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K. Junker · M. Thomas · K. Schulmann · F. Klinke
U. Bosse · K.-M. Müller

Tumour regression in non-small-cell lung cancer following neoadjuvant therapy. Histological assessment

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Abstract In the scope of a prospective multi-centre study after neoadjuvant combined chemotherapy (carboplatin, ifosfamide, etoposide, vindesine) and radiotherapy (45 Gy) 40 resection specimens of locally advanced non-small-cell lung cancer were analysed in order to establish reproducible pathological/anatomical results of tumour regression. Resection specimens of 28 squamous cell carcinomas and 12 adenocarcinomas were investigated using serial sections of the primary lesion. The mean age of the patients was 57 years. The results were compared to spontaneous regressive changes in a control group of 50 untreated non-small-cell lung cancers. Marked scarry fibrosis in the region of the former primary tumour, concentric foci of fresh tumour necroses and surrounding foam cell clusters with transition into vascular granulation tissue could be established as characteristic features of therapy-induced tumour regression, whereas untreated carcinomas revealed necroses with adjoining vital tumour tissue. Using a three-step regression system, 3 tumours could be classified as grade I (no or only slight tumour regression), 10 tumours as grade IIA (marked but incomplete tumour regression, more than 10% vital tumour tissue), 20 tumours as grade IIB (less than 10% vital tumour tissue) and 7 tumours as grade III (complete tumour regression without vital tumour tissue). After a median follow-up period of 32.3 months in patients with grade IIB or III tumour regression (''responders'') the median survival time of 27.9 months was found to be significantly longer than in patients with grade I or IIA tumour regression (''non-responders'') with a median survival period of 13.7 months (log-rank test, $P = 0.020$). The resection specimens analysed, which were obtained 7 weeks (on average) after the end of radiochemotherapy, did not show specific changes due to preoperative therapy, but quite characteristic histological alterations in the former tumour area were registered, which had been induced by combined neoadjuvant radiation and chemotherapy. The grade of therapy-induced tumour regression could be shown to be a significant prognostic factor in non-small-cell lung cancer.

Key words Non-small-cell lung cancer · Neoadjuvant therapy · Regression grading · Therapy-induced tumour regression · Spontaneous tumour regression

K. Junker (✉) · K. Schulmann · K.-M. Müller
Institute of Pathology, Bergmannsheil-University Hospital, Bürkle-de-la-Camp-Platz 1, 44789 Bochum, Germany
Tel.: 0049 234 3026620; Fax: 0049 234 3026671

M. Thomas
Department of Internal Medicine A, University Hospital, Münster, Germany

F. Klinke
Department of Thoracic Surgery, St. Elisabeth Hospital, Ibbenbüren, Germany

U. Bosse
Institute of Pathology, Osnabrück, Germany

Introduction

The overall prognosis of patients suffering from non-small-cell lung cancer is extremely poor, the 5-year survival rate being under 10%. The tumour stage at the time of diagnosis is of major importance for the prognostic outcome (Mould and Williams 1982). In a retrospective study including 3750 patients, Mountain (1986) found the 5-year survival rate of patients with stage I carcinoma to be between 36% and 72%; for patients in stage II it was between 23% and 34% and for patients in stage III it was between 5% and 8%; finally in stage IV it was only 2%.

While most patients with non-small-cell lung cancer stages I and II are subject to surgical therapy, and patients with stage IV carcinoma (distant metastases) receive only palliative or supportive treatment, therapeutic management of stage III tumours is still a matter of intense discussion (Mountain 1994; Rosell et al. 1994; Roth et al. 1994).

In order to improve the prognostic outcome of these patients suffering from locally advanced tumours, there have been attempts – mostly in controlled studies – to lower the rate of local recurrences and distant metastases by means of pre- or postsurgical chemoradiotherapy.

Keeping these facts in mind, in a phase II study, patients with locally advanced non-small-cell lung cancer received neoadjuvant combined chemoradiotherapy. In this study the regression grade achieved by presurgical therapy is to be seen as an indicator of individual sensitivity to chemotherapy, and thus of major prognostic importance for the respective patient.

On the basis of pathological/anatomical findings in the tumour specimens resected after neoadjuvant therapy, the type and extent of therapy-induced morphological alterations were analysed.

Materials and methods

After histological confirmation of the diagnosis, pre-therapy staging included the patient's history and clinical examination of the patient, an X-ray and a computed tomography (CT) scan of the thorax, CT scans of the abdomen and skull, bone scintigraphy, body plethysmography, mediastinoscopy with lymph node biopsy and bronchoscopy.

The present study included all patients fulfilling the following criteria.

Criteria leading to the inclusion of the patients

Histologically established non-small-cell lung cancer stages IIIA or IIIB
Functional operability
General condition according to Karnowsky at least 70%
Maximum patient age of 69 years
Sufficient liver and kidney function
Sufficient bone marrow reserve before onset of therapy
Patient's consent.

Criteria leading to the exclusion of the patients

Previous irradiation or chemotherapy
Resection of the primary lesion
Concurrent second malignant tumour
Manifest infection before onset of therapy.

Treatment protocol

The protocol consisted of three cycles of neoadjuvant therapy. Two cycles included combined chemotherapy with ifosfamide (1500 mg/m^2 body-surface area on days 1, 3 and 5 of each cycle) carboplatin (300 mg/m^2 body-surface area on day 1 of each cycle) and etoposide (100 mg/m^2 body-surface area on days 1, 3 and 5 of each cycle) (ICE protocol).

Radiotherapy consisted of twice-daily, accelerated irradiation. The overall dose of 45 Gy was applied over a period of 3 weeks. For 5 days a week, the patients received two daily doses of 1.5 Gy. At the same time, on days 1, 8 and 15, combined chemotherapy using carboplatin (100 mg/m^2 body-surface area) and vindesine (3 mg) was administered.

Two weeks after completion of the first two cycles of chemotherapy, interim staging I was performed, including an X-ray of the thorax, abdominal ultrasound and thoracic CT scan. In order to assess operability, 3 weeks after completion of radiochemotherapy interim staging II was performed, which, apart from the radiological techniques employed during the pre-therapy staging, included laboratory parameters and bronchoscopy. The clinical state of remission was established according to the definition of the Southwest Oncology Group (SWOG) (Green and Weiss 1992) (Table 1).

