



Lucy Kettlewell. *Boats, Michigan #3*. Oil on board. From the personal collection of John Horton.

Several molecules and profiles show promise as predictive and prognostic parameters in non-small cell lung cancer.

Molecular Analysis-Based Treatment Strategies for Non-Small Cell Lung Cancer

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Background: Lung cancer is the leading cause of cancer-related mortality. Improved understanding in the molecular biology and genetics of lung cancer has resulted in the identification of individual genes, gene expression profiles, and molecular pathways that may be useful for clinical management decisions.

Methods: We focused on recent molecules and platforms under evaluation for implementation into clinical decision making.

Results: Prognostic molecular parameters are defined as markers that impact overall outcome in terms of survival independent of therapeutic interventions. Predictive molecular parameters are defined as markers that impact therapeutic efficacy.

Conclusions: Several molecules and profiles are emerging with promising utility as predictive and prognostic parameters in non-small cell lung cancer independent of the standard clinical parameters, such as stage, performance status, and gender. These include the genes *ERCC1*, *RRM1*, and *BRCA1*, which are involved in nucleotide metabolism and DNA damage repair; epidermal growth factor receptor, which is involved in cell proliferation and survival, and oligonucleotide expression array profiles, which are signatures of global gene expression associated with specific tumor phenotypes.

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Abbreviations used in this paper: NSCLC = non-small cell lung cancer, NER = nucleotide excision repair, EGFR = epidermal growth factor receptor, TKI = tyrosine kinase inhibitor, RT-PCR = reverse-transcriptase polymerase chain reaction, MALDI-TOF-MS = matrix-assisted laser desorption ionization time-of-flight mass spectrometry.

Introduction

Lung cancer is the leading cause of cancer-related mortality worldwide. Approximately 85% of lung cancer cases are of the non-small cell type (NSCLC). Surgery, chemotherapy, and radiotherapy have been used in various combinations to improve survival and maximize the therapeutic benefit. However, despite advances in treatment, the 5-year survival rate for NSCLC across all stages is presently only 16%.

Gene mutations triggered by environmental exposure and intrinsic genome plasticity are thought to be responsible for cancer development. Recent advances in the identification of molecular determinants of poor

outcome and therapeutic benefit for patients with NSCLC hold the promise for rational clinical decisions based on each patient's molecular tumor profile. The impact of genes influencing drug activity offers the ability to tailor therapy for individual patients. The nucleotide excision repair (NER) pathway plays an important role in DNA damage repair. Impaired NER can result in genomic instability with an increased malignant phenotype (Fig 1A-E).¹ Molecular markers

such as the DNA repair genes ERCC1 (excision repair cross complementation) and RRM1 (the regulatory subunit of ribonucleotide reductase) have already been used to customize chemotherapy and improve outcome in lung cancer patients.^{2,3}

Many molecules have been studied as prognostic markers; here we focus on those more recently reported.

Prognostic and Predictive Markers

The terms *prognostic* and *predictive* are often used interchangeably. However, the term *prognostic* specifically refers to a marker/parameter that is useful for estimating a patient's prognosis (outcome) independent of therapeutic decisions; for example, survival is different in marker-positive and -negative patients. The term *predictive* refers to a marker/parameter that is useful to make therapeutic decisions, ie, the effect of treatment is different in marker-positive and -negative patients. For instance, female gender is prognostic of improved survival in NSCLC (women tend to have a better survival than men independent of therapy). Female gender is also predictive of improved response to epidermal growth factor receptor tyrosine kinase inhibitors (EGFR TKIs); women have a higher likelihood of response than men to these agents. In contrast, as described later, high ERCC1 is prognostic of improved survival and predictive of reduced response to platinum-based therapy.

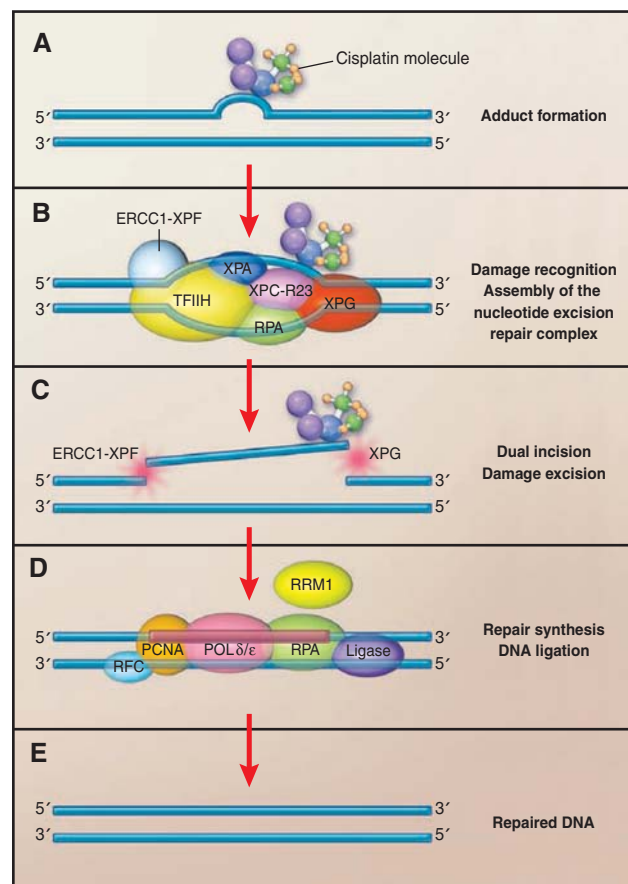


Fig 1A-E. — ERCC1 and RRM1 in DNA damage repair. (A) Cisplatin binds covalently to genomic DNA, forming a bulky, helix-distorting adduct. The most prevalent adduct is the intrastrand linkage of two adjacent guanine bases by the nitrogen atoms at position 7 (the GG adduct). In chemosensitive cells with low NER activity, apoptosis usually follows. In chemoresistant cells with high NER, the adduct may be excised and the DNA repaired. (B) The adduct is recognized, and proteins of the NER complex are assembled at the adduct site. The heterodimeric protein excision repair cross-complementation group 1 (ERCC1)-XPF is the last component to be assembled. It is the rate-limiting step. Unwinding of the DNA duplex in the immediate vicinity of the adduct results in the formation of a bubble. (C) Endonucleases create dual incisions flanking the damaged bases, with the protein XPG acting on the 3' side and the heterodimer ERCC1-XPF acting on the 5' side. A segment of about 22 to 32 nucleotides containing the adduct is removed. (D) The excised segment is repaired by polymerases and the accessory replication proteins PCNA, RPA, and RFC. The integrity of the damaged strand is restored by DNA ligase. (E) The repair process is complete, and the original state of the DNA is restored. Ribonucleotide reductase, although not an integral part of the repair complex, catalyzes the biosynthesis of deoxyribonucleotides from the corresponding ribonucleotides, providing the building blocks for reconstitution of the excised oligonucleotide. From Friedberg EC. How nucleotide excision repair protects against cancer. *Nat Rev Cancer*. 2001;1:22-33. Adapted by permission from Macmillan Publishers Ltd. <http://www.nature.com>.

