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Contents

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Editorial Board:

Preface

Dear colleagues,

This publication summarises content that was presented at the Lung Cancer International Preceptorship conference that took place in Vienna, Austria, on 20th and 21st June, 2016. The Medical University of Vienna and the Comprehensive Cancer Center Vienna jointly organised this conference, which was addressed to medical oncologists involved in the care of patients with lung cancer. Twenty-five specialists from nine European countries participated in 10 interactive sessions, and shared their experience in the course of discussions evolving around recent scientific findings that were presented by a multidisciplinary panel of experts.

The lectures initially focused on diagnostic aspects, such as pathology, liquid biopsy, and staging. Another topic of great importance was screening and early detection of lung cancer. Last but not least, aspects of treatment received broad attention and are summarised in this publication. Clinical research has provided us with a range of modern therapies necessitating algorithms that support physicians in the making of treatment decisions. We hope that both the Preceptorship and these summaries will provide doctors with a better understanding of lung cancer.

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Robert Pirker, MD, Medical University of Vienna, Austria

Pathology and WHO classification of tumours of the lung: what is new?

The WHO classification of tumours of the lung, pleura, thymus and heart that was published in 2004 [1] was revised in 2015 [2] **(Figure 1).** Leonhard Müllauer, MD, Institute of Pathology, Medical University of Vienna, Austria, summarised the changes.

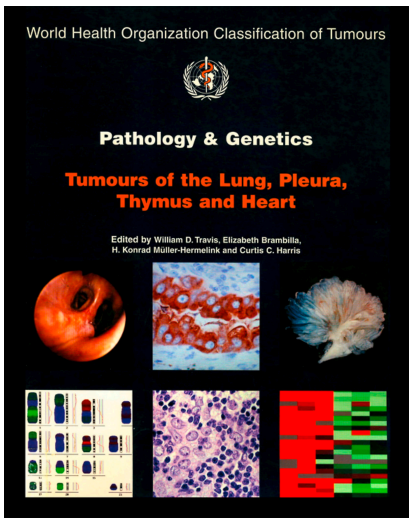

Figure 1: WHO classifications of tumours of the lung, pleura, thymus and heart published in 2004 (left) and 2015 (right)

Adenocarcinoma *in situ*

"The 2004 classification had only one type of pre-invasive lesion, called atypical adenomatous hyperplasia", Dr. Müllauer explained. "This type is still part of the new classification, but a new entity has been added, which is adenocarcinoma *in situ*." By definition, atypical adenomatous hyperplasia is a localised, small (≤ 0.5 cm) proliferation of mildly to moderately atypical type II pneumocytes and/or Clara cells that line the alveolar walls. Adenocarcinoma *in situ,* on the other hand, is localised and small (≤ 3 cm), with growth restricted to neoplastic cells along pre-existing alveolar structures. "This growth pattern is called pure lepidic growth," Dr. Müllauer said. The term "lepidic growth" replaces the older expression of bronchioloalveolar carcinoma (BAC). Adenocarcinoma *in situ* shows no stromal, vascular or pleural invasion, and no pattern of invasive growth. Most of these tumours are non-mucinous, although some are mucinous and some are mixed. After complete resection, good disease-specific long-term survival can be expected.

(Minimally) invasive adenocarcinoma

While the 2004 classification related to the term of adenocarcinoma only, the 2015 classification differentiates between minimally invasive adenocarcinoma and invasive adenocarcinoma.

Minimally invasive adenocarcinoma is small (≤ 3 cm) and solitary, with a predominantly lepidic growth pattern and ≤ 5 mm invasion. This type of tumour is usually non-mucinous. Invasion is defined by infiltration of the stroma or the presence of a non-lepidic growth pattern. Minimally invasive adenocarcinoma does not invade vessels or pleura; furthermore, neither tumour necrosis nor spread through air spaces are involved. Again, disease-specific survival after resection approaches 100 %.

The definition of invasive adenocarcinoma according to the 2015 classification differs from adenocarcinoma as defined in 2004, with regard to the subtypes. The entity of non-mucinous BAC has been replaced by the lepidic (non-mucinous) type. The other categories now included are the acinar, papillary, micropapillary and solid variants. "The micropapillary type was introduced as a new entity after it had been recognised as a specific pattern," Dr. Müllauer noted. "These tumours appear to convey unfavourable prognosis."

Further variants of invasive adenocarcinoma include the mucinous, colloid, foetal and enteric types. Almost all of these categories differ from those classified in the 2004 version. The former terms of mucinous adenocarcinoma and mucinous cystadenocarcinoma are now summarised as colloid adenocarcinoma. Enteric adenocarcinoma represents a novel subtype, which histologically resembles colorectal carcinoma. "In these cases, it is important to rule out primary tumours of the colon that have spread to the lung," Dr. Müllauer pointed out. "The pathologist should indicate this in his report." Moreover, the term of signet-ring/clear-cell adenocarcinoma has been removed. Adenocarcinoma *in situ,* minimally invasive adenocarcinoma, and predominantly lepidic adenocarcinoma are infrequent compared to invasive adenocarcinoma, according to an analysis of 514 patients with stage I tumours **(Figure 2).**

TABLE
Miscellaneous malignant epithelial tumours (WHO 2015)

Adenosquamous carcinoma (each component constitutes a least 10 % of the tumour)
Pleomorphic, spindle-cell, and giant-cell carcinoma (contains at least 10 % spindle and/or giant cells)
Carcinosarcoma (mixture of non–small-cell lung cancer and sarcoma elements)
Pulmonary blastoma (foetal adenocarcinoma and primitive mesenchymal stroma)
Lymphoepithelioma-like carcinoma (EBV-associated)
Nuclear-protein-in-testis (NUT) carcinoma
Salivary-gland-type tumours

Other histologies

For squamous-cell carcinoma, the new classification recognises pre-invasive lesions (squamous cell carcinoma *in situ*) on the one hand, and invasive squamous cell carcinoma on the other. The newly defined morphological subtypes of the invasive variant are keratinising, non-keratinising, and basaloid. Large-cell carcinoma, according to WHO 2015, is an undifferentiated, non–small-cell carcinoma that lacks the features of small-cell carcinoma, adenocarcinoma and squamous-cell carcinoma. "This type of tumour is diagnosed only in resection specimens, because lung cancer is very heterogeneous and large-cell structures in a biopsy might only represent a small component of the tumour," Dr. Müllauer stressed.

The **Table** lists the other malignant epithelial lung tumours that are mentioned in the new classification. Here, EBV-associated lymphoepithelioma-like carcinoma and nuclear-protein-in-testis (NUT) carcinoma are new entities. NUT tumours are very rare, but they might also be under-diagnosed. As specific immunohistochemistry is required for detection of the NUT protein, increased use of this type of analysis might increase the numbers of diagnosed cases. "NUT carcinoma is a very aggressive tumour with a poor prognosis", Dr. Müllauer explained. For neuroendocrine tumours, the classification differentiates between small-cell carcinoma, large-cell neuroendocrine carcinoma, and carcinoid tumours. Carcinoid tumours, which comprise both typical and atypical variants, are less aggressive than the other types.

