[Frontiers in Bioscience, Landmark, 20, 644-688, January 1, 2015]

Molecular diagnosis in lung diseases

Fiorella Calabrese[1], Francesca Lunardi[1], Helmut Popper[2]

[1]Department of Cardiac, Thoracic and Vascular Sciences; University of Padua Medical School, Padua, Italy, [2]Institute of Pathology; Medical University of Graz, Graz, Austria

TABLE OF CONTENTS

1. ABSTRACT

The development of different molecular biology techniques in the past decade has led to an explosion of new research in molecular pathology with consequent important applications to diagnosis, prognosis, and therapeutics, as well as a clearer concept of the disease pathogenesis. Many methods used in molecular pathology are now validated and used in several areas of pathological diagnosis, particularly on infectious and neoplastic diseases. The spectrum of infectious diseases, especially lung infective diseases, is now broadening and modifying, thus the pathologist is increasingly involved in the diagnosis of these pathologies. The precise tissue characterization of lung infections has an important impact on specific therapeutic treatment. Increased knowledge of significant alterations in lung cancer has led today to a better understanding of the pathogenic substrate underlying the development, progression and metastasis of neoplastic processes. Molecular tests are now routinely performed in different lung tumors allowing a more precise patient stratification in terms of prognosis and therapy. This review focuses on molecular pathology of the principal infective lung diseases and tumors.

2. INTRODUCTION

Molecular pathology, defined broadly as the use of nucleic acid probes to diagnose and study disease, is an emerging discipline of growing importance and promise (1). Molecular techniques from more simple to high throughput are becoming fundamental ancillary tools to improve final morphological diagnoses. Traditionally, snap-frozen samples are considered optimal for nucleic acid extractions. In the past, the capacity to extract high quality DNA and RNA from formalin-fixed paraffin-embedded (FFPE) tissue specimens was considered cumbersome and an important limiting factor for routine use of molecular methods (2,3). To overcome this problem, in the last few years a multitude of reliable protocols for DNA, RNA and protein extraction from FFPE tissue have been developed and the use of neutral buffered formalin for 24–48 hours can be regarded as a standard procedure for molecular approaches with high sensitivity and specificity. The current use of molecular tools across the entire spectrum of human diseases provides a more precise diagnostic characterization and overall important suggestions for better patient stratification and treatment.

The present review will briefly mention the most common molecular approaches used in routine pathological practice, and then will focus on the main infective and neoplastic lesions whose final diagnosis is currently associated with molecular analyses. Both the sections (infective and neoplastic lesions) will be preceded by a brief introduction reporting general aspects of molecular pathology and the main infective lung diseases and tumors.

3. MOLECULAR TECHNIQUES

3.1. *In situ* hybridization (ISH)

In situ techniques, particularly ISH, are techniques that allow precise localization of a specific segment of nucleic acid within a histological section. The underlying basis of ISH is that nucleic acids, if adequately preserved within a histological specimen, can be detected through the application of a complementary strand of nucleic acid to which a reporter molecule is attached. Visualization of the reporter molecule allows DNA or RNA sequences to be localized in a large variety of biological samples, both tissue and cytological specimens. This approach is particularly used when it is crucial to describe the exact cellular localization of DNA or RNA of interest (4). There are several limitations to the use of this approach, in particular the low sensitivity and reliability. ISH is cumbersome: specimen preparation consists of securing the sample to glass slides and denaturing the DNA without detaching or destroying the morphological identity of the cells. Moreover, when using fluorescence ISH, the fluorescent signal rapidly fades over time, a fluorescence microscope is needed for interpretation (which is not usually done in routine clinical works) and it is difficult to detect the overall morphology and tumor heterogeneity.

3.2. Polymerase chain reaction (PCR), reverse-transcriptase (RT)-PCR

Non-*in situ* techniques are emerging as the preferred approach for molecular diagnosis of both oncological and infectious diseases. PCR is a primer-mediated enzymatic amplification of specifically cloned or genomic DNA sequences (5). Different strategies have been introduced to perform this analysis also in archival tissues (6). Throughout the last decade numerous works have demonstrated that it is also possible to sensitively detect mRNA levels (i.e. profile gene expression) using FFPE as a source of RNA, despite the fact that RNA extracted from archival tissue is often present in fragments less than 300 bases in length (7,8).

The availability of adequate quality and quantity of RNA extracted and reverse-transcribed (by RT-PCR) in cDNA, also from FFPE tissues, is extremely important. The prognostic/predictive potential of gene expression profile, i.e. defining new subcategories of known diseases with different prognoses and possible dissimilar responses to drugs, is now evident and reported by several groups (9,10). PCR and RT/PCR, surely the most frequent diagnostic tools used in molecular labs, are characterized by high sensitivity and reliability but present some disadvantages such as possible contamination, since it leads to an exponential increase of the gene target copy number, and the laborious post-amplification processing (e.g. gel electrophoresis). Over the years several creative pre- and post-amplification methods have been developed to prevent or reduce amplicon carryover contamination.

3.3. Real-time PCR

A significant advance in PCR was the invention of real-time PCR, which allows for multiple analysis detection and quantification in a single tube (12). One approach for real-time monitoring of amplicon production is to use fluorescent DNA-intercalating dyes, such as SYBR Green I, which binds non-specifically to double-stranded DNA generated during amplification. A more popular alternative approach is to use a fluorescent-labeled internal DNA probe which specifically anneals within the target amplification region (13). Moreover it eliminates the need for laborious post-amplification processing (e.g. gel electrophoresis) conventionally needed for amplicon detection (14). Real-time PCR offers a detection method that is rapid, specific, and sensitive. In the past number of years, an abundance of multiplex real-time PCR and reverse transcriptase (RT) real-time PCR products have become available on the clinical diagnostic market (15). However, some limitations have been detected in this technique, such as the overlap of emission spectra and the increased risk of false-negative results (15). Different commercial kits both for infective agent detection (i.e. artus EBV TM PCR Kit, Qiagen) and for EGFR and KRAS mutation analysis (cobas® EGFR Mutation Test, Roche Molecular Systems Inc., therascreen® EGFR RGQ PCR Kit, Qiagen, therascreen® KRAS RGQ PCR Kit, Qiagen) are now available and are usually CE-IVD guaranteed, indeed real-time PCR shows a much higher successful detection rate and mutation rate of lung cancer tissues compared with that of Sanger sequencing. The spectrum of tested

agents and the technology used to reveal the PCR products as well as the laboratory organization are determinant for the selection of the most appropriate kit (16).

3.4. Gene sequencing

DNA sequencing has revolutionized biological and medical research and has now become an essential tool of diagnostic molecular pathology. Gene sequencing consists of an enzymatic reaction that polymerizes the DNA fragments complementary to the template DNA of interest (unknown DNA). DNA polymerase catalyzes polymerization of deoxynucleoside triphosphates (dNTP) onto the DNA until the enzyme incorporates a modified nucleoside into the growing chain. Automated DNA sequencing utilizes a fluorescent dye to label the nucleotides instead of a radioactive isotope, thus reducing the volume of low-level radioactive waste and providing more reliable research results than manual DNA sequencing (17). Sanger sequencing was long considered to be the "gold standard" method for mutational analyses. However this method has several limitations: low throughput (in the oncological field, requires at least 30%–40% of neoplastic to non-neoplastic cell ratios) and high cost making multigene panels laborious and expensive. A number of alternative technologies based on real-time PCR (PCR, allele-specific, melting curve, PNA clamp) strip assay, chip array, pyrosequencing, and next-generation sequencing have been developed to increase the sensitivity of mutational analyses, allowing investigation of poorly enriched tumor samples below the detection threshold for Sanger sequencing (18).

"Next-generation" and "massive-parallel" DNA sequencing are blanket terms used to refer collectively to the high throughput DNA sequencing technologies available which are capable of sequencing large numbers of different DNA sequences in a single reaction (19). Since this approach improves the detection of mutations, it should be promoted for the clinical diagnosis of mutations in specimens with unfavorable tumor cell content (20), e.g. next generation sequencing platforms by Life Technologies, Illumina-Solexa, Diatech, Roche and many others. These analyses should be performed by very experienced molecular lab. In these setting robust mechanisms of external quality assessment to the molecular procedures and standardized protocols should not be underestimated.

Comparative genomic hybridization is a molecular cytogenetic method for comparing of two genomic DNA samples arising from two sources, which are most often closely related, because it is suspected that they contain differences in terms of either gains or losses of either whole chromosomes or subchromosomal regions. Conventional and array CGH has been used mainly for the identification of genetic abnormalities that are recurrently lost or gained in tumors; in the future, their use could be extended for diagnostic, prognostic and therapeutic purposes.

High-throughput sequencing approaches are also a method of choice for a large spectrum of known and unknown viruses discovery but it will also become a tool for in depth or even routine diagnosis as soon as the cost and delays decrease significantly (9).

4. MOLECULAR PATHOLOGY OF INFECTIVE LUNG DISEASES

Infectious diseases account for the majority of human diseases and are still the leading cause of debilitating chronic disease and death. In the last few decades, medicine has made giant steps in the diagnosis and treatment of infection but it has also seen the emergence of new and deadly pathogens that continue to have a significant impact on modern medicine. New medical treatments such as potent immunosuppressive treatment that weaken host defenses have contributed to the emergence of new microorganisms and overall to the recrudescence of others. In this scenario the pathologist is increasingly needed to provide diagnoses from both cytology and biopsy specimens. Traditional microbiological investigations such as culture have several important limitations: they are not able to distinguish infection from colonization, nor can they determine the significance of isolated microorganisms. Only the presence of a pathogen *in situ* together with an inflammatory response by the host represents acceptable evidence of its role in infection. A high level of experience and expertise in this field together with the use of different ancillary tools, as molecular investigations, are mandatory for a sensitive final diagnosis. The use of molecular tools in routine pathology significantly increases the sensitivity of the diagnosis mainly for two reasons: 1) exiguous specimens are frequently available for pathological diagnosis due to limited applicability of invasive procedures in debilitated/immunocompromised patients, 2) a small number of pathogens, frequently

also modified in their morphology by antimicrobial therapy are present in specimens. Exact etiological characterization is extremely important for target therapy and molecular monitoring of infective agents is now adopted to verify the efficiency of treatment. Infective pneumonia is a frequent cause of morbidity and mortality in children, immunocompromised patients and in the elderly. Respiratory tract infections remain difficult to diagnose accurately as a broad range of pathogens and opportunistic microorganisms are involved in their etiology. Frequently, patients with signs of respiratory tract infections are treated empirically without an accurate diagnosis of the causative microorganism. Delays in accurate pathogen-specific diagnoses may result in the prescription of inappropriate medical treatment and poor outcome. The pathologist has an important role in the diagnosis or exclusion of infectious diseases mainly for those developing in immunocompromised patients such as lung recipients. Indeed lung allografts show increased susceptibility to bacterial and opportunistic infections, especially when compared with recipients of other solid organ transplants (21). Post-transplant lungs are continuously exposed to the environment, have impaired mucociliary clearance and a decreased cough mechanism because of denervation of the allograft, and are treated with high-dose immunosuppression, all of which increase the risk for infection (22). Numerous bacterial, viral, and fungal pathogens take advantage of these factors to cause invasive disease. Although some microorganisms or their cytopathic effects may be clearly visible on routine hematoxylin and eosin-stained sections, additional histochemical or immunohistochemical stains and overall molecular investigations are often needed for a definitive characterization.

Virus, mycobacteria and fungi, the most frequent pathogens investigated in lung specimens by using also molecular tools, will be more thoroughly discussed below.

4.1. Viral infection

Viral infections, including beta-herpes viruses, particularly cytomegalovirus (CMV) and community respiratory viruses-CARVD- (rhinovirus, influenza virus A/B, parainfluenza virus 1/2/3, respiratory syncitial virus) are frequent pathogens in immunocompromised patients, particularly in HIV-seropositive and lung transplant recipients. Notably in the lung transplant field the recent development of molecular methods for CMV detection has resulted in more rapid diagnosis and has played an important role in the management of CMV infection. Quantitative measurements of viral load in blood can trigger initiation of antiviral therapy or can be used to monitor response to therapy (23,24). While several works have emphasized the importance of molecular diagnostic tests on blood (25) it was also demonstrated that blood viral load appears useful but not completely predictive of CMV pneumonitis (26). Transbronchial biopsies and bronchoalveolar lavage specimens remain the best tools for the final diagnosis of infective viral pneumonitis. While bacterial, fungal or protozoal microorganisms are more easily detectable, viral infections are quite hard to characterize by routine histological and cytological examination. In post-transplant transbronchial biopsies it is important to note that cytological or histological interpretation appears fairly difficult due to three main aspects: precise identification of viral infections with histological patterns that may differ from those observed in the non-transplanted immune-competent patient, differentiation of acute rejection from viral infections and differentiation between colonization, subclinical infection and clinically significant disease. Viral cytopathic effects, including the typical cytomegalic changes, are rarely seen in post-transplant biopsies or cytological smears.

The use of immunohistochemistry, with both monoclonal and polyclonal antibodies, has significantly increased the sensitivity for the histological diagnosis of CMV pneumonitis compared to standard hematoxylin and eosin staining. ISH has been demonstrated to have similar sensitivity as immunohistochemistry (27,28) and this is also our recent experience (Calabrese *et al*., unpublished data). All our cases tested with CMV immunohistochemistry showed the same marked cells in the serial sections processed by CMV ISH. Thus ISH due to greater technical difficulties and higher cost is rarely used in routine CMV screening. Routine histology with immunohistochemistry and/or ISH show however low sensitivity in CMV detection often leading to under-diagnosis and/or delayed diagnosis of CMV pneumonitis (29). Increasing interest has focused on more sensitive molecular detection techniques both applied to transbronchial biopsy and overall to bronchoalveolar lavage.

Although the critical threshold of viral load ($4.6. \times 10^4$ and $5.0. \times 10^5$) is not yet completely validated (30), several works have proposed CMV quantification in bronchoalveolar lavage to have a higher sensitivity in the differentiation of

patients with active CMV disease from latently infected patients (31) and suggested that molecular quantitative assay on bronchoalveolar lavage should replace traditional culture and also CMV DNA assay in the blood. Regarding this aspect several authors have demonstrated a significantly higher sensitivity of CMV DNA in bronchoalveolar lavage compared with the same detection in plasma samples. High bronchoalveolar lavage CMV load (>64,000 copies/ML) has been reported to be associated not only with viral inclusions in lung allograft biopsies but also is more likely to be associated with specific symptomatic disease syndromes such as CMV pneumonitis (32). However, a low bronchoalveolar lavage cellularity (due to the technical procedure) could represent a bias in the interpretation of real-time PCR quantification results. Molecular tools, in particular the most sensitive PCR or quantitative PCR, have recently been reported to have an important impact also on the diagnosis of CARV, today recognized as an important cause of morbidity and sometimes mortality in immunocompromised patients. In healthy subjects these infections are generally limited to the upper respiratory tract with viral clearance occurring within a few days (33). In contrast, patients with impaired immune function are at higher risk of severe lower respiratory illness with prolonged shedding (34). Among CARV the most frequently detected virus is rhinovirus. Rhinovirus is mostly limited to the upper respiratory tract but experimental data have shown that it also replicates in the lower respiratory tract (35) and severe rhinoviral pneumonia has been described in immunocompromised hosts (36), including lung transplant recipients. Moreover molecular investigations using the RT-PCR and gene sequencing portion of the 5'NCR region and VP1 surface glycoprotein were also able to demonstrate rhinoviral persistence in lung transplant recipients with lung dysfunction (37) suggesting a possible influence of this pathogen on chronic allograft dysfunction (see below, section "contributing etiological role").

4.2. Mycobacterial infection

Mycobacterial infections have a high economic human and animal health impact. The incidence of nontuberculous and tuberculous mycobacterial diseases has increased worldwide over the past several decades. Symptomatic and severe disease commonly occurs in patients with structural lung diseases, such as chronic obstructive pulmonary disease, bronchiectasis and immunodepressed patients. Early diagnosis of mycobacterial infection, particularly tuberculosis (TB), is extremely important for starting prompt and specific antimycobacterial treatment. In terms of histopathological diagnosis, TB can be diagnosed only as "a chronic granulomatous inflammation, suggestive of tuberculosis" on a routine surgical pathology report. However, histopathological features of chronic granulomatous inflammation can be detected in different conditions and diseases other than TB, such as foreign body reaction, fungal infection, sarcoidosis, and Wegener's granulomatosis (38). Although additional morphological findings can help to perform a more accurate diagnosis (i.e. presence of vegetable material for foreign body reaction, or necrotizing vasculitis in Wegener's granulomatosis), the tissue reaction to mycobacteria, including TB, shows a spectrum of changes that may not involve the presence of pathognomonic granuloma. Chronic inflammation without distinct granulomatous lesions may be the only histological feature detected in several cases of mycobacterial infections including TB forms. In addition, since the size of histological specimens is usually small as bronchial or tranbronchial biopsy, all pathognomonic histopathological features of TB, such as a well-formed granuloma with caseous necrosis, are rarely seen. The conventional methodology of microscopic examination for acid-fast bacilli, culture, biochemical tests is laborious and time-consuming. In particular routine microscopic evaluation of lung tissue stained with both types of acid fast stains (Ziehl-Neelsen and rhodamine-auramine stains) shows a low sensitivity also when the lesion appears histologically active or many bacilli are detected in the sputum. Some organic solvents, which are used to make paraffin-embedded tissue samples, have been suggested to affect the stainability of mycobacteria by acid-fast staining. This hypothesis seems reasonable because the molecular target of acid-fast staining dyes (fuchsin, auramine, or rhodamine) is the mycolate on the bacterial surface (39). Mycolate is soluble in organic agents (40) and might be more or less extracted from the cell surface into the liquid. Similarly, immunohistochemical identification using polyclonal antibodies against mycobacteria proved to be not sensitive enough for this purpose (41). Moreover acid fast staining and immunohistochemistry are not able for distinguishing MT from non-tuberculous mycobacteria (NTM).

Over the last few years, new molecular methods have been introduced, including nested-PCR, RT-PCR, real-time PCR, DNA sequencing

and DNA strip technology, leading to considerable improvement in both the speed and accuracy of mycobacterial identification (42). All these could be used also in FFPE applying simple protocols that take into consideration DNA fragmentation and/or cross-linkage caused by formalin fixation (43). In addition to FFPE-related problems mycobacteria carry a further methodological obstacle because of their thick mycolate-rich outer cell wall, which requires special measures of cell disintegration without causing further damage to DNA (44). The highest sensitivity for MT and NTM detection is usually obtained amplifying *IS6110* and *HSP65* genes respectively (45,46). The study of Fukunaga *et al*. reported a significant low sensitivity of acid fast staining compared to real-time PCR. The estimated number of bacilli from the real-time PCR data was 10^3 to 10^9 times larger than the number counted with microscopy among the acid fast staining positive samples (47). In our experience less than half of TB-PCR–positive cases have the typical lesion of granulomatous inflammation with central necrosis. For this reason, if there is a high suspicion of mycobacterial infection based on both clinical and radiological findings, in our center we routinely perform nested-PCR method, especially in cases with small biopsy specimens or cases of immunosuppressed patients, often lacking of typical granulomatous lesions. Nested PCR is more sensitive than 1-step PCR, especially in some circumstances, such as amplification from FFPE tissue specimens. In view of the higher sensitivity of nested PCR using *IS6110*, special attention should be paid to the capacity of TB DNA to remain in the tissue specimen after drug therapy. Hernandez-Pando *et al*. (48) showed the persistence of DNA from TB in normal lung tissues during latent infection, and Salian *et al*. (49) suggested that healed tuberculous granulomas, which were culture and acid fast stain negative for TB, would sometimes be positive for TB DNA. This suggests that caution must be adopted in interpreting molecular data such as PCR or other similar molecular techniques because these may be too sensitive for distinguishing between active and inactive or infectious or noninfectious states of the lesion. Thus, when TB DNA is detected in lung tissue specimens, a further evaluation of other laboratory and clinical information is needed, especially before a final diagnosis of TB is performed.

4.3. Fungal infection

Fungi may cause serious morbidity in immunocompromised patients. In lung recipients lesions comprise colonization of bronchiectatic airways, cavitating pneumonia, bronchocentric granulomatosis, invasive infection with mediastinitis and infection of the airway anastomosis. Among fungi Aspergillus spp. are the most common cause of invasive fungal infection. Approximately 6% (range 3 to 15%) of lung transplant recipients develop aspergillosis (50). Early diagnosis of fungal infection is critical for optimized treatment and successful outcome. Molecular techniques appear promising tools for rapid and sensitive detection of fungal disease thus allowing a more timely initiation of antifungal therapy, when appropriate (51). However at present molecular approaches in this setting lack standardized protocols and are not able to distinguish fungal colonization from disease (52). A study by Cuenca-Estrella and colleagues (53) suggests that combining serial RT-PCR for invasive aspergillosis with galactomannan testing improves sensitivity and early diagnosis of invasive aspergillosis. Additional studies of PCR-based technologies in combination with other diagnostic tools should better clarify the feasibility and potential benefits of combination approaches to diagnosis of invasive fungal infections.

5. MOLECULAR PATHOLOGY OF OTHER LUNG LESIONS: CONTRIBUTING ROLE OF INFECTIVE AGENTS

In this paragraph a heterogeneous group of diseases (both inflammatory and neoplastic lung lesions) will be briefly covered. In these diseases infectious agents have been mostly detected by molecular methods and their etiological contributing role will be commented.

