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Imaging, Diagnosis, Prognosis

Clinical Cancer Research

MutS Homologue 2 and the Long-term Benefit of Adjuvant Chemotherapy in Lung Cancer

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Abstract

Purpose: We sought to determine the long-term (median follow-up, 7.5 years) predictive power of human MutS homologue 2 (MSH2) immunohistochemical expression in patients who enrolled in the International Adjuvant Lung Trial.

Experimental design: We tested the interaction between MSH2 and the allocated treatment (chemotherapy versus observation) in a Cox model adjusted on clinicopathologic variables. The significance level was set at 0.01.

Results: MSH2 levels were low in 257 (38%) and high in 416 (62%) tumors. The benefit from chemotherapy was likely different according to MSH2 (interaction test, $P = 0.06$): there was a trend for chemotherapy to prolong overall survival when MSH2 was low [hazard ratio (HR), 0.76; 95% confidence interval (95% CI), 0.59-0.97; $P = 0.03$], but not when MSH2 was high (HR, 1.12; 95% CI, 0.81-1.55; $P = 0.48$). In the control arm, the HR was 0.66 (95% CI, 0.49-0.90; $P = 0.01$) when MSH2 was high. When combining MSH2 with excision repair cross-complementing group 1 (ERCC1) into four subgroups, the benefit of chemotherapy decreased with the number of markers expressed at high levels ($P = 0.01$). A similar decrease was noted when combining MSH2 and P27 ($P = 0.01$). Chemotherapy prolonged overall survival in the combined low MSH2/low ERCC1 subgroup (HR, 0.65; 95% CI, 0.47-0.91; $P = 0.01$) and in the combined low MSH2/low P27 subgroup (HR, 0.65; 95% CI, 0.46-0.93; $P = 0.01$).

Conclusions: MSH2 expression is a borderline significant predictor of a long-term benefit from adjuvant cisplatin-based chemotherapy in patients with completely resected lung cancer. MSH2 combined with ERCC1 or P27 may identify patients most likely to benefit durably from chemotherapy. *Clin Cancer Res; 16(4); 1206–15. ©2010 AACR.*

Platinum compounds are a hallmark of chemotherapy against cancer due to their DNA binding capacity, which results in DNA damage and cell death. A single DNA cross-link, if not repairable, can be lethal. The processing of cross-links in mammalian cells is not clearly understood. However, it is known that their processing may involve components belonging to different DNA excision repair pathways, including the nucleotide excision repair (NER) pathway and the mismatch repair (MMR) pathway (1). The role that DNA excision repair pathways play in mediating platinum resistance has been studied for many years, first in the preclinical, and more recently, in the clinical setting (2). The underlying hypothesis of these studies is that a reduced capacity by tumor cells to excise platinum-DNA adducts increases cell sensitivity to chemotherapy, which in turn may translate into a clinical benefit.

In 2004, the International Adjuvant Lung Trial (IALT) was the first and still is the largest clinical trial to establish that patients with non–small cell lung cancer (NSCLC) benefited from adjuvant cisplatin-based chemotherapy after their tumors have been surgically completely removed (3). A benefit from platinum-based adjuvant chemotherapy was further validated by the results of the ANITA, CALGB, and JBR10 trials, and by their meta-analysis (4–7). When the survival data in the IALT were updated in 2007, however, an improvement of the long-term overall survival from chemotherapy could no longer be observed due to an excess of late deaths in patients who underwent chemotherapy (8).

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Translational Relevance

We report for the first time the ability of two DNA repair proteins MSH2 and ERCC1 and of the cyclin-dependent kinase inhibitor P27 to predict the long-term (median follow-up, 7.5 years) benefit from adjuvant cisplatin-based chemotherapy in the International Adjuvant Lung Trial. As the magnitude of benefit from chemotherapy decreased with time, the results emphasize the importance of long-term follow-up in clinical trials and translational studies. Our opinion is that ERCC1, MSH2, and P27 are the three most promising markers of the benefit of adjuvant cisplatin-based chemotherapy and should be tested in priority in validation studies. After validation, the most efficacious markers will be used in the clinical practice either alone or in combination for recognizing patients unlikely to benefit from conventional platinum-based therapies and to which new agents that are active in advanced stages of lung cancer can be proposed in the adjuvant setting.

The IALT-BIO study was designed to examine whether a limited number of markers predicted survival in relationship with chemotherapy. To reduce the risk of false-positive results due to multiple comparisons, the significance level was set at $P = 0.01$. Initially, 19 markers belonging to five groups were studied: drug transporters, DNA repair, cell cycle regulators, signal transduction, and apoptosis. Only excision repair cross-complementation group 1 (ERCC1), a key NER protein also involved in homologous recombination (9) and maintenance of telomeres (10), significantly predicted ($P = 0.009$) the short-term survival benefit of chemotherapy (11). Patients with low ERCC1 expression benefited from chemotherapy, whereas those with high ERCC1 expression did not. Among the other markers, the closest to significantly predict the benefit of chemotherapy was the cyclin-dependent kinase inhibitor P27 ($P = 0.02$; ref. 12). Furthermore, when ERCC1 and P27 were combined, the predictive values further increased (13). Other markers were either more weakly associated to the benefit of chemotherapy or not associated at all (14, 15).

The results with ERCC1 pointed to the potential of studying the expression of DNA repair genes to tailor the use of adjuvant chemotherapy in lung cancer. Except for *ERCC1*, no DNA repair gene was included in the initial IALT-Bio analysis. In a second phase of IALT-Bio, we thus tried to increase the predictive power of *ERCC1* by examining the expression of *MutS homologue 2* (*MSH2*), a gene that is crucially involved in the repair of cisplatin-DNA cross-links. *MSH2*, which is frequently mutated in hereditary nonpolyposis colon cancer, encodes a critical protein of the MMR pathway (16). MSH2 binds to DNA mismatches, thereby initiating DNA repair. In addition to its function in the MMR pathway, MSH2 also recognizes and binds to cisplatin-induced DNA interstrand cross-links, thereby initiating their excision and repair (17, 18). MSH2 is required to repair interstrand cross-links in mammalian cells, in which it physically interacts with the NER pathway component ERCC1 (19). During the recombinational repair processing of interstrand cross-links, MSH2 cooperates with several components of DNA damage repair pathways, including ERCC1, REV1, components of the Fanconi anemia pathway, and homologous recombination repair factors (20). Reduced MSH2 expression by tumor cells was reported in 10% to 58% of NSCLC (21–30). A recent study reported that the loss of MSH2 expression in tumors from patients with advanced NSCLC led to higher rates of response to oxaliplatin-based chemotherapy, but not to cisplatin-based chemotherapy (30). In a larger study of genetic polymorphisms among patients with advanced NSCLC, the *MSH2* gIV12-6T>C variant was associated with low MSH2 expression and better response to cisplatin, but the authors did not report a direct relationship between MSH2 expression and chemotherapy benefit (29).