Operable patients underwent radical surgery with curative intention, after haemogram normalization and regression of non-haematological side-effects, 7 weeks, on average, after completion of radiochemotherapy (von Eiff et al. 1994; Thomas et al. 1995, 1996).

From April 1992 to September 1995, 64 patients were elegible for this study according to the criteria mentioned above. Retrospectively, 10 patients had to be excluded because of the histological demonstration of small-cell areas or cervical lymph node metastases, corresponding to stage M1, leaving a total of 54 patients whose data were analysed. In 40 of these patients, resection therapy was possible after combined chemoradiotherapy. Of the remaining 14 patients, 3 were technically and 2 functionally inoperable after radiochemotherapy. Three patients had developed distant metastases during therapy. Another 4 patients now refused surgery. One patient had died of pneumonitis, 1 of tumour bleeding prior to surgery. The surgical specimens obtained were morphologically investigated for therapy-induced alterations of the tumour tissue.

The formalin-fixed resection samples were first viewed macroscopically. Areas with likely vital tumour growth or previous, now regressively altered, tumour tissue were paraffin-embedded and serial sections were prepared. Depending on the size of the tumour, up to 58 paraffin blocks were prepared. For further evaluation, histological slides of the surrounding, macroscopically tumour-free parenchymal lung tissue were also prepared. In each case, haematoxylin/eosin and Elastica/van Giesson stains were available.

In order to determine the degree of tumour regression, the type and extent of vital tumour tissue and tumour necroses as well as reactive alterations with foam cell reaction and fibrosis or scar formation were taken into account. The findings were correlated to the following *regression grading*:

Grade I: no or only minor, mostly spontaneous tumour regression
Grade II: morphological signs of therapy-induced tumour regression
  Grade IIa: more than 10% vital tumour tissue
  Grade IIb: less than 10% vital tumour tissue
Grade III: complete tumour regression, no evidence of vital tumour tissue.

Regression grades IIb and III suggested a good response to neoadjuvant therapy; the responses of these patients were subsequently summarized in the "responder" group. The presence of 10% or more of vital tumour tissue (regression grades I and IIa) indicated a poor response to presurgical therapy; the details of these patients were then summarized in the "non-responder" group. The median survival period of the corresponding patients was correlated with regression grades I and IIa versus IIb and III. Statistical significance was tested using the log-rank test.

The relative amount of vital tumour tissue was determined morphologically using the prepared histological sections.

In order to differentiate therapy-induced alterations from spontaneous tumour regression, which is a frequent finding in malignant pulmonary tumours, 50 unselected non-small-cell lung cancers without previous presurgical chemoradiotherapy, taken from our current patient collective, were analysed with regard to spontaneous regressive alterations of the tumour tissue.

Results

The 40 resection specimens included 28 squamous cell carcinomas and 12 adenocarcinomas, according to their

respective predominant histological tumour type. Twenty lesions were classified as stage IIIA, 20 as stage IIIB; 4 of the patients were female, 36 were male; the average age was 57 years.

In 7 of the lesions treated with neoadjuvant therapy, complete tumour regression with no evidence of vital tumour tissue was seen (regression grade III). Circumscribed residual tumour foci of less than 10% were seen in 20 samples (regression grade IIb). Ten specimens showed more than 10% vital tumour tissue (regression grade IIa). In 3 samples no evidence of therapy-induced tumour regression could be established (regression grade I) (Fig. 1). Three lesions classified as regression grade IIb showed small residual tumour foci only in mediastinal lymph nodes, whereas no vital tumour tissue was seen in the former primary lesion.

In order to determine the "vitality" of the residual tumour foci in the respective resection specimens after neoadjuvant chemoradiotherapy, immunohistochemistry using the proliferation markers proliferating cell nuclear antigen (PCNA) and MIB-1 (Ki-67) was performed (modified ABC method according to Hsu et al. 1981). Every tumour classified as demonstrating regression grades I–IIb showed a positive reaction in the area of the histologically identified tumour tissue.

The most important characteristics of the patients and the corresponding tumours are summarized in Table 1.

Morphology of therapy-induced tumour regression

In 24 cases classified as regression grades IIa–III, different-sized target-like foci with central necrosis, mostly a narrow foam cell rim, vascular granulation tissue and marked peripheral scar formation, were demonstrated in the tumour areas (Fig. 2A). Foam cell clusters without central necrosis were found in 4 additional specimens (Fig. 2B, C). Towards the periphery, the foam cell rims and nests showed a transition into vascular granulation tissue. Twenty-three samples revealed cholesterol clefts, sometimes in association with a multinucleate giant-cell reaction, mostly in the marginal areas of tumour necroses or foam cell clusters (Fig. 2B). Towards the periphery, the granulation tissue showed a transition into fibrous connective tissue with large areas of scar formation and a focally accentuated increase or condensation of elastic fibres in 36 specimens (Fig. 2D). This scarry fibrosis was a major feature in 8 resection samples of regression grades IIa–III (Table 2).

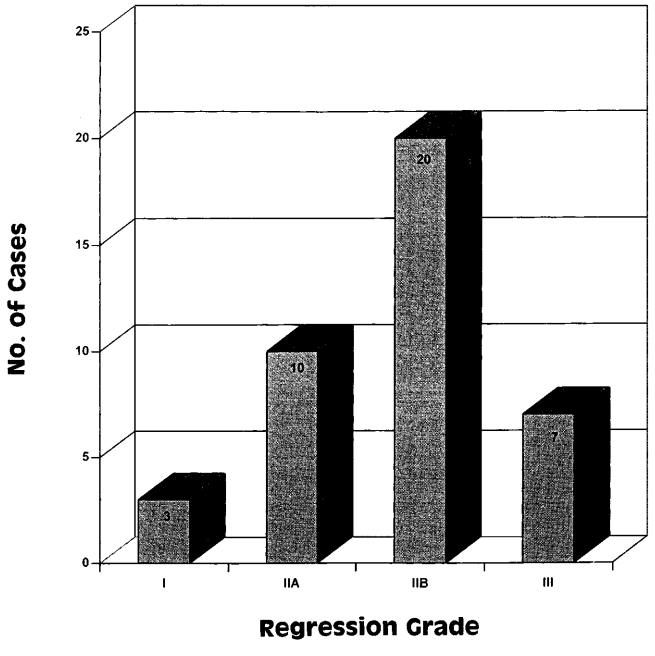

Fig. 1 Distribution of 40 non-small-cell lung cancers to the single grades of tumour regression following preoperative radiochemotherapy

In the area of the previous tumour or its surrounding tissue, all resection samples showed alterations in the vascular wall, ranging from endangiitis to complete scarry luminal obliteration. Several specimens even revealed a complete scarry bronchial obliteration. In these cases, the bronchial wall and peribronchial tissue were also affected by scar formation. Here, several areas with pneumocytic proliferation, sometimes going as far as to form tumourlets, were embedded in the surrounding scar tissue.