NOS is largely obsolete

The new stratification highlights the importance of accurate subtyping. Immunohistochemistry is mandatory in this context. Most laboratories use panels of antibodies against the following proteins: TTF-1, cytokeratin 7 and napsin for the diagnosis of adenocarcinoma; p40, p63 and cytokeratin 5/6 for squamous-cell carcinoma; and chromogranin A and synaptophysin for neuroendocrine tumours. Cytokeratin 20, CDX2 and other markers contribute to differentiation between primary tumours and metastatic lesions. Semi-quantitative subtyping of invasive adenocarcinoma is recommended. "All of the components of the tumour, such as acinar,

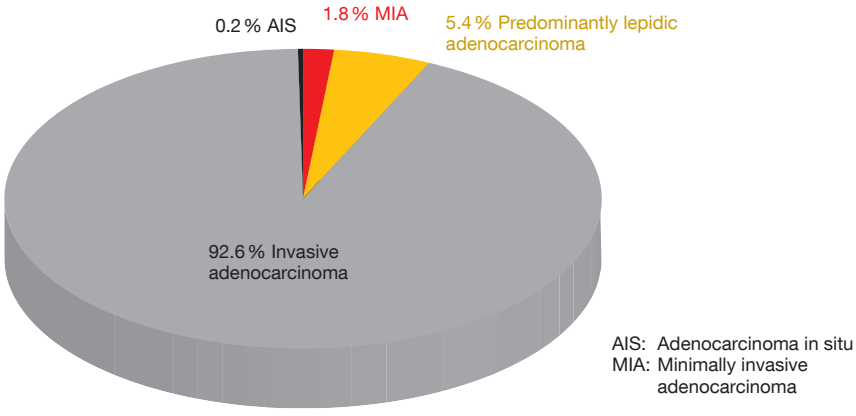

Figure 2: Frequency of adenocarcinoma *in situ*, minimally invasive adenocarcinoma, and predominantly lepidic adenocarcinoma, in 514 patients with stage I adenocarcinoma of the lung

papillary or solid, should be specified in the report, and the predominant morphological pattern should be defined," Dr. Müllauer emphasised. As immunohistochemistry basically allows for subtyping of all carcinomas, the term of NSCLC not otherwise specified (NOS) should be used as little as possible.

Also, the 2015 classification contains a specific terminology that relates the findings in small biopsies and cytological specimens to those in resection specimens, which are more likely to be correct. "For instance, the classification states that cells with the appearance of adenocarcinoma and a lepidic pattern according to a small biopsy or cytology might represent adenocarcinoma *in situ,* minimally invasive adenocarcinoma, or invasive adenocarcinoma with a lepidic component, according to the resection specimen," Dr. Müllauer said. Non-small-cell lung cancer (NSCLC) NOS tends to be large-cell carcinoma, and NSCLC with neuroendocrine morphology and positive neuroendocrine markers is large-cell neuroendocrine carcinoma of the lung in most cases. Only when there are unequivocal findings (i. e., distinct morphology, matching immunohistochemical staining) are the terminologies for small biopsies and cytology the same as for resection specimens. ■

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Screening and early detection of lung cancer

According to the principles stated by the World Health Organisation, screening programmes are aimed at timely detection of diseases that represent important health problems and for which there are accepted treatments [1]. Suitable and acceptable tests are required, and the cost of case finding should be economically balanced. "Lung cancer is definitively a relevant health problem, which is why we need early detection," stated Rudolf Maria Huber, MD, Division of Respiratory Medicine and Thoracic Oncology, Thoracic Oncology Centre Munich, University of Munich, Germany. The incidence rates are high, and 60 % of patients die within one year of diagnosis. Symptomatic lung cancer is almost always detected at an advanced stage, where cure is hardly ever achieved.

From X-ray to low-dose computed tomography

Lung-cancer screening in the 1970s, 1980s and 1990s involved chest radiography and sputum cytology. Ten prospective X-ray trials with and without cytology were conducted between 1951 and 1985, but none of these demonstrated any clinically relevant benefits of screening. For instance, the randomised, controlled Mayo Lung Project showed that more lung-cancer cases were detected in the screening group than in the control group, but the lung-cancer death rates did not differ between these two groups [2]. Based on the available evidence, almost all of the scientific societies and legal authorities recommend against screening by chest X-rays or sputum cytology.

The lack of benefit of screening using chest X-rays can be due to the advanced size of nodules at the time of their routine clinical detection, with the tumour cells having already gone through many doubling times. "The diameter of these lesions is usually 2 cm to 10 cm," noted Dr. Huber **(Figure 1).** Computed tomography (CT), on the other hand, enables identification of nodules of 4 mm, or even less. "This technique is much more sensitive than chest X-rays."

The National Lung Screening Trial

Many prospective studies have investigated lung-cancer screening using low-dose CT. The large randomised American National Lung Screening Trial

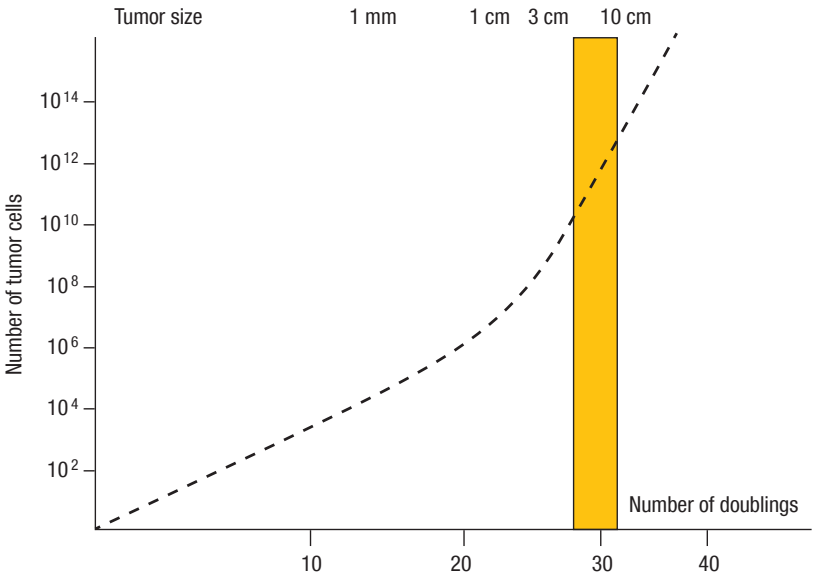

Figure 1: Typical tumour size at the time of routine clinical detection with X-rays (yellow box)

(NLST) included 53,464 high-risk subjects, and it yielded positive results. People aged 55 to 74 years who had a smoking history of 30 or more pack years were randomised to CT or chest X-rays. Former smokers had quit smoking within the previous 15 years. The participants underwent three annual screens. "As compared to radiography, low-dose CT screening promoted a relative reduction in lung-cancer mortality of 20 %," Dr. Huber reported **(Figure 2)** [3]. "Overall mortality was reduced by 6.7 %." As in the Mayo Project, the cumulative number of tumours detected by CT exceeded the number found in the control arm, but the patients benefited from it to a clinically relevant extent.

From a statistical point of view, the positive predictive value estimated in the NLST was higher for chest X-rays than for low-dose CT (5.7 % vs. 3.8 %), although X-rays were clearly less sensitive (74 % vs. 94 %) [4]. "On the other hand, a normal chest CT scan does not guarantee the absence of cancer," Dr. Huber pointed out. Interval cancers (tumours that emerged in-between screenings) occurred in 26 people participating in the NLST, and 42 % of these were stage IV [5]. Endobronchial tumours do not regularly show on CT. " Patients undergoing a screening programme have to be informed that there is the possibility of missed cases." Also, radiation exposure confers a slightly increased risk of leukaemia and brain cancer [6].

Potential confounders

Based on the NLST, researchers have estimated that 320 people need to be screened with low-dose CT to prevent one death [3]. Expenses for one life year gained were estimated at US$ 52,000 [7]. For one quality-adjusted life year gained, this was US$ 81,000 [7]. "These results depend one many assumptions, and accordingly vary widely across subgroups, and they have to be calculated differently for every country," Dr. Huber said.