Prognostic Molecular Markers in Patients With Resected NSCLC

ERCC1 as a Marker for Prognosis in NSCLC

ERCC1 is a DNA damage repair gene that encodes the 5' endonuclease of the NER complex. ERCC1 may serve as an intermediate biomarker of the extent of intratumoral DNA damage. Cisplatin causes cytotoxicity of cancer cells by forming adducts that result in DNA cross-links. The NER complex recognizes and removes these adducts and thus might trigger resistance to platinum agents.

We evaluated the effect of intratumoral ERCC1 expression on survival in 51 patients with NSCLC who underwent surgical resection for cure.⁴ ERCC1 mRNA expression was a continuous parameter, and the value of 50 (cohort median) was used to dichotomize patients into high and low ERCC1 expressors. A statistically significant difference ($P=.01$) in median survival was seen in patients with high ERCC1 expression (94.6 months) compared to patients with low ERCC1 expression (35.5 months). A significant relationship was also observed between ERCC1 and survival when the levels of ERCC1 were categorized into <30, 30 to 100, and >100 (mRNA expression values as determined by real-time reverse-transcriptase polymerase chain reaction [RT-PCR] have no unit; they are calculated by dividing the value of the target gene, here ERCC1, by the value of a housekeeping gene, here 18S rRNA). The

median survival in these groups was 35.5, 62.1, and 94.6 months, respectively (Fig 2A-B).

These findings were confirmed by Olausen et al² in a cohort of specimens from the International Adjuvant Cancer Trial (IALT). In this study immunohisto-

chemical (IHC) staining was used to determine the expression of ERCC1 protein in 761 resected NSCLC tumors. These patients had been randomized to treatment with cisplatin-based chemotherapy or observation. Patients were dichotomized into high and low

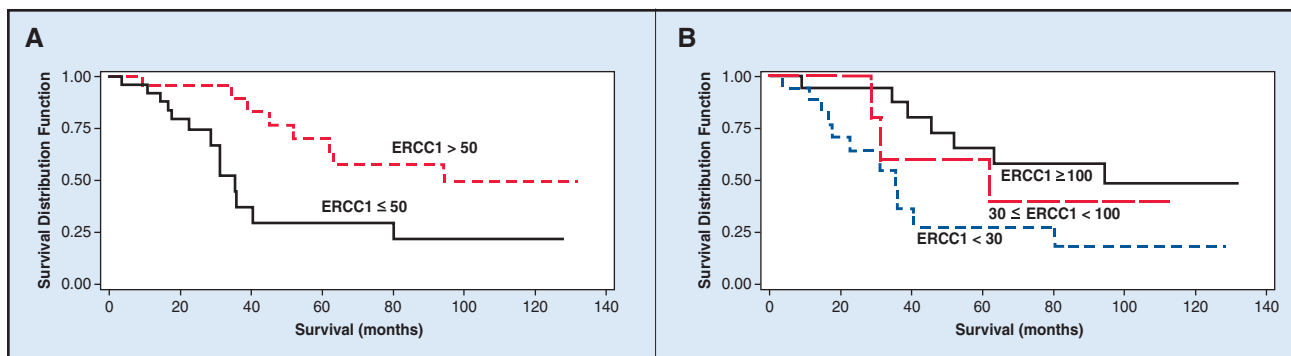


Fig 2A-B. — (A) Median survival of patients with ERCC1 of more than 50 (94.6 months) vs less than 50 (35.5 months) ($P=.01$). (B) Median survival of patients with ERCC1 of less than 30 (35.5 months), 30 to 100 (62.1 months) and >100 (94.6 months) ($P=.03$). From Simon GR, Sharma S, Cantor A, et al. ERCC1 expression is a predictor of survival in resected patients with non-small cell lung cancer. *Chest*. 2005;127:978-983. Reproduced with permission of the American College of Chest Physicians; permission conveyed through Copyright Clearance Center, Inc.

