION: A number of biologic factors associated with poor prognosis are found with a significantly increased frequency in breast tumors from Hispanic and, particularly, from black women. Tumors with a more aggressive biology could lead to a higher stage at diagnosis and a poorer survival for the group as a whole.

Emi, M., M. Yoshimoto, et al. (1999). "Allelic loss at 1p34, 13q12, 17p13.3, and 17q21.1 correlates with poor postoperative prognosis in breast cancer." Genes Chromosomes Cancer **26**(2): 134-41.

Allelic losses of tumor suppressor genes (TSGs), or the chromosomal regions harboring them, in tumor DNA may become useful postoperative prognostic indicators. To examine whether specific allelic losses might correlate with postoperative survival in a 5-year prospective follow-up, we tested tumors from a cohort of 264 breast cancer patients for allelic losses of 18 microsatellite markers representing either a known TSG or a region where genetic alterations are frequent in breast tumors. Patients whose tumors had lost an allele at 1p34, 13q12, 17p13.3, or 17q21.1 had significantly higher risks of postoperative mortality than those whose tumors retained both alleles at those loci (at 1p34, a 5-year mortality rate of 29% among patients with losses vs. 7% with retentions, $P = 0.0008$; at 13q12, 31% vs. 10%, $P = 0.0062$; at 17p13.3, 24% vs. 13%, $P = 0.026$; and at 17q21.1, 31% vs. 13%, $P = 0.0047$). Furthermore, combined losses at 13q12 and 17p13.3 increased the predicted postoperative mortality risks by a factor of 9.6 (5-year mortality rate of 42% vs. 5%

with retentions, P = 0.0001), and combined losses at 1p34 and 17p13.3 raised the predicted postoperative mortality risks by a factor of 8.6 (27% vs. 3%, P = 0.0064). We conclude that allelic losses at these loci can serve as negative prognostic indicators to guide postoperative management of patients. Genes Chromosomes Cancer 26:134-141, 1999.

Esteller, M., S. Gonzalez, et al. (2001). "K-ras and p16 aberrations confer poor prognosis in human colorectal cancer." J Clin Oncol **19**(2): 299-304.

PURPOSE: Mutations in the K-ras gene are frequent in human cancer. ras activation in primary cells results in a cellular senescence phenotype that is precluded by inactivation of p16. At the clinical level, this may imply a differential behavior for tumors with alternative or cooperative activation of K-ras function and impairment of p16 pathways. PATIENTS AND METHODS: We have determined the presence of mutations in the K-ras gene and the methylation status of p16 promoter in a series of 119 prospectively collected colorectal carcinomas. p53 mutations and p14 alternative reading frame methylation status were also assessed. Associations with survival were investigated. RESULTS: K-ras mutations were present in 44 (38%) of 115 cases, and p16 methylation was present in 42 (37%) of 113 cases. p53 mutations were detected in 50% (56 of 115) and p14 methylation in 29% (32 of 112) of cases. K-ras and p16 alterations were independent genetic events. Presence of K-ras or p16 genetic alterations (analyzed independently) was associated with shorter survival, although differences were not statistically significant. Cox analysis of the two variables combined showed a diminished survival as the results of an interaction between p16 and K-ras. Alternative alteration of K-ras and p16 genes was an independent prognostic factor in human colorectal cancer in univariate and multivariate analysis. Differences were maintained when cases undergoing radical surgery and without distant metastases were considered. CONCLUSION: These results suggest that the combined K-ras and p16 analyses may be of prognostic use in human colorectal cancer.

Ewers, S. B., B. Baldetorp, et al. (1989). "Flow cytometry DNA ploidy and number of cell populations in the primary breast cancer and their correlation to the prognosis." Acta Oncol **28**(6): 913-8.

In a prospective study on 516 breast cancer patients flow cytometry DNA ploidy and number of cell populations (defined as number of DNA stem lines) detected in the primary tumor were evaluated for prognostic purposes. The median follow-up time was about 5 years. In the 241 node negative cases, those patients with three or more cell populations had the worst prognosis, with a distant recurrence-free survival rate of about 60% at five years compared to 90% in cases with only one cell population detected in the primary tumor. The number of tumor involved axillary lymph nodes was the outstanding prognostic indicator which was confirmed in 275 node positive patients; DNA ploidy and number of cell populations did not give any significant prognostic information in this group of patients.

Fasching, P. A., C. R. Loehberg, et al. (2008). "Single nucleotide polymorphisms of the aromatase gene (CYP19A1), HER2/neu status, and prognosis in breast cancer patients." Breast Cancer Res Treat **112**(1): 89-98.

PURPOSE: Estrogen exposure is involved in both breast cancer susceptibility and the prognosis in patients with breast cancer. Aromatase is involved in the production of estrogens, and altered expression of it might be associated with the prognosis. The aim of this study was to examine the effect of single nucleotide polymorphisms (SNPs) in the aromatase gene, CYP19A1, on the prognosis, and in relation to tumor and patient characteristics in a cohort of breast cancer patients. PATIENTS AND METHODS: The cohort analyzed in this study consisted of 1,257 patients with invasive primary breast cancer. Polymorphisms rs10046, rs4646 and rs700519 were genotyped within this group. RESULTS: The variant genotypes of rs10046 and rs4646 were associated with a lower percentage of HER2-positive tumors. There was no association of rs700519 and rs4646 with disease-free survival (DFS) or overall survival (OS). The variant genotype of rs10046 was significantly associated with a better 5-year DFS (hazards ratio 0.51; 95% CI, 0.32 to 0.81; P=0.004) adjusted for age, nodal status, tumor size grading, and hormone receptor status. This effect appeared to be determined in the subgroup of premenopausal patients. CONCLUSION: SNPs rs10046 and rs4646 may influence the HER2 status of breast cancer tumors, and rs10046 genotypes are associated with an altered DFS. Genotypes of aromatase polymorphisms may influence the prognosis for breast cancer patients not only by affecting the extent of estrogen exposure but also through an alteration in tumor characteristics.

Fluge, O., K. Gravdal, et al. (2009). "Expression of EZH2 and Ki-67 in colorectal cancer and associations with treatment response and prognosis." Br J Cancer **101**(8): 1282-9.

BACKGROUND: Enhancer of zeste homologue 2 (EZH2) is a member of the Polycomb group of genes that is involved in epigenetic silencing and cell cycle regulation. METHODS: We studied EZH2 expression in 409 patients with colorectal

cancer stages II and III. The patients were included in a randomised study, and treated with surgery alone or surgery followed by adjuvant chemotherapy. RESULTS: EZH2 expression was significantly related to increased tumour cell proliferation, as assessed by Ki-67 expression. In colon cancer, strong EZH2 expression (P=0.041) and high proliferation (>or=40%; P=0.001) were both associated with better relapse-free survival (RFS). In contrast, no such associations were found among rectal cancers. High Ki-67 staining was associated with improved RFS in colon cancer patients who received adjuvant chemotherapy (P=0.001), but not among those who were treated by surgery alone (P=0.087). In colon cancers stage III, a significant association between RFS and randomisation group was found in patients with high proliferation (P=0.046), but not in patients with low proliferation (P=0.26). Multivariate analyses of colon cancers showed that stage III (hazard ratio (HR) 4.00) and high histological grade (HR 1.80) were independent predictors of reduced RFS, whereas high proliferation indicated improved RFS (HR 0.55). CONCLUSION: Strong EZH2 expression and high proliferation are associated features and both indicate improved RFS in colon cancer, but not so in rectal cancer.

Fordyce, C. A., C. M. Heaphy, et al. (2006). "Telomere content correlates with stage and prognosis in breast cancer." Breast Cancer Res Treat **99**(2): 193-202.

PURPOSE: To evaluate the hypothesis that telomere DNA content (TC) in breast tumor tissue correlates with TNM staging and prognosis. EXPERIMENTAL DESIGN: Slot blot assay was used to quantitate TC in 70 disease-free normal tissues from multiple organ sites, and two independent sets of breast tumors containing a total of 140 samples. Non-parametric Rank-Sums tests, logistic regression and Cox proportional hazards models were used to evaluate the relationships between TC and tumor size, nodal involvement, TNM stage, 5-year survival and disease-free interval. RESULTS: TC in 95% of normal tissues was 75-143% of that in the placental DNA standard, whereas only 50% of tumors had TC values in this range. TC was associated with tumor size (p=0.02), nodal involvement (p<0.0001), TNM stage (p=0.004), 5-year overall survival (p=0.0001) and 5-year disease-free survival (p=0.0004). A multivariable Cox model was developed using age at diagnosis, TNM stage and TC as independent predictors of breast cancer-free survival. Relative to the high TC group (>123% of standard), low TC (<101% of standard) conferred an adjusted relative hazard of 4.43 (95% CI 1.4-13.6, p=0.009). Receiver operating characteristic curves using thresholds defined by the TC distribution in normal tissues predicted 5-year breast cancer-free survival with 50% sensitivity and 95% specificity, and predicted death due to breast cancer with 75% sensitivity and 70% specificity. CONCLUSIONS: TC in breast cancer tissue is an independent predictor of clinical outcome and survival interval, and may discriminate by stage.

Gawrychowski, J., B. Lackowska, et al. (2003). "Prognosis of the surgical treatment of patients with non-small cell lung cancer (NSCLC)--relation to DNA ploidy." Eur J Cardiothorac Surg **23**(6): 870-7; discussion 877.

OBJECTIVE: The aim of this study was to evaluate prognostic importance of cell ploidy and proliferation activity in non-small cell lung cancers. Survivals were compared according to the following factors: sex, age, histology, grading, DNA ploidy, tumour size, T factor, N factor and operative procedure. METHODS: In a group of 191 patients in whom cytofluorometric examinations had been performed on archival tumour specimens, postoperative recurrences were observed. RESULTS: Postoperative recurrence was observed in 64 (64.6%) of 99 patients with aneuploid tumours and in 35 (38.0%) of 92 with diploid tumours (P<0.001). Overall survival (OS) rates for the group of 92 patients operated for diploid non-small cell lung cancer (NSCLC) at 5 and 10 years were 62 and 51.1%, whereas of other 99, operated for aneuploid tumours 33.3 and 25.9%, respectively (P<0.001). In the former group of patients disease-free survival (DFS) rates at 5 and 10 years were 58.7 and 51.4% but in the latter 29.3 and 26%, respectively (P=0.00014). Significant differences dependent on cell ploidy were also observed in OS and DFS rates of patients operated respectively for SCLC (P=0.0029; P=0.00318) and adenocarcinoma (AC; P=0.0241; P=0.02109). In general, the mean percentage of S-phase cells in non-small cell lung cancers was 14.0% (SD=13.1) in patients who survived 5 years, and 22.4% (SD=15.7) in those who had a recurrence or died (P<0.001). CONCLUSIONS: In our opinion the most important finding of our work is that determination of cell ploidy in NSCLC provides a valuable supplement to the TNM stage when evaluating late results of the surgical treatment. However, the paper demonstrates that aneuploidy, although unfavourable, is not an independent prognostic factor in the group of patients with NSCLC and in the subgroups - both with squamous cell carcinoma and adenocarcinoma. Our results show also that the percentage of S-phase cells is an independent, unfavourable prognostic factor in patients treated surgically for non-small cell lung cancer and in the subgroup with squamous cell lung carcinoma.

Gilchrist, K. W., R. Gray, et al. (1993). "High DNA content and prognosis in lymph node positive breast cancer. A case control study by the University of Leiden and ECOG. (Eastern Cooperative Oncology Group)." Breast Cancer Res Treat **28**(1): 1-8.

To investigate whether breast cancer cells with unusually high nuclear DNA content are associated with an adverse outcome, Eastern Cooperative Oncology Group investigators selected breast cancer trial patients who suffered an early death (ED) within two years after diagnosis to compare with other trial patients who had a survival of at least 7.5 years. Paraffin blocks of primary breast cancers were obtained from 93 evaluable patients who had been enrolled in two surgical adjuvant trials for lymph node positive (LN+) disease (T1-3N1M0). Single cell monolayer preparations from these blocks were stained with acriflavine-Feulgen and analyzed by image analysis for DNA content with the automated Leiden Television Analysis System (LEY-TAS). Standard prognostic variables (estrogen receptor (ER) status, number of lymph nodes with metastases, and size of the cancer) were compared with three DNA content characteristics: DNA ploidy status, number of nuclei with > 5C DNA content, and percent of nuclei with > 5 C. Estimates of the odds ratio in multivariate comparisons showed that ER negativity was associated with ED (p = 0.0005) and an odds ratio estimate using negative/positive of 4.87. The number of positive lymph nodes associated with ED had a p-value of 0.0005 and an odds ratio estimate of 4.63 when comparing the > 3 nodes group to the 1-3 nodes group. In contrast, the strongest association for any of the DNA content characteristics with ED had a p-value of 0.017 and an odds ratio estimate of 2.76. This power of association disappeared when stratified on ER status.(ABSTRACT TRUNCATED AT 250 WORDS)

Giltnane, J. M., C. B. Moeder, et al. (2009). "Quantitative multiplexed analysis of ErbB family coexpression for primary breast cancer prognosis in a large retrospective cohort." Cancer **115**(11): 2400-9.

BACKGROUND: Assessment of outcome using a single prognostic or predictive marker is the current basis of targeted therapy, but is inherently limited by its simplicity. Multiplexing has provided better classification, but has only been done quantitatively using RNA or DNA. Automated quantitative analysis is a new technology that allows quantitative in situ assessment of protein expression. The authors hypothesized that multiplexed quantitative measurement of ErbB receptor family proteins may allow better prediction of outcome. METHODS: The authors quantitatively assessed the expression of 6 proteins in 4 subcellular compartments in 676 patients using breast carcinoma tissue microarrays. Then using Cox proportional hazards modeling and unsupervised hierarchical clustering, they assessed the prognostic value of the expression singly and multiplexed. RESULTS: Epidermal growth factor receptor (EGFR), HER-2, and HER-3 expression were associated with decreased survival. Multivariate analysis showed high HER-2 and HER-3 expression maintained independence as prognostic markers. Hierarchical clustering of expression data defined a small class enriched for HER-2 expression with 40% 10-year survival, compared with 55% using conventional methods. Clustering also revealed a similarly poor-prognostic subgroup coexpressing EGFR and HER-3 (but low for estrogen receptor, progesterone receptor, and HER-2) with a 42% 10-year survival. CONCLUSIONS: This work shows that the combined analysis of protein expression improved prognostic classification, and that multiplexed models were superior to any single-marker-based method for prediction of 10-year survival. These methods illustrate a protein-based, multiplexed approach that could more accurately identify patients for targeted therapies.

Gnant, M. F., G. H. Blijham, et al. (1993). "Aneuploidy fraction but not DNA index is important for the prognosis of patients with stage I and II breast cancer--10-year results." Ann Oncol **4**(8): 643-50.

BACKGROUND: Individual assessment of the prognosis of patients with breast cancer is crucial for the selection of risk-adapted adjuvant therapy and in follow-up. Parameters from DNA flow-cytometry have been shown to provide significant prognostic information, but published results are in conflict and there are only a few investigations with long-term follow-up. The aim of this study is to clarify the impact of tumor DNA data on the clinical course of stage I and stage II breast cancer patients. PATIENTS AND METHODS: Several flow-cytometry DNA analyses were performed on tumor samples derived from 191 breast cancer patients entered in a controlled clinical trial after a median follow-up of more than 10 years. In addition to DNA index (DNI) and the percentage of cells in S phase (SPF), an index, designated aneuploidy fraction (AF), was determined. It ascertains the percentage of aneuploid cells out of all cells in the DNA flow-cytometry histogram, and its reproducibility has been tested by measurements of AF in two different samples of the same tumor. Univariate analyses and, in the 122 patients for whom complete information was available, a Cox model, were performed to investigate the individual prognostic impact of flow-cytometry parameters compared with established clinical factors. RESULTS:

AF proved to be a very valuable prognostic indicator both in univariate and multivariate analyses, whereas DNI and SPF failed to provide independent prognostic information. The combination of AF and lymph node status clearly identifies different prognostic subgroups in operable breast cancer. CONCLUSIONS: Routine evaluation of patients with breast cancer should include tumor DNA flow-cytometry. Aneuploidy fraction is a valuable tool in assessing an individual patient's prognosis and thus can help in the choice of the appropriate adjuvant treatment strategy. Whether it, rather than DNI and SPF should be used, as we found, needs to be validated in a larger prospective investigation.

Goh, H. S., J. R. Jass, et al. (1987). "Value of flow cytometric determination of ploidy as a guide to prognosis in operable rectal cancer: a multivariate analysis." Int J Colorectal Dis **2**(1): 17-21.

The DNA content of 203 cases of rectal cancer, with at least 15 years follow-up, was analysed by flow cytometry. One hundred and twenty-nine (64%) were DNA aneuploid and corrected survivals were significantly influenced by ploidy distribution (p less than 0.01). The DNA content and details of stage and grade were subjected to multivariate analysis using the Cox regression model. Ploidy was entered into models including Dukes' stage alone, tumour differentiation alone, Dukes' stage and differentiation in combination and a more comprehensive range of discrete stage- and grade-related parameters. All four models demonstrated its independent contribution to survival. However, its contribution was very small (5%, 18%, 5%, 4% respectively). These findings illustrate that results based on new technological developments should not be viewed in isolation, but their values must be assessed in combination with traditionally available data by means of multivariate analysis.

Goodheart, M. J., J. M. Ritchie, et al. (2005). "The relationship of molecular markers of p53 function and angiogenesis to prognosis of stage I epithelial ovarian cancer." Clin Cancer Res **11**(10): 3733-42.