Moreover, bias is prone to interfere with the interpretation of trials, such as lead-time bias, length-time bias, and over-diagnosis bias. For the NLST, there was also participation bias, as participants were younger and better educated than those who usually develop lung cancer [8]. Also, they were less likely to be current smokers. European studies such as the Danish Lung-Cancer Screening Trial [9] and the DANTE trial [10] prospectively assessed CT-based lung-cancer screening, with negative results so far. Dr. Huber cautioned against a meta-analysis due to differences in design.

Due to these mixed trial results, recommendations on the use of lung-cancer screening vary between countries. The American College of Chest Physicians and the American Society of Clinical Oncology recommend annual screening with low-dose CT for smokers and former smokers (of ≥ 30 pack years) aged 55 to 74 [11]. A joint statement of the German Respiratory and Radiological Societies says that native low-dose CT can be justified on an individual basis (as individual early detection) [12]. Intensive patient education is necessary, which includes smoking cessation advice, as well as interdisciplinary management and quality assurance. The French National Authority for Health commissioned experts to carry out a systematic review on the effectiveness, acceptability and safety of lung-cancer screening with low-dose CT in subjects highly exposed to tobacco [13]. "They concluded that screening should not be recommended in this population," Dr. Huber reported.

Figure 2: National Lung Screening Trial: diminished lung-cancer–related mortality due to low-dose CT screening, as compared to chest X-ray screening [3]

How to improve early detection

"We all agree that screening needs to be improved," Dr. Huber stressed. This particularly applies to the definition of risk populations and the work-up, with the aim being to avoid false-positive findings. General screening of the US population using the NLST criteria would detect 26.7 % of all lung cancers [14], but reimbursement represents an unsolved issue in this context. Another problem relating to screening programmes is the lack of tools for the assessment of never or light smokers.

With regard to the definition of risk populations, at the ASCO Congress, Aberle et al. presented eligibility criteria for population screening [15]. These are now being introduced in the US. "NLST-based risk factor scoring shows that the preventive effect is greatest in people with the highest risk," said Dr. Huber [16]. However, models involving multiple variables are not the only tests that make risk stratification possible. Lung function testing, which is simple and inexpensive, allows for very good discrimination. "The lung-cancer risk rises exponentially in men if FEV_1 is reduced", Dr. Huber emphasised [17] **(Figure 3).** Likewise, decreased carbon monoxide diffusion capacity indicates increased risk, which is true for both sexes [18]. A more sophisticated tool is exhaled breath analysis that differentiates between benign and malignant nodules [19]. Moreover, a blood-based proteomic classifier score was shown to characterise pulmonary nodules as either benign or malignant at the molecular level with high

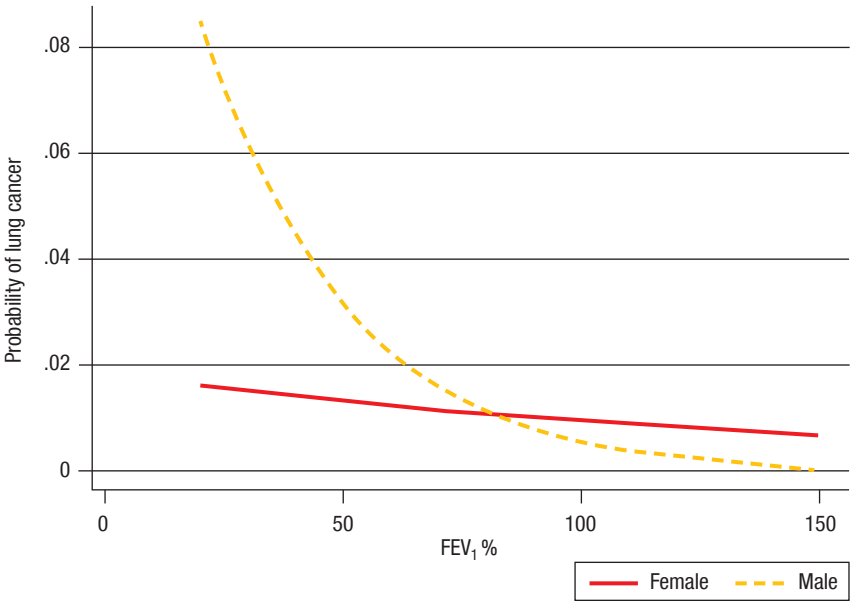

Figure 3: Association between loss of FEV_1 and probability of lung cancer in men [17]

confidence [20]. All of these tools can contribute to the improvement of lung-cancer diagnosis and can avoid unnecessary invasive procedures.

False positives and over-diagnosis

False-positive findings that give rise to pointless diagnostic and therapeutic measures are of great concern in the setting of lung-cancer screening. According to data from the NLST, 30 % of all surgical procedures were performed in patients with benign disease [4]. "This happened even though experienced centres were involved that routinely used CT-guided biopsy," Dr. Huber noted. Another important aspect relates to over-diagnosis. More than 18 % of all lung cancers detected by low-dose CT in the NLST appeared to be indolent [21]. The probability of over-diagnosis in a bronchioalveolar lung-cancer case detected by low-dose CT was 78.9 %. Statistically, for one patient who stayed alive due to screening in the NLST, 1.38 patients were over-diagnosed. As Dr. Huber pointed out, this needs to be kept in mind when implementation of screening is discussed.

The Lung CT Screening Reporting and Data System (Lung-RADS™), which was designed to standardise lung-cancer screening CT reporting and management recommendations, decreases the probability of false positives. Also, volumetric measurements of nodules help to decide whether invasive diagnosis should be used [22]. Online calculators can facilitate the assessment of the probability of malignancy in a given nodule (www.brocku.ca/cancerpredictionresearch).

Is prevention better than detection?

The NLST-based analysis by Tanner et al. suggested that smoking cessation is just as effective as early detection of lung cancer [23]. "Former smokers in the control arm who had abstained for seven years showed a 20 % mortality reduction," Dr. Huber noted. Compared to this group, current smokers showed increased lung-cancer–specific mortality and all-cause mortality, irrespective of screening arm. The maximum benefit occurred in patients who received CT screening and who had abstained from smoking for at least 15 years; here, a 38 % reduction in lung-cancer–specific mortality was seen.

These observations were even surpassed by an Italian trial that compared current smokers with ex-smokers [24]. "The benefit of smoking cessation appeared to be 3-fold to 5-fold greater than that achieved by early detection in the NLST trial." The survival curves separated throughout the follow-up period. "I believe that we have to focus on prevention," Dr. Huber summarised. "Nevertheless, we should try to improve detection techniques and algorithms." ■

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Staging of lung cancer: the 8th TNM classification

In 2009, the 7th Edition of the TNM classification of malignant tumours was published [1]. The proposals for the revised T, N and M categories will be implemented in the 8th Edition that is expected for late 2016. These proposals have been developed by the Staging and Prognostic Factors Committee of the International Association for the Study of Lung Cancer (IASLC) based on a large prospective database [2]. "It contains almost 100,000 cases and has been built up from 1990 onwards," explained Wilfried Eberhardt, MD, Department of Medical Oncology, University Hospital Essen, University of Duisburg-Essen, Germany.

