Early and Specific Prediction of the Therapeutic Efficacy in Non–Small Cell Lung Cancer Patients by Nucleosomal DNA and Cytokeratin-19 Fragments

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ABSTRACT: **Facing an era of promising new antitumor therapies, predictors of therapy response are needed for the individual management of treatment. In sera collected prospectively from 311 patients with advanced non-small cell lung cancer receiving first-line chemotherapy, changes in nucleosomal DNA fragments, cytokeratin-19 fragments (CYFRA 21–1), carcinoembryonic antigen (CEA), neuron-specific enolase (NSE), and progastrin-releasing peptide (ProGRP) were investigated and correlated with therapy response. In univariate analysis, high levels, slower and incomplete decline in nucleosomal DNA, CYFRA 21–1, and CEA predicted poor outcome. DNA concentrations at day 8 of the first therapeutic cycle and CYFRA 21–1 before start of the second cycle were identified as best predictive variables. In multivariate analysis, they predicted progression with a specificity of 100% in 29% of the cases earlier than imaging techniques. Thus, nucleosomal DNA and CYFRA 21–1 specifically identify a subgroup of patients with insufficient therapy response at the early treatment phase and showed to be valuable for disease management.**

KEYWORDS: **DNA; nucleosomes; cytokeratin-19 fragments; CYFRA 21–1; serum; plasma; prediction; chemotherapy; lung cancer**

INTRODUCTION

Lung cancer accounts for most deaths caused by cancer in males and has an increasing prevalence in women worldwide.[1,2] Often it is detected only in

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advanced stages when therapeutic options are restricted to systemic chemo- and radiotherapy. These therapies often are associated with insufficient success, but there are efforts to improve the situation by the introduction of new drugs.[3,4] Progress in recent years is mirrored by the 2003 therapy guidelines of the American Society of Clinical Oncology (ASCO), which include recommendations for the first-, second-, and third-line chemotherapy while in 1997 only one option for first-line therapy was given.[5] Therefore, it is important to detect as early as possible whether patients will benefit from a specific therapy or not in order to save time and costs by changing early the treatment strategy and by avoiding unnecessary side effects. Because the consequences will be most striking in cases of insufficient response to therapy, the predication has to be highly specific and sensitive.

Macroscopic alterations of the tumor mass are often detected only after several cycles of chemotherapy by imaging techniques. Promising candidates for the early estimation of therapeutic efficacy are biochemical parameters in blood that reflect the biochemical response of the tumor during the initial treatment phase. In lung cancer, various oncological biomarkers are used for diagnosis, prognosis, and therapy monitoring. Carcinoembryonic antigen (CEA) and cytokeratin-19 fragments (CYFRA 21–1) are known to be sensitive, however, not specific markers for non-small cell lung cancer.[6,7] Neuron-specific enolase (NSE) and progastrin-releasing peptide (ProGRP) have high specificity for small-cell lung cancer.[6,8] In addition, circulating DNA, which is supposed to be present in serum and plasma mainly in conjunction with histones as nucleosomes,[9] showed high potential for diagnosis, prognosis, and therapy monitoring.[10–15]

Recently, it was shown that in lung cancer nucleosomal DNA and CYFRA 21–1 during the initial phase of chemotherapy are able to discriminate between responders and nonresponders.[16] On the basis of these promising results, we analyzed in an extended sample of lung cancer patients undergoing first-line therapy whether these parameters and other relevant oncological biomarkers could predict early the response to chemotherapy with high specificity. If a subgroup of patients who do not respond to the treatment can be identified early by these blood markers prior to established imaging techniques, they might be useful in clinical practice as an indicator for the early adjustment of therapy. Here, a model with the best predictive clinical and biochemical parameters was developed that was tested under various therapeutic conditions.

MATERIALS AND METHODS

Patients

Consecutive patients ($n = 311$) with inoperable non-small cell lung cancer (stages III and IV) under the care of the Asklepios Clinics Gauting were

included in our study. All patients were investigated initially by whole body computed tomography, bone scan, and bronchoscopy. All patients received first-line chemotherapy regimens containing alternatively carboplatin, mitomycin c, and vinblastin (CMV) or mitomycin c and vinorelbin (MV) or gemcitabine and cisplatin (GC), which were given in three weekly cycles.

