DNA Repair Capacity in Peripheral Lymphocytes Predicts Survival of Patients With Non–Small-Cell Lung Cancer Treated With First-Line Platinum-Based Chemotherapy

Li-E Wang, Ming Yin, Qiong Dong, David J. Stewart, Kelly W. Merriman, Christopher I. Amos, Margaret R. Spitz, and Qingyi Wei

All authors: The University of Texas MD Anderson Cancer Center, Houston, TX.

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Corresponding author: Qingyi Wei, MD, PhD, Department of Epidemiology, Unit 1365, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030; e-mail: qwei@mdanderson.org.

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ABSTRACT

Purpose

Platinum-based regimens are the standard chemotherapy for patients with advanced non–small-cell lung cancer (NSCLC). DNA repair capacity (DRC) in tumor cells plays an important role in resistance to platinum-based drugs. We have previously reported that efficient DRC, as assessed by an in vitro lymphocyte-based assay, was a determinant of poor survival in patients with NSCLC in a relatively small data set. In this larger independent study of 591 patients with NSCLC, we further evaluated whether DRC in peripheral lymphocytes predicts survival of patients with NSCLC who receive platinum-based chemotherapy.

Patients and Methods

All patients were recruited at The University of Texas MD Anderson Cancer Center and donated blood samples before the start of any chemotherapy. We measured DRC in cultured T lymphocytes by using the host-cell reactivation assay, and we assessed associations between DRC in peripheral lymphocytes and survival of patients with NSCLC who were treated with first-line platinum-based chemotherapy.

Results

We found an inverse association between DRC in peripheral lymphocytes and patient survival. Compared with patients in the low tertile of DRC, patients with NSCLC in the high tertile of DRC had significantly worse overall and 3-year survival (adjusted hazard ratio [HR], 1.33; 95% CI, 1.04 to 1.71; $P = .023$; and HR, 1.35; 95% CI, 1.04 to 1.76; $P = .025$, respectively). This trend was more pronounced in patients with early-stage tumors, adenocarcinoma, or squamous cell carcinoma.

Conclusion

We confirmed that DRC in peripheral lymphocytes is an independent predictor of survival for patients with NSCLC treated with platinum-based chemotherapy.

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INTRODUCTION

Lung cancer remains the leading cause of cancer death, with an estimated 156,940 deaths in the United States in 2011. Non–small-cell lung cancer (NSCLC) accounts for approximately 90% of all lung cancer cases and is treated with platinum-based double-agent chemotherapy, particularly for advanced cases. Platinum causes platinum-DNA adducts that block transcription, leading to cytotoxicity and cell death, whereas increased removal of platinum from cancer cells and repair of platinum-DNA adducts by tolerance mechanisms induce drug resistance.¹ Therefore, efficient DNA repair can modulate platinum cytotoxicity and its anticancer efficacy.²⁻⁵

Among DNA repair pathways, nucleotide excision repair (NER) is an important mechanism underlying cisplatin resistance by removing platinum-DNA interstrand adducts.⁵⁻⁷ Excision repair cross-complementation group 1 (*ERCC1*) plays a key role in repairing the damaged DNA.^{2,8} The *ERCC1* expression levels have been widely investigated in lung cancer cell lines, tumors, and clinical trials, and high expression levels of *ERCC1* have been associated with reduced cisplatin efficacy.⁷⁻¹¹ A recent meta-analysis¹² indicated that low *ERCC1* expression levels were associated with higher objective response and longer median survival time in patients with advanced NSCLC. Genetic variations in *NER* genes, including *ERCC1*, have also been examined in several studies. However, our recent

meta-analysis¹³ on polymorphisms of *ERCC1* C118T/C8092A and *ERCC2* Lys751Gln/Asp312Asn did not support their use as prognostic predictors of platinum-based chemotherapy in NSCLC. Therefore, a phenotypic DNA repair marker that may represent the sum of all genetic variants is desirable.

Although malignancies may possess numerous mutations compared with host normal tissues, cancers do initially inherit their genome from the host, which may influence tumor sensitivity to therapy.² We have previously reported that efficient cellular DNA repair capacity (DRC, measured in vitro in lymphocytes by using the host-cell reactivation [HCR] assay) was a determinant of poor survival in patients with NSCLC in a relatively small data set with a 9% increase of risk of death for every unit increase in DRC in peripheral lymphocytes.¹⁴ In this larger independent study of 591 patients with NSCLC treated with first-line platinum-based chemotherapy, we further evaluated whether DRC in peripheral lymphocytes predicts survival of patients with NSCLC who are receiving platinum-based chemotherapy.

