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ORIGINAL REPORT

Tissue Platinum Concentration and Tumor Response in Non–Small-Cell Lung Cancer

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ABSTRACT

Purpose
Platinum resistance is a major limitation in the treatment of advanced non–small-cell lung cancer (NSCLC). Reduced intracellular drug accumulation is one of the most consistently identified features of platinum-resistant cell lines, but clinical data are limited. We assessed the effects of tissue platinum concentrations on response and survival in NSCLC.

Patients and Methods
We measured total platinum concentrations by flameless atomic absorption spectrophotometry in 44 archived fresh-frozen NSCLC specimens from patients who underwent surgical resection after neoadjuvant platinum-based chemotherapy. Tissue platinum concentration was correlated with percent reduction in tumor size on post- versus prechemotherapy computed tomography scans. The relationship between tissue platinum concentration and survival was assessed by univariate and multicovariate Cox proportional hazards regression model analysis and Kaplan-Meier analysis.

Results
Tissue platinum concentration correlated significantly with percent reduction in tumor size ($P < .001$). The same correlations were seen with cisplatin, carboplatin, and all histology subgroups. Furthermore, there was no significant impact of potential variables such as number of cycles and time lapse from last chemotherapy on platinum concentration. Patients with higher platinum concentration had longer time to recurrence ($P = .034$), progression-free survival ($P = .018$), and overall survival ($P = .005$) in the multicovariate Cox model analysis after adjusting for number of cycles.

Conclusion
This clinical study established a relationship between tissue platinum concentration and response in NSCLC. It suggests that reduced platinum accumulation might be an important mechanism of platinum resistance in the clinical setting. Further studies investigating factors that modulate intracellular platinum concentration are warranted.

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INTRODUCTION

Platinum-based chemotherapy is widely used in advanced non–small-cell lung cancer (NSCLC). For their mechanism of action, platinum drugs must form DNA adducts, which induce a cascade of signaling transduction pathways that culminate in activating p53-dependent and p53-independent apoptosis.[1] However, first-line platinum-containing doublets yield response rates in NSCLC of only 20% to 30%,[2,3] because a significant portion of tumors express intrinsic or de novo resistance. A better understanding of molecular mechanisms of intrinsic platinum resistance is necessary to develop new therapeutic approaches that induce greater platinum sensitivity and more durable responses.

There are several mechanisms by which NSCLC cells may express intrinsic resistance to platinum, such as, but not limited to, drug inactivation by detoxifying factors, alterations in checkpoint and apoptotic proteins, and alteration in intracellular drug accumulation.[4] Despite the multifactorial nature of platinum resistance, reduced intracellular drug accumulation is one of the most consistently identified features of cisplatin-resistant cell lines,[5,6] including resistant NSCLC cell lines.[7-11]

Studies in cell lines have enhanced our understanding of platinum resistance, but there are several limitations. For instance, each cell line represents a single phenotype, making it difficult to assess its clinical relevance. Also, cell line studies do not take into account tumor cell interactions with host factors that may constitute a unique microenvironment, and this limits the applicability of cell line data to biologically complex human tumors. Therefore, a tissue-based model is needed

to study platinum resistance that could be used to validate data from cell line studies and to identify specific impediments that correlate directly with clinical response.

In previous studies, total platinum and DNA adducts were detectable in autopsy tumor samples from patients who had last received cisplatin 6 to 15 months antemortem.[12,13] Because this suggests that platinum has a long half-life in tumors, we measured platinum concentrations in surgical NSCLC specimens from patients who had received neoadjuvant platinum-based chemotherapy to assess whether tumor platinum concentration correlated with outcome and to validate reduced platinum accumulation as a mechanism of clinical platinum resistance in NSCLC. To our knowledge, no clinical studies in any tumor type have examined the relationship between platinum concentration and clinical outcome. We hypothesized that total platinum concentrations in NSCLC specimens after neoadjuvant platinum-based chemotherapy would correlate with tumor response.

PATIENTS AND METHODS

Patients and Tissue Specimens

This study (approved by the Institutional Review Board of the University of Texas MD Anderson Cancer Center) used University of Texas Lung Cancer Specialized Program of Research Excellence Tissue Bank archived fresh-frozen NSCLC tumor specimens. Tumors examined were obtained from 44 patients with stage I to III NSCLC who had received neoadjuvant platinum-based chemotherapy without radiation followed by curative surgical resection between 2001 and 2009 at the MD Anderson Cancer Center. This represented all patients with NSCLC undergoing curative surgical resection (without radiation) during that time period who had undergone neoadjuvant platinum-based therapy and for whom sufficient archival fresh-frozen tumor tissue was available to permit platinum measurement. An additional four patients who underwent surgery alone and one patient who received pemetrexed without a platinum agent as neoadjuvant chemotherapy provided a control group. Various histopathologic features, including percentage of residual viable tumor cells, necrosis, and fibrosis, were assessed in all tumor samples as described previously.[14]

Tissue Platinum Measurement

Approximately 30 mg of tumor from each patient was weighed and digested overnight in benzethonium hydroxide at 55°C to achieve homogeneity.[15,16] After acidification, each sample was analyzed by flameless atomic absorption spectrophotometry (FAAS) to measure absorbance unit associated with platinum content, as previously described.[15] Validity of the assay was ensured with a linear standard curve (Appendix Fig A1, online only) that was generated from serial dilutions of certified stock platinum standard (987 μg/mL; Sigma, St Louis, MO). Most specimens were analyzed in at least two independent experiments where samples were taken from different parts of the tumor. The averaged platinum concentration was reported as absorbance unit per milligram of tissue.