The IASLC Lung Cancer Staging Project collects not just anatomical information, but also non-anatomical data. These include patient-related elements (e. g., age, sex, race, smoking history, laboratory analyses, lung function test results), tumour-related elements (e. g., maximum standardised uptake value for tumour and lymph nodes, histological type, vascular invasion, tumour markers) and environment-related elements (method of detection, treatment, residual tumour, geographic area). Dr. Eberhardt detailed the proposals for the T, N and M descriptors and the new stage groupings for patients with NSCLC **(Table).**

T descriptors

With respect to the T descriptors, the proposals underline the significance of tumour volume, which is one of the major prognostic factors. The tumour diameter can also be used as a surrogate marker for tumour volume. "This has always been part of staging, but it is becoming more and more important", Dr. Eberhardt said. T1 has been subclassified into T1a (≤ 1 cm), T1b (> 1 to ≤ 2 cm) and T1c (> 2 to ≤ 3 cm). Likewise, T2 now consists of T2a (> 3 to ≤ 4 cm) and T2b (> 4 to ≤ 5 cm). Tumours > 5 cm to ≤ 7 cm have been reclassified as T3, and those > 7 cm belong to the T4 category. "We are taking into account the poor prognosis of larger tumours," Dr. Eberhardt noted. As is typical of solid tumours, life expectancy decreases with increasing lung cancer size.

Another change pertains to the involvement of the main bronchus, which has been classified as T2 regardless of the distance from the carina. Moreover, partial and total atelectasis/pneumonitis are defined as T2, and diaphragm invasion has been reclassified as T4. Mediastinal pleura invasion has been removed as a T descriptor. On the other hand, various features have been maintained in the new edition, such as the definition of visceral pleural invasion as a T2 descriptor, and the subclassification of parietal pericardium, mediastinal pleura and chest-wall invasion, Pancoast tumour, parietal pleural invasion, and additional nodules in the same lobe as T3 descriptors. Also, involvement of the mediastinum, pulmonary artery, aortic wall, vena cava, vertebral body, trachea and carina, and separate nodules in other ipsilateral lobes, are still classified as T4 descriptors.

As Dr. Eberhardt pointed out, these proposed changes successfully serve the purpose of defining different prognostic groups more clearly. "Whereas the survival curves hardly differed between cT3 and cT4 according to the 7th Edition, the new T categories make for nice separation of all of the staging groups." This observation applies to both clinical and pathological staging.

N descriptors

The database did not provide enough information to warrant implementation of changes with regard to the N descriptors. For both clinical and pathological staging, the 5-year survival estimates vary distinctly according to the established categories of N0 to N3. "Therefore, the committee decided that the current N descriptors adequately predict prognosis and should be maintained in the forthcoming staging system," Dr. Eberhardt reported.

However, based on the large heterogeneity of stage III lung cancer, it has been recommended that physicians record the number of metastatic lymph nodes (or stations) and further classify the N category using the new descrip-

TABLE

Descriptors and T and M categories in the 7th Edition and as proposed for the 8th Edition. The resulting stage groupings proposed for the 8th Edition are highlighted in bold where changes have been implemented (the 7th Edition stage is given in parentheses). [1,2]

7th Edition descriptor	*Proposals for 8th Edition*				
	T/M	*N categories overall staging*			
		N0	*N1*	*N2*	*N3*
T1 ≤ 1 cm	T1a	**IA1** (IA)	**IIB** (IIA)	IIIA	IIIB
T1 > 1 – 2 cm	T1b	**IA2** (IA)	**IIB** (IIA)	IIIA	IIIB
T1 < 2 – 3 cm	T1c	**IA3** (IA)	**IIB** (IIA)	IIIA	IIIB
T2 > 3 – 4 cm	T2a	**IB**	**IIB** (IIA)	IIIA	IIIB
T2 > 4 – 5 cm	T2b	**IIA** (IB)	**IIB** (IIA)	IIIA	IIIB
T2 > 5 – 7 cm	T3	**IIB** (IIA)	**IIIA** (IIB)	**IIIB** (IIIA)	**IIIC** (IIIB)
T3 structures	T3	**IIB**	IIIA	**IIIB** (IIIA)	**IIIC** (IIIB)
T3 > 7 cm	T4	**IIIA** (IIB)	IIIA	**IIIB** (IIIA)	**IIIC** (IIIB)
T3 diaphragm	T4	**IIIA** (IIB)	IIIA	**IIIB** (IIIA)	**IIIC** (IIIB)
T3 endobronch: location/atelectasis 3 – 4 cm	T2a	**IB** (IIB)	**IIB** (IIIA)	IIIA	IIIB
T3 endobronch: location/atelectasis 4 – 5 cm	T2b	**IIA** (IIB)	**IIB** (IIIA)	IIIA	IIIB
T4	T4	**IIIA**	IIIA	IIIB	**IIIC** (IIIB)
M1a	M1a	**IVA** (IV)	**IVA** (IV)	**IVA** (IV)	**IVA** (IV)
M1b single lesion	M1b	**IVA** (IV)	**IVA** (IV)	**IVA** (IV)	**IVA** (IV)
M1c multiple lesions	M1c	**IVB** (IV)	**IVB** (IV)	**IVB** (IV)	**IVB** (IV)

tors of N1a (single), N1b (multiple), N2a1 (single without N1 – skip), N2a2 (single with N1), N2b (multiple), and N3. "This was felt to be necessary to define the heterogeneity of stage III patients more correctly in future classifications," Dr. Eberhardt said. These changes will hopefully provide more data that will enable the committee to implement new recommendations in the next revision.

M descriptors

Important changes have been proposed regarding the M descriptors, with the aim being to define oligometastatic disease. The restriction of analyses to long-term survivors in this group of patients naturally promotes selection bias, and the definition of oligometastatic disease itself has been very vague, with the lack of any evidence-based foundation. "Some scientists suggest only one metastatic lesion, others up to five," Dr. Eberhardt said.

In the current revision of the staging system, several features continue to be grouped as the M1a category (pleural/pericardial effusions, contralateral/bilateral lung nodules, contralateral/bilateral pleural nodules, or a combination of these parameters), whereas single metastatic lesions in a single distant organ have been newly designated to the M1b category. At the same time, multiple lesions in a single organ and in multiple organs have been reclassified as the new M1c category. "This division can serve as a first step towards providing rational definitions for staging of oligometastatic NSCLC," Dr. Eberhardt stated.

The maintenance of the M1a category is based on the lack of differences in survival according to the four parameters of pleural/pericardial nodules, contralateral/bilateral tumour nodules, pleural/pericardial effusion, and multiple M1a descriptors. Here, the patient numbers remain relatively small, which also applies to the new M1b category. For survival according to single lesions at single sites, only metastases to the adrenal gland stood out in the first analysis, although this difference did not hold true for other subsets. "In the future, more patients with metastatic disease should be included in the database," Dr. Eberhardt emphasised. Only the M1a and M1b categories are associated with long-term survival according to the 8th Edition, while the M1c category is not.

Similar staging changes in NSCLC and SCLC

Compared to the 7th Edition, the staging of NSCLC that is being proposed for the 8th Edition is more complicated, as it involves 11 categories rather than 7. "However, at the end of the day, these curves separate nicely," Dr. Eberhardt stressed **(Figure).** Patients with stage IA1 disease experience an average 60-month survival of 92 %; for stage IVB, at the other end of the range, this rate is as low as 0 %, while patients with stage IVA experience long-term survival at 10 %. This difference might have some implications with regard to prospective clinical trials that use multimodal treatments.

For small-cell lung cancer (SCLC), as opposed to NSCLC, stage IV is not being divided into subcategories due to insufficient patient numbers. Other than this, the same subsets have been defined as for NSCLC, and these differ clearly with regard to survival. "The prognosis of SCLC patients based on staging is comparable to that in patients with NSCLC."