Using the long-term overall survival IALT data that were updated in 2007, we looked again at ERCC1 and studied for the first time the predictive power of MSH2 and the potential of combining MSH2 and ERCC1 to identify patients who benefited durably from chemotherapy. We also report the results of the combined analysis of MSH2 and P27 and discuss the potential usefulness of combining these markers.

Materials and Methods

Patients and study design. All patients had participated in the IALT study (1,867 patients). The IALT-Bio study was designed by a steering committee to examine whether tumor markers assessed by immunohistochemical analysis could be used to predict survival in relationship with chemotherapy. The study was conducted according to a detailed protocol that stressed the importance of collecting all samples within the participating centers and required a large number of tumor samples to ensure adequate power for prognostic and predictive analyses. All 50 IALT centers which had enrolled 10 or more patients (total 1,468 patients) were solicited to participate in the IALT-Bio study. Twenty-eight centers in 14 countries agreed to participate. From their 1,045 patients, they contributed 867 blocks, which represents an overall completeness rate of 83%. Approval was obtained from the local institutional review boards, according to the legal regulations in each participating country. All tumors were reviewed centrally at the Centre Hospitalier Universitaire Albert Michallon, according to the histopathologic classification system adopted by the WHO in 2004 (31). After tissue collection and a pathologic review, 783 tissue blocks were included in the IALT-Bio study. The fixation procedure depended on each center and was not registered.

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The quality of slides after H&E staining, referred throughout the article as slide quality, was semiquantitatively classified into two categories (average and good) taking into account staining contrast as well as cell integrity and morphologic appearance.

Tissue microarray construction. Three representative tumor areas were selected for each case. Cores measuring 0.6 mm in diameter and 5 mm in length (spots) were arrayed following a map. Among the 783 IALT-Bio tissue blocks that contained tumor material, triplicate spots were obtained in 768 (98%) cases. Random spots were compared with the original blocks to verify the agreement between their coordinates and the original samples.

MSH2 immunostaining. Immunostaining was done following a standard procedure using the Vectastain Elite kit with NovaRED (Vector Laboratories) as the substrate and Mayer's hematoxylin as the counterstain. The primary antibody was the mouse monoclonal antibody FE11 (Calbiochem) raised against the COOH-terminal fragment of human MSH2. For epitope retrieval, slides were heated at 98°C for 1 h in 10 mmol/L citrate buffer (pH 7.3). Sections were incubated at room temperature for 90 min with the FE11 antibody at a dilution of 1:50. The freshly cut tissue microarray (TMA) sections were manually immunostained in a single experiment that included a tonsil section as an external control.

Evaluation of MSH2 immunostaining. Two investigators (N.S.K. and P.F.), who were blinded to clinical data, independently evaluated MSH2 nuclear reactivity. The TMA slides were scanned at high resolution (VM3 virtual scanner, Ziemens), enabling the study of an identical high-quality image at ×20 magnification for each spot for detailed evaluation.

The spots were carefully examined for reactive lung or stromal cells (endothelial cells and fibroblasts), which served as an internal positive control. Spots without a valid internal control were discarded. Cases for which no valid tumor spot could be evaluated were excluded.

Staining intensity was graded on a scale of 0 to 3, using the expression level in fibroblasts or endothelial cells as a reference (defined *a priori* as a score of 2). The percentage of reactive tumor cells was graded on a scale of 0% to 100%. A proportion score was assigned to the percentage of reactive tumor nuclei: 0 was assigned if 0% of reactive tumor nuclei were found; 0.1 was assigned if 1% to 9% were found; 0.5 was assigned if 10% to 49% were found; and 1 was assigned if ≥50% of reactive tumor nuclei were present. This proportion score was multiplied by the staining intensity to obtain a histology score (H-score) for each patient (11). All discordant cases were reviewed to reach a consensus.

Statistical analysis. Long-term IALT survival data were used, with median follow-up of 7.5 years (8). A logistic model stratified by center was used to compare patients with high MSH2 and low MSH2 tumors.

The prognostic values of the biomarker status and chemotherapy for overall survival were studied using the Cox model. As in the main IALT analysis (3), the Cox model included every factor used in the stratified randomization (center, tumor stage, and type of surgery) plus clinical and histologic prognostic factors (age, sex, WHO performance status, nodal status, lymphoid infiltration, and the revised histopathologic type). All other factors that were statistically related to the biomarker status in the multivariate logistic model ($P < 0.05$) were added to the Cox model.

The predictive value of the biomarker was studied by testing the interaction between the biomarker status and the allocated treatment (chemotherapy versus observation) in the same Cox model. Tests of homogeneity of the hazard ratios were done within the Cox model. For the joint analysis of MSH2 and ERCC1, the Cox model included the same variables as above together with three variables indicating the status of the combination of MSH2 and ERCC1 and a different treatment variable for each of the four combinations. All reported *P* values were two sided. In the IALT-Bio analysis plan, *P* values below 0.01 were considered as statistically significant to limit the risk of false-positive results. Survival rates were estimated using the Kaplan-Meier method (all *P* values indicated, besides Kaplan-Meier curves, were adjusted *P* values corresponding to the Cox analysis).

All analyses were done using the SAS software, version 9.1 (SAS Institute, Inc.) and curves were drawn with the Tigre software.

Results

MSH2 expression. Among the 768 patients whose tumor was included in the TMA, no tumor material could be analyzed after immunohistochemistry in 34 (4%) cases. After excluding the cases without valid internal controls, the H-scores were evaluated in 673 of 768 (88%) patients.