PATIENTS AND METHODS

Study Population

Patients were accrued from an ongoing, hospital-based, case-control study of epidemiologic and genetic risk factors for lung cancer without regard to age, sex, or tumor stage at The University of Texas MD Anderson Cancer Center between January 2000 and September 2008. Each consented patient had a personal interview that used a standardized questionnaire to elicit life-style factors, and each patient donated a one-time 30-mL sample of whole blood drawn into a heparinized tube that was cryopreserved to maintain lymphocyte viability. The protocol was approved by the MD Anderson Cancer Center institutional review board.

For this analysis, we included all patients who were newly diagnosed with histologically confirmed NSCLC and who were identified from the institutional pharmacy database as receiving first-line carboplatin, cisplatin, or oxaliplatin for at least one complete course of platinum-based chemotherapy. Resectional surgery was defined as a wedge resection, lobectomy, or pneumonectomy. Definitive radiotherapy was delivered in doses of 45 to 69 Gy to the chest. Follow-up was through April 14, 2009, for 591 patients with stage I to IV NSCLC whose DRC data were available.

HCR Assay for DRC in Peripheral Lymphocytes

DRC was measured in cultured peripheral lymphocytes by using the HCR assay. Details of the assay have been reported previously.¹⁵ Briefly, the assay used a BPDE (benzo[a]pyrene diol epoxide)-damaged nonreplicating recombinant plasmid (pCMVcat) harboring a chloramphenicol acetyltransferase (CAT) reporter gene that was transfected into cultured T lymphocytes stimulated by phytohemagglutinin.¹⁶ Because even a single unreplicated DNA adduct can block CAT transcription, any measurable CAT activity in the transfected cells will be proportional to the ability of the cells to remove BPDE-induced DNA adducts from the plasmids. We used the diethylaminoethyl-dextran method¹⁷ to transfect untreated plasmids and BPDE-treated plasmids for each sample in parallel and in duplicate. The cultures were then incubated for 40 hours after transfection, allowing for the CAT expression. Because transfection efficiencies for UV-damaged and undamaged pCMVcat plasmids were around 70% in DNA repair-proficient and -deficient lymphoblastoid cell lines, as we reported before,¹⁸ we assumed that the transfection efficiencies for BPDE-treated and untreated plasmids were equal or comparable.

The CAT activity was measured by adding chloramphenicol and [³H] acetyl coenzyme A and measuring the production of [³H] monoacetylated and [³H] diacetylated chloramphenicols with a scintillation counter. DRC was calculated as a ratio of the radioactivity (cpm) of cells transfected with BPDE-treated plasmids to the radioactivity of cells transfected with untreated plasmids, as a measure of treated versus baseline CAT expression levels.

Because certain laboratory characteristics can potentially affect DRC values, we also adjusted DRC in peripheral lymphocytes for sample storage time (the difference between the date of the DRC assay and date of blood collection), baseline CAT expression levels, and blastogenic rates (percentage of lymphocytes that responded to phytohemagglutinin stimulation) in the statistical models.^{15,19-22}

Statistical Analysis

We performed the univariate analysis on the distributions of continuous variables, including crude and natural logarithmic (log)-transformed DRC. Students's *t* test or analysis of variance (ANOVA) was used to compare the differences in DRC among groups defined by each variable. Since DRC values did not follow normal distribution both before and after log transformation (Kolmogorov-Smirnov test $P < .01$), we used the Cox proportional hazards regression model with both continuous (before and after log transformation) and discrete (in tertiles) DRC values to evaluate the effect of DRC on overall and 3-year survival calculated as hazard ratios (HRs) with their corresponding 95% CIs. We also calculated odds ratios and 95% CIs for risk of death by logistic regression analysis. The survival time was calculated from the date of diagnosis until the date of death or last follow-up. All HRs or odds ratios were adjusted for age, sex, ethnicity, smoking status, tumor histology, tumor stage, and treatment (radiation or surgical therapy), in addition to the blastogenic rate, the baseline CAT expression level, and cell storage time. The Kaplan-Meier curve was used to visualize the DRC effect on the cumulative probability of overall and 3-year survival. The log-rank test was used to compare differences among survival times of different groups. All reported *P* values were two-sided with $P < .05$ as the significance level. All analyses were performed by using SAS version 9.2 (SAS Institute, Cary, NC).