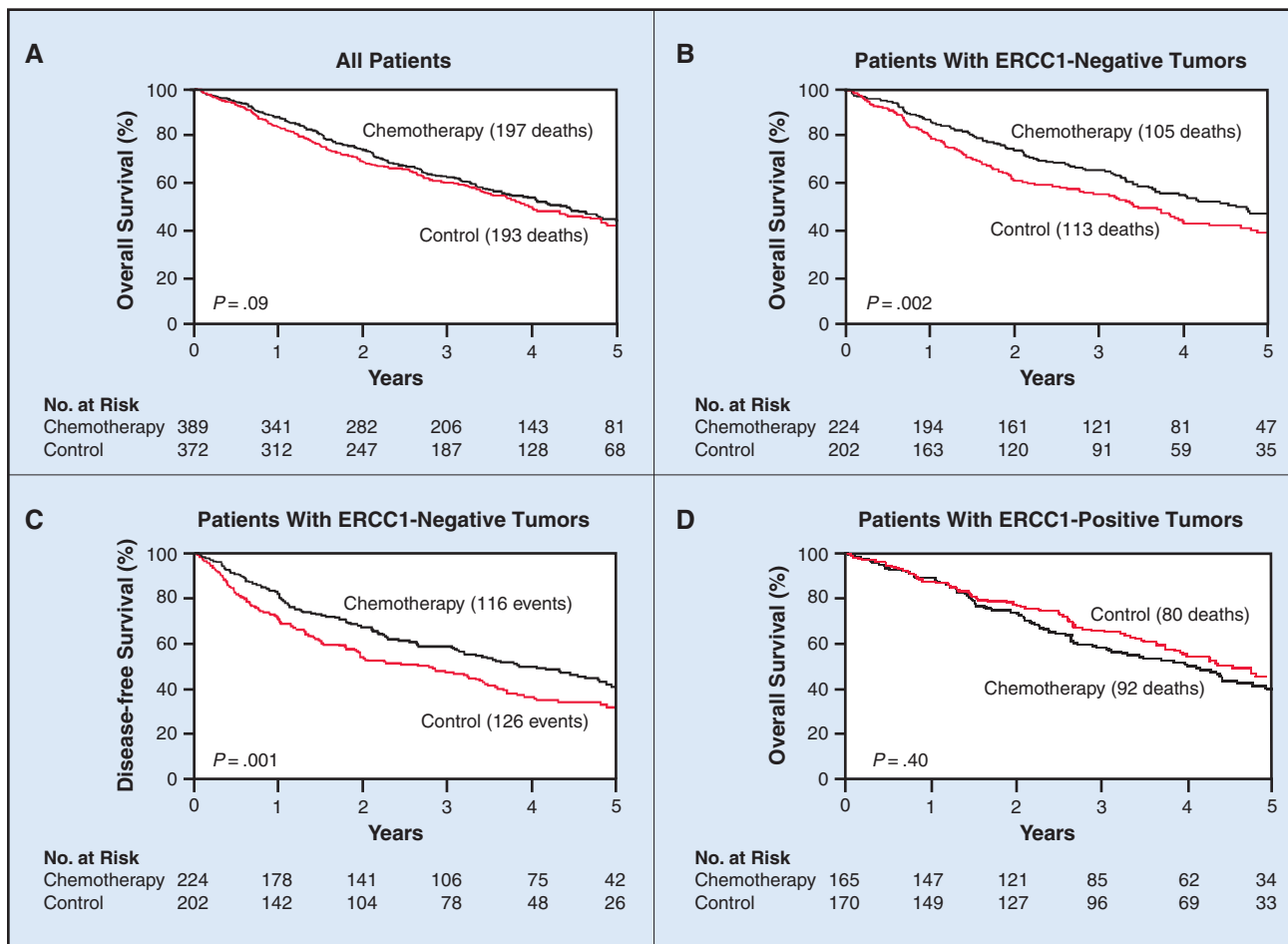


Fig 3A-D. — Overall survival of patients analyzed for ERCC1 expression from the International Adjuvant Lung Trial. (A) Overall survival of all 761 patients for whom ERCC1 expression data based on immunohistochemical analysis were obtained by assigned treatment. (B) Overall survival was significantly better in patients with low ERCC1 expression who received adjuvant chemotherapy vs observation ($P=.002$). (C) Disease-free survival was also significantly better in patients with low ERCC1 expression who received chemotherapy vs observation ($P=.001$). (D) Patients with high ERCC1 expression did not benefit from cisplatin-based chemotherapy compared to observation ($P=.40$). From Olausen KA, Dunant A, Fouret P, et al. DNA repair by ERCC1 in non-small-cell lung cancer and cisplatin-based adjuvant chemotherapy. *N Engl J Med*. 2006;355:983-991. Copyright © 2006 Massachusetts Medical Society. All rights reserved.

ERCC1 expressors by using the median level as the cut-off. Results of this study validated the mRNA expression-based findings of our previous study. Patients in the control group, who had not received adjuvant chemotherapy, with ERCC1-positive tumors (defined as ERCC1 expression determined by IHC above the median of the entire cohort) had longer survival compared to patients with ERCC1-negative tumors (defined as ERCC1 expression below the cohort median; adjusted hazard ratio [HR] 0.66; 95% confidence interval [CI] 0.49 to 0.90; $P=0.009$). Among patients who received adjuvant chemotherapy, a significantly prolonged survival was observed in ERCC1-negative tumors (adjusted HR 0.65; 95% CI 0.50 to 0.86; $P=0.002$) but not among patients with ERCC1-positive tumors (adjusted HR 1.14; 95% CI 0.84 to 1.55; $P=0.40$) (Fig 3A-D). The study concluded that low ERCC1 expression was associated with a survival benefit from cisplatin-based adjuvant chemotherapy for resected NSCLC. Patients with high ERCC1 in the control group had a better outcome than those with low ERCC1, yet their tumors are resistant to platinum-based therapy. However, a recent study by Rosell et al⁵ including 126 patients who underwent curative resection for NSCLC in Gdansk, Poland, between 2000 and 2004, did not show a statistically significant impact of ERCC1 mRNA expression on survival. In fact, there was a trend towards better survival for patients with low tumoral ERCC1 compared to those with high ERCC1 (median overall survival not reached vs 39.5 months; $P=0.89$).

RRM1 as a Marker for Prognosis in NSCLC

Another enzyme involved in DNA synthesis and repair is ribonucleotide reductase, which catalyzes the biosynthesis of deoxyribonucleotides from corresponding ribonucleotides.⁶ It is the molecular target of gemcitabine, an antimetabolite with activity in several malignancies including NSCLC. RRM1, the regulatory subunit of ribonucleotide reductase, plays a role in suppressing tumor cell migration and metastasis formation likely through the tumor suppression gene PTEN, which is involved in the attenuation of growth factor signaling through its lipid and protein phosphatase activity.⁷ Decreased survival has been observed in NSCLC patients with loss of one copy of the RRM1 gene compared to those with two copies.⁸

We previously assessed the prognostic importance of RRM1 expression at the mRNA level in patients with NSCLC.⁹ In this study of surgically resected patients with NSCLC, RRM1 expression in tumor tissue was prognostic of overall survival ($P=0.011$) and disease-free survival ($P=0.002$). Patients with high levels of expression survived longer and had delayed disease recurrence compared to patients with low levels of expression. High RRM1 expression was indicative of long-term survival independent of stage, performance status, and weight loss.

We confirmed this at the protein level in a subsequent study by means of an automated and quantitative estimation of RRM1 and ERCC1 in tumor specimens.³ The results of the study showed that overall survival in patients with completely resected stage I NSCLC with high RRM1 expression (>40.5 , the cohort median, a value without a unit on a scale from 0 to 255) was more than 120 months compared to 60.2 months in patients with low RRM1 expression (<40.5 ; HR 0.61; $P=0.02$) (Fig 4). RRM1 expression correlated with the expression of ERCC1, and the combination of both genes improved the prognostic utility. However, these results were not confirmed in the above-referenced study by Rosell et al.⁵ RRM1 mRNA expression did not show a statistically significant impact on survival. The median overall survival had not been reached in patients with low RRM1, and it was 33.9 months in patients with high RRM1 ($P=0.11$). The authors did report a significant correlation between ERCC1 and RRM1 mRNA expression ($\rho=0.33$, $P=0.0001$).

