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## **Introduction to prognosis of lung cancer**

Together with the performance status, the TNM-staging - based on anatomical spread - nowadays still is the most important tool to make estimates of prognosis for lung cancer patients, and to choose the best combination of treatment modalities such as surgery, radiation and chemotherapy. The TNM-staging gives an answer to the question “where in the body is the disease?”. The clinical, or even pathological, TNM-staging, however, does not always give a satisfactory explanation for differences in survival. Resected stage I NSCLC is a typical example: many patients are cured, but some have an early relapse and die.

In recent years, it has become clear that, in order to understand more about different patterns of survival in resected stage I NSCLC, and lung cancer in general, we should also answer the question “what is the character of the disease?”. Standard pathological typing and grading is only a rough answer to this question. Better understanding of the factors determining aggressiveness of lung cancer, would help us to determine which patients should be followed more closely, which patients are candidates for (neo)-adjuvant treatments, or even find out which patients are poor surgical candidates even if they have apparently localised disease.

In recent years, many groups have analysed different potentially important prognostic factors, especially in the field of molecular biology, and this has generated an overwhelming amount of data. A Medline search using the terms “prognostic factor” and “cancer” produced 2,393 papers according to a recent editorial [1]. A major challenge for future revisions of the TNM-system will be to what extent the influence on prognosis of the rapidly evolving data on these prognostic factors will be integrated in the staging system for lung cancer, in order to implement new techniques in the best interest of the patient, without losing the widespread applicability of the TNM-system.

It is not and cannot be the aim of this contribution to give a comprehensive overview of all potentially relevant prognostic factors in non-small cell lung cancer (NSCLC), but rather to put the different contributions of this symposium into clinical perspective.

### **Clinical factors in stage III NSCLC**

#### *Pre-treatment prognostic factors*

Apart from the TNM and performance status, some other clinical characteristics have also been reported to be of prognostic relevance. Probably one of the most important is weight loss, but even other symptoms such as haemoptysis, cough, or chest pain might also have some importance. In a study on 289 consecutive stage I NSCLC patients, it was reported that patients without symptoms had a 5-year survival of 74%, while this was only 41% in case of symptoms ( $P=0.001$ ), and that this factor remained significant in a multivariate analysis ( $P<0.05$ ) [2]. The use of clinical factors for staging non-metastatic lung cancer nevertheless is of limited value, first because of the low prevalence of symptoms in these patients, and second because of the subjective nature of symptoms.

When looking at prognostic factors in locally advanced NSCLC, we should realise that the recently revised staging system has mainly been derived from a patient population database where the majority of patients were treated with surgery as the dominant treatment modality [3]. As we have learned from other solid tumour models as well as from other systemic malignancies (such as lymphomas or leukaemia's), prognostic factors are also closely related to the treatment that is performed. Any introduction of new or different, e.g. more aggressive treatment strategies, may thus lead to a change in the pattern of prognostic factors being of significance within these patient populations. With the treatment of stage III NSCLC being currently under a rapidly changing influence of new developments from combined modality

treatment protocols, one has to keep in mind this background when looking at any evaluation of prognostic factors [4].

At the meeting, **Eberhardt** presented an overview on possible relevant prognostic factors in stage III disease NSCLC by carefully reviewing the current literature concerning that issue. A major impact was laid on the availability of long-term survival data (at least 3-, 4- or 5-year survival rates) from analysis of different treatment strategies and within distinct patients populations. Analysis was separated into a) surgical treatment alone (table 1); b) radiation or combined chemoradiation treatment (table 2); and c) combined modality protocols including surgery (table 3). As said above, one has to be cautious to differentiate *pre-treatment* prognostic factors from prognostic factors *with any relation/dependence on treatment effects* (e.g. clinical or pathological treatment response, as explained in the paragraph below) that cannot be used as parameters to be potentially included into a conventionally intended pre-treatment staging system but possibly into patients selections procedures during complex treatment protocols.

In tables 1, 2 and 3, a brief overview on potential pre-treatment prognostic factors under investigation in the different treatment strategies is given. Factors, which have a significant influence on long-term survival in individual publications only are listed in italics.

Only a very small group of patients of stages III is nowadays still considered for any *upfront surgical approach* (table 1): patients with either minimal mediastinal N2 involvement and some selected T4N0-1 patients are taken to thoracotomy in different institutions with experience in such extensive surgical approaches [5-7].

Patients who are taken *onto radiotherapy or combined chemoradiation trials* have been analysed on possible prognostic factors in large databases (e.g. the Radiation Therapy Oncology Group (RTOG) database). With radiotherapy included, factors of tumour

size/tumour volume or tumour burden become of critical importance (table 2). Interestingly, pre-treatment haemoglobin levels seem to be of major impact once radiotherapy is to be included into treatment (table 2). The RTOG and his personal experience was put into perspective by **Jeremic**. Although gender was occasionally examined as potential prognostic factor in inoperable NSCLC, results obtained are conflicting. While surgical studies and some studies using chemotherapy alone in metastatic disease did not indicate clear impact of gender, International Association for the Study of Lung Cancer (IASLC) indicated gender as possible prognostic factor [8]. If so, then impact of gender needs to be better explained since it was observed that females have better outcome, even when stratified for stage and other adverse risk factors [9]. This has been observed in a number of studies which used chemotherapy with or without radiotherapy or in the studies using radiotherapy alone [10,11]. Age was not consistently shown to have an independent influence on survival. Possible difference in biological aggressiveness, or lower survival in older (e.g. >70 years) patients due to pre-existing co-morbidity, may be the reason. The latter case is well proven in surgical series, but not in unresectable disease. Some indicated only a minor influence of age as prognostic factor in studies that used chemotherapy with or without radiotherapy [11], sometimes favouring older patients [10]. Others indicated that age may be an important prognostic factor [12-14], favouring younger patients. In a quality-adjusted survival analysis of the RTOG, patients <70 years had improved survival with more aggressive therapy (induction chemotherapy and radical standard radiotherapy or concurrent chemotherapy and hyperfractionated radiotherapy, in contrast to patients >70 years who qualitatively benefited mostly from less aggressive therapy such as standard fraction radiotherapy alone [15]. IASLC indicated probable lack of independent influence in their statement in 1994 [8]. With respect to performance status (PS), almost all series that evaluated the impact of this potential prognostic factor reported a significant difference in survival favouring patients with higher

PS [16-23]. Recent IASLC consideration of prognostic factors in NSCLC, clearly indicated PS as one of the two definite prognostic factors influencing survival [8]. Recently, RTOG [24] noted that of all patients with unresectable NSCLC treated with either radiotherapy alone or various chemoradiotherapy regimens, those with poor PS were at high risk for death without progression, so that they should probably be removed from the context of current clinical trial and possibly be investigated as a separate entity requiring a different approach. In another RTOG report [15], quality-adjusted survival showed that patients with poor KPS (50-70) had the lowest median survival (7.8 months) and the lowest quality-adjusted survival time (6.7 months). Influence of weight loss was evaluated in many studies and almost uniformly it was found that patients having marked weight loss did worse than those with less pronounced weight loss [11,16-23]. Weight loss indicates a generalised influence of the disease, having interrelation with the stage of the disease and performance status and it is not surprising that patients with previous weight loss have a worse prognosis. International Association for Study of Lung Cancer (IASLC) indicated the weight loss as probable prognostic factor in NSCLC [8]. An issue remains on the table when dealing with weight loss as a potential prognostic factor for NSCLC: weight loss definitions are many and different, and may have influenced the value attributed to such a prognostic factor. A recent study, evaluating the most commonly adopted definitions (i.e., less or more than 10 pounds, less or more than 5 or 10 percent of the weight measured at 6 months prior to diagnosis), seems to suggest that the percentage of the total weight loss -with no time boundaries- is the most prognostically meaningful way to record it.

Considering the primary tumour, squamous histology was usually considered as more “localised” form of NSCLC, while there were observations that this may not be the case for adenocarcinoma and large cell carcinoma. RTOG [24] observed that when treated with chemoradiotherapy, adenocarcinomas was less likely to progress at the primary than either

squamous cell or large cell carcinomas, but adenocarcinomas were more likely to spread to brain and other distant sites than squamous cell carcinomas. The authors concluded that squamous cell carcinomas should be treated with more “local” forms of treatment. The significantly higher risk of brain metastasis in patients harbouring either adenocarcinoma or large cell carcinoma may warrant for new strategies, including prophylactic cranial radiotherapy in selected cases and more efficient chemotherapy, needed also to combat distant spread. Finally, an interesting finding that squamous histology carried increased risk of death without progression, may suggest that this histological subtype of NSCLC might require a different approach.

In those patients put on *preoperative chemotherapy or chemoradiotherapy trials*, patient selection to an inclusion of possible surgical resection becomes important. Therefore, patients subgroups with T4N0-1 disease and without any mediastinal involvement seem to have the best prognosis among those with stage IIIB (table 3). Within stage IIIA, of major importance seems to be the detailed extent of mediastinal involvement, especially the number of involved mediastinal lymph nodes (levels) [5,7]. Pre-treatment LDH seems to reflect the overall tumour burden and may therefore be of major influence on long-term survival for these groups (table 3). In general, it has to be critically remarked, that most investigations on prognostic factors in stage III patients groups did not perform multivariate statistical analyses. Most factors have only been investigated by univariate analyses, mainly because the patients numbers of individual investigations were mostly very small.