RESULTS

Patient Characteristics

This analysis included 591 patients with NSCLC who were treated with first-line platinum-based chemotherapy (Table 1). The patients were between age 28 and 85 years at diagnosis with a mean age of 59.3 years and standard deviation of 10.9 years. There were slightly more men than women (54.8% v 45.2%), and 76.5% were non-Hispanic whites, 18.9% were African American, and 4.6% were of Hispanic origin. Ever-smokers (smoked more than 100 cigarettes in their lifetime) accounted for 80% of the study population. In addition to chemotherapy, 28.6% of patients underwent surgery and 65.5% received radiotherapy. At the last follow-up (April 2009), 438 (74.1%) of the 591 patients had died, with a median survival time of 19.2 months. Of all patients, 36.6% presented with stages I to IIIA and 63.4% with stages IIIB to IV. The distribution of tumor histology type was 55.7% adenocarcinoma, 22.8% squamous cell carcinoma, and 21.5% NSCLC not otherwise specified. When all of these variables were included in a Cox hazards regression model with adjustment to calculate HRs, being male (HR, 1.30; 95% CI, 1.07 to 1.58; $P = .009$), undergoing surgery (HR, 0.47; 95% CI, 0.35 to 0.63; $P < .001$), and having late-stage disease (HR, 1.80; 95% CI, 1.40 to 2.32; $P < .001$) all remained statistically significant prognostic indicators (log-rank $P < .05$; Table 1). Although the log-rank *P* value was .007 for mean survival time among histology types, adjusted HRs were not statistically significant for squamous cell and NSCLC not otherwise specified subgroups, compared with adenocarcinoma. Table 1 also presents the DRC data within each stratum. Older patients ($P = .002$), women ($P = .009$), and whites ($P = .03$) exhibited significantly lower DRC in peripheral lymphocytes than others in their respective strata. The differences among histologic subgroups were marginally significant with patients who had squamous cell carcinoma in the highest mean

Table 1. Association of Host and Clinical Characteristics With Patient Survival and DNA DRC in Peripheral Lymphocytes in Patients With NSCLC Treated With First-Line Platinum-Based Chemotherapy

Variable	Patients		Deaths		MST (months)	95% CI	Log-Rank <i>P</i> for Death	Cox Regression Crude HR	95% CI	<i>P</i> *	Cox Regression Adjusted HR†	95% CI	<i>P</i> *	DRC Mean ± SD	<i>P</i> ‡ for DRC
	No.	%	No.	%											
All patients	591	100	438	74.1	19.2	16.8 to 21.6									
Age, years							.098								
≤ 60	301	50.9	231	52.7	16.8	14.4 to 20.4		1 (ref)			1 (ref)			9.19 ± 2.81	
> 60	290	49.1	207	47.3	20.4	16.8 to 26.4		0.86	0.71 to 1.03	.105	0.94	0.78 to 1.14	.940	8.48 ± 2.57	.002
Sex							.003								
Female	267	45.2	182	41.6	22.8	19.2 to 27.6		1 (ref)			1 (ref)			8.52 ± 2.79	
Male	324	54.8	256	58.4	16.8	14.4 to 18.0		1.34	1.10 to 1.62	.003	1.30	1.07 to 1.58	.009	9.11 ± 2.63	.009
Race/ethnicity							.142								
White	452	76.5	333	76.0	19.2	16.8 to 22.8		1 (ref)			1 (ref)			8.69 ± 2.60	
Other	139	23.5	105	24.0	16.8	12.0 to 21.6		1.18	0.94 to 1.46	.151	0.99	0.79 to 1.24	.914	9.31 ± 3.03	.030
Smoking status							.820								
No	118	20.0	88	20.1	21.6	15.6 to 28.8		1 (ref)			1 (ref)			8.45 ± 2.68	
Yes	473	80.0	350	79.9	18.0	15.6 to 21.6		1.03	0.81 to 1.30	.825	1.15	0.89 to 1.47	.285	8.94 ± 2.72	.086
Surgery							< .001								
No	422	71.4	355	81.1	14.4	13.2 to 15.6		1 (ref)			1 (ref)			8.81 ± 2.76	
Yes	169	28.6	83	19.9	54.0	39.6 to 69.6		0.33	0.26 to 0.42	< .001	0.47	0.35 to 0.63	< .001	8.92 ± 2.62	.668
Radiation							< .001								
No	204	34.5	129	29.5	30.0	22.8 to 39.6		1 (ref)			1 (ref)			8.58 ± 2.71	
Yes	387	65.5	309	70.5	16.8	15.6 to 18.0		1.52	1.24 to 1.87	< .001	1.11	0.88 to 1.13	.358	8.98 ± 2.72	.092
Tumor stage							< .001								
I to IIIA	216	36.6	123	28.1	39.6	30.0 to 56.4		1 (ref)			1 (ref)			8.82 ± 2.63	
IIIB to IV	375	63.4	315	71.9	13.2	12.0 to 15.6		2.57	2.08 to 3.17	< .001	1.80	1.40 to 2.32	< .001	8.85 ± 2.77	.875
Histology type							.007								
Adenocarcinoma	329	55.7	239	54.6	21.6	16.8 to 26.4		1 (ref)			1 (ref)			8.68 ± 2.78	
Squamous cell	135	22.8	95	21.7	19.2	16.8 to 22.8		0.96	0.75 to 1.21	.717	0.96	0.75 to 1.23	.750	9.33 ± 2.52	
NSCLC NOS	127	21.5	104	23.7	14.4	12.0 to 18.0		1.39	1.11 to 1.75	.005	1.21	0.96 to 1.54	.108	8.72 ± 2.71	.055

Abbreviations: DRC, DNA repair capacity; HR, hazard ratio; MST, median survival time; NOS, not otherwise specified; NSCLC, non-small-cell lung cancer; SD, standard deviation.