All resection samples investigated showed various numbers of intraalveolar macrophages with brownish pigmentation in the remaining pulmonary parenchymal tissue, independent of the regression grade. These macrophages were predominantly seen in the immediate neighbourhood of vital or by now regressively altered tumour tissue and revealed a positive Prussian blue reaction. Also, accompanying fresh haemorrhages of the pulmonary parenchyma were seen, ranging from small erythrocyte extravasates to larger haemorrhagic areas.

Apart from the therapy-induced histological alterations demonstrated, 26 resection samples also revealed cytological changes in the remaining tumour tissue, mainly a marked swelling of tumour cells with a sometimes honeycomb-like cytoplasm. Apart from nuclear pyknoses, mostly irregularly shaped, markedly enlarged and sometimes vacuolated cell nuclei were seen. These changes were seen in 1 tumour of regression grade I, 9 tumours of regression grade IIa and 16 tumours of regression grade IIb. 9 adenocarcinomas and 17 squamous cell carcinomas were affected. In lesions with regression grades I and IIa the cytological changes of the tumour tissue, described above, were always observed focally. In regression grade IIb the swollen tumour cells were generally arranged in a mesh-like pattern, embedded in a fibrous scar tissue.

Three resection specimens, which were classified as being of regression grade I, revealed major tumour areas. The necrotic tumour areas, which were present here too, did not exceed those seen in spontaneous tumour regression. Lesions with regression grade IIa showed parallel signs of spontaneous and therapy-induced tumour regression after neoadjuvant therapy.

Morphology of spontaneous tumour regression

In order to differentiate therapy-induced alterations reliably from spontaneous tumour regression, 50 non-small-cell lung cancers, which had not been subject to neoadjuvant irradiation or chemotherapy before, were analysed. These lesions included 31 squamous cell carcinomas and 19 adenocarcinomas. Six of the patients were female, 44 were male; the mean age was 61 years.

When these 50 cases of non-small-cell lung cancer were analyzed, no regressive foci corresponding to those described above, i.e. with a recurrent sequence of central necrosis, foam cell rim, granulation tissue, and peripheral scar formation, were seen.

However, 43 lesions of our control collective (86%) revealed vital tumour tissue as well as focal necrotic areas. Unlike therapy-induced alterations, these were characterized by immediately adjoining vital tumour rims (Fig. 3A, B). In several cases, vital tumour tissue, growing bud-like along capillary vessels, could be established in the marginal areas of relatively large necrotic zones (Fig. 3C). Rims of vital tumour tissue surrounding capillary vessels, with small necrotic areas in between, sometimes gave the impression of a comedo-like histological feature (Fig. 3D). Almost two-thirds (65%) of the tumours revealing signs of spontaneous tumour regression, or 56% of the complete control group, showed a granulocyte reaction, mostly in the marginal areas of the necrotic zones (Fig. 3B).

Six resection specimens without presurgical therapy showed small foam cell accumulations in the tumour areas. Only one case of the control group revealed a medium-sized necrotic area, partly with a vital tumour rim, partly with a rim-like foam cell reaction.

In only one case granuloma-like cholesterol clefts with giant-cell reaction were seen. This sample showed no further signs of spontaneous tumour regression.

Predominantly central scarry fibrosis of the tumour stroma was registered in 8 samples (Table 2).

Table 1 Tabulated survey of patients and tumour characteristics. *CA* clinical assessment, *RG* regression grade, *SCC* squamous cell carcinoma, *ADC* adenocarcinoma, *CR* complete response, *PR* partial response, *NC* no change

No.	Sex	Age (years)	Histology	TNM	Stage	CA	RG
1	M	67	SCC	T3N2	IIIA	PR	IIB
2	M	53	SCC	T3N2	IIIA	PR	III
3	M	59	SCC	T2N2	IIIA	PR	IIA
4	F	64	ADC	T2N3	IIIB	PR	III
5	M	57	SCC	T2N3	IIIB	PR	IIB
6	M	58	SCC	T2N2	IIIA	CR	IIB
7	M	47	ADC	T4N2	IIIB	NC	IIA
8	M	57	SCC	T3N2	IIIA	PR	IIB
9	M	49	SCC	T3N2	IIIA	PR	IIA
10	M	47	ADC	T4N0	IIIB	PR	III
11	M	55	SCC	T2N3	IIIB	PR	IIB
12	M	63	SCC	T4N2	IIIB	NC	IIB
13	M	64	SCC	T4N2	IIIB	PR	IIA
14	M	56	ADC	T4N1	IIIB	PR	IIB
15	M	59	SCC	T3N2	IIIA	PR	IIA
16	M	49	ADC	T2N2	IIIA	NC	III
17	M	43	SCC	T3N3	IIIB	PR	I
18	M	45	SCC	T2N2	IIIA	PR	IIB
19	M	62	SCC	T3N2	IIIA	PR	III
20	M	63	SCC	T3N3	IIIB	NC	IIB
21	M	51	SCC	T3N2	IIIA	PR	IIB
22	M	65	SCC	T3N2	IIIA	CR	IIB
23	M	58	SCC	T2N2	IIIA	NC	IIA
24	M	60	ADC	T2N2	IIIA	PR	IIB
25	M	61	ADC	T4N2	IIIB	NC	IIB
26	M	69	SCC	T2N2	IIIA	CR	I
27	F	37	SCC	T2N2	IIIA	PR	IIB
28	M	59	SCC	T2N2	IIIA	NC	IIB
29	M	52	SCC	T2N2	IIIA	CR	IIB
30	M	60	SCC	T4N3	IIIB	PR	III
31	M	60	SCC	T4N2	IIIB	PR	IIB
32	M	58	ADC	T3N3	IIIB	NC	I
33	F	52	ADC	T4N2	IIIB	PR	III
34	M	52	ADC	T2N3	IIIB	PR	IIA
35	M	37	SCC	T4N2	IIIB	PR	IIA
36	M	66	SCC	T3N1	IIIA	PR	IIB
37	M	65	SCC	T4N3	IIIB	PR	IIB
38	M	65	SCC	T2N3	IIIB	PR	IIB
39	F	51	ADC	T2N3	IIIB	PR	IIA
40	M	64	ADC	T2N2	IIIA	PR	IIA