In all patients, staging investigations consisting of clinical examination, whole body computed tomography, and laboratory examinations were performed before start of the third cycle of chemotherapy. The response to therapy was classified according to the World Health Organization classifications as follows: "partial remission" as tumor reduction $\geq$50%, "progression" as tumor increase $\geq$25% or appearance of new tumor manifestations, and "no change" as tumor reduction <50% or increase <25%. Of the 311 patients, 126 patients had partial remission (40.5%), 92 showed progression (29.6%), and 93 had no change of disease (29.9%). Patients with no change of disease were followed up until staging before the fifth treatment cycle. Those who presented with partial remission or no change at that time ($n = 52$) were added to the responsive group (in total $n = 178$; 57.2%), whereas those with progression at that time ($n = 19$) joined the nonresponsive group (in total $n = 111$; 35.7%); 22 patients (7.1%) with no change terminated the therapy before cycle 5 or were lost to follow-up and could not be considered for the evaluation (TABLE 1).

Materials and Methods

Blood samples were collected prospectively before the first and second cycle of therapy for determination of the baseline values (BV1 and BV2), and during the first week of the first cycle, at days 1 (before start of the therapy), 3, 5, and 8.

The samples for nucleosomal DNA determination were centrifugated at 3000 g for 15 min and treated with 10 mM EDTA (pH 8) immediately after centrifugation. Samples were stored at −70°C and analyzed in batches. All samples from a single patient were analyzed in one batch. The details of the preanalytic handling of the samples are described in Holdenrieder *et al.*[17] Nucleosomal DNA fragments were determined by the Cell Death Detection-ELISA^{plus} (Roche Diagnostics, Mannheim, Germany) which was modified for its use in serum matrix as specified in Holdenrieder *et al.*[17] Nucleosomal DNA fragments were quantified using the calibration curve generated from known amounts of DNA.

The baseline values of the oncological biomarkers CEA, CYFRA 21–1, NSE (by Elecsys 2010; Roche Diagnostics), and ProGRP (by ELISA; [ALSI, Japan/IBL, Germany]) were determined before each therapeutic cycle (BV1 and BV2) at the day of sample collection. In addition, CEA and CYFRA 21–1 were determined more frequently during the first week of therapy.

TABLE 1. Characteristics of the patients investigated

	Median	Range
Age		
Years	63.0	25–86
	Number	Percentage
Gender		
Female	99	(31.8%)
Male	212	(68.2%)
Stage		
III A	13	(4.2%)
III B	100	(32.2%)
IV	198	(63.7%)
Performance score		
ECOG 1	110	(35.4%)
ECOG 2	160	(51.5%)
ECOG 3	38	(12.2%)
ECOG 4	3	(0.9%)
Histology		
Squamous cell CA	89	(28.6%)
Adeno cell CA	129	(41.5%)
Large cell CA	8	(2.6%)
Not classified NSCLC	85	(27.3%)
Mode of therapy		
CMV	119	(38.3%)
MV	37	(11.9%)
GC	105	(33.8%)
Others	50	(16.0%)
Therapy response before cycle 3		
PR	126	(40.5%)
NC	93	(29.9%)
PD	92	(29.6%)
Therapy response of NC patients before cycle 5		
PR and NC	52	(16.7%)
PD	19	(6.1%)
Lost to follow-up	22	(7.1%)

Statistics

For all parameters, the baseline values before the first and second cycle (BV1 and BV2), and the percent changes (BV1–2) were considered for statistical analysis. In addition, nucleosomal DNA, CYFRA 21–1, and CEA values at day 8 (d8) of the first therapeutic cycle, the changes in the values between days 1 and 8 (d1–8), and the area under the curve of the values from days 1 to 8 (AUC1–8/d) were evaluated for their predictive power. To calculate the AUC1–8/d, values on days 1 and 8 and at least one of the days 3 or 5 were required.