5.1. Kaposi's sarcoma, pleural effusion lymphoma, Castleman's disease and granulomatous-lymphocytic interstitial lung disease

Human herpesvirus 8 (HHV-8) has been implicated in the etiology of Kaposi's sarcoma (KS), pleural effusion lymphoma (PEL), multicentric Castleman's disease (CD) and granulomatous-lymphocytic interstitial lung disease (54). These pathological processes rarely involve lung parenchyma or pleura and occur frequently in immunocompromised patients (KS, PEL and CD) or in patients with common variable immunodeficiency –CVID- (granulomatous-lymphocytic interstitial lung disease). Unlike KS and PEL, not all CD (except for those forms arising in HIV patients) or lung lesions associated with CVID have been found to contain HHV-8 DNA thus, there is likely more

than one etiology for this somewhat diverse group of lesions. One hypothesis that would account for different pathological manifestations of infection by the same virus is that viral genes (lytic, latent forms) are differentially expressed in heterogeneous cell types (55).

As the exact frequency of HHV8 infection in these diseases is unknown, particularly in MCD and PEL, molecular investigations for this virus should be performed when these lesions are diagnosed. Moreover in such lesions the use of molecular techniques may be of help to follow the therapy efficacy. Finally, the identification of latent/replicative status could help to better understand their role in the development/progression of those diseases.

In immunocompromised patients the detection of HHV8 DNA in the bronchoalveolar lavage fluid has been considered highly specific and sensitive for the diagnosis of pulmonary Kaposi's sarcoma. Symptoms of pulmonary Kaposi's sarcoma include cough, dyspnea, hemoptysis and pleural pain due to pleural effusion. These symptoms as well as radiological exams (nodules, tumor masses, bronchovascular pathway thickening, and pleural effusions) are nonspecific and can mimic different lesions as those caused by other opportunistic infections. The histology is surely the gold standard for the final diagnosis of Kaposi's sarcoma but there is a considerable risk of major bleeding after biopsy of these heavily vascularized tumors. Open lung biopsy and video-assisted thoracoscopic surgery to obtain large tissue samples are invasive procedures and rarely performed for this indication. The detection of HHV8 DNA in bronchoalveolar lavage may be considered a very useful noninvasive test to confirm suggested Kaposi's sarcoma (56,57). As these KSHV/HHV-8-related diseases cause significant morbidity and mortality in affected patients, the identification of the virus within pathological tissue will allow for more targeted therapy. This is extremely important in lung recipients whose treatment should be changed with tapering of standard immunosuppression and/or switching to other immunosuppressive drugs and overall association of specific antiviral therapy.

5.2. Lymphomatoid granulomatosis (LYG), post-transplant proliferative disorder (PTLD) and lymphoid interstitial pneumonia (LIP)

Epstein-Barr virus (EBV) has been thought to be an important contributing factor in several lymphoproliferative lesions such as LYG, PTLD and LIP. LYG, an uncommon pulmonary lesion consisting of atypical angiocentric lymphoreticular infiltrates with variable amounts of necrosis, may occur in both immunocompetent and immunocompromised patients. PTLD occurs in 3-5% of lung recipients with frequent involvement of the allograft, often as one or multiple nodules.

Pulmonary LYG appears to be associated with EBV infection in most but not all cases. A small number of cases show no demonstrable evidence of EBV infection, and may represent a distinct form of peripheral T cell lymphoma (58). Alternatively; these cases may reflect grade 1 disease with infrequent EBV-positive cells (59).

PTLDs, morphologically characterized by lymphocyte aggregates, mainly B-lymphocytes, are also but not invariably associated with EBV. The reported incidence, however, of EBV-negative PTLDs varies widely, and it is uncertain whether they should be considered analogous to EBV-positive PTLDs and whether they have any distinctive features. A few years ago Izadi *et al*. demonstrated relatively better histopathological features of EBV-positive cases than EBV-negative even if the overall survival rate of EBV-positive PTLD patients was not inferior to that of the EBV-negative subjects (60).

The diagnosis of these conditions is often difficult as the physical signs, chest X-ray, and routine laboratory investigations are usually non-specific. The diagnosis requires lung tissue samples, and rapid and precise characterization is mandatory as these lymphoproliferative disorders can be refractory to treatment and overall progress to overt lymphoma. EBV in lung samples should be sensitively investigated using molecular investigations as *in situ* hybridization for EBV-encoded small RNA (mRNA *EBER*) and the result should always be reported in a final diagnostic report. LYG can be subclassified using a grading system based on the number of EBV-positive large B-cell malignant cells and should be reported to grade LYG. This is critical in selecting appropriate management strategies (61). Although the value of non *in situ* recognition of EBV as DNAmia is quite discussed to monitor PTLD in lung recipients it is extremely important to note that this disorder may also occur in the absence of detectable EBV DNAmia in contrast with strong EBER positivity in lung samples (62).

LIP, a rare interstitial lung disease characterized by diffuse interstitial inflammatory cell infiltrate, is frequently associated with immunological

disorders. In immunocompromised patients (e.g. AIDS) several infective agents, in particular EBV, has been considered a potential etiological factor (63).

EBV seems to play a role also in carcinogenesis of lymphoepithelial-like large cell carcinoma (see below its tumor altered genes) only in patients from Southeast Asia, whereas in Caucasians these carcinomas are negative (64,65).

5.3. Squamous cell papilloma

Papilloma is a benign lung tumor covered by metaplastic epithelium, most often composed of squamous cells, sometimes with certain degrees of dysplasia. Koilocytes are sometimes seen, and by immunohistochemistry, ISH, or by PCR human papillomavirus (HPV) can be detected (66). A bizarre outline of nuclei is usually detected in cases of HPV 16 or 18 positive cases. Many HPV types are known; in general they are divided into oncogenic and non-oncogenic types. They all have in common to promote growth of the epithelial cells when integrated into the cell genome. Also recurrence of papillomas is common. The most important function of HPV genome is its interference with the cell cycle control proteins p53 and RB protein. Specific parts of the HPV genome induce stabilization of the p53 and RB proteins preventing apoptosis induced by genetic aberrations. Therefore the DNA repair machinery is ineffective and mitosis progress despite DNA defects and gene aberrations (67,68). Integration of oncogenic HPV types (16 and 18) will induce a rapid progression of the papilloma into invasive squamous cell carcinoma (66).

5.4. Acute and chronic rejection

Along with such direct effects as acute pneumonitis different viruses may indirectly contribute to acute rejection and the development of bronchiolitis obliterans syndrome (BOS), the major limiting factor in long-term survival after lung transplantation. CMV is one of the major viruses detected in bronchoalveolar lavage/ transbronchial biopsies and strictly correlated with graft dysfunction. The majority of studies focused on this topic have investigated viral infections using sensitive molecular tools (69). The recent CMV prophylaxis, sometimes associated with the use of CMV immunoglobulin, has reduced/delayed but did not eliminate CMV disease or CMV-complications. This is also the experience of our centre: patients treated with combined immunoprophylaxis had a significantly less incidence of CMV disease and less acute rejection than those without (Calabrese *et al*., unpublished data) at least in the first year after lung transplantation but it seems to have less impact on chronic dysfunction. Human herpesvirus-6, another herpesvirus frequently detected in bronchoalveolar lavage and transbronchial biopsies, has also been associated with chronic lung dysfunction (70). The overall frequency of herpesvirus-6 DNA detection in bronchoalveolar lavage was about 20%, and it was usually detected together with the other opportunistic herpesviruses CMV, herpesvirus-7 and EBV (71). However, the clinical significance of the herpesvirus-6 finding in BAL has remained unclear, probably also ascribable to the concomitance of other viral infections. Over the years the improvement of molecular tools, including real-time PCR technology, has contributed to increase the sensitivity of diagnostic procedures and new species (HMpV, coronavirus NL63 and HKU1 and bocavirus) other than CMV and CARV have also been detected in bronchoalveolar lavage or transbronchial biopsy. The results of the various papers in the literature are quite discordant and a relatively recent review which examined 34 different studies highlighted the concept that the clinical link between respiratory viruses and acute lung rejection or BOS needs to be characterized in prospective and appropriately designed cohort studies (72).

5.5. Idiopathic pulmonary fibrosis (IPF)

IPF is a progressive disease of unknown etiology characterized by a deregulated wound healing response that leads to progressive accumulation of fibroblasts and extracellular matrix which compromises tissue architecture and lung function capacity. Injury to type II alveolar epithelial cells is thought to be the key event for the initiation of the disease, and so far both genetic factors, such as mutations in telomerase (73) surfactant protein C (74) and *MUC5B* genes (75,76) as well as environmental components, like cigarette smoking, dust exposure, gastroesophageal reflux and viral infections have been implicated as potential initiating triggers (77). Among viral agents herpes viruses, in particular EBV, have been suggested as principal cofactors (as initiating or exacerbating agents) of fibrotic lung disease (78,79). The majority of studies investigated the presence of viruses in lung samples using sensitive molecular tools (PCR or nested-PCR) and reported a frequency of herpes viruses in IPF lungs ranging from 30 to 100%. Only a few works have demonstrated an adverse impact of herpes virus infection in IPF patients (79,80). Tsukamoto *et al*. reported a more rapid disease progression in EBV

positive cases. The majority of viral cases died from respiratory failure at a mean of 41 months of follow up (80). Our group has recently demonstrated a high frequency of viral infection in advanced forms of IPF and we have showed, for the first time, a different phenotype of virus-positive IPF patients. In particular virus-positive IPF cases showed more pronounced vessel remodeling and a higher mPAP, a complication that severely affects prognosis of the disease. The presence of herpesviral DNA in IPF native lungs also has a significant impact on the graft in the early post-transplant period. We have recently shown a significant higher risk of primary graft dysfunction in virus-positive IPF compared to negative cases who underwent lung transplantation (79). Molecular viral investigation in IPF lung samples, involving larger case series from different centers, could give in the future more information on the influence of these viruses on the disease.

6. FINAL REMARKS

Molecular techniques are now an important armamentarium for pathologists to establish the diagnosis of infective pneumonia. Precise characterization of infective pneumonia now requires appropriate morphological and molecular analyses and at the same time interlaboratory communication and collaboration with other specialists as pulmonologists, radiologists and microbiologists involved daily in the diagnosis and care. The contributing role of infective agents in different forms of diseases requires to be more consistently investigated particularly in those diseases with multifactorial etiology in order to weigh their role in the development/progression of the disease.

7. MOLECULAR PATHOLOGY OF LUNG TUMORS

Within the last decade many important discoveries were made in the regulation of growth, differentiation, apoptosis, and metastasis of lung cancers. These findings have dramatically changed the awareness in the oncology community about the classification of lung carcinomas. A decade ago oncologists were mainly interested to get the differentiation between small cell (SCLC) and non-small cell carcinomas (NSCLC) of the lung. With the findings of different responses for cisplatin and anti-angiogenic treatment in adenocarcinomas versus squamous cell carcinomas this simple clinical lung carcinoma classification schema was abolished. Now oncologists want to know the differentiation within NSCLC, and the near future will even increase subtyping of different NSCLC entities. In this review we will first focus on general aspects of molecular pathology in lung carcinomas and then discuss different genetic abnormalities within the different entities. These abnormalities will be ordered according to their importance such as targeted therapy and impact on outcome.

7.1. Therapy relevant molecular changes in pulmonary carcinomas

7.1.1. Non small cell lung carcinoma and angiogenesis

Angiogenesis, better neoangiogenesis is a process by which primary tumors get access to nutrients and oxygen. The process of neoangiogenesis is still not fully understood. In some cases the tumor cells themselves produce angiogenic factors such as vascular endothelial growth factors (*VEGFs*), in other cases these growth factors are produced by macrophages present in the tumor microenvironment (81). However, once new blood vessels (capillaries, small arteries, veins) are formed, this provides advantage for the tumor cells over their normal neighbor cells in getting better oxygen and nutrient supply. Nutrients and oxygen are not the only important factor for better growth, also purine and pyrimidine bases are essential for a dividing tumor cell (82,83). A good example how this can influence the progression from preinvasive to invasive lesions is the vascular variant of squamous cell dysplasia. It seems that the early access to blood vessels promote rapid progression into squamous cell carcinoma (84). In another preneoplasia atypical adenomatous hyperplasia (AAH) vascularization is a late event, usually at the transition from in-situ to invasive adenocarcinoma (85). This might explain why AAH can persist for several years without progression (86). In addition there seems to be a difference between mucinous and non-mucinous adenocarcinomas with respect to neoangiogenesis (87). Increased angiogenesis itself in invasive adenocarcinomas has a negative impact on survival and progression of these patients (88).

Angiogenesis is essential for the primary tumor as well as for metastasis. The secretion of *VEGFs* facilitates most often neoangiogenesis. Tumor blood vessels are fragile, and are prone to rupture.

In the last decade humanized antibodies, such as those against *VEGF* (Bevacizumab), have been developed to interfere with the neoangiogenesis

in primary as well as metastatic carcinomas (89,90). However, anti-angiogenetic drugs can cause severe bleeding, especially in patients with centrally located squamous cell carcinomas. However, it is still not clear, if the reported bleeding episodes in these patients are due to the squamous histology or to the centrally located tumors, usually supported by arteries and veins arising from large branches. In addition it was reported that cavitation within the tumor is prone to hemorrhage, again something more common in central tumors located close to large blood vessels (91). Thus the use of Bevacizumab is recommended for adenocarcinomas and large cell carcinomas, but squamous cell carcinomas are excluded, since they are most often centrally located tumors.

Different strategies are being developed to inhibit the *VEGF* pathway, *HIF* signaling and hypoxia in tumor development and metastasis. In several studies the importance of the VEGF and VEGFR axis was noted for vascular invasion and metastasis, mainly involving *VEGF-C* and *VEGFR3 (*88, 92-94). Studies aiming to target this axis showed positive results in experimental settings (95-97). Bringing these targeted therapies into clinical trials is still in its infancy (98). A major problem in targeting *VEGF-VEGFR* is the fact that its regulation is under the major influence of the hypoxia pathway. Hypoxia is an important factor in invasion and angioinvasion, and *HIF1α* signaling results in the upregulation of *VEGF* (99,100). Thus the hypoxia pathway might constantly overrule a blockade of *VEGF-VEGFR* unless also *HIF1α* production is also inhibited (101).

7.1.2. Non small cell lung carcinoma and cisplatin drugs, the effect of anti-apoptotic signaling

In a large multi-institutional study the effect of cisplatin chemotherapy was investigated. High expression of DNA repair enzymes, especially nucleotide excision repair enzyme (*ERCC1*) was found to be responsible for failure of cisplatin chemotherapy and this expression correlated predominantly with squamous cell histology (102). *ERCC1* is part of the excision repair machinery involved in the repair of damaged DNA. In NSCLC showing a high expression of this enzyme, the action of cisplatin-based chemotherapeutics is inefficient, most probably because DNA damage induced by the drug is immediately repaired. Therefore *ERCC1* should be investigated by immunohistochemistry to predict response to therapy especially in squamous cell carcinomas.

7.1.3. Thymidylate synthase blocker

Pemetrexed is an inhibitor of *thymidylate* synthase (TS) less for the other enzymes in the thymidine cycle. Thymidine uptake is essential for rapidly dividing carcinoma cells. In tumors with low expression of TS pemetrexed can block the enzyme resulting in growth inhibition. TS expression most often is low in adenocarcinomas, but is highly expressed in many squamous cell carcinomas. Thus pemetrexed is efficient in most adenocarcinomas and not in squamous cell carcinomas (103). However, the action of pemetrexed is still not entirely clear. Thymidylate metabolism does not only rely on enzymes of the thymidylate cycle, but also needs active and passive uptake mechanisms; and thymidine uptake might also be influenced by pemetrexed (104).

7.1.4. Receptor tyrosine kinases in lung carcinomas

Receptor tyrosine kinases (*RTK*) are membrane-bound protein receptor composed of an external receptor domain, a transmembrane spanning portion, and an internal domain, which at its C-terminal end contains the kinase domain. The external receptor domain has a specific configuration for the binding of growth factors, where usually two molecules form homo- or heterodimer with the receptor domain. This specific binding changes the configuration of the whole receptor and leads to the activation of the kinase domain. There are two ways of activation of receptor tyrosine kinases in lung cancer: overproduction of ligands either by the tumor cell or by cells within the microenvironment, such as macrophages, or activation by a mutation of the receptor gene, most often within the kinase domain. The receptor kinase itself can act also in two different ways: one is transfer of phosphorylation to transfer molecules (105,106), like *GAB1* or *GRB2*, or the kinase splits into fragments, where one activated protein fragment translocates into the nucleus and binds to specific DNA elements and induces transcription of proteins (107). In lung cancer *RTKs* can be constantly activated by different mechanisms: amplification of the *RTK* gene, mutations of the *RTK* gene, gene rearrangements (translocation/inversion) with constant activation or inactivation of regulatory proteins. Another mechanism is downregulation of regulatory proteins by miRNAs, so a tumor suppressor or a negative feedback protein is not synthesized because of mRNA inactivation by miRNA (108-113).

7.1.5. TP53 the tumor suppressor gene

TP53 was one of the first tumor suppressor genes detected as being mutated in almost every cancer type. *TP53* is located on chromosome 17p13-12, contains 11 exons, has two promoters (one upstream of noncoding first exon, and another within first intron) (114). The protein p53 functions as a cell cycle control, which can send cells with defective DNA directly into apoptosis (115). *TP53* is either mutated or methylated in most lung carcinomas, especially in all tobacco smoke associated variants. Analysis of these types of mutations highlighted some non-functional mutations most probably due to interaction with some tobacco genotoxic compounds (116-119), whereas other mutations resulted in truncation of the protein, defect of protein degradation, and loss of function (120-122). Mutations and methylation-induced silencing is common in SCLC, squamous cell carcinomas, less frequently in adenocarcinomas (117,123,124). There is still an ongoing debate if p53 inactivation is related to a metastatic phenotype (125,126).

Next we will focus more specifically on tumor entities and what molecular profiles are known in each entity.

7.2. Non small cell lung cancer

7.2.1. Adenocarcinoma

Adenocarcinomas in highly industrialized countries are the most common lung carcinomas, with a percentage of 40% of all lung carcinomas. In addition, what was previously regarded as a single entity has become a huge diversity of carcinomas. Adenocarcinomas in never-smokers most probably represent a separate entity with different gene signatures and a slower progression rate compared to adenocarcinomas in smokers. Also gene signatures have contributed to a more heterogeneous picture. Morphologically adenocarcinomas can show a variety of patterns, which in part correlate with gene signatures, although our knowledge in this respect is still in its infancy. Adenocarcinoma is defined by the formation of papillary, micropapillary, cribriform, acinar, and solid structures, the latter with mucin synthesis and mucin-containing vacuoles in at least 10% of the tumor cells. Adenocarcinomas can be either mucinous or non-mucinous. Both will show the above-mentioned patterns. Some rare variants are fetal, colloid, and enteric adenocarcinomas. Most often a mixed pattern is seen with a predominance of at least one component. Tumor cells in adenocarcinomas can show differentiations along well-known cell types as Clara cells, type II pneumocytes, columnar cells, and goblet cells. Due to the importance of targeted therapy the exact classification of adenocarcinomas and their differentiation from other NSCLC has become a major task in pulmonary pathology. Differentiation factors are used to prove the nature of the carcinoma especially in less well-differentiated examples. A variety of useful markers have been tested, the most important ones are *TTF1* and Napsin A.

7.2.1.1. EGFR

In 2004 an epidermal growth factor receptor (*EGFR*) mutation was detected in a patient with lung adenocarcinoma and responded to tyrosine kinase inhibitor (TKI) treatment - a new era of targeted therapy in NSCLC was invented (127,128). Mutations of *EGFR* have been detected in a small percentage of lung cancer patients of the Caucasian population. These are activating mutations found in exons 18, 19, 20, and 21 of the *EGFR* gene (kinase domain) (129). Mutations are most often found in never smokers, females, and in patients with adenocarcinoma histology. Mutations change the configuration of the kinase, which does not need anymore the ligand-based activation from the receptor domain. The receptor stays in an activated stage and constantly signals downstream. Carcinomas with this activating mutation can be growth inhibited by small receptor *TKI* such as Gefitinib, Erlotinib, and Afatinib. These *TKIs* bind either reversible or irreversible into the ATP pocket of the mutated *EGFR* kinase and thus inhibit phosphor-transfer to downstream molecules, thus blocking the signaling cascade (130). The most common mutations are deletions within exon 19 with a variation of 9-18 nucleotides, and a point mutation at exon 21. Other less common mutations are point mutations in exon 18, and insertions in exon 20. However, mainly within exon 20 there are also resistance mutations, the best known is T790M. This type of mutation inhibits or reverses the binding of the *TKIs* Gefitinib and Erlotinib and prevents the receptor blockade. The irreversible *TKI* Afatinib might overrule some of these resistance mutations, but more data are needed to proof this (131). For targeted therapy with TKIs tissue samples of NSCLC have to be analyzed for these mutations. Within the different subtypes of adenocarcinomas some will show a higher percentage of *EGFR* mutations, whereas other not. In Caucasian population adenocarcinomas with acinar or papillary pattern are up to 27% mutated, whereas mucinous adenocarcinomas are constantly negative for *EGFR* mutations.

Carcinomas with biphasic morphology such as adenosquamous carcinomas and mixed small cell and adenocarcinomas can show mutations but usually in a very small percentage. Another therapy approach was tested with humanized monoclonal antibodies for EGF. By competitive binding to the receptor, this antibody replaces EGF and thus inhibits transactivation of the kinase. This type of therapy seems to be especially promising in *EGFR*-naïve (wild-type) adenocarcinomas and in addition also in squamous cell carcinomas (132,133).