PURPOSE: Multiple angiogenic factors may influence tumor progression and metastasis. Several are modified by the p53 gene. We sought to identify molecular markers for high-risk stage I epithelial ovarian cancers. EXPERIMENTAL DESIGN: Seventy-seven consecutive stage I epithelial ovarian cancers were evaluated for p53, CD31 microvessel density, thrombospondin-1, vascular endothelial growth factor (VEGF), p21 immunohistochemical staining, and p53 gene mutations. Molecular marker impact upon disease-specific survival, disease recurrence, and distant recurrence was evaluated with Cox regression. RESULTS: There were 12 deaths from disease. Twelve of the 77 tumors contained p53 mutations-10 missense and 3 null (one tumor had two mutations). Fesddration Internationale des Gynaecologistes et Obstetristes substage (IA/IB versus IC; $P < 0.001$) and VEGF staining ($P = 0.02$) were significant in bivariate models with relationship to disease-specific survival. Stage ($P = 0.0004$), grade ($P = 0.008$), histology ($P = 0.0025$), p53 dysfunction (positive stain and/or mutation; $P = 0.048$), and microvessel density ($P = 0.04$) were significant in bivariate models with relationship to time to recurrence. In multivariate analyses among stage IC patients, failure to receive chemotherapy and microvessel density were associated with disease-specific survival, time to recurrence, and time to distant recurrence with hazard ratios of 4.8 to 44.1. CONCLUSIONS: The p53-dependent molecular markers of angiogenesis are of limited utility in developing a clinical strategy for postoperative management of stage I ovarian carcinoma. Microvessel density impacts survival and metastasis for high-risk stage IC disease. Adjuvant chemotherapy is necessary, but not sufficient, for cure of high-risk stage I epithelial ovarian cancers.

Hall, P., A. Ploner, et al. (2006). "Hormone-replacement therapy influences gene expression profiles and is associated with breast-cancer prognosis: a cohort study." BMC Med **4**: 16.

BACKGROUND: Postmenopausal hormone-replacement therapy (HRT) increases breast-cancer risk. The influence of HRT on the biology of the primary tumor, however, is not well understood. METHODS: We obtained breast-cancer gene expression profiles using Affymetrix human genome U133A arrays. We examined the relationship between HRT-regulated gene profiles, tumor characteristics, and recurrence-free survival in 72 postmenopausal women. RESULTS: HRT use in patients with estrogen receptor (ER) protein positive tumors ($n = 72$) was associated with an altered regulation of 276 genes. Expression profiles based on these genes clustered ER-positive tumors into two molecular subclasses, one of which was associated with HRT use and had significantly better recurrence free survival despite lower ER levels. A comparison with external data suggested that gene regulation in tumors associated with HRT was negatively correlated with gene regulation induced by short-term estrogen exposure, but positively correlated with the effect of tamoxifen. CONCLUSION: Our findings suggest that post-menopausal HRT use is associated with a distinct gene expression profile related to better recurrence-free survival and lower ER protein levels. Tentatively,

HRT-associated gene expression in tumors resembles the effect of tamoxifen exposure on MCF-7 cells.

Harima, Y., S. Sawada, et al. (2001). "Chromosome 6p21.2, 18q21.2 and human papilloma virus (HPV) DNA can predict prognosis of cervical cancer after radiotherapy." Int J Cancer **96**(5): 286-96.

Loss of heterozygosity (LOH) is one of the most important mechanisms for inactivation of tumor-suppressor genes. Studies of LOH in patients with cervical carcinoma have reported a high frequency of LOH on 3p21.3, 6p21.2, 17p13.1, and 18q21.2. Our study explored whether p53 status, human papilloma virus (HPV), and LOH on chromosome 3p21.3, 6p21.2, 17p13.1, and 18q21.2 are associated with treatment outcome in 65 patients with cervical cancer after radiotherapy. Tumors and normal DNA were analyzed by polymerase chain reaction (PCR) for genetic losses at 10 polymorphic microsatellite loci. The presence of HPV and its type were analyzed by PCR-based assay using the consensus primers for E6, E7, and L1 region. Mutations of the p53 gene were identified by a single-strand conformation polymorphism analysis. Chromosomes 3p21.3, 6p21.2, 17p13.1, and 18q21.2 were involved in the LOH in 23.1%, 41.5%%, 33.8%, and 23.1% of the tumors in our study, respectively. HPV-positive tumors were found in 73.8% of the patients and p53 mutation in 10.8%. The patients with LOH on chromosome 6p21.2 and 18q21.2 survived significantly shorter compared with those without LOH on chromosome 6p21.2 and 18q21.2 in both the overall survival (P = 0.006 and P = 0.007) and the disease-free survival (P = 0.005 and P = 0.008). The HPV-negative patients survived significantly shorter compared with the HPV-positive patients in both the overall survival (P = 0.01) and the disease-free survival (P = 0.04). According to multivariate analysis, HPV status (P = 0.0004, P = 0.01), LOH on 6p21.2 (P = 0.006, P = 0.02), and LOH on 18q21.2 (in both P = 0.01) is a significant predictor of both overall and disease-free survival time. The results of our study suggest that absence of HPV infection, LOH on 6p21.2, and LOH on 18q21.2 are the most important determinants of outcome of patients with cervical carcinoma after radiotherapy.

Harima, Y., S. Sawada, et al. (2002). "Human papilloma virus (HPV) DNA associated with prognosis of cervical cancer after radiotherapy." Int J Radiat Oncol Biol Phys **52**(5): 1345-51.

PURPOSE: The importance of human papilloma virus (HPV) infection in the outcome of cervical cancer after radiotherapy remains unknown. Our study explored whether the HPV status of tumors is associated with the outcome of radiotherapy in patients with cervical cancer. METHODS AND MATERIALS: A total of 84 patients with cervical cancer (6 Stage I, 10 Stage II, 49 Stage III, and 19 Stage IV) who underwent definitive radiotherapy between January 1995 and June 2000 were included in this study. Tumor samples were obtained from all patients by punch biopsy before radiotherapy. The presence of HPV and its type were analyzed by polymerase chain reaction (PCR) based assay using the consensus primers for E6 and L1 regions. Actuarial methods were used to calculate overall survival and disease-free survival. RESULTS: A total of 42 patients (50%) had cancer recurrence after radiotherapy. HPV-positive tumors were found in 76.2% (64 cases) of patients. HPV-negative patients survived for significantly shorter time periods compared to the HPV-positive patients in the overall survival (p = 0.007) and the disease-free survival (p = 0.005). According to multivariate analysis, HPV status is a significant predictor of both overall (p = 0.02) and disease-free survival time (p = 0.005). CONCLUSION: The results of this study suggest that HPV-negative patients with cervical carcinoma have a significantly poorer prognosis after radiotherapy, and HPV status may be used as a marker to optimize the treatment of patients with this type of cancer.

Hebbar, M., A. Adenis, et al. (2009). "E-selectin gene S128R polymorphism is associated with poor prognosis in patients with stage II or III colorectal cancer." Eur J Cancer **45**(10): 1871-6.

Some host-related factors may predict the risk of metastasis after surgery of colorectal cancer (CRC). The endothelial adhesion molecule E-selectin is implicated in the metastatic spread of CRC. We postulated that some polymorphisms within the E-selectin gene, especially the S128R polymorphism, may increase the risk of metastases by facilitating adhesion of tumour cells to the endothelium. We collected blood samples for DNA extraction from 264 patients treated for stage II or III CRC and from 310 healthy controls in order to assess three polymorphisms within the E-selectin gene (S128R, G98T and L554F) and one within the P-selectin gene (V640L). Genotypes were analysed by the allelic discrimination TaqMan real-time PCR assay. The S128R polymorphism was detected in 59 patients (22.3%) and was strictly correlated with the G98T polymorphism. In multivariate analysis, the S128R polymorphism was associated with shorter event-free survival (EFS) and overall survival (OS) in the whole population (EFS: P=.003, HR 1.82, 95% CI 1.23-2.70; OS: P<10(-4), HR 4.31, 95% CI 2.46-10.99), in patients with stage II CRC(EFS: P=.04, HR 1.92, 95% CI 1.02-3.60; OS: P=.02, HR 4.44, 95% CI 1.16-17.03), and in patients with stage III CRC (EFS:

P=.04, HR 1.68, 95% CI 1.01-2.80; OS: P=.001, HR 4.04, 95% CI 1.73-9.46). L554F and V640L polymorphisms had no prognostic value. The S128R polymorphism is a constitutional factor associated with a higher risk of relapse and death in patients treated for CRC. This polymorphism detection may permit better selection of patients suitable for adjuvant therapy, especially among those with stage II disease.

Ho-Pun-Cheung, A., E. Assenat, et al. (2007). "Cyclin D1 gene G870A polymorphism predicts response to neoadjuvant radiotherapy and prognosis in rectal cancer." Int J Radiat Oncol Biol Phys **68**(4): 1094-101.

PURPOSE: To investigate whether CCND1 genetic variations associated with a constitutive nuclear protein may influence either the pathologic response to preoperative RT or the prognosis in a series of rectal cancer patients. METHODS AND MATERIALS: Seventy rectal cancer patients treated by neoadjuvant radiotherapy were included in the study. CCND1 exon 5 mutations were screened, and the G870A polymorphism was assessed for correlation with clinical variables, tumor response, and patient outcome. RESULTS: No exon 5 mutation was found. Concerning the G870A polymorphism, the A/A variant was significantly associated with radiosensitivity (p = 0.022). Moreover, patients harboring the A allele were correlated with a lower risk of local failure (p = 0.017). Also, combination of the G870A polymorphism with the post-therapeutic lymph node status allowed the elaboration of a prognostic index, which accurately distinguished subgroups of patients with predictable recurrence-free (p = 0.003) and overall (p = 0.044) survival. CONCLUSIONS: Although CCND1 exon 5 mutations are rare in rectal cancer, G870A polymorphism is a frequent variation that may predict radiosensitivity and prognosis.

Huerta, S. (2008). "Recent advances in the molecular diagnosis and prognosis of colorectal cancer." Expert Rev Mol Diagn **8**(3): 277-88.

Colon cancer remains a leading cause of mortality worldwide despite the well-characterized molecular events in the adenoma-to-carcinoma sequence. There has been a strong emphasis on early detection of colon cancer, and fecal DNA-based methods have been developed to assist with early screening. Tissue-based assays have been utilized for many years to assess tumor aggressiveness and to determine prognosis and response to chemotherapeutic interventions. The most widely used serum marker for colon cancer (carcinoembryonic antigen) remains a useful modality to assess for occult disease following curative resection. Identification of tumor mutations in circulating tumor cells and microarray analysis holds a great deal of promise in the diagnosis and prognosis of patients with colorectal cancer. The inhibitors of apoptosis may be important markers to determine resistance to radiation cytotoxicity in rectal cancer. This report presents a summary of the current status of the molecular markers of colorectal cancer to establish a diagnosis, determine prognosis and chemoradiotherapeutic interventions, and assess relapse following curative surgery.

Ikoma, H., D. Ichikawa, et al. (2006). "Correlation between serum DNA methylation and prognosis in gastric cancer patients." Anticancer Res **26**(3B): 2313-6.

BACKGROUND: Gastric carcinogenesis is thought to involve multiple genetic and epigenetic changes. The relationships between the promoter methylation status of relevant genes in the serum and outcomes in patients undergoing curative gastrectomy for cancer were investigated. MATERIALS AND METHODS: Pre-operative serum samples obtained from 97 gastric cancer patients, who underwent radical gastrectomy, were subjected to methylation-specific polymerase chain reaction (MSP) assays for the p16, E-cadherin and retinoic acid receptor beta (RARbeta) genes. RESULTS: Promoter hypermethylation of p16, E-cadherin and the RARbeta gene was detected in sera from 18 (19%), 24 (25%) and 24 patients (25%), respectively. Altogether, 47 patients (48%) showed hypermethylation of at least one gene analyzed. Survival curves differed significantly between groups defined by the methylation status of E-cadherin ($p<0.05$), but not those defined by p16 or RARbeta (p =0. 77 and 0. 19, respectively). CONCLUSION: Serum MSP assays can provide not only diagnostic, but also prognostic information in gastric cancer.

Ishida, Y., K. Kawakami, et al. (2002). "Association of thymidylate synthase gene polymorphism with its mRNA and protein expression and with prognosis in gastric cancer." Anticancer Res **22**(5): 2805-9.

Thymidylate synthase (TS) is a target enzyme of 5-fluorouracil (5-FU). TS has a polymorphic repeated sequence in the 5'-UTR and the polymorphism is associated with TS protein expression. TS polymorphism has been reported to link with the efficacy of 5-FU-based chemotherapy in colorectal cancer. In this study, we examined whether the association among TS polymorphism, TS mRNA expression and TS protein expression is also observed in gastric cancer tissues and whether the TS polymorphism is a prognostic factor for patients with gastric cancer. We quantified TS mRNA isolated from

115 gastric cancer tissues by real-time reverse transcription PCR and TS protein in 72 available samples by the fluoro-dUMP binding assay. These values were compared with TS genotypes of the samples determined by a PCR assay. The tumor with the 3R/3R genotype had higher TS protein expression than that with the 2R/3R genotype, although there is no association between the TS genotype and mRNA expression. The result suggests that mRNA translation is responsible for the genotype-dependent difference of TS protein expression, as is consistent with our previous observation in colorectal cancer. This association encouraged us to further examine the link of the TS genotype with the stage of disease and the prognosis of the patients. The clinicopathological analysis showed that gastric cancers with the 3R/3R genotype are at a more advanced stage than those with the combined 2R/2R and 2R/3R genotype. We observed a longer survival in those patients with the combined 2R/2R and 2R/3R genotype, compared with the 3R/3R genotype, although it did not reach significance when the patients who had received the oral fluoropyrimidines therapy were analyzed. These results warrant further large-scale clinical study of the role of the TS genotyping for the prediction of efficacy using 5-FU-based chemotherapy and prognosis in gastric cancer.

Isola, J., T. Visakorpi, et al. (1992). "Association of overexpression of tumor suppressor protein p53 with rapid cell proliferation and poor prognosis in node-negative breast cancer patients." J Natl Cancer Inst **84**(14): 1109-14.

BACKGROUND: Recent evidence indicates that a subset of axillary node-negative (ANN) breast cancer patients can benefit from adjuvant therapy. Reliable prognostic markers are needed, however, to help clinicians identify these patients and arrive at more rational treatment decisions. PURPOSE: Mutations of the p53 tumor suppressor gene often result in overexpression of the p53 protein. In this study, we evaluated the prognostic significance of p53 protein overexpression in patients with ANN breast cancer. We also studied the association between the tumor cell proliferation rate and overexpression of the p53 and c-erbB-2 proteins, both of which have been implicated in cell cycle control. The c-erbB-2 protein is the product of the ERBB2 gene. METHODS: Two hundred eighty-nine ANN cases were randomly selected from a population-based cohort of patients who had not received any kind of adjuvant chemotherapy or endocrine therapy. Overexpression of the p53 and c-erbB-2 proteins was studied immunohistochemically in archival paraffin-embedded tumor samples, using the CM-1 polyclonal and the TAb 250 monoclonal antibodies, respectively. The tumor cell proliferation rate was measured as the S-phase fraction by DNA flow cytometry. Statistical analyses were performed using BMDP software. RESULTS: High-level p53 protein overexpression, found in 41 of the 289 tumors, was most common in tumors with high histologic grade, negative estrogen receptor status, c-erbB-2 protein overexpression, DNA index greater than 1.3, or high S-phase fraction. The lowest S-phase levels were found in tumors with neither p53 nor c-erbB-2 protein overexpression; the highest levels were seen in tumors showing overexpression of both proteins (P less than .0001). Both p53 and c-erbB-2 overexpression, as well as tumor size, had independent prognostic value in multivariate analysis. Eight-year survival of patients with p53 protein overexpression was 56% compared with 81% in patients with no overexpression (relative risk, 3.7; P less than .0001). If the S-phase fraction was included in a Cox regression analysis, however, only the tumor size and the S-phase fraction emerged as independent predictors of survival. CONCLUSIONS: Overexpression of the p53 and c-erbB-2 proteins indicates a high malignant potential in ANN breast cancer, but it is not a significant prognostic factor independent of the cell proliferation rate. The correlation between overexpression of these proteins and an increased S-phase fraction suggests that they may confer a proliferative advantage to cancer cells in vivo.

Jeffrey, S. S., P. E. Lonning, et al. (2005). "Genomics-based prognosis and therapeutic prediction in breast cancer." J Natl Compr Canc Netw **3**(3): 291-300.

Breast cancer is a heterogeneous disease. DNA microarray technology is being applied to breast cancer to identify new prognostic biomarkers, to predict response to therapy, and to discover targets for the development of novel therapies. New diagnostic assays based on global gene expression are being introduced into clinical practice or tested in large-scale clinical trials. This review focuses on translational studies using microarray analyses and discusses best practice features and pitfalls. We note that factors that predict metastatic disease are not necessarily the same factors that predict therapeutic response. We believe that the characterization and discernment of different systems among breast cancers is crucial for understanding drug sensitivity and resistance mechanisms and for guiding therapy.

Jen, J., H. Kim, et al. (1994). "Allelic loss of chromosome 18q and prognosis in colorectal cancer." N Engl J Med **331**(4): 213-21.

BACKGROUND: Colorectal cancer occurs in approximately 150,000 people each year in the United States. Prognostic assessment influences the

treatment of patients with colorectal cancer, including decisions about adjuvant therapy. We evaluated chromosome 18q allelic loss, a genetic event associated with tumor progression, as a prognostic marker for this disease. METHODS: We developed procedures to examine the status of chromosome 18q with microsatellite markers and DNA from formalin-fixed, paraffin-embedded tumors. Allelic loss of chromosome 18q was assessed in 145 consecutively resected stage II or III colorectal carcinomas. RESULTS: Among patients with stage II disease, the five-year survival rate was 93 percent in those whose tumor had no evidence of allelic loss of chromosome 18q and 54 percent in those with allelic loss; among patients with stage III disease, survival was 52 and 38 percent, respectively. The overall estimated hazard ratio for death in patients whose tumor had chromosome 18q allelic loss was 2.83 (P = 0.008) according to univariate analysis. Furthermore, chromosome 18q allelic loss remained a strong predictive factor (hazard ratio for death, 2.46; 95 percent confidence interval, 1.06 to 5.71; P = 0.036) after adjustment for all other evaluated factors, including tumor differentiation, vein invasion, and TNM stage. CONCLUSIONS: The status of chromosome 18q has strong prognostic value in patients with stage II colorectal cancer. The prognosis in patients with stage II cancer and chromosome 18q allelic loss is similar to that in patients with stage III cancer, who are thought to benefit from adjuvant therapy. In contrast, patients with stage II disease who do not have chromosome 18q allelic loss in their tumor have a survival rate similar to that of patients with stage I disease and may not require additional therapy.

Kaiser, P. C., M. Korner, et al. (2005). "Retinoid receptors in ovarian cancer: expression and prognosis." Ann Oncol **16**(9): 1477-87.