Statistical Analysis

The primary outcome was tumor response, determined by measuring percent reduction in the largest tumor diameter from pre- to postchemotherapy computed tomography scans, blinded to the results from platinum analysis. In addition, we determined tumor response using the standard RECIST classification.[17] Pearson correlation coefficients were used to assess correlations between tissue platinum concentration and variables of interest, including percent reduction in tumor size. Two-sided $P < .05$ was considered statistically significant. The t test was used to compare platinum concentrations between two groups. Kaplan-Meier curves and log-rank tests were used to evaluate differences in time to recurrence (TTR), progression-free survival (PFS), and overall survival (OS) between two risk groups dichotomized by median platinum concentration. Recurrence was defined as evidence of local recurrence or new sites of involvement in lymph nodes or distant organs after curative resection. PFS was defined as the time from date of surgery until recurrence or last follow-up or death from any cause. OS was defined as the time from date of surgery until death from any cause or last follow-up. Four patients who died without any information on the status of recurrence were excluded from TTR analysis. Hazard ratios (HRs) and 95% CIs were estimated using univariate and multicovariate Cox proportional hazards regression models to assess for the association between platinum concentration and clinical parameters including TTR, PFS, and OS. In addition, odds ratio for

Table 1. Demographic and Clinical Characteristics of Evaluable Patients

Characteristic	No. of Patients (N = 44)	%
Age, years		
Median	64	
Range	44-78	
Sex		
Male	24	55
Female	20	45
Ethnicity		
White	36	82
African American	7	16
Hispanic	1	2
Clinical stage		
IA	1	2
IB	3	7
IIA	3	7
IIB	13	30
IIIA	20	45
IIIB	4	9
Histology		
Adenocarcinoma	24	55
Squamous cell carcinoma	12	27
Other	8	18
Neoadjuvant chemotherapy*		
Cisplatin	16	36
Plus taxane	14	
Plus other	3	
< 3 cycles	5	
≥ 3 cycles	11	
Carboplatin†	27	61
Plus taxane	25	
Plus other	2	
< 3 cycles	9	
≥ 3 cycles	17	
Smoking status		
Current smoker	13	30
Former smoker	18	41
Never smoker	7	16
Undocumented	6	14
Tumor response by RECIST		
Stable disease	30	68
Partial response	14	32
Tobacco use, pack-years		
Median	33	
Range	0-145	
Time since last chemotherapy, days		
Median	37	
Range	22-85	

*One patient who received both cisplatin and carboplatin was not categorized into cisplatin or carboplatin group.
†One patient in carboplatin group who received five weekly doses of carboplatin was not categorized into < three cycles or ≥ three cycles group.

response by covariates according to RECIST was calculated using logistic regression analysis. Multicovariate analysis was used to include variables with $P < .20$ for any of the three time-to-event end points in the univariate analysis as the entry criterion. However, only the variables that were significant in any of the end points were retained in the final model. The statistical analyses were performed using GraphPad PRISM (version 5.04; GraphPad Software, La Jolla, CA) and R (version 2.13.1; http://www.r-project.org/) software.

RESULTS

Patient Characteristics

Table 1 lists the patient characteristics of 44 evaluable patients with early-stage NSCLC who received neoadjuvant platinum-based chemotherapy before undergoing surgical resection. Median age was 64 years; 55% of patients were men, and 45% were women. A majority of patients had either stage IIB (30%) or IIIA (45%) disease. All 44 patients received a doublet consisting of cisplatin (n = 16), carboplatin (n = 27), or cisplatin followed by carboplatin (n = 1). Most patients received taxanes as the second agent. Median time from last dose of chemotherapy to surgery was 37 days. There were 24 adenocarcinomas (55%), 12 squamous cell carcinomas (27%), and eight other histology types (18%).