BRCA1 as a Marker for Prognosis in NSCLC

The BRCA1 (breast cancer 1) gene was originally described as a tumor suppressor gene in breast and ovarian cancer.¹⁰ It is involved in DNA damage response pathways. Low levels of mRNA expression of BRCA1 have recently been shown to be associated with long survival in patients with NSCLC who had a complete resection of their tumor, while patients with high tumoral BRCA1 expression had a short survival.⁵ In this study of 129 patients, 40 had BRCA1 expression >5 and 83 had levels ≤ 5 . The median overall survival was 29

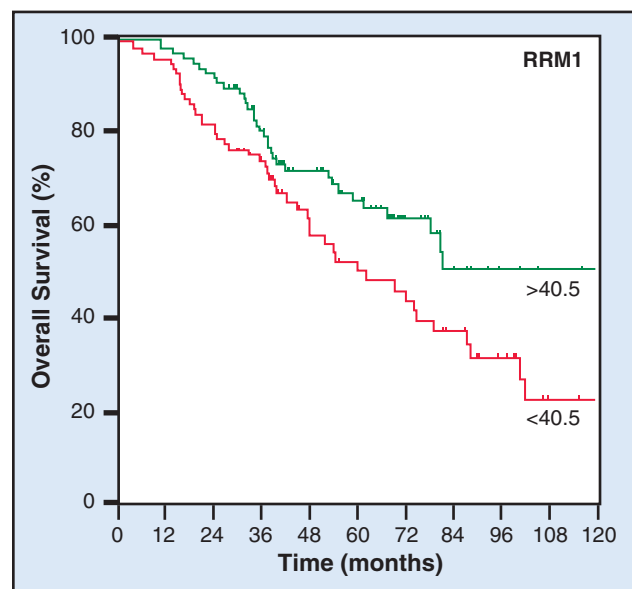


Fig 4. — Kaplan-Meier estimates of overall survival among 187 patients with completely resected stage I NSCLC according to RRM1 in situ protein expression levels. Modified from Zheng Z, Chen T, Li X, et al. DNA synthesis and repair genes RRM1 and ERCC1 in lung cancer. *N Engl J Med*. 2007;356:800-808. Copyright © 2007 Massachusetts Medical Society. All rights reserved.

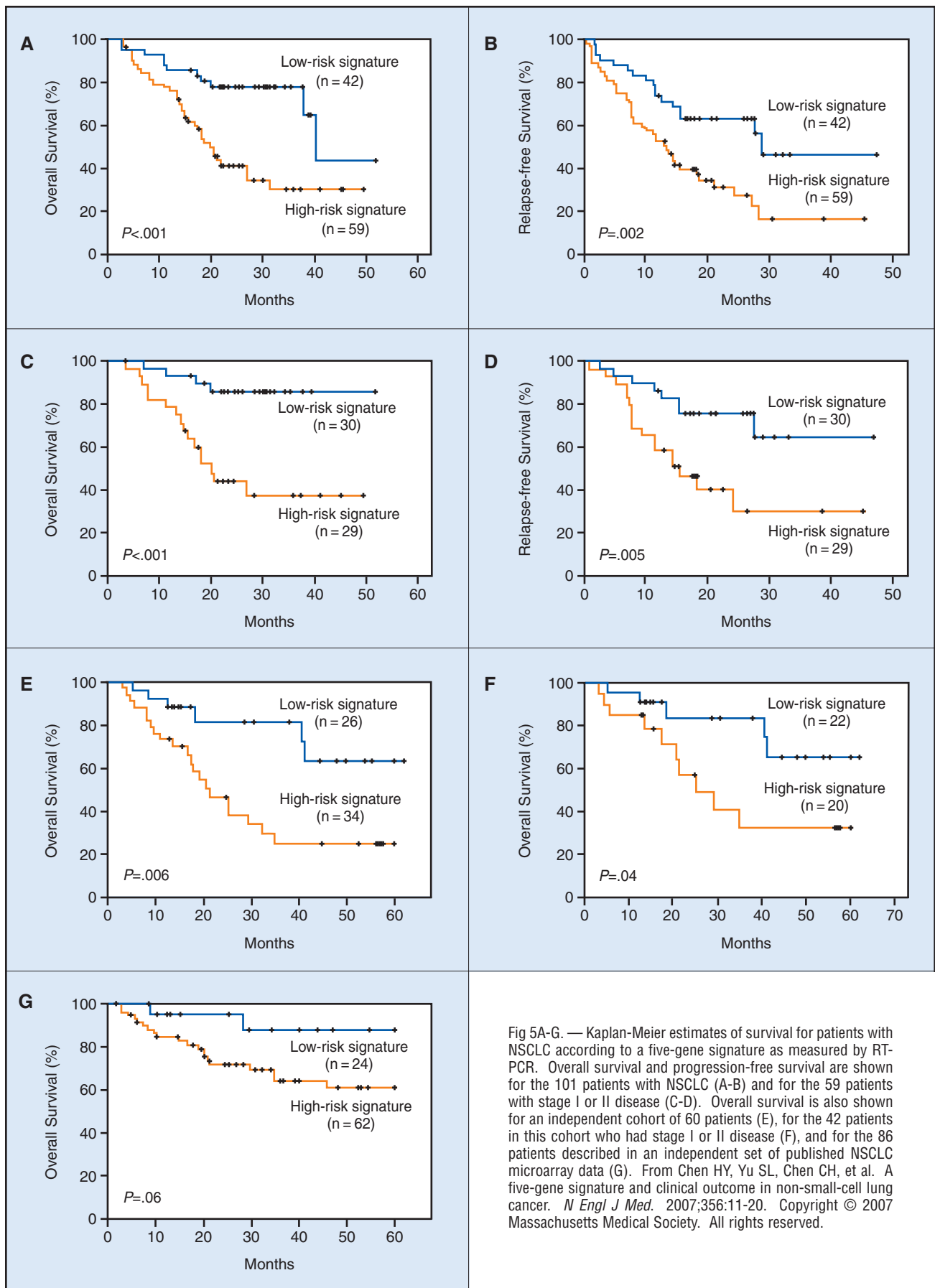


Fig 5A-G. — Kaplan-Meier estimates of survival for patients with NSCLC according to a five-gene signature as measured by RT-PCR. Overall survival and progression-free survival are shown for the 101 patients with NSCLC (A-B) and for the 59 patients with stage I or II disease (C-D). Overall survival is also shown for an independent cohort of 60 patients (E), for the 42 patients in this cohort who had stage I or II disease (F), and for the 86 patients described in an independent set of published NSCLC microarray data (G). From Chen HY, Yu SL, Chen CH, et al. A five-gene signature and clinical outcome in non-small-cell lung cancer. *N Engl J Med.* 2007;356:11-20. Copyright © 2007 Massachusetts Medical Society. All rights reserved.

months in the high BRCA1 group, and it was not reached in the low BRCA1 group ($P=.01$). The authors reported a highly significant correlation between BRCA1 and ERCC1 expression ($\rho=0.62$; $P=.0001$) and between BRCA1 and RRM1 expression ($\rho=0.62$; $P=.0001$).

Oligonucleotide-Based Gene Expression Signatures of Improved Survival in Resected NSCLC Patients

With the advent of whole genomic and/or proteomic approaches to classify cancers, the development of molecular profiles as prognostic markers of outcome or predictive markers of response to therapy has increased substantially.