BACKGROUND: Ovarian cancer is frequently lethal despite aggressive multimodal therapy, and new therapies are therefore needed. Retinoids are potential candidate drugs: they prevent the development of ovarian carcinoma and enhance the efficacy of cytotoxic drugs in ovarian cancer cells. At present, little is known about the retinoid receptor expression in ovarian cancer. PATIENTS AND METHODS: The retinoid receptors comprise two classes, retinoic acid receptors (RARs) and retinoid X receptors (RXRs), each with three subclasses, alpha, beta and gamma. We investigated the expression of the subtypes RARalpha, RARgamma, RXRalpha and RXRbeta by immunohistochemistry in ovarian cancers of 80 patients, and assessed their prognostic significance. In addition, we quantified the expression of retinoid receptor mRNA using real-time PCR and correlated the results with clinical characteristics. RESULTS: RARalpha and RXRbeta were highly expressed in a majority of ovarian cancers, particularly in advanced stages. High expression of RARalpha was an independent negative prognostic factor of survival in addition to FIGO stage, age and p53 accumulation. The mRNA expression of retinoid receptors did not correlate with clinical properties of the tumors. CONCLUSIONS: Retinoic acid receptors are frequently and strongly expressed in epithelial ovarian cancer and may be indicators of an adverse prognosis. This study provides the molecular basis for the therapeutic use of retinoids in ovarian cancer.

Kakolyris, S., A. Giatromanolaki, et al. (2001). "Thioredoxin expression is associated with lymph node status and prognosis in early operable non-small cell lung cancer." Clin Cancer Res **7**(10): 3087-91.

PURPOSE: Thioredoxin (TRX), a low molecular weight protein, exerts reduction-oxidation control over a number of transcription factors involved in cell activation and proliferation. High TRX mRNA levels have been found in lung carcinomas, a trait associated with a growth and survival advantage. EXPERIMENTAL DESIGN: In this study, we examined the immunohistochemical expression of human TRX in normal lung and in 102 primary non-small cell lung carcinomas. RESULTS: In normal lung, the staining for TRX was cytoplasmic in the respiratory bronchial epithelium, alveolar epithelium, and alveolar macrophages. Bronchial glandular cells demonstrated a mixed nuclear and cytoplasmic staining. In lung carcinomas, the pattern of expression for TRX was predominantly cytoplasmic and only occasionally nuclear. A strong association between absence of TRX expression and regional lymph node negativity was observed (P = 0.004). High proliferation index, as detected with Ki-67 antibody, was associated with high TRX expression (P = 0.02). A significant correlation between high cytoplasmic p53 reactivity and low TRX expression was observed (P = 0.04). No association with grade, tumor stage, histology, or bcl-2 was noted. A significant coexpression of TRX with human activator protein endonuclease 1 was recorded (P = 0.04). Absence of TRX expression was associated with a better outcome (P < 0.05). CONCLUSIONS: We conclude that overexpression of TRX in non-small cell lung carcinomas is indicative of a more aggressive tumor phenotype and is associated with bad prognostic features and possibly with a poorer outcome.

Kang, B., R. F. Guo, et al. (2008). "Expression status of ataxia-telangiectasia-mutated gene correlated with

prognosis in advanced gastric cancer." Mutat Res **638**(1-2): 17-25.

Many studies have revealed the ATM alterations involved in cancer development and progression. In order to elucidate ATM deficiency in advanced GC and its clinical significance, a total of 20 exons of ATM gene, including frequently reported variations, were screened in 40 advanced primary GC and matched normal tissues using denaturing high performance liquid chromatography (DHPLC) and DNA sequencing analysis. Furthermore, ATM mRNA level was analyzed using Real-time RT-PCR and in situ hybridization, and protein expression and phosphorylation at Ser1981 were measured by immunohistochemical assessment in tissue microarray of GC. Five variants were identified in 6 of 40 cases (15%), but no hot spot of variation was detected. However, decreased expression and phosphorylation of ATM were consistently presented in tumors. In a cohort of 70 GC samples, low level of phosphorylated ATM was significantly correlated with poor differentiation, lymph node metastasis and poor 5-year survival ($P<0.05$). These results indicated that ATM phosphorylation status might be a prognostic marker for individual therapy in advanced GC patients.

Kanters, S. D., J. W. Lammers, et al. (1995). "Molecular and biological factors in the prognosis of non-small cell lung cancer." Eur Respir J **8**(8): 1389-97.

For patients with non-small cell lung cancer the tumour/node/metastasis (TNM) staging system and other conventional prognostic factors fail to predict the outcome of treatment and survival accurately. New prognostic factors are urgently needed to improve understanding of the biological behaviour of the different subtypes of non-small cell lung cancer and to recognize patients with a good or poor prognosis. This review will focus on molecular and biological factors published in the English language literature between 1988 and 1994. To be included in this survey, the predictive value of a specific prognostic factor had to be confirmed by multivariate analysis in at least two different studies. Blood group antigen expression, ras oncogenes, microvessel density, and factors reflecting the proliferative state of the tumour may be important determinants of outcome of treatment. The search for new determinants of prognosis has provided insight in the complex tumour biology of non-small cell lung cancer and indicated possible targets for tumour therapy. Several promising prognostic factors have now been recognized. To validate these factors, prospective studies of a large patient population are needed. This ultimately serves the recognition of subsets of patients who may benefit from adjuvant therapy.

Kashihara, M., K. Azuma, et al. (2009). "Nuclear Y-box binding protein-1, a predictive marker of prognosis, is correlated with expression of HER2/ErbB2 and HER3/ErbB3 in non-small cell lung cancer." J Thorac Oncol **4**(9): 1066-74.

INTRODUCTION: Nuclear expression of Y-box binding protein-1 (YB-1) is closely associated not only with global drug resistance and expression of several growth factor receptors in various human malignancies but also with overall patient survival. METHODS: The effect of YB-1 knockdown on expression of epidermal growth factor receptor (EGFR) family proteins was examined by Western blot using human lung cancer cell lines. Immunohistochemistry was used to evaluate the expression of nuclear YB-1 and EGFR family proteins in patients with non-small cell lung cancer (NSCLC) (n = 104). RESULTS: In the five NSCLC cell lines, expressions of EGFR, human epidermal growth factor receptor 2 (HER2), HER3, and hepatocyte growth factor receptor (c-Met) in PC-9 cells; of HER2 and c-Met in EBC-1 cells; and of HER3 in QG56 cells were down-regulated by YB-1 knockdown. By immunohistochemical analysis, we observed that HER3 expression was significantly negatively correlated with nuclear YB-1 expression in squamous cell carcinoma ($p = 0.038$). HER2 expression was positively correlated with nuclear YB-1 expression in adenocarcinoma ($p = 0.052$). Nuclear expression of YB-1 correlated with overall survival of all patients ($p = 0.028$) and of patients with adenocarcinoma ($p = 0.007$). Furthermore, there was a significant difference in therapeutic efficacies of gefitinib between patients with nuclear YB-1 expression and those with non-nuclear YB-1 expression in patients with NSCLC ($p = 0.004$, $n = 26$) but not between those with high and those with low expression of EGFR, HER2, HER3, and c-Met. CONCLUSION: Nuclear YB-1 expression might be essential for the malignant phenotype in lung cancer patients and might be an important biomarker for the development of therapeutic strategy against NSCLC.

Kato, K., S. Iida, et al. (2008). "Methylated TMS1 and DAPK genes predict prognosis and response to chemotherapy in gastric cancer." Int J Cancer **122**(3): 603-8.

Gastric cancer is the second most common cause of cancer deaths worldwide. The identification of molecular genetic parameters that are associated with response to chemotherapy and prognosis is of utmost interest. We examined methylation of the apoptosis-related genes, TMS1 and DAPK, in 81

primary gastric cancers using methylation-specific PCR and compared their methylation status with clinicopathological findings. Aberrant methylation of TMS1 and DAPK genes was detected in 26 (32.1%) tumors and in 18 (22.2%) tumors, respectively. The overall survival of patients with both methylated genes was significantly shorter compared with those with only one methylated gene or no methylated genes ($p = 0.0003$). Neither gene methylation had any relation to other clinicopathological findings. Next, we examined 43 patients treated by 5-fluorouracil-based chemotherapy, who had distant metastasis or recurrence after radical resection, to determine the relation between chemosensitivity and methylation. The response rate was lower in patients with either methylation than without (TMS1: 22.2% vs. 48.0%; DAPK: 21.4% vs. 44.8%). Overall survival tended to be shorter in the patients with both methylations compared with either or no methylations ($p = 0.0806$). The time to progression of patients with methylation of TMS1 or DAPK was significantly shorter than patients without methylation (TMS1: $p = 0.0123$; DAPK: $p = 0.0464$). Furthermore, the time to progression of patients with both methylated genes was significantly shorter than patients with one methylation or no methylation ($p = 0.0082$). In conclusion, TMS1 and DAPK methylation might predict the prognosis and response to chemotherapy in gastric cancer.

Kato, M., S. Saji, et al. (1997). "Clinical study of the relationship between cytological behavior and postoperative prognosis in colorectal cancer cases with special reference to nuclear DNA content and nucleolar organizer regions." J Surg Oncol **64**(1): 36-41.

BACKGROUND: We studied the usefulness of nuclear DNA patterns and argyrophylic nucleolar organizer regions (AgNORs) for evaluating the malignant potential of colorectal cancers, which is increasingly being regarded as important in predicting patients' prognosis and for their appropriate postoperative management. METHODS: We measured these two factors in curatively resected specimens of 91 colorectal cancer cases, which were followed up for 1,549 +/- 788 days postoperatively. Ploidy pattern was either diploid or aneuploid, and AgNORs score was either low (LS) or high (HS). Thus, we classified our cases into Group I (diploid, LS). Group II (aneuploid, LS), Group III (diploid, HS), and Group IV (aneuploid, HS). Postoperative survival curves in the cases belonging to these groups were analyzed. RESULTS: Survival rates in Groups I and II were significantly higher than those in Group IV. Correlation between subgroups and clinicopathological factors such as average age, histologic type, depth of invasion, and histologic stage were observed. Incidence of lymph node metastasis at the time of operation and that of postoperative recurrence were higher in group IV than that in groups I and II. CONCLUSIONS: Measurement of DNA ploidy patterns and AgNORs score were found to be useful in evaluating malignant potential of colorectal cancers.

Kim, J., H. A. Reber, et al. (2006). "Unfavourable prognosis associated with K-ras gene mutation in pancreatic cancer surgical margins." Gut **55**(11): 1598-605.

BACKGROUND: Despite intent to cure surgery with negative resection margins, locoregional recurrence is common in pancreatic cancer. AIMS: To determine whether detection of K-ras gene mutation in the histologically negative surgical margins of pancreatic cancer reflects unrecognised disease. PATIENTS: Seventy patients who underwent curative resection for pancreatic ductal adenocarcinoma were evaluated. METHODS: All patients had surgical resection margins (pancreatic transection and retroperitoneal) that were histologically free of invasive cancer. DNA was extracted from these paraffin embedded surgical margins and assessed by quantitative real time polymerase chain reaction to detect the K-ras gene mutation at codon 12. Detection of K-ras mutation was correlated with standard clinicopathological factors. RESULTS: K-ras mutation was detected in histologically negative surgical margins of 37 of 70 (53%) patients. A significant difference in overall survival was demonstrated between patients with margins that were K-ras mutation positive compared with negative (median 15 v 55 months, respectively; $p = 0.0008$). By univariate and multivariate analyses, detection of K-ras mutation in the margins was a significant prognostic factor for poor survival (hazard ratio (HR) 2.8 (95% confidence interval (CI) 1.5-5.3), $p = 0.0009$; and HR 2.8 (95% CI 1.4-5.5), $p = 0.004$, respectively). CONCLUSIONS: Detection of cells harbouring K-ras mutation in histologically negative surgical margins of pancreatic cancer may represent unrecognised disease and correlates with poor disease outcome. The study demonstrates that molecular-genetic evaluation of surgical resection margins can improve pathological staging and prognostic evaluation of patients with pancreatic ductal adenocarcinoma.

Kim, J. G., S. K. Sohn, et al. (2009). "TP53 codon 72 polymorphism associated with prognosis in patients with advanced gastric cancer treated with paclitaxel and cisplatin." Cancer Chemother Pharmacol **64**(2): 355-60.

PURPOSE: The present study analyzed the polymorphisms of apoptosis-related genes and their impact on the response to chemotherapy and survival of patients with advanced gastric cancer. PATIENTS AND METHODS: Fifty-seven patients with advanced gastric cancer treated with paclitaxel and cisplatin combination chemotherapy were enrolled in the present study. The genomic DNA was extracted from paraffin-embedded tissue, and the single nucleotide polymorphisms (SNPs) of ten apoptosis-related genes [LTA, TP53, BCL2L11, BID, FASL, caspase 3, caspase 6, caspase 7, and caspase 9] determined using a polymerase chain reaction-restriction fragment length polymorphism assay. RESULTS: The Arg/Pro and Pro/Pro genotypes of TP53 codon 72 were significantly correlated with a lower response rate to the combination chemotherapy when compared to the Arg/Arg genotype (35.7 vs. 66.7%, P-value 0.019) in a logistic regression analysis. A multivariate survival analysis also showed that the time to progression for the patients with the Arg/Pro and Pro/Pro genotypes of TP53 codon 72 was worse than for the patients with the Arg/Arg genotype (Hazard ratio = 3.056, P-value = 0.047), whereas the overall survival was not significantly different. CONCLUSION: The TP53 codon 72 SNP was found to be predictive of the response to chemotherapy and correlate with the time to progression in patients with advanced gastric cancer treated with paclitaxel and cisplatin chemotherapy.

Kim, J. Y., S. Park, et al. (2009). "Low initial human papilloma viral load implicates worse prognosis in patients with uterine cervical cancer treated with radiotherapy." J Clin Oncol **27**(30): 5088-93.

PURPOSE: To evaluate whether human papillomavirus (HPV) viral load measured in cervical smear and HPV type 18 are associated with radiotherapy outcomes in uterine cervical cancer. PATIENTS AND METHODS HPV DNA: was semiquantitatively measured in the cervical smears of 169 radiotherapy patients. HPV viral load was classified as low or high according to median HPV DNA titer and examined for its prognostic value. The multivariable Cox proportional hazards model was used to adjust for covariates. A relapse-predicting model was constructed to classify three risk groups for disease-free survival (DFS), which were used for internal validation. RESULTS: Patients with lower HPV viral load showed worse DFS in univariate analysis. HPV type 18, younger patient age, stage group, nodal status, histologic grade, and histologic type were other prognostic factors for poor DFS. Among these factors, all except stage group were associated with HPV viral load. Multivariate analysis showed the strong influence of HPV viral load for poor DFS. The prognostic model developed using our outcome data performed well in predicting the risk of relapse. CONCLUSION: Our data suggest that HPV viral load is a strong independent prognostic factor for DFS. HPV type 18 showed a significant relationship with poor radiotherapy outcome in univariate analysis, but not in multivariate analysis.

Kioulafa, M., I. Balkouranidou, et al. (2009). "Methylation of cystatin M promoter is associated with unfavorable prognosis in operable breast cancer." Int J Cancer **125**(12): 2887-92.

The methylation status of cystatin M (CST6) gene in breast tumors was investigated and its prognostic significance as a novel breast cancer biomarker was evaluated. Using methylation-specific PCR (MSP), CST6 promoter methylation was examined in 134 formalin fixed paraffin-embedded tissues (FFPEs): 10 pairs of breast tumors and their surrounding normal tissues, 10 breast fibroadenomas, 11 normal breast tissues and 93 breast tumors. Methylation of CST6 promoter was observed in 2/21 (9.5%) noncancerous breast tissues, 1/10 (10%) benign breast tumors (fibroadenomas) and 52 (55.9%) operable breast cancer tumor samples. CST6 was rarely methylated in the normal tissue surrounding the tumor (10%). During the follow-up period, 24 (25.8%) patients relapsed and 19 (20.4%) died. CST6 methylation was detected in 19 (79.2%) of patients who relapsed and in 15 (78.9%) of patients who died. Disease-free-interval (DFI) and overall survival (OS) were significantly associated with CST6 promoter methylation (p=0.004 and p=0.001 respectively). Multivariate analysis revealed that CST6 methylation is an independent prognostic factor for DFI (HR=3.484; 95% CI: 1.155-10.511; p=0.027). and OS (HR=9.190; 95% CI: 1.989-42.454; p=0.004). CST6 promoter methylation status in tumor cells seems to provide important prognostic information in operable breast cancer and merits to be further evaluated and validated in a larger cohort of patients.

Klozar, J., V. Kratochvil, et al. (2008). "HPV status and regional metastasis in the prognosis of oral and oropharyngeal cancer." Eur Arch Otorhinolaryngol **265 Suppl 1**: S75-82.

Prognostic factors are important for treatment decisions as they help adapt the therapy on a case-to-case basis. Nodal status, number of positive nodes, and presence of extracapsular spread are considered to be the important prognostic factors in head and neck cancer. Some studies suggest that human papillomavirus (HPV) status also influences the outcome of the treatment. This influence can be explained by the variation in tendency to develop regional metastases and by variation in the type of neck node involvement. The study objectives were to

compare patients with HPV positive and HPV-negative tumors for survival and prevalence and type of regional metastasis, to identify prognostic factors and to test whether HPV presence is an independent factor of survival. The study included 81 patients treated by surgery including neck dissection for oral or oropharyngeal squamous cell cancer. A computerized medical report was completed for each patient. Analysis of the tumor specimen for the HPV DNA presence was done on paraffin-fixed tissue. HPV DNA detection and typing were performed by PCR with GP5+/GP6+BIO primers and reverse line blot hybridization. Overall, 64% (52/81) of tumors were HPV positive with 80% in the tonsillar site. HPV-positive patients had significantly better both overall (73 vs. 35%) (P=0.0112) and disease-specific (79 vs. 45%) (P=0.0015) survival rates than HPV-negative patients. No significant differences were found in the pN classification, in the number of positive nodes and the presence of extracapsular spread in the involved nodes between HPV positive and HPV-negative tumors. Multivariate analysis showed that significant prognostic factors of survival were the presence of HPV in the tumor, extracapsular spread and tumor size. HPV was the most significant prognostic factor in the studied group of patients with oropharyngeal tumors (HR=0.27, 95%CI 0.12-0.61) and possibly should be considered in treatment decisions.

Kohonen-Corish, M. R., J. J. Daniel, et al. (2005). "Low microsatellite instability is associated with poor prognosis in stage C colon cancer." J Clin Oncol **23**(10): 2318-24.