Tissue Platinum Concentration and Tumor Response

By FAAS, absorbance values from tissues of our five controls who had not received a platinum were similar to the background value

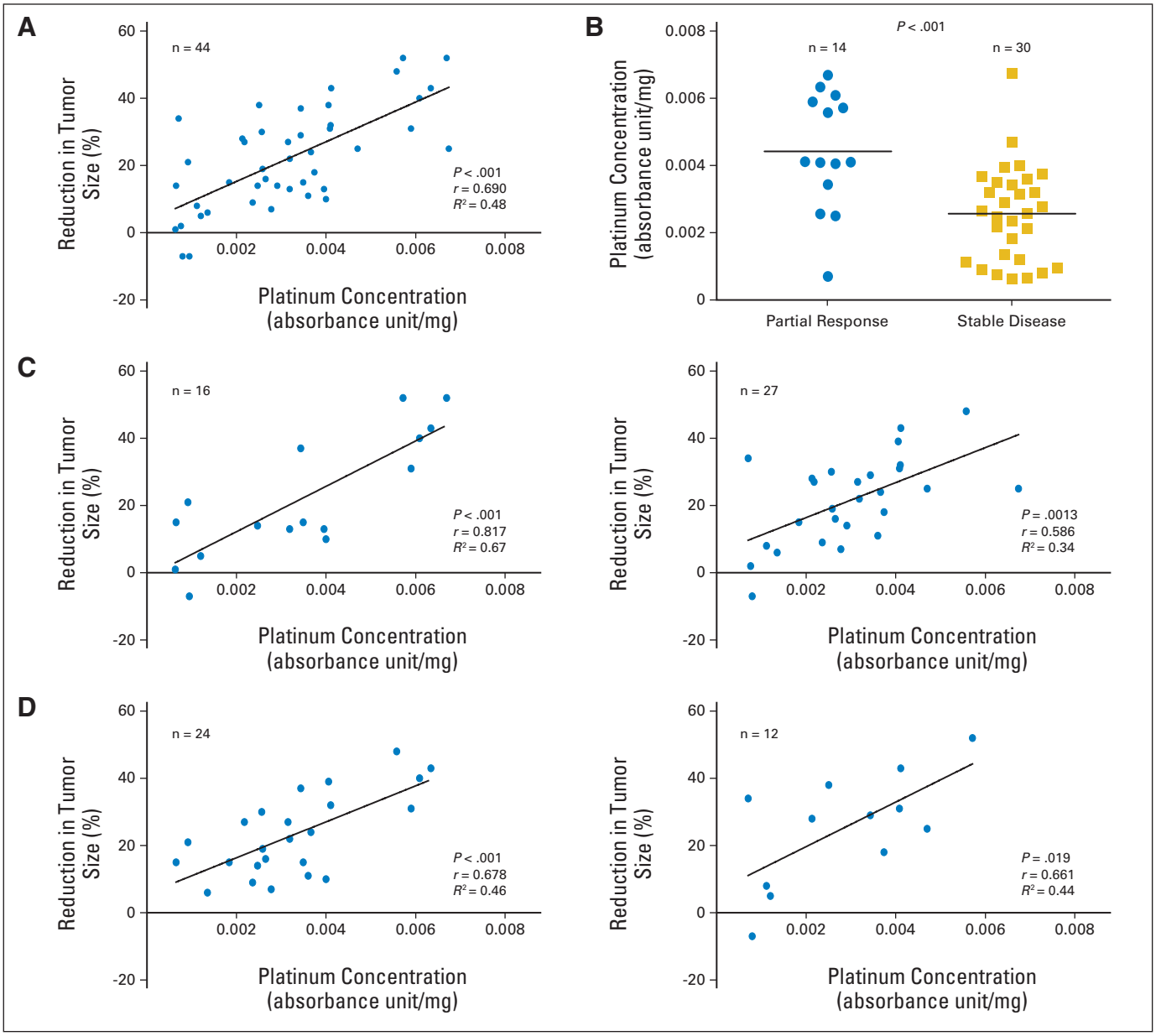

Fig 1. Pearson's correlation between tissue platinum concentration and tumor response. (A) A highly significant correlation was observed between total tissue platinum concentration and percent reduction in tumor size. (B) In addition, a significantly higher platinum concentration was detected in patients who achieved a partial response compared with those who achieved stable disease. The correlation between platinum concentration and percent reduction in tumor size was not specific for (C) cisplatin (left) versus carboplatin (right) subgroups or (D) adenocarcinoma (left) versus squamous cell carcinoma (right) subgroups.

obtained with hydrochloric acid (Appendix Table A1, online only). As illustrated in Figure 1A, all except two platinum-treated patients had at least some degree of tumor shrinkage with therapy, and there was a significant correlation (Pearson $r = 0.69$, $P < .001$) between tumor platinum concentration and percent change in tumor size. Furthermore, as demonstrated in Figure 1B, patients who achieved a partial response had a significantly higher platinum concentration compared with patients who had stable disease ($P < .001$). Separate analyses were also performed for each of the following subgroups: patients treated with cisplatin, patients treated with carboplatin, patients with adenocarcinomas, and patients with squamous cell carcinomas; tumor shrinkage correlated significantly with tumor platinum concentration for each subgroup (Figs 1C and 1D). The correlation between tissue platinum concentration and tumor response in eight specimens from the other histology subgroup was also statistically significant (Table 2; not shown in Fig 1). The same analyses using Spearman correlation coefficients (data not shown) also revealed significant correlations between platinum concentration and tumor response with the exception of the squamous cell carcinoma subgroup, which demonstrated a borderline significance (Spearman $\rho = 0.511$, $P = .090$) possibly because of a small sample size.