**P* values from Cox regression model.

†Adjusted for age, sex, ethnicity, smoking status, surgery, radiation, clinical stage, and histology type accordingly.

‡Two-sided Student's *t* test or analysis of variance (ANOVA) test for DRC differences among groups in each category.

DRC ($P = .055$). Never-smokers also exhibited poorer DRC in peripheral lymphocytes than ever-smokers, and patients who did not have radiotherapy had lower DRC in peripheral lymphocytes than those who had radiotherapy, but the difference did not reach statistical significance ($P = .086$ and $P = .092$, respectively; Table 1).

Association Between DRC in Peripheral Lymphocytes and Survival

We constructed a Cox hazards regression model to evaluate the effect of DRC in peripheral lymphocytes on both overall and 3-year survival. When DRC was included in the model as a continuous variable, the HR increased by 4% for both overall survival ($P = .055$) and 3-year survival ($P = .045$) with every 1% increase in DRC in peripheral lymphocytes (Table 2). The log-transformed DRC was qualitatively similar, with 38% increased HR for overall survival ($P = .040$) and 43% increased HR for 3-year survival ($P = .031$) per unit increase of log-transformed DRC after adjustment (Table 2). We then trichotomized DRC in peripheral lymphocytes into three groups by using the DRC tertiles of the entire study population as cutoff values (< 7.4 , 7.4 - 9.9 , and > 9.9) to facilitate further analysis in the Kaplan-Meier curve and Cox hazards regression model. Across all three DRC tertiles, we found an inverse trend ap-

proaching statistical significance for an association between DRC in peripheral lymphocytes and survival. Median survival times were 24, 18, and 16.8 months, respectively, as the DRC tertile increased (log-rank $P = .08$; Table 2). This pattern was more evident for patients with early-stage tumors (log-rank $P = .067$) and for those with adenocarcinomas (log-rank $P = .039$). In patients with stages IIIB to IV disease, the effect of DRC level was also approaching statistical significance (log-rank $P = .061$, which bases survival comparisons on differences across the entire length of the Kaplan-Meier curve). Despite the minimal differences in median survival times, the survival curves split over much of their lengths (Figs 1A to 1D).

In the Cox regression analysis, patients with the most efficient DRC (high tertile) in peripheral lymphocytes exhibited a significantly increased hazard of death in both univariate and multivariate models compared with those with the least efficient DRC (low tertile HR, 1.33; 95% CI, 1.04 to 1.71; $P = .023$) after adjustment for age, sex, ethnicity, smoking status, blastogenic rates, baseline *CAT* expression levels, cell storage time, surgery, radiation, clinical stage, and histology type. As with comparisons across all three DRC tertiles in the stratification analysis, this trend was more pronounced in patients with stages I to IIIA NSCLC (adjusted HR, 1.71; 95% CI, 1.07 to 2.74; $P = .025$ for the high tertile; adjusted HR, 1.83; 95% CI, 1.13 to 2.97; $P = .014$ for the

Table 2. DRC Effects on HR from Cox Hazards Proportional Models in the Stratification Analysis for Patients With NSCLC Treated with First-Line Platinum-Based Chemotherapy