As Dr. Eberhardt pointed out, the participation of new centres in the next staging round would be greatly welcomed. "We desperately need more patients, and we would appreciate it if additional centres joined us." ■

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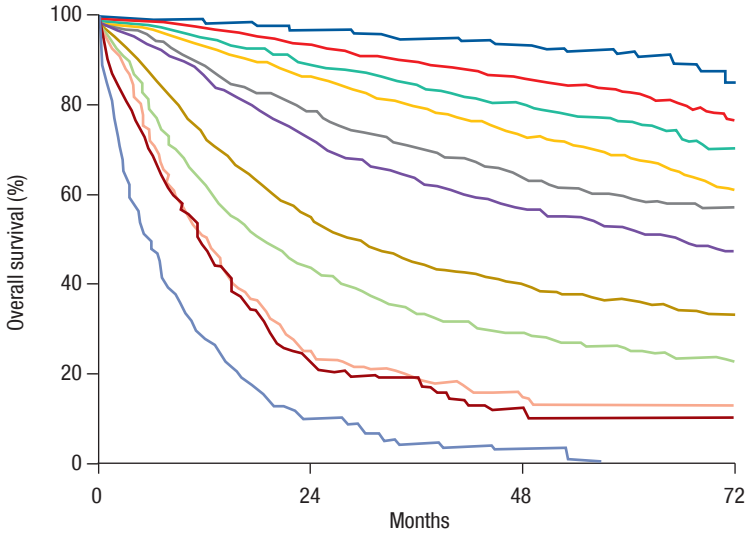


Staging	60-month overall survival (%)
IA1	92
IA2	83
IA3	77
IB	68
IIA	60
IIB	53
IIIA	36
IIIB	26
IIIC	13
IVA	10
ICB	0

Figure: Overall survival according to the 8th Edition IASCL staging proposals [2]

Treatment of lung cancer: *status quo* & news from ASCO 2016

Robert Pirker, MD, Department of Internal Medicine I, Medical University of Vienna, Austria, discussed evidence from clinical trials for the treatment of lung cancer, with a focus on the findings presented at the ASCO 2016 Congress. As Dr. Pirker emphasised, a variety of topics was discussed at the conference, although none of the clinical trial results reported on this year have heralded fundamental changes in clinical practice.

Adjuvant chemotherapy of completely resected NSCLC

The implementation of adjuvant chemotherapy in lung cancer marks one of the major achievements over the last decades. One of the current goals is the identification of predictive biomarkers for patient selection. However, this approach has not been successful to date. "The IALT-Bio trial tried to establish biomarkers, and LACE-Bio aimed to validate them, but the validation has failed," Dr. Pirker reported. At present, no predictive biomarkers are available. Another approach is customised chemotherapy, which is being tested in an Italian trial (NCT01784549). "Customised chemotherapy is complex, and it remains to be seen if this concept works," Dr. Pirker said.

Thus far, the integration of targeted therapies into the adjuvant setting has also failed. This applies to the addition of bevacizumab to chemotherapy, to the MAGE-A3 vaccine, and to EGFR tyrosine kinase inhibitor (TKI) therapy in patients unselected for *EGFR* mutations. Two phase III trials are assessing adjuvant gefitinib compared to chemotherapy in patients with *EGFR*-mutation-positive tumours: ADJUVANT (NCT01405079), and WJOG6410L [1]. Disease-free survival is the endpoint in both studies. "These results will be available soon," Dr. Pirker noted.

Also, the ongoing ADAURA study is investigating the third-generation EGFR TKI osimertinib *versus* placebo in patients with completely resected stage IB-IIIA NSCLC and exon 19 deletion or L858R mutation (NCT02511106). Patients with or without adjuvant chemotherapy are eligible. The ALCHEMIST trial aims at the identification of molecular markers in the adjuvant setting (NCT02194738) and evaluates adjuvant therapy with erlotinib in *EGFR*-mutation-positive patients, as well as treatment with crizotinib in *ALK*-positive patients.

Therapeutic options in stage III NSCLC

Locally advanced (stage III) NSCLC is a heterogeneous disease, the treatment of which usually involves radiotherapy and chemotherapy. These two strategies can be applied either sequentially or concomitantly **(Figure 1)**. Induction chemotherapy can precede chemoradiotherapy, or a combined schedule can be followed by consolidation chemotherapy. Surgery is included for selected patients.

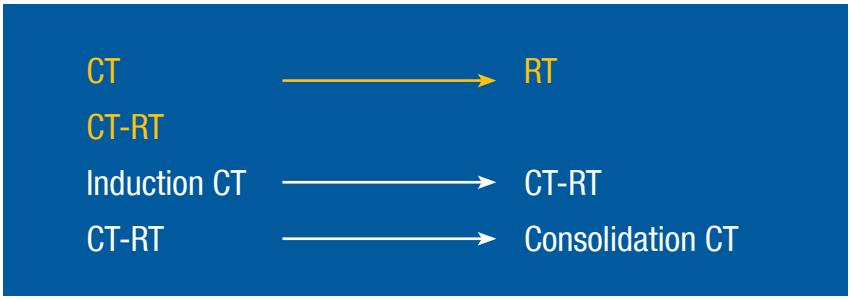
Optimisation of stage III therapy has been attempted in different areas, with limited success. Prophylactic brain irradiation was tested in a trial based on the rationale that the central nervous system is frequently the first metastatic site. "This study had to be stopped because the patients declined receiving another treatment after completion of chemoradiotherapy," Dr. Pirker reported. Vaccination did not meet expectations, either. The randomised, placebo-controlled, phase III START study revealed no overall survival (OS) advantage of tecemotide maintenance after chemoradiation [2].

A new concept is proton therapy, which allows for circumscribed application of irradiation doses, thus sparing other organs such as the heart. At the ASCO Congress, Liao et al. presented a Bayesian randomised trial comparing intensity-modulated radiation therapy and passively scattered proton therapy for locally advanced NSCLC [3]. Time to treatment failure as the primary endpoint did not differ between the two techniques. "A major issue with proton therapy is its costs," Dr. Pirker stated. "Accordingly, the patients who resort to proton therapy are mostly wealthy, better educated, and live in urban areas that provide access to this method."

Anti-EGFR agents in untreated patients with advanced NSCLC

EGFR mutations are present in 30 % to 60 % of Asian patients with adenocarcinomas, and in 10 % to 15 % of their Caucasian counterparts. For *ALK* rearrangement, these percentages range from 3 % to 5 %. While all EGFR TKIs prolong PFS, afatinib is the only EGFR TKI that has shown improved survival in patients with advanced *EGFR*-mutated NSCLC, compared to chemotherapy. The combined analysis of the LUX-Lung 3 and 6 trials yielded median survival times for patients with deletion 19 of 31.7 vs. 20.7 months (HR, 0.59; p = 0.0001; **Figure 2**) [4]. In contrast, for those with L858R mutations, the median survival times were 22.1 vs. 26.9 months (HR, 1.25; p = 0.16).