7.2.1.2. KRAS

KRAS was one of the early-detected oncogenes in adenocarcinomas of the lung. *KRAS* belongs to the family of small GTPases located close to the inner cell membrane. They can be activated by tyrosine receptor kinases either membrane bound as *EGFR* or also by cytosolic kinases as SRC. Usually phosphorylated transfer molecules activate them as *GRB2 (*134-136). Once activated they can signal downstream into three major cascades: *RAL-RAF, MEK-ERK*, or *PI3K-AKT*. These different activation cascades have different effects on tumor cells, however, the exact interaction and the mechanisms, which select a specific signaling pathway are not clear (137,138). Mutations of *KRAS* in lung adenocarcinomas are found in the codons 12, 13, and 61. These mutations result in constant activation of *KRAS* and consecutively activation of the downstream cascades. *KRAS* in this situation does not need an upstream activation. *KRAS* mutations occur at an average of 30% of all pulmonary adenocarcinomas, but this percentage rises to 50% in mucinous adenocarcinomas (Geles *et al*. under review). In one study *KRAS* mutations were more frequently seen in solid adenocarcinomas (161). Whereas *KRAS* mutations are frequent in Caucasians, they are rare in Southeast Asian populations, opposite to the situation of the frequency of *EGFR* mutations (139-141).

At present time targeted therapy does not exist for patients with mutated adenocarcinomas, but there are trials going on, which aim to inhibit the downstream signaling pathway with *MEK* and *ERK* inhibitors (98).

7.2.1.3. EML4ALK1 and additional fusion partners

Inversion (erroneously called translocation) of the *ALK1* kinase gene and fusion with the *EML4* gene has been recently shown in patients with NSCLC, especially in solid adenocarcinomas with focal differentiation into signet ring cells. Subsequently other patterns have been associated with this type of gene rearrangement, such as micropapillary. Both genes are on chromosome 2; the chromosomal break is inversely rearranged whereby the kinase domain of *ALK* and *EML4* are fused together. The *ALK* kinase thus is under the control of *EML4*, which results in a constant activation of the kinase. *ALK*, similarly to *EGFR*, stimulates proliferation and inhibits apoptosis. Patients with this inversion respond excellently to crizotinib treatment, which is now the second example of targeted therapy in NSCLC (142). Proof of *EML4ALK1* inversion can be done with different methods. The most common is FISH where two probes (3' and 5') detecting the *ALK* gene on both sides of the breakpoint are used. In the normal situation these probes will detect the two portions close together or overlapping within the tumor nucleus. In cases of rearrangement, the probes will highlight each of the split portions of the *ALK1* gene, so instead of two overlapping signals the signals split apart. In the Caucasian population *EML4ALK1* rearrangement is usually found in 4-6% of NSCLC; in adenocarcinomas this might be increased to 8%. Other genes joining the *ALK1* gene in the same way can replace the *EML4* gene. If *KIF5B* joins to *ALK1* the overexpression of *KIF5B-ALK* (111) in mammalian cells led to the activation of signal transducer and activator of transcription 3 (*STAT3*) and protein kinase B and enhanced cell proliferation, migration, and invasion (111). Another fusion partner recently described is *ALK-KLC1 (*143). These other ALK1 fusions are rare; the incidence is about 1%. Resistance mechanisms in *EML4ALK* rearranged lung adenocarcinomas do exist, however, the exact mechanisms are still under investigation (144,145).

7.2.1.4. ROS1

ROS1 is another kinase involved as a driver gene in adenocarcinomas of the lung (146). Usually the rearrangement of ROS1 is evaluated by two FISH probes for the 3'- and the 5' ends. Only few fusion partners have been identified so far, *CD74, SLC34A2, EZR*, and *GOPC/FIG* (147,148). This gene rearrangement has no influence on outcome, but similar to ALK1 this is usually a younger population of cancer patients (149). The incidence of *ROS1* rearrangement is in the range of 1%. The function of one of the fusion genes *EZR-ROS* was studied in a mouse model and showed that in this experimental setting the fusion gene acted as an oncogene inducing multiple tumor nodules in mice (150). Most important patients with this type

of gene aberrations responded well to the *ALK1* inhibitor crizotinib (151-153).

7.2.1.5. KIF5B and RET

KIF5B is one of the fusion partners for either *ALK1* or *RET*. The *KIF5B-RET* fusion gene is caused by a pericentric inversion of 10p11.2.2-q11.2.1. This fusion gene overexpresses chimeric *RET* receptor tyrosine kinase, which can spontaneously induce cellular transformation. Besides *KIF5B*, *CCDC6*, and *NCOA4* can form fusion genes with *RET*. Patients with lung adenocarcinomas with *RET* fusion gene had more poorly differentiated tumors, are younger, and more often never-smokers. Solid adenocarcinomas predominate, tumors are smaller but lymph node incidence is higher. The incidence of *RET* fusion is about in 1% of NSCLCs and almost 2% of adenocarcinomas (154-156).

7.2.1.6. MET

MET is another receptor tyrosine kinase bound to cell membranes in NSCLC. The ligand for MET is HGF, originally found in hepatic carcinomas. This receptor came into consideration in NSCLC because amplification of *MET* or alternatively upregulation of *HGF* was identified as a mechanism of the resistance in *EGFR* mutated adenocarcinomas (109,157). A search for the role of *MET* in other NSCLC excluding *EGFR* mutated adenocarcinomas showed that *MET* amplification was rare, but upregulation of *MET* a common event: approximately 20% of NSCLC including adenocarcinomas and squamous cell carcinomas showed high protein expression, but only 2% *MET* amplification (Popper *et al*., unpublished data). Clinical studies are in progress to evaluate the possibility to interfere with *MET* signaling using monoclonal antibodies. Other studies use small molecule inhibitors for *MET*. Since *MET* expression is common in *EGFR* mutated adenocarcinomas these studies aim to inhibit both *EGFR* and *MET* signaling pathways (158).

7.2.1.7. Other genes

Histone acetylases and deacetylases (*HAT*, *HDAC*) regulate the access of the DNA by methylation and demethylation. Thus these enzymes are important for DNA silencing (159,160). In addition heavily methylated DNA tends to switch into a supercoiled form, which cannot be read the transcription machinery. Histones themselves are in addition important for the correct positioning of the DNA, fixing the DNA to specific areas of the nuclear membrane (161,162). Attempts to interfere with this system were made quite a while ago, but the results were not convincing, probably because there are several types of *HATs* and *HDACs* with different functions. Recently treatment with *HDAC* inhibitors in combination with other drugs has shown promising results resulting in apoptosis and tumor cell necrosis (163-165).

In a study focusing on molecular alterations in pulmonary adenocarcinomas many additional genes were identified: well known are losses of one allele of the tumor suppressor *PTEN* in 9%, often associated with upregulation of *PIK3CA*, however *PI3KCA* mutations were also detected in 5%. Two other genes mutated in 3% and 2% were identified as *STK11* and *BRAF*, respectively (166). Interestingly these gene alterations could be sorted by smoking habits to either smokers (*STK11*) or non-smokers (*PIK3CA*). In addition squamous cell carcinoma morphology was associated with *PTEN*, *STK11*, and *PIK3CA* (166).

In a study analyzing Korean lung cancer patients by transcriptome sequencing the authors identified the well known candidates *EGFR*, *KRAS*, *NRAS*, *BRAF*, *PIK3CA*, *MET*, and *CTNNB1*, but also new driver mutations such as *LMTK2*, *ARID1A*, *NOTCH2*, and *SMARCA4 (*167). Besides these mutated genes also fusion genes were detected as *ALK*, *RET*, *ROS1*, and new ones as *FGFR2*, *AXL*, and *PDGFRA*. We also found an association between lymph node metastasis and somatic mutations in TP53. Elevated levels of insulin-like growth factor (*IGF*)-II are associated with a poor prognosis in human pulmonary adenocarcinoma. Moorehead *et al*. succeeded in establishing pulmonary adenocarcinoma in mice by transgenic overexpression of *IGF-II* in lung epithelium. These tumors expressed *TTF-1, SP-B* and *proSP-C*. Activation of *IGF-IR* resulted in the downstream activation of either the *ERK1/ERK2* or *p38 MAPK* pathways (168). Within this *IGF/IGFR* system also binding proteins play a prominent role: *IGFBP3* expression resulted in up-regulation of *VEGF* and *HIF1*, underlying the importance in neoangiogenesis and consequently in tumor growth and invasion (169). In contrast *IGFBP1* seems to act like a tumor suppressor decreasing colony formation of cell cultures and increasing apoptosis. *IGFBP1* has been found methylated in pulmonary adenocarcinomas (170). The *IGFR1* pathway is also involved in resistance mechanisms in EGFR mutated adenocarcinomas (171). Treatment with monoclonal antibodies for *IGFR1* also occurs in clinical studies (172,173).

7.2.2. Squamous cell carcinoma

Squamous cell carcinoma is defined by a plate-like layering of cells, keratinization of at least single cells, intercellular gaps and bridges (represented by desmosomes and hemidesmosomes), and positive staining for high molecular weight cytokeratins (*CK* 3/5, 13/14). There are some morphological variants as small cell and basaloid, but these have not been associated with gene signatures and therefore are only important in diagnostics. The incidence of squamous cell carcinoma has dropped in the last three decades from a major entity representing 35% of lung carcinomas to around 17%. One of the major reasons is the shift from filter-less to filter cigarettes. This has resulted in the reduction of particle-bound carcinogens and increase of vaporized carcinogens, which more easily reach the bronchioloalveolar terminal unit, inducing mainly adenocarcinomas. In the past squamous cell carcinoma was mainly a diagnosis required to exclude several therapeutic options in the clinic: no pemetrexed therapy, no antiangiogenic drugs, less responsiveness to cisplatin treatment. However, this has changed within the last three years.

7.2.2.1. FGFR1

Fibroblast growth factor receptor 1 was identified being amplified in about 20% of squamous cell carcinomas (174 and Popper *et al*., unpublished data). In experimental studies as well as in ongoing clinical trials it was found that only amplification, proven by ISH identified patients, that responded to small molecule inhibitor treatment (175) (and unpublished communication from R. Thomas, Cologne, Germany).

7.2.2.2. DDR2 and FGFR2

DDR2 and *FGFR2* mutations are found exclusively in squamous cell carcinomas, however, in a small percentage, 4% and 2%, respectively (166). For FGFR2 multikinase inhibitors might be an option for specific treatment (176,177).

7.2.2.3. SOX2 amplification

SOX2 gene located on chromosome 3q26.3. is a factor for the maintenance of stem cell like properties in lung cancer cells (178). *SOX2* amplification has been reported to be specifically associated with squamous cell carcinoma morphology (179-181). However, other investigations have also claimed an importance for small cell carcinomas and adenocarcinomas (178,182). Amplification in squamous cell carcinoma was associated with better prognosis (183) whereas with poor prognosis in adenocarcinomas and SCLC. So far no specific therapies do exist for patients with this genetic abnormality.

7.2.2.4. PTEN mutation-deletion

PTEN deletions are quite common in NSCLC, usually associated with the subsequent upregulation of *PI3K* and downstream activation of the *AKT* pathway (184). In the past therapies were conducted with inhibitors of mTOR, but failed due to the subsequent upregulation of a negative feedback loop of *mTOR*, which in turn activates *AKT* and this time results in a circumvention of *mTOR* via other pathways (185-187). Recent works, however, have shown a benefit of *mTOR* inhibition, when applied in the right context (188,189). *PTEN* mutations are rare, but can be found more often in squamous cell carcinomas (166). If patients with this genetic abnormality can also be treated by a combined modality is still an unsolved issue.

7.2.2.5. PDGFRA amplification

Amplification of the *PDGFR* alpha was predominantly found in squamous cell carcinomas (190). Although this is not a frequent event in these carcinomas, specific inhibitors have shown a growth inhibiting effect in cell lines and might be considered in patient treatment (191).

7.2.2.6. CDKN2A (p16) mutation, deletion, and methylation

Another uncommon genetic modification is found in the *CDKN2A* gene coding for the p16 protein. P16 is regarded as a tumor suppressor protein and is involved in cell cycle regulation in many pulmonary carcinomas including squamous cell carcinoma and adenocarcinoma (192-194). It closely interacts with Rb and p53 proteins.

7.2.2.7. NOTCH1 mutation

NOTCH1 regulates cell specification and homeostasis of stem cell compartments, and it is counteracted by the cell fate determinant *Numb*. *NOTCH* signaling is altered in approximately one third of NSCLCs. Loss of Numb expression results in increased *NOTCH* activity; in a smaller fraction of cases gain-of-function mutations of the *NOTCH1* gene are present. Inhibitors of Notch can selectively kill epithelial cell cultures harboring constitutive activation of the *NOTCH* pathway (195). In a subsequent study *NOTCH1* and 2 frame shift and nonsense mutations were identified in pulmonary squamous cell carcinomas (196). More importantly

NOTCH1 has an important role in carcinogenesis by suppressing p53-mediated apoptosis and regulating the stability of the p53 protein. *NOTCH1* also plays an important role in *KRAS* induced mouse adenocarcinoma models (197).

7.2.2.8. REL amplification

In a mouse model it was shown that different downstream activation of *RAS* pathways is necessary to induce an invasive and more important angioinvasive phenotype. Only the combined activation of the *PI3K, RAS-MAPK-ERK*, and the *RAL* and *REL* pathway could induce an aggressive phenotype (138). Whereas *REL-A* seems to be exclusively expressed in squamous cell lung carcinoma, *REL-B* was shown to be processed in NSCLC cell lines. *REL-B* was shown to suppress the expression of ß1-integrin and thus prevented adherence of the carcinoma cell (198).

7.2.3. Large cell carcinoma

Large cell carcinoma is defined by large cells (> 25 mµ) devoid of any cytoplasmic differentiation, and large vesicular nuclei. They have a well-ordered solid structure and ultrastructural tumor cells show hemidesmosomes, tight junctions, intracytoplasmic vacuoles with microvilli, and ill-formed cilia. This clearly fits into the concept of a carcinoma, at the doorstep of adenocarcinoma and squamous cell carcinoma differentiation. Large cell carcinoma numbers have dramatically decreased due to the use of immunohistochemistry for differentiating. Using *TTF1* low-molecular cytokeratins, as well as p63 and cytokeratin 5/6 most cases of large cell carcinoma were either shifted into adenocarcinoma or squamous cell carcinoma, respectively (199). These recent changes make an evaluation of genetic aberrations in large cell carcinoma quite difficult, since genetic studies were based on previous classifications.

Not surprisingly *EGFR* mutations, *MET* amplifications, and *EML4ALK1* fusions have been reported in large cell carcinoma (200). *LKB1* a gene mutated in a small percentage of adenocarcinomas was also shown in squamous and large cell carcinomas (201). *LKB1*, also known as *STK11*, is involved in the negative regulation of *mTOR* and closely cooperates with *TSC1* and *2* genes (202).

In contrast to large cell carcinoma, which is negatively defined by exclusion criteria, the variants are positively defined.

7.2.3.1. Large cell carcinoma with rhabdoid phenotype

Large cell carcinoma with rhabdoid phenotype is characterized by a solid growth pattern, often overlaid by a reactive proliferation of pneumocytes, which can give these tumors a pseudoalveolar pattern and a pseudo-composition of two cell populations. Within the cytoplasm of the tumor cells eosinophilic inclusion bodies can be found, similar to those seen in rhabdomyosarcomas. These inclusion bodies are stained by eosin, are negative for striated muscle markers, but positive for vimentin. The production of vimentin filaments, which seem to have no function because of package into a cytoplasmic vacuole, is the only known abnormality for this tumor type. In a single study *KRAS* mutations were found in some cases of this tumor type (199).

Sheets of undifferentiated tumor cells embedded in a lymphocyte rich stroma characterize *lymphoepithelial-like large cell carcinoma*. The carcinoma cells are positive for cytokeratins 13/14 and the lymphocytes in most cases are B-lymphocytes. Only one study looked up genetic changes in this tumor entity. They found unusual mutations in *TP53* at exon 8, and *EGFR* mutations in few patients within their large series. These were exon 21 (not L858R) and exon 18 and 20 mutations (203).

7.2.3.2. Pulmonary clear cell carcinoma

Pulmonary clear cell carcinoma is another rare variant of large cell carcinoma, defined by abundant glycogen in the cytoplasm of the tumor cells. Tissue processing usually dissolves glycogen, thus leaving the impression of a clear/empty cytoplasm. Only one study examined molecular changes in this tumor type and found predominantly *KRAS* mutations (199).

7.3. The neuroendocrine tumors

7.3.1. Small cell neuroendocrine carcinoma

Small cell lung carcinoma (SCLC) is defined by nuclear size of 16-23 mµ (not so small!), dark stained nuclei (mainly composed of heterochromatin), inconspicuous or lacking nucleoli, small cytoplasmic rim, often invisible in light microscopy, and fragile nuclei. SCLC is regularly positive for the neuroendocrine markers NCAM and synaptophysin, but most often negative for chromogranin A. The best marker is NCAM with a strong membranous staining. SCLC is positive for low molecular weight cytokeratins. SCLC produces hormones, such as adrenocorticotropin (ACTH), but also substances interfering with the blood coagulation

system. In contrast to carcinoids SCLC more often are positive for heterotopic hormones (i.e. hormones usually not found in adult lung). In our experience a positive reaction for gastrin-releasing peptide (GRP) and ACTH is most often seen. The secretion of ACTH can cause Cushing syndrome. Some of the hormones especially GRP act as an autocrine loop: the peptide is produced by the cancer cells, released and bind back to their respective membrane bound receptors, which themselves signal back into the nucleus with a growth stimulation (204).

Genetic abnormalities in SCLC are quite common, usually over 50% of the SCLC chromosomes are affected (205,206). These many genomic alterations made a search for driver gene mutations/alterations complicated, which consequently resulted, that besides classical chemotherapy, no targeted therapy has emerged up to now. Some genetic alterations are known for a long time, however, not resulting in a therapeutic intervention strategy. Two genetic alterations have been long known, *RB* loss or mutation, and *TP53* mutation. Since both genes are involved in cell cycle checkpoint controls, this might explain the high numbers of genetic alterations (207-209). Other genes involved in SCLC are the tumor suppressor *FHIT*, *RASSF1*, both on chromosome 3p, *RARbeta*, and *Myc* genes (209). *RASSF1* mRNA expression was lost in all tested SCLC cell lines, whereas its promoter was methylated in some NSCLC cell lines (210). In SCLC also apoptotic and immunogenic mechanisms seem to be inactivated. In a study by Senderowicz *FasL* was overexpressed in almost all SCLC cases examined. The ratio of *Fas/FasL* was decreased. The authors concluded, that *FasL* overexpression in the context of *Fas* downregulation might allow tumor cells to induce paracrine killing of cytotoxic T cells (211). Since the *PI3K-Akt* signaling pathway is activated in almost all cases of SCLC, this system might also be associated with inhibition of apoptosis via upregulation of *TNFRSF4*, *DAD1*, *BCL2L1*, and *BCL2L2*, and with chemoresistance (212,213). These data demonstrate that several systems are involved in SCLC growth, survival, and resistance to chemotherapy. ASH1 was identified as the gene responsible for the neuroendocrine phenotype in both high-grade carcinomas (214). Together with other genes (*ATOH1, NEUROD1* and 4) involved in neurogenic differentiation, they are also expressed in NSCLC with neuroendocrine phenotype. As SCLC, these NSCLC cases expressed mRNA for dopa decarboxylase and stained positively for neuroendocrine markers (215). *ASH1* seems to be an early differentiation gene in the developing lung. In embryonic development *ASH1* was found in neuroepithelial bodies and solitary neuroendocrine cells, but vanishes with maturation of the lung. Therefore *ASH1* might be an early program for neuroendocrine cell differentiation (216). If there is another function of *ASH1* is not entirely clear. *ASH1* seems to repress tumor suppressor such as *DKK1* and 3, which are regulators of the *Wnt-β* catenin pathway. *ASH1* also inactivates E-cadherin and integrinß1 by de-acetylation and methylation of the *DKK1* and *E-cadherin* promoters (217). In an animal lung cancer model the expression of *ASH1* enhances the carcinogenic effect of SV40 large T antigen, suggesting that *ASH1* might cooperate with *pRB* (218).

7.3.2. Large cell neuroendocrine carcinoma (LNEC)

LCNEC is defined by a neuroendocrine pattern, e.g. rosettes, trabecules. On low power they look similar to carcinoids, but on higher magnification abundant mitoses are obvious. The prognosis of LCNEC is similar to that of SCLC, both are grouped as the high-grade neuroendocrine carcinomas.

Genetic analysis of LCNEC showed similar alterations as found in SCLC. However, allelic losses at 5q and abnormalities in the p16 gene may differentiate large cell neuroendocrine carcinoma from SCLC (219). Another difference between both high-grade neuroendocrine carcinomas is seen at chromosome 3q: gains of 3q are frequently seen in SCLC, whereas were absent in LCNEC. However, gains of 6p were frequent in LCNEC; deletions within 10q, 16q, and 17p were more common in SCLC (220). *ASH1* mRNA was found higher in SCLC, whereas its counteracting gene *HES1* was more frequently expressed in LCNEC (221).

7.3.3. Carcinoids

Typical carcinoid is defined by neuroendocrine structures, such as rosettes, trabecules, and solid nests, 0 or 1 mitosis per 2 mm^2, and absence of necrosis. Atypical carcinoid is defined by 2-10 mitoses per 2 mm^2, and/or presence of necrosis, and again neuroendocrine structures. In both carcinoids there is an invasive growth into the lung, and lymphatic and blood vessel invasion can be found in some cases. Some carcinoids can metastasize, but so far there are no predictive markers for the biological behavior.

Those carcinoids, which have more than two losses on distal chromosome 11q, and those with multiple chromosomal losses (<10), also show

a worse outcome. Based on genetic studies it can be speculated, that the 140 kDa isoform precursor of NCAM, Zinc-finger protein-like 1, and sorting Nexin 15 might be involved in the genesis of carcinoids (222).

7.4. Other rare lung carcinomas

7.4.1. Salivary gland type carcinomas

Salivary gland tumors can occur in the lung, usually in a central location. Within the spectrum are mucoepidermoid carcinomas, adenoid cystic carcinomas, and epithelial-myoepithelial carcinomas. These are rare carcinomas with a wide range of affected ages, from children as early as 3 years of age, as well as in elderly patients.