In conclusion, the existence of different multimodality strategies at the moment does not allow to define a more generally available prognostic index for stage III NSCLC. Therefore, planned improvements in pre-treatment staging systems for these patients groups will probably be hampered by the existing different treatment approaches and will also be difficult

to be generalised [25]. This will even more depend on the use of newer and more sensitive staging investigations such as more widespread use of mediastinoscopy or FDG-PET. Therefore, any group presenting data on treatment in stage III NSCLC has to define clearly their upfront staging investigations, their prospectively planned treatment protocol, and has to record prospectively different pre-treatment parameters of their included patient population.

#### *Therapy-related prognostic factors in locally advanced NSCLC*

With the advent of several combined modality treatment strategies, these prognostic factors, usually parameters that reflect the clinical and even more the pathological response to any induction therapy have become of special importance (table 4). It seems that the performance of a complete resection of any residual (vital) tumour into the treatment is one of the major independent factors determining long-term survival both in the IIIA as well as the IIIB subset. The importance of the *morphology of tumour response* to chemo- and radiotherapy in correlation with prognosis was presented in more detail by **Junker**.

Studies on neoadjuvant therapy have demonstrated that the degree of tumour regression in resection specimens of primary tumour and lymph nodes is suggestive for long-term survival [26,27]. To determine whether reproducible pathological-anatomical findings of therapy-induced tumour regression could be demonstrated, resection samples of locally advanced NSCLC after neoadjuvant multimodality treatment were analysed. The neoadjuvant treatment, consisting of 2 cycles of carboplatin-etoposide-ifosfamide chemotherapy, followed by a third cycle of concurrent chemoradiotherapy (carboplatin-vindesine plus twice daily 1.5 Gy radiation therapy up to 45 Gy), has been described in detail elsewhere [28]. After completion of combined chemoradiotherapy, patients who remained free of distant metastases underwent resection with extensive mediastinal lymph node sampling. For the first time in

this treatment approach, a regression grading scheme (table 5) was applied and its impact on survival was assessed [29].

Of the 54 patients (25 stage IIIA and 29 stage IIIB) treated in this multimodality protocol, 40 went on to resection. Examination of these 40 resection specimens revealed type III regression in 7 (17.5%), type IIa regression in 20 (50%), type IIb in 10 (25%), and type I in 3 (7.5%). Grouped together, 27 tumours (67.5%) showed regression grades IIb or III, 13 (32.5%) regression grades I or IIa.

In 24 patients with regression grades IIa to III, different-sized target like foci with central necrosis, narrow foam cell rim, vascular granulation tissue and marked peripheral scarring were demonstrated in the tumour region. In addition, foam cell nests without central necrosis were present in four resection specimens. Towards the periphery, the foam cell rims and nests showed transition into vascular granulation tissue and adjoining cicatrization, sometimes forming large scar areas. Comparatively large tumour areas were present in the three samples classified as regression grade I. The areas of tumour necrosis, seen in these cases, did not exceed the extent of spontaneous tumour regression. In tumours after neoadjuvant therapy classified as regression grade IIa, signs of spontaneous and therapy-induced tumour regression were found simultaneously.

Correlation between pre-surgical clinical response (based on CT) and therapy-induced tumour regression in the resection specimen was poor ( $P=1.0$ ). Of 8 patients with “no change” on CT, five had less than 10% vital tumour tissue (regression grades IIb/III).

Patients with regression grades IIb and III ( $n=27$ ) showed a significantly longer survival compared to those classified as regression grades I and IIa ( $n=13$ ) (median survival, 36 vs. 14 months; 3-year survival rate 52% vs. 9%;  $P=0.02$ ). In a multivariate analysis according to the Cox proportional hazards regression model, independent prognostic factors were the grade of tumour regression (I/IIa vs. IIb/III,  $P=0.007$ ), the resection status (complete vs. incomplete,

P=0.009), but not clinical response as assessed on CT (response vs. no change or progression, P=0.1).

In multimodality induction protocols, spontaneous and therapy-induced tumour regression thus can be distinguished with high certainty [29]. Several morphological changes which are due to neoadjuvant chemoradiotherapy were demonstrated. The described regression foci may be seen as the morphological expression of therapy-induced tumour regression. However, it has to be pointed out that the single changes are merely non specific, but, in their entirety, enable us to draw reliable conclusions regarding the response to pre-surgical therapy.

Several groups have reported a positive correlation between histologically confirmed complete tumour regression after neoadjuvant chemotherapy, alone or in combination with radiotherapy, and favourable prognosis [30-36]. Moreover, several trials demonstrated a significant survival advantage according to the extent of histomorphological response after chemoradiation [26-28,33]. A high rate of pathological complete response (39%) was reported by Eberhardt et al., but no differences in long-term survival were found between patients who had a pathological complete response and those with persistent viable tumour [37]. In contrast to the trials mentioned above, no standardised histological examination of the entire tumour area was carried out in the study of Eberhardt et al.

The determination of complete or predominant tumour regression immediately after completion of neoadjuvant therapy may offer information on the potential benefits of a therapy concept. So, this parameter may be included as an intermediate end point in future neoadjuvant trials on patients with stage III NSCLC [26,33]. However, complete step-by-step processing of the primary tumour area in the resection specimens and the resected mediastinal lymph nodes is mandatory. A favourable or unfavourable value of the therapy concept may possibly be determined immediately after completion of the therapy and before reaching a certain follow-up period. Thus, following studies may be started earlier. Standardised

classification of tumour regression may also assist in creating an unambiguous description and subsequently better comparability of this parameter in different studies.

The finding that response on CT is a poor predictor for histomorphologically determined tumour regression has been discussed by several other authors [38,39]. This can be explained by the fact that vital tumour can not be differentiated from already necrotic tumour tissue or scar formation on CT. After neoadjuvant therapy, "tumours" of several centimetres may well show marked or even complete tumour regression, so that a generous indication for surgery is justified in cases of "no change" under therapy. Recent pilot data have suggested that FDG-PET might be a better imaging technique to assess pathological tumour response [40].

### **Standard biological factors**

Amongst the different standard biological tests of potential relevance to the prognosis of NSCLC, the role of leukocytes and tumour markers was highlighted at this workshop.

#### *Leukocytes*

The experience of the European Lung Cancer Working Party about the usefulness of leukocyte and neutrophil counts as additional independent prognostic factor in NSCLC was reviewed by **Paesmans**.

A prognostic factor analysis on a series of 1052 patients with advanced NSCLC included in seven clinical trials performed by this Group during a ten years period from December 1980 to August 1991 was carried out [41]. These trials were mainly randomised trials testing chemotherapy regimens based on platinum derivatives. By uni- and multivariate parametric and non-parametric methods, the prognostic value of 23 routinely assessed variables including clinical factors related to the patient or to the tumour (sex, age, body weight loss,

histology, prior therapy, type of lesions, disease extent and metastatic sites) and biological factors (leukocytes, neutrophils and platelets counts, haemoglobin, serum alkaline phosphatases, serum LDH, bilirubin, creatinine and calcium) were assessed. Survival was measured from the day of registration in the trials. All biological covariates were dichotomised. Pre-therapeutic absolute white blood cell and relative neutrophil counts were respectively considered as abnormal if  $>10 \times 10^3$  cells/ $\mu\text{l}$  and  $>75\%$  of the total white blood cells. At the time of analysis, median follow-up was 270 weeks and 951 events (90%) were observed. Both abnormal white blood cell and neutrophil counts were identified as poor prognostic factors (median survival times of 24 versus 32 weeks for white blood cells,  $P<0.0001$  and 22 versus 32 weeks for neutrophils,  $P<0.0001$ ). Rates of abnormal values were 40% and 36%. On parametric multivariate analysis, an independent prognostic value was found for these two covariates with hazard ratios of 1.33 (95% CI 1.10-1.63,  $P=0.0003$ ) for abnormal white blood cells count and of 1.27 (95% CI 1.08-1.59,  $P=0.02$ ) for abnormal rate of neutrophils. The other variables explaining heterogeneity in survival were disease extent, Karnofsky performance index, age, sex, presence of skin metastases and serum calcium level. Using non-parametric recursive partitioning and amalgamation algorithms, we constructed a patients classification into four groups with different survival distributions. White blood cell and neutrophil counts were implied in the definition of the two intermediate groups.

In a second step, the data relative to the clinical trials performed during the last decade were analysed, in order to see if this prognostic value found for leukocyte count and neutrophil count were reproducible. The first trial was one in patients with unresectable locoregional NSCLC, who were treated with an induction chemotherapy regimen (mitomycin C- ifosfamide-cisplatin). The objective responders to this induction treatment were randomised to three further courses of the same chemotherapy or to chest radiation. Four hundred sixty-two eligible patients were registered. The univariate prognostic value of both haematological

counts were confirmed with median survival times of 36 weeks vs. 48 ( $P=0.001$ ) in case of elevated leukocytosis and of 33 weeks vs. 51 ( $P<0.001$ ) in case of abnormal neutrophil count. Using Cox multivariate regression models, the independent value of neutrophil count was found again with a hazard ratio of 1.48 (95% CI 1.19-1.83,  $P<0.001$ ) adjusted for performance status, age, platelets count and combination of T3-4 tumour stage and N3 nodal status. The second trial was done in stage IV NSCLC and compared a treatment with mitomycin-ifosfamide plus standard dose cisplatin to mitomycin-ifosfamide plus moderate dose cisplatin and carboplatin. Two hundred ninety-seven patients were eligible. The results were similar to those found for locoregional disease. Univariate analysis confirmed the prognostic value of both counts and in multivariate Cox regression. A hazard ratio significantly different from 1 (1.73, 95% CI 1.32-2.28,  $P<0.001$ ) for abnormal neutrophil count was found, with adjustment for performance index, sex and prior therapy.