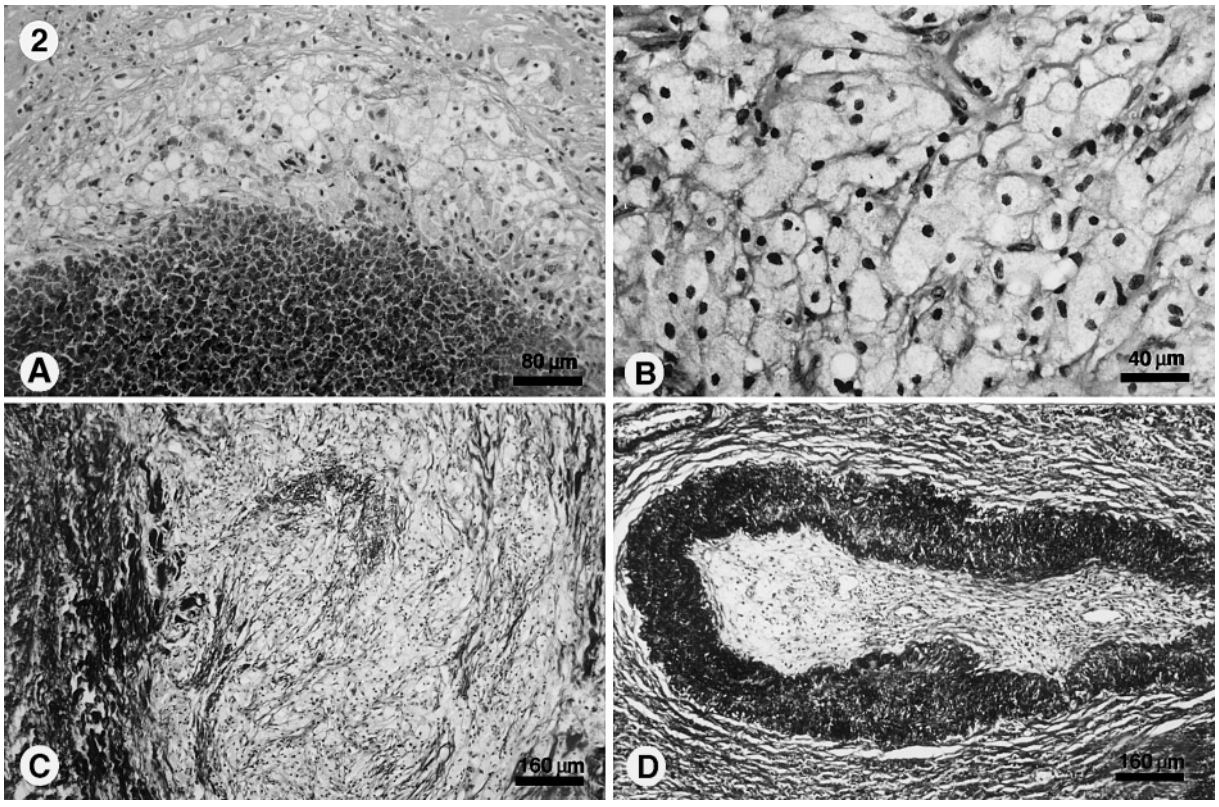

Fig. 2A–D Therapy-induced tumour regression in non-small cell lung cancer. **A** Segment of a regression area with centrally located therapy-induced necrosis and adjoining foam cell rim (haematoxylin/eosin, H&E). **B** Accumulation of foam cells without central necrosis (H&E). **C** Foam cell nest with transition into collagenous scar formation Elastica/van Giesson (EvG). **D** Complete scarry luminal obliteration of a medium-sized pulmonary artery in the region of the former primary tumour (EvG)

Clinical relevance of regression grading

The surgically treated patients included in this study, suffering from locally advanced lung cancers and having received neoadjuvant therapy, at present have a median follow-up period of 32.3 months (range: 5.8–53.9 months). The median survival time for this collective is 23.1 months (range: 5.8–53.9 months).

Table 2 Comparison of morphological changes caused by tumour regression in neoadjuvantly treated and untreated non-small-cell lung cancers (*NSCLC*). Whereas therapy-induced and spontaneous tumour regression overlap in treated tumours, untreated carcinomas show exclusively therapy-independent alterations

	Occurence (%)	
Morphological change	Treated NSCLC ($n = 40$)	Untreated NSCLC ($n = 50$)
Necrosis with foam cell reaction	60	2
Necrosis with adjacent vital tumour tissue	32.5	86
Cholesterol clefts	57.5	2
Giant cell reaction	55	2
Foam cells	70	12
Granulocyte reaction	15	56
Desmoplastic reaction/ scarry fibrosis	90	16

In these 40 surgically treated cases it was possible to employ a system of morphological regression grading based on the criteria described above. A total of 27 patients were classified as manifesting regression grades III or IIb. These patients had less than 10% vital tumour tissue in the former tumour area. In contrast, 13 patients had to be classified as having regression grades IIa or I, showing more than 10% vital tumour tissue in the former tumour area after combined chemoradiotherapy.