7.4.1.1. Mucoepidermoid carcinoma

Due to their central location they may grow as a polypoid mass occluding large bronchi and thus causing obstruction, with often poststenotic bronchopneumonia. In low-grade carcinomas cystic and solid areas are found. Within the glandular areas squamous epithelium is interspersed, the tumor cells are usually non-keratinizing. In high-grade carcinomas the distinction from adenosquamous carcinoma might sometimes be impossible, due to overlapping features. The proof is the mixture of squamous and mucin-producing columnar cells within a gland, endobronchial growth, and absence of keratin pearls and an in-situ component. The prognosis of high-grade mucoepidermoid carcinomas is similar to other NSCLC.

EGFR was studied in mucoepidermoid carcinomas of the lung. Protein expression was frequently found, whereas mutations in exon 18-21 were absent. Polysomy of the *EGFR* was seen in a small percentage of cases (223). In another study the authors reported *EGFR* mutations and also a response to tyrosine kinase inhibitor treatment. One of the patients in this study showed a response to treatment without alterations of the *EGFR* (224). A third study probably presents the explanation for this behavior: the authors showed a translocation t(11;19) resulting in a fusion oncogene *CRTC1-MAML2*. *CRTC1-MAML2* was shown to up-regulate the *EGFR* ligand, amphiregulin and thus activated the receptor (225).

7.4.1.2. Adenoid cystic carcinoma

Similar to the salivary counterpart adenoid cystic carcinoma of the bronchus is a slowly growing tumor with late lymph node and distant organ involvement. However, recurrence is frequent. They occur in the trachea and the large bronchi as far as normal bronchial glands are found. The tumor forms pseudotubules filled with mucin-like material, which is composed of matrix proteins of the basal lamina as collagen type 4 and fibronectin.

In an experimental model of adenoid cystic carcinoma it was shown that dual inhibition of *EGFR* and *VEGFR2* resulted in apoptosis, decreased angiogenesis and metastasis (226). In an array-CGH study a gain was found on chromosome 4q12, which contains a cluster of receptor tyrosine kinases such as *KIT*, *PDGFRA* (196), however, in another study looking at this area *KIT* mutations or amplifications were not found (227). Also mutations of EGFR could not be demonstrated in adenoid cystic carcinomas, but upregulation of the protein was a constant feature (223,228). In another study experimental inhibition of phospho-*ERK1/2* resulted in inhibition of tumor cell growth and metastasis (229) and in a clinical study growth of adenoid cystic carcinoma could be inhibited by a multikinase inhibitor (230). However, in a subsequent clinical trial this inhibition could not be confirmed (231). Recently new investigations came up with genetic aberrations in these carcinomas. The *MYB-NFIB* fusion resulting in overexpression of *MYB* was found in the majority of these carcinomas (232). Copy number alterations including down-regulated suppressor genes were in addition associated with behavior of these carcinomas (232). Newly identified mutations in adenoid cystic carcinomas, some of which might lead to a targeted therapy are *FGF-IGF-PI3K* mutations and mutations in *NOTCH1/2* signaling regulator *SPEN*, which might be a new cancer gene in these types of tumors, and finally within a subset of these carcinomas also *FGFR2* mutations (233,234).

Another rare salivary gland tumor is acinus cell carcinoma. Uniform tumor cells showing acidophilic/azurophilic granules in their cytoplasm characterize it. So far no specific genetic abnormality has been reported in pulmonary variants.

7.4.2. Sarcomatoid carcinomas

The sarcomatoid carcinomas are a group of carcinomas with sarcomatoid features. This group is composed of pleomorphic carcinoma, spindle and giant cell carcinoma, pulmonary blastoma, and carcinosarcoma. Pleomorphic carcinoma is defined as a carcinoma with either a spindle or giant cell component and any other NSCLC component.

Pulmonary blastoma is composed by a fetal type adenocarcinoma and an embryonic type of stroma. Most often this stroma component is benign, however, in rare cases the stroma can exhibit atypia and even malignancy. Finally carcinosarcoma is composed of any type of NSCLC with a sarcoma with osseous, rhabdomyoblastic, or chondroblastic differentiation.

Few molecular studies have focused on these tumor types. In the study by Pelosi the epithelial components of sarcomatoid carcinomas showed expression of epithelial markers and cell cycle inhibitors *P21WAF1* and *P27KIP1*, and the loss of *FHIT*, whereas the sarcomatoid components expressed mesenchymal markers and differently reacted for molecules involved in cell differentiation, cell cycle control, and tumor cell growth and motility (235). In another study the mechanisms of epithelial-mesenchymal transition was explored. The classical pathways NOTCH1, *SNAIL*, and the *WNT*-pathway could be excluded, but a possible alternative pathway including *JUN-VIMENTIN-FASCIN* was found (236). Looking up *EGFR* and *KRAS* mutations in sarcomatoid carcinomas no mutations were found for *EGFR*, but *KRAS* in almost half of the cases. However, *EGFR* was constantly overexpressed at the protein level, which might suggest the possibility of an *EGF* antibody treatment (237).

8. MOLECULAR PATHOLOGY OF PRE-NEOPLASTIC LESIONS

Actually our knowledge is very small on preneoplastic lung lesions: for a few carcinomas the preneoplastic lesion is known, but for most carcinomas discussed above we know nothing. And most importantly even for those well known lesions we cannot predict, if these lesions will progress into carcinoma or not. The factor(s) responsible for progression and invasion are unknown as well as are the ways for their sensitive detection. From our current understanding the neoplastic transformation has some important requirements, which usually precede morphological changes: nutrition, nucleotides for DNA replication, and oxygen for metabolism. Preneoplastic cells need increased access to nutrients for building up an energy reserve for the increase of cell divisions, they need to get access to higher levels of purine and pyrimidine bases to facilitate their increased DNA replications during mitosis, and for an increased metabolism also an increase of oxygen might be required. Increased oxygen consumption, however, is controversially discussed: hypoxia seems to be critical for invasion and metastasis, so probably increased oxygen and oxidative metabolism might be required only in the early intraepithelial expansion of the preneoplastic cells, whereas glycolysis seems more common in invasive carcinomas (238).

8.1. Hyperplasia of goblet cells and squamous metaplasia/dysplasia

For squamous cell carcinomas the preneoplastic lesion has been known for a while. In the large bronchi there is a protection program for toxin/carcinogen exposure, which starts with goblet cell hyperplasia and proceeds to squamous cell metaplasia, and further on to squamous cell dysplasia or intraepithelial neoplasia. A vascular variant of dysplasia has been described characterized by an ingrowth of capillaries into the squamous epithelium. Atypia and mitosis might be mild or absent, respectively, however this represents a rapid progressive lesion (84) most probably due to the expression and release of *VEGF* (Popper *et al.*, unpublished data).

A few factors influencing this progression have been identified, such as a *p63* splice variant, amplification of *PI3KCA*, increased *NFkB p65* nuclear expression, gradual increases of *caspase-8*, *STAT* 5, and *p70s6K* or decrease of *E-cadherin* (239-242) Angiogenic stimuli seem to play a role in progression, since it is upregulated in rapid progressive squamous cell dysplasia with prominent vascular intraepithelial growth pattern (84). However, the interrelation of genes upregulated and downregulated has not been elucidated, so we are presently limited to single studies looking for different genetic abnormalities.

8.2. Atypical adenomatous hyperplasia and bronchial columnar cell dysplasia

They are two preneoplastic lesions confined to the alveolar and bronchiolar periphery, respectively. Atypical alveolar hyperplasia is visible already at low power magnification. The normal epithelium is replaced by pneumocyte type II like atypical cells. Between the cells, lined up as a single row, there are usually gaps left. When these gaps are lost, and cell pile up close to each other, or form cell papillae without stroma this already fulfills the criteria of adenocarcinoma in-situ. Atypical adenomatous hyperplasia is regarded as the precursor for some non-mucinous adenocarcinomas, especially acinar and papillary types.

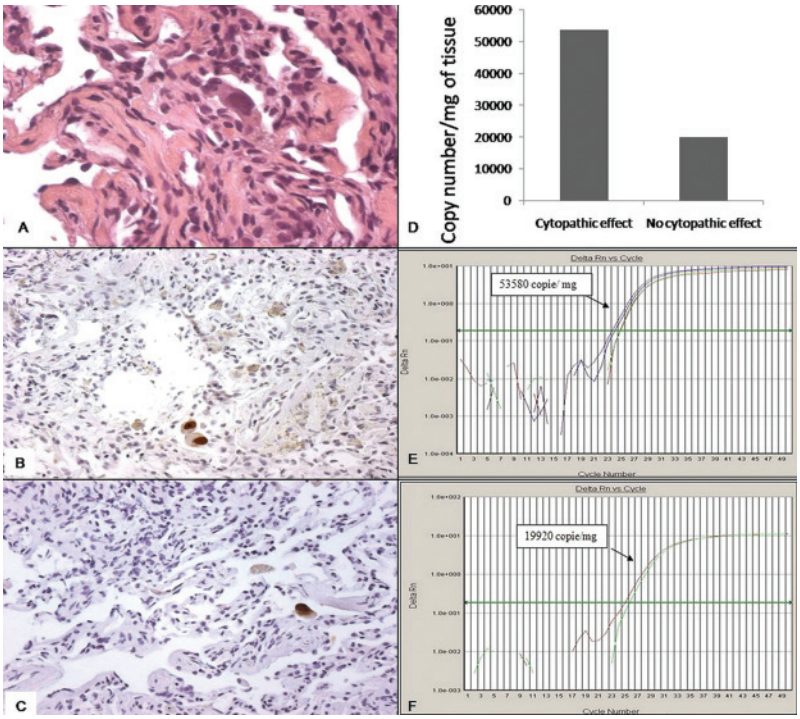

Figure 1. A) Histological section of scheduled TBB from lung recipient: note atypical CMV inclusion. Original magnification: X 300. B, C) Immunohistochemistry for CMV and in situ hybridization showed similar sensitivity in CMV detection. Original magnification: X 150. D) Data obtained from Real Time PCR analysis with Artus CMV PCR Kit in TBB from CMV positive cases (both in BAL and in blood): a high number of copies was detected also in cases lacking CMV related cytopathic changes. E, F) Emblematic amplification plot obtained by Real Time PCR in 2 cases with CMV cytopathic changes and in one case without CMV-related cytopathic effects (mean CMV copy numbers are reported in the plots).

Genomic aberrations were found to increase from atypical adenomatous hyperplasia to adenocarcinoma in-situ and invasive adenocarcinoma; the chromosomes involved in the atypical adenomatous hyperplasia lesions were found to be present in the high grade lesions, suggesting an atypical adenomatous hyperplasia to adenocarcinoma in-situ to invasive adenocarcinoma sequence (243,244). *KRAS* mutations were detected in one third of atypical adenomatous hyperplasia studied and seem to be an early event in carcinogenesis (245). A loss of either *TSC1* or *2* genes was another finding in well differentiated adenocarcinomas and their associated atypical adenomatous hyperplasia lesions (246,247). *EGFR* mutations as well as amplifications could not be detected in atypical adenomatous hyperplasia, but were found in adjacent well differentiated adenocarcinomas, and less frequently in less differentiated ones (248). In another study comparing *KRAS* and *EGFR* mutations in atypical adenomatous hyperplasia, adenocarcinoma in-situ, and invasive adenocarcinomas the percentage of *KRAS* mutations decreased from atypical adenomatous hyperplasia to adenocarcinoma in-situ to invasive adenocarcinomas, whereas *EGFR* mutations increased from atypical adenomatous hyperplasia to adenocarcinoma in-situ and to invasive adenocarcinomas. This study concluded that two different pathways of atypical adenomatous hyperplasia exist, one driven by *KRAS*, the other by *EGFR* mutations, respectively (249).

In contrast to atypical adenomatous hyperplasia bronchial columnar cell dysplasia can only be identified at high magnification. In bronchial columnar cell dysplasia atypical cells gradually replace normal bronchial epithelium. In early stages of bronchial columnar cell dysplasia a monomorphic proliferation of cells replace these differentiated cells and gradually expand up to the surface. If completely replaced, more atypia is recognized.

In a study of genetic aberrations Ullmann found an increase of genetic alterations from 2.6.

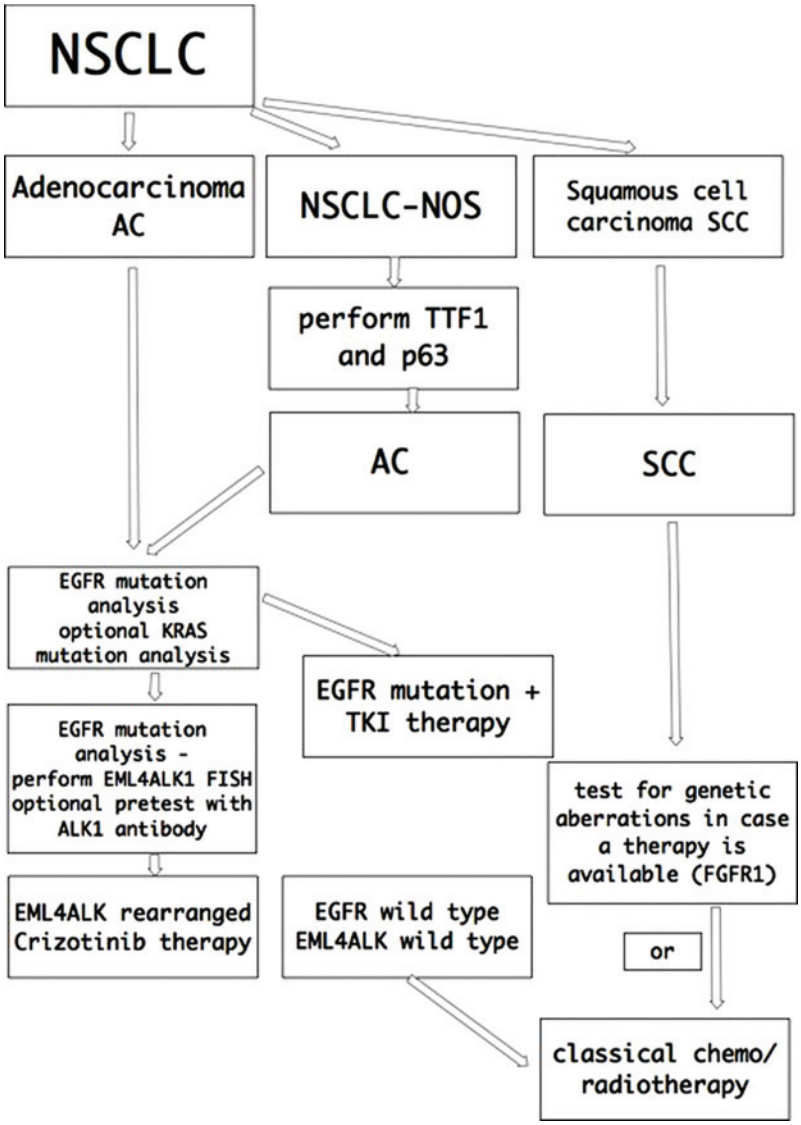

Figure 2. Algorithm of diagnostic decisions and following molecular analysis of NSCLC.

in bronchial columnar cell dysplasia to 14.7. in the concomitant adenocarcinoma. Unbalanced numerical aberrations were losses of 3p, 9, 13, 14 and gains of 1q, 17, 19q and 20q (250). Another study demonstrated loss of *P16INK4a* protein in 70% of cases. p53 accumulation was found in 26% of the cases (251).

Atypical goblet cell hyperplasia is much more difficult to recognize: the nuclei are compressed at the cell border and the chromatin structure is invisible. Atypical, signet-ring-like cells replace the normal epithelium resulting in a monotonous pattern. Atypical goblet cell hyperplasia might give rise to the different mucinous adenocarcinomas of the lung. Atypical goblet cell hyperplasia was studied in cystic pulmonary adenomatous malformation. In this study, chromosomal gains on chromosomes 2 and 4 were found as the major genetic alteration (252). The same genetic alterations were demonstrated in three associated adenocarcinomas of children. Thus most probably oncogenes located on these chromosomes are involved in carcinogenesis.

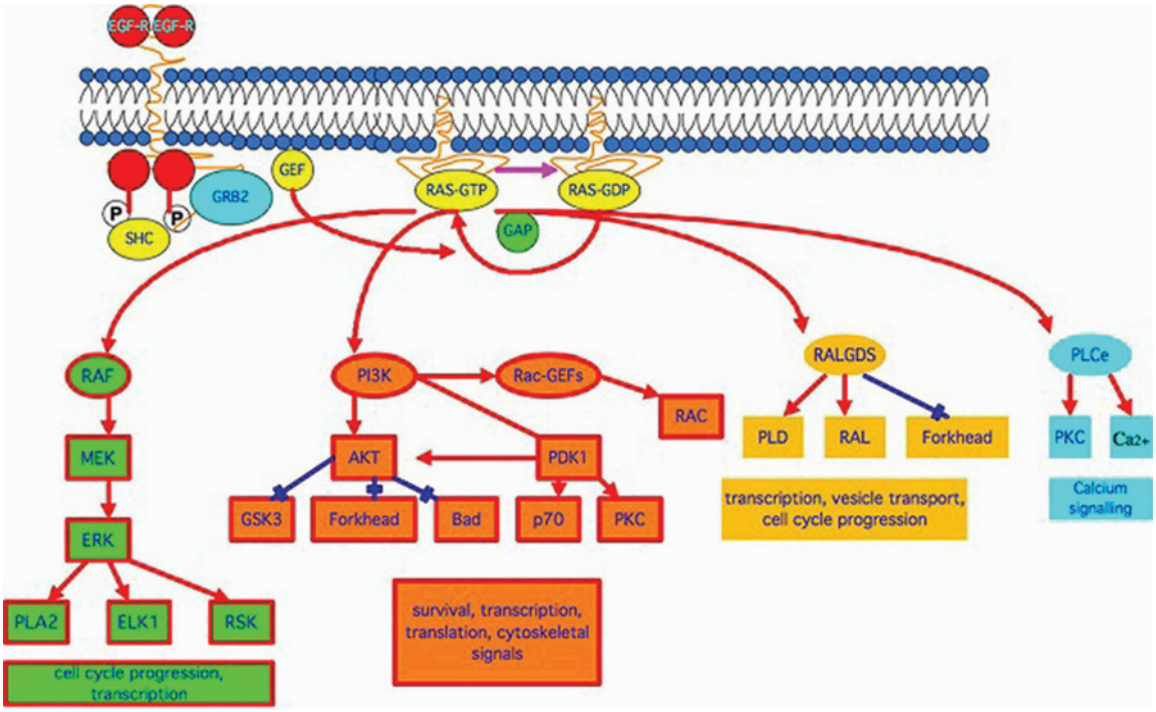

Figure 3. Schema of possible downstream pathways in RAS activated carcinomas. This schema illustrates the possibility for tumor cells to shift from a dominant to another downstream activation.

8.3. Neuroendocrine cell hyperplasia

It is divided into neuroendocrine hyperplasia associated with fibrosis and bronchiectasis or in the vicinity of a carcinoid, and diffuse neuroendocrine hyperplasia without known cause; in addition a nodular proliferation is described and finally there is a tumorlet, which is different from carcinoid by size and separation of tumor cell groups by fibrous bands. Tumorlets as well as diffuse neuroendocrine hyperplasias are often found in the vicinity of carcinoids, and therefore it is speculated that they may represent preneoplastic lesions for carcinoids. A few studies focused on molecular changes in these lesions. Since neuroendocrine lesions and low-grade tumors are usually rich in capillaries, one study focused on angiogenetic factors.

VEGF was found highly expressed in diffuse proliferations, but there were no correlations with progression, but rather an association with fibrosis (253). N-Cadherin was found in a high proportion of diffuse hyperplasias and carcinoids, but less in SCLC and LCNEC. In carcinoids N-Cadherin expression was associated with negative lymphnode status (254).

9. MALIGNANT TUMORS OF CHILDHOOD

9.1. Pleuropulmonary blastoma

Pleuropulmonary blastoma is a malignant mesenchymal tumor, which can arise in the pleura, the lung, or both. It can present as a predominant cystic, a mixed cystic and solid, or a pure solid lesion, which is also reflected by increased aggressive behavior. The tumor cells form layers of immature small cells (so called germinal layer) with relatively large nuclei and dense chromatin. In addition interspersed giant cells with rhabdomyoblast differentiation and areas of chondrosarcoma are usually seen (255).

Genetically gains of large parts or whole chromosome 8 are often found in pleuropulmonary blastoma (256,257). A few other aberrations have been reported: losses on 6q13-qter, 10pter-p13, 10q22-qter, and 20p13, and gains of chromosomes 2 and 8p11-p12 (258). In some reports mutations of the *TP53* gene were described (259). Recently *DICER 1* syndrome has been linked to pleuropulmonary blastoma: *DICER* is a protein involved in the processing of small inhibitory microRNAs. A mutation has been reported in several families and this was

linked to the development of pleuropulmonary blastoma and other childhood tumors (260).

9.2. Congenital myofibroblastic tumor

Congenital myofibroblastic tumor is another rare childhood tumor composed of proliferating myofibroblastic cells, sometimes with marked cellularity and mitotic activity. The tumor cells proliferate within the interstitium and peribronchiolar area, and thus cause bronchiolar obstruction. They can behave biologically like malignant tumors (261). A single genetic analysis was reported, under the other name of the tumor, namely childhood leiomyosarcoma. The authors reported gains of chromosomes 2 and 11, and losses of chromosomes 9, 19, 20, and 22 (262).