PURPOSE: The significance of low microsatellite instability (MSI-L) in colorectal cancer is poorly understood. No clear biologic distinction has been found between MSI-L and microsatellite stable (MSS) colorectal cancer, and these two phenotypes are usually combined when analyzed against the well-defined high MSI (MSI-H) phenotype. Evidence is emerging that an O(6)-methylguanine DNA methyltransferase (MGMT) gene defect is associated with MSI-L. Therefore, to further define this phenotype, we undertook a detailed analysis of the prognostic significance of MSI-L and loss of MGMT expression in colon cancer. PATIENTS AND METHODS: The study cohort was 183 patients with clinicopathologic stage C colon cancer who had not received adjuvant therapy. We analyzed MSI status, MGMT, and mismatch repair protein expression, as well as MGMT and p16 promoter hypermethylation. RESULTS: We showed that MSI-L defines a group of patients with poorer survival (P = .026) than MSS patients, and that MSI-L was an independent prognostic indicator (P = .005) in stage C colon cancer. Loss of MGMT protein expression was associated with the MSI-L phenotype but was not a prognostic factor for overall survival in colon cancer. p16 methylation was significantly less frequent in MSI-L than in MSI-H and MSS tumors and was not associated with survival. CONCLUSION: MSI-L characterizes a distinct subgroup of stage C colon cancer patients, including the MSI-L subset of proximal colon cancer, who have a poorer outcome. Neither the MGMT defect nor p16 methylation are likely to contribute to the worse prognosis of the MSI-L phenotype.

Kruschewski, M., A. Noske, et al. (2002). "Is reduced expression of mismatch repair genes MLH1 and MSH2 in patients with sporadic colorectal cancer related to their prognosis?" Clin Exp Metastasis **19**(1): 71-7.

The majority of mutations in hereditary nonpolyposis colon carcinoma (HNPCC) patients affect the mismatch-repair genes (MMRG) MLHI and MSH2. In addition, mutations of these genes were found in about 15% of sporadic colorectal carcinomas which appear to be related to microsatellite instability (MSI). However, mutations in MMRG were not found in all MSI-positive carcinomas, but MMRG mutations may be relevant for the assessment of tumor characteristics and patients' prognosis. Therefore, we investigated the relationship between expression of MMRG, tumor biology and patients' survival. In 127 patients with sporadic colorectal carcinomas and a minimum of 5 years follow-up after curative surgery immunohistochemical detection of MLHI and MSH2 was analyzed semiquantitatively. Lost expression of MLHI has been found in tumor specimens from 10 patients, whereas MSH2 expression was missing in 5 patients. This reduced expression did not correlate with tumor stage, lymph node involvement, grading or tumor invasion into blood vessels. However, a significant correlation was found for lymphovascular invasion (P = 0.02) and localization within the colorectum (P = 0.003) in MLH1-negative carcinomas. In addition, although there was a clear tendency for longer overall survival (72 vs. 63 months) for patients with MLH1-negative carcinomas, significant differences for overall and recurrence-free survival were not seen. In conclusion of our results and a critical review of literature, the prognostic importance of the MMR genes in sporadic colorectal carcinomas remains controversial.

Kute, T. E., H. B. Muss, et al. (1990). "The use of flow cytometry for the prognosis of stage II adjuvant treated breast cancer patients." Cancer **66**(8): 1810-6.

Characterization of breast cancer cells by histology, flow cytometry, and steroid receptors was performed on 197 Stage II breast node positive cancer

patients given adjuvant chemotherapy, plus tamoxifen for patients with positive hormone receptors. Histologic and steroid receptor assays were performed using standard techniques; flow cytometric analysis was performed from paraffin-embedded blocks obtained from the primary tumor. Quality control studies on reproducibility, tissue heterogeneity, and analysis procedures have been included. Of the 197 patients studied, aneuploidy was found in 102 (52%); the median %S value was 8% with a range of 0.4% to 38%. Our results demonstrated that number of positive nodes, receptor status, and grade were of prognostic value. Cell cycle kinetic data were not of independent prognostic value in this series. However, ploidy could differentiate in prognosis in the receptor-negative subgroup. Patients with receptor-negative tumors had a significantly better overall survival if the tumor was diploid in nature. Cell kinetics was not significantly prognostic for either receptor subgroup, although patients with higher %S tended to have better relapse-free and overall survival. This is in disagreement with other studies and may demonstrate that treatment has confounded our results and diminished the ability of flow cytometry data to help predict outcome.

Lai, C. H., C. J. Chang, et al. (2007). "Role of human papillomavirus genotype in prognosis of early-stage cervical cancer undergoing primary surgery." J Clin Oncol **25**(24): 3628-34.

PURPOSE: Our aim was to evaluate the prognostic significance of human papillomavirus (HPV) genotype in early-stage cervical carcinoma primarily treated with surgery in a large tertiary referral medical center. PATIENTS AND METHODS: Consecutive patients who underwent primary surgery for invasive cervical carcinoma of International Federation of Gynecology and Obstetrics (FIGO) stage I to IIA between 1993 and 2000 were retrospectively reviewed. Polymerase chain reaction (PCR) using a general primer set followed by reverse-blot detection of 38 types of HPV DNA in a single reaction was performed for genotyping. E6 type-specific PCR was performed to validate multiple types. RESULTS: A total of 1,067 eligible patients were analyzed. HPV DNA sequences were detected in 95.1% of the specimens, among which 9.6% contained multiple types. HPV 16 was detected in 63.8% of the samples, and HPV 18 was detected in 16.5% of the samples. The median follow-up time of surviving patients was 77 months. By multivariate analysis, FIGO stage, lymph node metastasis, depth of cervical stromal invasion, grade of differentiation, and HPV 18 positivity were significantly related to cancer relapse. FIGO stage II, deep stromal invasion, parametrial extension, HPV 18 positivity, and age older than 45 years were significant predictors for death. Using the seven selected variables from either recurrence-free or overall survival analysis, death-predicting ($P < .0001$) and relapse-predicting ($P < .0001$) models classifying three risk groups (low, intermediate, and high risk) were constructed and endorsed by internal validation. CONCLUSION: The independent prognostic value of HPV genotype is confirmed in this study. The prognostic models could be useful in counseling patients and stratifying patients in future clinical trials.

Lanigan, F., E. McKiernan, et al. (2009). "Increased claudin-4 expression is associated with poor prognosis and high tumour grade in breast cancer." Int J Cancer **124**(9): 2088-97.

The role of intercellular tight junctions in breast epithelial cells is traditionally thought to be in maintaining polarity and barrier function. However, claudin-4, a tight junction protein, is overexpressed in breast tumour cells compared to normal epithelial cells, which generally corresponds to a loss in polarity. The aim of this study was to investigate the distribution and potential clinical value of claudin-4 in breast cancer, and to evaluate its usefulness as a prognostic and predictive biomarker. Expression of claudin-4 was initially examined by Western blot analysis in a cohort of 88 breast tumours, and was found to correlate positively with tumour grade and negatively with ER. Claudin-4 expression was then evaluated by immunohistochemistry in a larger cohort of 299 tumours represented on a tissue microarray. Claudin-4 expression correlated positively with tumour grade and Her2, and negatively with ER. High claudin-4 expression was also associated with worse breast cancer-specific survival ($p = 0.003$), recurrence-free survival ($p = 0.025$) and overall survival ($p = 0.034$). Multivariate analysis revealed that claudin-4 independently predicted survival in the entire cohort (HR 1.95; 95%CI 1.01-3.79; $p = 0.047$) and in the ER positive subgroup treated with adjuvant tamoxifen (HR 4.34; 95%CI 1.14-16.53; $p = 0.032$). This relationship between increased claudin-4 expression and adverse outcome was validated at the mRNA level in a DNA microarray dataset of 295 breast tumours. We conclude that high levels of claudin-4 protein are associated with adverse outcome in breast cancer patients, including the subgroup of patients treated with adjuvant tamoxifen.

Lanza, G., M. Matteuzzi, et al. (1998). "Chromosome 18q allelic loss and prognosis in stage II and III colon cancer." Int J Cancer **79**(4): 390-5.

The prognostic significance of chromosome 18q allelic loss was evaluated in a series of 118 patients with curatively resected TNM stage II or stage III colon cancer. Chromosome 18q status was determined on frozen tumour samples, using

microsatellite markers and the polymerase chain reaction (PCR). Mean follow-up in surviving patients was 75.9 months. Chromosome 18q allelic loss was significantly related to tumour site, extramural venous invasion, flow cytometric nuclear DNA content and p53 protein expression. Patients whose tumour had no evidence of chromosome 18q allelic loss showed a better disease-free and overall survival than patients whose tumour demonstrated 18q allelic loss. When patients were stratified by tumour stage, a significant survival advantage for patients whose tumour had no allelic loss on chromosome 18q was observed in stage II as well as in stage III disease. In particular, patients with stage II disease whose tumour had no chromosome 18q allelic loss demonstrated an excellent clinical outcome, with a 5-year disease-free survival rate of 96%. In contrast, the 5-year disease-free survival rate of patients with stage II disease and chromosome 18q allelic loss was only 54%. In multivariate analysis, status of chromosome 18q was the only significant independent prognostic factor for both disease-free and overall survival. These results indicate that assessment of chromosome 18q status provides relevant prognostic information in colon cancer and might be employed in the selection of patients for adjuvant therapy.

Li, Y., X. Li, et al. (2009). "Thymidylate synthase was associated with patient prognosis and the response to adjuvant therapy in bladder cancer." BJU Int **103**(4): 547-52.

OBJECTIVE: To investigate the expression of thymidylate synthase (TS), a key enzyme in DNA synthesis that is over-expressed in several cancer cells, in bladder cancer and its association with patient prognosis and the response to adjuvant therapy. PATIENTS AND METHODS: In all, 67 bladder tissue specimens were obtained from patients who had undergone transurethral resection (TUR). TS expression in bladder cancer and normal bladder tissue was analysed by immunohistochemistry. RESULTS: Of the 67 bladder tissue specimens, 47 (70%) and 10 (15%) had positive expression for TS in cancer and normal tissues, respectively. TS expression was greater in patients with Grade 3 (16/17, 94%) than in Grade 1 and 2 (31/50, 64%; P = 0.002). It was also greater in Stage T1 (14/14) than in Stage Ta (33/53, 62%; P = 0.001). Furthermore, patients with negative TS expression had a longer postoperative recurrence-free survival (RFS) than those with positive expression during the 5 year follow-up (P = 0.028). In the patients with positive TS-expressing tumours, adjuvant therapy significantly improved RFS (P < 0.001). CONCLUSIONS: High TS expression might be a marker of poor prognosis for patients with bladder cancer. In addition, patients with high TS expression might also be benefit from adjuvant therapy.

Linder, N., C. Haglund, et al. (2006). "Decreased xanthine oxidoreductase is a predictor of poor prognosis in early-stage gastric cancer." J Clin Pathol **59**(9): 965-71.

BACKGROUND: Xanthine oxidoreductase (XOR) is a key enzyme in the degradation of DNA, RNA and high-energy phosphates. About half of the patients with breast cancer have a decrease in XOR expression. Patients with breast cancer with unfavourable prognosis are independently identified by the loss of XOR. AIM: To assess the clinical relevance of XOR expression in gastric cancer. METHODS: XOR levels were studied by immunohistochemistry in tissue microarray specimens of 337 patients with gastric cancer and the relation between XOR expression and a series of clinicopathological variables, as well as disease-specific survival, was assessed. RESULTS: XOR was moderately decreased in 41% and was undetectable in another 14% of the tumours compared with the corresponding normal tissue. Decreased XOR was associated with advanced stage, deep tumour penetration, diffusely spread tumour location, positive lymph node status, large tumour size, non-curative disease, cellular aneuploidy, high S-phase fraction and high cyclooxygenase-2 expression, but not with p53 expression or Borrmann classification. Down regulation of XOR was associated with unfavourable outcome, and the cumulative 5-year gastric cancer-specific survival in patients with strong XOR expression was 47%, compared with 22% in those with moderate to negative expression ($p<0.001$). This was also true in patients with stage I-II ($p = 0.01$) and lymph node-negative ($p = 0.02$) disease, as well as in patients with smaller (< or =5 cm) tumours ($p = 0.02$). CONCLUSION: XOR expression in gastric cancer may be a new marker for a more aggressive gastric cancer biology, similar to that previously reported for breast cancer.

Mader, R. M. (2006). "Links between biology, prognosis and prediction of response to chemotherapy in colorectal cancer." Onkologie **29**(7): 334-41.

Compared with the progress achieved in breast cancer, the use of prognostic and predictive parameters in colorectal cancer is lagging behind. One of the reasons is the limited information provided by 'classic' mutations as markers for response to therapy. To bridge this gap, prospective clinical trials need to be conducted to evaluate the usefulness of gene expression profiling and candidate markers, such as DNA repair proteins, onset of the methylator phenotype, neo-angiogenetic pathways related to

inflammation, matrix metalloproteinases, tumor suppressors and cell signaling pathways (e.g. Akt). In parallel, the unrivalled sensitivity of molecular techniques may be applied to diagnostic parameters, such as cytokeratin 20 mRNA, for the detection of lymph node metastases. This article reviews the current knowledge on prognostic and predictive parameters in order to highlight the close link between tumor biology and tumor pharmacology. The increasing number of candidate markers together with recently introduced therapeutic options offer novel opportunities for a stepwise approach to the ambitious goal of individualized therapy in colorectal cancer.

Masters, J. R., R. S. Camplejohn, et al. (1989). "DNA ploidy and the prognosis of stage pT1 bladder cancer." Br J Urol **64**(4): 403-8.

The histopathological grade, proportion of "S"-phase nuclei and DNA ploidy values were linked and of prognostic significance in a retrospective series of stage pT1 bladder cancers. Nuclei were extracted from paraffin sections of 75 biopsies (56 patients). DNA ploidy and the proportion of "S"-phase nuclei were measured using flow cytometry. Progressive disease (pT2 or greater) developed within 3 years in 35% (6/17) of patients with poorly differentiated tumours, 35% (8/23) with aneuploid tumours and 35% (7/20) of those with a high proportion of "S"-phase nuclei. Of 8 tumours with all 3 features, progressive disease developed in 6 cases (75%). Of 9 patients who developed progressive disease, 8 (89%) had aneuploid tumours. Progressive disease did not develop in 11 patients with well differentiated tumours, compared with 4% (1/24) in diploid/tetraploid tumours and 7% (2/27) in those with a low/medium percentage of "S"-phase nuclei. In contrast to muscle-invasive disease, recurrent superficial tumours developed with a high incidence in all groups. Only 6/56 patients (11%) remained alive and disease-free for 3 years. It is concluded that these 3 features are of similar prognostic significance and accuracy in identifying patients requiring more aggressive therapy.

Matsumoto, H., H. Matsuyama, et al. (2004). "Allelic imbalance at 1p36 may predict prognosis of chemoradiation therapy for bladder preservation in patients with invasive bladder cancer." Br J Cancer **91**(6): 1025-31.

Invasive bladder cancers have been treated by irradiation combined with cis-platinum (CDDP) as a bladder preservative option. The aim of this study was to find a marker for predicting patient outcome as well as clinical response after chemoradiation therapy (CRT) by investigating allelic loss of apoptosis-related genes. A total of 67 transitional cell carcinomas of the bladder treated by CRT (median dose: 32.4 Gy of radiation and 232 mg of CDDP) were studied. We investigated allelic imbalances at 14 loci on chromosomes 17p13 and 1p36 including the p53 and p73 gene regions by fluorescent multiplex PCR based on DNA from paraffin-embedded tumour specimens and peripheral blood. The response to CRT was clinical response (CR) in 21 patients (31%), partial response (PR) in 31 (46%), and no change(NC) in 15 (22%). There was no statistical correlation between treatment response and clinical parameters, such as tumour grade, stage, radiation dose, or CDDP dose. The frequencies of allelic imbalance for TP53 and TP73 were 21 and 56%, respectively; neither was correlated with clinical treatment response and tumour stage or grade. There was no statistical correlation between treatment response and allelic imbalance at the other 12 loci. We found a significant correlation between cancer-specific survival and an imbalance of D1S243 (P=0.0482) or TP73 (P=0.0013) using a Log-rank test, although other loci including TP53 did not correlate with survival (P=0.4529 Multivariate analysis showed performance status (P=0.0047), recurrence (P=0.0017), and radiation doses (P=0.0468) were independent predictive factors for cancer-specific survival. However, an allelic imbalance of TP73 was the most remarkable independent predictive factor of poor patient survival (P=0.0002, risk ratio: 3382). Our results suggest that the allelic loss of the p73 gene predicts a clinical outcome of locally advanced bladder cancer when treated by CRT.

Menon, A. G., H. Morreau, et al. (2002). "Down-regulation of HLA-A expression correlates with a better prognosis in colorectal cancer patients." Lab Invest **82**(12): 1725-33.

To evaluate the prognostic impact of human leukocyte antigen class I (HLA-I) expression on immune surveillance in colorectal cancer, we studied 88 curatively resected tumors for HLA-A and HLA-B/C expression and correlated these data to clinical and histopathological parameters. HLA-A was normal (all tumor cells had HLA expression) in 32%, reduced (HLA-negative and -positive tumor cells coexisted) in 56%, or absent (no tumor cells expressed HLA) in 12% of evaluable cases. HLA-B/C was normal in 47%, reduced in 47%, and absent in 7% of the cases. Considering both markers, total HLA-I expression was normal in 27%, reduced in 63%, absent in 7%, and could not be evaluated in 3% of the cases due to absent HLA-A expression in tumor and normal cells. Down-regulation of HLA-A expression significantly correlated with a lower tumor stage ($p = 0.005$), mucinous tumors ($p = 0.05$), a lower incidence of recurrences ($p = 0.03$), and a longer disease-free survival ($p = 0.02$). Down-regulation of HLA-B/C

expression correlated with a lower tumor stage ($p < 0.001$) and a longer disease-free survival ($p = 0.04$). In multivariate analysis, HLA-A down-regulation was the only prognostic factor correlated with a longer disease-free survival ($p = 0.02$). Six tumors were negative for HLA-A and -B/C and did not recur during follow-up. Therefore, we analyzed microsatellite instability (MSI) in these cases. Three of these six tumors indeed showed down-regulation of MLH-1, MSH-2, or MSH-6, indicating a MSI-high phenotype. Beta-2-microglobulin protein expression was lost in five of six of the HLA-I-negative cases, but frame shift mutations in three repetitive sequences in beta2-microglobulin were absent. In contrast, loss of MLH-1, MSH-2, and MSH-6-protein expression was only observed in two of nine matched controls with reduced or normal HLA-A and -B/C expression. Our data showed that HLA-I was down-regulated in 72% of colorectal cancers and provided independent prognostic information for a longer disease-free survival. The better prognosis may be caused by elimination of HLA-negative cells by natural killer cells or by an attenuated tumor aggressiveness, as is seen in tumors with a MSI-high phenotype.