The H-scores were as follows: 0.2 in 1 case (0%), 1 in 52 cases (8%), 2 in 363 cases (54%), and 3 in 257 cases (38%). The median H-score was 2, which was chosen to separate high MSH2 cases (H-score = 3) and low MSH2 cases (H-score < 3). There were 257 (38%) high MSH2 cases and 416 (62%) low MSH2 cases. Figure 1 shows examples of high MSH2 and low MSH2 cases.

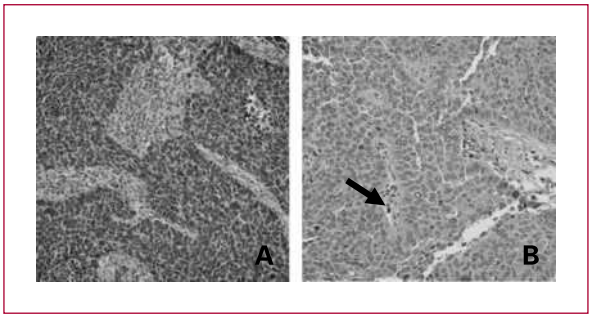

Fig 1. Examples of high MSH2 and low MSH2 NSCLS. A, high MSH2. The staining was intense and diffuse. B, low MSH2. Arrow, positive stromal cells (internal control).

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Table 1. Patient characteristics

Characteristic	Patients with high MSH2 tumors (n = 257)		Patients with low MSH2 tumors (n = 416)		All patients (n = 673)		P*
	Number	Percent	Number	Percent	Number	Percent	
Sex							0.05
Male	221	86	326	78	547	81	
Female	36	14	90	22	126	19	
Age							0.99
<55 years	70	27	127	31	197	29	
55-64 years	116	45	181	44	297	44	
>64 years	71	28	108	26	179	27	
Pathological TNM stage†							0.79
Stage I	83	32	150	36	233	35	
Stage II	53	21	102	25	155	23	
Stage III	121	47	164	39	285	42	
Tumor							0.83
T1	36	14	63	15	99	15	
T2	145	56	263	63	408	61	
T3	74	29	82	20	156	23	
T4	2	1	8	2	10	1	
Nodes							0.99
N0	122	47	188	45	310	46	
N1	69	27	125	30	194	29	
N2	66	26	103	25	169	25	
Histological type							0.002
Adenocarcinoma	51	20	154	37	205	30	
Squamous-cell carcinoma	174	68	217	52	391	58	
Other NSCLC	32	12	45	11	77	11	
Surgery							0.34
Pneumonectomy	118	46	161	39	279	41	
Lobectomy or segmentectomy	139	54	255	61	394	59	
Performance status scored‡							0.37
0	147	57	222	53	369	55	
1	84	33	165	40	249	37	
2	26	10	29	7	55	8	
Lymphoid infiltration							0.20
Not intense	235	91	360	87	595	88	
Intense	22	9	56	13	78	12	
Pleural invasion							0.19
No	232	90	386	93	618	92	
Yes	25	10	30	7	55	8	
Vascular invasion							0.44
No	181	70	295	71	476	71	
Yes	76	30	121	29	197	29	
Lymphatic invasion							0.19
No	71	28	137	33	208	31	
Yes	186	72	279	67	465	69	
Quality after H&E staining							0.01
Average	37	14	28	7	65	10	
Good	220	86	388	93	608	90	

NOTE: Percentages may not total 100 because of rounding.

*P values testing the difference between positive and negative tumors were calculated using logistic regression stratified on centre.

†TNM denotes tumor-node-metastases.

‡World Health Organization scores.

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MSH2 expression and baseline clinical characteristics. The relationships between MSH2 expression and the clinical characteristics are provided in Table 1. The proportion of adenocarcinomas was lower ($P = 0.002$) for high MSH2 cases (20%) than for low MSH2 cases (37%). The morphologic slide quality after H&E staining was associated with MSH2 expression ($P = 0.01$).

In a logistic model adjusted on sex and slide quality, histology differed according to MSH2 expression, with fewer adenocarcinomas among patients with high MSH2 tumors ($P = 0.006$).

The 673 cases included in the MSH2 analysis differed from the 95 cases excluded in terms of histology ($P < 0.001$), type of surgery ($P = 0.02$), and slide quality ($P = 0.01$; there were fewer squamous cell carcinomas, fewer pneumonectomies, and lower slide quality in excluded cases).

Overall survival and adjuvant chemotherapy. For the group of patients included in the MSH2 analysis (673 patients), the adjusted hazard ratio for death associated with chemotherapy compared with observation was 0.88 [95% confidence interval (95% CI), 0.72-1.07; $P = 0.21$]. The 5-year overall survival rates were 47% (95% CI, 41%-52%) in the chemotherapy arm, and 44% (95% CI, 39-49%) in the control arm. The 8-year overall survival rates were 36% (95% CI, 31-42%) in the chemotherapy arm and 37% (95% CI, 32-43%) in the control arm.

Effect of chemotherapy on overall survival according to MSH2 expression. Overall, the test for interaction between chemotherapy and MSH2 gave a P value of 0.06 in the Cox model including slide quality and histologic type. In the low-MSH2 group, there was a trend for overall survival to be longer in the chemotherapy arm than in the control arm (adjusted hazard ratio for death, 0.76; 95% CI, 0.59-0.97; $P = 0.03$; Table 2; Fig. 2A). The 5-year overall survival rates among patients with low MSH2 tumors were 49% (95% CI, 43-56%) in the chemotherapy arm and 41% (95% CI, 34-48%) in the control arm. The 8-year overall survival rates among patients with low MSH2 tumors were 38% (95% CI, 32-45%) in the chemotherapy arm and 36% (95% CI, 30-43%) in the control arm. Among patients with low MHS2 tumors, median overall survival tended to be 16 months longer in the chemotherapy arm (58 months) than in the control arm (42 months). In the high MSH2 group, there was no difference in the overall survival between the chemotherapy arm and the control arm (adjusted hazard ratio for death, 1.12; 95% CI, 0.81-1.55; $P = 0.48$; Table 2, Fig. 2B). The 5-year overall survival rates among patients with high MSH2 tumors were 42% (95% CI, 34-51%) in the chemotherapy arm and 49% (95% CI, 40-58%) in the control arm. The 8-year overall survival rates among patients with high MSH2 tumors were 34% (95% CI, 25-43%) in the chemotherapy arm and 39% (95% CI, 31-49%) in the control arm.