10. FINAL REMARKS

Several other topics, which are more general and not organ based in the development of cancer, are not discussed herein, such as the stem cell theory in cancer development and metastasis. Another aspect is invasion and metastasis of lung cancer. Up to now the picture in lung cancer is unclear: it seems there is no single metastasis gene for the different entities, and also the mechanisms of invasion do not follow a general rule for each carcinoma type. Survival in the blood stream and adherence on endothelial venules at the metastatic site is diverse, as well as the preference of metastasis. Some carcinomas prefer brain, others bone marrow, and others liver and adrenal glands as their primary metastatic site outside the thorax. Moreover it seems there are different genes associated with this metastatic preference. The reader is therefore referred to special reviews on these subjects.

During the last few years a high number of genes have been identified as potential biomarkers of different lung tumors. Some of these are routinely used in targeted therapy approaches. Morphology remains the gold standard for the diagnosis. New technologies, as molecular tools, should always appropriately applied and the pathologist plays a crucial role both in the selection of the most representative tissue and in the correlation between molecular signature and histotypes.

11. ACKNOWLEDGEMENTS

The authors thank Judith Wilson for English revision. The authors apologize to those colleagues whose outstanding work could not be cited due to space constraints or to unintended omissions. There is no conflict of interest to declare.

12. REFERENCES

1. W.W. Grody, R.A. Gatti, F. Naeim: Diagnostic molecular pathology. Mod Pathol 2, 553-568 (1989)
2. J.A. Fairley, K. Gilmour, K. Walsh: Making the most of pathological specimens: molecular diagnosis in formalin-fixed, paraffin embedded tissue. Curr Drug Targets 13, 1475-1487 (2012) DOI: 10.2174/138945012803530125
3. M. Dietel, K. Jöhrens, M. Laffert, M. Hummel, H. Bläker, B.M. Müller, A. Lehmann, C. Denkert, F.L. Heppner, A. Koch, C. Sers, I. Anagnostopoulos: Predictive molecular pathology and its role in targeted cancer therapy: a review focusing on clinical relevance. Cancer Gene Ther 20, 211-221 (2013) DOI: 10.1038/cgt.2013.13
4. J. Bhatnagar, T. Jones, D.M. Blau, W.J. Shieh, C.D. Paddock, C. Drew, A.M. Denison, D.C. Rollin, M. Patel, S.R. Zaki: Localization of pandemic 2009 H1N1 influenza A virus RNA in lung and lymph nodes of fatal influenza cases by in situ hybridization: New insights on virus replication and pathogenesis. J Clin Virol 56, 232–237 (2013) DOI: 10.1016/j.jcv.2012.11.014
5. M.A. Innes, D.H. Gelfand, J.J. Sninsky, T.J. White: PCR Protocols, A Guide to Methods and Application. Eds Academic, San Diego, CA (1990)
6. S. Paireder, B. Werner, J. Bailer, W. Werther, E. Schmid, B. Patzak, M. Cichna-Mark: Comparison of protocols for DNA extraction from long-term preserved formalin fixed tissues. Anal Biochem 439, 152-160 (2013) DOI: 10.1016/j.ab.2013.04.006
7. G. Stanta, S. Bonin: RNA quantitative analysis from fixed and paraffin-embedded tissues: membrane hybridization and

capillary electrophoresis. Biotechniques 24, 271–276 (1998)

8. M. Cronin, M. Pho, D. Dutta, J.C. Stephans, S. Shak, M.C. Kiefer, J.M. Esteban, J.B. Baker: Measurement of Gene Expression in Archival Paraffin-Embedded Tissues. Am J Pathol 164, 35-42 (2004)
DOI: 10.1016/S0002-9440(10)63093-3

9. J. Cheval, V. Sauvage, L. Frangeul, L. Dacheux, G. Guigon, N. Dumey, K. Pariente, C. Rousseaux, F. Dorange, N. Berthet, S, Brisse, I. Moszer, H. Bourhy, C.J. Manuguerra, M. Lecuit, A. Burguiere, V. Caro, M. Eloit: Evaluation of high-throughput sequencing for identifying known and unknown viruses in biological samples. J Clin Microbiol 49:3268-3275 (2011)
DOI: 10.1128/JCM.00850-11

10. L.J. Van't Veer, H. Dai, M.J. van de Vijver, Y.D. He, A.A. Hart, M. Mao, H.L. Peterse, K .van der Kooy, M.J. Marton, A.T. Witteveen, G.J. Schreiber, R.M. Kerkhoven, C. Roberts, P.S. Linsley, R. Bernards, S.H. Friend. Gene expression profiling predicts clinical outcome of breast cancer. Nature 415, 530–536 (2002)
DOI: 10.1038/415530a

11. D. Wang, L. Coscoy, M. Zylberberg, P.C. Avila, H.A. Boushey, D. Ganem, J.L. DeRisi: Microarray-based detection and genotyping of viral pathogens. Proc Natl Acad Sci USA 99,15687–15692 (2002)
DOI: 10.1073/pnas.242579699

12. J.D. Fox: Nucleic acid amplification tests for detection of respiratory viruses. J Clin Virol 40, S15–S23 (2007)
DOI: 10.1016/S1386-6532(07)70005-7

13. S. Yang, R.E. Rothman: PCR-based diagnostics for infectious diseases: uses, limitations, and future applications in acute-care settings. Lancet Infect Dis 4, 337-348 (2004)
DOI: 10.1016/S1473-3099(04)01044-8

14. D. Klein: Quantification using real-time PCR technology: applications and limitations. Trends Mol Med 8, 257-260 (2002)
DOI: 10.1016/S1471-4914(02)02355-9

15. S. Yang, R.E. Rothman: PCR-based diagnostics for infectious diseases: uses, limitations, and future applications in acute-care settings. Lancet Infect Dis 4, 337-348 (2004)
DOI: 10.1016/S1473-3099(04)01044-8

16. S. Pillet, M. Lardeux, J. Dina, F. Grattard, P. Verhoeven, J. Le Goff, A. Vabret, B. Pozzetto: Comparative evaluation of six commercialized multiplex PCR kits for the diagnosis of respiratory infections. PLoS One 8, e72174 (2013)
DOI: 10.1371/journal.pone.0072174

17. L.T. França, E. Carrilho, T.B. Kist: A review of DNA sequencing techniques. Q Rev Biophys 35, 169-200 (2002)
DOI: 10.1017/S0033583502003797

18. A. Altimari, D. de Biase, G. De Maglio, E. Gruppioni, E. Capizzi, A. Degiovanni, A. D'Errico, A. Pession, S. Pizzolitto, M. Fiorentino, G. Tallini: 454 next generation-sequencing outperforms allele-specific PCR, Sanger sequencing, and pyrosequencing for routine KRAS mutation analysis of formalin-fixed, paraffin-embedded samples. Onco Targets Ther 6:1057-1064 (2013)

19. J.M. Rizzo, M.J. Buck: Key principles and clinical applications of "Next-generation" DNA sequencing. Cancer Prev Res (Phila) 5, 887-900 (2012)
DOI: 10.1158/1940-6207.CAPR-11-0432

20. D. de Biase, M. Visani, U. Malapelle, F. Simonato, V. Cesari, C. Bellevicine, A. Pession, G. Troncone, A. Fassina, G. Tallini: Next-Generation Sequencing of Lung Cancer EGFR Exons 18-21 Allows Effective Molecular Diagnosis of Small Routine Samples (Cytology and Biopsy) PLoS One 8, e83607 (2013)
DOI: 10.1371/journal.pone.0083607

21. J.A. Fishman, R.H. Rubin: Infection in organ-transplant recipients. N Engl J Med 338, 1741-1751 (1998)
DOI: 10.1056/NEJM199806113382407

22. K.D. Sims, E.A. Blumberg: Common infections in the lung transplant recipient. Clin Chest Med 32, 327–341 (2011)
DOI: 10.1016/j.ccm.2011.02.010

23. I.G. Sia, J.A. Wilson, C.M. Groettum, M.J. Espy, T.F. Smith, C.V. PayA: Cytomegalovirus (CMV) DNA load predicts relapsing CMV infection after solid organ transplantation. J Infect Dis 181, 717–720 (2000)
DOI: 10.1086/315242

24. W.L. Drew: Ganciclovir resistance: a matter of time and titer. Lancet 356, 609–610 (2000)
DOI: 10.1016/S0140-6736(00)02597-6

25. S.M. Bhorade, C. Sandesara, E.R. Garrity, W.T. Vigneswaran, L. Norwick, S. Alkan, A.N. Husain, M.A. McCabe, V. Yeldandi: Quantification of cytomegalovirus (CMV) viral load by the hybrid capture assay allows for early detection of CMV disease in lung transplant recipients. J Heart Lung Transplant 20, 928–934 (2001)
DOI: 10.1016/S1053-2498(01)00283-2

26. J.L. Sanchez, R.M. Kruger, S. Paranjothi, E.P. Trulock, J.P. Lynch, C. Hicks, W.D. Shannon, G.A. Storch: Relationship of cytomegalovirus viral load in blood to pneumonitis in lung transplant recipients. Transplantation 72: 733–735 (2001)
DOI: 10.1097/00007890-200108270-00030

27. E. Arbustini, M. Grasso, M. Diegoli, E. Percivalle, P. Grossi, M. Bramerio, C. Campana, C. Goggi, A. Gavazzi, M. Vigano: Histopathologic and molecular profile of human cytomegalovirus infections in patients with heart transplants. Am J Clin Pathol 98: 205-213 (1992)

28. D.Y. Lu, J. Qian, K.A. Easley, S.M. Waldrop, C. Cohen: Automated in situ hybridization and immunohistochemistry for cytomegalovirus detection in paraffin-embedded tissue sections. Appl Immunohistochem Mol Morphol 17, 158-164 (2009)
DOI: 10.1097/ PAI.0b013e318185d1b5

29. L. Wilkens, M. Werner, M. Nolte, R.V. Wasielewski, W. Verhagen, J. Flik, J. Klempnauer, A. Georgii: Influence of formalin fixation on the detection of cytomegalovirus by polymerase chain reaction in immunocompromised patients and correlation to in situ hybridization, immunohistochemistry, and serological data. Diagn Mol Pathol 3, 156-162 (1994)
DOI: 10.1097/00019606-199409000-00004

30. G.C. Riise, R. Andersson, T. Bergstrom, A. Lundmark, F.N. Nilsson, S. Olofsson: Quantification of cytomegalovirus DNA in BAL fluid: a longitudinal study in lung transplant recipients. Chest 118, 1653–1660 (2000)
DOI: 10.1378/chest.118.6.1653

31. C. Liesnard, L. De Wit, S. Motte, F. Brancart, J. Content: Rapid diagnosis of cytomegalovirus lung infection by DNA amplification in bronchoalveolar lavages. Mol Cell Probes 8, 273–283 (1994)
DOI: 10.1006/mcpr.1994.1039

32. G.P. Westall, A. Michaelides, T.J. Williams, G.I. Snell, T.C. Kotsimbos: Human Cytomegalovirus Load in Plasma and Bronchoalveolar Lavage Fluid: A Longitudinal Study of Lung Transplant Recipients. J Infect Dis 190, 1076-1083 (2004)
DOI: 10.1086/422327

33. C.B. Hall. Respiratory syncytial virus and parainfluenza virus. N Engl J Med 344, 1917-1928 (2001)
DOI: 10.1056/NEJM200106213442507

34. E. Malcolm, E. Arruda, F.G. Hayden, L. Kaiser: Clinical features of patients with acute respiratory illness and rhinovirus in their bronchoalveolar lavages. J Clin Virol 21: 9-16 (2001)
DOI: 10.1016/S1386-6532(00)00180-3

35. N.G. Papadopoulos, P.J. Bates, P.G. Bardin, A. Papi, S.H. Leir, D.J. Fraenkel,

J. Meyer, P.M. Lackie, G. Sanderson, S.T. Holgate, S.L. Johnston: Rhinoviruses infect the lower airways. J Infect Dis 181, 1875-1884 (2000)
DOI: 10.1086/315513

36. M.G. Ison, F.G Hayden, L. Kaiser, L. Corey, M. Boeckh: Rhinovirus infections in hematopoietic stem cell transplant recipients with pneumonia. Clin Infect Dis 36:1139-1143 (2003)
DOI: 10.1086/374340

37. L. Kaiser, J.D. Aubert, J.C. Pache, C. Deffernez, T. Rochat, J. Garbino, W. Wunderli, P. Meylan, S. Yerly, L. Perrin, I. Letovanec, L. Nicod, C. Tapparel, P.M. Soccal: Chronic rhinoviral infection in lung transplant recipients. Am J Respir Crit Care Med 174, 1392-1399 (2006)
DOI: 10.1164/rccm.200604-489OC

38. O.A. EL Zammar; A. Katzenstein: Pathological diagnosis of granulomatous lung disease: a review. Histopathology 50, 289–310 (2007)
DOI: 10.1111/j.1365-2559.2006.02546.x

39. G. Cserni: Auramine fluorescence for acid-fast bacilli in formalin-fixed paraffin-embedded tissues. Am J Clin Pathol 103, 114 (1995)

40. D.E. Minnikin, S.M. Minnikin, J.H. Parlett, M. Goodfellow, M. Magnusson: Mycolic acid patterns of some species of Mycobacterium. Arch Microbiol 139, 225-231 (1984)
DOI: 10.1007/BF00402005

41. P. Seiler, T. Ulrichs, S. Bandermann, L. Pradl, S. Jörg, V. Krenn, L. Morawietz, S.H. Kaufmann, P. Aichele: Cell-wall alterations as an attribute of Mycobacterium tuberculosis in latent infection. J Infect Dis 188, 1326-1331 (2003)
DOI: 10.1086/378563

42. I.K.Neonakis, Z. Gitti, E. Krambovitis, D.A. Spandidos: Molecular diagnostic tools in mycobacteriology. J Microbiol Methods 75, 1-11 (2008)
DOI: 10.1016/j.mimet.2008.05.023

43. A.R. Zink, A.G. Nerlich: Molecular strain identification of the M. tuberculosis complex in archival tissue samples. J Clin Pathol 57, 1185-1192 (2004)
DOI: 10.1136/jcp.2003.015719

44. T. Goldmann, E. Vollmer: PCR-based diagnosis of mycobacteria: use and pitfalls of formalin-fixed, paraffin-embedded specimens. Eur J Pathol 8,4–03 (2002)

45. C. Schewe, T. Goldmann, M. Grosser, A. Zink, K. Schlüns, S. Pahl, T. Ulrichs, S.H. Kaufmann, A. Nerlich, G.B. Baretton, M. Dietel, E. Vollmer, I. Petersen: Inter-laboratory validation of PCR-based detection of Mycobacterium tuberculosis in formalin-fixed, paraffin-embedded tissues. Virchows Arch 447, 573-585 (2005)
DOI: 10.1007/s00428-005-1233-3

46. N. Escobar-Escamilla, J.E. Ramírez-González, M. González-Villa, P. Torres-Mazadiego, A. Mandujano-Martínez, C. Barrón-Rivera, C.E. Bäcker, D.E. Fragoso-Fonseca, H. Olivera-Díaz, P. Alcántara-Pérez, A. Hernández-Solís, R. Cícero-Sabido, I.A. Cortés-Ortíz: hsp65 Phylogenetic Assay for Molecular Diagnosis of Nontuberculous Mycobacteria Isolated in Mexico. Arch Med Res 45, 90-97 (2014)
DOI: 10.1016/j.arcmed.2013.12.004

47. H. Fukunaga, T. Murakami, T. Gondo, K. Sugi, T. Ishihara: Sensitivity of acid-fast staining for Mycobacterium tuberculosis in formalin-fixed tissue. Am J Respir Crit Care Med 166, 994-997 (2002)
DOI: 10.1164/rccm.2111028

48. R. Hernández-Pando, M. Jeyanathan, G. Mengistu, D. Aguilar, H. Orozco, M. Harboe, G.A. Rook, G. Bjune: Persistence of DNA from Mycobacterium tuberculosis in superficially normal lung tissue during latent infection. Lancet. 356 : 2133-2138 (2000)
DOI: 10.1016/S0140-6736(00)03493-0

49. N.V. Salian, J.A. Rish, K.D. Eisenach, M.D. Cave, J. H. Bates: Polymerase chain reaction to detect Mycobacterium

tuberculosis in histologic specimens. Am J Respir Crit Care Med 158, 1150–1155 (1998)
DOI: 10.1164/ajrccm.158.4.9802034

50. N. Singh, D.L. Paterson: Aspergillus infections in transplant recipients. Clin Microbiol Rev 18: 44 (2005)
DOI: 10.1128/CMR.18.1.44-69.2005

51. R. Scerif, B.H. Segal: Pulmonary aspergillosis: clinical presentation, diagnostic tests, management and complications. Curr Opin Pulm Med 16, 242-250 (2010)

52. J.L. Hsu, S.J. Ruoss, N.D. Bower, M. Lin, M. Holodniy, D.A. Stevens: Diagnosing invasive fungal disease in critically ill patients. Crit Rev Microbiol 37, 277-312 (2011)
DOI: 10.3109/1040841X.2011.581223

53. M. Cuenca-Estrella, Y. Meije, C. Diaz-Pedroche, A. Gomez-Lopez, M.J. Buitrago, L. Bernal-Martinez, C. Grande, R.S. Juan, M. Lizasoain, J.L. Rodriguez-Tudela, J.M. Aguado: Value of serial quantification of fungal DNA by a real-time PCR-based technique for early diagnosis of invasive aspergillosis in patients with febrile neutropenia. J Clin Microbiol 47, 379–384 (2009)
DOI: 10.1128/JCM.01716-08

54. W.H. Wheat, C.D. Cool, Y. Morimoto, P.R. Rai, C.H. Kirkpatrick, B.A. Lindenbaum, C.A. Bates, M.C. Ellison, A.E. Serls, K.K. Brown, J.M. Routes: Possible role of human herpesvirus 8 in the lymphoproliferative disorders in common variable immunodeficiency. J Exp Med 202, 479-484 (2005)
DOI: 10.1084/jem.20050381

55. K.A. Staskus, R.Sun, G. Miller, P. Racz, A. Jaslowski, C. Metroka, H. Brett-Smith, A.T. Haase: Cellular tropism and viral interleukin-6 expression distinguish human herpesvirus 8 involvement in Kaposi's sarcoma, primary effusion lymphoma, and multicentric Castleman's disease. J Virol 73, 4181-4187 (1999)

56. M. Tamm, F. Reichenberger, C.E. McGandy, A. Stalder, A. Tietz, P. Dalquen, A.P. Perruchoud, G. Cathomas: Diagnosis of Pulmonary Kaposi's Sarcoma by Detection of Human Herpes Virus 8 in Bronchoalveolar Lavage. Am J Resp Crit Care Med 157, 458-463 (1998)
DOI: 10.1164/ajrccm.157.2.9707068

57. B. Tarp, S. Jensen-Fangel, R. Dahl, N. Obel: Herpesvirus type 1–8 in BAL fluid from HIV-1-infected patients with suspected pneumonia and from healthy individuals. Eur Respir J 18, 146-150 (2001)
DOI: 10.1183/09031936.01.00090601

58. G.P. Cosgrove, M.I. Schwartz: Lymphocy toplasmic infiltrations of the lung. In: Interstitial Lung Disease. Eds: Schwarz MI, King TE Jr. People's Medical Publishing House, Hamilton, USA (2011)

59. W.G. Morice, P.J. Kurtin, J.L. Myers: Expression of cytolytic lymphocyte-associated antigens in pulmonary lymphomatoid granulomatosis. Am J Clin Pathol 118, 391 (2002)
DOI: 10.1309/PMR7-7XLY-F10U-4V1Q

60. M. Izadi, S. Taheri: Significance of in situ hybridization results for EBV-encoded RNA in post-transplantation lymphoproliferative disorder setting: report from the PTLD. Int Survey. Ann Transplant 15, 102-109 (2010)

61. E.S. Jaffe, W.H. Wilson: Lymphomatoid granulomatosis: Pathogenesis, pathology and clinical implications. Cancer Surv 30, 233 (1997)

62. I.P. Neuringer: Postransplant lympho proliferative disorder after lung transplantation. Clin Dev Immunol 2013, 430209 (2013)
DOI: 10.1155/2013/430209

63. P.M. Kaan, R.G. Hegele, S. Hayashi, J.C. Hogg: Expression of bcl-2 and Epstein-Barr virus LMP1 in lymphocytic interstitial pneumonia. Thorax 52,12-16 (1997)
DOI: 10.1136/thx.52.1.12

64. W. Wockel, G. Hofler, H. H. Popper and A. Morresi: Lymphoepithelioma-like carcinoma of the lung. Pathol Res Pract 191, 1170-1174 (1995)
DOI: 10.1016/S0344-0338(11)80665-5

65. Y. L. Chang, C. T. Wu, J. Y. Shih and Y. C. Lee: New aspects in clinicopathologic and oncogene studies of 23 pulmonary lymphoepithelioma-like carcinomas. Am J Surg Pathol 26, 715-723 (2002)
DOI: 10.1097/00000478-200206000-00004

66. H. H. Popper, Y. el-Shabrawi, W. Wockel, G. Hofler, L. Kenner, F. M. Juttner-Smolle and M. G. Pongratz: Prognostic importance of human papilloma virus typing in squamous cell papilloma of the bronchus: comparison of in situ hybridization and the polymerase chain reaction. Hum Pathol 25, 1191-1197 (1994)
DOI: 10.1016/0046-8177(94)90036-1

67. S. Solinas-Toldo, M. Durst and P. Lichter: Specific chromosomal imbalances in human papillomavirus-transfected cells during progression toward immortality. Proc Natl Acad Sci U S A 94, 3854-3859 (1997)
DOI: 10.1073/pnas.94.8.3854

68. S. M. Lele, A. M. Pou, K. Ventura, Z. Gatalica and D. Payne: Molecular events in the progression of recurrent respiratory papillomatosis to carcinoma. Arch Pathol Lab Med 126, 1184-1188 (2002)