Oligonucleotide array-based gene expression patterns have been studied to evaluate and develop the use of the gene expression profiles as a means to stratify risk and treatment. In a study by Potti et al¹¹ from a cohort of 89 patients with early-stage NSCLC, a profile was developed and validated independently in two groups of 25 patients from the American College of Surgeons Oncology Group (ACOSOG) Z0030 study and 84 patients from the Cancer and Leukemia Group B (CALGB) 9761 study. The authors concluded that their expression profile was prognostic of recurrence for individual patients with an accuracy of 72% and 79% in the two groups, respectively. It was a more accurate prognostic marker of 5-year survival than clinical or pathologic stage. Thus, the profile may provide a method to identify patients at high risk of recurrence who would benefit from adjuvant chemotherapy.

Chen et al¹² developed a signature that was associated with survival in 125 randomly selected patients. By using oligonucleotide microarray data and risk scores, 16 genes were identified that correlated with survival. Of these 16 genes, 5 genes (DUSP6, MMD, STAT1, ERBB3, and LCK) were selected for RT-PCR and decision tree analysis, and they were independent predictors of progression-free and overall survival. A significant correlation was observed between the microarray-based and real-time RT-PCR-based expression levels for the 5 genes in 101 patients. The use of the authors' profiles resulted in separating patients into high-risk (59 patients) and low-risk (42 patients) groups. The 5-gene signature was strongly associated with overall survival with 96% accuracy, 98% sensitivity, 93% specificity, and 98% positive predictive value. Patients with a high-risk gene signature had a shorter overall survival than did patients with a low-risk signature (20 months vs 40 months; $P<.001$) (Fig 5A-G). Median progression-free survival in the high-risk group was 13 months, whereas the low-risk group had a median progression-free survival of 29 months ($P=.002$).

Serum Proteomic Profiles as Prognostic Markers of Improved Prognosis in NSCLC

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) holds the promise

to produce high-quality, cancer-specific protein profiles.¹³ Proteomic patterns obtained directly from small amounts of fresh-frozen lung tumor tissues have been used to study associations with tumor type, nodal involvement, and survival of cancer patients. In a study by Yanagisawa et al,¹⁴ proteomic spectra were obtained from 79 lung tumors and 14 normal lung tissues to build and test a class-prediction profile. Profiles based on differentially expressed peaks were able to classify lung cancers by histology, distinguish primary lung cancers from cancers metastatic to the lung, and determine nodal involvement with 85% accuracy. Based on a profile of 15 distinct mass spectrometry peaks, patients were differentiated into those with poor prognosis (median survival 6 months, $n = 25$) and those with good prognosis (median survival 33 months, $n = 41$, $P<.0001$).

Markers That Predict for Therapeutic Efficacy

The current standard of treatment for patients with incurable metastatic NSCLC is a doublet chemotherapy regimen.¹⁵ The principal agents used are cisplatin or carboplatin, docetaxel or paclitaxel, gemcitabine, and vinorelbine. The reported response rates to such therapy are 17% to 37%, the median overall survival ranges from 6.7 to 11.3 months, and the 1-year and 2-year survival rates are 31% to 46% and 9% to 21%, respectively. After patients fail initial therapy, response to further systemic treatment is approximately 10% for single-agent therapy.¹⁶ This suggests that resistance to systemic therapy does not appear to be an all-or-none phenomenon but rather a function of the molecular characteristics of the individual tumor.

ERCC1 as a Predictor for Platinum Resistance in Advanced NSCLC

Platinum compounds are heavy metal complexes that form adducts with and cross-links between DNA molecules and thus effectively block DNA replication and transcription. Repair of these adducts and cross-links is dependent on ERCC1. In gastric cancer, ERCC1 mRNA levels are inversely associated with response and survival to platinum-containing treatment.¹⁷ The median survival in patients with low ERCC1 was estimated to be more than 24 months. On the other hand, patients with high ERCC1 expression had a median survival of 5.4 months ($P=.034$). Similar observations have been reported for malignancies of the ovaries,¹⁸ esophagus, colorectum,¹⁹ lung,²⁰ and breast.²¹ In these studies, ERCC1 levels were evaluated by RT-PCR, on gel-based or real-time-based platforms, using specimens that were frozen or preserved in formalin.

Lord et al²⁰ correlated response and survival with the level of ERCC1 expression in 56 patients with advanced NSCLC treated with gemcitabine and cisplatin. They isolated mRNA from formalin-fixed tumor speci-

mens prior to therapy, and relative expression levels of ERCC1 were determined by real-time RT-PCR. The overall response rate was 44.7%, and there was no significant correlation with ERCC1 levels. Median overall survival was significantly longer in patients with low ERCC1 expression (61.6 weeks; 95% CI 42.4 to 80.7) compared to patients with high expression (20.4 weeks, 95% CI 6.9 to 33.9 weeks; $P = .046$). As a result, it is unclear if there was an interaction between ERCC1 expression and treatment, ie, the predictive utility of ERCC1.

As noted earlier, Olausson et al² found that ERCC1 protein expression was associated with benefit from adjuvant cisplatin-based therapy ($P = .009$) in a large group of patients with surgically resected NSCLC. ERCC1 was predictive of the benefit of adjuvant cisplatin-based chemotherapy. Only patients with low tumoral ERCC1 protein levels benefited from adjuvant chemotherapy (adjusted HR for death, 0.65; 95% CI 0.50 to 0.86; $P = .002$).

We had studied prospectively if an association exists between ERCC1 mRNA levels as determined by real-time RT-PCR in fresh-frozen tumor specimens and response to two cycles of gemcitabine/carboplatin. In a group of 35 patients, we found an inverse correlation between ERCC1 levels and tumor shrinkage, ie, tumors with low ERCC1 expression had a better response compared to tumors with high ERCC1 expression ($r = -0.283$; $P = .099$).²² Wachters et al²³ did not find a significant correlation between treatment benefit from gemcitabine/platinum or epirubicin/platinum and ERCC1 expression as determined by immunohistochemistry in 33 patients with advanced-stage NSCLC.

RRM1 as a Predictor of Gemcitabine Efficacy

Several studies have demonstrated that RRM1 is a mol-

ecular target of gemcitabine and thus a key cellular determinant of its therapeutic efficacy.

Davidson et al²⁴ generated in vitro resistance to gemcitabine through exposure of two NSCLC lines to increasing doses of drug. Using oligonucleotide expression arrays, they identified RRM1 as the gene with the most consistent and reproducible increase in expression in resistant cell lines compared to sensitive cell lines. The increase in RRM1 expression was confirmed by RT-PCR regardless of growth time and absence or presence of gemcitabine.