DRC in Peripheral Lymphocytes (%)	Overall Survival												3-Year Survival						
	Patients		Deaths		MST (months)	95% CI	Log-Rank <i>P</i> for Death	Crude HR	95% CI	Adjusted HR*	95% CI	<i>P</i> *	Alive		Dead		Adjusted HR*	95% CI	<i>P</i> *
	No.	%	No.	%									No.	%	No.	%			
All platinum-treated patients																			
Continuous, by 1% increment	591	100	438	74.1	19.2	16.8 to 21.6		1.03	1.00 to 1.07	1.04	1.00 to 1.07	.055	205	34.7	386	65.3	1.04	1.00 to 1.08	.045
Log transformed DRC tertile	591	100	438	74.1	19.2	16.8 to 21.6		1.32	0.98 to 1.78	1.38	1.02 to 1.89	.040	205	34.7	386	65.3	1.43	1.03 to 1.98	.031
< 7.4	186	31.5	126	28.8	24.0	16.8 to 31.2		1 (ref)		1 (ref)			80	43.0	106	27.4	1 (ref)		
7.4-9.9	201	34.0	153	34.9	18.0	15.6 to 20.4		1.19	0.94 to 1.50	1.13	0.89 to 1.45	.317	66	32.8	135	35.0	1.21	0.93 to 1.57	.156
9.9+	204	34.5	159	36.3	16.8	15.6 to 21.6	.080	1.30	1.03 to 1.64	1.33	1.04 to 1.71	.023	59	28.8	145	37.6	1.35	1.04 to 1.76	.025
Tumor stage I to IIIA																			
< 7.4	72	33.3	32	26.0	58.8	37.2 to N/A		1 (ref)		1 (ref)			48	40.7	24	24.5	1 (ref)		
7.4-9.9	65	30.1	41	33.3	30.0	21.6 to 56.4	.067	1.65	1.04 to 2.62	1.83	1.13 to 2.97	.014	30	25.4	35	35.7	2.07	1.20 to 3.56	.009
9.9+	79	36.6	50	40.7	34.8	26.4 to 48.0		1.54	0.99 to 2.40	1.71	1.07 to 2.74	.025	40	33.9	39	39.8	1.72	1.00 to 2.95	.049
IIIB to IV																			
< 7.4	114	30.4	94	29.8	13.2	10.8 to 19.2	.061	1 (ref)		1 (ref)			32	36.8	82	28.5	1 (ref)		
7.4-9.9	136	36.3	112	35.6	14.4	12.0 to 16.8		0.94	0.71 to 1.24	1.03	0.78 to 1.37	.838	36	41.4	100	34.7	1.09	0.81 to 1.48	.573
9.9+	125	33.3	109	34.6	13.2	9.6 to 15.6		1.27	0.96 to 1.67	1.23	0.91 to 1.65	.176	19	21.8	106	36.8	1.26	0.93 to 1.72	.135
Additional treatment																			
Surgery																			
< 7.4	53	31.4	20	24.1	67.2	49.2 to N/A	.130	1 (ref)		1 (ref)			41	37.6	12	20.0	1 (ref)		
7.4-9.9	59	34.9	31	37.4	54.0	26.4 to 78.0		1.64	0.93 to 2.87	1.58	0.87 to 2.88	.133	34	31.2	25	41.7	2.26	1.08 to 4.74	.032
9.9+	57	33.7	32	38.5	39.6	31.2 to 69.6		1.69	0.97 to 2.96	1.59	0.86 to 2.95	.141	34	31.2	23	38.3	1.99	0.91 to 4.36	.086
No surgery																			
< 7.4	133	31.5	106	29.8	14.4	12.0 to 19.2	.305	1 (ref)		1 (ref)			39	40.6	94	28.8			
7.4-9.9	142	33.7	122	34.4	14.4	12.0 to 16.8		1.10	0.84 to 1.42	1.10	0.84 to 1.44	.494	32	33.3	110	33.8	1.13	0.85 to 1.50	.405
9.9+	147	34.8	127	35.8	14.4	10.8 to 15.6		1.22	0.94 to 1.58	1.23	0.93 to 1.61	.147	25	26.1	122	37.4	1.25	0.94 to 1.67	.121
Radiation																			
< 7.4	110	28.4	80	25.9	16.8	13.2 to 25.2	.244	1 (ref)		1 (ref)			37	35.2	73	25.9	1 (ref)		
7.4-9.9	139	35.9	113	36.6	15.6	13.2 to 19.2		1.18	0.89 to 1.57	1.13	0.84 to 1.53	.408	37	35.2	102	36.2	1.13	0.83 to 1.55	.438
9.9+	138	35.7	116	37.5	16.8	14.4 to 20.4		1.27	0.95 to 1.69	1.34	0.99 to 1.82	.060	31	29.6	107	37.9	1.30	0.95 to 1.79	.106
No radiation																			
< 7.4	76	37.3	46	35.7	40.8	25.2 to 49.2	.684	1 (ref)		1 (ref)			43	43.0	33	31.7	1 (ref)		
7.4-9.9	62	30.4	40	31.0	22.8	16.8 to 54.0		1.06	0.69 to 1.62	1.39	0.88 to 2.19	.156	29	29.0	33	31.7	1.86	1.12 to 3.10	.017
9.9+	66	32.3	43	33.3	26.4	13.2 to 37.2		1.20	0.79 to 1.82	1.25	0.79 to 1.96	.340	28	28.0	38	36.6	1.43	0.87 to 2.36	.160
Histology type																			
Adenocarcinoma																			
< 7.4	119	36.2	76	31.8	27.6	16.8 to 40.8	.039	1 (ref)		1 (ref)			57	45.6	62	30.4	1 (ref)		
7.4-9.9	107	32.5	80	33.5	19.2	15.6 to 26.4		1.23	0.90 to 1.69	1.25	0.90 to 1.73	.177	39	31.2	68	33.3	1.36	0.95 to 1.94	.094
9.9+	103	36.3	83	34.7	16.8	14.4 to 25.2		1.49	1.09 to 2.03	1.47	1.05 to 2.05	.024	29	23.2	74	36.3	1.49	1.04 to 2.13	.031
Squamous cell																			
< 7.4	29	21.5	17	17.9	33.6	13.2 to N/A	.295	1 (ref)		1 (ref)			14	29.8	15	17.0	1 (ref)		
7.4-9.9	47	34.8	35	36.8	16.8	12.0 to 20.4		1.54	0.86 to 2.75	1.51	0.81 to 2.82	.197	15	31.9	32	36.4	1.70	0.88 to 3.27	.112
9.9+	59	43.7	43	45.3	19.2	15.6 to 26.4		1.47	0.84 to 2.58	1.88	1.05 to 3.37	.035	18	38.3	41	46.6	1.99	1.08 to 3.68	.028
NSCLC NOS																			
< 7.4	38	29.9	33	31.7	13.8	7.2 to 19.2	.823	1 (ref)		1 (ref)			9	27.2	29	30.9	1 (ref)		
7.4-9.9	47	37.0	38	36.6	15.6	10.8 to 20.4		0.87	0.54 to 1.38	0.75	0.45 to 1.25	.270	12	36.4	35	37.2	0.77	0.45 to 1.31	.330
9.9+	42	33.1	33	31.7	13.2	10.8 to 25.2		0.92	0.56 to 1.49	0.89	0.52 to 1.50	.649	12	36.4	30	31.9	0.90	0.52 to 1.57	.716