Biological interpretation of the prognostic relevance of white blood cell indices is not obvious, but we can hypothesise that increased neutrophilia might be a marker of a respiratory infection linked to the bronchial tumour and therefore associated with a poorer prognosis. The assessment of the prognostic value of leukocytosis and neutrophil count has not been extremely frequent in the literature, at least on large series of patients, but some other authors, but not systematically, identified hyperleukocytosis as an independent factor associated with a worse prognosis in NSCLC [42,43].

#### *Standard serum tumour markers*

The potential use of serum tumour markers was summarised by **Buccheri**. Neoplastic cells produce and release several substances. Such substances are tumour-specific and can be produced by one, few, or several types of cancer. Other substances are produced by tumour cells in larger amounts than by normal cells. Occasionally, normal cells release abnormal

quantities of their products in response to invasion by cancer cells. Independently of the mechanism of production, an array of biological substances “marks” the existence, clinical course, and the destiny of certain types of cancer. These substances are called “tumour markers”.

The term tumour marker is sometimes broadly used to include any tumour cell surface antigen, intracellular protein, or even chromosomal or genetic abnormality detectable in the patients’ body fluids, tissue or cytological specimens [44]. In this contribution, serum tumour marker refers exclusively to substances present in the blood of patients with lung cancer, that are suitable for an easy and inexpensive serum test.

The expression of lung tumour markers is known since many years [45-47]. Lung tumour markers fall into several categories, including oncofetal proteins, structural proteins, enzymes, membrane antigens, peptide and non-peptide hormones, and other tumour-associated antigens [46]. A list of lung tumour markers that were used frequently over the past 10 years has been published recently [48]. Lung tumour markers may play different roles in clinical practice including the assessment of prognosis [48].

At least 3 classes of tumour markers have prognostic significance in NSCLC: CEA [7,49-53] and the two cytokeratin-derived markers TPA [54-58] and Cyfra 21-1 [59-61]. Of course, these tumour markers may predict clinical outcome mainly because their evaluation correlates with the tumour mass [48]. However, there are examples in which the correlation with prognosis directly reflects the malignant potential of the tumour. In a recent French study on the prognostic value of 6 different tumour markers, the analysis was based on multivariate models of survival [62]. It was found that, besides metastases ( $P=0.017$ ), Cyfra 21-1 ( $P=0.017$ ) and CA125 ( $P=0.03$ ) were significantly correlated with the outcome of 88 non-surgical NSCLC patients. Furthermore, elevated levels of Cyfra 21-1 during the course of disease were also independent predictors of poor survival. In a recent study on lung cancer

prognosis, 1296 consecutive patients seen over a 16-year period (1983-1998) were analysed by Cox regression models [63]. In every multivariate test, TPA emerged among the most important predictors of survival. Depending on the combination of variables in the model, TPA proved to be the second most important factor after either stage or performance status, but preceding other important clinical factors such as the number and type of metastatic sites, the T- and N-factor, or the weight loss.

### **Pathological factors**

#### *T- and N-status*

The prognostic importance of the T- and N-stages, and even substages, was detailed in the first report of this working group.

#### *Subtypes*

According to the WHO histological subtyping, there are 4 major subtypes of lung cancer (squamous cell carcinoma, adenocarcinoma, large cell carcinoma, and small cell lung carcinoma). Whether there is a difference in survival between the 3 major types of NSCLC still remains controversial, but many groups suggest a difference between squamous versus non-squamous tumours. In a multivariate model of recurrence of the Lung Cancer Study Group based on 392 stage I NSCLC cases with final pathological review, it was reported that patients with squamous cell tumours had a lower risk of recurrence and tumour-related death [64]. A review of several large series on the treatment outcome in patients with pathological stage I or II came to the same conclusion [65]. A Japanese group reported a more favourable outcome for squamous cell tumours in stage II [66]. In the Leuven Lung Cancer Group experience on 140 surgically treated patients with IIIA-N2 NSCLC, patients with non-

squamous cell tumours had a significantly increased risk of tumour-related death (relative risk 1.29, 95%CI 1.02-1.63, P=0.03) [7]. The same group reported a similar finding in IIIA-N2 patients treated with induction chemotherapy followed by surgery [67]. Regarding relapse patterns, different authors suggested that squamous cell tumours are more prone to locoregional recurrence and adenocarcinomas to distant recurrence [68-70].

In contrast with all this evidence, some other groups reported no survival differences across the 3 major subtypes of NSCLC [2,71].

#### *Differentiation and invasion*

Absence of differentiation and presence of lymphatic or blood vessel invasion are considered to be pointing at aggressiveness of lung tumours. Although this seems quite obvious, the data on this matter are far from unequivocal. This is probably due to the limited sensitivity of standard light microscopy to describe these findings, and to the important influence of the pathologist in the interpretation, and thus the possible intra- and inter-observer variability. Nevertheless, it must be decided whether vascular or lymphatic invasion should be implemented in a next staging revision.

Based on light microscopy, a distinction can be made between well, moderate, poor, or non differentiated lung tumours. Less differentiation has been associated with decreased survival in NSCLC in some series [2,66]. In a UK experience based on 479 consecutive resections, undifferentiated carcinoma had a significantly worse survival than the other NSCLC types [72]. In a Japanese series on 151 patients with p-stage I NSCLC, grade of differentiation was one of the predominant prognostic factors in the multivariate analysis [73].

In some studies, the presence of blood vessel invasion was associated with decreased survival. In a US study on 289 consecutive stage I NSCLC patients, vascular invasion proved to be of significant prognostic value both in univariate (P<0.01) and multivariate (P<0.05) analysis

[2]. Japanese investigators found that venous invasion in different stages of resected NSCLC was of prognostic significance [66]. In a French study on 593 patients with completely resected NSCLC, it was found that major blood vessel invasion was the most important prognostic factor in the multivariate analysis, T-stage and lymph node metastasis being the remaining independent prognostic factors [74]. In a modern prognosis study, using different molecular-biological markers (bcl-2, p53, Ki-67, angiogenesis) in resected stage I or II NSCLC, vascular invasion was the only independent prognostic factor for survival and recurrence in the patients without lymph node involvement ( $P=0.02$ ) [75]. An Italian study looking at the prognostic impact of both traditional and newer tumour parameters in 95 patients with resected T1N0M0 NSCLC, mentioned blood vessel invasion by tumour cells to be of independent prognostic value, both for survival ( $P=0.0001$ ) as for disease-free survival ( $P=0.0004$ ) [76].

In a recent retrospective study on 244 patients with resected stage I NSCLC, lymphatic invasion was of independent prognostic significance besides other standard and molecular tumour characteristics [77]. A French group reported that venous but not arterial vascular invasion correlated with the T-factor and p-TNM, whereas lymphatic vessel invasion correlated with the N-factor and p-TNM [78]. In their multivariate model on 96 resected NSCLC patients, lymphatic vessel invasion and p-TNM were important predictors for poor disease-free and overall survival.

Finally, a Japanese group reported a significant correlation between survival and blood vessel ( $P=0.044$ ) or lymphatic vessel invasion ( $P=0.042$ ) in a group of 66 patients with resectable NSCLC with intrapulmonary metastases [79].

#### *Neuro-endocrine differentiation*

The prognostic importance of neuro-endocrine differentiation was reported by **Verbeken**.

The most simple classification of the common lung carcinomas makes a distinction between two types, SCLC and NSCLC. This has paramount therapeutic and prognostic consequences. Yet, even between expert pathologists, the inter-observer agreement in classifying tumours into those two categories is not higher than 89 to 94% [80,81]. For the classification of the major subtypes and WHO-variants of NSCLC, the agreement is not better in particular for adenocarcinomas [82] and large cell carcinomas [83].

The classification of the 4 common lung neoplasm's is simple and clinically useful, with regard to diagnosis and treatment. Also, each tumour is conceptually associated with a single cell of origin, the SCLC being derived from the neuroendocrine Kulchitsky cell.

There are, however, major arguments against the morphologic discontinuity between SCLC and NSCLC. In a well conducted morphometric study, Brämer et al. showed a continuity and impressive overlap between both nuclear diameters and cell diameters between SCLC (formerly oat-cell and intermediate cell carcinomas), adeno-, squamous- and large cell carcinomas [84].

Secondly, it is well appreciated that lung carcinomas are heterogeneous tumours. Roggli et al. reported that only 34 of 100 lung tumours were homogenous, when assessed by 5 expert pathologists. In their study, 45% showed a major heterogeneity according to the WHO-classification [85], a figure that is now widely accepted [86]. Tumour heterogeneity is also documented ultrastructurally [87]. It reflects that lung carcinomas are derived from pluripotential cells, and that different differentiation levels within an individual case do occur and determine morphology. This is in keeping with Yesner's diagram [88] proposing a common histogenesis and a continuity between small cell undifferentiated and large cell undifferentiated carcinoma (with in between an intermediate small cell type), and even squamous carcinoma at the one and adenocarcinoma at the other end. Yesner thus proposed a more dynamic interpretation of tumour morphology. As pointed out by Colby et al. [89], it is

at present not clear how heterogeneity should impact on routine practice and diagnosis of lung carcinomas.