Moreno, V., F. Gemignani, et al. (2006). "Polymorphisms in genes of nucleotide and base excision repair: risk and prognosis of colorectal cancer." Clin Cancer Res **12**(7 Pt 1): 2101-8.

OBJECTIVES: We have undertaken a comprehensive study of common polymorphisms in genes of DNA repair, exploring both the risk of developing colorectal cancer and the prognosis of patients. METHODS: Subjects from a case-control study (377 cases and 329 controls) designed to assess gene-environment interactions were genotyped by use of an oligonucleotide microarray and the arrayed primer extension technique. Twenty-eight single nucleotide polymorphisms in 15 DNA repair genes were included. The candidate genes belong to different DNA repair pathways: base excision repair (OGG1, LIG3, APEX, POLB, XRCC1, PCNA, and MUTYH), nucleotide excision repair (ERCC1, ERCC2, ERCC4, and ERCC5), double-strand breaks repair (XRCC2, XRCC3, and XRCC9), and reversion repair (MGMT) genes. RESULTS: Polymorphism OGG1 S326C was associated with an increased risk of colorectal cancer [odds ratio (OR), 2.3; 95% confidence interval (95% CI), 1.1-5.0], the risk being higher in younger individuals. A haplotype of ERCC1 was associated with increased risk (OR, 2.3; 95% CI, 1.0-5.3). POLB P242R was also associated with decreased risk (OR, 0.23; 95% CI, 0.05-0.99), although the number of variant allele carriers was low. In the univariate analysis, adjusted for age, sex, and Dukes' stage, three polymorphisms were significantly associated with better prognosis: XRCC1 R399Q [hazard ratio (HR), 0.38; 95% CI, 0.17-0.85], XRCC3 T141M (HR, 0.66; 95% CI, 0.45-0.97), and MGMT L84F (HR, 0.14; 95% CI, 0.02-0.99). ERCC1 19007T>C was associated with worse prognosis (HR, 1.51; 95% CI, 1.01-2.27). In a multivariate analysis, only XRCC1 R399Q and ERCC1 19007T>C remained significant. These associations were stronger among patients receiving adjuvant chemotherapy. CONCLUSIONS: Although the overall effect of DNA repair genes in colorectal cancer etiology seems limited, their influence in the response to chemotherapy and prognosis may be more relevant. This knowledge may help to clarify the utility of specific adjuvant treatments according to the individual genetic background.

Nagai, S., K. Takenaka, et al. (2006). "A novel classification of MUC1 expression is correlated with tumor differentiation and postoperative prognosis in non-small cell lung cancer." J Thorac Oncol **1**(1): 46-51.

BACKGROUND: MUC1 is a transmembrane mucin that plays an important role in tumor progression. Many clinical studies have suggested that the expression pattern of MUC1 core protein can be a useful prognostic marker in various malignancies, but the prognostic significance in non-small cell lung cancer (NSCLC) remains uncertain. We performed a study to assess clinical significance, especially prognostic impact, of MUC1 expression in NSCLC. METHODS: A total of 62 patients with completely resected pathologic stage I to IIIA NSCLC were retrospectively reviewed. Histologic sections cut from primary tumors were immunohistochemically stained with an anti-MUC1 monoclonal antibody (CA15-3, clone DF3), which recognizes unglycosylated epitope of MUC1 core protein. According to MUC1 expression pattern, each patient was classified into the high-grade polarized expression (HP), the low-grade polarized expression (LP), or the depolarized expression (D) group. RESULTS: Twenty-four (38.7%), 21 (33.9%), and 17 (27.4%) patients were classified into the HP group, the LP group, and the D group, respectively. HP was exclusively seen in adenocarcinoma, mostly in well-differentiated adenocarcinoma. D was correlated with progressive stage and lymph node metastasis. Postoperative survival of the D group seemed to be poorer than that of the HP group for all NSCLC patients, and the difference was enhanced in adenocarcinoma patients. CONCLUSION: A novel classification of MUC1 expression pattern (HP, LP, and D) was correlated with tumor differentiation and postoperative survival in NSCLC, especially in lung adenocarcinoma.

Nagler, R. M. (2009). "Saliva as a tool for oral cancer diagnosis and prognosis." Oral Oncol **45**(12): 1006-10.

Saliva testing, a non-invasive alternative to serum testing, may be an effective modality for diagnosis and for prognosis prediction of oral cancer, as well as for monitoring post therapy status, by measuring specific salivary macromolecules, examining proteomic or genomic targets such as enzymes, cytokines, growth factors, metalloproteinases, endothelin, telomerase, cytokeratines, mRNA's and DNA transcripts. Salivary analysis has been shown to be a useful diagnostic tool also for distant malignancies such as breast cancer. In recent years, significant alterations have been demonstrated in the saliva of oral cancer patients in the epithelial tumor markers--Cyfra 21-1, TPS and CA12, various oxidative stress-related salivary parameters as ROS and RNS, biochemical and immunological parameters as IGF and MMP's and RNA transcripts of IL8, IL-1B, DUSP1, HA3, OAZ1, S100P, and SAT. Collectively these accumulated data are predicted to alter the field of oral cancer diagnosis by employing highly sensitive new tools which will enable both medical professionals and the patients themselves to monitor their saliva for diagnosis and prognosis prediction, as they relate to oral cancer. At this point however, the aim of salivary analysis is mainly for screening which may be helpful in the future.

Naka, T., M. Kobayashi, et al. (1998). "Aberrant p16INK4 expression related to clinical stage and prognosis in patients with pancreatic cancer." Int J Oncol **12**(5): 1111-6.

The p16 tumor suppressor gene is thought to play an important role in cell cycle regulation by encoding for protein products that can inhibit the progression from G1 to S phase in the cell cycle. Recently, the p16 gene has been found to be mutated or deleted in a variety of different types of primary human malignant tumors and human-derived malignant tumor cell lines. In this study, primary ductal pancreatic adenocarcinomas from 32 human patients were analyzed immunohistochemically for expression of p16 protein, with emphasis on the role of abberant p16 protein expression as a prognostic indicator. In addition, the same tumors were also assessed for p53 protein expression, AgNOR counts, and DNA ploidy. Nineteen out of the 32 cases (59%) showed positive immunoreactivity for p16 protein in their tumors and a significant association was found between lack of p16 protein expression, and both advancing clinical stage classification of disease, and poorer survival ($p<0.05$). The rate of positive immunoreactivity for p53 protein expression was 59%, however, no clear association was found between p53 protein expression, and either clinical stage of disease, or survival. These findings suggest that alteration of the p53 gene may be a relatively early event in pancreatic tumorigenesis, whereas alteration of the p16 gene is more likely to be correlated with tumor progression in pancreatic malignancies. Further survival analysis revealed that all five of the 32 cases that survived for three years or longer had positive immunostaining for p16 protein, and a relatively low level of AgNOR counts. In four out of five of these patients, the tumors also exhibited negative immunostaining for p53 protein and DNA diploidy. These findings suggest that molecular analysis of patient tumor sections may yield potentially useful prognostic indicators for patients undergoing surgical resection for pancreatic cancer.

Ng, A. B. and N. B. Atkin (1973). "Histological cell type and DNA value in the prognosis of squamous cell cancer of uterine cervix." Br J Cancer **28**(4): 322-31.

Based on the evaluation of 362 cases of squamous cell carcinoma of the uterine cervix, the distribution of the tumours in relation to their modified Broders' grade, histological cell type as proposed by Wentz and Reagan, and the clinical stage of disease was evaluated. The morphological characteristics of the 3 cell types-large cell non-keratinizing, keratinizing, and small cell cancers-were described. The 5 year survival in relation to Broders' grade, cell type, extent and DNA values of the malignant cells were evaluated and compared. Broders' grading system was not useful in predicting the biological behaviour of cervical squamous cancer. The histological cell type and extent of the tumour were important factors in prognosis. The 5 year survival for large cell cancer was 51.8%, keratinizing cancer 34.7% and small cell cancer 10.0%. The 5 year survival was 63.3% for stage I neoplasms, 52.9% for stage II neoplasms, 30.7% for stage III neoplasms and 15.0% for stage IV neoplasms. When the DNA values of neoplastic cells were considered in relation to cell type and extent of disease, the biological behaviour of cervical squamous cell cancers was determined more accurately. The 5 year survival of women with cervical cancer in which the DNA values of the neoplastic cells exceeded 155 was more favourable than those with DNA values of less than 155. This difference in 5 year survival was evident for comparable cell type and clinical stage of disease.

Nimmrich, I., A. M. Sieuwerts, et al. (2008). "DNA hypermethylation of PITX2 is a marker of poor prognosis in untreated lymph node-negative hormone

receptor-positive breast cancer patients." Breast Cancer Res Treat **111**(3): 429-37.

BACKGROUND: In this study, we evaluated if PITX2 DNA methylation is a marker for disease recurrence in lymph node-negative (LNN), steroid hormone receptor-positive (HR+) breast cancer patients. In addition, we studied the association between PITX2 DNA methylation and PITX2 gene expression. PATIENTS AND METHODS: PITX2 DNA-methylation was measured in tumor tissue from 412 LNN/HR+ breast cancer patients who had not received any adjuvant systemic treatment. In addition, PITX2 DNA-methylation and mRNA expression was evaluated in 32 breast cancer cell lines. RESULTS: In univariate Cox regression analysis, DNA-methylation of PITX2 as a continuous variable was associated with early distant metastasis (HR = 1.71; P < 0.01) and poor overall survival (HR = 1.71; P < 0.01). In multivariate analysis together with the established prognostic factors age, tumor size and tumor grade, and steroid hormone receptor levels, both associations retained their significance (for MFS, HR = 1.74; P < 0.01; for OS, HR = 1.46; P = 0.02). In the breast cancer cell lines, PITX2 DNA methylation was inversely association with PITX2A and PITX2B mRNA expression (P < 0.01). CONCLUSIONS: Hypermethylation of PITX2 is, in cell lines, negatively associated with PITX2 mRNA expression and, in clinical specimens, positively associated with breast cancer disease progression.

Nobeyama, H., T. Sumi, et al. (2004). "Association of HPV infection with prognosis after neoadjuvant chemotherapy in advanced uterine cervical cancer." Int J Mol Med **14**(1): 101-5.

Whether the human papillomavirus (HPV) status of the tumor affects the sensitivity to neoadjuvant chemotherapy, and the prognosis in advanced uterine cervical cancer (FIGO stage III or higher) remains unknown. We examined the HPV status of 43 patients who had received CDDP therapy by balloon-occluded arterial infusion (BOAI), as neoadjuvant chemotherapy for advanced uterine cervical cancer (squamous cell carcinoma) stage III or higher. DNA was extracted from formalin-fixed, paraffin-embedded tumor samples obtained by punch biopsy before the neoadjuvant chemotherapy. The detection of HPV and its typing were analyzed by a polymerase chain reaction (PCR)-based assay using consensus primers for the L1 consensus regions. HPV DNA was detected in all 43 patients (100%): 29 cases with HPV 16 (67.4%), 5 cases with HPV 33 (11.6%), 4 cases with HPV 31 (9.3%), 3 cases with HPV 35 (7.0%), 1 case with HPV 18 (2.3%) and 1 case with HPV 58 (2.3%). The HPV types were divided into 3 groups, HPV 16, HPV 33 and other HPV types (HPV 18, 31, 35, 58), and comparisons and examinations were performed among the 3 groups. Although the rates of tumor reduction and operation accomplishment after 3 courses of BOAI showed no significant differences among the 3 groups, there were significant differences in the survival rates. The survival rate of advanced uterine cervical cancer patients with HPV 33 infection was the highest, followed by that of patients with HPV 16 infection. The survival rates of patients with the other types of HPV infection were the worst among the 3 groups and significantly lower than those of patients with HPV 16 or HPV 33 infection. The differences in the curative effect after BOAI may depend on the different characters of the HPV types.

Noike, T., S. Miwa, et al. (2008). "Increased expression of thioredoxin-1, vascular endothelial growth factor, and redox factor-1 is associated with poor prognosis in patients with liver metastasis from colorectal cancer." Hum Pathol **39**(2): 201-8.

We examined whether the expression of thioredoxin-1 (Trx-1) was associated with patient prognosis after liver resection for metastatic colorectal cancer. Eighty-four patients underwent resection of liver metastases from colorectal cancer, leaving no macroscopic evidence of residual tumor. Immunohistochemical study was performed to evaluate the relation among Trx-1, vascular endothelial growth factor (VEGF), and redox factor-1 (Ref-1) expression and the clinicopathologic characteristics and patient survival. Thirty-seven patients (44.0%) with Trx-1-positive metastases had shorter survival after primary liver resection (P = .0003) than the 47 patients (56.0%) with Trx-1-negative metastases. The percentage VEGF-positive and Ref-1-positive metastases was significantly higher in patients with Trx-1 expression (P = .0009 and .0002, respectively). Multivariate analysis revealed that Trx-1 expression was an independent prognostic factor. Expression of VEGF and Ref-1 is associated with Trx-1 overexpression, which is related to a poor prognosis in patients with liver metastases from colorectal cancer.

O'Connell, M. J., D. J. Schaid, et al. (1992). "Current status of adjuvant chemotherapy for colorectal cancer. Can molecular markers play a role in predicting prognosis?" Cancer **70**(6 Suppl): 1732-9.

BACKGROUND: Recent clinical trials establish a beneficial effect for adjuvant chemotherapy after surgical resection of the primary tumor (1) as single treatment for patients with colonic cancer and (2) combined with radiation therapy for patients with rectal cancer. Because adjuvant chemotherapy is not universally effective and is associated with toxicity

and some degree of risk, it would be desirable to supplement standard pathologic staging criteria to define more precisely the subset of patients at high risk for tumor recurrence who would benefit most from adjuvant therapy. Tumor cell DNA content and cell proliferation measured by flow cytometry were identified as important and independent prognostic factors for patients undergoing curative resection of colorectal cancer. Basic laboratory investigations show a series of more specific molecular and genetic abnormalities that might provide better prognostic discrimination. Recent molecular studies suggest that the process of tumorigenesis in colorectal cancer proceeds through a series of genetic alterations that include both dominant and recessive protooncogenes. Characterization of these molecular genetic abnormalities may provide valuable prognostic information for use in patient management. METHODS: Allelic loss was studied for chromosomes 5, 17, and 18, and immunohistochemical analysis was done of the p53 protein product in tumors from 91 patients with colorectal cancer. RESULTS: Preliminary analysis of disease-free survival after surgical resection in 60 patients with Dukes' B or C tumors suggests a poorer prognosis associated with allelic loss on chromosome 18q (P = 0.08). CONCLUSIONS: Additional studies involving a much larger population of patients with Dukes' B and C colorectal cancer are needed to define the true prognostic significance of these molecular markers.

Okada, K., T. Shimura, et al. (2006). "Reduced galectin-3 expression is an indicator of unfavorable prognosis in gastric cancer." Anticancer Res **26**(2B): 1369-76.

BACKGROUND: Galectin-3 (gal-3) participates in a variety of biological events, including cell adhesion, proliferation, differentiation and apoptosis. The aim of this study was to determine the relationship of gal-3 expression with clinicopathological findings and prognosis in patients with gastric cancer. PATIENTS AND METHODS: Gal-3 and Ki-67 expressions were assessed by immunohistochemistry in 115 patients with gastric cancer. PCR-single strand conformation polymorphism (SSCP)-sequence analysis and the levels of gal-3 mRNA were also examined. RESULTS: The present study demonstrated that gal-3 expression was correlated with nodal status, lymphatic inivasion, pathological stage and histological parameters. On the other hand, gal-3 expression did not correlate with the expression of Ki-67. Reduced expression of gal-3 was significantly associated with a poor prognosis and multivariate analysis showed that gal-3 expression was an independent prognostic factor. On PCR-SSCP-sequence analysis, 2 single nucleotide polymorphisms (SNPs) were detected in the gal-3 gene, but none showed mutations. CONCLUSION: Reduced gal-3 expression was associated with lymph node metastasis, advanced stage and tumor differentiation in gastric cancer. Gal-3 expression could be a useful prognostic factor in gastric cancer.

Okuda, K., H. Sasaki, et al. (2008). "Met gene copy number predicts the prognosis for completely resected non-small cell lung cancer." Cancer Sci **99**(11): 2280-5.

The Met oncogene encodes the tyrosine kinase receptor for hepatocyte growth factor (HGF). Uncontrolled activation of Met is oncogenic and has been implicated in the growth, invasion and metastasis in a variety of tumors. Several distinct mechanisms including amplification, translocation or mutation of Met may underlie uncontrolled Met activation. In several solid tumors, amplification and mutation of Met were reported to be associated with tumorigenesis, invasion and metastasis. The present study evaluated the amplification and mutation of Met in a large number of non-small cell lung cancer (NSCLC). Among 213 NSCLC patients, increased Met copy number was identified in 12 patients (5.6%) and associated with a worse prognosis (P = 0.0414). The mutation of Met in 534 NSCLC patients was also evaluated. In these patients there were no previously reported mutations within the juxtamembrane (JM) domain (R988C, T1010I, S1058P and G1085X). However, a somatic exon 14 deleting splice variant in 3 (1.7%) of 178 NSCLC samples was identified for which sequencing was performed. Met amplification and mutation were rare in Japanese NSCLC. However, the results support a critical role of Met gene dose in NSCLC, suggesting that Met may be a specific molecular therapeutic target in selected NSCLC patients with increased Met copy number.

Okumura, M., Y. Kajiyama, et al. (2008). "Correlation between loss of Bcl-XL expression and improved prognosis in advanced esophageal cancer treated by preoperative chemoradiotherapy." Eur Surg Res **41**(3): 260-6.