To evaluate the impact of intratumoral RRM1 expression on the efficacy of gemcitabine and carboplatin in previously untreated NSCLC patients, we conducted a prospective phase II clinical trial²² to confirm these in vitro data. Findings of this study confirmed that tumoral levels of RRM1 expression were significantly and inversely correlated with the magnitude of tumor shrinkage ($r = -0.498$; $P = .002$), ie, tumors with low RRM1 expression responded better to treatment compared to tumors with high levels of expression (Fig 6A-B). In addition, we generated NSCLC lines with different levels of RRM1 expression through genetic modification and found that in vitro cytotoxicity of gemcitabine was directly associated with the level of RRM1 expression.

The experimental evidence for RRM1 as the dominant determinant of gemcitabine efficacy is not limited to NSCLC. Bergman et al²⁵ generated in vivo resistance to gemcitabine by serial subcutaneous transplantation of the tumor colon 26 in BALB/c mice under repetitive intraperitoneal treatment with gemcitabine for 1 year. The authors found that RRM1 was the gene with the most striking increase in expression (25-fold) in the resistant tumor compared with the parent tumor in expression arrays, by RT-PCR and by immunoblotting.

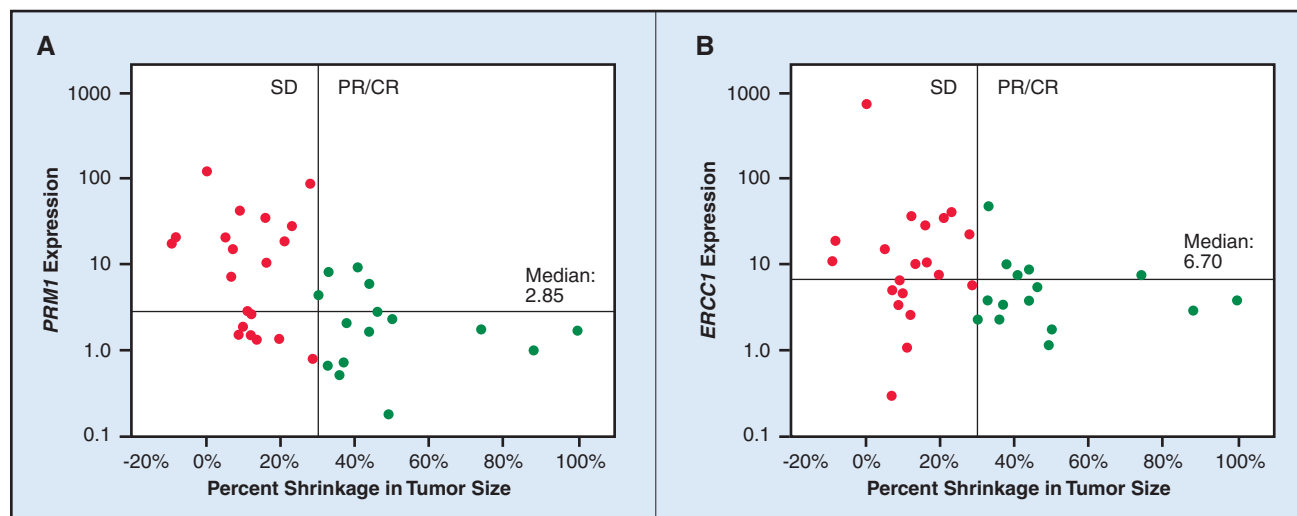


Fig 6A-B. — Scatter plot of (A) RRM1 and (B) ERCC1 expression in relation to the percent change in tumor size after two cycles of gemcitabine and carboplatin chemotherapy in 35 patients. Red dots indicate patients with less than 30% tumor shrinkage (stable disease [SD]), and green dots indicate patients with greater than 30% tumor shrinkage (partial remission/complete remission [PR/CR]). The Spearman correlation coefficient was $r = -0.498$ ($P = .002$) for RRM1 and $r = -0.283$ ($P = .099$) for ERCC1. From Bepler G, Kusmartseva I, Sharma S, et al. RRM1 modulated in vitro and in vivo efficacy of gemcitabine and platinum in non-small-cell lung cancer. *J Clin Oncol*. 2006;24:4731-4737. Reprinted with permission from the American Society of Clinical Oncology.

Other genes involved in the intracellular metabolism of gemcitabine were only minimally changed (deoxycytidine kinase and deoxycytidine deaminase).

Finally, Nakahira et al²⁶ evaluated the mechanism of gemcitabine resistance in 18 patients with pancreatic cancer. Patients treated by gemcitabine were divided into two groups based on RRM1 levels. A significant association was seen between gemcitabine response and RRM1 expression ($P=.018$). In addition, survival in patients with high RRM1 levels was worse after gemcitabine treatment than in patients with low RRM1 levels ($P=.016$).

BRCA1 as a Predictor of Chemotherapy Efficacy

BRCA1 plays an important role in DNA repair. Present knowledge suggests that it has a differential effect on chemotherapeutic efficacy, depending on the class of agents used. It functions as a sensitizer to apoptosis induced by antimicrotubulin agents such as taxanes and vinca alkaloids; however, it also mediates resistance to DNA-damaging agents such as platinum compounds.

Taron et al²⁷ reported the first clinical study on the potential predictive value of BRCA1 mRNA levels in NSCLC patients treated with gemcitabine and cisplatin. BRCA1 levels were detected in all 55 resected tumors, ranging from 0.28 to 10.43. Patients were grouped based on the BRCA1 expression (<0.61, .65 to 2.37, >2.45). Median survival was longer in the low-expression group than in the high-expression group (not reached in the low-expression group, 12.7 months in the high-expression group (95% CI 0.28 to 28.8 months; $P=.01$). Because of the study design, the impact of BRCA1 expression on treatment efficacy could not be assessed in detail. As a result, the predictive value of BRCA1 levels on chemotherapeutic efficacy in lung cancer remains unclear.

Wachters et al²³ did not find a significant correlation between treatment benefit from gemcitabine/platinum or epirubicin/platinum and BRCA1 expression as determined by immunohistochemistry in 33 patients with advanced-stage NSCLC.

Predictors of Improved Response or Survival With EGFR TKIs

EGFR is a member of the HER (also referred to as erb-B) family of receptor tyrosine kinases that are involved in the pathogenesis of many cancers including NSCLC.²⁸ Gefitinib and erlotinib, the orally active, selective EGFR TKIs, have shown a response rate of 9% to 26% in advanced-stage NSCLC.

A phase III trial by Shepherd et al²⁹ comparing erlotinib and placebo as a second- or third-line therapy in 731 patients with advanced NSCLC, reported a survival benefit for the EGFR TKI group. Patients were stratified based on performance status, response to prior chemotherapy, number of prior regimens, and prior platinum-based therapy. They were randomly assigned in a 2:1 ratio to receive oral erlotinib or placebo. The response rate was

8.9% in the erlotinib group and less than 1% in the placebo group ($P<.001$). Progression-free survivals were 2.2 months and 1.8 months in erlotinib and placebo groups, respectively (HR 0.61; $P<.001$). Overall survival was 6.7 months compared with 4.7 months in the placebo group (HR 0.70; $P<.001$), in favor of erlotinib.