Table 2. Association Between Tissue Platinum Concentration and Various Clinicopathologic Parameters

Parameter	Pearson Correlation	
	r	*P*
Age	−0.010	.95
Sex: male *v* female		.69
Histology: adenocarcinoma *v* other		.59
Tobacco pack-years	−0.014	.93
Pretreatment diameter	−0.244	.11
Clinical stage: III *v* I/II		.91
Percent reduction in tumor size	0.690	< .001
Cisplatin	0.817	< .001
Carboplatin	0.586	.0013
Adenocarcinoma	0.678	< .001
Squamous cell carcinoma	0.661	.019
Other histology	0.889	.0031
Time from last treatment to surgery	−0.157	.33
Lactate dehydrogenase*	−0.044	.78
Total protein*	−0.079	.62
Albumin*	0.197	.21
Creatinine*	−0.030	.85
Hemoglobin*	0.275	.075
Cisplatin	0.147	.59
Carboplatin	0.434	.027
Cumulative dose of platinum†	0.061	.72
Body-surface area	−0.019	.91
Pathologic response		
Percent viable tumor	−0.248	.10
Percent viable tumor (after removing three specimens with sarcomatoid features)	−0.383	.014
Percent necrosis	−0.026	.87
Percent fibrosis	−0.053	.73
Percent inflammation	0.170	.27

NOTE. For the discrete variables including sex, histology, and clinical stage, *t* tests were used. The mean (median) platinum concentrations (absorbance unit/mg in 10^{-3}) for the overall population and the discrete variables were as follows: overall, 3.2 (3.2); male, 3.3 (3.3); female, 3.0 (3.0); adenocarcinoma, 3.3 (3.2); other histology, 3.0 (3.0); stage III, 3.1 (3.5); and stage I/II, 3.2 (2.9).

*Based on blood collections prior to first dose of chemotherapy.

†Total milligrams of platinum metal administered; in patients who received carboplatin = total carboplatin dose in milligrams × 195/371; in patients who received cisplatin = total cisplatin dose in milligrams × 195/300.

Association Between Platinum Concentrations and Clinicopathologic Parameters

In addition to establishing the correlation between tissue platinum concentration and percent reduction in tumor size, we determined the association between platinum concentration and other variables (Table 2). No single parameter beside percent reduction in tumor size correlated significantly ($P < .05$) with platinum concentration in our group of 44 patients. Notably, hemoglobin level and percent viable tumor demonstrated an association with platinum concentration at borderline significance. Hemoglobin levels in patients who received carboplatin but not cisplatin correlated significantly with platinum concentration (Table 2). There were three specimens with sarcomatoid features on pathology that seemed to be outliers for the correlation between platinum concentration and percent viable tumor. These three specimens demonstrated 80% viable tumor despite radiologic response to neoadjuvant chemotherapy (data not shown). The correlation between platinum concentration and percent viable tumor became significant ($P = .014$) after removing these three specimens.

Effects of Different Platinum Compounds and Number of Cycles on Platinum Concentration

Tumor platinum concentrations were similar between cisplatin and carboplatin subgroups (Fig 2A). Furthermore, there was no significant impact of number of cycles ($\geq v <$ three cycles) on platinum concentration in the overall population (Fig 2B) or within cisplatin or carboplatin subgroups (Figs 2C and 2D).

Effects of Tissue Platinum Concentration on Recurrence and Survival

The Cox proportional hazards regression model was used to assess the effect of high versus low platinum concentration, dichotomized by the median, on TTR, PFS, and OS. The univariate analysis showed that high platinum concentration was a protective factor for TTR (HR, 0.39; 95% CI, 0.16 to 0.97; $P = .043$), PFS (HR, 0.44; 95% CI, 0.20 to 0.96; $P = .038$), and OS (HR, 0.39; 95% CI, 0.16 to 0.95; $P = .038$; Table 3). In addition to the platinum concentration, percent reduction in tumor size ($P = .16$) and number of cycles ($P = .022$) demonstrated some association ($P < .20$) with OS and were included in multicovariate analysis initially. High platinum concentration remained a significant protective factor for TTR (HR, 0.36; 95% CI, 0.14 to 0.93; $P = .034$), PFS (HR, 0.37; 95% CI, 0.17 to 0.85; $P = .018$), and OS (HR, 0.26; 95% CI, 0.10 to 0.67; $P = .005$) after multicovariate analysis (Table 3). Number of cycles ($\geq v <$ three cycles) was also significantly associated with OS ($P = .003$) but not with TTR ($P = .35$) or PFS ($P = .071$). Percent reduction in tumor size was no longer significant in any of the end points and, hence, was dropped in the final multicovariate models. We also performed logistic regression analysis to determine the association between platinum concentration and response by RECIST. Patients with high platinum concentration were more likely to achieve a partial response compared with patients with low platinum concentration (odds ratio, 7.07; 95% CI, 1.51 to 33.09; $P = .013$) after adjusting for number of cycles. Kaplan-Meier curves