69. M. Paraskeva, M. Bailey, B.J. Levvey, A.P. Griffiths, T.C. Kotsimbos, T.P. Williams, G. Snell, G. Westall: Cytomegalovirus replication within the lung allograft is associated with bronchiolitis obliterans syndrome. Am J Transplant 11:2190-2196 (2011)
DOI: 10.1111/j.1600-6143.2011.03663.x

70. C. Neurohr, P. Huppmann, H. Leuchte, M. Schwaiblmair, I. Bittmann, G. Jaeger, R. Hatz, L. Frey, P. Uberfuhr, B. Reichart, J. Behr; Munich Lung Transplant Group: Human herpesvirus 6 in bronchalveolar lavage fluid after lung transplantation: a risk factor for bronchiolitis obliterans syndrome? Am J Transplant 5, 2982-2991 (2005)
DOI: 10.1111/j.1600-6143.2005.01103.x

71. C. Costa, L. Delsedimide, P. Solidoro, A. Curtoni, M. Bergallo, D. Libertucci, S. Baldi, M. Rinaldi, R. Cavallo: Herpesviruses detection by quantitative real-time polymerase chain reaction in bronchoalveolar lavage and transbronchial biopsy in lung transplant: viral infections and histopathological correlation. Transplant Proc 42,1270 (2010)
DOI: 10.1016/j.transproceed.2010.03.086

72. D.L. Vu, P.O. Bridevaux, J.D. Aubert, P.M. Soccal, L. Kaiser:. Respiratory viruses in lung transplant recipients: a critical review and pooled analysis of clinical studies. Am J Transplant 11:1071-1078 (2011)
DOI: 10.1111/j.1600-6143.2011.03490.x

73. M.Y. Armanios, J.J. Chen, J.D. Cogan, J.K. Alder, R.G. Ingersoll, C. Markin, W.E. Lawson, M. Xie, I. Vulto, J.A. Phillips 3rd, P.M. Lansdorp, C.W. Greider, J.E. Loyd: Telomerase mutations in families with idiopathic pulmonary fibrosis. N Engl J Med 356:1317-1326 (2007)
DOI: 10.1056/NEJMoa066157

74. A.Q. Thomas, K. Lane, J. Phillips 3rd, M. Prince, C. Markin, M. Speer, D.A. Schwartz, R. Gaddipati, A. Marney, J. Johnson, R. Roberts, J. Haines, M. Stahlman, J.E. Loyd: Heterozygosity for a surfactant protein C gene mutation associated with usual interstitial pneumonitis and cellular nonspecific interstitial pneumonitis in one kindred. Am J Respir Crit Care Med 165, 1322-1328 (2002)
DOI: 10.1164/rccm.200112-123OC

75. M.A. Seibold, A.L. Wise, M.C. Speer, M.P. Steele, K.K. Brown, J.E. Loyd, T.E. Fingerlin, W. Zhang, G. Gudmundsson, S.D. Groshong, C.M. Evans, S. Garantziotis, K.B. Adler, B.F. Dickey, R.M. du Bois, I.V. Yang, A. Herron, D. Kervitsky, J.L. Talbert, C. Markin, J. Park,

A.L. Crews, S.H. Slifer, S. Auerbach, M.G. Roy, J. Lin, C.E. Hennessy, M.I. Schwarz, D.A. Schwartz: A common MUC5B promoter polymorphism and pulmonary fibrosis. N Engl J Med 364:1503-1512 (2011)
DOI: 10.1056/NEJMoa1013660

76. A.L. Peljto, Y. Zhang, T.E. Fingerlin, S.F. Ma, J.G. Garcia, T.J. Richards, L.J. Silveira, K.O. Lindell, M.P. Steele, J.E. Loyd, K.F. Gibson, M.A. Seibold, K.K. Brown, J.L. Talbert, C. Markin, K. Kossen, S.D. Seiwert, E. Murphy, I. Noth, M.I. Schwarz, N. Kaminski, D.A. Schwartz: Association between the MUC5B promoter polymorphism and survival in patients with idiopathic pulmonary fibrosis. JAMA 309:2232-2239 (2013)
DOI: 10.1001/jama.2013.5827

77. T.E.King Jr, A. Pardo, M. Selman: Idiopathic pulmonary fibrosis. Lancet 378:1949-1961 (2011)
DOI: 10.1016/S0140-6736(11)60052-4

78. J.P. Stewart, J.J. Egan, A.J. Ross, B.G. Kelly, S.S. Lok, P.S. Hasleton, A.A.Woodcock: The detection of Epstein-Barr virus DNA in lung tissue from patients with idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 159:1336-1341 (1999)
DOI: 10.1164/ajrccm.159.4.9807077

79. F. Calabrese, A. Kipar, F. Lunardi, E. Balestro, E. Perissinotto, E. Rossi, N. Nannini, G. Marulli, J.P. Stewart, F. Rea: Herpes virus infection is associated with vascular remodeling and pulmonary hypertension in idiopathic pulmonary fibrosis. PLoS One 8, e55715 (2013)
DOI: 10.1371/journal.pone.0055715

80. K. Tsukamoto, H. Hayakawa, A. Sato, K. Chida, H. Nakamura, K. Miura K: Involvement of Epstein-Barr virus latent membrane protein 1 in disease progression in patients with idiopathic pulmonary fibrosis. Thorax 55, 958-961 (2000)
DOI: 10.1136/thorax.55.11.958

81. M. Decaussin, H. Sartelet, C. Robert, D. Moro, C. Claraz, C. Brambilla and E. Brambilla: Expression of vascular endothelial growth factor (VEGF) and its two receptors (VEGF-R1-Flt1 and VEGF-R2-Flk1/KDR) in non-small cell lung carcinomas (NSCLCs): correlation with angiogenesis and survival. J Pathol 188, 369-377 (1999)
DOI:10.1002/(SICI)1096-9896(199908)188:4<369::AID-PATH 381> 3.0. CO;2-X
DOI:10.1002/(SICI)1096-9896(199 908)188:4<369::AID-PATH381>3.3.CO; 2-O

82. A. A. Adjei: Pharmacology and mechanism of action of pemetrexed. Clin Lung Cancer 5, S51-55 (2004)
DOI: 10.3816/CLC.2004.s.003

83. M. I. Koukourakis, A. Giatromanolaki, E. Sivridis, K. C. Gatter and A. L. Harris: Pyruvate dehydrogenase and pyruvate dehydrogenase kinase expression in non small cell lung cancer and tumor-associated stroma. Neoplasia 7, 1-6 (2005)
DOI: 10.1593/neo.04373

84. R. L. Keith, Y. E. Miller, R. M. Gemmill, H. A. Drabkin, E. C. Dempsey, T. C. Kennedy, S. Prindiville and W. A. Franklin: Angiogenic squamous dysplasia in bronchi of individuals at high risk for lung cancer. Clin Cancer Res 6, 1616-1625 (2000)

85. M. Takahama, M. Tsutsumi, T. Tsujiuchi, K. Nezu, K. Kushibe, S. Taniguchi, Y. Kotake and Y. Konishi: Enhanced expression of Tie2, its ligand angiopoietin-1, vascular endothelial growth factor, and CD31 in human non-small cell lung carcinomas. Clin Cancer Res 5, 2506-2510 (1999)

86. A. M. Maeshima, N. Tochigi, A. Yoshida, H. Asamura, K. Tsuta and H. Tsuda: Clinicopathologic analysis of multiple (five or more) atypical adenomatous hyperplasias (AAHs) of the lung: evidence for the AAH-adenocarcinoma sequence. J Thorac Oncol 5, 466-471 (2010)
DOI: 10.1097/JTO.0b013e3181ce3b73

87. N. Guedj, A. Couvelard, G. Arcangeli,

S. Dubois, G. Thabut, G. Leseche, M. Fournier, C. Degott and O. Groussard: Angiogenesis and extracellular matrix remodelling in bronchioloalveolar carcinomas: distinctive patterns in mucinous and non-mucinous tumours. Histopathology 44, 251-256 (2004)
DOI: 10.1111/j.1365-2559.2004.01803.x

88. H. Kojima, N. Shijubo, G. Yamada, S. Ichimiya, S. Abe, M. Satoh and N. Sato: Clinical significance of vascular endothelial growth factor-C and vascular endothelial growth factor receptor 3 in patients with T1 lung adenocarcinoma. Cancer 104, 1668-1677 (2005)
DOI: 10.1002/cncr.21366

89. C. J. Langer and R. B. Natale: The emerging role of vascular endothelial growth factor receptor tyrosine kinase inhibitors. Semin Oncol 32, S23-29 (2005)
DOI: 10.1053/j.seminoncol.2005.10.005

90. M. Mae, T. P. O'Connor and R. G. Crystal: Gene transfer of the vascular endothelial growth factor receptor flt-1 suppresses pulmonary metastasis associated with lung growth. Am J Respir Cell Mol Biol 33, 629-635 (2005)
DOI: 10.1165/rcmb.2005-0092OC

91. M. D. Hellmann, J. E. Chaft, V. Rusch, M. S. Ginsberg, D. J. Finley, M. G. Kris, K. A. Price, C. G. Azzoli, M. G. Fury, G. J. Riely, L. M. Krug, R. J. Downey, M. S. Bains, C. S. Sima, N. Rizk, W. D. Travis, N. A. Rizvi and P. K. Paik: Risk of hemoptysis in patients with resected squamous cell and other high-risk lung cancers treated with adjuvant bevacizumab. Cancer Chemother Pharmacol 72, 453-461 (2013)
DOI: 10.1007/s00280-013-2219-5

92. T. Kajita, Y. Ohta, K. Kimura, M. Tamura, Y. Tanaka, Y. Tsunezuka, M. Oda, T. Sasaki and G. Watanabe: The expression of vascular endothelial growth factor C and its receptors in non-small cell lung cancer. Br J Cancer 85, 255-260 (2001)
DOI: 10.1054/bjoc.2001.1882

93. T. Niki, S. Iba, T. Yamada, Y. Matsuno, B. Enholm and S. Hirohashi: Expression of vascular endothelial growth factor receptor 3 in blood and lymphatic vessels of lung adenocarcinoma. J Pathol 193, 450-457 (2001)
DOI: 10.1002/path.828

94. M. Arinaga, T. Noguchi, S. Takeno, M. Chujo, T. Miura and Y. Uchida: Clinical significance of vascular endothelial growth factor C and vascular endothelial growth factor receptor 3 in patients with nonsmall cell lung carcinoma. Cancer 97, 457-464 (2003)
DOI: 10.1002/cncr.11073

95. Y. Li, M. N. Wang, H. Li, K. D. King, R. Bassi, H. Sun, A. Santiago, A. T. Hooper, P. Bohlen and D. J. Hicklin: Active immunization against the vascular endothelial growth factor receptor flk1 inhibits tumor angiogenesis and metastasis. J Exp Med 195, 1575-1584 (2002)
DOI: 10.1084/jem.20020072

96. A. Abdollahi, K. E. Lipson, A. Sckell, H. Zieher, F. Klenke, D. Poerschke, A. Roth, X. Han, M. Krix, M. Bischof, P. Hahnfeldt, H. J. Grone, J. Debus, L. Hlatky and P. E. Huber: Combined therapy with direct and indirect angiogenesis inhibition results in enhanced antiangiogenic and antitumor effects. Cancer Res 63, 8890-8898 (2003)

97. J. Lin, A. S. Lalani, T. C. Harding, M. Gonzalez, W. W. Wu, B. Luan, G. H. Tu, K. Koprivnikar, M. J. VanRoey, Y. He, K. Alitalo and K. Jooss: Inhibition of lymphogenous metastasis using adeno-associated virus-mediated gene transfer of a soluble VEGFR-3 decoy receptor. Cancer Res 65, 6901-6909 (2005)
DOI: 10.1158/0008-5472.CAN-05-0408

98. O. Takahashi, R. Komaki, P. D. Smith, J. M. Jurgensmeier, A. Ryan, B. N. Bekele, Wistuba, II, J. J. Jacoby, M. V. Korshunova, A. Biernacka, B. Erez, K. Hosho, R. S. Herbst and M. S. O'Reilly: Combined MEK and VEGFR inhibition

in orthotopic human lung cancer models results in enhanced inhibition of tumor angiogenesis, growth, and metastasis. Clin Cancer Res 18, 1641-1654 (2012) DOI: 10.1158/1078-0432.CCR-11-2324

99. B. Das, H. Yeger, R. Tsuchida, R. Torkin, M. F. Gee, P. S. Thorner, M. Shibuya, D. Malkin and S. Baruchel: A hypoxia-driven vascular endothelial growth factor/Flt1 autocrine loop interacts with hypoxia-inducible factor-1alpha through mitogen-activated protein kinase/extracellular signal-regulated kinase 1/2 pathway in neuroblastoma. Cancer Res 65, 7267-7675 (2005) DOI: 10.1158/0008-5472.CAN-04-4575

100. S. R. Boreddy, R. P. Sahu and S. K. Srivastava: Benzyl isothiocyanate suppresses pancreatic tumor angiogenesis and invasion by inhibiting HIF-alpha/VEGF/Rho-GTPases: pivotal role of STAT-3. PLoS ONE 6, e25799 (2011) DOI: 10.1371/journal.pone.0025799

101. A. Goyal, C. Poluzzi, C. D. Willis, J. Smythies, A. Shellard, T. Neill and R. V. Iozzo: Endorepellin affects angiogenesis by antagonizing diverse vascular endothelial growth factor receptor 2 (VEGFR2)-evoked signaling pathways: transcriptional repression of hypoxia-inducible factor 1alpha and VEGFA and concurrent inhibition of nuclear factor of activated T cell 1 (NFAT1) activation. J Biol Chem 287, 43543-4356 (2012) DOI: 10.1074/jbc.M112.401786

102. K. A. Olaussen, A. Dunant, P. Fouret, E. Brambilla, F. Andre, V. Haddad, E. Taranchon, M. Filipits, R. Pirker, H. H. Popper, R. Stahel, L. Sabatier, J. P. Pignon, T. Tursz, T. Le Chevalier and J. C. Soria: DNA repair by ERCC1 in non-small-cell lung cancer and cisplatin-based adjuvant chemotherapy. N Engl J Med 355, 983-991 (2006) DOI: 10.1056/NEJMoa060570

103. R. G. Zinner, S. Novello, G. Peng, R. Herbst, C. Obasaju and G. Scagliotti: Comparison of patient outcomes according to histology among pemetrexed-treated patients with stage IIIB/IV non-small-cell lung cancer in two phase II trials. Clin Lung Cancer 11, 126-131 (2010) DOI: 10.3816/CLC.2010.n.017

104. E. Giovannetti, C. Lemos, C. Tekle, K. Smid, S. Nannizzi, J. A. Rodriguez, S. Ricciardi, R. Danesi, G. Giaccone and G. J. Peters: Molecular mechanisms underlying the synergistic interaction of erlotinib, an epidermal growth factor receptor tyrosine kinase inhibitor, with the multitargeted antifolate pemetrexed in non-small-cell lung cancer cells. Mol Pharmacol 73, 1290-1300 (2008) DOI: 10.1124/mol.107.042382

105. S. Grant, L. Qiao and P. Dent: Roles of ERBB family receptor tyrosine kinases, and downstream signaling pathways, in the control of cell growth and survival. Front Biosci 7, 376-389 (2002) DOI: 10.2741/grant

106. C. V. Rajadurai, S. Havrylov, K. Zaoui, R. Vaillancourt, M. Stuible, M. Naujokas, D. Zuo, M. L. Tremblay and M. Park: Met receptor tyrosine kinase signals through a cortactin-Gab1 scaffold complex, to mediate invadopodia. J Cell Sci 125, 2940-2953 (2012) DOI: 10.1242/jcs.100834

107. J. Kim, S. Ahn, R. Guo and Y. Daaka: Regulation of epidermal growth factor receptor internalization by G protein-coupled receptors. Biochemistry 42, 2887-2894 (2003) DOI: 10.1021/bi026942t

108. M. Nomura, H. Shigematsu, L. Li, M. Suzuki, T. Takahashi, P. Estess, M. Siegelman, Z. Feng, H. Kato, A. Marchetti, J. W. Shay, M. R. Spitz, Wistuba, II, J. D. Minna and A. F. Gazdar: Polymorphisms, mutations, and amplification of the EGFR gene in non-small cell lung cancers. PLoS Med 4, e125 (2007) DOI: 10.1371/journal.pmed.0040125

109. S. Yano, W. Wang, Q. Li, K. Matsumoto,

H. Sakurama, T. Nakamura, H. Ogino, S. Kakiuchi, M. Hanibuchi, Y. Nishioka, H. Uehara, T. Mitsudomi, Y. Yatabe and S. Sone: Hepatocyte growth factor induces gefitinib resistance of lung adenocarcinoma with epidermal growth factor receptor-activating mutations. Cancer Res 68(22), 9479-9487 (2008) DOI: 10.1158/0008-5472.CAN-08-1643

110. R. H. Palmer, E. Vernersson, C. Grabbe and B. Hallberg: Anaplastic lymphoma kinase: signalling in development and disease. Biochem J 420, 345-361 (2009) DOI: 10.1042/BJ20090387

111. D. W. Wong, E. L. Leung, S. K. Wong, V. P. Tin, A. D. Sihoe, L. C. Cheng, J. S. Au, L. P. Chung and M. P. Wong: A novel KIF5B-ALK variant in nonsmall cell lung cancer. Cancer 117, 2709-18 (2011) DOI: 10.1002/cncr.25843

112. B. Zhang, X. Pan, G. P. Cobb and T. A. Anderson: microRNAs as oncogenes and tumor suppressors. Dev Biol 302, 1-12 (2007) DOI: 10.1016/j.ydbio.2006.08.028

113. M. S. Kumar, J. Lu, K. L. Mercer, T. R. Golub and T. Jacks: Impaired microRNA processing enhances cellular transformation and tumorigenesis. Nat Genet 39, 673-677 (2007) DOI: 10.1038/ng2003

114. J.-C. Bourdon, K. Fernandes, F. Murray-Zmijewski, G. Liu, A. Diot, D. P. Xirodimas, M. K. Saville and D. P. Lane: p53 isoforms can regulate p53 transcriptional activity. Genes Dev 19, 2122-2137 (2005) DOI: 10.1101/gad.1339905

115. B. Vogelstein and K. W. Kinzler: p53 function and dysfunction. Cell 70, 523-526 (1992) DOI: 10.1016/0092-8674(92)90421-8

116. K. Husgafvel-Pursiainen, P. Boffetta, A. Kannio, F. Nyberg, G. Pershagen, A. Mukeria, V. Constantinescu, C. Fortes and S. Benhamou: p53 mutations and exposure to environmental tobacco smoke in a multicenter study on lung cancer. Cancer Res 60, 2906-2911 (2000)

117. S. N. Rodin and A. S. Rodin: Human lung cancer and p53: the interplay between mutagenesis and selection. Proc Natl Acad Sci U S A 97, 12244-12249 (2000) DOI: 10.1073/pnas.180320897

118. M. Sanchez-Cespedes, S. A. Ahrendt, S. Piantadosi, R. Rosell, M. Monzo, L. Wu, W. H. Westra, S. C. Yang, J. Jen and D. Sidransky: Chromosomal alterations in lung adenocarcinoma from smokers and nonsmokers. Cancer Res 61, 1309-1313 (2001)

119. S. Zienolddiny, D. Ryberg, M. O. Arab, V. Skaug and A. Haugen: Loss of heterozygosity is related to p53 mutations and smoking in lung cancer. Br J Cancer 84, 226-231 (2001) DOI: 10.1054/bjoc.2000.1528

120. J. Bartkova, Z. Horejsi, K. Koed, A. Kramer, F. Tort, K. Zieger, P. Guldberg, M. Sehested, J. M. Nesland, C. Lukas, T. Orntoft, J. Lukas and J. Bartek: DNA damage response as a candidate anti-cancer barrier in early human tumorigenesis. Nature 434, 864-870 (2005) DOI: 10.1038/nature03482

121. M. Hollstein, D. Sidransky, B. Vogelstein and C. C. Harris: p53 mutations in human cancers. Science 253, 49-53 (1991) DOI: 10.1126/science.1905840

122. T. Takahashi, D. D'Amico, I. Chiba, D. L. Buchhagen and J. D. Minna: Identification of intronic point mutations as an alternative mechanism for p53 inactivation in lung cancer. J. Clin. Invest 86, 363-369 (1990) DOI: 10.1172/JCI114710

123. R. Iggo, K. Gatter, J. Bartek, D. Lane and A. L. Harris: Increased expression of mutant forms of p53 oncogene in primary lung cancer. Lancet, 335, 675-679 (1990) DOI: 10.1016/0140-6736(90)90801-B

124. J. Yokota and T. Kohno: Molecular footprints of human lung cancer

progression. Cancer Sci 95, 197-204 (2004)
DOI: 10.1111/j.1349-7006.2004.tb02203.x

125. J. Yokota, M. Nishioka, M. Tani and T. Kohno: Genetic alterations responsible for metastatic phenotypes of lung cancer cells. Clin Exp Metastasis 20, 189-193 (2003)
DOI: 10.1023/A:1022978932215

126. D. L. Gibbons, W. Lin, C. J. Creighton, S. Zheng, D. Berel, Y. Yang, M. G. Raso, D. D. Liu, Wistuba, II, G. Lozano and J. M. Kurie: Expression signatures of metastatic capacity in a genetic mouse model of lung adenocarcinoma. PLoS ONE 4, e5401 (2009)
DOI: 10.1371/journal.pone.0005401

127. J. G. Paez, P. A. Janne, J. C. Lee, S. Tracy, H. Greulich, S. Gabriel, P. Herman, F. J. Kaye, N. Lindeman, T. J. Boggon, K. Naoki, H. Sasaki, Y. Fujii, M. J. Eck, W. R. Sellers, B. E. Johnson and M. Meyerson: EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science 304, 1497-1500 (2004)
DOI: 10.1126/science.1099314