Lynch et al³⁰ and Paez et al³¹ reported missense mutations and deletions in the tyrosine kinase domain of the EGFR gene that are highly associated with gefitinib response. These somatic mutations were small, in-frame deletions or amino acid substitutions clustered around the adenosine triphosphate (ATP)-binding pocket of the tyrosine kinase domain, and they were predominantly identified in patients with gefitinib responsive tumors. These data, and other subsequently reported studies, demonstrate the predictive utility of EGFR mutations for EGFR TKIs.

Hirsch et al²⁸ assessed EGFR and HER2 gene copy numbers by fluorescence in situ hybridization (FISH) in 81 patients with advanced bronchioloalveolar carcinoma treated with gefitinib. Tumors were dichotomized into EGFR FISH-positive and -negative groups. Among the FISH-positive patients, 63% had nonprogressive disease as best response compared with 39% of FISH-negative patients ($P=.087$). Improved survival was also observed in patients with increased EGFR gene copy numbers. The study demonstrates the predictive utility of EGFR gene copy numbers for gefitinib efficacy.

Oligonucleotide-Based Gene Expression Profiles as Predictors of Response in NSCLC Patients

Using in vitro drug sensitivity and oligonucleotide expression data, Potti et al³² developed a gene signature that predicts sensitivity to individual chemotherapeutic drugs. Independent sets of cell lines were identified and each signature was validated with response data. A gene expression-based predictor was developed consisting of 50 genes that classified cell lines based on docetaxel sensitivity. The docetaxel signature predicted sensitivity with an accuracy of 80% in an independent data set ($P<.001$). The study also integrated chemotherapy response signatures with oncogenic pathway deregulation to obtain information on potential drug efficacy in the context of specific pathways involved in tumorigenesis. Oligonucleotide expression array data from 17 NSCLC cell lines predicted to be resistant to docetaxel also had profiles suggestive of phosphatidylinositol 3 kinase (PI3K) pathway activation. Cell lines that had the PI3K activation profile responded to PI3K inhibitors. This suggests that cells resistant to docetaxel may be sensitive to PI3K inhibition.

Serum Proteomic Profiles as Predictors of Response in NSCLC Patients

MALDI-TOF-MS analysis has also been used to identify patients with NSCLC who are likely to benefit from

treatment with EGFR TKIs.³³ The MALDI-TOF-MS profile was developed from a training set of 139 patients from three cohorts. The profile was tested in two independent validation cohorts of 67 and 96 patients treated with gefitinib and erlotinib, respectively, and in three control cohorts of patients who were not treated with EGFR TKIs. The profile identified patients who had improved outcomes after EGFR TKI treatment in both validation cohorts. The authors concluded that the profile could classify NSCLC patients into good or poor outcomes after treatment with EGFR TKIs and may be useful for selection of patients for treatment with EGFR TKIs.

Individualizing Treatment for Advanced-Stage NSCLC Using ERCC1 and RRM1

We recently reported results from a prospective phase II clinical trial that was designed to test the feasibility and efficacy of molecular analysis-directed individualized therapy in patients with advanced NSCLC.³⁴ ERCC1 and RRM1 mRNA expression levels were determined by real-time RT-PCR in fresh-frozen tumor specimens from 53 eligible patients. Predetermined values for ERCC1 and RRM1 were used for decisions regarding treatment with gemcitabine and carboplatin. Gemcitabine was used in the treatment doublet if RRM1 expression was ≤ 16.5 , and carboplatin was used in the doublet if ERCC1 expression was ≤ 8.7 . This strategy resulted in four gene expression groups. Patients in the low RRM1 and low ERCC1 group were treated with gemcitabine and carboplatin, those in the low RRM1 and high ERCC1 group were treated with gemcitabine and docetaxel, those in the high RRM1 and low ERCC1 group were treated with docetaxel and carboplatin, and those in the high RRM1 and high ERCC1 group were treated with vinorelbine and docetaxel. The disease response rate was 44%. Overall and progression-free survival rates were 59% and 14% at 12 months with medians of 13.3 and 6.6 months, respectively. The conclusions from this trial are that gene expression analysis for treatment decisions of individual patients with advanced NSCLC is feasible and safe and that it has produced favorable response and survival data, which require confirmation in a randomized phase III trial.

Cobo et al³⁵ recently reported results from the first customized and randomized trial in patients with advanced NSCLC. In this trial, ERCC1 mRNA expression, determined by real-time RT-PCR in routinely collected diagnostic tumor specimens, was used to determine if cisplatin was to be included in a doublet regimen. The primary study endpoint was best disease response. Of 366 patients who had a successful gene expression analysis and received at least one cycle of therapy, 346 were evaluable for response. The response rate was 39.3% in the control arm (docetaxel and carboplatin) and 50.7% in the experimental arm (docetaxel and gem-

citabine if the ERCC1 level was high and docetaxel and carboplatin if the ERCC1 level was low).

Conclusions

The level of ERCC1 expression appears to be prognostic of survival, and it is a predictive marker for platinum efficacy. A low level of ERCC1 is associated with short survival; however, it is also predictive of increased platinum efficacy. Likewise, the level of RRM1 appears to be prognostic of survival, and it is a predictive marker for gemcitabine efficacy. A low level of RRM1 is associated with long survival; however, it is also predictive of high gemcitabine efficacy. A low level of BRCA1 expression appears to be prognostic of long survival; it also appears to be predictive of good platinum efficacy and of poor taxane efficacy. EGFR mutations and gene copy numbers are prognostic of survival and predictive for EGFR TKI efficacy. The presence of mutations and high gene copy numbers are predictive of high EGFR TKI efficacy. Oligonucleotide expression array and proteomic profiles are promising alternative strategies for outcomes prognostication and prediction of therapeutic efficacy.