The 27 patients summarized in the ''responder'' group, having regression grades III and IIb, had a median survival period of 27.9 months (range: 6.5–53.9 months). The 13 patients summarized in the ''non-responder'' group, on the other hand, having regression grades IIa and I, had a median survival period of only 13.7 months (range: 5.8–32.3 months). In the log-rank test, these two groups showed a statistically significant difference in their median survival periods ($P = 0.020$). The corresponding Kaplan-Meier estimates can be seen in Fig. 4 (Kaplan and Meier 1958). In multivariate analysis according to the Cox regression model this could be shown to be an independent prognostic factor ($P = 0.0097$).

On the basis of the remission criteria of the Southwest Oncology Group (SWOG) (Green and Weiss 1992) 32 of the 40 surgically treated patients had partial or complete remission; 8 lesions showed ''no change''. Of these 8 lung tumours, 5 revealed less than 10% vital tumour

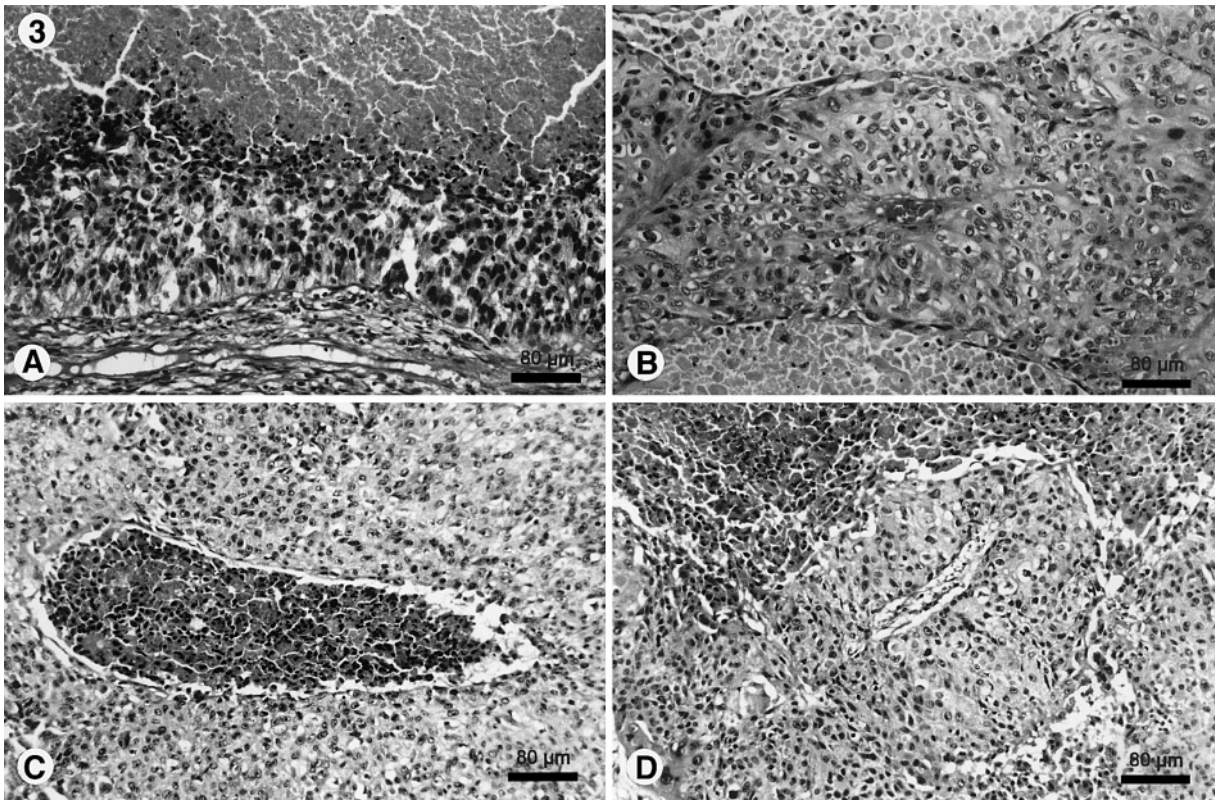

Fig. 3A–D Spontaneous tumour regression in non-small-cell lung cancer. **A** Zone of tumour necrosis with immediately adjoining vital tumour tissue revealing capillary-dependent growth (H&E). **B** Centrally located capillary with surrounding vital tumour tissue and tumour necrosis in the periphery (H&E). **C** Comedo-like aspect of spontaneous tumour regression with central necrosis and surrounding rim of vital tumour tissue (H&E). **D** Bud-like pericapillary tumour growth on the margin of an extensive tumour necrosis (H&E)

tissue in the resection specimens (regression grades IIb or III). Only 3 tumours showed regression grades I or IIa with more than 10% vital tumour tissue. All in all, the presurgical remission status after completion of neoadjuvant multimodal therapy had no predictive value for the evaluation of therapy-induced tumour regression in the corresponding resection specimens (Fisher's exact test: $P = 1.0$).

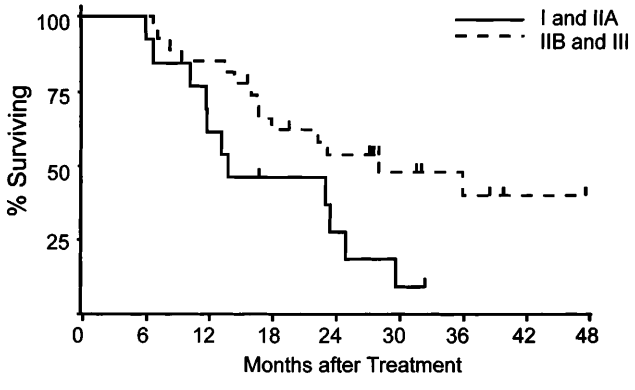

Fig. 4 Survival curves according to Kaplan and Meier for regression grades I and IIa ("non-responders") versus regression grades IIb and III ("responders") with a statistically significantly longer median survival period for patients with regression grade IIb or III (log-rank test, $P = 0.020$)

Discussion

In a phase II study, patients with locally advanced non-small-cell lung cancer (stages IIIA and IIIB) were subject to neoadjuvant combined chemoradiotherapy. The final evaluation included data for 54 patients. In this patient collective with its poor prognosis, the treatment protocol was found to be of high efficiency, with a remission rate of 72% and only 12% having progressive tumours.

After completion of the combined chemoradiotherapy, resection was possible in 40 patients, corresponding to nearly 75% of the cases. Of these 40 patients, tissue for determining the regression grade and for further histological processing was available.