Thirdly, and not unexpectedly, the accuracy of histological subclassification is influenced by the technique used to make the diagnosis. Of a total of 96 light microscopic large cell carcinomas, 18% showed ultrastructurally squamous, 34% adenocarcinomatous, 19% adenosquamous and 14% neuroendocrine differentiation, whereas 14% remained large cell carcinomas [89]. Transmission electron microscopy obviously contributes to accurately diagnose a lung tumour. In consequence, the need for a double standard, a light optical and an ultrastructural one, may be questioned [90].

Neuroendocrine lung tumours include with increasing malignancy: typical carcinoid (TC) atypical carcinoid (AC), large cell neuroendocrine carcinoma (LCNEC) and SCLC.

In the WHO classification, LCNEC is considered a variant of large cell carcinoma, in which neuro-endocrine differentiation is confirmed by immunohistochemistry and/or electron-microscopy.

TC and AC are straight forward neuroendocrine tumours occurring within and outside the lung, that can be accurately diagnosed by light microscopy alone. This does not account for SCLC and large cell carcinoma. The latter tumours represent heterogeneous groups: some 25% of the SCLC fails to stain with an immunohistochemical panel of neuroendocrine markers, and, in an identical proportion, ultrastructural evidence of neuroendocrine differentiation is lacking. Furthermore, LCNEC may be closely related to a neuro-endocrine type small cell lung carcinoma: 30% of cultured SCLC cell lines develop features of large cell carcinoma [91]. Przygodski et al. [92] found that TC and AC were genetically distinct from the higher grade neuroendocrine SCLC and LCNEC. Additionally, they concluded that, although LCNEC is categorised as a NSCLC, it is more akin genetically to SCLC.

Neuroendocrine differentiation can also be documented often in carcinomas without neuroendocrine (so called organoid) morphology, most often encountered in adenocarcinomas. It is doubtful whether the latter has significant prognostic implications. The phenomenon of scattered neuroendocrine differentiation is also observed in adenocarcinomas outside the lung (breast, gastrointestinal tractus, oesophagus, ovary, prostate) without being a prognostic factor. It may be wise to conclude today, that in the lung also, neuroendocrine differentiation in those cases is ranking in the lowest category of prognostic factors, that are not yet sufficiently studied.

### **Molecular biological factors**

The last decade has been characterised by an overwhelming amount of new information on the molecular-biological processes that take place in lung cancer. A large scala of potentially important prognostic factors has emerged from this research. As mentioned above, a recent Medline search using the terms “prognostic factor” and “cancer” produced no less than 2,393 papers [1]. Some aspects of 2 important fields in molecular biology, neo-angiogenesis and changes in tumour suppressor genes, were reported at this workshop.

#### *Neo-angiogenesis*

**Fontanini** started her contribution with the fact that the first observation that neo-angiogenesis occurs around tumours, was already made by Goldman about 100 years ago [93]. A great amount of data has been collected up to now about the role of neo-angiogenesis in cancer, and, at the beginning of the third millennium, we are still discussing whether neo-angiogenesis may really be a crucial point in the clinical evaluation of cancer [94]. Much data in a great number of human tumours, emphasise that the angiogenic phenomenon is a useful

indicator of clinical outcome. The observation of increased microvessel density in tumours not only serves as an independent prognostic indicator, but also suggests that anti-angiogenic therapy may be an important component of treatment regimens for cancer patients [95]. Tumour angiogenesis is a complex process arising through sprouting and intussusception from pre-existing vessels, which involves both positive and negative regulators. Although complex, the angiogenic phenomenon provides a number of targets for therapy. Many positive regulators, including growth factor receptors, matrix metalloproteinases, and integrins, have been correlated with increased vascularity of tumours and poor prognosis for patients survival. Thus, these mediators may represent ideal targets for anti-angiogenic therapy [96]. In tumour samples, neo-angiogenesis may be evaluated as vascular density and as expression of angiogenic regulators, and both these methods may provide useful indications from a prognostic point of view [97].

In the last ten years, more than 100 papers have been published [98] concerning the role of angiogenic phenomenon in the outcome of lung carcinoma, and despite the large amount of data, no confirmatory data is available, nor has the clinical practice been altered up to now. However, interesting results have been obtained in a number of these studies, since a great number of patients have been analysed and some of these analyses enrolled consecutive and prospective series of patients. In a series of 407 NSCLC, the number of microvessels was significantly associated with poor prognosis in terms of overall survival [99]. Angiogenesis was quantified as microvessel count and the median value of this series was 20 vessels; the counts were categorised as low vs. high, or in five categories of increasing microvessels. In the univariate analysis, patients with larger tumours, more advanced stage, greater degree of regional lymph node involvement, or more vascular tumours experienced reduced overall survival. When microvessel count was analysed in five categories, a highly significant trend towards a worse prognosis with increasing tumour vascularity was observed. Moreover, the

probability of two years' survival was significantly influenced by microvessel counting, adding strong prognostic information to tumour-node metastasis staging. In multivariate analysis microvessel count retained its prognostic role on overall survival, with a relative risk of 8.38 associated with the highest number of microvessels. Other important studies demonstrated the same prognostic impact of neo-angiogenesis in clinical outcome of NSCLC. In particular, Giatromanolaki et al. [100] and Harpole et al. [101] analysed large retrospective series of patients, although using slightly different methodologies.

However, even if in lung cancer the majority of studies made evident that neo-angiogenesis has an important prognostic role, there has been negative data reported. A large number of stage I NSCLC has been reviewed by Pastorino et al. [102], in order to perform a biological assessment of tumours including neo-angiogenesis. The authors analysed 515 cases, with a median follow-up of 102 months. In that series, tumour extension represented the most powerful prognostic factor for survival and relapse. Among the immunohistochemical markers investigated, none emerged as an independent prognostic indicator for survival. On the basis of this discrepancy it appears particularly useful that neo-angiogenesis should be appropriately evaluated in further prospective and multi-center studies, able to provide confirmatory data in this important field.

Vascular endothelial growth factor (VEGF) is one of the most important tumour-derived cytokines which contributes to the increased permeability of tumour vasculature, and which shows a mitogenic activity on endothelial cells. A great number of studies demonstrated its influence in lung cancer progression, with both VEGF-protein and mRNA expression being associated with bad overall survival of NSCLC in univariate and in multivariate analyses. O'Byrne et al., [103] in a retrospective study of 223 NSCLC, pointed out the prognostic role of VEGF and PD-ECGF protein expression, and similar results have been obtained by our group in a series of 105 NSCLC patients, in which the expression of VEGF protein showed

an independent prognostic role on overall and disease-free survival [104]. Important results have also been obtained by other groups, who demonstrated the necessity of evaluating angiogenic mediators in lung cancer, either in relation to their prognostic impact or for the possibility that they represent relevant therapeutic targets in this type of cancer [105,106]. This data may thus also be a basis for conducting an extensive investigation into the utility of treating cancer patients with anti-angiogenic agents.

#### *Prognostic value of p53 and p16 gene abnormalities*

Advances in molecular biology have provided clues to the pathogenesis of cancer and shown the involvement of oncogene activation and tumour-suppressor gene inactivation. Recent evidence suggests that epigenetic regulations are also of critical importance during tumorigenesis. Among several genetic aberrations that have been implicated in lung cancer, alterations in the p53 and p16 tumour suppressor genes are the most common.

At the workshop, **Niklinski** explained that mutations in the p53 gene usually result in increased steady-state levels of p53, which may play a role in carcinogenesis through trans-dominant mechanisms, perhaps involving oligomerisation between mutant and wild-type proteins. During the last ten years, a large number of studies have evaluated p53 alterations in lung cancer [107]. However, no general conclusions have been reached with respect to their clinical impact. Considering the rates of detected mutations in published reports, these are very variable, from 20 to 60% [107]. With respect to prognosis after surgical treatment of NSCLC, some studies have demonstrated that a p53 mutation is associated with poor prognosis [108-110], while others have reported no significant effect [111], or have even concluded that p53 protein overexpression can be a good prognostic characteristic [112]. It has been suggested that these discrepancies may be due to the methods used for the

determination of p53 function. Most studies relied on screening tests such as SSCP, or DGGE, which are burdened with about 20% false-negative and false-positive results – as much as immunohistochemistry [113].

Recently, another method based on a yeast functional assay was developed to detect p53 abnormalities [114]. Recent data indicates that a functional assay combined with molecular analysis is the most powerful and useful approach for the study of p53 alterations [115]. In this assay, loss of DNA binding and transcriptional transactivation function in mutant p53 is detected by the colony colour of the yeast [114].

Genetically, the p16 gene can be inactivated by point mutation or homozygous deletion, as observed in various human primary tumours, including lung cancer. Furthermore, recent studies have demonstrated that de novo methylation, i.e., hypermethylation, of 5' CpG islands of the promoter region represented another important mechanism for the transcriptional inactivation of the p16 gene. It has been shown that the methylation profile commands 2 major mechanisms responsible for the process of tumorigenesis. First, DNA methylation is a significant contributor of point mutations at CpG dinucleotides in a variety of growth-regulatory genes. Secondly, methylation controls the regulation of gene expression and its presence in DNA is correlated with gene silencing [116]. Several studies have demonstrated that CpG islands within the p16 promoter are frequently hypermethylated in lung cancer, with rates of 30-50% [117,118]. Recently Belinsky et al. [119] for the first time have shown that inactivation of the p16 gene by aberrant methylation is an early and likely critical event in the development of lung cancer.

At the workshop, more details on the prognostic significance of p53 mutations (by direct sequencing through exons 5 to 8), p53 alterations (by Yeast Functional Assay) and p16

abnormalities (mutations by sequencing and hypermethylation by methylation-specific PCR) in radically resected NSCLC were reported. The exact methodology of these different assays has been reported elsewhere [110,120].