Prognostic effect of MSH2 expression on overall survival. In the control arm, high MSH2 compared with low MSH2 was associated with an adjusted hazard ratio for death of 0.66 (95% CI, 0.49-0.90; $P = 0.01$). Median overall survival tended to be 16 months longer in the high MSH2 group (58 months) than in the low MSH2 group (42 months).

In the chemotherapy arm, there was no difference in overall survival between high MSH2 and low MSH2 tumors (adjusted hazard ratio for death, 0.99; 95% CI, 0.74-1.32; $P = 0.93$).

Effect of chemotherapy on overall survival according to MSH2 and ERCC1 expression. The expression levels of MSH2 and ERCC1 were both available for 658 patients (84% of the 783 patients included in IALT-Bio). In a subanalysis of these 658 patients, the test for interaction between chemotherapy and the markers gave borderline P values ($P = 0.05$ for MSH2 and $P = 0.04$ for ERCC1).

In the low MSH2 group (404 patients), the adjusted hazard ratio for death associated with chemotherapy versus observation was 0.75 (95% CI, 0.58-0.98; $P = 0.03$; Table 3). In the high MSH2 group (254 patients), there was no difference in the overall survival between the chemotherapy arm and the control arm (adjusted hazard ratio for death, 1.14; 95% CI, 0.82-1.57; $P = 0.44$).

Among these 658 patients, the adjusted hazard ratio for death associated with chemotherapy versus observation

Table 2. Overall survival according to allocated treatment and MSH2 status

	Chemotherapy group (No deaths / No patients)	Control group (No deaths / No patients)	Hazard ratio for death (95% CI)*	*P*
Patients with low MSH2 tumors N = 416	131 / 215	130 / 201	0.76 (0.59-0.97)	$P = 0.03$
Patients with high MSH2 tumors N = 257	83 / 131	75 / 126	1.12 (0.81-1.55)	$P = 0.48$
Hazard ratio for death (95% CI)†	0.99 (0.74-1.32)	0.66 (0.49-0.90)	-	-
P Value	$P = 0.93$	$P = 0.01$	-	$P = 0.06$‡

*Hazard ratios are for the comparison of the chemotherapy group with the control group.
†Hazard ratios are for the comparison of patients with MSH2 positive tumors with those with MSH2 negative tumors.
‡The *P* value is for the interaction between MSH2 expression and treatment.

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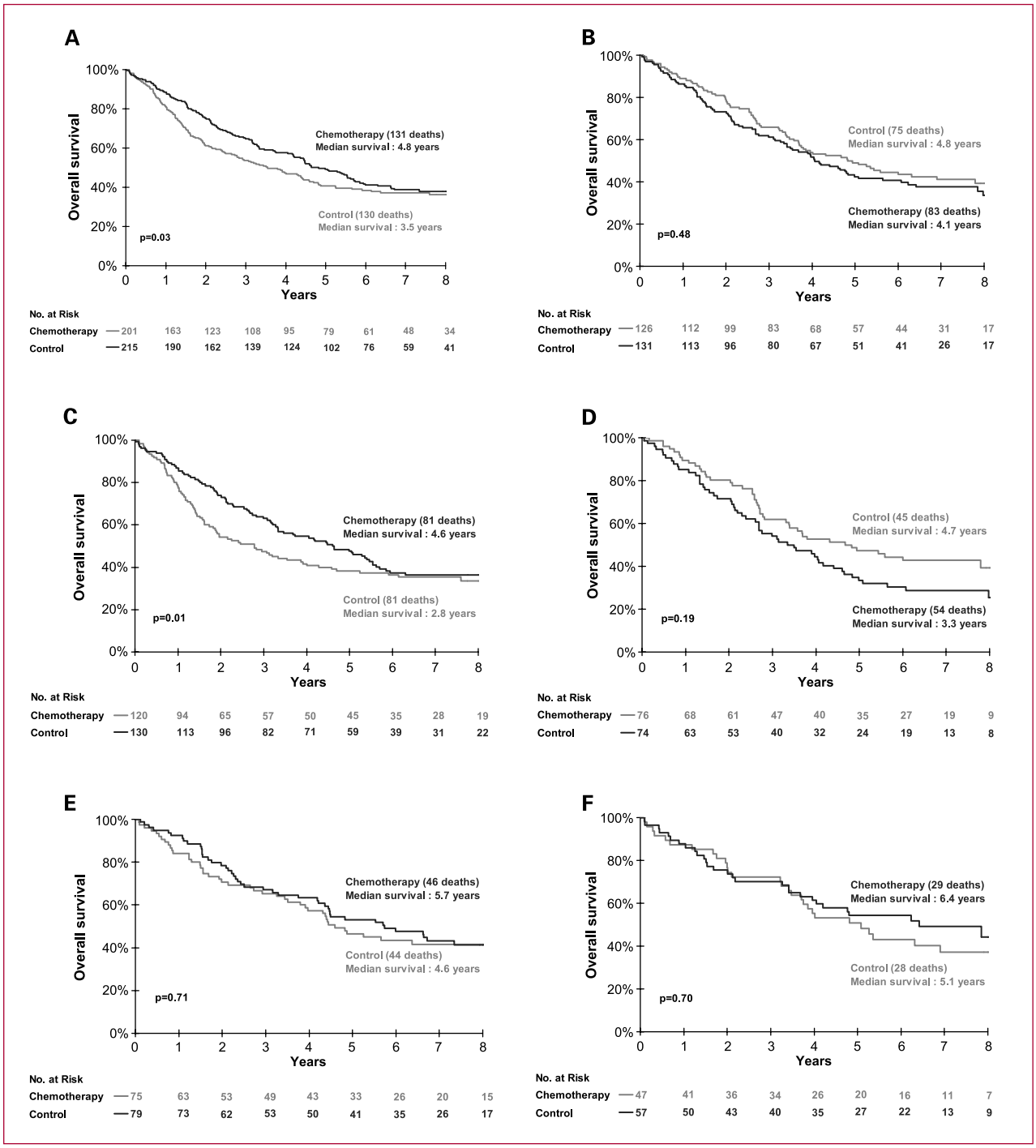