The fully human anti-EGFR monoclonal antibody necitumumab was investigated in the SQUIRE trial in 1,093



CT: chemotherapy RT: radiotherapy

Figure 1: Administration of chemotherapy and radiotherapy in stage III NSCLC

patients with advanced squamous NSCLC [5]. This study compared necitumumab added to cisplatin plus gemcitabine with cisplatin plus gemcitabine alone. "OS was significantly prolonged in the necitumumab arm," Dr. Pirker pointed out (11.5 vs. 9.9 months; stratified HR, 0.84; p = 0.01; **Figure 3**). Based on this pivotal trial, necitumumab was approved as first-line therapy for squamous *EGFR*-expressing NSCLC in combination with gemcitabine and cisplatin. Non-squamous patients were included in the INSPIRE trial that investigated cisplatin plus pemetrexed with or without necitumumab [6]. However, enrolment was prematurely stopped due to a lack of survival benefit of the combination, in addition to an increased rate of severe adverse events.

Later lines and *EGFR* mutation positivity

The LUX-Lung 8 trial compared afatinib with erlotinib in patients with advanced squamous NSCLC who had progressed after at least four cycles of a first-line platinum-based doublet. Afatinib therapy led to improved survival compared to erlotinib (median, 7.9 vs. 6.8 months; HR, 0.81; p = 0.0077) [7].

Patients with *EGFR*-mutation-positive tumours who undergo EGFR TKI therapy will ultimately develop resistance. At that time, re-biopsy is recommended. Treatment options at the time of resistance to the first-generation and second-generation TKIs include a switch to chemotherapy, possibly followed by TKI re-challenge, use of third-generation EGFR TKIs, and continuation of the original TKI therapy. Local interventions can also be added to the TKI treatment. Another option is combined administration of afatinib and cetuximab.

At present, the third-generation EGFR TKIs are osimertinib, rociletinib and olmutinib. These agents target *EGFR*-activating mutations and the T790M mutation, while sparing wild-type *EGFR*. "The sparing of wild-type *EGFR* ensures improved tolerability due to decreased side effects, such as rash or diarrhoea," Dr. Pirker explained. Osimertinib has already been approved in locally advanced or metastatic T790M-positive disease. The AURA3 trial is comparing osimertinib with platinum-based chemotherapy after progression on EGFR TKI therapy in T790M-positive disease (NCT02151981). "The OS results will be available soon, and these will provide information on the choice between TKI therapy and chemotherapy after disease progression," Dr. Pirker noted.

Encouraging results have been obtained with olmutinib in a phase I/II trial in Korean patients [8]. "Tumour responses in T790M-positive patients occurred in 61 %, and disease control was achieved in 90 %." In the meantime, the clinical development of rociletinib has been stopped due to toxicity and modest efficacy.

Next-generation agents in *ALK*-positive disease

Alectinib is an ALK inhibitor with activity against *ALK*-resistance mutations. In the Japanese J-ALEX study, it gave rise to improved PFS compared to crizotinib as first-line treatment in patients with *ALK*-positive disease (not reached vs. 10.2 months; HR, 0.34; p > 0.0001) [9]. "We are waiting for the results of the global ALEX trial, which is comparing alectinib with crizotinib." Dr. Pirker explained (NCT02075840). "These findings will shed light on the optimal first-line strategy in *ALK*-positive NSCLC."

Brigantinib, another next-generation ALK inhibitor with broad inhibitory activity, was tested at two doses in the ALTA trial [10]. Patients who had experienced progression on crizotinib received either 180 mg or 90 mg brigantinib. The objective response rates were 54 % and 45 %, respectively; disease control was achieved in 86 % and 82 %, respectively. In the 180 mg dose group, the median PFS exceeded 1 year (12.9 months).

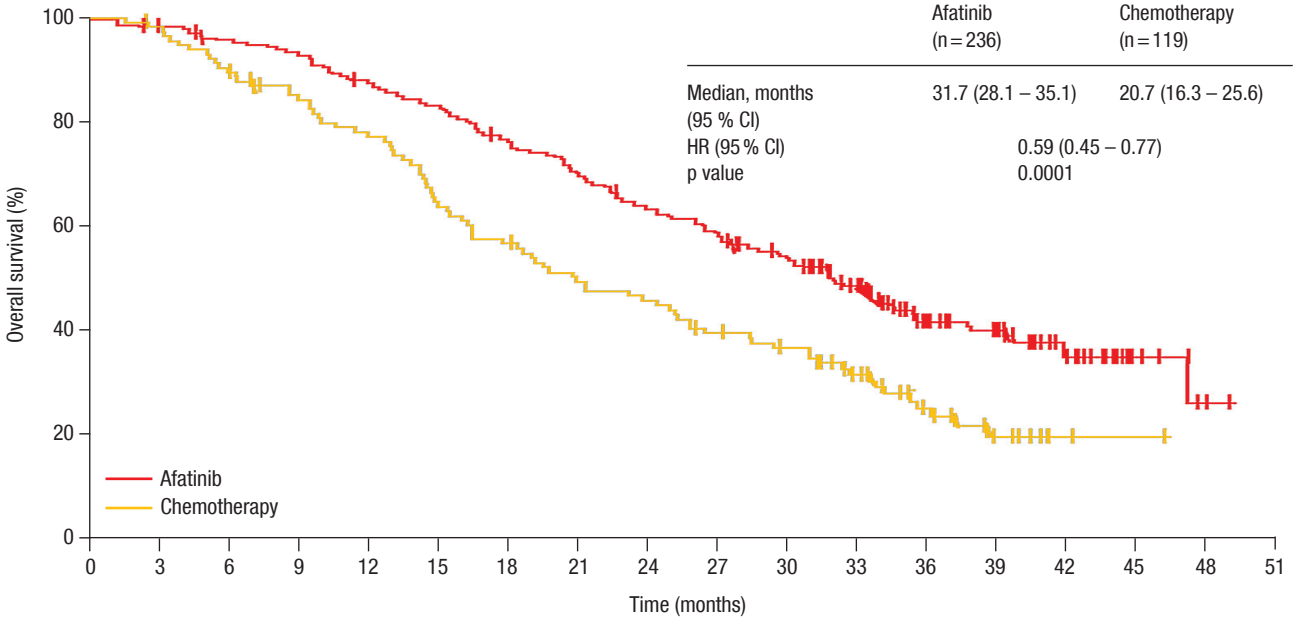


Figure 2: Highly significant overall survival improvement with first-line afatinib over chemotherapy according to the combined analysis of LUX-Lung 3 and LUX-Lung 6 [4]

Ramucirumab and nintedanib in second-line treatment of advanced NSCLC

In the second-line setting of advanced NSCLC, treatments with ramucirumab, the triple angiokinase inhibitor nintedanib, the irreversible ErbB family blocker afatinib, and immune checkpoint inhibitors constitute major recent advances. Ramucirumab was tested in addition to docetaxel in the REVEL trial [11]. As compared to the control arm, where the patients received docetaxel only, the combination gave rise to a 14 % reduction in mortality risk. Nintedanib was assessed in two phase III trials. The LUME-Lung 1 study investigated docetaxel with or without nintedanib in patients with all histological subtypes [12]. "While the addition of nintedanib significantly improved PFS in the total cohort, a significant OS benefit occurred only in the subgroup with adenocarcinoma," Dr. Pirker reported (10.9 vs. 7.9 months; HR, 0.75; p = 0.0073).

LUME-Lung 2 compared nintedanib plus pemetrexed with pemetrexed alone in patients with non-squamous NSCLC [13]. The results favoured the combination in terms of both progression-free survival (PFS; 4.4 vs. 3.6 months; HR, 0.83; p = 0.04) and disease control (61 % vs. 53 %; p = 0.039), whereas OS did not differ between the two arms. "Nintedanib has been approved in Europe for second-line treatment of locally advanced or metastatic adenocarcinoma in combination with docetaxel," Dr. Pirker said.