We investigated the clinical significance of the apoptosis- related molecule expression of tumor cells in patients with advanced esophageal cancer treated with preoperative chemoradiotherapy (CRT). Preoperative CRT reduced Bcl-X(L) expression in a significant proportion of the group responding to CRT but not in the group resisting CRT, although Bcl-2 expression was reduced in both groups. The mean survival time of the patients with cancers that lost Bcl-X(L) following CRT was significantly longer

compared to those with cancers expressing Bcl-X(L). These results suggested that CRT reduced Bcl-X(L) expression, and this decrease closely correlated with the prolonged survival of advanced esophageal cancer patients treated with preoperative CRT.

Ott, K., F. Lordick, et al. (2008). "Glutathione-S-transferase P1, T1 and M1 genetic polymorphisms in neoadjuvant-treated locally advanced gastric cancer: GSTM1-present genotype is associated with better prognosis in completely resected patients." Int J Colorectal Dis **23**(8): 773-82.

OBJECTIVE: Neoadjuvant chemotherapy in gastric cancer is now standard in the Western world; however, only 30-40% of the patients respond to induction therapy. Pretherapeutic predictors of response and prognosis would be of utmost interest to individualize treatment. Glutathione-S-transferase enzymes detoxify therapeutic drugs such as platin derivates and may influence outcome of the treated patients. Therefore, glutathione-S-transferase (GST) polymorphisms were assessed as predictive markers in cisplatinum-based neoadjuvant-treated gastric cancer. MATERIALS AND METHODS: DNA was isolated from 139 patients with locally advanced gastric cancer (cT3/4 anyN cM0) before chemotherapy. Multiplex polymerase chain reaction was used for GSTT1 and GSTM1 genes, and allelic discrimination assay with the TaqMan system for the GSTP1 gene. RESULTS: One hundred ten patients could be analyzed for GSTT1 (T-:23; T+87), 112 for GSTM1 (M-:52; M+:60) and 132 for GSTP1 (Ile/Ile: 55; Ile/Val: 59; Val/Val: 18). There was no significant correlation between any of the GSTT1, GSTM1, or GSTP1 genotypes and patients' characteristics or histopathological data; only the GSTM1+ genotype was associated with the non-intestinal subtype of the Lauren classification (p=0.045). GSTT1, GSTM1, and GSTP1 genotypes were not correlated with response to chemotherapy (p=0.57, p=0.38, p=0.33). In R0 resected patients, we found an improved survival for patients with the GSTM1-present genotype compared to patients with the GSTM1-null genotype (p=0.017). Moreover, the GSTM1-present genotype showed a significantly better tumor-related (p=0.017) and disease-free survival (p=0.029). CONCLUSION: None of the common GST polymorphisms predicts response in our study, but the GSTM1+ genotype was associated with a better prognosis in completely resected patients. Further investigations on chemotherapy-associated gene polymorphisms are warranted.

Pietersen, A. M., H. M. Horlings, et al. (2008). "EZH2 and BMI1 inversely correlate with prognosis and TP53 mutation in breast cancer." Breast Cancer Res **10**(6): R109.

INTRODUCTION: PolycombGroup (PcG) proteins maintain gene repression through histone modifications and have been implicated in stem cell regulation and cancer. EZH2 is part of Polycomb Repressive Complex 2 (PRC2) and trimethylates H3K27. This histone mark recruits the BMI1-containing PRC1 that silences the genes marked by PRC2. Based on their role in stem cells, EZH2 and BMI1 have been predicted to contribute to a poor outcome for cancer patients. METHODS: We have analysed the expression of EZH2 and BMI1 in a well-characterised dataset of 295 human breast cancer samples. RESULTS: Interestingly, although EZH2 overexpression correlates with a poor prognosis in breast cancer, BMI1 overexpression correlates with a good outcome. Although this may reflect transformation of different cell types, we also observed a functional difference. The PcG-target genes INK4A and ARF are not expressed in tumours with high BMI1, but they are expressed in tumours with EZH2 overexpression. ARF expression results in tumour protein P53 (TP53) activation, and we found a significantly higher proportion of TP53 mutations in tumours with high EZH2. This may explain why tumours with high EZH2 respond poorly to therapy, in contrast to tumours with high BMI1. CONCLUSIONS: Overall, our data highlight that whereas EZH2 and BMI1 may function in a 'linear' pathway in normal development, their overexpression has different functional consequences for breast tumourigenesis.

Pinto, A. E., S. Andre, et al. (2001). "C-erbB-2 oncoprotein overexpression identifies a subgroup of estrogen receptor positive (ER+) breast cancer patients with poor prognosis." Ann Oncol **12**(4): 525-33.

PURPOSE: To investigate the predictive value of c-erbB-2 oncoprotein expression as compared with established histopathological and cytometric indicators of disease evolution in breast carcinoma. PATIENTS AND METHODS: A short-term retrospective study was conducted on a series of 306 breast cancer patients. Classic prognostic factors included tumour size, nodal involvement, histological grading, and hormone receptor status. Flow cytometric DNA ploidy and S-phase fraction (SPF) were also assessed. A Cox proportional hazards regression model was used for multivariate statistical analysis. RESULTS: c-erbB-2 overexpression was present in 43 out of 295 (14.6%) tumours, and showed a statistically significant correlation with high histological grade, DNA aneuploidy, high SPF and lack of estrogen receptors (ER). Univariate analysis revealed its association with worse disease-free survival (DFS)

and overall survival (OS). The combined evaluation of c-erbB-2 with ploidy and SPF defines a variable (P + S + c) that showed a significant correlation with disease outcome. By multivariate analysis, only nodal status ($P < 0.001$) and P + S + c subgrouping (group 2: $P = 0.002$; group 3: $P = 0.001$) in relation to DFS, and nodal status ($P = 0.001$) and DNA ploidy ($P = 0.006$) in relation to OS, retained independent prognostic significance. Subset analyses showed that cytometric parameters, P + S + c subgrouping and hormone receptors were significantly correlated with disease outcome in node-positive patients, whereas in node-negative subgroup no prognostic indicators were found. c-erbB-2 overexpression exhibited a trend in node-positive breast cancer (DFS: $P = 0.068$; OS: $P = 0.086$), and significant correlation with poor clinical evolution in ER positive patients (DFS: $P = 0.015$; OS: $P = 0.004$), mostly receiving tamoxifen. CONCLUSIONS: c-erbB-2 is an independent prognostic indicator of DFS when evaluated in conjunction with ploidy and SPE. It also seems to predict response to tamoxifen therapy, by identifying a subgroup of ER positive (ER+) breast cancer patients with poor prognosis.

Piperi, C., F. Vlastos, et al. (2008). "Epigenetic effects of lung cancer predisposing factors impact on clinical diagnosis and prognosis." J Cell Mol Med **12**(5A): 1495-501.

Lung carcinogenesis is a complex process requiring the acquisition of genetic mutations that confer the malignant phenotype as well as epigenetic alterations that may be both manipulated in the course of therapy. Aberrant gene function and transcriptional silencing by CpG island hypermethylation has become a critical component in the initiation and progression of lung cancer. Growing evidence shows that acquired epigenetic abnormalities participate with genetic alterations to cause this dysregulation. Human and animal studies have fostered significant advances in elucidating the role of gene-specific methylation in cancer initiation and progression, the modulation of DNA methylation by carcinogen exposure and the ability of pharmacologic agents to reverse promoter hypermethylation, making it an attractive target to pursue for prevention of lung cancer. This review focuses on how lung cancer predisposing factors participate in epigenetic alterations of lung neoplasia, and discusses the growing implications of these alterations for strategies to control cancer.

Rainwater, L. M. and H. Zincke (1988). "Radical prostatectomy after radiation therapy for cancer of the prostate: feasibility and prognosis." J Urol **140**(6): 1455-9.

A total of 30 patients underwent an operation (radical prostatectomy in 27 and cystoprostatectomy in 3) for prostate cancer. In 17 patients radiotherapy with curative intent (range 5,700 to 7,500 cGy., mean 6,130 cGy.) had failed locally at 7 to 150 months (mean 48.8 months) later; 13 underwent the operation 2 months or less after planned radiotherapy (4,000 to 7,000 cGy., mean 5,431 cGy.). Followup was 1 to 16 years (mean 6.7 years). There was no perioperative death. The most significant complications were vesical neck contracture (17 per cent), lymphedema (10 per cent) and incontinence (10 per cent). Pathological staging revealed 8 patients with stage D1 disease. Of tumor grade, stage, adjuvant hormonal treatment and ploidy pattern only the last was associated with progression and survival. An increasing number of patients with local, albeit often symptomatic, radiation failure may present during the next years and they must be treated. Salvage prostatectomy is feasible and its associated morbidity seems to be acceptable; alternative (nonoperative) treatment modalities have proved not to be effective. Deoxyribonucleic acid ploidy pattern can provide valuable prognostic information in this difficult patient population for decision-making regarding radical surgery and adjuvant hormonal treatment.

Rakowicz-Szulczynska, E. M., M. Markowski, et al. (1997). "New protein and PCR markers RAK for diagnosis, prognosis and surgery guidance for breast cancer." Cancer Lett **112**(1): 93-101.

Breast cancer antigens RAK-p120, -p42, -p25 were detected in 100% of breast cancer cases tested (71 cases). Only 10% of adjacent tissue cases tested positive for all three cancer antigens, and 17.5% of the cases tested positive for two antigens only. Eighty-five percent of histologically normal breast tissue samples, isolated either from breast cancer patients or patients with advanced fibrocystic disease, tested RAK-negative, with the exception of low expression of p25, observed in some patients. Polymerase chain reaction (PCR) with HIV-1 gp 41-derived primers revealed cancer-associated DNA fragments of similar size (140 bp) as in HIV-1 genome. Fifty-four percent of cancer adjacent tissues, and 50% of malignancy-free breast tissue samples, tested PCR-negative. It is suggested that genetic predisposition to cancer may be associated with the presence of RAK genes, while expression of RAK antigens marks an already ongoing process of malignant changes.

Ravaioli, A., L. Bagli, et al. (1998). "Prognosis and prediction of response in breast cancer: the current role of the main biological markers." Cell Prolif **31**(3-4): 113-26.

In the medical literature there are frequently conflicting reports on the utility of biological tumour markers available in the clinical management of breast cancer. In this review we analyse current information on the relationships between the most widely investigated breast cancer biological markers including oestrogen and progesterone receptors, p53, Bcl-2, c-erbB-2, cyclin expression, proliferative activity, DNA ploidy and the urokinase plasminogen activation system, as well as their relevance to prognosis and response to clinical treatment. By biological prognostic indicator, we mean a marker that correlates with survival and disease-free survival; the term predictor marker indicates a marker that is capable of predicting tumour sensitivity or resistance to various therapies. Similarly to other authors' experiences, our analysis suggests that oestrogen receptors are weak prognostic indicators and good predictors of response to endocrine therapy. Furthermore, there are consistent data suggesting that proliferation indices are good indicators of prognosis, and that they are directly related to response to chemotherapy and closely related to response to hormonotherapy. On the contrary, there is no evidence or conflicting data for all of the other biological markers. These should be considered in the context of randomized trials in order to precisely define their prognostic and predictive roles. p53 and c-erbB-2 seem to be the most promising factors, but their use in routine practice still needs validation.

Richman, S. D., M. T. Seymour, et al. (2009). "KRAS and BRAF mutations in advanced colorectal cancer are associated with poor prognosis but do not preclude benefit from oxaliplatin or irinotecan: results from the MRC FOCUS trial." J Clin Oncol **27**(35): 5931-7.

PURPOSE: Activating mutation of the KRAS oncogene is an established predictive biomarker for resistance to anti-epidermal growth factor receptor (anti-EGFR) therapies in advanced colorectal cancer (aCRC). We wanted to determine whether KRAS and/or BRAF mutation is also a predictive biomarker for other aCRC therapies. PATIENTS AND METHODS: The Medical Research Council Fluorouracil, Oxaliplatin and Irinotecan: Use and Sequencing (MRC FOCUS) trial compared treatment sequences including first-line fluorouracil (FU), FU/irinotecan or FU/oxaliplatin in aCRC. Tumor blocks were obtained from 711 consenting patients. DNA was extracted and KRAS codons 12, 13, and 61 and BRAF codon 600 were assessed by pyrosequencing. Mutation (mut) status was assessed first as a prognostic factor and then as a predictive biomarker for the benefit of adding irinotecan or oxaliplatin to FU. The association of BRAF-mut with loss of MLH1 was assessed by immunohistochemistry. RESULTS: Three hundred eight (43.3%) of 711 patients had KRAS-mut and 56 (7.9%) of 711 had BRAF-mut. Mutation of KRAS, BRAF, or both was present in 360 (50.6%) of 711 patients. Mutation in either KRAS or BRAF was a poor prognostic factor for overall survival (OS; hazard ratio [HR], 1.40; 95% CI, 1.20 to 1.65; P < .0001) but had minimal impact on progression-free survival (PFS; HR, 1.16; 95% CI, 1.00 to 1.36; P = .05). Mutation status did not affect the impact of irinotecan or oxaliplatin on PFS or OS. BRAF-mut was weakly associated with loss of MLH1 staining (P = .012). CONCLUSION: KRAS/BRAF mutation is associated with poor prognosis but is not a predictive biomarker for irinotecan or oxaliplatin. There is no evidence that patients with KRAS/BRAF mutated tumors are less likely to benefit from these standard chemotherapy agents.

Rudas, M., M. F. Gnant, et al. (1994). "Thymidine labeling index and Ki-67 growth fraction in breast cancer: comparison and correlation with prognosis." Breast Cancer Res Treat **32**(2): 165-75.

In situ determination of proliferative activity was performed on 184 consecutive primary invasive breast cancers. Methods used were monoclonal antibody Ki-67 in immunohistochemistry and thymidine labeling index. Tumor proliferation correlated between both methods (p = 0.0001). For thymidine labeling index and Ki-67, respectively, significant correlations existed with histologic tumour grade and steroid hormone receptors (Tumor grade: TLI p = 0.0001; Ki-67 p = 0.0001. ER-ICA: TLI = 0.0001; Ki-67 p = 0.014. PgR-ICA: TLI p = 0.0001; Ki-67 p = 0.0008). For thymidine labeling index a significant correlation was demonstrated for overall survival (p = 0.001) and recurrence free survival (p = 0.01). No statistical significance was observed for clinical outcome and Ki-67 (overall survival p = 0.18; recurrence free survival p = 0.1). None of the factors, TLI or Ki-67, was an independent prognostic factor as demonstrated by multivariate analysis.

Scarpa, A., C. Di Pace, et al. (2000). "Cancer of the ampulla of Vater: chromosome 17p allelic loss is associated with poor prognosis." Gut **46**(6): 842-8.

BACKGROUND: Cancer of the ampulla of Vater kills 60% of affected patients. Local spread of the tumour (T stage) is the only reliable prognostic factor. Nevertheless, any cancer stage includes long term survivors and patients dying from the disease. The molecular anomalies involved in this process have the potential to serve as additional prognostic markers. AIM: To evaluate if allelic losses (LOH) of chromosomes 17p and 18q may be of prognostic value in multivariate survival analysis. METHODS: We

examined 53 ampullary cancers for chromosome 17p and 18q LOH using microsatellite markers and DNA from paraffin embedded tumours. All patients were treated by surgery alone (pancreaticoduodenectomy). Multivariate survival analysis included age, sex, tumour size, macroscopic appearance, grade of differentiation, T stage, lymph node metastasis, and chromosome 17p and 18q status. RESULTS: Chromosome 17p and 18q LOH were detected in 28 (53%) and 18 (34%) cancers, respectively. Multivariate survival analysis indicated chromosome 17p status as an independent prognostic factor together with T stage. The five year survival for chromosome 17p retention and 17p loss was 80% and 7%, respectively. The risk of death from cancer within the five year follow up period for patients with cancers harbouring chromosome 17p LOH was 11 times higher than that of patients with cancers retaining chromosome 17p ($p<0.0001$), regardless of the tumour stage at diagnosis. CONCLUSIONS: Chromosome 17p status is an independent prognostic factor among ampullary cancers at the same stage. The combined use of T stage and chromosome 17p status may help in deciding whether ampullary cancer patients require additional therapy other than surgery alone.

Schneider, P. M., H. W. Praeuer, et al. (2000). "Multiple molecular marker testing (p53, C-Ki-ras, c-erbB-2) improves estimation of prognosis in potentially curative resected non-small cell lung cancer." Br J Cancer **83**(4): 473-9.

A prospective study was performed in patients with non-small cell lung cancer (NSCLC) to evaluate the prognostic importance of multiple molecular marker (p53, c-Ki-ras, c-erbB-2) testing. 103 patients with potentially curative resections (RO resection) for NSCLC in histopathological stages I-IIIA were included. SSCP analysis and DNA sequencing for p53 and c-Ki-ras genes were performed on paired tumour and normal lung tissue samples and immunohistochemistry (c-erbB-2) was done on frozen tissue sections with a specific anti-c-erbB-2 monoclonal antibody. 46/103 (44.6%) NSCLC showed p53 mutations and 17/103 (16.5%) c-Ki-ras mutations including 12/37 (32.4%) adenocarcinomas. Overexpression of c-erbB-2 (p185) was detected in 56/103 (54.4%) tumours. 24/103 (23.3%) NSCLC were negative for alterations in all 3 parameters (c-Ki-ras, p53 and p185) whereas 79/103 (76.7%) were positive for at least one of the 3 parameters. In a regression model including a multiple molecular marker parameter (negative for all 3 markers versus positive for at least one marker), histopathological stage ($P<0.00001$), respectively the pT ($P<0.01$) and pN ($P<0.00001$) categories and the multiple molecular marker parameter ($P<0.01$) were of significant prognostic importance. This study demonstrates that testing 3 molecular markers (c-Ki-ras, p53 and c-erbB-2) improves estimation of prognosis compared to single marker testing and appears to define low (82.6%+/-7.9% 5-year survival) and high risk (40.2%+/-5.5% 5-year survival) groups for treatment failure in potentially curative (RO) resected NSCLC.

Schuuring, E., E. Verhoeven, et al. (1992). "Amplification of genes within the chromosome 11q13 region is indicative of poor prognosis in patients with operable breast cancer." Cancer Res **52**(19): 5229-34.

Amplification of the chromosome 11q13 region, which harbors the BCL1 region and the PRAD1, EMS1, HSTF1, and INT2 genes, was found in 36 (16%) of a series of 226 breast carcinomas. In the 153 patients with stage I-IIIa disease who had received no therapy prior to surgery and who were treated with curative intent, 11q13 amplification was associated with the presence of lymph node metastases (P less than 0.002). The presence of an 11q13 amplification was associated with a significantly shorter relapse-free survival (P less than 0.002) and a higher breast cancer-specific mortality (P less than 0.003). Stepwise multivariate analysis showed that, in addition to lymph node status, 11q13 amplification was the best predictor for short survival. Stratified log-rank analysis indicated that, within the group of lymph node-positive breast cancer patients, 11q13 amplification identifies a subgroup at high risk.