In the first instance, biochemical parameters were analyzed by Wilcoxon test on their power to discriminate between patients with remission and progression

TABLE 2. Summary of all markers and variables investigated on their discriminating power between patients with remission, no change, and progression of disease

Biochemical parameters		Units	Remission		No change		Progression		*P*-value
			Median	Range	Median	Range	Median	Range	
Nucleosomal DNA	BV1	ng/mL	196.8	9.2–3450	176.2	13.7–6471	256.3	13.7–1936	0.0222
	BV2	ng/mL	89.2	9.2–638.4	140.1	9.2–1439	164.7	11.4–1758	<0.0001
	BV1–2	DEC %	54.7	(–2700)–97.9	27.5	(–825)–98.7	31.5	(–1186)–96.0	0.0265
	d8	ng/mL	64.1	9.2–1135	121.3	22.9–1721	169.3	11.4–1588	<0.0001
	d1–8	DEC %	63.5	(–1650)–99.7	44.6	(–1571)–97.3	40.8	(–334)–94.8	0.0015
	AUC1–8/d	ng/mL	139.6	18.0–1058	205.3	34.3–1910	243.1	35.5–1066	0.0001
CYFRA 21–1	BV1	ng/mL	3.7	0.2–67.0	4.5	0.1–266.2	7.3	0.4–1018	0.0001
	BV2	ng/mL	1.9	0.1–23.0	3.5	0.4–73.5	6.2	0.4–1721	<0.0001
	BV1–2	DEC %	47.1	(–570)–93.0	22.7	(–300)–91.2	22.0	(–13779)–79.3	<0.0001
	d8	ng/mL	4.3	1.1–79.71	6.4	2.0–87.6	10.2	0.7–1114	0.0019
	d1–8	DEC %	2.4	(–446)–83.0	–16.2	(–317)–50.8	–5.5	(–336)–78.9	0.3812
	AUC1–8/d	ng/mL	4.7	0.8–51.9	5.6	1.7–68.7	7.9	0.7–632.0	0.0244
CEA	BV1	ng/mL	6.4	0.2–438.2	5.7	0.5–1437	9.0	0.2–77695	0.4001
	BV2	ng/mL	6.4	0.9–353.6	5.8	0.5–1426	12.5	0.9–1171	0.0665
	BV1–2	DEC %	10.6	(–1050)–73.3	–6.7	(–920)–58.7	–8.9	(–3054)–74.0	<0.0001
	d8	ng/mL	3.9	0.7–52.4	6.7	1.9–220.2	6.1	0.7–174.8	0.1129
	d1–8	DEC %	12.8	(–15,4)–58.2	–5.7	(–77.2)–44.1	–1.1	(–53,5)–38.1	0.0228
	AUC1–8/d	ng/mL	3.6	0.7–42.5	5.5	1.8–179.1	4.0	0.7–121.2	0.4177
NSE	BV1	ng/mL	14.8	5.4–355.0	14.2	3.6–63.0	15.6	8.1–275.3	0.1592
	BV2	ng/mL	12.5	7.0–35.6	11.6	4.2–38.0	16.2	7.8–52.9	0.0118
	BV1–2	DEC %	10.6	(–198)–91.8	7.4	(–144)–90.1	11.5	(–154)–62.8	0.9187

(*continued*)

TABLE 2. (*Continued*)

Biochemical parameters		Units	Remission		No change		Progression		*P*-value
			Median	Range	Median	Range	Median	Range	
ProGRP	BV1	pg/mL	15	2.0–4292	15	3.0–66.0	13	2.0–822	0.2560
	BV2	pg/mL	14	3.0–89.0	15	3.0–789	17	3.0–174	0.4740
	BV1–2	DEC %	13.3	(−1033)–99.6	14.2	(−8667)–93.3	−8.3	(−650)–88.0	0.2851
Clinical parameters									
Age			62	39–78	65	39–86	63	25–83	0.3352
			%	N	%	N	%	N	
Gender	Male		63.5	80	72.0	67	70.6	65	0.2686
	Female		36.5	46	28.0	26	29.4	27	
Performance status	**ECOG 1**		50.8	64	33.3	31	16.3	15	1 vs. 2: **0.0004**
	ECOG 2		46.0	58	61.3	57	48.9	45	2 vs. 3: **<0.0001**
	ECOG 3		3.2	4	5.4	5	31.5	29	3 vs. 4: 0.5224
	ECOG 4		0	0	0	0	3.3	3	
Stage	**M0**		46.0	58	38.7	36	20.6	19	**0.0001**
	M1		54.0	68	61.3	57	79.4	73	
Histology	**SC**		38.1	48	26.9	25	17.4	16	SC vs. (AC + NCC)
	AC		37.3	47	46.2	43	42.4	39	**0.0009**
	NCC		24.6	31	26.9	25	40.2	37	
Therapy	**GC**		52.8	56	35.2	25	23.4	18	GC vs. (CMV + MV)
	CMV		38.7	41	50.7	36	54.5	42	**<0.0001**
	MV		8.5	9	14.1	10	22.1	17	