Seven patients revealed complete tumour regression with no demonstration of residual tumour tissue, corresponding to regression grade III according to our grading system. All in all, together with another 20 resection specimens which showed only minor residual tumour foci, 27 cases (67.5%) showed less than 10% vital tumour tissue (regression grades IIb and III).

Citing the amount of vital tumour tissue as more or less than 10% must not be seen as an exact statement, but rather as an estimate based on numerous slides after complete histological preparation of the former tumour region. Even higher methodical efforts would not have

led to a more exact quantification since, in some cases, especially in those with good response to pre-surgical chemoradiotherapy, in tissue with marked scarry fibrosis it was not possible to determine exactly the border of former tumour tissue. In this context, the size of the tumour as determined radiologically or by CT scan prior to therapy and known to the examiner is not a reliable parameter, since perifocal oedema and tumour-associated inflammatory alterations can not be clearly differentiated from the tumour lesion itself.

Morphology of tumour regression

In a number of cases, the present study revealed comparatively typical morphological alterations, which may be explained by presurgical chemoradiotherapy.

In 24 resection specimens classified as regression grades IIa–III, different-sized, slightly target-like foci with a recurrent sequence of central eosinophilic necrosis, a surrounding resorption zone with numerous foam cells, transition into vascular granulation tissue and marked peripheral scar formation were observed. Another 4 resection samples showed basically similar areas, but without central tumour necrosis.

As reported in earlier studies on small-cell lung cancer (Junker et al. 1994, 1995; Müller et al. 1995) the observed regression foci may be seen as the morphological correlate of therapy-induced tumour regression. It has, however, to be pointed out that these are merely unspecific alterations.

The granulation tissue observed in the regression area showed a transition into fibrous collagenous connective tissue towards the periphery, sometimes forming large scar areas. In 8 resection samples classified as regression grades IIa–III, these fibrotic areas were the predominant feature of therapy-induced alterations.

Foam cells are a very striking and characteristic feature seen in regressively altered regions. As in so-called ''fatty streaks'' found in atherosclerotic lesions, their rim-like arrangement around the necrotic area suggests that, because of the sudden formation of tumour necrosis induced by chemotherapy, resorbing histiocytes are overloaded with lipids with subsequent transformation into foam cells (Gerrity 1981). The possible origin of these cells is seen either in the monocyte/macrophage system or in smooth muscle cells (Gerrity 1981; Klurfeld 1985; Schaffner et al. 1980). Our own studies, carried out in resection samples of small-cell lung cancer lesions after neoadjuvant therapy, revealed a continuously positive foam cell labelling with an anti-CD68 antibody, underlining the presumed derivation of foam cells from the monocyte/macrophage system (Dabbs 1993; Klurfeld 1985).

The demonstration of cholesterol clefts mostly in the marginal regions of tumour necrosis or foam cell nests may be explained by the fact that the degradation of foam cells releases stored lipids into the tissue, which will then crystallize. The free cholesterol crystals trigger a giant-cell foreign-body reaction, which we were able to demonstrate. All resected specimens revealed a marked siderophage reaction in the remaining lung parenchyma, independent of the regression grade, as well as fresh intraalveolar haemorrhages. However, both features showed no direct link to therapy-induced tumour regression.

Comparing therapy-induced and spontaneous regressive alterations of the tumour tissue, we found that they can be differentiated comparatively well. The most important differentiating criterion is the varying morphology of spontaneous and therapy-induced necrotic areas. Therapy-induced necroses mostly show a typical organisation with small foam cell rims, granulation tissue and peripheral scar formation; whereas spontaneously occurring necroses always reveal vital tumour rims. The features seen in the marginal areas of the necrotic fields are of major importance in differentiating spontaneous and therapy-induced tumour necroses.

So far, only a few studies have included a systematic analysis of histologically demonstrated findings in tumours after neoadjuvant therapy, with the aim of detecting regression patterns characteristic of therapy response. Such studies have been performed on osteosarcoma (Huvos et al. 1977; Picci et al. 1985; Rosen et al. 1982; Salzer-Kuntschik et al. 1983), Ewing's sarcoma (Roessner et al. 1986), carcinoma of the prostate (Dhom and Degro 1982; Helpap et al. 1985), oesophageal carcinoma (Mandard et al. 1994), and squamous cell carcinoma of the head and neck region (Al-Kourainy et al. 1987; Böheim et al. 1982; Sulfaro et al. 1989). In several of these studies statistically significant correlations between response to neoadjuvant therapy and survival period could be demonstrated, corresponding to our results. Most findings reported in these analyses, however, may not be transferred to malignant pulmonary lesions (Junker et al. 1995).

Morphologically established alterations after presurgical chemotherapy of locally advanced gastric carcinoma (Becker et al. 1996) show similarities to our own findings, especially regarding foam cell reactions. In one study on malignant primary and secondary liver tumours after high-dose regional cytostatic therapy, Fischer (1985) investigated therapy-induced tumour regression. According to his study, large necrotic areas in tumours with a high rate of spontaneous regression should only be seen as therapy-induced if they are localized close to the tumour margin or if the tumour margin is involved. However, this limitation can not be generally transferred to malignant pulmonary neoplasms. In these lesions, the different necrotic patterns seem to be more important.

Prognostic relevance of regression grading in non-small-cell lung cancer

Statistical evaluation using the log-rank test showed that patients with vital tumour tissue of less than 10%

(regression grades III and IIb) had a statistically significantly longer median survival period of 27.9 months than patients classified as manifesting regression grades IIa or I, who had a median survival period of 13.7 months (log-rank test, $P = 0.020$).

Bromley and Szur (1955) reported on 66 resected non-small-cell lung cancers after presurgical radiotherapy; 29 of these showed no vital tumour tissue. However, this was not connected with a longer survival period. Shields et al. (1970) too were unable to prove longer survival times for patients suffering from lung cancer who had undergone radiotherapy prior to surgery and shown histologically complete tumour regression.