Scott, N. A., H. S. Wieand, et al. (1987). "Colorectal cancer. Dukes' stage, tumor site, preoperative plasma CEA level, and patient prognosis related to tumor DNA ploidy pattern." Arch Surg **122**(12): 1375-9.

Flow cytometric DNA histograms of colorectal carcinomas from 264 patients were evaluated for the association of tumor site, Dukes' stage, tumor grade, and preoperative carcinoembryonic level with patient survival. The DNA nondiploid carcinomas were significantly more common from the left (descending and sigmoid) colon and the rectum. A poorer prognosis was found for patients with DNA nondiploid cancers than for patients with DNA diploid cancers. This was particularly true for patients with Dukes' stages B2 and C tumors with a small number (one to three) of lymph nodes with metastatic deposits. The DNA nondiploid cancers also had a relatively poorer prognosis in patients with unresectable disease. In a Cox multivariate analysis model, the DNA pattern was an independent prognostic variable for this group of 264 patients with resected colorectal carcinoma.

Sendler, A., K. P. Gilbertz, et al. (2001). "Proliferation kinetics and prognosis in gastric cancer after resection." Eur J Cancer **37**(13): 1635-41.

The influence of proliferation and proliferation kinetics on prognosis in gastric cancer after complete resection are controversial. In a prospective study we investigated the tumour specimens of 111 patients after resection of gastric cancer, who received 200 mg intravenous (i.v.) bromodeoxyuridine (BrdU) pre-operatively. The following biological parameters were analysed in the tumour tissue using flow-cytometry: DNA ploidy, proportion of S-phase cells, BrdU labelling index (LI), DNA synthesis time (T(s)), potential tumour doubling time (T(pot)), proliferating cell nuclear antigen (PCNA) and Ki-67 LI. The median follow-up time was 40 months (range 19-62 months). Besides the established pathohistological prognostic factors, univariate analysis revealed a prognostic influence on survival for BrdU LI, T(pot) and the proportion of S-phase cells. By multivariate Cox analysis of the completely resected cases, only tumour stage and T(pot) had a significant, independent influence on survival. By classification and regression trees (CART) analysis, resection status, tumour stage and T(pot) defined risk groups with significantly different outcomes. A short T(pot) was a predictor of better survival in stage I, II and IIIA tumours. Ploidy and the other investigated proliferation-related parameters failed to demonstrate any influence on prognosis after resection of gastric cancer.

Sher, Y. P., J. Y. Shih, et al. (2005). "Prognosis of non-small cell lung cancer patients by detecting circulating cancer cells in the peripheral blood with multiple marker genes." Clin Cancer Res **11**(1): 173-9.

PURPOSE: Current lung cancer staging and prognosis methods are based on imaging methods, which may not be sensitive enough for early and accurate detection of metastasis. This study aims to validate the use of a panel of markers for circulating cancer cell detection to improve the accuracy of cancer staging, prognosis, and as a rapid assessment of therapeutic response. EXPERIMENTAL DESIGN: We analyzed the National Cancer Institute-Cancer Genome Anatomy Project database to identify potential marker genes for the detection of circulating cancer cells in peripheral blood. Nested real-time quantitative PCR and a scoring method using cancer cell load Lc were employed to correlate the amount of circulating cancer cells with clinical outcomes in 54 non-small cell lung cancer (NSCLC) patients. The Kaplan-Meier method was employed for analysis of prognostic variables. RESULTS: A panel of four marker genes was identified and experimentally validated. With these marker genes, we achieved an overall positive detection rate of 72% for circulating cancer cells in the peripheral blood of NSCLC patients. Patients who had higher Lc values had worse outcomes and shorter survival times. Patients with poor therapeutic response were revealed by positive detection of circulating cancer cells after therapy. The results correlated well with the patients' survival time. CONCLUSION: Circulating cancer cell detection by a panel of markers and the Lc scoring method can supplement the current tumor, node, metastasis staging method for improved prognosis and for rapid assessment of therapeutic response. Together, they may facilitate the design of better therapeutic strategies for the treatment of NSCLC patients.

Shyr, Y. M., C. H. Su, et al. (1999). "The role of MIB-1 index in the prognosis of resectable pancreatic head cancer." Hepatogastroenterology **46**(29): 2968-73.

BACKGROUND/AIMS: Cell kinetics are important indicators of the biological behavior of various human tumors. There were 21 resectable pancreatic head cancers. By univariate analysis MIB-1 index, cell differentiation and lymphovascular invasion were significant prognostic factors. The 5-year survival rate was 22.2% for overall patients and 29.2% for patients with MIB-1 < or = 11%, while it was 0% for MIB-1 index > 11%, p=0.011. Tumors without lymphovascular invasion had significantly better prognosis than those with lymphovascular invasion (median survival: 38 vs. 10 months, p=0.009). The median survival was significantly longer for well-differentiated cancers than for moderately and poorly differentiated cancers (44 vs. 11 and 9 months, p=0.038). There was no correlation between the MIB-1 index and the other 2 conventional prognostic factors. After multivariate analysis, only the MIB-1 index emerged as the independent prognostic factor. CONCLUSIONS: MIB-1 index played a significant role in the prognosis of the resectable pancreatic head cancer and could potentially complement the conventional factors in predicting the prognosis and determining the optimal treatment strategy. MIB-1 index was also an important independent prognostic factor.

Sigurdsson, H., B. Baldetorp, et al. (1990). "Indicators of prognosis in node-negative breast cancer." N Engl J Med **322**(15): 1045-53.

Measures of the proliferative activity of tumor cells have prognostic value in patients with node-negative breast cancer. We studied 367 women in southern Sweden who had undergone surgical resection for such cancer. Tumor specimens were analyzed with DNA flow cytometry in order to estimate both the DNA content (ploidy) and the fraction of cells in the synthetic phase of the cell cycle

(S phase). The median duration of follow-up was four years; 28 percent of the patients received adjuvant therapy, usually with tamoxifen (n = 83). A multivariate analysis based on complete data on 250 patients included the following covariates: age (greater than or equal to 75, 50 to 74, and less than or equal to 49 years), tumor size (less than or equal to 20 vs. greater than 20 mm), concentration of estrogen and progesterone receptors (less than 10 vs. greater than or equal to 10 fmol per milligram of protein), ploidy (diploid vs. nondiploid), and S-phase category (fraction of cells in S phase: less than 7.0 percent, 7.0 to 11.9 percent, and greater than or equal to 12 percent). The S-phase fraction yielded the most prognostic information, followed by progesterone-receptor status and tumor size. A prognostic model based on these three variables identified 37 percent of the patients as constituting a high-risk group with a fourfold increased risk of distant recurrence. In the remaining 63 percent of the patients, the five-year overall survival rate (92 +/- 4 [+/- SE] percent) did not differ from the expected age-adjusted rate for Swedish women. We conclude that a prognostic index that includes indicators of the proliferative activity of tumor cells may be able to identify women with node-negative breast cancer in whom the risk of recurrence is sufficiently low that adjuvant chemotherapy can be avoided.

Silverstein, M. J., E. D. Gierson, et al. (1990). "Breast cancer diagnosis and prognosis in women augmented with silicone gel-filled implants." Cancer **66**(1): 97-101.

From 1981 through 1988, 35 patients with prior augmentation mammoplasty were treated for breast carcinoma. Thirty-two patients had unilateral infiltrating carcinomas; three had noninvasive (in situ) lesions. Thirty-four of 35 (97%) lesions were palpable. One noninvasive cancer was occult, discovered mammographically in the absence of physical findings. Prebiopsy mammography was performed in 29 patients with palpable masses and failed to reveal an abnormality in 12 patients, a false-negative rate of 41%. Fifteen patients were treated with mastectomy; the remaining 20 with breast preservation. Thirty-two patients underwent axillary node dissection; 15 (47%) patients had lymph node metastases. There have been seven (20%) recurrences: one local and six metastatic. Four (11%) patients have died. The median follow-up time is 48 months. Women, previously augmented with silicone gel-filled implants, who develop breast cancer are similar in terms of nodal positivity and prognosis, to nonaugmented breast cancer patients who present with palpable masses. When compared with nonaugmented women whose cancers were found with screening mammography, augmented patients with breast cancer present with a higher percentage of invasive lesion and involved axillary lymph nodes, resulting in a poorer prognosis.

Silvestrini, R., A. Costa, et al. (1996). "Biological prospectives to define prognosis and treatment strategies in liver metastases from colorectal cancer." Ann Ital Chir **67**(6): 733-7.

Liver metastases arise in about a third of patients with colorectal cancer. Although important clinical results have been obtained by surgical treatment in patients with limited liver involvement, other intra-arterial or systemic therapies do not provide important long term clinical benefits in patients with unresectable liver metastases. Better knowledge of the biology of liver metastases could imply a more appropriate use of the available therapeutic approaches and a retrospective definition the biologic subgroups of patients who benefit from them. Phenotypic and molecular aspects of tumor cells have been investigated and have proven to be important determinants of clinical outcome in patients with different human tumor types. Liver metastases from colorectal cancer have been scarcely studied, but cell proliferation has been shown to be a discriminant of freedom from progression and even more of long-term clinical outcome in subsets in patients treated with radical surgery. Moreover, in patients with resectable liver metastases, DNA and entity of DNA abnormalities are significantly associated with patient survival. A few recent reports have indicated a potential prognostic relevance of abnormal activation or expression of the p53 tumor suppressor gene, bel-2 protein and ras oncogene. In conclusion, prognostic biologic factors are acquiring an important role as indicators of clinical outcome in patients with liver metastases. However, all information is derived from retrospective analyses heterogeneous for patient population and biomarkers analyzed. Therefore, the comparison among results from different studies is difficult, and prospective studies are needed to develop a prognostic classification which integrates biologic and pathologic factors.

Sivaraksa, M. and D. Lowe (2008). "Predictive gene lists for breast cancer prognosis: a topographic visualisation study." BMC Med Genomics **1**: 8.

BACKGROUND: The controversy surrounding the non-uniqueness of predictive gene lists (PGL) of small selected subsets of genes from very large potential candidates as available in DNA microarray experiments is now widely acknowledged 1. Many of these studies have focused on constructing discriminative semi-parametric models and as such are also subject to the issue of random correlations of

sparse model selection in high dimensional spaces. In this work we outline a different approach based around an unsupervised patient-specific nonlinear topographic projection in predictive gene lists. METHODS: We construct nonlinear topographic projection maps based on inter-patient gene-list relative dissimilarities. The Neuroscale, the Stochastic Neighbor Embedding(SNE) and the Locally Linear Embedding(LLE) techniques have been used to construct two-dimensional projective visualisation plots of 70 dimensional PGLs per patient, classifiers are also constructed to identify the prognosis indicator of each patient using the resulting projections from those visualisation techniques and investigate whether a-posteriori two prognosis groups are separable on the evidence of the gene lists.A literature-proposed predictive gene list for breast cancer is benchmarked against a separate gene list using the above methods. Generalisation ability is investigated by using the mapping capability of Neuroscale to visualise the follow-up study, but based on the projections derived from the original dataset. RESULTS: The results indicate that small subsets of patient-specific PGLs have insufficient prognostic dissimilarity to permit a distinction between two prognosis patients. Uncertainty and diversity across multiple gene expressions prevents unambiguous or even confident patient grouping. Comparative projections across different PGLs provide similar results. CONCLUSION: The random correlation effect to an arbitrary outcome induced by small subset selection from very high dimensional interrelated gene expression profiles leads to an outcome with associated uncertainty. This continuum and uncertainty precludes any attempts at constructing discriminative classifiers.However a patient's gene expression profile could possibly be used in treatment planning, based on knowledge of other patients' responses.We conclude that many of the patients involved in such medical studies are intrinsically unclassifiable on the basis of provided PGL evidence. This additional category of 'unclassifiable' should be accommodated within medical decision support systems if serious errors and unnecessary adjuvant therapy are to be avoided.

Span, P. N., J. Bussink, et al. (2003). "Carbonic anhydrase-9 expression levels and prognosis in human breast cancer: association with treatment outcome." Br J Cancer **89**(2): 271-6.

Here, we set out to assess CA9 expression levels by real-time quantitative RT-PCR in breast cancer tissue samples obtained from 253 patients, and correlated those with relapse-free (RFS) survival. The median follow-up time was 75 months (range 2-168 months). CA9 expression was mainly found in high-grade, steroid receptor negative cancer tissues. CA9 levels were not significantly associated with RFS (P=0.926, hazard ratio (HR)=0.99, 95% CI=0.80-1.22) in the total cohort of 253 patients. In multivariate analysis with other clinicopathological factors, CA9 (P=0.018, HR=0.77, 95% CI=0.62-0.96), the interaction of adjuvant chemotherapy with CA9 (P=0.009, HR=1.31, 95% CI=1.07-1.61) and the interaction of adjuvant endocrine therapy with CA9 (P<0.001, HR=1.41, 95% CI=1.20-1.66) all contributed significantly to the final model. These results indicate that patients with low CA9 levels benefit more from adjuvant treatment than do patients with high levels. Thus, the determination of CA9 levels could aid in the selection of patients who will not benefit from adjuvant therapy, and whose prognosis will more likely improve with other treatment modalities.

Streeter, E. H. and J. P. Crew (2001). "Angiogenesis, angiogenic factor expression and prognosis of bladder cancer." Anticancer Res **21**(6B): 4355-63.

Angiogenesis is the process by which tumours induce a blood supply from their surrounding tissues and it has been shown to be necessary for tumour growth. Evidence is accumulating for both the prognostic usefulness of measures of angiogenesis and its potential as a target for anticancer therapy. This review discusses the evidence concerning the association between angiogenesis and bladder cancer, focusing on the mechanisms behind the angiogenic process and the quantification of factors believed to be involved, relating these clinically to their prognostic use and to the antiangiogenic strategies so far described in vitro and in vivo.

Suzuki, M., S. Mohamed, et al. (2008). "Aberrant methylation of CXCL12 in non-small cell lung cancer is associated with an unfavorable prognosis." Int J Oncol **33**(1): 113-9.

Chemokines play an important role in the pathogenesis of non-small cell lung cancer (NSCLC). However, aberrant methylation of CXCL12 has not been examined in NSCLC. CXCL12 mRNA expression and methylation were examined in 17 NSCLC cell lines by RT-PCR and methylation-specific PCR (MSP). MSP was performed on 236 tumor specimens from NSCLC patients who received curative intent surgery. CXCL12 and CXCR4 protein expression was examined in 90 of the 236 NSCLC specimens by immunohistochemistry. Down-regulation of CXCL12 expression was found in 10 of 17 (59%) NSCLC cell lines compared with normal bronchial cells. Treatment of 8 expression-negative cell lines with a demethylating agent restored expression in all cases. Twelve cell lines (71%)

showed aberrant methylation, and good concordance between methylation and expression was present. Aberrant methylation occurred in 85 out of 236 (36%) primary NSCLCs in a tumor-specific manner. In multivariate analysis, CXCL12 methylation correlated strongly and independently with prognosis both in all patients with NSCLCs and in those with stage I NSCLCs (hazard ratio=1.68, P=0.015 and hazard ratio=3.58, P=0.017). Secreted protein CXCL12 and its receptor CXCR4 were abundant in NSCLC cells (72 out of 90, 80%; 57 out of 90, 63%) and correlated with the progression of NSCLCs. In conclusion, epigenetic silencing of CXCL12 is a frequent event in NSCLCs, and could be an independent and powerful prognostic marker in patients with NSCLCs and those with stage I disease. Analysis for CXCL12 may provide novel opportunities for prognosis and therapy of resected NSCLCs.

Thompson, A. M., R. A. Hawkins, et al. (1993). "pS2 is an independent factor of good prognosis in primary breast cancer." Br J Cancer **68**(1): 93-6.

In breast cancer, oestrogen regulated genes, such as pS2, may be expressed in well differentiated tumours with a good prognosis. We have examined pS2 mRNA expression in 78 primary, untreated breast cancers and related pS2 expression to disease behaviour and known prognostic factors. pS2 mRNA expression was detected in 25/78 (32%) of cancers and was significantly associated with a moderate/high oestrogen receptor content (P = 0.045, Chi Square test). pS2 mRNA expression was associated with freedom from disease at median 31 months clinical and radiological follow-up (P = 0.015, Fisher's exact test, odds ratio 8.6). Using multiple logistic regression analysis of six potential prognostic factors only pathological axillary node status (P < 0.01) and pS2 mRNA expression (P < 0.05) provided independent prognostic information. Furthermore, pS2 was associated with a good prognosis in the axillary node positive patients where only 1/13 (8%) with pS2 mRNA expression compared with 13/29 (45%) without detectable expression had recurrence of their disease. These data provides strong support for pS2 as a useful independent prognostic factor in primary breast cancer.

Troyer, D. A., J. Mubiru, et al. (2004). "Promise and challenge: Markers of prostate cancer detection, diagnosis and prognosis." Dis Markers **20**(2): 117-28.

Approximately 1 man in 6 will be diagnosed with prostate cancer during his life lifetime, and over 200,000 men in the U.S. are diagnosed with prostate cancer annually. Since the widespread adoption of PSA testing, about 60-70% of men at risk in the U.S. have had a blood test for prostate cancer. With this, prostate cancer death rates have decreased, yet only slightly. Thirty thousand men still die each year from this disease. PSA testing fails to identify a small but significant proportion of aggressive cancers, and only about 30% of men with a "positive" PSA have a positive biopsy. Additionally, of men who are treated for prostate cancer, about 25% require additional treatment, presumably due to disease recurrence. Also of concern is the growing evidence that there are some prostate cancers for which treatment may not be necessary. Very long-term studies from the U.S. and Europe, following men with prostate cancer have found that some tumors do not progress over time. In these individuals, prostate cancer treatment is unnecessary and harmful as these men do not benefit from treatment but will be at risk of treatment-related side effects and complications. They suggest a fundamental problem with prostate cancer: it is not possible, at this time, to predict the natural history of the disease. It is for these reasons that the most important challenge in prostate cancer today is the inability to predict the behavior of an individual tumor in an individual patient. Here we review issues related to performance and validation of biomarkers with a focus on "doing no harm", and bearing in mind that it is the ultimate goal of early detection to save lives. Improved diagnostic and prognostic biomarkers are needed for prostate cancer, and the use of these markers should ultimately translate into increased life span and quality of life. The ultimate goal would be to not only have accurate biomarkers suitable for early diagnosis, but also biomarkers that identify men at greatest risk of developing aggressive disease. Technology has been brought to bear on this problem, and the major approaches are genomics, expression analysis, and proteomics. Proteomics and DNA methylation assays may soon be used in sensitive and specific diagnostic testing of serum and tissues for cancer. Expression arrays may be used to establish both a more specific diagnosis and prognosis for a particular tumor. The proteome is only beginning to be understood, and alternative splicing and post-translational modifications of proteins such as glycosylation and phosphorylation are challenging areas of study. Finally, risk assessment and prognosis are being pursued through analysis of genomic polymorphisms (single nucleotide polymorphisms, SNPs). This huge task is only beginning, and requires the combined expertise of molecular epidemiologists, oncologists, surgeons, pathologists, and basic scientists.