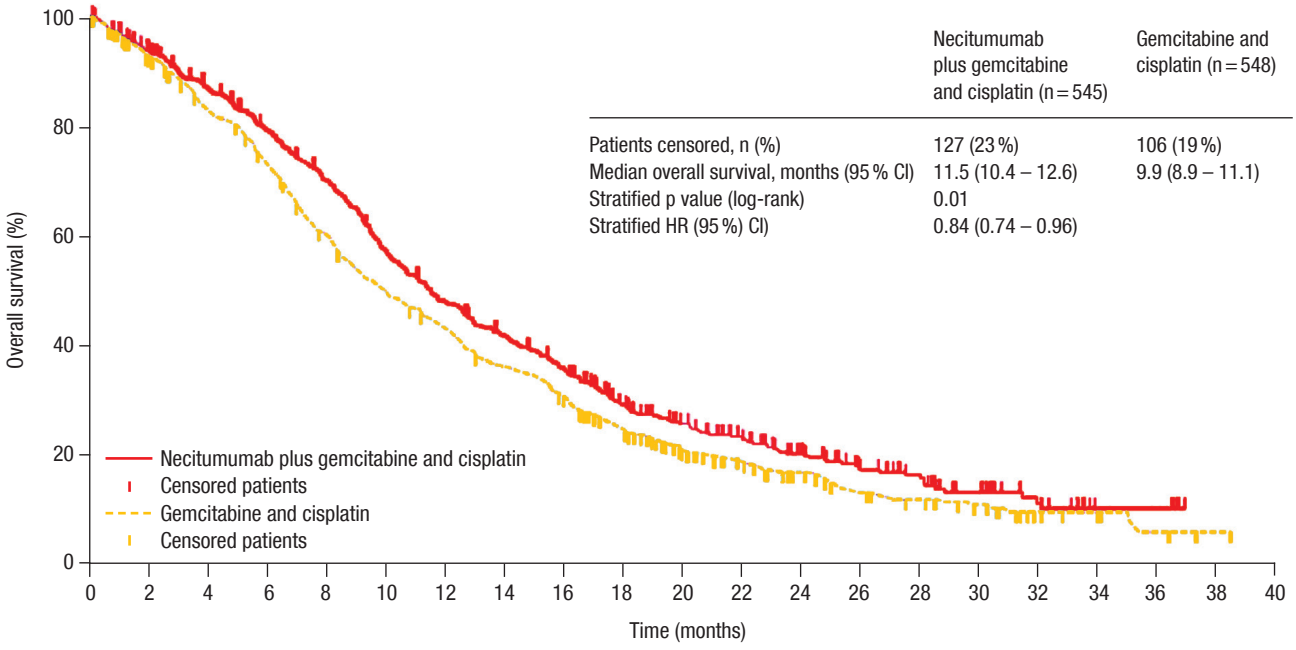
Immune checkpoint inhibitors in advanced NSCLC

Immune checkpoint inhibitors have demonstrated superior efficacy compared to docetaxel in second-line treatment of patients with advanced NSCLC. Side effects include immune-related pneumonitis, colitis, hepatitis, nephritis, endocrinopathies and infusion-related events. "Approval and reimbursement of nivolumab and pembrolizumab have been implemented in many countries," Dr. Pirker said.

The anti-PD-1 antibody nivolumab has shown OS benefits compared to docetaxel in advanced squamous NSCLC (CheckMate 017) [14] and adenocarcinomas (CheckMate 057) [15]. The degree of PD-L1 expression appears to predict the benefit of immune checkpoint inhibitors. The anti-PD-1 antibody pembrolizumab showed superiority over docetaxel in the KEYNOTE-010 trial, which enrolled patients with PD-L1 expression on at least 1 % of tumour cells [16]. OS was significantly longer for both doses of pembrolizumab (HRs, 0.71 and 0.61, for 2 mg/kg and 10 mg/kg, respectively). "This difference was even more pronounced in patients with at least 50 % of tumour cells expressing PD-L1," Dr. Pirker noted.

Similarly, the efficacy of the anti-PD-L1 antibody atezolizumab is greatest in the biomarker-enriched population, according to the POPLAR trial [17]. Here, baseline PD-L1 expression on tumour cells and tumour-infiltrating immune cells was assessed using immunohistochemistry (IHC). Atezolizumab significantly improved OS compared with docetaxel. "The survival advantage correlated with PD-L1 assessment that considered expression on both cell types," Dr. Pirker reported.

Phase III trials on first-line treatment with immune checkpoint inhibitors are ongoing in advanced NSCLC. KEYNOTE-024, which includes 305 patients with PD-L1 expression of ≥ 50 %, is comparing pembrolizumab with platinum-based chemotherapy (NCT02142738). "According to a press release issued in June 2016, pembrolizumab treatment has demonstrated superior PFS and OS," Dr. Pirker said [18]. KEYNOTE-042 is also testing pembrolizumab *versus* chemotherapy, although in patients with a broader range of PD-L1 expression (≥ 1 %) (NCT02220894). The primary outcome is OS.



	Necitumumab plus gemcitabine and cisplatin (n = 545)	Gemcitabine and cisplatin (n = 548)
Patients censored, n (%)	127 (23 %)	106 (19 %)
Median overall survival, months (95 % CI)	11.5 (10.4 – 12.6)	9.9 (8.9 – 11.1)
Stratified p value (log-rank)	0.01	
Stratified HR (95 %) CI)	0.84 (0.74 – 0.96)	

Figure 3: SQUIRE trial: overall survival benefit with necitumumab plus gemcitabine and cisplatin *versus* gemcitabine plus cisplatin alone [5]

TABLE
Evidence-based treatment options in advanced NSCLC

NSCLC feature	*First line*	*Maintenance*	*Second line*
Non-squamous	Platinum doublet ± bevacizumab	Pemetrexed Bevacizumab	Docetaxel ± nintedanib Docetaxel ± ramucirumab Pemetrexed, erlotinib Nivolumab, pembrolizumab
Squamous	Platinum doublet + necitumumab	Necitumumab	Docetaxel ± ramucirumab Erlotinib, afatinib Nivolumab; pembrolizumab
High EGFR IHC score or FISH positivity	Platinum doublet + cetuximab*	Cetuximab*	Docetaxel ± ramucirumab Pemetrexed Erlotinib
EGFR-*mutation positivity*	Afatinib, erlotinib, gefitinib	Afatinib Erlotinib Gefitinib	Chemotherapy EGFR TKIs** 3rd-generation EGFR TKIs
ALK *positivity*	Platinum doublet ± bevacizumab Crizotinib	Crizotinib Pemetrexed Bevacizumab	Crizotinib** Pemetrexed Ceritinib

* Not approved in the European Union
** If not given in earlier lines

Suggested algorithm for advanced NSCLC

Dr. Pirker presented his algorithm for first-line, maintenance and second-line treatments of advanced NSCLC **(Table).** The choice of treatment varies according to histological features (non-squamous, squamous) and the presence of EGFR IHC score or FISH positivity, EGFR-mutation or ALK positivity. As Dr. Pirker pointed out, the choices presented in the Table are evidence based, although some have not been approved in the European Union.

Small-cell lung cancer (SCLC)

The management of SCLC poses specific challenges. "Available treatments are often not being administered to patients," Dr. Pirker stressed. "According to the SEER data, only 50 % of patients with SCLC receive chemotherapy."

SCLC is radiosensitive, but the ideal radiotherapy is under discussion. Here, the CONVERT trial that was presented at the ASCO Congress has shown that once-daily thoracic irradiation with 66 Gy resulted in similar survival compared to twice-daily radiotherapy with 45 Gy [19]. The authors concluded that both regimens can be used.