In a group of 98 surgically treated stage I-IIIa NSCLC patients, p53 mutations were detected in 46 (47%) cases and p16 abnormalities in 42 (43%) (point mutations in 8 and promoter hypermethylation in 34). No correlation was found between p53 and p16 abnormalities and various clinicohistological factors, including age, sex, histological type of tumour and TNM stage.

Survival analysis revealed that both the patients with p53 and p16 abnormalities tended to have a poorer prognosis than the patients without p53 ( $P= 0.02$ ) and p16 ( $P= 0.01$ ) abnormalities. In the multivariate analysis, however, when the types of p16 inactivation were analysed, p16 hypermethylation rather than point mutation was associated with poor prognosis.

Evaluation of p53 by Yeast Functional Assay was performed in 42 patients. Twenty-seven of the 42 (64%) NSCLC samples contained mutant p53 in the yeast functional assay with a higher frequency in squamous cell carcinoma 16/22 (73%) than in large cell carcinoma 4/7 (57%) and adenocarcinoma 7/13 (54%) ( $P< 0.02$ ). There were no significant differences in the frequency of positive test with respect to sex and TNM stage. Preliminary prognostic analysis showed that patients scoring positive for yeast test had significantly shorter disease-free survival (median 11 months) than those that scored negative (median 23+ months).

p53 point mutation and p16 hypermethylation thus could be useful molecular markers for the prognosis of patients with surgically resected NSCLC. Yeast functional assay for p53 is not only an improved methodology to examine the status of p53, but might hopefully improve understanding of the role of mutant p53 in the prognosis of NSCLC.

### *Glucose metabolism (FDG-PET)*

NSCLC is characterised by carbohydrate metabolic derangement's, which have been identified as independent prognostic factors correlated with poor treatment response and survival [42]. Increased glycolysis results in upregulation of glucose transporter proteins (especially subtype Glut-1) and increased hexokinase activity [121]. Overexpression of the Glut-1 transporter, an important feature of the glucose disturbance in NSCLC, was linked to a worse prognosis [122]. FDG-uptake in NSCLC cells has also been correlated with growth rate and proliferation capacity [123]. These glucose metabolism derangement's can be measured quantitatively in vivo by positron emission tomography (PET) after administration of <sup>18</sup>F-fluoro-2-deoxy-glucose (FDG).

Two larger studies have analysed whether the Standardised Uptake Value (SUV), a semi-quantitative measurement of FDG-uptake in NSCLC on PET is useful for prognostic purposes.

One study by the Leuven Lung Cancer Group reported on 125 potentially operable NSCLC patients, of whom 91 underwent complete resection [124]. In a univariate analysis, it was found that performance status (P=0.002), stage (P=0.001), tumour diameter (P=0.06), tumour cell type (P=0.03) and SUV>7 (P=0.001) were correlated with survival. In a multivariate Cox-analysis, performance status (P=0.02), stage (P=0.01), and SUV (P=0.007) were retained as independent prognostic factors. In the operated patients, those with a tumour <3 cm had an expected 2-year survival of 86% if the SUV was below 7, and 60% if above 7; nearly all resected tumours >3 cm had SUV's of more than 7, and an expected 2-year survival of 43%. The SUV seemed to be more important than the tumour diameter. It was concluded that the FDG-uptake in primary NSCLC on PET has an important and independent prognostic value, and might be of help in the decision on adjuvant treatment protocols.

A study from Duke University reported an SUV of more than 10 to be of significant adverse prognostic importance [125]. When they used the cut-off point of 10, seventy-six percent of the patients fell below this value. In the Leuven experience, using the cut-off point of 7, seventy-five percent of the patients were above this value. It seems reasonable to hypothesise that there is no true cut-off point, but rather a transition zone, where the prognosis gradually worsens.

At present, it remains intriguing whether there is a relationship regarding prognosis between FDG-uptake and other molecular-biological factors such as proliferation, apoptosis or angiogenesis. Future study must concentrate on the question whether the glucose disturbances and other molecular-biological aberrations in NSCLC are independent or linked findings, and what is the contribution of each of these features to the prognosis.

### **The concept of molecular-biological staging**

The massive amount of new information on the molecular biology from the last decade has certainly improved our knowledge and understanding of lung cancer tumorigenesis to a large extent. The prognostic information of these new molecular markers, however, is not always unequivocal. Indeed, for many of these markers, conflicting results have been reported, with either worse prognosis, no significant effect, or even a more favourable prognosis. Two major factors might account for the contradictions in these results. First, differences in methodology and assays for the determination of these markers. Second, the complexity of the multi-step process of tumorigenesis makes it very hazardous to make prognostic speculations based on one factor only.

Some groups have therefore examined the concept of so-called “molecular-biological staging”. Based on the Boston experience, for instance, where angiogenesis, proto-oncogene

erbB-2, suppressor gene p53, and the proliferation marker KI-67 were included in a prognostic model, Harpole et al. reported a 5-year survival of 81% in resected stage I NSCLC patients without any adverse marker, but only 49% in those with 3 or more markers [101,126]. Similar findings were reported by Apolinario et al. based on the series from Amsterdam, where p53, bcl-2, bax, and neo-angiogenesis were included in a model on 116 radically resected NSCLC. p53 status, assessed with one monoclonal antibody, was not predictive for survival. However, a combination of expression p53+(antibody PAb1801) and bcl2- had the worst survival in stage I patients (P=.034). In multivariate analysis including all patients, the presence of p53+/ bcl2- tumour expression and large tumour diameter ( $\geq 4$  cm) were independent prognostic factors for shorter survival duration. For stage I, only the presence of bax+/ bcl2- Tumor expression had a significant negative influence on survival.

It was strongly suggested in this working group that these so-called “molecular-biological staging” systems should *not* be included in the general staging system at this moment. Indeed, these systems are not unequivocal (e.g. differences in methodology and contradictions in prognostic consequence) and are not based on straightforward techniques accessible to all, two vital conditions of any good staging system. They may serve, however, as very interesting prognostic tools in highly specialised centres, but will probably not find their way to general clinical staging in the near future, where the TNM remains the central tool.

Table 1 : Potential prognostic factors in stage III NSCLC : surgery only

Stage IIIA

- *microscopic involvement of one/two vs. multi-level lymph node involvement*
- peripheral T3N1 versus centrally located T3N1
- superior sulcus T3N1 vs non-superior sulcus T3 N1
- tumour size

Stage IIIB

- *T4 – carinal involvement*
- *T4 – spine involvement*
- *T4 – vena cava involvement*
- T4 - involvement of right atrium
- *selected T4N0/1 versus any N3*

Table 2 : Potential prognostic factors in stage III NSCLC : radiotherapy or combined chemoradiotherapy

#### Stage III general

- stage IIIA vs. stage IIIB
- *performance status (WHO)*
- *weight loss*
- *tumour size/tumour volume*
- pre-treatment serum LDH
- *pre-treatment haemoglobin values*
- age

#### Stage IIIA

- bulky vs. non-bulky N2
- centrally located T3 vs. peripherally located T3
- superior sulcus tumours vs. “non-superior sulcus” tumours

#### Stage IIIB

- T4 – carinal involvement
- T4 – spine involvement
- T4 – vena cava involvement
- T4 – any involvement of the heart
- T4 – oesophageal involvement
- any N3 – contralateral mediastinal nodes
- N3 – supraclavicular nodes
- *T4N0-N1 vs. T4N2 vs. T1-2N3 vs. T3-4N3*
- *T4 – any pleural effusion*
- *post-obstructive pneumonia/infection*

Table 3 : Potential prognostic factors in stage III NSCLC : chemo(radio-)therapy followed by surgery

#### Stage III general

- stage IIIA vs. stage IIIB
- performance status (WHO)
- weight loss
- histology
- tumour size/tumour volume
- *pre-treatment serum LDH*
- pre-treatment haemoglobin values
- age
- post-obstructive pneumonia/infection

#### Stage IIIA

- bulky vs. non-bulky N2
- *microscopic involvement of one/two vs. multi-level lymph node involvement*
- centrally located T3 vs. peripherally located T3
- superior sulcus tumours vs. “non-superior sulcus” tumours

#### Stage IIIB

- T4 – carinal involvement
- T4 – spine involvement
- T4 - vena cava involvement
- T4 – pulmonary artery involvement
- T4 – involvement of the right atrium
- *any N3 – contralateral mediastinal nodes*
- *N3 – supraclavicular nodes*
- *T4N0-N1 vs. T4N2 vs. T1-2N3 vs. T3-4N3*

Table 4. : Potential prognostic factors in stage III NSCLC : treatment related factors

Stage III general

- objective clinical response (chest X-ray/chest CT scan)
- *“functional response” of primary tumour on repeat PET-scan*
- *pathological response (pathological CR) at the time of thoracotomy*
- *amount of pathological response at time of thoracotomy (major pathological. response)*
- complete resection possible following induction treatment (R0 vs R1/2)
- mediastinal “downstaging” (PET/repeat mediastinoscopy)
- pulmonary risk of radiotherapy (mean lung dose depending on tumour volume/location)

Stage IIIB

- selective T4 – potentially resectable vs not potentially resectable

Table 5 : Regression grading system used for the assessment of resection specimens after neoadjuvant therapy

Grade I : no or only spontaneous tumour regression in the primary lesion and mediastinal lymph nodes