Fig. 2. Kaplan-Meier estimates of the probability of overall survival according to treatment with chemotherapy. Survival rates were estimated with the use of the Kaplan-Meier method. The *P* values beside the curves are those of the adjusted Cox model. A, overall survival according to treatment in patients with low MSH2 tumors. The adjusted hazard ratio for death in the chemotherapy group, compared with the control group, was 0.76 (95% CI, 0.59-0.97; *P* = 0.03). B, overall survival according to treatment in high MSH2 tumors. The adjusted hazard ratio for death in the chemotherapy group, compared with the control group, was 1.12 (95% CI, 0.81-1.55; *P* = 0.48). C, overall survival according to the combined low MSH2/low ERCC1 subgroup. The adjusted hazard ratio for death in the chemotherapy group, compared with the control group, was 0.65 (95% CI, 0.47-0.91; *P* = 0.01). D, overall survival according to the combined high MSH2/high ERCC1 subgroup. The adjusted hazard ratio for death associated with chemotherapy was 1.32 (95% CI, 0.88-1.99; *P* = 0.19). E, overall survival according to the combined low MSH2/high ERCC1 subgroup. The hazard ratio for death in the chemotherapy group, compared with the control group, was 1.32 (95% CI 0.88-1.99; *P* = 0.71). F, overall survival according to the combined high MSH2/low ERCC1 subgroup. The hazard ratio for death in the chemotherapy group, compared with the control group, was 0.90 (95% CI, 0.52-1.55; *P* = 0.70).

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Table 3. Overall survival according to allocated treatment and MSH2/ERCC1 subgroups

	Patients with low MSH2 tumors	Patients with high MSH2 tumors	Total
Patients with low ERCC1 tumors			
Number of death / number of patients	162 / 250	57 / 104	
Hazard ratio for death (95% CI)	0.65 (0.47-0.91)*	0.90 (0.52-1.55)*	0.73 (0.55-0.96)*
P Value	*P* = 0.01	*P* = 0.70	*P* = 0.03
Patients with high ERCC1 tumors			
Number of death / number of patients	90 / 154	99 / 150	
Hazard ratio for death (95% CI)	0.92 (0.60-1.42)*	1.32 (0.88-1.99)*	1.11 (0.83-1.50)*
P Value	*P* = 0.71	*P* = 0.19	*P* = 0.49
Total			
Hazard ratio for death (95% CI)	0.75 (0.58-0.98)*	1.14 (0.82-1.57)*	-
	P = 0.03	*P* = 0.44	

*Hazard ratios are for the comparison of the chemotherapy group with the control group.

was 0.73 (95% CI, 0.55-0.96; *P* = 0.03) in the low ERCC1 group (354 patients; Table 3). In the high ERCC1 group (304 patients), there was no difference in overall survival between the chemotherapy arm and the control arm (adjusted hazard ratio for death, 1.11; 95% CI, 0.83-1.50; *P* = 0.49).

Relationships between MSH2 and ERCC1 expression. The proportion of high MSH2 cases was 29% (104 of 354 cases) in the low ERCC1 group and 49% (150 of 304 cases) in the high ERCC1 group (*P* < 0.001). The association of MSH2 with ERCC1 remained in a model adjusted on slide quality and histologic type (*P* < 0.001).

Predictive value of combining MSH2 and ERCC1. The effect of chemotherapy was examined in the following subgroups defined by combining MSH2 and ERCC1: low MSH2/low ERCC1, high MSH2/high ERCC1, low MSH2/high ERCC1, and high MSH2/low ERCC1 (Fig. 2C-F). The test for heterogeneity when considering these four subgroups was not significant (*P* = 0.07). The differential effect of chemotherapy for patients with high MSH2 tumors compared with those with low MSH2 lesions was similar among patients with high ERCC1 and among patients with low ERCC1 tumors (*P* = 0.94), i.e., the ratio of the hazard ratios associated with MSH2 among patients with low ERCC1 (0.90/0.65) was equal to that among patients with high ERCC1 tumors (1.32/0.92; Table 3).

In the combined low MSH2/low ERCC1 subgroup, overall survival was 21 months longer in the chemotherapy arm (55 months) than in the control arm (34 months; adjusted hazard ratio for death, 0.65; 95% CI, 0.47-0.91; *P* = 0.01; Fig. 2C). In the high MSH2/high ERCC1 subgroup, the hazard ratio for death associated with chemotherapy was 1.32 (95% CI, 0.88-1.99; *P* = 0.19; Fig. 2D). The adjusted hazard ratio for death associated with chemotherapy when one marker was expressed at high levels (either MSH2 or ERCC1 but not both) was 0.91 (95% CI, 0.65-1.28; *P* = 0.60). When we defined a score by counting the number of high MSH2 and ERCC1 markers in the above-defined subgroups (allocating a score of 0 for no marker at high levels, 1 for 1 marker at high levels, and 2 for 2 markers at high levels), the test for trend of a decreasing effect of chemotherapy with an increasing score was significant (P_{trend} = 0.01; $P_{heterogeneity}$ = 0.02).

Predictive value of combining MSH2 and P27. Like for MSH2 and ERCC1, the effect of chemotherapy was examined in the following subgroups defined by combining MSH2 and P27: low MSH2/low P27, high MSH2/high P27, low MSH2/high P27, and high MSH2/low P27. The test for heterogeneity when considering these four subgroups was not significant (*P* = 0.11). The differential effect of chemotherapy for patients with high MSH2 tumors compared with those with low MSH2 tumors was similar among patients with high P27 and among patients with low P27 tumors (*P* = 0.91), i.e., the ratio of the hazard ratios associated with MSH2 among patients with low P27 (0.92/0.65) was equal to that among patients with high P27 tumors (1.31/0.88).