Parameters which discriminated significantly ($p < 0.05$) according response to therapy were indicated by bold letters. BV = baseline values; d = day; AUCI-8/d = area under the curve of days 1 to 8; DEC = decrease (negative values indicating increases).

of diseases. Clinical variables that were available in defined categories were tested by the chi-square test. In order to identify the best predictive markers, cutoffs for each variable were defined at the 90% specificity for patients with remission, and sensitivities and positive predictive values for having progression of disease were calculated. Among all parameters, those with the best profile of sensitivity and positive predictive value were included in a multivariate analysis. Mantel–Haenszel statistics was used to test whether the predictive power of the markers was independent of relevant clinical parameters. Within the group of patients with clinically good performance status (ECOG1 + 2), the additive effect of the best predictive markers was shown by receiver operating characteristic (ROC) curves. A P value of $P < 0.05$ was considered statistically significant. All statistical analyses were performed with software of SAS (version 8.2, SAS Institute Inc., Cary, NC).

RESULTS

In lung cancer patients undergoing first-line chemotherapy, those with remission could be distinguished from those with progression by the pretherapeutic concentration of nucleosomal DNA fragments ($P = 0.022$), the baseline value before cycle 2 ($P < 0.0001$), values during the first therapeutic week at day 8 ($P < 0.0001$), and the area under the curve of the values from days 1 to 8 ($P = 0.0001$). In addition, the time course of nucleosomal DNA fragments showed a faster decline in patients with remission than in those with progressive disease, between the baseline values of cycles 1 and 2 ($P = 0.027$), and also during the first cycle between the pretherapeutic BV1 and the value at day 8 ($P = 0.002$). Similarly, the following CYFRA 21–1 values discriminated clearly between the groups: the pretherapeutic BV1 ($P = 0.0001$), the baseline value before cycle 2 ($P < 0.0001$), the value at day 8 ($P = 0.002$), the area under the curve of the values from days 1 to 8 ($P = 0.024$), and the kinetics from cycle 1 to 2 ($P < 0.0001$). However, changes in CYFRA 21–1 from days 1 to 8 during the first week of therapy were not significant ($P = 0.381$). Concerning other oncological biomarkers, CEA discriminated patients according to therapy response for the courses from cycle 1 to 2 ($P < 0.0001$) and from day 1 to 8 during the first cycle ($P = 0.023$); NSE for the baseline value before cycle 2 ($P = 0.012$). ProGRP was not capable to distinguish between the groups. Regarding clinical factors, stage (M0 vs. M1: $P = 0.0001$), performance status (ECOG 1 vs. 2: $P = 0.0004$; 2 vs. 3: <0.0001), histology (SC vs. AC + NCC: $P = 0.0009$), and mode of therapy (GC vs. CMV + MV: <0.0001) showed predictive potential, however, not age ($P = 0.3352$) or gender ($P = 0.2686$) (TABLE 2).

In order to identify the best predictive markers for insufficient response to therapy, cutoffs for all parameters were calculated at the 90% specificity for patients with remission, and profiles of sensitivity and positive predictive