Truelson, J. M., S. G. Fisher, et al. (1992). "DNA content and histologic growth pattern correlate with prognosis in patients with advanced squamous cell carcinoma of the larynx. The Department of Veterans

Affairs Cooperative Laryngeal Cancer Study Group." Cancer **70**(1): 56-62.

BACKGROUND: Alterations in DNA content, nuclear morphologic characteristics, and histologic grading have been associated with prognosis in several types of solid malignant neoplasms. METHODS: To determine the potential usefulness of these factors in predicting tumor behavior in patients with laryngeal squamous cell carcinoma, tumor specimens from 88 previously untreated patients with Stage III or IV cancers were studied. The DNA content and nuclear area (NA) were measured for individual nuclei of each tumor with the use of Azure A-stained frozen sections. An adjusted DNA index (aDI) for each patient was calculated from the slope of the linear regression analysis of nuclear DNA index on NA. Hematoxylin and eosinstained sections were examined and graded systematically for histologic growth pattern. All patients were enrolled in a prospective clinical trial and had laryngectomy and postoperative radiation therapy. RESULTS: The disease-free survival length was longer and the relapse rates were lower in patients with a low aDI (P less than 0.005) and with tumors exhibiting low-grade growth patterns (P less than 0.001). CONCLUSIONS: These parameters were independent of staging variables and were better predictors of tumor relapse than traditional clinical staging classifications.

Tseng, C. J., C. C. Pao, et al. (1999). "Detection of human papillomavirus types 16 and 18 mRNA in peripheral blood of advanced cervical cancer patients and its association with prognosis." J Clin Oncol **17**(5): 1391-6.

PURPOSE: To evaluate the feasibility of detecting human papillomavirus E6 (HPVE6) gene mRNA in the peripheral blood of patients with locally advanced cervical cancer, and the relationship of the circulating HPV viral-specific mRNA with clinicopathologic factors and prognosis of locally advanced cervical cancer. PATIENTS AND METHODS: The presence of types 16 and 18 HPVE6 gene mRNA was determined by reverse transcription followed by nested polymerase chain reaction. Thirty-five patients with locally advanced cervical cancer who were positive for HPV type 16 or 18 DNA were included in the study. All patients received external-beam radiation therapy followed by intracavitary brachytherapy. RESULTS: Eighteen (51.4%) of 35 HPV DNA-positive cervical cancer patients had HPV-specific mRNA in their peripheral blood cells, compared with none of 17 HPV DNA-negative cervical cancer patients and none of 12 control volunteers. The presence of HPVE6 gene mRNA in peripheral blood was associated with bulky tumor volume (> 4 cm) and pelvic lymph node metastasis (tumor volume, P = .03; lymph node status, P = .03). After a median follow-up of 22 months, patients who were positive for peripheral-blood HPVE6 gene mRNA had a significantly higher risk of recurrence than those who were negative (10 of 18 v three of 17, P = .02; mean recurrent time, 20.7 months v 12.6 months, P = .02). There was also a statistically significant association of peripheral-blood HPVE6 gene mRNA positivity with distant metastasis (eight of 18 vone of 17; P = .01). CONCLUSION: Results of this study seem to suggest that the presence of HPVE6 gene mRNA in peripheral blood may provide an early marker that identifies patients who are at risk for metastasis.

Tsuchiya, A., N. Katagata, et al. (1993). "Immunohistochemical overexpression of C-erbB-2 in the prognosis of breast cancer." Surg Today **23**(10): 885-90.

Immunohistochemical c-erbB-2 protein overexpression was detected in 34 of 124 (27.4%) paraffin-embedded breast cancer specimens. Although no difference was seen between the c-erbB-2 positive and negative groups in 5-year disease-free survival, 5-year overall survival was significantly less favorable in the c-erbB-2 positive group. Furthermore, patients graded as having positive c-erbB-2 staining and aneuploid DNA showed significantly poorer survival than those in other categories. The significant prognostic factors, determined by a multivariate analysis, were nodal status and c-erbB-2 overexpression. Our findings therefore suggest that c-erbB-2 expression is a prognostic factor in breast cancer and that it could be useful in the determination of postoperative adjuvant therapy.

Tsuda, H., T. Takarabe, et al. (1999). "der(16)t(1;16)/der(1;16) in breast cancer detected by fluorescence in situ hybridization is an indicator of better patient prognosis." Genes Chromosomes Cancer **24**(1): 72-7.

By two-color fluorescence in situ hybridization (FISH), der(16)t(1;16) or der(1;16) was frequently detected in low-grade papillary carcinoma but not in benign intraductal papilloma of the breast. In order to clarify the incidence and clinicopathological significance of der(16)t(1;16)/der(1;16) in common breast cancers, der(16)t(1;16)/der(1;16) was examined by two-color FISH in breast cancers resected from 51 patients by using DNA probes for 16cen, 16q11.2, and 1q12 labeled with biotin or digoxigenin. der(16)t(1;16)/der(1;16) was clonally detected in 16 cancers (31%), being more frequent in ductal carcinomas of lower grade and invasive lobular carcinoma than in high-grade invasive ductal

carcinoma (P<0.001). der(16)t(1;16)/der(1;16) was also correlated with a higher amount of hormone receptors in the tumor (P<0.05). Disease-free and overall survival rates of the patient group with der(16)t(1;16)/der(1;16)-positive cancer were higher (88% and 94%) than those of the group with der(16)t(1;16)/der(1; 16)-negative cancer (39% and 68%) (P<0.05). Among the 16 patients with lymph node metastasis who received one of two similar forms of postsurgical adjuvant chemo-endocrine therapy, the prognosis of those with der(16)t(1;16)/der(1;16)-positive cancer was better than that of those with der(16)t(1;16)/der(1;16)-negative cancer (P<0.05). der(16)t(1;16)/der(1;16) detected by FISH is considered helpful in identifying patients with a better prognosis and for stratification of patients in randomized clinical trials of adjuvant chemo-endocrine therapies.

Uramoto, H., K. Sugio, et al. (2004). "Expression of deltaNp73 predicts poor prognosis in lung cancer." Clin Cancer Res **10**(20): 6905-11.

PURPOSE: DeltaNp73 is an isoform of the p53 homologue p73, which lacks an NH(2)-terminal transactivation domain and antagonizes the induction of gene expression by p53/p73. The aim of this study was to detect DeltaNp73 expression in lung cancer and to evaluate the relationship between the DeltaNp73 expression level and the prognosis of patients with resected lung cancer. EXPERIMENTAL DESIGN: We used immunohistochemistry to analyze the protein expression of DeltaNp73 in paraffin-embedded tumor samples from 132 well-characterized lung cancer patients and compared the expression level of DeltaNp73, clinical variables, and survival outcome. RESULTS: Positive expression of DeltaNp73 was detected mainly in the cytoplasm of tumor cells in 77 of 132 patients (58.3%) with lung cancer. The incidence of positive expression of DeltaNp73 was 52.2, 50.0, and 70.2% in patients with stage I, II, and III, respectively (P = 0.04). Positive expression of DeltaNp73 was associated with gender but not associated with age, histologic type, pathological stage, pathological T status, and pathological N status. Lung cancer patients with positive DeltaNp73 expression had a poorer prognosis than those with negative DeltaNp73 expression. In addition, multivariate analysis of the clinicopathological characteristics of lung cancer indicated that positive expression of DeltaNp73 was a significant independent factor for predicting poor prognosis (P < 0.0001, risk ratio = 3.39). CONCLUSIONS: Expression of DeltaNp73 may be a useful marker for predicting poor prognosis of patients who underwent resection of lung cancer.

Utada, Y., M. Emi, et al. (2000). "Allelic loss at 1p34-36 predicts poor prognosis in node-negative breast cancer." Clin Cancer Res **6**(8): 3193-8.

Allelic losses of specific chromosomal regions in the DNA of tumor cells, which imply loss of tumor suppressor genes normally resident at those loci, may become useful postoperative prognostic indicators for breast cancers that have not yet metastasized to lymph nodes. To examine whether specific allelic losses might correlate with postoperative disease-free survival, we tested tumors from a cohort of 228 node-negative breast cancer patients for allelic losses at 18 microsatellite loci chosen to represent either a known tumor suppressor gene or a region where genetic alterations are frequent in breast tumors. We followed the patients clinically for 5 years or until death (if patient death occurred before completion of 5 years of follow-up). Patients whose tumors had lost an allele at 1p34-36 bore significantly higher risks of postoperative recurrence than those whose tumors retained both alleles of the markers in that region [the 5-year recurrence rate was 15% among patients with losses versus 2% among patients with retention (P = 0.001)]. Multivariate analysis demonstrated that allelic loss at 1p34-36 was an independent postoperative predictor of shorter disease-free survival (hazard ratio, 5.8; P = 0.0117). Thus, allelic losses at 1p34-36 in a tumor might have a potential to serve as a negative prognostic indicator to guide postoperative management of breast cancer patients, especially in the selection of high-risk women who will benefit from adjuvant chemotherapy and endocrine therapy.

Walker, J. and P. Quirke (2002). "Prognosis and response to therapy in colorectal cancer." Eur J Cancer **38**(7): 880-6.

Colorectal cancer is Europe's second biggest cancer killer. Yet despite advances in knowledge and changes in chemotherapy practice, we have not seen great strides in improved survival. Histopathological staging is at present the most accurate prognostic factor for survival and recurrence. Improvements in staging have led to the recognition of the importance of the circumferential resection margin (CRM) and how the quality of surgery influences local recurrence rates. Further refinements in staging and increasing knowledge of tumour biology will have a large contribution to play in the future.

Walsh, C. S., S. Ogawa, et al. (2008). "ERCC5 is a novel biomarker of ovarian cancer prognosis." J Clin Oncol **26**(18): 2952-8.

PURPOSE: To identify a biomarker of ovarian cancer response to chemotherapy. PATIENTS AND METHODS Study: participants had epithelial

ovarian cancer treated with surgery followed by platinum-based chemotherapy. DNA and RNA were isolated from frozen tumors and normal DNA was isolated from matched peripheral blood. A whole-genome loss of heterozygosity (LOH) analysis was performed using a high-density oligonucleotide array. Candidate genomic areas that predicted enhanced response to chemotherapy were identified with Cox proportional hazards methods. Gene expression analyses were performed through microarray experiments. Candidate genes were tested for independent effects on survival using Cox proportional hazards models, Kaplan-Meier survival curves, and the log-rank test. RESULTS: Using a whole-genome approach to study the molecular determinants of ovarian cancer response to platinum-based chemotherapy, we identified LOH of a 13q region to predict prolonged progression-free survival (PFS; hazard ratio, 0.23; P = .006). ERCC5 was identified as a candidate gene in this region because of its known function in the nucleotide excision repair pathway, the unique DNA repair pathway that removes platinum-DNA adducts. We found LOH of the ERCC5 gene locus and downregulation of ERCC5 gene expression to predict prolonged PFS. Integration of genomic and gene expression data shows a correlation between 13q LOH and ERCC5 gene downregulation. CONCLUSION: ERCC5 is a novel biomarker of ovarian cancer prognosis and a potential therapeutic target of ovarian cancer response to platinum chemotherapy.

Walther, A., R. Houlston, et al. (2008). "Association between chromosomal instability and prognosis in colorectal cancer: a meta-analysis." Gut **57**(7): 941-50.

BACKGROUND: Several studies have suggested that microsatellite instability (MSI) resulting from defective DNA mismatch repair confers a better prognosis in colorectal cancer (CRC). Recently, however, data have suggested this is secondary to the effects of ploidy/chromosomal instability (CIN). To estimate the prognostic significance of CIN for survival, data from published studies have been reviewed and pooled. METHODS: Studies stratifying survival in CRC by CIN status were identified by searching PubMed and hand-searching bibliographies of identified studies. Two reviewers confirmed study eligibility and extracted data independently, and data were pooled using a fixed-effects model. The principal outcome measure was the HR for death. RESULTS: 63 eligible studies reported outcome in 10 126 patients, 60.0% of whom had CIN+ (aneuploid/polyploid) tumours. The overall HR associated with CIN was 1.45 (95% CI 1.35 to 1.55, p<0.001). In patients with stage II-III CRCs, the HR was 1.45 (95% CI 1.27 to 1.65, p<0.001). The effect was similar for progression-free survival (HR = 1.71, 95% CI 1.51 to 1.94, p<0.001). There was no evidence of significant interstudy heterogeneity. CONCLUSION: CIN is associated with a worse prognosis in CRC, and should be evaluated as a prognostic marker, together with MSI status, in all clinical trials, particularly those involving adjuvant therapies.

Wiley, A., D. Katsaros, et al. (2006). "Methylation of the insulin-like growth factor binding protein-3 gene and prognosis of epithelial ovarian cancer." Int J Gynecol Cancer **16**(1): 210-8.

Insulin-like growth factor binding protein-3 (IGFBP-3) is a member of the IGFBP family, which regulates the mitogenic and antiapoptotic effects of insulin-like growth factors. Hypermethylation of the IGFBP-3 promoter has been found to suppress its expression. To evaluate the role of IGFBP-3 in ovarian cancer progression, we examined the survival of 235 consecutively selected epithelial ovarian cancer patients in association with IGFBP-3 promoter methylation and IGFBP-3 expression in tumor tissue. IGFBP-3 promoter methylation was analyzed using methylation-specific polymerase chain reaction. Cytosol protein was extracted and measured using a bicinchoninic acid assay; IGFBP-3 was measured by enzyme linked immunosorbent assay. Promoter methylation of the IGFBP-3 gene was detected in 44% (104/235) of patients. IGFBP-3 promoter methylation was associated with disease progression and death after adjusting for clinical and pathologic variables. The association was more evident in patients with early-stage disease: RR = 2.87 (95% CI: 0.78-10.63) for disease progression and RR = 3.94 (95% CI: 0.91-15.78) for death. Tissue levels of IGFBP-3 did not differ by methylation status but were inversely associated with disease stage and residual tumor size. These results suggest that IGFBP-3 promoter methylation may be a useful prognostic marker for disease progression and death in early-stage ovarian cancer.

Wu, M. S., C. W. Lee, et al. (2002). "Alterations of BAT-26 identify a subset of gastric cancer with distinct clinicopathologic features and better postoperative prognosis." Hepatogastroenterology **49**(43): 285-9.

BACKGROUND/AIMS: Gastrointestinal tumors with microsatellite instability represent a replication error-positive phenotype. BAT-26, a repeat of 26 deoxyadenosine localized in intron 5 of hMSH2 gene, has been reported as a reliable indicator of replication error phenotype in colorectal cancers. This study investigated whether BAT-26 is a useful marker for a mutator phenotype with distinct

clinicopathologic features in gastric cancer. Gastric cancer with BAT-26 alterations was highly correlated with multiple microsatellite alterations (> or = 3 loci) and frameshift mutations of transforming growth factor-beta type II receptor, and predominantly showed antral location, intestinal histologic subtype, advanced stage, a higher rate of Helicobacter pylori infection, a better postoperative survival and less lymph node metastasis. CONCLUSIONS: These results show testing of BAT-26 alterations is a convenient and rapid screening method for identifying a subset of gastric cancer with a mutator phenotype and better prognosis.

Wyatt, J. I., P. Quirke, et al. (1989). "Comparison of histopathological and flow cytometric parameters in prediction of prognosis in gastric cancer." J Pathol **158**(3): 195-201.

Flow cytometric analysis was performed retrospectively on material from 76 patients having potentially curative resection for gastric carcinoma between 1968 and 1984. The prognostic significance of DNA aneuploidy was compared with that of conventional histological grading and staging of the tumour. The presence of DNA aneuploidy was associated with a significantly poorer prognosis when compared with diploid tumours (P less than 0.02), but was not found to be predictive of survival when the presence of lymph node metastases (P less than 0.0001) and resection margin involvement (P less than 0.003) were allowed for using multiple regression analysis. When intestinal and diffuse types of gastric carcinoma were analysed separately, DNA aneuploidy was associated with a significantly shorter survival only in patients with intestinal type tumours.

Yamaguchi, A., Y. Kurosaka, et al. (1992). "Expression of p53 protein in colorectal cancer and its relationship to short-term prognosis." Cancer **70**(12): 2778-84.

BACKGROUND AND METHODS: The expression of p53 protein in 100 large bowel cancers was studied immunohistochemically by use of a monoclonal antibody (PAb1801). RESULTS: Immunoreactivity was found in 61.0% of specimens from 100 patients with colorectal cancer. The pattern of p53 expression was mainly detected in the nuclei of the cancer cells. There was no significant correlation between the expression of p53 and the histologic grade, tumor size, serosal invasion, lymphatic invasion, venous invasion, lymph node metastasis, or liver metastasis. However, patients with p53-positive tumors had a greater relative risk of death compared with those with p53-negative tumors. The p53 negative-tumors showed a recurrence rate of 5.9%; for the p53 positive-tumors, a recurrence rate of 23.8% was recorded. The 3-year survival rate was 96.7% of 39 patients with p53-negative carcinomas and 61.8% for the patients with p53-positive tumors; there was a significant difference in the rate between the two groups of patients ($P < 0.05$). The growth fraction of p53-positive tumors determined with a monoclonal antibody against DNA polymerase alpha (49.0%) was significantly higher than that of p53-negative tumors (40.7%, $P < 0.01$). CONCLUSIONS: These results suggest that the immunoreactivity of p53 may be a biologic marker of prognostic significance.

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