Immunotherapy has found its way into the treatment of SCLC. The CheckMate 032 trial has established the activity of nivolumab plus ipilimumab in the second-line setting [20]. CheckMate 451 is currently assessing this combination as maintenance therapy in extensive-stage SCLC after first-line platinum-based doublet chemotherapy (NCT02538666).

Promising results have been obtained with the DLL3-targeted antibody–drug conjugate rovalpituzumab tesirine (Rova-T™). In the SCRX16-001 study, clinical benefit rates were 68 % and 89 % in the total cohort and in the biomarker-selected group (DLL3 expression of ≥ 50 %), respectively [21]. Thirty-two percent of patients with pronounced DLL3 expression were alive at 1 year.

Tiseo et al. showed that the addition of bevacizumab to cisplatin and etoposide in first-line treatment of extensive SCLC gives rise to significantly improved PFS compared to the chemotherapy regimen alone, but OS did not differ between the two arms [22]. ■

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Disease monitoring using circulating cell-free tumour DNA

In Caucasian patients, mutations of the *EGFR* gene occur in 10 % to 15 % of adenocarcinomas of the lung. "These tumours depend on EGFR signalling for growth and survival," explained Anna Buder, MSc, Institute of Cancer Research, Department of Medicine I, Comprehensive Cancer Center, Medical University of Vienna, Vienna, Austria. As these patients are sensitive to treatment with EGFR tyrosine kinase inhibitors (TKIs), identification of oncogenic driver mutations in adenocarcinoma has become a standard procedure in diagnostic testing.

Access to mutations via blood sampling

The *EGFR* gene contains 28 exons. Activating *EGFR* mutations are found in exons 18 to 21, which code for the tyrosine kinase domain of *EGFR*. "Although mutations occur throughout this domain, only some of them confer sensitivity to EGFR TKIs," Ms. Buder noted. The most prevalent mutations are lesions in exon 19, which make up 45 % of sensitising mutations. Point mutations in exon 21 are also very common, especially L858R. Nucleotide substitutions can be identified in exon 18, and there can be in-frame insertions in exon 20. Acquired resistance caused by the T790M mutation develops in more than half of TKI-treated patients, necessitating changes in their treatment. According to an analysis conducted at the Institute of Cancer Research in Vienna, 35 % of 167 patients tested negative for the T790M mutation and also stayed negative during follow-up, while 65 % developed the T790M mutation over time.

Patient case: rising copy numbers of T790M and deletion 19 preceding progression

The accuracy of liquid biopsy is illustrated by the case of a female patient with NSCLC who was monitored using ddPCR. As her tumour tested EGFR-mutation-positive, she received treatment with the EGFR TKI afatinib. In August 2015, while she was still clinically stable, ddPCR already revealed elevated copy numbers for both T790M (1,969/mL) and deletion 19 (1,072/mL). By the end of October, the numbers had risen to 3,925/mL and 6,146/mL, respectively, and radiological progression became obvious. Treatment was therefore switched to carboplatin and pemetrexed. This change resulted in a drop in copy numbers (to 204/mL and 282/mL, respectively, as measured on 10 December, 2015), although a steep rise was observed again by the end of December (1,671/mL and 2,626/mL, respectively). The analysis that was performed one month later revealed copy numbers of 38,093/mL and 33,560/mL, respectively. Correspondingly, the patient showed massive radiological progression and severe dyspnoea. Treatment with the third-generation TKI osimertinib, which targets both EGFR-activating mutations and the T790M mutation, was initiated, as it had recently become available. Subsequently, copy numbers decreased very rapidly, paralleled by improved clinical status. One week after the start of therapy, the copy numbers were 299/mL and 430/mL, respectively, and by the end of April 2016, they had decreased further to 2/mL and 16/mL, respectively.

Mutations are highly specific, as they are present in cancer cells, but not in normal body cells. Evaluation of somatic mutations is done either by conventional tumour tissue examination or by analysis of blood or body fluids; here, the testing can be performed using circulating cell-free tumour DNA, circulating tumour cells, or exosomes. Of course, liquid biopsies offer the advantage of minimal invasiveness, as they are based on normal blood sampling.

Circulating cell-free tumour DNA (ctDNA) consists of DNA fragments with a half-life of approximately 2 hours. Only traces of cell-free DNA are present in the plasma, but they are highly specific for the tumour. The length of these fragments is typically 120 – 200 base pairs, with 180 base pairs representing the length of the DNA chain within one nucleosome [1].

Measuring systems

Several options are available for ctDNA testing. Digital polymerase chain reaction (PCR) is one possibility, such as droplet digital PCR (ddPCR), although this only allows for the search for known mutations **(Table).** "Primers that target specific mutations have to be used for this test," Ms. Buder explained. Next-generation sequencing (NGS), on the other hand, can be used to evaluate entire genomic regions, and to detect *de-novo* mutations. On the downside, NGS has a long turnaround time of several days, and a high false-discovery

TABLE
Analysis of circulating ctDNA: advantages and drawbacks of digital PCR and next-generation sequencing

Digital PCR	Next-generation sequencing
Individual point mutations, deletions	Evaluation of genomic sequencing by PCR or capture-based methods
Only known mutations	Genomic amplifications, rearrangements, aneuploidy, whole-exome sequencing
Sensitivity dependent on specific mutation and assay optimisation	High false-discovery rate
Fast and highly reproducible results	Turnaround time 1 to 2 days
Low cost	
Minimal bioinformatic expertise	

rate. "Complicated bioinformatic techniques are required to exclude artefacts of false-positives," Ms. Buder said.

At the Institute of Cancer Research, activating and resistance *EGFR* mutations are analysed from base-line blood samples and follow-up blood samples that are drawn every 1 to 3 months. "This allows for minimally invasive assessment of the response to treatment and the development of resistance." For ddPCR, cell-free tumour DNA is extracted from the plasma and partitioned into droplets, each of which contains 0 or 1 molecule of the target DNA [2]. PCR is performed on each droplet. Droplets containing mutant and wild-type DNA emit differently coloured signals, which enables analysis and quantification.

High sensitivity of ddPCR

As the amount of ctDNA frequently correlates with tumour load, increasing copy numbers of activating mutations or resistance mutations might be indicative of disease progression and can be observed prior to radiological and/or clinical deterioration (see Box). Likewise, decreases suggest response to therapy. Copy numbers can range from zero to tens of thousands.

"We compared the T790M test results obtained with ddPCR with the results of the real-time-PCR-based Cobas test, which is being used by many Austrian hospitals," Ms. Buder reported. Patients with very low numbers of DNA-mutated fragments in particular tended to be positive according to ddPCR, but negative according to Cobas. "It appears that Cobas is less sensitive than ddPCR, which detects EGFR T790M with high specificity and sensitivity," concluded Ms. Buder. This is an important finding, because a considerable proportion of patients carries only very small amounts of mutated DNA in their plasma. For patients with 1 to 20 copies/mL, the detection rate is 37 % with ddPCR, while nearly all of these will test negative with the currently used Cobas test. Moreover, the researchers compared liquid biopsy and tissue re-biopsy with regard to the T790M mutation rate. This analysis yielded a high concordance rate of approximately 80 %.

Overall, liquid biopsy appears to be an appropriate method for identification of actionable alterations and selection of TKI therapy. Plasma ddPCR is a powerful tool for early detection of resistance mechanisms. "Liquid biopsy has the potential to replace tissue biopsy in the future," Ms. Buder summarised. ■

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