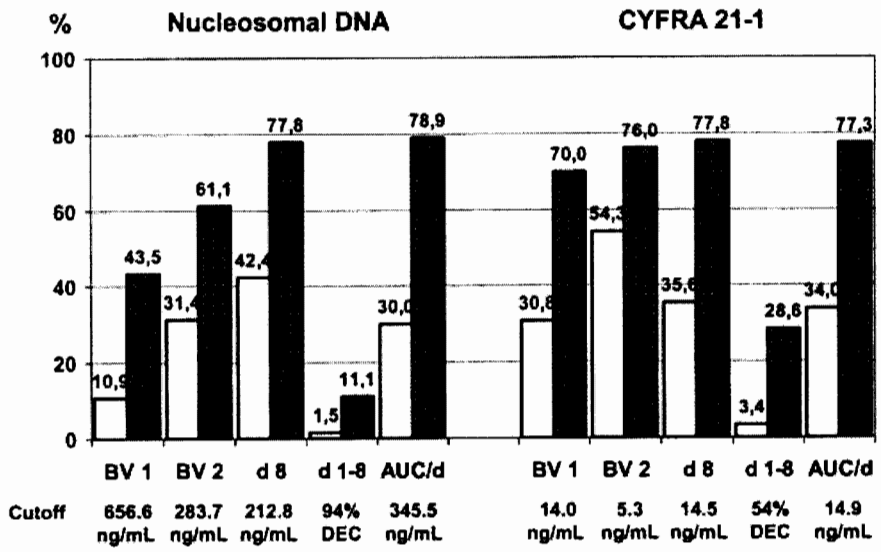

FIGURE 1. Profiles of sensitivity □ and positive predictive values ▩ (PPV) for prediction of insufficient response to therapy in patients with newly diagnosed NSCLC undergoing first-line chemotherapy for nucleosomal DNA and CYFRA 21–1 using cutoffs defined at the 90% specificity for patients with remission. Nucleosomal DNA at day 8 and the BV2 of CYFRA 21–1 exhibited the best profiles.

value (PPV) were established. Among all univariately predictive parameters, nucleosomal DNA at day 8 (sensitivity: 42.4%; confidence interval 30.6–55.2%; PPV: 77.8%; confidence interval 60.4–89.3%) and the BV2 of CYFRA 21–1 (sensitivity: 54.3%; confidence interval 42.0–66.1%; PPV: 76.0%; confidence interval 61.5–86.5%) exhibited the best profiles (FIG. 1).

Nucleosomal DNA fragments were found to be independent predictive markers with respect to CYFRA 21–1 ($P = 0.0012$), stage ($P = 0.0003$), and performance status ($P < 0.0001$) using Mantel–Haenszel statistics; and also CYFRA 21–1 predicted response to therapy independently from nucleosomal DNA ($P < 0.0001$), stage ($P < 0.0001$), and performance status ($P < 0.0001$) (TABLE 3).

In a multivariate model including nucleosomal DNA fragments and CYFRA 21–1, both biochemical parameters showed additive information for prediction of insufficient therapy response, particularly in a subgroup of patients with good clinical status (ECOG 1 + 2). As most of the patients with poorer performance status (ECOG 3 + 4) suffered from rapid progression of disease, the application of additional biochemical markers appeared to be superfluous in this setting. However, if, in patients with initially good clinical status, nucleosomal DNA fragments, and CYFRA 21–1 were combined, 100% specificity for prediction of progression was achieved with a sensitivity of 29% (FIG. 2). In a further subgroup of patients with pretherapeutic CYFRA

TABLE 3. Mantel–Haenszel statistics showing independency of nucleosomal DNA values of day 8 (d8) and baseline value 2 of CYFRA 21–1 (BV2) on each other, on stage, and performance status for the prediction of progressive disease

		Nucleosomal DNA (d8)		CYFRA 21–1 (BV2)	
Parameters	Subgroups	Relative risk	*P* value	Relative risk	*P* value
Nucleosomal DNA (d8)	<212.8 ng/mL			3.81	<0.0001
	≥212.8 ng/mL			2.37	
CYFRA 21–1 (BV2)	<5.3 ng/mL	2.51	0.0012		
	≥5.3 ng/mL	1.53			
Stage	UICC III	5.48	0.0003	2.04	<0.0001
	UICC IV	1.84		3.88	
Performance status	ECOG 1	3.00	<0.0001	6.33	<0.0001
	ECOG 2	3.08		3.35	
	ECOG 3	1.10		1.25	

21–1 values >3.3 ng/mL, the sensitivity for prediction of progression could be enhanced to 39.1% at 100% specificity. Although the various treatment protocols showed differences concerning therapeutic success, the power of prediction by nucleosomal DNA and CYFRA 21–1 was comparable in the groups receiving gemcitabine + cisplatin (GC) and carboplatin + mitomycin + vinblastin (CMV) with a sensitivity of 33.3% and 31.1%, respectively, at 100% specificity.

If patients with no change before cycle 3 were added to the "responsive" patient group if remission or stable disease was achieved before cycle 5 and added to the "non-responsive" group if progression occurred before cycle 5, the sensitivity was still 27% at a slightly lower specificity of 98%. The drop in specificity was on account of two patients with high concentrations of nucleosomal DNA and CYFRA 21–1 but nominal "no change" before cycle 5. However, both patients suffered from progressive disease 2 and 4 weeks, respectively, after this staging investigation was done.