As in our own study, positive correlation between histologically confirmed complete tumour regression and a more favourable prognostic outcome could be established in cases of neoadjuvant chemotherapy for the treatment of malignant lung tumours – either alone or in combination with radiotherapy (Faber et al. 1989; Weiden and Piantanosi 1988; Strauss et al. 1988; Sherman et al. 1987; Skarin et al. 1989; Eagan et al. 1987; Pisters et al. 1990; Burkes et al. 1992). Here, in up to 20% of the cases, complete tumour regression could be achieved (Faber et al. 1989).

In the series of Pisters et al. (1993), which included 21 cases of locally advanced non-small-cell lung cancer with histologically complete tumour regression after presurgical poly-chemotherapy, the patients showed significantly better survival periods with good functional results. Here, histologically confirmed complete tumour regression occurred only in those patients having a major clinical response to induction chemotherapy. The conclusion drawn from the results of this study, i.e. that histologically confirmed complete tumour regression should be considered as a major criterion or end-point in future neoadjuvant therapy trials, seems to be very problematic to us, if binding criteria concerning the extent of histological preparation of the tumours analysed are not defined at the same time. Our own experience has shown that, even after complete step-by-step processing of the former or remaining tumour tissue, continuous transition from complete to subtotal tumour regression may exist (our regression grade III and IIb), so that we favour the separation of responders and non-responders at a cut-off level of 10% vital tumour tissue, as we have done in our own study.

Several authors (Edelmann et al. 1996; Rusch 1993) discuss a discrepancy between the clinical response of the tumour to neoadjuvant multimodal therapy and the extent of histomorphologically determined tumour regression. In our own study too, we were able to show that the presurgical remission status is of no predictive value for indicating the extent of tumour regression in the resection specimen after chemoradiotherapy.

All in all, the available data show that, in patients suffering from non-small-cell lung cancer stage III, after neoadjuvant therapy the demonstration of a marked tumour regression in the resection specimen with less than 10% remaining vital tumour tissue (regression grades IIb or III) is significantly predictive of a longer survival period.

The demonstration of complete or predominating tumour regression may offer information concerning the expected benefits of a therapy protocol directly after completion of neoadjuvant treatment. Thus, this parameter may be included as an end-point in further neoadjuvant therapy studies for patients suffering from non-small-cell lung cancer stage III, if complete histological step-by-step processing of the resection specimens and resected mediastinal lymph nodes is carried out. It might be possible to assess whether the therapy concept employed has had a favourable or unfavourable influence after completion of the therapy, and well before the end of a long follow-up period, with faster consideration of the results in further studies. A standardized classification of tumour regression may also contribute to a clear description and better comparability of this parameter between different studies.

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References

Al-Kourainy, Kish J, Ensley J, Tapazoglou E, Jacobs J, Weaver A, Crissman J, Cummings G, Al-Sarraf (1987) Achievement of superior survival for histologically negative versus positive clinically complete responders to cisplatin combination in patients with locally advanced head and neck cancer. Cancer 59:233–238

Becker K, Fink U, Siewert JR, Höfler H (1996) Morphological effects of preoperative chemotherapy in locally advanced adenocarcinoma of the stomach (T3/T4) with EAP (etoposid, Adriamycin, cis-platin). Pathol Res Pract 192:341

Böheim K, Mikuz G, Böheim C (1982) Pathohistologische Veränderungen nach zytostatischer Induktionstherapie mit Methotrexat, Bleomycin und Cis-Platinum. Laryngol Rhinol Otol 61:246–250

Bromley LL, Szur L (1955) Combined radiotherapy and resection for carcinoma of the bronchus. Lancet 1955:937–941

Burkes RL, Ginsberg RJ, Shepherd FA (1992) Induction chemotherapy with mitomycin, vindesine, and cisplatin for stage III unresectable non-small cell lung cancer: results of the Toronto phase II trial. J Clin Oncol 10:580–586

Dabbs DJ (1993) Mammary ductal foam cells: macrophage immunophenotype. Hum Pathol 24:977–981

Dhom G, Degro S (1982) Therapy of prostatic cancer and histopathologic follow-up. Prostate 3:531–542

Eagan R, Ruud C, Lee R (1987) Pilot study of induction therapy with cyclophosphamide, doxorubicin, and cisplatin (CAP) and chest irradiation prior to thoracotomy in initially inoperable stage III M0 non-small cell lung cancer. Cancer Treat Rep 71:895–900

Edelmann MJ, Gandara DR, Roach M, Benfield JR (1996) Multimodality therapy in stage III non-small cell lung cancer. Ann Thorac Surg 47:669–675

Eiff M von, Geiger A, Wagner W, Rübe C, Klinke V, Thomas M, Loo J van de (1994) Neoadjuvant chemo-/radiotherapy in locally advanced non-small cell lung cancer. In: Rao RS, Deo MG, Sanghvi LD (eds) Proceedings of the 16th International Cancer Congress, Monducci, Bologna 1994. pp 1187–1190

Faber LD, Kittle CF, Warren WT (1989) Preoperative chemotherapy and irradiation for stage III non-small cell lung cancer. Ann Thorac Surg 47:669–677

Fischer HP (1985) Therapieinduzierte Tumorregression – Morphologische Befunde an malignen primären und sekundären Lebertumoren nach hochdosierter regionaler Zytostase. Pathologe 6:16–23

Gerrity RG (1981) The role of monocyte in atherosclerosis. I. Transition of blood-borne monocytes into foam cells in fatty lesions. Am J Pathol 103:181–190

Green S, Weiss GR (1992) Southwest Oncology Group standard response criteria, endpoint definitions and toxicity criteria. West New Drugs 10:243–253

Helpap B, Böcking A, Dhom G, Faul P, Kastendieck H (1985) Klassifikation, histologisches und zytologisches Grading sowie Regressionsgrading des Prostatakarzinoms. Pathologe 6:3–7

Hsu SM, Raine L, Fanger H (1981) Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and PAP. J Histochem Cytochem 29:577–580

Huvos AG, Rosen G, Marcove RC (1977) Primary osteogenic osteosarcoma. Arch Pathol Lab Med 101:14–18

Junker K, Krapp D, Müller KM (1994) Morphological changes in small cell bronchial carcinoma after neoadjuvant chemotherapy. In: Antypas G (ed) Proceedings of the 16th International Cancer Congress, Monducci, Bologna 1994. pp 133–136