Grade II : morphological evidence of therapy-induced tumour regression

- Grade IIa at least 10% residual tumour cells in the sections of the primary lesion and/or mediastinal lymph nodes presenting more than focal microscopic disease
- Grade IIb less than 10% residual tumour cells in the sections of the primary lesion and/or mediastinal lymph nodes presenting focal microscopic disease

Grade III : complete tumour regression with no evidence of vital tumour tissue in the sections of the primary lesion and mediastinal lymph nodes

## References

1. Cannistra SA. When is a "prognostic factor" really prognostic? *J Clin Oncol* 2000; 18:3745-3747.
2. Harpole DH, Herndon JE, Young WG, Wolfe WG, Sabiston DC. Stage I non-small cell lung cancer. A multivariate analysis of treatment methods and patterns of recurrence. *Cancer* 1995; 76:787-796.
3. Mountain CF. Revisions in the international system for staging lung cancer. *Chest* 1997; 111:1710-1717.
4. Feld R, Abratt R, Graziano S, et al. Pretreatment minimal staging and prognostic factors for non-small cell lung cancer. *Lung Cancer* 1997; 17 Suppl 1:S3-S10.
5. Andre F, Grunenwald D, Pignon JP, et al. Survival of patients with resected N2 non-small cell lung cancer: Evidence for a subclassification and implications. *J Clin Oncol* 2000; 18:2981-2989.
6. Van Raemdonck DE, Schneider A, Ginsberg RJ. Surgical treatment for higher stage non-small cell lung cancer. *Ann Thorac Surg* 1992; 54:999-1013.
7. Vansteenkiste JF, De Leyn PR, Deneffe GJ, et al. Survival and prognostic factors in resected N2 non-small cell lung cancer: A study of 140 cases. The Leuven Lung Cancer Group. *Ann Thorac Surg* 1997; 63:1441-1450.
8. Feld R, Borges M, Giner V, et al. Prognostic factors in non-small cell lung cancer. *Lung Cancer* 1994; 11 Suppl 3:19-23.
9. O'Connell JP, Kris MG, Gralla RJ, et al. Frequency and prognostic importance of pretreatment clinical characteristics in patients with advanced non-small cell lung cancer treated with combination chemotherapy. *J Clin Oncol* 1986; 4:1604-1614.
10. Jeremic B, Shibamoto Y. Pre-treatment prognostic factors in patients with stage III non-small cell lung cancer treated with hyperfractionated radiation therapy with or without concurrent chemotherapy. *Lung Cancer* 1995; 13:21-30.
11. Sorensen JB, Osterlind K. Prognostic factors. From clinical parameters to new biologic markers. In Van Houtte P, Klastersky J, Rocmans P., eds. Progress and perspective in the treatment of lung cancer. Berlin: Springer, 2000; 1-21.
12. Graham MV, Geitz LM, Byhardt R, et al. Comparison of prognostic factors and survival among black patients and white patients treated with irradiation for non-small cell lung cancer. *J Natl Cancer Inst* 1992; 84:1731-1735.
13. Scott C, Sause WT, Byhardt R, et al. Recursive partitioning analysis of 1592 patients on four Radiation Therapy Oncology Group studies in inoperable non-small cell lung cancer. *Lung Cancer* 1997; 17 Suppl 1:S59-S74.
14. Volm M, Mattern J, Muller T, Drings P. Flow cytometry of epidermoid lung carcinomas: relationship of ploidy and cell cycle phases to survival. A five-year follow up study. *Anticancer Res* 1988; 8:105-112.
15. Movsas B, Scott C, Sause W, et al. The benefit of treatment intensification is age and histology-dependent in patients with locally advanced non-small cell lung cancer (NSCLC): a quality-adjusted survival analysis of radiation therapy oncology group (RTOG) chemoradiation studies. *Int J Radiat Oncol Biol Phys* 1999; 45:1143-1149.
16. Schaake-Koning C, Van den Bogaert W, Dalesio O, et al. Effects of concomitant cisplatin and radiotherapy on inoperable non-small-cell lung cancer. *N Engl J Med* 1992; 326:524-530.
17. Jeremic B, Shibamoto Y, Acimovic L, Djuric L. Randomized trial of hyperfractionated radiation therapy with or without concurrent chemotherapy for stage III non-small-cell lung cancer. *J Clin Oncol* 1995; 13:452-458.

18. Jeremic B, Shibamoto Y, Acimovic L, Milisavljevic S. Hyperfractionated radiation therapy with or without concurrent low-dose daily carboplatin/etoposide for stage III non-small cell lung cancer: a randomized study. *J Clin Oncol* 1996; 14:1065-1070.
19. Jeremic B, Shibamoto Y, Milicic B, Nikolic N, Dagovic A, Milisavljevic S. Concurrent radiochemotherapy for patients with stage III non-small cell lung cancer (NSCLC): long-term results of a phase II study. *Int J Radiat Oncol Biol Phys* 1998; 42:1091-1096.
20. Bonomi P, Gale M, Rowland K, et al. Pre-treatment prognostic factors in stage III non-small cell lung cancer patients receiving combined modality treatment. *Int J Radiat Oncol Biol Phys* 1991; 20:247-252.
21. Pater JL, Loeb M. Nonanatomic prognostic factors in carcinoma of the lung: a multivariate analysis. *Cancer* 1982; 50:326-331.
22. Furuse K, Fukuoka M, Kawahara M, et al. Phase III study of concurrent versus sequential thoracic radiotherapy in combination with mitomycin, vindesine, and cisplatin in unresectable stage III non-small cell lung cancer. *J Clin Oncol* 1999; 17:2692-2699.
23. Jeremic B, Shibamoto Y, Acimovic L, et al. Hyperfractionated radiation therapy and concurrent low-dose, daily carboplatin/etoposide with or without weekend carboplatin/etoposide chemotherapy in stage III non-small cell lung cancer: A randomized trial. *Int J Radiat Oncol Biol Phys* 2001; 50:19-25.
24. Cox JD, Scott CB, Byhardt RW, et al. Addition of chemotherapy to radiation therapy alters failure patterns by cell type within non-small cell carcinoma of lung (NSCCL): analysis of Radiation Therapy Oncology Group (RTOG) trials. *Int J Radiat Oncol Biol Phys* 1999; 43:505-509.
25. Grunenwald D, Le Chevalier T. Stage IIIA category of non-small cell lung cancer: a new proposal [letter]. *J Natl Cancer Inst* 1997; 89:88-89.
26. Choi NC, Carey RW, Daly W, et al. Potential impact on survival of improved tumor downstaging and resection rate by preoperative twice-daily radiation and concurrent chemotherapy in stage IIIA non-small-cell lung cancer. *J Clin Oncol* 1997; 15:712-722.
27. Albain KS, Rusch VW, Crowley JJ, et al. Concurrent cisplatin/etoposide plus chest radiotherapy followed by surgery for stages IIIA (N2) and IIIB non-small-cell lung cancer: mature results of Southwest Oncology Group phase II study 8805. *J Clin Oncol* 1995; 13:1880-1892.
28. Thomas M, Rube C, Semik M, et al. Impact of preoperative bimodality induction including twice-daily radiation on tumor regression and survival in stage III non-small cell lung cancer. *J Clin Oncol* 1999; 17:1185
29. Junker K, Thomas M, Schulmann K, Klinke F, Bosse U, Muller KM. Tumour regression in non-small-cell lung cancer following neoadjuvant therapy. Histological assessment. *J Cancer Res Clin Oncol* 1997; 123:469-477.
30. Burkes R, Ginsberg RJ, Shepherd FA, et al. 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* 1992; 10:580-586.
31. Eagan RT, Ruud C, Lee RE, Pairolero PC, Gail MH. Pilot study of induction therapy with cyclophosphamide, doxorubicin, cisplatin (CAP) and chest irradiation prior to thoracotomy in initially inoperable stage III M0 non-small cell lung cancer. *Cancer Treat Rep* 1987; 71:895-900.
32. Faber LP, Kittle CF, Warren WH, et al. Preoperative chemotherapy and irradiation for stage III non-small cell lung cancer. *Ann Thorac Surg* 1989; 47:669-675.
33. Pisters KM, Kris MG, Gralla RJ, Zaman MB, Heelan RT, Martini N. 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* 1993; 11:1757-1762.