DISCUSSION

Along with the development of new therapeutics in oncology, there is a growing need for diagnostic tools for estimating prognosis, treatment monitoring, and early prediction of response to therapy in order to optimize disease management on an individual basis. In patients with non-small cell lung cancer, a panel of clinical and biochemical parameters showed prognostic relevance.[6,7,10,14,18–20] Among them, CYFRA 21–1 was shown to have the strongest evidence as prognostic marker in the early, operable stages as well

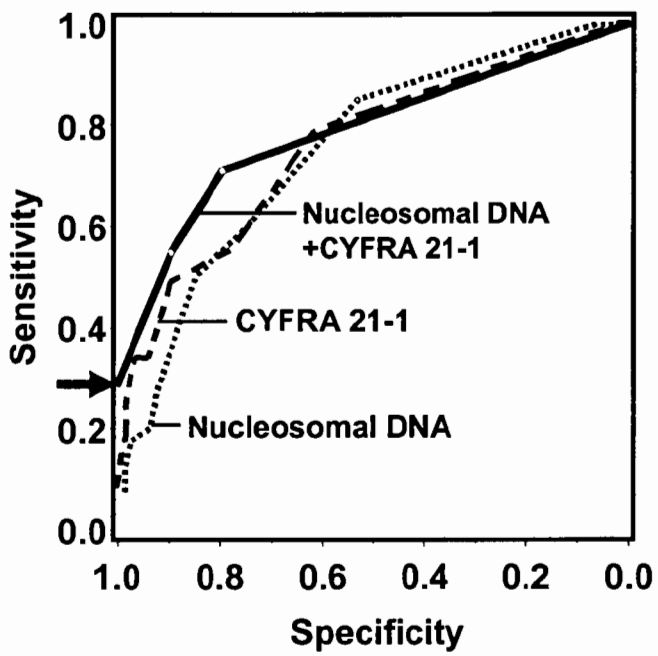

FIGURE 2. Prediction of therapy response by nucleosomal DNA and CYFRA 21–1 in patients with ECOG 1 + 2. ROC curves indicate the predictive power of nucleosomal DNA, CYFRA 21–1, and both markers revealing a clear additive effect. The combination curve meets the 100% specificity axis at a sensitivity level of 29%.

as in the late stages of non-small cell lung cancer.[19] In addition, independent prognostic value of lactate dehydrogenase, albumin, calcium, NSE, CEA, and DNA have been observed in various studies.[7,14,18–20] For monitoring systemic therapy and the early detection of recurrent disease in non-small cell lung cancer patients, CYFRA 21–1 and circulating DNA were frequently reported to be useful.[6,12–15,21–23] However, little is known about the relevance of biochemical markers for the *early* prediction of therapy response prior to imaging techniques.

Recently, we demonstrated that circulating nucleosomal DNA fragments and CYFRA 21–1 at the initial phase of therapy were able to distinguish between responders and nonresponders to chemotherapy.[16] On the basis of these results, we investigated in the present study the predictive power of nucleosomal DNA and CYFRA 21–1, and other relevant lung cancer biomarkers CEA, NSE, and ProGRP in a large sample of patients. As early prediction of insufficient therapy response would result in an early change of disease management in clinical practice, the predication had to be highly specific and sensitive. Therefore, we analyzed the predictive power of these parameters for poor therapy efficacy, especially during the initial treatment phase.

In univariate analysis, we confirmed our previous results that nucleosomal DNA and CYFRA 21–1 were able to distinguish patients according to their response to therapy: Patients with remission had lower levels for the pretherapeutic value and the baseline value before cycle 2, and greater decrease of the baseline values in the kinetic investigations than patients with progression of disease. Already during the first treatment cycle, nucleosomal DNA levels discriminated highly significantly between both groups. Patients with remission exhibited lower values at day 8 and smaller (AUC1–8/d) than patients with progressive disease. This might be on account of less aggressive tumors in responsive patients being related to lower rates of cellular turnover and cell death and more effective elimination of cell death products from circulation. Similar differences between patient groups were also observed concerning early CYFRA 21–1 courses, however, not as pronounced as for nucleosomal DNA during the first week. As the half-life of CEA in serum is considerably longer than the half-life of nucleosomal DNA and CYFRA 21–1, CEA did not show characteristic changes during the initial phase of therapy and discriminated according to response to chemotherapy only before start of the second cycle. As expected, small-cell cancer markers NSE and ProGRP were associated with weak or no predictive potential.