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References

1. de Laat WL, Jaspers NG, Hoeijmakers JH. Molecular mechanism of nucleotide excision repair. *Genes Dev.* 1999;13:768-785.
2. Olausson KA, Dunant A, Fouret P, et al. DNA repair by ERCC1 in non-small-cell lung cancer and cisplatin-based adjuvant chemotherapy. *N Engl J Med.* 2006;355:983-991.
3. Zheng Z, Chen T, Li X, et al. DNA synthesis and repair genes RRM1 and ERCC1 in lung cancer. *N Engl J Med.* 2007;356:800-808.
4. Simon GR, Sharma S, Cantor A, et al. ERCC1 expression is a predictor of survival in resected patients with non-small cell lung cancer. *Chest.* 2005;127:978-983.
5. Rosell R, Skrzypski M, Jassem E, et al. BRCA1: a novel prognostic factor in resected non-small-cell lung cancer. *PLoS ONE.* 2007;2:e1129.
6. Elledge SJ, Zhou Z, Allen JB. Ribonucleotide reductase: regulation, regulation, regulation. *Trends Biochem Sci.* 1992;17:119-123.
7. Gautam A, Li ZR, Bepler G. RRM1-induced metastasis suppression through PTEN-regulated pathways. *Oncogene.* 2003;22:2135-2142.
8. Bepler G, Gautam A, McIntyre LM, et al. Prognostic significance of molecular genetic aberrations on chromosome segment 11p15.5 in non-small-cell lung cancer. *J Clin Oncol.* 2002;20:1353-1360.
9. Bepler G, Sharma S, Cantor A, et al. RRM1 and PTEN as prognostic parameters for overall and disease-free survival in patients with non-small-cell lung cancer. *J Clin Oncol.* 2004;22:1878-1885.
10. Futreal PA, Liu Q, Shattuck-Eidens D, et al. BRCA1 mutations in primary breast and ovarian carcinomas. *Science.* 1994;266:120-122.
11. Potti A, Mukherjee S, Petersen R, et al. A genomic strategy to refine prognosis in early-stage non-small-cell lung cancer. *N Engl J Med.* 2006;355:570-580.
12. Chen HY, Yu SL, Chen CH, et al. A five-gene signature and clinical outcome in non-small-cell lung cancer. *N Engl J Med.* 2007;356:11-20.
13. Amann JM, Chaurand P, Gonzalez A, et al. Selective profiling of proteins in lung cancer cells from fine-needle aspirates by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Clin Cancer Res.* 2006;12:5142-5150.

14. Yanagisawa K, Shyr Y, Xu BJ, et al. Proteomic patterns of tumour subsets in non-small-cell lung cancer. *Lancet*. 2003;362:433-439.
15. Schiller JH, Harrington D, Belani CP, et al. Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. *N Engl J Med*. 2002;346:92-98.
16. Hanna N, Shepherd FA, Fossella FV, et al. Randomized phase III trial of pemetrexed versus docetaxel in patients with non-small-cell lung cancer previously treated with chemotherapy. *J Clin Oncol*. 2004;22:1589-1597.
17. Metzger R, Leichman CG, Danenberg KD, et al. ERCC1 mRNA levels complement thymidylate synthase mRNA levels in predicting response and survival for gastric cancer patients receiving combination cisplatin and fluorouracil chemotherapy. *J Clin Oncol*. 1998;16:309-316.
18. Dabholkar M, Vionnet J, Bostick-Bruton F, et al. Messenger RNA levels of XPAC and ERCC1 in ovarian cancer tissue correlate with response to platinum-based chemotherapy. *J Clin Invest*. 1994;94:703-708.
19. Shirota Y, Stoecklacher J, Brabender J, et al. ERCC1 and thymidylate synthase mRNA levels predict survival for colorectal cancer patients receiving combination oxaliplatin and fluorouracil chemotherapy. *J Clin Oncol*. 2001;19:4298-4304.
20. Lord RV, Brabender J, Gandara D, et al. Low ERCC1 expression correlates with prolonged survival after cisplatin plus gemcitabine chemotherapy in non-small cell lung cancer. *Clin Cancer Res*. 2002;8:2286-2291.
21. Bewick MA, Conlon MS, Lafrenie RM. Polymorphisms in XRCC1, XRCC3, and CCND1 and survival after treatment for metastatic breast cancer. *J Clin Oncol*. 2006;24:5645-5651.
22. Bepler G, Kusmartseva I, Sharma S, et al. RRM1 modulated in vitro and in vivo efficacy of gemcitabine and platinum in non-small-cell lung cancer. *J Clin Oncol*. 2006;24:4731-4737. Epub 2006 Sep 11.
23. Wachters FM, Wong LS, Timens W, et al. ERCC1, hRad51, and BRCA1 protein expression in relation to tumour response and survival of stage III/IV NSCLC patients treated with chemotherapy. *Lung Cancer*. 2005;50:211-219. Epub 2005 Sep 16.
24. Davidson JD, Ma L, Flagella M, et al. An increase in the expression of ribonucleotide reductase large subunit 1 is associated with gemcitabine resistance in non-small cell lung cancer cell lines. *Cancer Res*. 2004;64:3761-3766.
25. Bergman AM, Eijk PP, Ruiz van Haperen VW, et al. In vivo induction of resistance to gemcitabine results in increased expression of ribonucleotide reductase subunit M1 as the major determinant. *Cancer Res*. 2005;65:9510-9516.
26. Nakahira S, Nakamori S, Tsujie M, et al. Involvement of ribonucleotide reductase M1 subunit overexpression in gemcitabine resistance of human pancreatic cancer. *Int J Cancer*. 2007;120:1355-1363.
27. Taron M, Rosell R, Felip E, et al. BRCA1 mRNA expression levels as an indicator of chemoresistance in lung cancer. *Hum Mol Genet*. 2004;13:2443-2449. Epub 2004 Aug 18.
28. Hirsch FR, Varella-Garcia M, McCoy J, et al. Increased epidermal growth factor receptor gene copy number detected by fluorescence in situ hybridization associates with increased sensitivity to gefitinib in patients with bronchioloalveolar carcinoma subtypes. *J Clin Oncol*. 2005;23:6838-6845. Epub 2005 Jul 5.
29. Shepherd FA, Rodrigues Pereira J, Ciuleanu T, et al. Erlotinib in previously treated non-small-cell lung cancer. *N Engl J Med*. 2005;353:123-132.
30. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med*. 2004;350:2129-2139. Epub 2004 Apr 29.
31. Paez JG, Jänne PA, Lee JC, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science*. 2004;304:1497-1500. Epub 2004 Apr 29.
32. Potti A, Dressman HK, Bild A, et al. Genomic signatures to guide the use of chemotherapeutics. *Nat Med*. 2006;12:1294-1300. Epub 2006 Oct 22.
33. Taguchi F, Solomon B, Gregorc V, et al. Mass spectrometry to classify non-small-cell lung cancer patients for clinical outcome after treatment with epidermal growth factor receptor tyrosine kinase inhibitors: a multicohort cross-institutional study. *J Natl Cancer Inst*. 2007;99:838-846.
34. Simon G, Sharma A, Li X, et al. Feasibility and efficacy of molecular analysis-directed individualized therapy in advanced non-small-cell lung cancer. *J Clin Oncol*. 2007;25:2741-2746.
35. Cobo M, Isla D, Massuti B, et al. Customizing cisplatin based on quantitative excision repair cross-complementing 1 mRNA expression: a phase III trial in non-small-cell lung cancer. *J Clin Oncol*. 2007;25:2747-2754.