128. R. Sordella, D. W. Bell, D. A. Haber and J. Settleman: Gefitinib-sensitizing EGFR mutations in lung cancer activate anti-apoptotic pathways. Science 305, 1163-1167 (2004)
DOI: 10.1126/science.1101637

129. T. J. Lynch, D. W. Bell, R. Sordella, S. Gurubhagavatula, R. A. Okimoto, B. W. Brannigan, P. L. Harris, S. M. Haserlat, J. G. Supko, F. G. Haluska, D. N. Louis, D. C. Christiani, J. Settleman and D. A. Haber: Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 350, 2129-2139 (2004)
DOI: 10.1056/NEJMoa040938

130. T. Isobe, R. S. Herbst and A. Onn: Current management of advanced non-small cell lung cancer: targeted therapy. Semin Oncol 32, 315-328 (2005)
DOI: 10.1053/j.seminoncol.2005.02.016

131. H. A. Yu and W. Pao: Targeted therapies: Afatinib--new therapy option for EGFR-mutant lung cancer. Nat Rev Clin Oncol 10, 551-552 (2013)
DOI: 10.1038/nrclinonc.2013.154

132. M. P. Morelli, T. Cascone, T. Troiani, C. Tuccillo, R. Bianco, N. Normanno, M. Romano, B. M. Veneziani, G. Fontanini, S. G. Eckhardt, S. De Pacido, G. Tortora and F. Ciardiello: Anti-tumor activity of the combination of cetuximab, an anti-EGFR blocking monoclonal antibody and ZD6474, an inhibitor of VEGFR and EGFR tyrosine kinases. J Cell Physiol 208, 344-353 (2006)
DOI: 10.1002/jcp.20666

133. R. Pirker, J. R. Pereira, J. von Pawel, M. Krzakowski, R. Ramlau, K. Park, F. de Marinis, W. E. Eberhardt, L. Paz-Ares, S. Storkel, K. M. Schumacher, A. von Heydebreck, I. Celik and K. J. O'Byrne: EGFR expression as a predictor of survival for first-line chemotherapy plus cetuximab in patients with advanced non-small-cell lung cancer: analysis of data from the phase 3 FLEX study. Lancet Oncol 13, 33-42 (2012)
DOI: 10.1016/S1470-2045(11)70318-7

134. J. M. Siegfried, A. T. Gillespie, R. Mera, T. J. Casey, P. Keohavong, J. R. Testa and J. D. Hunt: Prognostic value of specific KRAS mutations in lung adenocarcinomas. Cancer Epidemiol Biomarkers Prev 6, 841-847 (1997)

135. M. Wislez, N. Fujimoto, J. G. Izzo, A. E. Hanna, D. D. Cody, R. R. Langley, H. Tang, M. D. Burdick, M. Sato, J. D. Minna, L. Mao, I. Wistuba, R. M. Strieter and J. M. Kurie: High expression of ligands for chemokine receptor CXCR2 in alveolar epithelial neoplasia induced by oncogenic kras. Cancer Res 66, 4198-4207 (2006)
DOI: 10.1158/0008-5472.CAN-05-3842

136. M. D. To, C. E. Wong, A. N. Karnezis, R. Del Rosario, R. Di Lauro and A. Balmain:

Kras regulatory elements and exon 4A determine mutation specificity in lung cancer. Nat Genet 40, 1240-1244 (2008) DOI: 10.1038/ng.211

137. Y. Zhou, W. M. Rideout, 3rd, T. Zi, A. Bressel, S. Reddypalli, R. Rancourt, J. K. Woo, J. W. Horner, L. Chin, M. I. Chiu, M. Bosenberg, T. Jacks, S. C. Clark, R. A. Depinho, M. O. Robinson and J. Heyer: Chimeric mouse tumor models reveal differences in pathway activation between ERBB family- and KRAS-dependent lung adenocarcinomas. Nat Biotechnol 28, 71-78 (2010) DOI: 10.1038/nbt.1595

138. M. Musteanu, L. Blaas, R. Zenz, J. Svinka, T. Hoffmann, B. Grabner, D. Schramek, H. P. Kantner, M. Muller, T. Kolbe, T. Rulicke, R. Moriggl, L. Kenner, D. Stoiber, J. M. Penninger, H. Popper, E. Casanova and R. Eferl: A mouse model to identify cooperating signaling pathways in cancer. Nat Methods 9, 897-900 (2012) DOI: 10.1038/nmeth.2130

139. C. Li, R. Fang, Y. Sun, X. Han, F. Li, B. Gao, A. J. Iafrate, X. Y. Liu, W. Pao, H. Chen and H. Ji: Spectrum of oncogenic driver mutations in lung adenocarcinomas from East Asian never smokers. PLoS ONE 6, e28204 (2011) DOI: 10.1371/journal.pone.0028204

140. B. Gao, Y. Sun, J. Zhang, Y. Ren, R. Fang, X. Han, L. Shen, X. Y. Liu, W. Pao, H. Chen and H. Ji: Spectrum of LKB1, EGFR, and KRAS mutations in chinese lung adenocarcinomas. J Thorac Oncol 5, 1130-1135 (2010) DOI: 10.1097/JTO.0b013e3181e05016

141. S. Ren, P. Kuang, L. Zheng, C. Su, J. Li, B. Li, X. Chen, Y. Wang, V. Kimcurran, L. Liu, Q. Hu, J. Zhang, L. Tang and C. Zhou: Analysis of driver mutations in female non-smoker asian patients with pulmonary adenocarcinoma. Cell Biochem Biophys 64, 155-160 (2012) DOI: 10.1007/s12013-012-9384-8

142. G. Scagliotti, R. A. Stahel, R. Rosell, N. Thatcher and J. C. Soria: ALK translocation and crizotinib in non-small cell lung cancer: an evolving paradigm in oncology drug development. Eur J Cancer 48, 961-973 (2012) DOI: 10.1016/j.ejca.2012.02.001

143. K. Ikeda, H. Nomori, T. Mori, J. Sasaki and T. Kobayashi: Novel germline mutation: EGFR V843I in patient with multiple lung adenocarcinomas and family members with lung cancer. Ann Thorac Surg 85, 1430-1432 (2008) DOI: 10.1016/j.athoracsur.2007.10.012

144. M. Nakao, J. Yoshida, K. Goto, G. Ishii, A. Kawase, K. Aokage, T. Hishida, M. Nishimura and K. Nagai: Long-term outcomes of 50 cases of limited-resection trial for pulmonary ground-glass opacity nodules. J Thorac Oncol 7, 1563-1566 (2012) DOI: 10.1097/JTO.0b013e3182641b5c

145. S. Zhang, F. Wang, J. Keats, X. Zhu, Y. Ning, S. D. Wardwell, L. Moran, Q. K. Mohemmad, R. Anjum, Y. Wang, N. I. Narasimhan, D. Dalgarno, W. C. Shakespeare, J. J. Miret, T. Clackson and V. M. Rivera: Crizotinib-resistant mutants of EML4-ALK identified through an accelerated mutagenesis screen. Chem Biol Drug Des 78, 999-1005 (2011) DOI: 10.1111/j.1747-0285.2011.01239.x

146. P. A. Janne and M. Meyerson: ROS1 rearrangements in lung cancer: a new genomic subset of lung adenocarcinoma. J Clin Oncol 30, 878-879 (2012) DOI: 10.1200/JCO.2011.39.4197

147. Y. Suehara, M. Arcila, L. Wang, A. Hasanovic, D. Ang, T. Ito, Y. Kimura, A. Drilon, U. Guha, V. Rusch, M. G. Kris, M. F. Zakowski, N. Rizvi, R. Khanin and M. Ladanyi: Identification of KIF5B-RET and GOPC-ROS1 fusions in lung adenocarcinomas through a comprehensive mRNA-based screen for tyrosine kinase fusions. Clin Cancer Res 18(24), 6599-6608 (2012) DOI: 10.1158/1078-0432.CCR-12-0838

148. V. M. Rimkunas, K. E. Crosby, D. Li, Y. Hu, M. E. Kelly, T. L. Gu, J. S. Mack, M. R. Silver, X. Zhou and H. Haack: Analysis of receptor tyrosine kinase ROS1-positive tumors in non-small cell lung cancer: identification of a FIG-ROS1 fusion. Clin Cancer Res 18, 4449-4457 (2012) DOI: 10.1158/1078-0432.CCR-11-3351

149. K. Bergethon, A. T. Shaw, S. H. Ou, R. Katayama, C. M. Lovly, N. T. McDonald, P. P. Massion, C. Siwak-Tapp, A. Gonzalez, R. Fang, E. J. Mark, J. M. Batten, H. Chen, K. D. Wilner, E. L. Kwak, J. W. Clark, D. P. Carbone, H. Ji, J. A. Engelman, M. Mino-Kenudson, W. Pao and A. J. Iafrate: ROS1 rearrangements define a unique molecular class of lung cancers. J Clin Oncol 30, 863-870 (2012) DOI: 10.1200/JCO.2011.35.6345

150. Y. Arai, Y. Totoki, H. Takahashi, H. Nakamura, N. Hama, T. Kohno, K. Tsuta, A. Yoshida, H. Asamura, M. Mutoh, F. Hosoda, H. Tsuda and T. Shibata: Mouse model for ROS1-rearranged lung cancer. PLoS ONE 8, e56010 (2013) DOI: 10.1371/journal.pone.0056010

151. L. P. Chin, R. A. Soo, R. Soong and S. H. Ou: Targeting ROS1 with anaplastic lymphoma kinase inhibitors: a promising therapeutic strategy for a newly defined molecular subset of non-small-cell lung cancer. J Thorac Oncol 7, 1625-1630 (2012) DOI: 10.1097/JTO.0b013e31826baf83

152. T. Komiya, A. Thomas, S. Khozin, A. Rajan, Y. Wang and G. Giaccone: Response to crizotinib in ROS1-rearranged non-small-cell lung cancer. J Clin Oncol 30(27), 3425-6; author reply 3426 (2012) DOI: 10.1200/JCO.2012.42.4556

153. H. Yasuda, L. L. de Figueiredo-Pontes, S. Kobayashi and D. B. Costa: Preclinical rationale for use of the clinically available multitargeted tyrosine kinase inhibitor crizotinib in ROS1-translocated lung cancer. J Thorac Oncol 7, 1086-1090 (2012) DOI: 10.1097/JTO.0b013e3182570919

154. R. Wang, H. Hu, Y. Pan, Y. Li, T. Ye, C. Li, X. Luo, L. Wang, H. Li, Y. Zhang, F. Li, Y. Lu, Q. Lu, J. Xu, D. Garfield, L. Shen, H. Ji, W. Pao, Y. Sun and H. Chen: RET fusions define a unique molecular and clinicopathologic subtype of non-small-cell lung cancer. J Clin Oncol 30, 4352-4359 (2012) DOI: 10.1200/JCO.2012.44.1477

155. K. Takeuchi, M. Soda, Y. Togashi, R. Suzuki, S. Sakata, S. Hatano, R. Asaka, W. Hamanaka, H. Ninomiya, H. Uehara, Y. Lim Choi, Y. Satoh, S. Okumura, K. Nakagawa, H. Mano and Y. Ishikawa: RET, ROS1 and ALK fusions in lung cancer. Nat Med 18, 378-381 (2012) DOI: 10.1038/nm.2658

156. Y. S. Ju, W. C. Lee, J. Y. Shin, S. Lee, T. Bleazard, J. K. Won, Y. T. Kim, J. I. Kim, J. H. Kang and J. S. Seo: A transforming KIF5B and RET gene fusion in lung adenocarcinoma revealed from whole-genome and transcriptome sequencing. Genome Res 22, 436-445 (2012) DOI: 10.1101/gr.133645.111

157. J. Bean, C. Brennan, J. Y. Shih, G. Riely, A. Viale, L. Wang, D. Chitale, N. Motoi, J. Szoke, S. Broderick, M. Balak, W. C. Chang, C. J. Yu, A. Gazdar, H. Pass, V. Rusch, W. Gerald, S. F. Huang, P. C. Yang, V. Miller, M. Ladanyi, C. H. Yang and W. Pao: MET amplification occurs with or without T790M mutations in EGFR mutant lung tumors with acquired resistance to gefitinib or erlotinib. Proc Natl Acad Sci U S A, 104, 20932-20937 (2007) DOI: 10.1073/pnas.0710370104

158. D. R. Spigel, T. J. Ervin, R. A. Ramlau, D. B. Daniel, J. H. Goldschmidt, Jr., G. R. Blumenschein, Jr., M. J. Krzakowski, G. Robinet, B. Godbert, F. Barlesi, R. Govindan, T. Patel, S. V. Orlov, M. S. Wertheim, W. Yu, J. Zha, R. L. Yauch, P. H. Patel, S. C. Phan and A. C. Peterson: Randomized phase II trial of Onartuzumab

in combination with erlotinib in patients with advanced non-small-cell lung cancer. J Clin Oncol 31, 4105-4114 (2013) DOI: 10.1200/JCO.2012.47.4189

159. A. Villar-Garea and M. Esteller: Histone deacetylase inhibitors: understanding a new wave of anticancer agents. Int J Cancer 112, 171-178 (2004) DOI: 10.1002/ijc.20372

160. N. Sengupta and E. Seto: Regulation of histone deacetylase activities. J Cell Biochem 93, 57-67 (2004) DOI: 10.1002/jcb.20179

161. T. A. Melhuish, C. M. Gallo and D. Wotton: TGIF2 interacts with histone deacetylase 1 and represses transcription. J Biol Chem 276, 32109-32114 (2001) DOI: 10.1074/jbc.M103377200

162. C. Beisel, A. Imhof, J. Greene, E. Kremmer and F. Sauer: Histone methylation by the Drosophila epigenetic transcriptional regulator Ash1. Nature 419(6909), 857-862 (2002) DOI: 10.1038/nature01126

163. H. Sasaki, S. Moriyama, Y. Nakashima, Y. Kobayashi, M. Kiriyama, I. Fukai, Y. Yamakawa and Y. Fujii: Histone deacetylase 1 mRNA expression in lung cancer. Lung Cancer 46, 171-8 (2004) DOI: 10.1016/j.lungcan.2004.03.021

164. M. C. Chen, C. H. Chen, J. C. Wang, A. C. Tsai, J. P. Liou, S. L. Pan and C. M. Teng: The HDAC inhibitor, MPT0E028, enhances erlotinib-induced cell death in EGFR-TKI-resistant NSCLC cells. Cell Death Dis 4, e810 (2013) DOI: 10.1038/cddis.2013.330

165. S. K. Pal, R. A. Figlin and K. Reckamp: Targeted therapies for non-small cell lung cancer: an evolving landscape. Mol Cancer Ther 9, 1931-1944 (2010) DOI: 10.1158/1535-7163.MCT-10-0239

166. S. J. An, Z. H. Chen, J. Su, X. C. Zhang, W. Z. Zhong, J. J. Yang, Q. Zhou, X. N. Yang, L. Huang, J. L. Guan, Q. Nie, H. H. Yan, T. S. Mok and Y. L. Wu: Identification of enriched driver gene alterations in subgroups of non-small cell lung cancer patients based on histology and smoking status. PLoS ONE 7, e40109 (2012) DOI: 10.1371/journal.pone.0040109

167. J. S. Seo, Y. S. Ju, W. C. Lee, J. Y. Shin, J. K. Lee, T. Bleazard, J. Lee, Y. J. Jung, J. O. Kim, S. B. Yu, J. Kim, E. R. Lee, C. H. Kang, I. K. Park, H. Rhee, S. H. Lee, J. I. Kim, J. H. Kang and Y. T. Kim: The transcriptional landscape and mutational profile of lung adenocarcinoma. Genome Res 22, 2109-2119 (2012) DOI: 10.1101/gr.145144.112

168. R. A. Moorehead, O. H. Sanchez, R. M. Baldwin and R. Khokha: Transgenic overexpression of IGF-II induces spontaneous lung tumors: a model for human lung adenocarcinoma. Oncogene 22, 853-857 (2003) DOI: 10.1038/sj.onc.1206188

169. T. G. Gharib, G. Chen, C. C. Huang, D. E. Misek, M. D. Iannettoni, S. M. Hanash, M. B. Orringer and D. G. Beer: Genomic and proteomic analyses of vascular endothelial growth factor and insulin-like growth factor-binding protein 3 in lung adenocarcinomas. Clin Lung Cancer 5, 307-312 (2004) DOI: 10.3816/CLC.2004.n.011

170. Y. Chen, M. Pacyna-Gengelbach, F. Ye, T. Knosel, P. Lund, N. Deutschmann, K. Schluns, W. F. Kotb, C. Sers, H. Yasumoto, T. Usui and I. Petersen: Insulin-like growth factor binding protein-related protein 1 (IGFBP-rP1) has potential tumour-suppressive activity in human lung cancer. J Pathol 211, 431-438 (2007) DOI: 10.1002/path.2132

171. F. Morgillo, W. Y. Kim, E. S. Kim, F. Ciardiello, W. K. Hong and H. Y. Lee: Implication of the insulin-like growth factor-IR pathway in the resistance of non-small cell lung cancer cells to treatment with gefitinib. Clin Cancer Res 13, 2795-2803 (2007) DOI: 10.1158/1078-0432.CCR-06-2077

172. A. Gualberto and D. D. Karp: Development of the monoclonal antibody figitumumab, targeting the insulin-like growth factor-1 receptor, for the treatment of patients with non-small-cell lung cancer. Clin Lung Cancer 10, 273-80 (2009)
DOI: 10.3816/CLC.2009.n.038

173. C. Gridelli, A. Rossi, M. A. Bareschino, C. Schettino, P. C. Sacco and P. Maione: The potential role of insulin-like growth factor receptor inhibitors in the treatment of advanced non-small cell lung cancer. Expert Opin Investig Drugs 19, 631-639 (2010)
DOI: 10.1517/13543781003767434

174. J. Weiss, M. L. Sos, D. Seidel, M. Peifer, T. Zander, J. M. Heuckmann, R. T. Ullrich, R. Menon, S. Maier, A. Soltermann, H. Moch, P. Wagener, F. Fischer, S. Heynck, M. Koker, J. Schottle, F. Leenders, F. Gabler, I. Dabow, S. Querings, L. C. Heukamp, H. Balke-Want, S. Ansen, D. Rauh, I. Baessmann, J. Altmuller, Z. Wainer, M. Conron, G. Wright, P. Russell, B. Solomon, E. Brambilla, C. Brambilla, P. Lorimier, S. Sollberg, O. T. Brustugun, W. Engel-Riedel, C. Ludwig, I. Petersen, J. Sanger, J. Clement, H. Groen, W. Timens, H. Sietsma, E. Thunnissen, E. Smit, D. Heideman, F. Cappuzzo, C. Ligorio, S. Damiani, M. Hallek, R. Beroukhim, W. Pao, B. Klebl, M. Baumann, R. Buettner, K. Ernestus, E. Stoelben, J. Wolf, P. Nurnberg, S. Perner and R. K. Thomas: Frequent and focal FGFR1 amplification associates with therapeutically tractable FGFR1 dependency in squamous cell lung cancer. Sci Transl Med 2, 62-93 (2010)
DOI: 10.1126/scitranslmed.3001451

175. A. Dutt, A. H. Ramos, P. S. Hammerman, C. Mermel, J. Cho, T. Sharifnia, A. Chande, K. E. Tanaka, N. Stransky, H. Greulich, N. S. Gray and M. Meyerson: Inhibitor-Sensitive FGFR1 Amplification in Human Non-Small Cell Lung Cancer. PLoS ONE 6, e20351 (2011)
DOI: 10.1371/journal.pone.0020351

176. Z. W. Cai, Y. Zhang, R. M. Borzilleri, L. Qian, S. Barbosa, D. Wei, X. Zheng, L. Wu, J. Fan, Z. Shi, B. S. Wautlet, S. Mortillo, R. Jeyaseelan, Sr., D. W. Kukral, A. Kamath, P. Marathe, C. D'Arienzo, G. Derbin, J. C. Barrish, J. A. Robl, J. T. Hunt, L. J. Lombardo, J. Fargnoli and R. S. Bhide: Discovery of brivanib alaninate ((S)-((R)-1-(4-(4-fluoro-2-methyl-1H-indol-5-yloxy)-5-methylpyrrolo(2,1-f)(1,2,4)triazin-6-yloxy)propan-2-yl)2-aminopropanoate), a novel prodrug of dual vascular endothelial growth factor receptor-2 and fibroblast growth factor receptor-1 kinase inhibitor (BMS-540215) J Med Chem 51, 1976-1980 (2008)
DOI: 10.1021/jm7013309

177. S. A. Antoniu and M. R. Kolb: Intedanib, a triple kinase inhibitor of VEGFR, FGFR and PDGFR for the treatment of cancer and idiopathic pulmonary fibrosis. IDrugs 13, 332-345 (2010)

178. M. Nakatsugawa, A. Takahashi, Y. Hirohashi, T. Torigoe, S. Inoda, M. Murase, H. Asanuma, Y. Tamura, R. Morita, Y. Michifuri, T. Kondo, T. Hasegawa, H. Takahashi and N. Sato: SOX2 is overexpressed in stem-like cells of human lung adenocarcinoma and augments the tumorigenicity. Lab Invest 91, 1796-804 (2011)
DOI: 10.1038/labinvest.2011.140

179. K. Mantripragada and H. Khurshid: Targeting genomic alterations in squamous cell lung cancer. Front Oncol 3, 195 (2013)
DOI: 10.3389/fonc.2013.00195

180. L. Li, H. Yu, X. Wang, J. Zeng, D. Li, J. Lu, C. Wang, J. Wang, J. Wei, M. Jiang and B. Mo: Expression of seven stem-cell-associated markers in human airway biopsy specimens obtained via fiberoptic bronchoscopy. J Exp Clin Cancer Res 32, 28 (2013)
DOI: 10.1186/1756-9966-32-28