34. Skarin A, Jochelson M, Sheldon T, et al. Neoadjuvant chemotherapy in marginally resectable stage III M0 non-small cell lung cancer: long-term follow-up in 41 patients. *J Surg Oncol* 1989; 40:266-274.
35. Strauss GM, Herndon JE, Sherman DD, et al. Neoadjuvant chemotherapy and radiotherapy followed by surgery in stage IIIA non-small-cell carcinoma of the lung: report of a Cancer and Leukemia Group B phase II study. *J Clin Oncol* 1992; 10:1237-1244.
36. Weiden PL, Piantadosi S. Preoperative chemotherapy (cisplatin and fluorouracil) and radiation in stage III non-small cell lung cancer: a phase II study of the Lung Cancer Study Group. *J Natl Cancer Inst* 1991; 83:266-272.
37. Eberhardt W, Wilke H, Stamatis G, et al. Preoperative chemotherapy followed by concurrent chemoradiation therapy based on hyperfractionated accelerated radiotherapy and definitive surgery in locally advanced non-small-cell lung cancer: mature results of a phase II trial. *J Clin Oncol* 1998; 16:622-634.
38. Edelman MJ, Gandara DR, Roach M, Benfield JR. Multimodality therapy in stage III non-small cell lung cancer. *Ann Thorac Surg* 1996; 61:1564-1572.
39. Rusch VW. Neoadjuvant therapy for stage III lung cancer. *Semin Thor Cardiovasc Surg* 1993; 5:258-267.
40. Vansteenkiste JF, Stroobants SG, De Leyn PR, et al. Potential use of FDG-PET scan after induction chemotherapy in surgically staged IIIA-N2 non-small cell lung cancer : A prospective pilot study. *Ann Oncol* 1998; 9:1193-1198.
41. Paesmans M, Sculier JP, Libert P, et al. Prognostic factors for survival in advanced non-small-cell lung cancer: univariate and multivariate analyses including recursive partitioning and amalgamation algorithms in 1,052 patients. The European Lung Cancer Working Party. *J Clin Oncol* 1995; 13:1221-1230.
42. Stanley KE. Prognostic factors for survival in patients with inoperable lung cancer. *J Natl Cancer Inst* 1980; 65:25-32.
43. Sorensen JB, Badsberg JH, Olsen J. Prognostic factors in inoperable adenocarcinoma of the lung: a multivariate regression analysis of 259 patients. *Cancer Res* 1989; 49:5748-5754.
44. Suresh MR. Classification of tumor markers. *Anticancer Res* 1996; 16:2273-2277.
45. Rasmuson T, Bjork GR, Damber L, et al. Tumor markers in bronchogenic carcinoma. An evaluation of carcinoembryonic antigen, tissue polypeptide antigen, placental alkaline phosphatase and pseudouridine. *Acta Radiol Oncol* 1983; 22:209-214.
46. Ferrigno D, Buccheri G, Biggi A. Serum tumour markers in lung cancer: history, biology and clinical applications. *Eur Respir J* 1994; 7:186-197.
47. Ferrigno D, Buccheri G. Clinical applications of serum markers for lung cancer. *Respir Med* 1995; 89:587-597.
48. Buccheri G. Tumor markers: clinical meaning and use. In Brambilla C, Brambilla E., eds. Lung tumors. New-York: Marcel Dekker Inc, 1999; 435-452.
49. Concannon JP, Dalbow MH, Hodgson SE, et al. Prognosis value of preoperative carcinoembryonic antigen plasma levels in patients with bronchogenic carcinoma. *Cancer* 1978; 42:1477-1483.
50. Icard P, Regnard JF, Essomba A, Panebianco V, Magdeleinat P, Levasseur P. Preoperative carcinoembryonic antigen level as a prognostic indicator in resected primary lung cancer. *Ann Thorac Surg* 1994; 58:811-814.
51. Niklinski J, Furman M, Laudanski J, Kozlowski M. Prognostic value of pretreatment CEA, SCC-Ag and CA 19-9 levels in sera of patients with non-small cell lung cancer. *Eur J Cancer Prev* 1992; 1:401-406.

52. Rubins JB, Dunitz J, Rubins HB, Maddaus MA, Niewoehner DE. Serum carcinoembryonic antigen as an adjunct to preoperative staging of lung cancer. *J Thorac Cardiovasc Surg* 1998; 116:412-416.
53. Salgia R, Harpole D, Herndon JE, Pisick E, Elias A, Skarin AT. Role of serum tumor markers CA 125 and CEA in non-small cell lung cancer. *Anticancer Res* 2001; 21:1241-1246.
54. Buccheri GF, Violante B, Sartoris AM, Ferrigno D, Curcio A, Vola F. Clinical value of a multiple biomarker assay in patients with bronchogenic carcinoma. *Cancer* 1986; 57:2389-2396.
55. Buccheri GF, Ferrigno D, Sartoris AM, Violante B, Vola F, Curcio A. Tumor markers in bronchogenic carcinoma. Superiority of tissue polypeptide antigen to carcinoembryonic antigen and carbohydrate antigenic determinant 19-9. *Cancer* 1987; 60:42-50.
56. Buccheri G, Ferrigno D. Usefulness of tissue polypeptide antigen in staging, monitoring, and prognosis of lung cancer. *Chest* 1988; 93:565-570.
57. Buccheri G, Ferrigno D. Prognostic value of the tissue polypeptide antigen in lung cancer. *Chest* 1992; 101:1287-1292.
58. Buccheri G, Ferrigno D, Vola F. Carcinoembryonic antigen (CEA), tissue polypeptide antigen (TPA) and other prognostic indicators in squamous cell lung cancer. *Lung Cancer* 1993; 10:21-33.
59. Niklinski J, Furman M, Burzykowski T, et al. Preoperative CYFRA 21-1 level as a prognostic indicator in resected primary squamous cell lung cancer. *Br J Cancer* 1996; 74:956-960.
60. Hirashima T, Takada M, Komiya T, et al. Prognostic significance of CYFRA 21-1 in non-small cell lung cancer. *Anticancer Res* 1998; 18:4713-4716.
61. Niklinski J, Burzykowski T, Niklinska W, et al. Preoperative CYFRA 21-1 level as a prognostic indicator in resected non-small cell lung cancer. *Eur Respir J* 1998; 12:1424-1428.
62. Brechot JM, Chevret S, Nataf J, et al. Diagnostic and prognostic value of Cyfra 21-1 compared with other tumour markers in patients with non-small cell lung cancer: a prospective study of 116 patients. *Eur J Cancer* 1997; 33:385-391.
63. Buccheri G, Ferrigno D. Prognostic value of stage grouping and TNM descriptors in lung cancer. *Chest* 2000; 117:1247-1255.
64. Gail MH, Eagan RT, Feld R, et al. Prognostic factors in patients with resected stage I non-small cell lung cancer. A report from the Lung Cancer Study Group. *Cancer* 1984; 54:1802-1813.
65. Nesbitt JC, Putnam JB, Walsh GL, Roth JA, Mountain CF. Survival in early-stage non small cell lung cancer. *Ann Thorac Surg* 1995; 60:466-472.
66. Ichinose Y, Yano T, Asoh H, Yokoyama H, Yoshino I, Katsuda Y. Prognostic factors obtained by a pathologic examination in completely resected non-small-cell lung cancer. An analysis in each pathologic stage. *J Thorac Cardiovasc Surg* 1995; 110:601-605.
67. Vansteenkiste JF, De Leyn PR, Deneffe GJ, et al. Vindesine-Ifosfamide-Platinum (VIP) induction chemotherapy in surgically staged IIIA-N2 non-small cell lung cancer: A prospective study. *Ann Oncol* 1998; 9:261-267.
68. Martini N, Burt H, Bains MS, et al. Survival after resection of stage II non-small cell lung cancer. *Ann Thorac Surg* 1992; 54:460-466.
69. Cangemi V, Volpino P, D'Andrea N, et al. Local and/or distant recurrences in T1-2/N0-1 non-small cell lung cancer. *Eur J Cardiothorac Surg* 1995; 9:473-478.

70. Sawyer TE, Bonner JA, Gould PM, Deschamps C, Lange CM, Li H. Patients with stage I non-small cell lung carcinoma at postoperative risk for local recurrence, distant metastasis, and death: implications related to the design of clinical trials. *Int J Radiat Oncol Biol Phys* 1999; 45:315-321.
71. Padilla J, Calvo V, Penalver JC, Sales G, Morcillo A. Surgical results and prognostic factors in early non-small cell lung cancer. *Ann Thorac Surg* 1997; 63:324-326.
72. Kadri MA, Dussek JE. Survival and prognosis following resection of primary non-small cell bronchogenic carcinoma. *Eur J Cardiothorac Surg* 1991; 5:132-136.
73. Ichinose Y, Hara N, Ohta M, et al. Is T factor of the TNM staging system a predominant prognostic factor in pathologic stage I non-small cell lung cancer ? A multivariate prognostic factor analysis of 151 patients. *J Thorac Cardiovasc Surg* 1993; 106:90-94.
74. Kessler R, Gasser B, Massard G, et al. Blood vessel invasion is a major prognostic factor in resected non-small cell lung cancer. *Ann Thorac Surg* 1996; 62:1489-1493.
75. Cagini L, Monacelli M, Giustozzi G, et al. Biological prognostic factors for early stage completely resected non-small cell lung cancer. *J Surg Oncol* 2000; 74:53-60.
76. Macchiarini P, Fontanini G, Hardin MJ, et al. Blood vessel invasion by tumor cells predicts recurrence in completely resected T1 N0 M0 non-small cell lung cancer. *J Thorac Cardiovasc Surg* 1993; 106:80-89.
77. Kwiatkowski DJ, Harpole DH, Godleski J, et al. Molecular pathologic substaging in 244 stage I non-small-cell lung cancer patients: clinical implications. *J Clin Oncol* 1998; 16:2468-2477.
78. Brechot JM, Chevret S, Charpentier MC, et al. Blood vessel and lymphatic vessel invasion in resected non-small cell lung carcinoma. Correlation with TNM stage and disease free and overall survival. *Cancer* 1996; 78:2111-2118.
79. Fujisawa T, Yamaguchi Y, Saitoh Y, Hiroshima K, Ohwada H. Blood and lymphatic vessel invasion as prognostic factors for patients with primary resected non small cell carcinoma of the lung with intrapulmonary metastases. *Cancer* 1995; 76:2464-2470.
80. Stanley KE, Matthews MJ. Analysis of a pathology review of patients with lung tumors. *J Natl Cancer Inst* 1981; 66:989-992.
81. Vollmer RT, Ogden L, Crissman JD. Separation of small cell from non-small cell lung cancer. The Southeastern Cancer Study Group pathologists' experience. *Arch Pathol Lab Med* 1984; 108:792-794.
82. Sorensen JB, Hirsch FR, Gazdar A, Olsen JE. Interobserver variability in histopathologic subtyping and grading of pulmonary adenocarcinoma. *Cancer* 1993; 71:2971-2976.
83. Wertzel H, Grahmann PR, Bansbach S, Lange W, Hasse J, Bohm N. Results after surgery in undifferentiated large cell carcinoma of the lung: the role of neuroendocrine expression. *Eur J Cardiothorac Surg* 1997; 12:698-702.
84. Braemer U. Morphometric study of the heterogeneity of malignant lung tumors (in German). 1984. (Thesis)
85. Roggli VL, Vollmer RT, Greenberg SD, McGavran MH, Spjut HJ, Yesner R. Lung cancer heterogeneity: a blinded and randomized study of 100 consecutive cases. *Hum Pathol* 1985; 16:569-579.
86. Travis WD, Colby TV, Corrin B, Shimosato Y, Brambilla E. Histological typing of lung and pleural tumours. WHO international classification of lung tumours. Berlin: Springer, 1999.
87. Mooi WJ, Dingemans KP, Wagenaar SS, Hart AA, Wagenvoort CA. Ultrastructural heterogeneity of lung carcinomas: representativity of samples for electron microscopy in tumor classification. *Hum Pathol* 1990; 21:1227-1234.