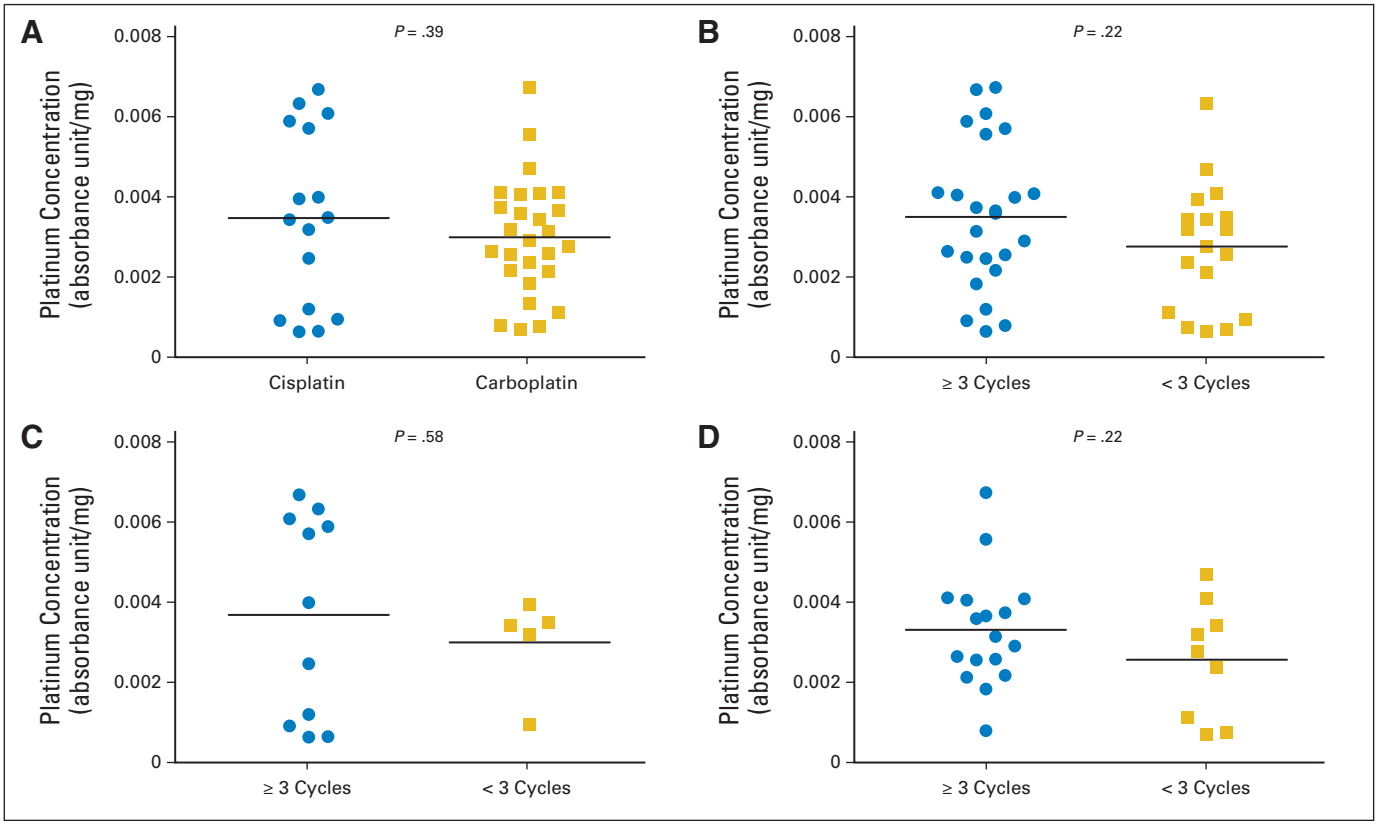

Fig 2. Effects of different platinum compounds and number of cycles on tissue platinum concentration. There was no significant difference in mean platinum concentration (A) between cisplatin and carboplatin subgroups or (B) between patients who received ≥ three cycles and < three cycles. This lack of significant difference between the subgroups who received ≥ three cycles and < three cycles was also observed in patients who received (C) cisplatin or (D) carboplatin. The dark horizontal line represents the mean.

and log-rank tests comparing high versus low platinum concentrations for TTR, PFS, and OS are shown in Figures 3A, 3B, and 3C, respectively, with Figure 3D reinforcing the observation that the difference in tumor response between high versus low tissue platinum groups is significant ($P < .001$). Furthermore, in the high platinum group, 11 patients (50%) achieved a partial response compared with three patients (14%) in the low platinum group (Fig 3D).

DISCUSSION

We report herein the proof-of-principle demonstration that tissue platinum level can be reliably measured using FAAS in resected NSCLC specimens from patients who received neoadjuvant platinum-based chemotherapy. Furthermore, using this approach, we report the novel findings that tissue platinum concentration is significantly associated with tumor response and survival in NSCLC, supporting reduced drug accumulation as a significant mechanism of platinum resistance in clinical tumor specimens.