Junker K, Krapp D, Müller KM (1995) Kleinzelliges Bronchialkarzinom nach Chemotherapie – Morphologische Befunde. Pathologe 16:217–222

Kaplan EL, Meier P (1958) Non-parametrics estimation from incomplete observations. J Am Statist Assoc 53:457–481

Klurfeld DM (1985) Identification of foam cells in human atherosclerotic lesions as macrophages using monoclonal antibodies. Arch Pathol Lab Med 109:445–449

Mandard AM, Dalibard F, Mandard JC, Marnay J, Henry-Amar M, Petiot JF, Roussel A, Jacob JH, Segol P, Samama G, Ollivier JM, Bonvalot S, Gignoux M (1994) Pathologic assessment of tumor regression after preoperative chemoradiotherapy of esophageal carcinoma. Cancer 73:2680–2686

Mould RF, Williams RJ (1982) Survival of histologically proven carcinoma of the lung registered in the North-West-Thames region. Br J Cancer 40:999–1003

Mountain CF (1986) A new international staging system for lung cancer. Chest [Suppl] 89:225s–232s

Mountain CF (1994) Surgery for stage IIIa-N2 non-small cell lung cancer. Cancer 73:2589–2598

Müller KM, Junker K, Wiethege T (1995) Nichtkleinzellige Bronchialkarzinome – Morphologie, Tumorregression, Molekularpatholgie. Onkologe 1:429–440

Picci P, Bacci G, Campanacci M, Gasparini M, Pilotti S, Cerasoli S, Bertoni F, Guerra A, Capanna R, Albisinni U, Galletti S, Gherlinzoni F, Calderoni P, Sudanese A, Baldini N, Bernini M, Jaffe N (1985) Histologic evaluation of necrosis in osteosarcoma induced by chemotherapy. Cancer 56:1515–1521

Pisters KMW, Kris MG, Gralla RJ (1990) Preoperative chemotherapy in stage IIIA non-small cell lung cancer: an analysis of a trial in patients with clinically apparent mediastinal node involvement. In: Salmon SE (ed) Adjuvant therapy of cancer. Saunders, Philadelphia, pp 133–137

Pisters KMW, Kris MG, Gralla RJ, Zaman MB, Heelan RT, Martini N (1993) Pathologic complete response in advanced non-small cell lung cancer following preoperative chemotherapy: implications for the design of future non-small cell lung cancer combined modality trials. J Clin Oncol 11:1757–1762

Roessner A, Krüger KH, Ritter J, Urbanitz D (1986) Zur Morphologie des Ewing-Sarkoms nach primärer Chemotherapie. GBK-Mitteilungsdienst 49:28–33

Rosell R, Gomez-Codina J, Camps C, Maestre J, Padille J, Canto A, Mate JL, Li S, Roig J (1994) A randomized trial comparing preoperative chemotherapy plus surgery with surgery alone in patients with non-small cell lung cancer. N Engl J Med 330:153–158

Rosen G, Caparros B, Huvos AG, Kosloff C, Nirenberg A, Cacavio A, Marcove RC, Lane JM, Mehta B, Urban C (1982) Preoperative chemotherapy for osteogenic sarcoma: selection of postoperative adjuvant chemotherapy based on the response of tumor to preoperative chemotherapy. Cancer 49:1221–1230

Roth JA, Fossella F, Komaki R, Ryan MB, Putnam JB, Lee JS, Dhingra H, De Caro L, Chasen M, McGavran M, Atkinson EN, Hong WK (1994) A randomized trial comparing perioperative chemotherapy and surgery with surgery alone in resectable stage IIIA non-small cell lung cancer. J Natl Cancer Inst 86:673–680

Rusch VW (1993) Neoadjuvant chemotherapy for stage III lung cancer. Semin Thorac Cardiovasc Surg 5:258–267

Salzer-Kuntschik M, Brand G, Delling G (1983) Bestimmung des morphologischen Regressionsgrades nach Chemotherapie bei malignen Knochentumoren. Pathologe 4:135–141

Schaffner T, Taylor K, Bartucci EJ (1980) Arterial foam cells with distinctive immunomorphologic and histologic features of macrophages. Am J Pathol 100:57–80

Sherman D, Strauss G, Schwartz J (1987) Combined modality therapy for regionally advanced stage III non-small cell carcinoma of the lung (NSCLC) employing neo-adjuvant chemotherapy (CT), radiotherapy (RT), and surgery (S). Proc Am Soc Clin Oncol 6:167

Shields TW, Higgins GA, Lawton R (1970) Preoperative X-ray therapy as an adjuvant in the treatment of bronchogenic carcinoma. J Thorac Carciovasc Surg 59:49–61

Skarin A, Jochelson M, Sheldon T (1989) Neoadjuvant chemotherapy in marginally resectable stage III M0 non-small cell lung cancer: long-term follow-up in 41 patients. J Surg Oncol 40:266–274

Strauss G, Sherman L, Mathisen D (1988) Concurrent chemotherapy (CT) and radiotherapy (RT) followed by surgery (S) in marginally resectable stage III non-small cell carcinoma of the lung (NSCLC): a Cancer and Leukemia Group B study. Proc Am Soc Clin Oncol 7:203

Sulfaro S, Frustaci S, Volpe R, Barzan L, Comoretto R, Monfardini S, Carbone A (1989) A pathologic assessment of tumor residue and stromal changes after intraarterial chemotherapy for head and neck carcinomas. Cancer 64:994–1001

Thomas M, Macha HN, Rübe C, Loo J van de (1995) Stellenwert der Chemotherapie in der multimodalen Behandlung des nichtkleinzelligen Bronchialkarzinoms. Dtsch Med Wochenschr 120:1627–1630

Thomas M, Eiff M von, Rübe C, Klinke V, Diederich S, Loo J van de (1996) Erfolgreiche multimodale Behandlung eines lokal weit fortgeschrittenen nicht-kleinzelligen Bronchialkarzinoms. Dtsch Med Wochenschr 121:658–662

Weiden P, Piantadosi S (1988) Preoperative Chemoradiotherapy in stage III non-small cell lung cancer (NSCLC): A phase II study of the lung cancer study group (LCSG). Proc Am Soc Clin Oncol 7:197