In the combined low MSH2/low P27 subgroup, the hazard ratio for death was reduced by chemotherapy (adjusted hazard ratio for death, 0.65; 95% CI, 0.46-0.93; *P* = 0.01). In the high MSH2/high P27 subgroup, the hazard ratio for death associated with chemotherapy was 1.31 (95% CI, 0.85-2.01; *P* = 0.22). The adjusted hazard ratio for death associated with chemotherapy when one marker was expressed at high levels (either MSH2 or P27 but not both) was 0.90 (95% CI, 0.67-1.21; *P* = 0.50). When we defined a score by counting the number of high MSH2 and P27 markers in the above-defined subgroups (allocating a score of 0 for no marker at high levels, 1 for 1 marker at high levels, and 2 for 2 markers at high levels), the test for trend of a decreasing effect of chemotherapy with an increasing score was significant (P_{trend} = 0.01; $P_{heterogeneity}$ = 0.05).

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Discussion

Complete resection of tumor followed by adjuvant platinum-based chemotherapy plays a central role as a curative treatment for NSCLC. However, in close relationship with pathologic stage, only 23% to 67% of patients are still alive at 5 years after the initial treatment with curative intent (32). Recurrences that account for mortality occur most commonly at distant extrathoracic sites. Beyond 5 years, the mortality rate decreases strongly, such that survivors may thereafter entertain a substantial hope to be cured. Here, we report that the expression by tumors of two crucial DNA excision repair proteins, MSH2 and ERCC1, were associated, albeit not significantly considering the significance level that was set at 0.01, with patients who benefited durably (with median follow-up of over 7 years) from cisplatin-based chemotherapy in the largest clinical trial of adjuvant chemotherapy for lung cancer. Considering the early and possible late toxicity of platinum-based chemotherapy (3, 8), the evaluation of MSH2 and ERCC1 expression may have a strong potential to be useful to tailor individual chemotherapy after complete surgical removal of NSCLC.

In the present study, we could show that the combination of MSH2 and ERCC1 was superior to the use of either one of the marker alone for the prediction of the long-term chemotherapy benefit. When low levels of MSH2 or ERCC1 were considered separately, there was a trend for chemotherapy to reduce the hazard for death by ~25% for each of the two markers. However, when MSH2 and ERCC1 were considered jointly and when the proteins were both expressed at low levels, the hazard for death was reduced by 35% in favor of chemotherapy. In this subgroup (38% of patients) defined by both low MSH2 and low ERCC1, patients who underwent chemotherapy had a median survival of 55 months, whereas those who were left to observation after surgery had a median survival of 34 months, which translates into a gain of 21 months in favor of chemotherapy ($P = 0.01$). The tests for heterogeneity for the two markers combined were not significant, but consistent with their independence ($P = 0.07$ and $P = 0.02$), suggesting that the loss of MSH2 and that of ERCC1 may act cumulatively. Because assessments of the function of the proteins are not possible in archival material, we can only assume that the low protein levels were indeed associated with impaired DNA repair activity. Although experimental evidence supports that MSH2 and ERCC1 proteins cooperate in a common process of excision and repair of cisplatin-induced DNA adducts (19, 20), the broad substrate range of the MMR and the NER, on the other hand, may suggest that one pathway may take care of lesions that are not repaired by the other (33). The combined use of the two markers may give a better evaluation of the repair of cisplatin-induced cross-links. The study of other DNA repair factors may still improve the accuracy of the prediction.

Among the 19 protein markers that were initially planned for study in the first phase of IALT-Bio, P27 had emerged as a borderline significant predictor of the short-term benefit of chemotherapy ($P = 0.02$). Here, we also report that MSH2 could be combined with P27 to predict the long-term benefit of chemotherapy, although the tests for heterogeneity for the combinations of MSH2 and P27 ($P = 0.10$ and $P = 0.05$) could not establish that they were independent. Data showing that ERCC1 and P27 could also be combined for a better prediction of the benefit of chemotherapy have been previously shown by our group (13). All together, our results are consistent with the view that ERCC1, MSH2, and P27 function in a common pathway in response to cisplatin-DNA adducts, as cell cycle arrest may be required for DNA repair to occur. The numbers for the joint analysis of MSH2 with P27 and that of MSH2 with ERCC1 were similar. With regard to the future use of predictive biomarkers alone or in combination in the clinical practice, the choice between the three most promising markers that were uncovered by IALT-Bio will be determined by the results of validation studies.

Before our report, there were only few indirect hints that MSH2 expression could predict the benefit of platinum-based chemotherapy (29, 30). The discovery of predictive markers requires their study in a large cohort of patients enrolled in a randomized trial such as the IALT. Then, the markers can be correlated with sufficient statistical power to survival data and to treatment. Here, the results were strengthened by the use of two-sided statistical tests in a Cox model that was adjusted on cancer-specific and trial-specific factors, including potential confounding variables, such as histologic type, slide quality, and center. The relative protein expression levels were assessed by directly comparing the levels in tumor cells to those in surrounding pulmonary or stromal cells. The primary antibody for MSH2 is a well-known reagent used in the clinical setting to help for the pathologic diagnosis of MMR defect in colon cancer. Our hypothesis was that low levels of MSH2 would predict a benefit from chemotherapy because our previous analysis of ERCC1 using the short-term IALT survival data have shown such relationship between low levels of the DNA repair protein and the short-term benefit from chemotherapy. However, it is known that MMR-defective tumors such as hereditary nonpolyposis colon cancer may be resistant to treatment with alkylating agents (34). The MMR defect results in microsatellite instability that inactivates many proapoptotic and DNA damage response genes (35). The frequent loss of MSH2 in lung cancer cells is well documented (21–30) but MMR defects and the resulting microsatellite instability appear rare in lung cancer (36). The consequences of MSH2 loss are organ specific (37), which may explain why MSH2 loss may have opposite consequences on sensitivity to chemotherapy in lung and colon cancers.