Abbreviations: DRC, DNA repair capacity; HR, hazard ratio; MST, median survival time; N/A, not applicable; NSCLC, non-small-cell lung cancer; ref, reference.

*Adjusted for age, sex, ethnicity, smoking status, blastogenic rates, baseline chloramphenicol acetyltransferase (CAT) expression levels, cell storage time, surgery, radiation, clinical stage, and histology type.

middle tertile) and those with adenocarcinoma (adjusted HR, 1.47; 95% CI, 1.05 to 2.05; $P = .024$) and squamous cell carcinoma (adjusted HR, 1.88; 95% CI, 1.05 to 3.37; $P = .035$; Table 2). We also analyzed the impact of DRC on 3-year survival (Table 2). Results were similar to those seen for overall survival.

To further assess interactions of DRC in peripheral lymphocytes with other factors, we derived interaction terms by multiplying the

DRC in tertiles and each variable listed in Table 1, and performed univariate and multivariate Cox hazards regression analyses. In multivariate analyses, we adjusted for all of the covariates in the same model, including the two main factors that formed the interaction term. The interaction term was significant in the univariate analysis for overall survival or 3-year survival with sex, smoking status, surgery, radiation, histology, or stage. However, only the interaction of DRC in

Fig 1. Overall survival curves by the tertile of DNA repair capacity (DRC) phenotype in selected patient groups. *P* values were obtained from the log-rank test. (A) Overall patients; (B) patients with stages I to IIIA; (C) patients with stages IIIB to IV; and (D) patients with adenocarcinoma.

peripheral lymphocytes with smoking status remained significant in the multivariate analysis (*P* for trend = .029 and *P* for trend = .008 for overall and 3-year survival, respectively).

Association Between DRC in Peripheral Lymphocytes and Short-Term Survival Versus Long-Term Survival

We further evaluated the influence of DRC phenotype in peripheral lymphocytes on the likelihood of survival of ≤ 3 years (referred to as short-term survival [STS]) versus survival longer than 3 years (long-term survival) by using a logistic regression model. We excluded patients who were lost to follow-up within 3 years (*n* = 69) because of uncertainty regarding their survival status. Therefore, the final sample size for this analysis was 522, and the median survival times for STS and long-term survival were 12 and 82.8 months, respectively. There was a statistically significant trend to an increased risk of STS with an increasing DRC tertile for the overall population, as well as for stages IIIB to IV and adenocarcinoma subgroups (Table 3). Patients with the most efficient DRC phenotype ($> 9.9\%$) had a more than two-fold increased risk of STS compared with those with the least efficient DRC phenotype ($< 7.4\%$). For patients with early-stage tumors or those who had surgery, the middle tertile of DRC groups (7.4% to 9.9%) was also associated with a three-fold increased risk of STS compared with those with the least efficient DRC in peripheral lymphocytes ($< 7.4\%$; Table 3).