Tumor platinum concentrations were similar in patients treated with carboplatin versus cisplatin despite platinum doses being considerably higher with carboplatin. This is in keeping with cellular uptake being slower for carboplatin than for cisplatin.[18] The weak correlation between time from last treatment and platinum concentration is in keeping with the long half-life previously noted for platinum in human tissues,[13] including dorsal root ganglion,[19] liver,[20] and kidney cortex,[21] and in earlier studies of human tumor platinum concentrations.[22,23]

The weak correlation between the number of cycles of platinum therapy and tumor platinum concentrations is similar to previous observations with respect to human autopsy kidney cortex platinum concentrations after cisplatin, where tissue concentrations varied with dose given in the first cycle but correlated only weakly with cumulative dose.[20,21] This suggests that for kidney and tumor, there may be adaptive response/resistance-inducing transport factors that limit further net platinum accumulation after initial exposure. In this regard, uptake transporters, such as the copper transporters CTR1 and CTR2, and various efflux transporters, such as the multidrug resistance protein (MRP), ABCB1, and ATP7B, are potential contributors.[4] Interestingly, more is known about the function of efflux transporters than uptake transporters. MRP expression is associated with decreased cellular drug accumulation of cisplatin.[24] In autopsy NSCLC tumor tissues, mRNA expression levels of MRP3[25] and MRP5[26] were significantly higher in patients who were exposed to platinum drugs compared with patients who had not received platinum drugs. However, it is not certain whether tumor MRP expression correlates with clinical outcome in NSCLC.[4] Conversely, the role of ABCB1 in transport of platinum drugs seems to be less significant, because its expression in NSCLC cell lines did not correlate significantly with sensitivity to cisplatin or intracellular platinum accumulation.[9,27] Likewise, in NSCLC tissues, ABCB1 expression by immunohistochemistry did not

Table 3. Logistic Regression Analysis for Tumor Response and Univariate and Multicovariate Cox Proportional Hazards Regression Analysis for Prediction of TTR, PFS, and OS After Surgical Resection (N = 44)

Variable	Response (PR *v* SD)			TTR			PFS			OS		
	OR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
Univariate analysis												
Age	1.02	0.95 to 1.10	.55	1.03	0.97 to 1.09	.34	1.02	0.97 to 1.07	.42	1.02	0.96 to 1.07	.58
Sex: male *v* female	1.17	0.32 to 4.19	.81	1.02	0.42 to 2.45	.97	1.27	0.59 to 2.74	.55	1.26	0.54 to 2.96	.59
Histology: adenocarcinoma *v* other	1.17	0.32 to 4.19	.81	1.71	0.68 to 4.31	.25	1.63	0.74 to 3.57	.23	1.37	0.58 to 3.24	.47
Smoking status: ever *v* never-smoker	1.19	0.20 to 7.23	.85	1.53	0.43 to 5.38	.51	1.49	0.51 to 4.39	.47	2.58	0.59 to 11.20	.21
Ethnicity: white *v* other	3.96	0.44 to 35.80	.22	1.97	0.45 to 8.50	.37	1.76	0.53 to 5.85	.36	1.19	0.35 to 4.02	.78
Stage: III *v* I/II	1.17	0.32 to 4.19	.81	1.69	0.68 to 4.25	.26	1.46	0.68 to 3.14	.34	0.90	0.39 to 2.08	.80
Time from last treatment to surgery	0.98	0.93 to 1.04	.55	1.02	0.99 to 1.04	.21	1.01	0.99 to 1.04	.30	1.02	0.99 to 1.04	.28
Percent reduction in tumor size	NA*			0.99	0.96 to 1.02	.42	0.99	0.96 to 1.01	.28	0.98	0.95 to 1.01	.16
Response by RECIST: PR *v* SD	NA*			0.81	0.31 to 2.12	.68	0.74	0.31 to 1.74	.48	0.75	0.29 to 1.91	.54
Number of cycles: $\geq$ *v* $<$ 3 cycles	2.24	0.51 to 9.85	.29	0.80	0.30 to 2.10	.65	0.59	0.27 to 1.28	.18	0.38	0.16 to 0.87	.022
Platinum concentration: high *v* low†	6.33	1.45 to 27.74	.014	0.39	0.16 to 0.97	.043	0.44	0.20 to 0.96	.038	0.39	0.16 to 0.95	.038
Multicovariate analysis												
Number of cycles: $\geq$ *v* $<$ 3 cycles	3.08	0.61 to 15.54	.17	0.62	0.23 to 1.68	.35	0.47	0.21 to 1.06	.071	0.27	0.11 to 0.65	.003
Platinum concentration: high *v* low†	7.07	1.51 to 33.09	.013	0.36	0.14 to 0.93	.034	0.37	0.17 to 0.85	.018	0.26	0.10 to 0.67	.005

NOTE. The median follow-up time was 59.7 months as calculated by the reverse Kaplan-Meier method. Fourteen patients achieved PR, and 30 patients had SD. The numbers of events were as follows: TTR, n = 20; PFS, n = 27; and OS, n = 23.

Abbreviations: HR, hazard ratio; NA, not available; OR, odds ratio; OS, overall survival; PFS, progression-free survival; PR, partial response; SD, stable disease; TTR, time to recurrence.

*Not available because these covariates are indicators of response.