The specificity of the 8F1 monoclonal antibody has been debated (38). We have shown (HeLa and A549) that 8F1 could discriminate cells in which *ERCC1* was expressed and isogenic strains in which *ERCC1* expression was knocked down by small interfering RNA (39). Recently, Bhagwat et al. (40) showed that 8F1 recognized a second antigen

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in skin fibroblasts from patients with *XPF* deficiency—an antigen that migrated as a second band closely above the ERCC1 band in Western blot. Unfortunately, the authors did not bring further data that might clearly identify the nature of the additional band, and therefore, the possibility that it is an unknown *ERCC1* isoform remains open. Further, it was proposed, because HeLa cells do not have appreciable amounts of the additional band, that these cells would be inappropriate for use as either positive or negative control for validating 8F1. Therefore, in addition to A549 and HeLa, we investigated several other carcinoma cell lines and found similar results (the data are part of an ongoing investigation), meaning that the additional band with a slightly higher molecular weight in skin fibroblasts, and discussed in Bhagwat et al. (40), actually might have limited interest in the study of most lung carcinomas.

As previously mentioned, when the IALT cohort was studied with median follow-up of 5 years, chemotherapy reduced the mortality by 4% (3), and ERCC1 low levels were found significantly ($P < 0.01$) associated with a reduction of the hazard for death of 35% in favor of chemotherapy (11). Regarding the long-term benefit from chemotherapy, the numbers for MSH2 were almost exactly those seen for ERCC1 (Table 3), suggesting that the long-term predictive abilities of MSH2 and ERCC1 are similar. As the magnitude of benefit from chemotherapy decreases with time, the predictive ability of the markers seems likewise to decrease. Possible unexplained late chemotherapy-related mortality and other factors may be involved (8). The results emphasize the importance of long-term follow-up of patients enrolled in clinical trials and translational studies (41).

It is also noteworthy that in the control arm, patients whose tumors expressed high levels of the DNA excision repair proteins survived longer than those whose tumors did not. A high capacity to repair DNA seems to act as a barrier against the occurrence of a more aggressive cancer phenotype but may not allow patients to benefit from DNA damaging drugs such as cisplatin (42).

Several predictors for the benefit from adjuvant chemotherapy for lung cancer have now been published, including factors involved in DNA synthesis (43) or regulation of the cell cycle (12) as well as mutations in *KRAS* and *TP53* (44), and a lung metagene model reported by Potti and colleagues (45). A comprehensive view of all these predictors is needed together with a validation of the most interesting ones on other completed trials. The most promising markers will be incorporated in future trials to select patients unlikely to benefit from conventional platinum-based therapies and to which new agents that are active in advanced stages of lung cancer can be proposed in the adjuvant setting (41). In our opinion, expression of genes that are directly involved in the repair of cisplatin-DNA adducts such as *MSH2* and *ERCC1* as well as genes involved in cell events that are required for DNA repair to occur, including genes involved in cell cycle arrest such as *P27* and DNA damage response genes, should be tested in priority in future trials.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Wu Q, Christensen LA, Legerski RJ, Vasquez KM. Mismatch repair participates in error-free processing of DNA interstrand crosslinks in human cells. EMBO Rep 2005;6:551–7.
2. Martin LP, Hamilton TC, Schilder RJ. Platinum resistance: the role of DNA repair pathways. Clin Cancer Res 2008;14:1291–5.
3. Arriagada R, Bergman B, Dunant A, Le Chevalier T, Pignon JP, Vansteenkiste J. Cisplatin-based adjuvant chemotherapy in patients with completely resected non-small-cell lung cancer. N Engl J Med 2004;350:351–60.
4. Winton T, Livingston R, Johnson D, et al. Vinorelbine plus cisplatin vs. observation in resected non-small-cell lung cancer. N Engl J Med 2005;352:2589–97.
5. Douillard JY, Rosell R, De Lena M, et al. Adjuvant vinorelbine plus cisplatin versus observation in patients with completely resected stage IB-IIIA non-small-cell lung cancer (Adjuvant Navelbine International Trialist Association [ANITA]): a randomised controlled trial. Lancet Oncol 2006;7:719–27.
6. Strauss GM, Herndon JE II, Maddaus MA, et al. Adjuvant paclitaxel plus carboplatin compared with observation in stage IB non-small-cell lung cancer: CALGB 9633 with the Cancer and Leukemia Group B, Radiation Therapy Oncology Group, and North Central Cancer Treatment Group Study Groups. J Clin Oncol 2008;26:5043–51.
7. Pignon JP, Tribodet H, Scagliotti GV, et al. Lung adjuvant cisplatin evaluation: a pooled analysis by the LACE Collaborative Group. J Clin Oncol 2008;26:3552–9.
8. Le Chevalier T DA, Arriagada R, Bergman B, et al. IALT Collaborative Group. Long-term results of the International Adjuvant Lung Cancer Trial (IALT) evaluating adjuvant cisplatin-based chemotherapy in non-small cell lung cancer (NSCLC). J Clin Oncol 2008;26:398s.
9. Bergstralh DT, Sekelsky J. Interstrand crosslink repair: can XPF-ERCC1 be let off the hook? Trends Genet 2008;24:70–6.
10. Zhu XD, Niedernhofer L, Kuster B, Mann M, Hoeijmakers JH, de Lange T. ERCC1/XPF removes the 3′ overhang from uncapped telomeres and represses formation of telomeric DNA-containing double minute chromosomes. Mol Cell 2003;12:1489–98.
11. Olaussen KA, Dunant A, Fouret P, et al. DNA repair by ERCC1 in