DISCUSSION

In this larger, independent prognostic analysis, we confirmed that DRC in peripheral lymphocytes was associated with poor survival in

patients with NSCLC who had received first-line platinum-based chemotherapy. We adjusted for some known confounders, such as sex, tumor stage, histology, surgery, or radiotherapy in both overall and stratified analyses, because they had a significant impact on either DNA repair phenotype or patient survival. The DRC remained to be an independent prognostic biomarker for patients with NSCLC in this heterogeneous study population.

Our results further suggest that DNA repair is important in the response of patients with NSCLC to the platinum-based chemotherapy,^{6,23} a finding consistent with that in animal models. In a murine lung cancer model, treatment with cisplatin was initially effective, but prolonged treatment promoted the emergence of resistant tumors with an enhanced repair capacity.²⁴ Cisplatin-resistant lung cancer cell lines exhibited higher DRC compared with cisplatin-sensitive cell lines,²⁵ whereas downregulation of NER components, such as the *XPF-ERCC1* complex, significantly decreased cisplatin-treated cancer cell viability and was correlated with decreased DNA repair phenotypes measured by the enzyme-linked immunosorbent assay, the alkaline comet assay, and the gamma-*H2AX* focus formation assay.²⁶ These results suggest that an efficient DRC plays an important role in both naive and induced platinum resistance and, therefore, assessing the DNA repair phenotype before platinum treatment could be of value in clinical management of chemotherapeutic agents or modalities.

Both here and in our previous studies in patients with cancers of the lung, skin, or head and neck and in cancer-free controls,^{15,19,21,22} we found that DRC in peripheral lymphocytes was lower in females than in males. It is tempting to speculate that the sex influence on prognosis may be mediated in part by DRC levels. The role of contraceptives and estrogen therapies in women may also be implicated.²⁷

Table 3. Risk of STS Versus LTS by Logistic Regression Analysis

DRC in Peripheral Lymphocytes (%)	STS (≤ 3 years)		LTS (> 3 years)		<i>P</i> *	Logistic Regression OR†	95% CI	<i>P</i> †	<i>P</i> † Trend
	No.	%	No.	%					
All platinum-treated patients	387	100	135	100					
DRC tertile					.013				.012
< 7.4	106	27.4	54	40.0		1 (ref)			
7.4-9.9	136	35.1	45	33.3		1.46	0.85 to 2.51	.166	
9.9+	145	37.5	36	26.7		2.11	1.18 to 3.78	.012	
Tumor stage									
I to IIIA					.044				.108
< 7.4	24	24.5	34	41.5		1 (ref)			
7.4-9.9	35	35.7	20	24.4		3.16	1.27 to 7.84	.013	
9.9+	39	39.8	28	34.1		2.18	0.90 to 5.30	.084	
IIIB to IV					.009				.047
< 7.4	82	28.4	20	37.7		1 (ref)			
7.4-9.9	101	34.9	25	47.2		1.05	0.51 to 2.16	.890	
9.9+	106	36.7	8	15.1		2.69	1.06 to 6.84	.038	
Additional treatment									
Surgery					.041				.104
< 7.4	12	19.7	29	39.8		1 (ref)			
7.4-9.9	26	42.6	22	30.1		2.97	1.03 to 8.58	.045	
9.9+	23	37.7	22	30.1		2.64	0.87 to 8.05	.087	
No surgery					.058				.070
< 7.4	94	28.83	25	40.32		1 (ref)			
7.4-9.9	110	33.74	23	37.10		1.16	0.59 to 2.25	.673	
9.9+	122	37.42	14	22.58		2.05	0.95 to 4.38	.066	
Radiation					.236				.225
< 7.4	73	25.9	25	35.2		1 (ref)			
7.4-9.9	102	36.2	25	35.2		1.19	0.60 to 2.37	.619	
9.9+	107	37.9	21	29.8		1.57	0.76 to 3.26	.228	
No radiation					.125				.046
< 7.4	33	31.43	29	45.31		1 (ref)			
7.4-9.9	34	32.38	20	31.25		2.44	0.88 to 6.79	.088	
9.9+	38	36.19	15	23.44		2.95	0.95 to 9.16	.062	
Histology type									
Adenocarcinoma					.031				.040
< 7.4	62	30.4	37	45.7		1 (ref)			
7.4-9.9	68	33.3	25	30.9		1.68	0.85 to 3.32	.136	
9.9+	74	36.3	19	23.4		2.15	1.01 to 4.56	.047	
Squamous cell					.218				.054
< 7.4	15	16.9	10	30.3		1 (ref)			
7.4-9.9	33	37.1	12	36.4		1.72	0.34 to 8.71	.514	
9.9+	41	46.0	11	33.3		4.62	0.84 to 25.34	.078	
NSCLC NOS					.952				.957
< 7.4	29	30.9	7	33.3		1 (ref)			
7.4-9.9	35	37.2	8	38.1		0.78	0.19 to 3.19	.732	
9.9+	30	31.9	6	28.6		1.06	0.22 to 5.12	.940	

NOTE. Patients who lost contact within 3 years were excluded from current analysis.