†High indicates greater than median platinum concentration, and low indicates less than median platinum concentration.

correlate with response to cisplatin.[28-30] The copper transporter ATP7B is also thought to play a role in efflux of platinum drugs. Human tumor cells transfected with ATP7B acquired significant resistance to cisplatin, mainly as a result of increased cisplatin efflux.[31] Furthermore, ATP7B mRNA and immunohistochemistry expression significantly correlated with cisplatin resistance in NSCLC xenografts.[32] However, in clinical NSCLC specimens, only the copper uptake transporter CTR1, but not ATP7A or ATP7B, predicted clinical outcome after platinum-based chemotherapy.[33] In addition, the observation that NSCLC dose-response curves flatten at higher platinum dose-intensities[34] would be more in keeping with resistance being related to reduced uptake rather than increased efflux.[35] Validating functions of transport factors in NSCLC and incorporating them as biomarkers for platinum sensitivity into future clinical trials would be of significant clinical value. Furthermore, designing a novel platinum complex that is not subject to reduced platinum accumulation in resistant cells could be another potential strategy to overcome platinum resistance.

There are factors other than transporters that may modulate intratumoral platinum concentration. A low extracellular pH favors uptake of weak acids such as aquated cisplatin and enhances cytotoxicity.[36] Serum levels of lactate dehydrogenase, which converts pyruvate to lactate in tumors,[37] did not correlate with tissue platinum concentration. Contrary to the potential importance of low pH on platinum uptake, we observed a positive correlation between hemoglobin level and tumor platinum concentration, despite the fact that higher hemoglobin level would have been expected to be associated with higher tissue pH. Because pretreatment hemoglobin level was used for correlation, chemotherapy-induced anemia does not explain this observation. It is possible that anemia contributes to tumor hypoxia affecting drug delivery of platinum agents. This would be consistent with a previous report in which prevention of anemia using darbepoetin alfa (an erythropoiesis-stimulating protein) resulted in reduced tumor hypoxia, higher intracellular platinum concentration, and greater tumor response in a murine model of Lewis lung carcinoma.[38]

ERCC1 may also play a role in modulating intratumoral platinum concentration. Platinum-DNA adducts can be removed by the nucleotide excision repair pathway in which ERCC1 plays an important role.[39,40] It is unclear what happens once adducts get removed from DNA by nucleotide excision repair, but they could potentially leave the cell, thereby contributing to reduced intracellular platinum concentration. Against ERCC1 being the major factor driving tumor platinum concentrations is the weak correlation with cumulative drug dose. If an active resistance factor such as ERCC1 (or an efflux pump) were responsible, one might expect little drug accumulation at lower drug doses and then a relatively steep increase in drug concentration with increasing dose as one saturated the capacity of the resistance-inducing factor at higher doses.[35]

Our sample size was relatively small because only a small proportion of all patients with NSCLC are candidates for neoadjuvant chemotherapy and because we were limited by the availability of sufficient quantities of archived fresh-frozen tumor to permit analysis in this study. Despite these small patient numbers, we achieved adequate statistical power to establish a correlation between tumor platinum concentration and therapeutic efficacy that has proven our hypothesis to be correct. Nevertheless, it is still appropriate to stress that this is a single-institution, retrospective study that, therefore, will require independent validation with a larger number of patients.

Once platinum gets into tumor cells and accumulates to adequate levels, platinum-DNA adducts are generated.[1] We attempted to measure DNA adduct levels in our specimens, but the analytic sensitivity proved limiting, and it was impractical to increase the amount of tissue for extracting an adequate amount of DNA for reliable detection