Published OnlineFirst February 15, 2010; DOI: 10.1158/1078-0432.CCR-09-2204

non-small-cell lung cancer and cisplatin-based adjuvant chemotherapy. N Engl J Med 2006;355:983–91.
12. Filipits M, Pirker R, Dunant A, et al. Cell cycle regulators and outcome of adjuvant cisplatin-based chemotherapy in completely resected non-small-cell lung cancer: the International Adjuvant Lung Cancer Trial Biologic Program. J Clin Oncol 2007;25:2735–40.
13. Pirker R, Filipits M, Dunant A, et al. IALT-Bio: a challenging research to improve adjuvant chemotherapy of completely resected NSCLC. J Thorac Oncol 2007;2:S397–8.
14. Filipits M, Haddad V, Schmid K, et al. Multidrug resistance proteins do not predict benefit of adjuvant chemotherapy in patients with completely resected non-small cell lung cancer: International Adjuvant Lung Cancer Trial Biologic Program. Clin Cancer Res 2007; 13:3892–8.
15. Brambilla E, Soria J, Haddad V, et al. Prognostic and predictive value of apoptosis related factors Fas, FasL and survivin in non small cell lung carcinoma patients enrolled in the IALT Trial. J Thorac Oncol 2007;2:S444–5.
16. Fishel R, Lescoe MK, Rao MR, et al. The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer. Cell 1993;75:1027–38.
17. Duckett DR, Drummond JT, Murchie AI, et al. Human MutSα recognizes damaged DNA base pairs containing O6-methylguanine, O4-methylthymine, or the cisplatin-d(GpG) adduct. Proc Natl Acad Sci U S A 1996;93:6443–7.
18. Zdraveski ZZ, Mello JA, Farinelli CK, Essigmann JM, Marinus MG. MutS preferentially recognizes cisplatin- over oxaliplatin-modified DNA. J Biol Chem 2002;277:1255–60.
19. Lan L, Hayashi T, Rabeya RM, et al. Functional and physical interactions between ERCC1 and MSH2 complexes for resistance to *cis*-diamminedichloroplatinum(II) in mammalian cells. DNA Repair (Amst) 2004;3:135–43.
20. Zhang N, Liu X, Li L, Legerski R. Double-strand breaks induce homologous recombinational repair of interstrand cross-links via cooperation of MSH2, ERCC1-XPF, REV3, and the Fanconi anemia pathway. DNA Repair (Amst) 2007;6:1670–8.
21. Xinarianos G, Liloglou T, Prime W, et al. hMLH1 and hMSH2 expression correlates with allelic imbalance on chromosome 3p in non-small cell lung carcinomas. Cancer Res 2000;60:4216–21.
22. Brooks KR, To K, Joshi MB, et al. Measurement of chemoresistance markers in patients with stage III non-small cell lung cancer: a novel approach for patient selection. Ann Thorac Surg 2003;76:187–93; discussion 93.
23. Wang YC, Lu YP, Tseng RC, et al. Inactivation of hMLH1 and hMSH2 by promoter methylation in primary non-small cell lung tumors and matched sputum samples. J Clin Invest 2003;111:887–95.
24. Zhu LQ, Diao LM, Chen DJ, et al. [Relationship between hMSH2 and FHIT gene expression in non-small cell lung cancer]. Ai Zheng 2003; 22:571–4.
25. Hsu HS, Wen CK, Tang YA, et al. Promoter hypermethylation is the predominant mechanism in hMLH1 and hMSH2 deregulation and is a poor prognostic factor in nonsmoking lung cancer. Clin Cancer Res 2005;11:5410–6.
26. Skarda J, Fridman E, Plevova P, et al. Prognostic value of hMLH1 and hMSH2 immunohistochemical expression in non-small cell lung cancer. A tissue microarray study. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 2006;150:255–9.
27. Kanellis G, Chatzistamou I, Koutselini H, et al. Expression of DNA mismatch repair gene MSH2 in cytological material from lung cancer patients. Diagn Cytopathol 2006;34:463–6.
28. Scartozzi M, Franciosi V, Campanini N, et al. Mismatch repair system (MMR) status correlates with response and survival in non-small cell lung cancer (NSCLC) patients. Lung Cancer 2006;53:103–9.
29. Hsu HS, Lee IH, Hsu WH, Kao WT, Wang YC. Polymorphism in the hMSH2 gene (gISV12-6T > C) is a prognostic factor in non-small cell lung cancer. Lung Cancer 2007;58:123–30.
30. Cooper WA, Kohonen-Corish MR, Chan C, et al. Prognostic significance of DNA repair proteins MLH1, MSH2 and MGMT expression in non-small-cell lung cancer and precursor lesions. Histopathology 2008;52:613–22.
31. Brambilla E, Lantuejoul S, Dunant A. IALT (International Adjuvant Lung Cancer Trial):quality assessment and histopathological review according to the WHO 2004 classification and assessment of prognostic and predictive role of pathological criteria. Lung Cancer 2005;49;suppl2:S44.
32. Mountain CF. Revisions in the International System for Staging Lung Cancer. Chest 1997;111:1710–7.
33. Yang W. Structure and mechanism for DNA lesion recognition. Cell Res 2008;18:184–97.
34. Claij N, te Riele H. Microsatellite instability in human cancer: a prognostic marker for chemotherapy? Exp Cell Res 1999;246:1–10.
35. Duval A, Hamelin R. Mutations at coding repeat sequences in mismatch repair-deficient human cancers: toward a new concept of target genes for instability. Cancer Res 2002;62:2447–54.
36. Forgacs E, Wren JD, Kamibayashi C, et al. Searching for microsatellite mutations in coding regions in lung, breast, ovarian and colorectal cancers. Oncogene 2001;20:1005–9.
37. Weinberg RA. The biology of cancer. Garland Science Eds 2007: 463–556.
38. Niedernhofer LJ, Bhagwat N, Wood RD. ERCC1 and non-small-cell lung cancer. N Engl J Med 2007;356:2538–40, author reply 40–1.
39. Olaussen KA, Fouret P, Kroemer G. ERCC1-specific immunostaining in non-small-cell lung cancer. N Engl J Med 2007;357:1559–61.
40. Bhagwat NR, Roginskaya VY, Acquafondata MB, Dhir R, Wood RD, Niedernhofer LJ. Immunodetection of DNA repair endonuclease ERCC1-XPF in human tissue. Cancer Res 2009;69:6831–8.
41. Chhatwani L, Cabebe E, Wakelee HA. Adjuvant treatment of resected lung cancer. Proc Am Thorac Soc 2009;6:194–200.
42. Gazdar AF. DNA repair and survival in lung cancer-the two faces of Janus. N Engl J Med 2007;356:771–3.
43. Zheng Z, Chen T, Li X, Haura E, Sharma A, Bepler G. DNA synthesis and repair genes RRM1 and ERCC1 in lung cancer. N Engl J Med 2007;356:800–8.
44. Tsao MS, Aviel-Ronen S, Ding K, et al. Prognostic and predictive importance of p53 and RAS for adjuvant chemotherapy in non small-cell lung cancer. J Clin Oncol 2007;25:5240–7.
45. 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;355:570–80.

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