88. Yesner R. Spectrum of lung cancer and ectopic hormones. *Pathol Annu* 1978; 13:1207-40.
89. Colby TV, Koss MN, Travis WD. Tumors of the lower respiratory tract. Washington DC: Armed Forces Institute of Pathology, 1995.
90. Sobin LH. The histologic classification of lung tumors: the need for a double standard. *Hum Pathol* 1983; 14:1020-1021.
91. Gazdar AF, Carney DN, Nau MM, Minna JD. Characterisation of variant subclasses of cell lines derived from small cell lung cancer having distinctive biochemical, morphological, and growth properties. *Cancer Res* 1995; 45:2924-2930.
92. Przygodzki RM, Finkelstein SD, Langer JC, et al. Analysis of p53, K-ras-2, and C-raf-1 in pulmonary neuroendocrine tumors. Correlation with histological subtype and clinical outcome. *Am J Pathol* 1996; 148:1531-1541.
93. Goldman E. The growth of malignant disease in man and lower animals with special reference to the vascular system. *Lancet* 1907; II:1236-1240.
94. Weidner N. Angiogenesis as a predictor of clinical outcome in cancer patients. *Hum Pathol* 2000; 31:403-405.
95. Folkman J. Angiogenesis and angiogenesis inhibition: an overview. *EXS* 1997; 79:1-8.
96. Cherrington JM, Strawn LM, Shawver LK. New paradigms for the treatment of cancer: the role of anti-angiogenesis agents. *Adv Cancer Res* 2000; 79:1-38.
97. Vermeulen PB, Gasparini G, Fox SB, et al. Quantification of angiogenesis in solid human tumours: an international consensus on the methodology and criteria of evaluation. *Eur J Cancer* 1996; 32A:2474-2484.
98. Cox G, Jones JL, Walker RA, Steward WP, O'Byrne KJ. Angiogenesis and non-small cell lung cancer. *Lung Cancer* 2000; 27:81-100.
99. Fontanini G, Lucchi M, Vignati S, et al. Angiogenesis as a prognostic indicator of survival in non-small cell lung carcinoma: a prospective study. *J Natl Cancer Inst* 1997; 89:881-886.
100. Giatromanolaki A, Koukourakis M, O'Byrne K, et al. Prognostic value of angiogenesis in operable non-small cell lung cancer. *J Pathol* 1996; 179:80-88.
101. Harpole DH, Richards WG, Herndon JE, Sugarbaker DJ. Angiogenesis and molecular biologic substaging in patients with stage I non-small cell lung cancer. *Ann Thorac Surg* 1996; 61:1470-1476.
102. Pastorino U, Andreola S, Tagliabue E, et al. Immunocytochemical markers in stage I lung cancer: relevance to prognosis. *J Clin Oncol* 1997; 15:2858-2865.
103. O'Byrne KJ, Koukourakis MI, Giatromanolaki A, et al. Vascular endothelial growth factor, platelet-derived endothelial cell growth factor and angiogenesis in non-small cell lung cancer. *Br J Cancer* 2000; 82:1427-1432.
104. Fontanini G, Vignati S, Boldrini L, et al. Vascular endothelial growth factor is associated with neovascularization and influences progression of non-small cell lung carcinoma. *Clin Cancer Res* 1997; 3:861-865.
105. Yuan A, Yu CJ, Chen WJ, et al. Correlation of total VEGF mRNA and protein expression with histologic type, tumor angiogenesis, patient survival and timing of relapse in non-small cell lung cancer. *Int J Cancer* 2000; 89:475-483.
106. Yano T, Tanikawa S, Fujie T, Masutani M, Horie T. Vascular endothelial growth factor expression and neovascularisation in non-small cell lung cancer. *Eur J Cancer* 2000; 36:601-609.

107. Salgia R, Skarin AT. Molecular abnormalities in lung cancer. *J Clin Oncol* 1998; 16:1207-1217.
108. Mitsudomi T, Oyama T, Kusano T, Osaki T, Nakanishi R, Shirakusa T. Mutations of the p53 gene as a predictor of poor prognosis in patients with non-small cell lung cancer. *J Natl Cancer Inst* 1993; 85:2018-2023.
109. Fukuyama Y, Mitsudomi T, Sugio K, Ishida T, Akazawa K, Sugimachi K. K-ras and p53 mutations are an independent unfavourable prognostic indicator in patients with non-small-cell lung cancer. *Br J Cancer* 1997; 75:1125-1130.
110. Niklinska W, Burzykowski T, Chyczewski L, et al. p53 gene mutation and protein expression in operable non-small cell lung cancer in Poland. *Eur J Cancer Prev* 2000; 9:81-87.
111. Passlick B, Izbicki JR, Haussinger K, Thetter O, Pantel K. Immunohistochemical detection of P53 protein is not associated with a poor prognosis in non-small cell lung cancer. *J Thorac Cardiovasc Surg* 1995; 109:1205-1211.
112. Lee JS, Yoon A, Kalapurakal SK, et al. Expression of p53 oncoprotein in non-small-cell lung cancer: a favorable prognostic factor. *J Clin Oncol* 1995; 13:1893-1903.
113. Soussi T, Legros Y, Lubin R, Ory K, Schlichtholz B. Multifactorial analysis of p53 alteration in human cancer: a review. *Int J Cancer* 1994; 57:1-9.
114. Flaman JM, Frebourg T, Moreau V, et al. A simple p53 functional assay for screening cell lines, blood, and tumors. *Proc Natl Acad Sci U S A* 1995; 92:3963-3967.
115. de Cremoux P, Salomon AV, Liva S, et al. p53 mutation as a genetic trait of typical medullary breast carcinoma. *J Natl Cancer Inst* 1999; 91:641-643.
116. Herman JG. Hypermethylation of tumor suppressor genes in cancer. *Semin Cancer Biol* 1999; 9:359-367.
117. Gazzeri S, Gouyer V, Vour'ch C, Brambilla C, Brambilla E. Mechanisms of p16INK4A inactivation in non small-cell lung cancers. *Oncogene* 1998; 16:497-504.
118. Sekido Y, Fong KM, Minna JD. Progress in understanding the molecular pathogenesis of human lung cancer. *Biochim Biophys Acta* 1998; 1378:F21-59.
119. Belinsky SA, Nikula KJ, Palmisano WA, et al. Aberrant methylation of p16(INK4a) is an early event in lung cancer and a potential biomarker for early diagnosis. *Proc Natl Acad Sci U S A* 1998; 95:11891-11896.
120. Laudanski J, Niklinska W, Burzykowski T, Chyczewski L, Niklinski J. Prognostic significance of p53 and bcl-2 abnormalities in operable nonsmall cell lung cancer. *Eur Respir J* 2001; 17:660-666.
121. Nelson CA, Wang JQ, Leav I, Crane PD. The interaction among glucose transport, hexokinase, and glucose-6-phosphatase with respect to 3H-2-deoxyglucose retention in murine tumor models. *Nuclear Medicine & Biology* 1996; 23:533-541.
122. Younes M, Brown RW, Stephenson M, Gondo M, Cagle PT. Overexpression of Glut1 and Glut3 in stage I non-small cell lung carcinoma is associated with poor survival. *Cancer* 1997; 80:1046-1051.
123. Duhaylongsod FG, Lowe VJ, Patz EF, Vaughn AL, Coleman RE, Wolfe WG. Lung tumor growth correlates with glucose metabolism measured by fluoride-18 fluorodeoxyglucose positron emission tomography. *Ann Thorac Surg* 1995; 60:1348-1352.
124. Vansteenkiste JF, Stroobants SG, De Leyn PR, et al. Prognostic importance of the Standardized Uptake Value on FDG-PET-scan in non-small cell lung cancer: An analysis of 125 cases. *J Clin Oncol* 1999; 17:3201-3206.
125. Ahuja V, Coleman RE, Herndon J, Patz EF. The prognostic significance of fluorodeoxyglucose positron emission tomography imaging for patients with nonsmall cell lung carcinoma. *Cancer* 1998; 83:918-924.

126. Harpole DH, Herndon JE, Wolfe WG, Iglehart JD, Marks JR. A prognostic model of recurrence and death in stage I non-small cell lung cancer utilizing presentation, histopathology, and oncoprotein expression. *Cancer Res* 1995; 55:51-56.