The best predictive markers for insufficient response to therapy were nucleosomal DNA at day 8 and the BV2 of CYFRA 21–1. This finding underlines the importance of the early changes in nonspecific circulating nucleosomal DNA fragments during therapy and the strong impact of somewhat later alterations in more specific CYFRA 21–1. Both variables were shown to provide additive information for therapy prediction independently from each other and from the most relevant clinical factors—stage and performance score. As shown by ROC curves, the combination of both markers reached 100% specificity for prediction of insufficient treatment response at a sensitivity level of 29% in the group of patients with pretherapeutic good clinical status (ECOG 1 + 2). This means that in about one-third of these patients, therapeutic response was predicted with a specificity of 100% after the first application of chemotherapy. In consequence, this information could have enabled an early change of the therapeutic regimen to avoid unnecessary side effects and enable more effective treatments in time. These results are not restricted to one specific chemotherapy but showed similar values in various protocols.

The correct classification of patients with stable disease in advanced non-small cell lung cancer is controversial. On the one hand it is important to stop the progression of the often already metastasized tumor disease. On the other hand, one would wish to achieve at least a partial remission during first-line therapy to prolong the survival of the patients. To take both aspects into account in our setting, patients with "no change" at cycle 3 were followed until staging before cycle 5. Those who showed no progression at that time were added to the responsive group while those with tumor progression joined the nonresponsive group. Following this procedure, the sensitivity for early prediction of progressive disease by combination of DNA and CYFRA 21–1

was still 27%; however, the specificity dropped to 98% because two patients with high levels of DNA and CYFRA 21–1 were in the "no change" group before cycle 5 but later found to show progressive disease, respectively.

It is worth noting that the levels of DNA and CYFRA 21–1 after the first application of chemotherapy were the strongest predictive markers. Many chemotherapeutic drugs are known to induce apoptotic cell death, which may result in a considerable release of these intracellular markers.[24,25] Thus, the increase in serum levels of DNA and CYFRA 21–1 reflect the spontaneous and the induced cell death, which might be the highest in less differentiated, very aggressive, and well-perfused tumors. The slower and incomplete decrease in nonresponsive patients could be influenced in addition by impaired elimination mechanisms and/or newly proliferating cell clones. An additional mechanism for DNA release, the active secretion by lymphocytes is still debated.[26] During apoptosis, most of cellular DNA is cleaved by endonucleases into mono- and oligonucleosomal fragments.[24] In this form, serum and plasma DNA seems to be better conserved from further digestion.[27] Methods that measure all cell-free DNA and those quantifying nucleosomal DNA showed a quite good correlation,[28] confirming earlier observations that most of the circulating DNA is bound to histones in nucleosomal complexes.[9] At least a substantial part of circulating DNA is of cancerous origin. Qualitative changes, such as specific mutations, microsatellite alterations, loss of heterozygosity, and epigenetic modifications were found in cell-free DNA as well as in tumor DNA.[15,29–31] In addition, tumor cells were found to be more susceptible to moderate radiation doses than normal epithelial cells resulting in higher release of nucleosomal DNA *in vitro*.[32] Thus, quantitative and qualitative aspects of circulating DNA have been shown to be helpful for diagnosis, prognosis, and therapy monitoring of various cancers.[10–15,24,29–31,33–36]

CYFRA 21–1 and CEA are oncological biomarkers that are more tumor specific and are currently used in the diagnosis and monitoring of non-small cell lung cancer.[6–8,21–24] However, neither their *early* predictive value for therapy response nor their additive effect to circulating DNA in diagnosis, prognosis, and therapy prediction have yet been shown.

The present study is to our knowledge the most comprehensive one in a welldefined patient population showing the high relevance of the combination of circulating nucleosomal DNA fragments and CYFRA 21–1 in predicting response to chemotherapy during the initial treatment phase. If these findings are confirmed by other prospective trials, the defined use of these parameters could contribute to improve the management of cancer patients.

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