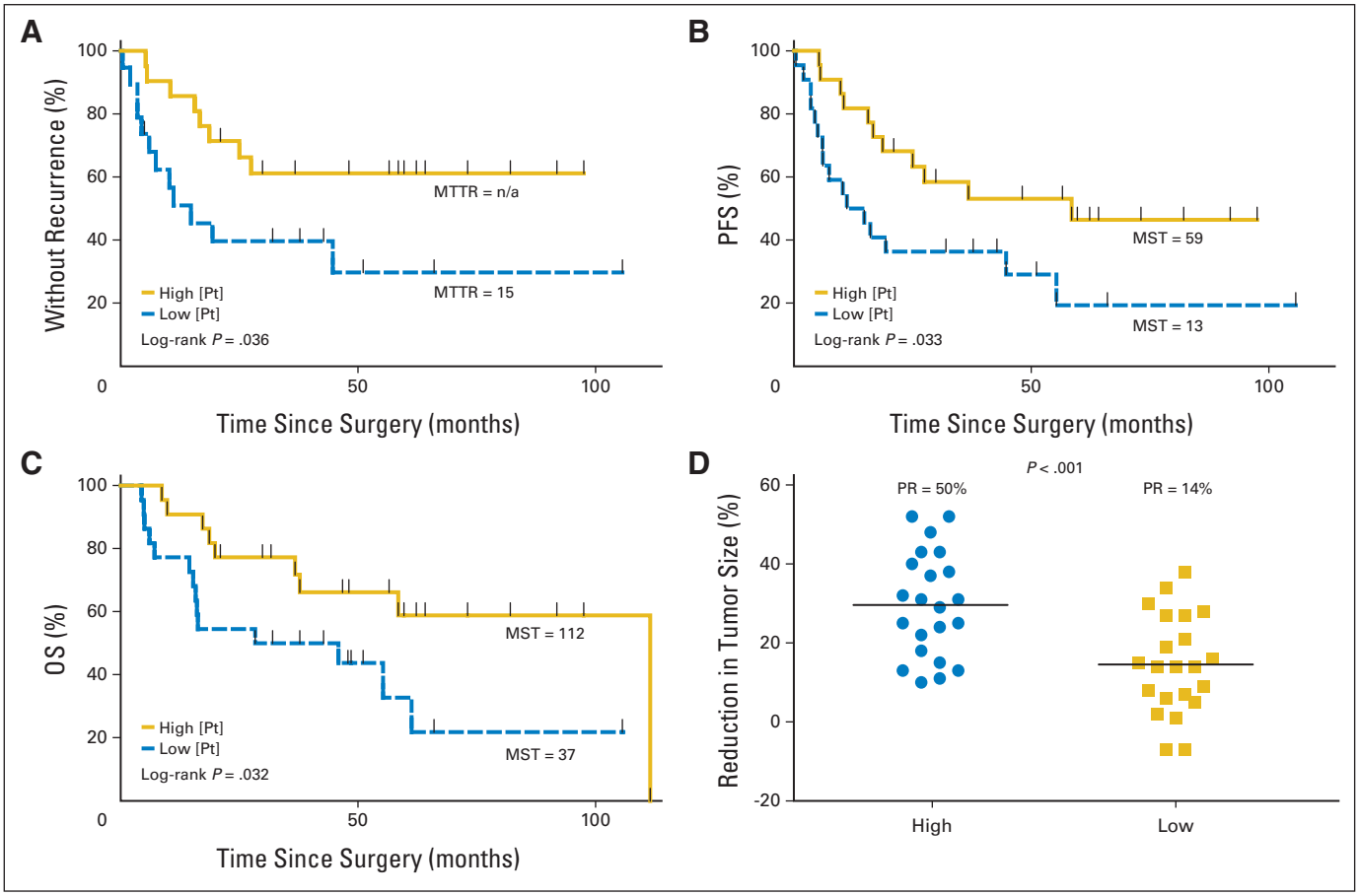

Fig 3. Kaplan-Meier curves for (A) time to recurrence, (B) progression-free survival (PFS), and (C) overall survival (OS) of patients with high versus low tissue platinum concentrations after neoadjuvant platinum-based chemotherapy. (D) A significant difference in tumor response was observed between high versus low tissue platinum groups. In the high platinum group, 11 patients (50%) achieved a partial response versus three patients (14%) in the low platinum group. The dark horizontal line represents the mean. MST, median survival time; MTTR, median time to recurrence; n/a, less than half the patients experienced recurrence; PR, partial response; [Pt], platinum concentration.

of adducts by FAAS. Furthermore, there is ample evidence that the amount of adduct formation is significantly associated with intracellular platinum accumulation.[41] However, we believe that total intratumoral platinum concentration is still the most accurate variable that can be used to validate reduced drug accumulation as a significant mechanism of platinum resistance.

Another limitation of our study is the potential influence of second agents (mostly taxanes) that were given concurrently with platinum agents. The factors that confer resistance to one agent may render tumors resistant to several other agents.[4] Alternating multiple agents with different mechanisms of action, including cisplatin and paclitaxel, does not improve clinical outcome in NSCLC.[42] However, there were a few outlying patients in our study whose tumors demonstrated clinically significant shrinkage despite low detectable levels of intratumoral platinum (Fig 1A). This shrinkage may have been a result of the concurrent taxane, although this remains uncertain. Additionally, number of cycles was associated with OS. However, the clinical significance of number of cycles cannot be definitely answered using our data.

In conclusion, to our knowledge, this is the first study evaluating impact of tissue platinum concentration on NSCLC response, recurrence, and survival. Our data strongly support reduced drug accumulation as a significant mechanism of platinum resistance. Because transport-related mechanisms of resistance are likely to persist or are acquired after platinum treatment in a variety of cancer tissue types,[1,4] we anticipate that our novel approach of measuring platinum concentration in resected specimens after neoadjuvant chemotherapy by FAAS could potentially be used to investigate the relationship between intratumoral platinum concentration and tumor response in the metastatic setting or in other tumor types, such as advanced ovarian carcinoma, in which neoadjuvant platinum-based chemotherapy could be considered before debulking surgery.[43] Finally, enhanced understanding of the molecular mechanism of platinum accumulation by tumor cells will be necessary to identify surrogate biomarkers for platinum accumulation that could be developed prospectively for individualizing therapy.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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Data analysis and interpretation: Eric S. Kim, J. Jack Lee, Guangan He, David J. Stewart, Zahid H. Siddik
Manuscript writing: All authors
Final approval of manuscript: All authors

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