Abbreviations: DRC, DNA repair capacity; LTS, long-term survival; NOS, not otherwise specified; NSCLC, non-small-cell lung cancer; OR, odds ratio; STS, short-term survival.

*Two-sided χ^2 test.

†Adjusted for age, sex, ethnicity, smoking status, blastogenic rates, baseline chloramphenicol acetyltransferase (CAT) expression levels, cell storage time, surgery, radiation, clinical stage, and histology type.

We had previously found that smoking can upregulate the DRC level, especially in patients with cancer.^{15,21}

The impact of DRC in peripheral lymphocytes appeared to be somewhat less in advanced-stage patients than in early-stage patients, possibly because of a dilution by an increasing number of additional tumor-specific resistance factors² that may emerge as tumors grow and metastasize. Logistic regression models suggested that late-stage patients in the high-tertile DRC had a nearly three-fold increased risk

for poor 3-year survival compared with those in the low-tertile DRC who survived more than 3 years.

In interpreting our results, several issues need to be considered and clarified. First, the DRC was measured in peripheral blood lymphocytes that were used as a surrogate for lung tissue; however, it has been reported that levels of carcinogen-induced DNA adducts in lymphocytes and in lung tissue are highly correlated.^{28,29} Second, NER is inducible by platinum and oxidants,³⁰ which may be responsible for

the induced platinum resistance in patients with cancer. However, our study used blood samples drawn before chemotherapy, which allowed us to measure the genetically determined interindividual difference in systemic NER ability. Third, the HCR assay uses BPDE-damaged plasmids as a surrogate for cisplatin-damaged human DNA. The DNA adducts produced by BPDE and cisplatin are slightly different: BPDE induces adducts at the N2 position of guanine,^{31,32} whereas cisplatin induces N7 adducts between guanines.³³ Although they both result in DNA bulky distortion and elicit the same repair mechanism (ie, NER),³⁴⁻³⁷ which recognizes and repairs platinum-DNA adducts consisting of approximately 90% platinum-induced DNA damage, platinum treatment also induces interstrand cross links (approximately 6% in linear DNA) that are repaired by the Fanconi pathways (during S phase).^{38,39} Furthermore, other cellular repair mechanisms, such as recombination or mismatch repair, can affect antitumor efficiency of cisplatin.^{2,3,38} Therefore, our HCR assay has some limitations in completely evaluating all relevant pathways involved in repair of platinum-induced DNA damage.

This HCR assay could be developed for general clinical practice, because this assay can be performed with a one-time blood sample of 30 mL or less, and the result can be delivered in a week, but the cost could be as high as \$300 per sample. Although the assay performs reliably in our laboratory, its further application in accredited clinical laboratories needs to be developed. Our previous case-control studies^{15,19,21,22} showed that both cell growth after stimulation and baseline reporter (*CAT*) gene expression of undamaged plasmids after transfection were similar in cases and controls, implying that cells from cases and controls had similar transfection efficiency. Furthermore, even repair-deficient cells, such as xeroderma pigmentosum cells, do not have a reduced transfection efficiency.¹⁸ Therefore, it is unlikely that the DRC values were influenced by differences in cell growth, differential response to mitogen stimulation, or variation in transfection efficiency.^{15,20}

In summary, we found that efficient DRC phenotype, as measured by the BPDE-induced HCR assay in cultured peripheral blood lymphocytes, was significantly associated with a reduced

survival in patients with NSCLC who have been treated with platinum-based chemotherapy. Therefore, it is promising to use DRC in peripheral lymphocytes as a prognostic factor to guide tailored individual therapeutics for patients with NSCLC. We are also studying genetic determinants that may predict the DRC phenotype. If identified and validated, testing for the predictive genotypes would be even cheaper and much more rapid, high throughput, and reliable.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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AUTHOR CONTRIBUTIONS

Conception and design: Li-E Wang, Margaret R. Spitz, Qingyi Wei

Financial support: Margaret R. Spitz, Qingyi Wei

Administrative support: Margaret R. Spitz, Qingyi Wei

Provision of study materials or patients: Kelly W. Merriman, Margaret R. Spitz, Qingyi Wei

Collection and assembly of data: Li-E Wang, Ming Yin, Kelly W. Merriman, Margaret R. Spitz, Qingyi Wei

Data analysis and interpretation: Li-E Wang, Ming Yin, Qiong Dong, David J. Stewart, Christopher I. Amos, Margaret R. Spitz, Qingyi Wei

Manuscript writing: All authors

Final approval of manuscript: All authors

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