181. V. D. Martinez, K. L. Thu, E. A. Vucic, R. Hubaux, M. Adonis, L. Gil, C. MacAulay,

S. Lam and W. L. Lam: Whole-genome sequencing analysis identifies a distinctive mutational spectrum in an arsenic-related lung tumor. J Thorac Oncol 8, 1451-1455 (2013)
DOI: 10.1097/JTO.0b013e3182a4dd8e

182. C. M. Rudin, S. Durinck, E. W. Stawiski, J. T. Poirier, Z. Modrusan, D. S. Shames, E. A. Bergbower, Y. Guan, J. Shin, J. Guillory, C. S. Rivers, C. K. Foo, D. Bhatt, J. Stinson, F. Gnad, P. M. Haverty, R. Gentleman, S. Chaudhuri, V. Janakiraman, B. S. Jaiswal, C. Parikh, W. Yuan, Z. Zhang, H. Koeppen, T. D. Wu, H. M. Stern, R. L. Yauch, K. E. Huffman, D. D. Paskulin, P. B. Illei, M. Varella-Garcia, A. F. Gazdar, F. J. de Sauvage, R. Bourgon, J. D. Minna, M. V. Brock and S. Seshagiri: Comprehensive genomic analysis identifies SOX2 as a frequently amplified gene in small-cell lung cancer. Nat Genet 44, 1111-1116 (2012)
DOI: 10.1038/ng.2405

183. Y. Chen, Y. Huang, J. Chen, S. Wang and J. Zhou: The prognostic value of SOX2 expression in non-small cell lung cancer: a meta-analysis. PLoS ONE 8, e71140 (2013)
DOI: 10.1371/journal.pone.0071140

184. K. A. Olaussen, J. C. Soria, L. Morat, A. Martin, L. Sabatier, J. F. Morere, D. Khayat and J. P. Spano: Loss of PTEN expression is not uncommon, but lacks prognostic value in stage I NSCLC. Anticancer Res 23, 4885-4890 (2003)

185. D. D. Sarbassov, D. A. Guertin, S. M. Ali and D. M. Sabatini: Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. Science 307, 1098-1101 (2005)
DOI: 10.1126/science.1106148

186. F. Zhang, X. Zhang, M. Li, P. Chen, B. Zhang, H. Guo, W. Cao, X. Wei, X. Cao, X. Hao and N. Zhang: mTOR complex component Rictor interacts with PKCzeta and regulates cancer cell metastasis. Cancer Res 70, 9360-9370 (2010)
DOI: 10.1158/0008-5472.CAN-10-0207

187. D. D. Sarbassov, S. M. Ali, S. Sengupta, J. H. Sheen, P. P. Hsu, A. F. Bagley, A. L. Markhard and D. M. Sabatini: Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. Mol Cell 22, 159-168 (2006)
DOI: 10.1016/j.molcel.2006.03.029

188. J. T. Fong, R. J. Jacobs, D. N. Moravec, S. B. Uppada, G. M. Botting, M. Nlend and N. Puri: Alternative Signaling Pathways as Potential Therapeutic Targets for Overcoming EGFR and c-Met Inhibitor Resistance in Non-Small Cell Lung Cancer. PLoS ONE 8, e78398 (2013)
DOI: 10.1371/journal.pone.0078398

189. D. Ishikawa, S. Takeuchi, T. Nakagawa, T. Sano, J. Nakade, S. Nanjo, T. Yamada, H. Ebi, L. Zhao, K. Yasumoto, T. Nakamura, K. Matsumoto, H. Kagamu, H. Yoshizawa and S. Yano: mTOR inhibitors control the growth of EGFR mutant lung cancer even after acquiring resistance by HGF. PLoS ONE 8, e62104 (2013)
DOI: 10.1371/journal.pone.0062104

190. E. Pros, S. Lantuejoul, L. Sanchez-Verde, S. D. Castillo, E. Bonastre, A. Suarez-Gauthier, E. Conde, J. C. Cigudosa, F. Lopez-Rios, J. Torres-Lanzas, J. Castellvi, S. Ramon y Cajal, E. Brambilla and M. Sanchez-Cespedes: Determining the profiles and parameters for gene amplification testing of growth factor receptors in lung cancer. Int J Cancer 133, 898-907 (2013)
DOI: 10.1002/ijc.28090

191. U. McDermott, R. Y. Ames, A. J. Iafrate, S. Maheswaran, H. Stubbs, P. Greninger, K. McCutcheon, R. Milano, A. Tam, D. Y. Lee, L. Lucien, B. W. Brannigan, L. E. Ulkus, X. J. Ma, M. G. Erlander, D. A. Haber, S. V. Sharma and J. Settleman: Ligand-dependent platelet-derived growth factor receptor (PDGFR)-alpha activation sensitizes rare lung cancer and sarcoma cells to PDGFR kinase inhibitors. Cancer Res 69, 3937-3946 (2009)
DOI: 10.1158/0008-5472.CAN-08-4327

192. M. Hou, Y. Morishita, T. Iljima, Y. Inadome,

K. Mase, Y. Dai and M. Noguchi: DNA methylation and expression of p16(INK4A) gene in pulmonary adenocarcinoma and anthracosis in background lung. Int J Cancer 84, 609-613 (1999)
DOI:10.1002/(SICI)1097-0215(19991222)84:6<609::AID-IJC12>3.0.CO;2-Q
DOI: 10.1002/(SICI)1097-0215(19991222)84:6<609::AID-IJC12>3.3.CO;2-H

193. H. Osada and T. Takahashi: Genetic alterations of multiple tumor suppressors and oncogenes in the carcinogenesis and progression of lung cancer. Oncogene 21, 7421-7434 (2002)
DOI: 10.1038/sj.onc.1205802

194. S. Toyooka, K. O. Toyooka, R. Maruyama, A. K. Virmani, L. Girard, K. Miyajima, K. Harada, Y. Ariyoshi, T. Takahashi, K. Sugio, E. Brambilla, M. Gilcrease, J. D. Minna and A. F. Gazdar: DNA methylation profiles of lung tumors. Mol Cancer Ther 1, 61-67 (2001)

195. S. Licciulli, J. L. Avila, L. Hanlon, S. Troutman, M. Cesaroni, S. Kota, B. Keith, M. C. Simon, E. Pure, F. Radtke, A. J. Capobianco and J. L. Kissil: Notch1 is required for Kras-induced lung adenocarcinoma and controls tumor cell survival via p53. Cancer Res 73, 5974-5984 (2013)
DOI: 10.1158/0008-5472.CAN-13-1384

196. A. Bernheim, S. Toujani, P. Saulnier, T. Robert, O. Casiraghi, P. Validire, S. Temam, P. Menard, P. Dessen and P. Fouret: High-resolution array comparative genomic hybridization analysis of human bronchial and salivary adenoid cystic carcinoma. Lab Invest 88, 464-473 (2008)
DOI: 10.1038/labinvest.2008.18

197. J. Osanyingbemi-Obidi, I. Dobromilskaya, P. B. Illei, C. L. Hann and C. M. Rudin: Notch signaling contributes to lung cancer clonogenic capacity in vitro but may be circumvented in tumorigenesis in vivo. Mol Cancer Res 9, 1746-1754 (2011)
DOI: 10.1158/1541-7786.MCR-11-0286

198. T. Saito, C. Y. Sasaki, L. J. Rezanka, P. Ghosh and D. L. Longo: p52-Independent nuclear translocation of RelB promotes LPS-induced attachment. Biochem Biophys Res Commun 391, 235-241 (2010)
DOI: 10.1016/j.bbrc.2009.11.039

199. G. Rossi, M. C. Mengoli, A. Cavazza, D. Nicoli, M. Barbareschi, C. Cantaloni, M. Papotti, A. Tironi, P. Graziano, M. Paci, A. Stefani, M. Migaldi, G. Sartori and G. Pelosi: Large cell carcinoma of the lung: clinically oriented classification integrating immunohistochemistry and molecular biology. Virchows Arch 464, 61-68 (2014)
DOI: 10.1007/s00428-013-1501-6

200. M. S. Tsao, N. Liu, J. R. Chen, J. Pappas, J. Ho, C. To, J. Viallet, M. Park and H. Zhu: Differential expression of Met/hepatocyte growth factor receptor in subtypes of non-small cell lung cancers. Lung Cancer 20, 1-16 (1998)
DOI: 10.1016/S0169-5002(98)00007-5

201. M. Strazisar, V. Mlakar, T. Rott and D. Glavac: Somatic alterations of the serine/threonine kinase LKB1 gene in squamous cell (SCC) and large cell (LCC) lung carcinoma. Cancer Invest 27, 407-416 (2009)
DOI: 10.1080/07357900802427919

202. B. C. Mak and R. S. Yeung: The tuberous sclerosis complex genes in tumor development. Cancer Invest 22, 588-603 (2004)
DOI: 10.1081/CNV-200027144

203. Y. L. Chang, C. T. Wu, J. Y. Shih and Y. C. Lee: Unique p53 and epidermal growth factor receptor gene mutation status in 46 pulmonary lymphoepithelioma-like carcinomas. Cancer Sci 102, 282-287 (2011)
DOI: 10.1111/j.1349-7006.2010.01768.x

204. K. Uchida, A. Kojima, N. Morokawa, O. Tanabe, C. Anzai, M. Kawakami, Y. Eto and K. Yoshimura: Expression

of progastrin-releasing peptide and gastrin-releasing peptide receptor mRNA transcripts in tumor cells of patients with small cell lung cancer. J Cancer Res Clin Oncol 128, 633-640 (2002)
DOI: 10.1007/s00432-002-0392-8

205. N. A. Levin, P. M. Brzoska, M. L. Warnock, J. W. Gray and M. F. Christman: Identification of novel regions of altered DNA copy number in small cell lung tumors. Genes Chromosomes Cancer 13, 175-185 (1995)
DOI: 10.1002/gcc.2870130307

206. R. Ullmann, A. Schwendel, H. Klemen, G. Wolf, I. Petersen and H. H. Popper: Unbalanced chromosomal aberrations in neuroendocrine lung tumors as detected by comparative genomic hybridization. Hum Pathol 29, 1145-1149 (1998)
DOI: 10.1016/S0046-8177(98)90428-2

207. J. W. Harbour, S. L. Lai, J. Whang-Peng, A. F. Gazdar, J. D. Minna and F. J. Kaye: Abnormalities in structure and expression of the human retinoblastoma gene in SCLC. Science 241, 353-7 (1988)
DOI: 10.1126/science.2838909

208. S. L. Lai, H. Brauch, T. Knutsen, B. E. Johnson, M. M. Nau, T. Mitsudomi, C. M. Tsai, J. Whang-Peng, B. Zbar, F. J. Kaye and et al.: Molecular genetic characterization of neuroendocrine lung cancer cell lines. Anticancer Res 15, 225-232 (1995)

209. Wistuba, II, A. F. Gazdar and J. D. Minna: Molecular genetics of small cell lung carcinoma. Semin Oncol 28, 3-13 (2001)
DOI: 10.1053/sonc.2001.25738
DOI: 10.1016/S0093-7754(01)90072-7

210. D. G. Burbee, E. Forgacs, S. Zochbauer-Muller, L. Shivakumar, K. Fong, B. Gao, D. Randle, M. Kondo, A. Virmani, S. Bader, Y. Sekido, F. Latif, S. Milchgrub, S. Toyooka, A. F. Gazdar, M. I. Lerman, E. Zabarovsky, M. White and J. D. Minna: Epigenetic inactivation of RASSF1A in lung and breast cancers and malignant phenotype suppression. J Natl Cancer Inst 93, 691-699 (2001)
DOI: 10.1093/jnci/93.9.691

211. A. M. Senderowicz, I. Viard-Leveugle, S. Veyrenc, L. E. French, C. Brambilla and E. Brambilla: Small-molecule cyclin-dependent kinase modulators - Frequent loss of Fas expression and function in human lung tumours with overexpression of FasL in small cell lung carcinoma. Oncogene 22, 6609-6620 (2003)
DOI: 10.1038/sj.onc.1206954

212. Y. H. Kim, L. Girard, C. P. Giacomini, P. Wang, T. Hernandez-Boussard, R. Tibshirani, J. D. Minna and J. R. Pollack: Combined microarray analysis of small cell lung cancer reveals altered apoptotic balance and distinct expression signatures of MYC family gene amplification. Oncogene 25, 130-138 (2006)

213. G. W. Krystal, G. Sulanke and J. Litz: Inhibition of phosphatidylinositol 3-kinase-Akt signaling blocks growth, promotes apoptosis, and enhances sensitivity of small cell lung cancer cells to chemotherapy. Mol Cancer Ther 1, 913-922 (2002)

214. D. W. Ball, C. G. Azzoli, S. B. Baylin, D. Chi, S. Dou, H. Donis-Keller, A. Cumaraswamy, M. Borges and B. D. Nelkin: Identification of a human achaete-scute homolog highly expressed in neuroendocrine tumors. Int J Cancer 54, 784-792 (1993)

215. B. A. Westerman, R. H. Breuer, A. Poutsma, A. Chhatta, L. A. Noorduyn, M. G. Koolen, P. E. Postmus, M. A. Blankenstein and C. B. Oudejans: Basic helix-loop-helix transcription factor profiling of lung tumors shows aberrant expression of the proneural gene atonal homolog 1 (ATOH1, HATH1, MATH1) in neuroendocrine tumors. Int J Biol Markers 22, 114-123 (2007)

216. M. Miki, D. W. Ball and R. I. Linnoila: Insights into the achaete-scute homolog-1 gene (hASH1) in normal and neoplastic human lung. Lung Cancer 75, 58-65 (2012)
DOI: 10.1016/j.lungcan.2011.05.019

217. H. Osada, S. Tomida, Y. Yatabe, Y. Tatematsu, T. Takeuchi, H. Murakami, Y. Kondo, Y. Sekido and T. Takahashi: Roles of achaete-scute homologue 1 in DKK1 and E-cadherin repression and neuroendocrine differentiation in lung cancer. Cancer Res 68, 1647-1655 (2008) DOI: 10.1158/0008-5472.CAN-07-5039

218. R. I. Linnoila, B. Zhao, J. L. DeMayo, B. D. Nelkin, S. B. Baylin, F. J. DeMayo and D. W. Ball: Constitutive achaete-scute homologue-1 promotes airway dysplasia and lung neuroendocrine tumors in transgenic mice. Cancer Res 60, 4005-4009 (2000)

219. K. Hiroshima, A. Iyoda, K. Shibuya, Y. Haga, T. Toyozaki, T. Iizasa, T. Nakayama, T. Fujisawa and H. Ohwada: Genetic alterations in early-stage pulmonary large cell neuroendocrine carcinoma. Cancer 100, 1190-1198 (2004) DOI: 10.1002/cncr.20108

220. R. Ullmann, S. Petzmann, A. Sharma, P. T. Cagle and H. H. Popper: Chromosomal aberrations in a series of large-cell neuroendocrine carcinomas: unexpected divergence from small-cell carcinoma of the lung. Hum Pathol 32, 1059-1063 (2001) DOI: 10.1053/hupa.2001.28248

221. R. Nasgashio, Y. Sato, T. Matsumoto, T. Kageyama, M. Hattori, A. Iyoda, Y. Satoh, S. Ryuge, N. Masuda, S. X. Jiang and M. Saegusa: The balance between the expressions of hASH1 and HES1 differs between large cell neuroendocrine carcinoma and small cell carcinoma of the lung. Lung Cancer 74, 405-410 (2011) DOI: 10.1016/j.lungcan.2011.04.012

222. S. Petzmann, R. Ullmann, I. Halbwedl and H. H. Popper: Analysis of chromosome-11 aberrations in pulmonary and gastrointestinal carcinoids: an array comparative genomic hybridization-based study. Virchows Arch 445, 151-159 (2004) DOI: 10.1007/s00428-004-1052-y

223. R. S. Macarenco, T. S. Uphoff, H. F. Gilmer, R. B. Jenkins, S. N. Thibodeau, J. E. Lewis, J. R. Molina, P. Yang and M. C. Aubry: Salivary gland-type lung carcinomas: an EGFR immunohistochemical, molecular genetic, and mutational analysis study. Mod Pathol 21, 1168-1175 (2008) DOI: 10.1038/modpathol.2008.113

224. S. W. Han, H. P. Kim, Y. K. Jeon, D. Y. Oh, S. H. Lee, D. W. Kim, S. A. Im, D. H. Chung, D. S. Heo, Y. J. Bang and T. Y. Kim: Mucoepidermoid carcinoma of lung: Potential target of EGFR-directed treatment. Lung Cancer 61, 30-34 (2008) DOI: 10.1016/j.lungcan.2007.11.014

225. I. D. O'Neill: Gefitinib as targeted therapy for mucoepidermoid carcinoma of the lung: possible significance of CRTC1-MAML2 oncogene. Lung Cancer 64, 129-130 (2009) DOI: 10.1016/j.lungcan.2009.01.003

226. M. N. Younes, Y. W. Park, Y. D. Yazici, M. Gu, A. A. Santillan, X. Nong, S. Kim, S. A. Jasser, A. K. El-Naggar and J. N. Myers: Concomitant inhibition of epidermal growth factor and vascular endothelial growth factor receptor tyrosine kinases reduces growth and metastasis of human salivary adenoid cystic carcinoma in an orthotopic nude mouse model. Mol Cancer Ther 5, 2696-2705 (2006) DOI: 10.1158/1535-7163.MCT-05-0228

227. M. C. Aubry, M. C. Heinrich, J. Molina, J. E. Lewis, P. Yang, S. D. Cassivi and C. L. Corless: Primary adenoid cystic carcinoma of the lung: absence of KIT mutations. Cancer 110, 2507-2510 (2007) DOI: 10.1002/cncr.23075

228. R. Dahse, O. Driemel, S. Schwarz, K. Kromeyer-Hauschild, A. Berndt and H. Kosmehl: KRAS status and epidermal growth factor receptor expression as determinants for anti-EGFR therapies in salivary gland carcinomas. Oral Oncol 45, 826-829 (2009) DOI: 10.1016/j.oraloncology.2009.01.013

229. J. A. Hu, Y. Li and J. Fang: Effect of ERK inhibitor on pulmonary metastasis

of inoculated human adenoid cystic carcinoma cells in nude mice. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 109, 117-123 (2010)
DOI: 10.1016/j.tripleo.2009.07.052

230. D. J. Dammrich, E. S. Santos and L. E. Raez: Efficacy of sorafenib, a multi-tyrosine kinase inhibitor, in an adenoid cystic carcinoma metastatic to the lung: case report and review of literature. J Med Case Rep 5, 483 (2011)
DOI: 10.1186/1752-1947-5-483

231. N. G. Chau, S. J. Hotte, E. X. Chen, S. F. Chin, S. Turner, L. Wang and L. L. Siu: A phase II study of sunitinib in recurrent and/or metastatic adenoid cystic carcinoma (ACC) of the salivary glands: current progress and challenges in evaluating molecularly targeted agents in ACC. Ann Oncol 23, 1562-1570 (2012)
DOI: 10.1093/annonc/mdr522

232. M. Persson, Y. Andren, C. A. Moskaluk, H. F. Frierson, Jr., S. L. Cooke, P. A. Futreal, T. Kling, S. Nelander, A. Nordkvist, F. Persson and G. Stenman: Clinically significant copy number alterations and complex rearrangements of MYB and NFIB in head and neck adenoid cystic carcinoma. Genes Chromosomes Cancer 51, 805-817 (2012)
DOI: 10.1002/gcc.21965

233. A. S. Ho, K. Kannan, D. M. Roy, L. G. Morris, I. Ganly, N. Katabi, D. Ramaswami, L. A. Walsh, S. Eng, J. T. Huse, J. Zhang, I. Dolgalev, K. Huberman, A. Heguy, A. Viale, M. Drobnjak, M. A. Leversha, C. E. Rice, B. Singh, N. G. Iyer, C. R. Leemans, E. Bloemena, R. L. Ferris, R. R. Seethala, B. E. Gross, Y. Liang, R. Sinha, L. Peng, B. J. Raphael, S. Turcan, Y. Gong, N. Schultz, S. Kim, S. Chiosea, J. P. Shah, C. Sander, W. Lee and T. A. Chan: The mutational landscape of adenoid cystic carcinoma. Nat Genet 45, 791-798 (2013)
DOI: 10.1038/ng.2643

234. P. J. Stephens, H. R. Davies, Y. Mitani, P. Van Loo, A. Shlien, P. S. Tarpey, E. Papaemmanuil, A. Cheverton, G. R. Bignell, A. P. Butler, J. Gamble, S. Gamble, C. Hardy, J. Hinton, M. Jia, A. Jayakumar, D. Jones, C. Latimer, S. McLaren, D. J. McBride, A. Menzies, L. Mudie, M. Maddison, K. Raine, S. Nik-Zainal, S. O'Meara, J. W. Teague, I. Varela, D. C. Wedge, I. Whitmore, S. M. Lippman, U. McDermott, M. R. Stratton, P. J. Campbell, A. K. El-Naggar and P. A. Futreal: Whole exome sequencing of adenoid cystic carcinoma. J Clin Invest 123, 2965-2968 (2013)
DOI: 10.1172/JCI67201

235. G. Pelosi, F. Fraggetta, O. Nappi, U. Pastorino, P. Maisonneuve, F. Pasini, A. Iannucci, P. Solli, H. S. Musavinasab, G. De Manzoni, A. Terzi and G. Viale: Pleomorphic carcinomas of the lung show a selective distribution of gene products involved in cell differentiation, cell cycle control, tumor growth, and tumor cell motility: a clinicopathologic and immunohistochemical study of 31 cases. Am J Surg Pathol 27, 1203-1215 (2003)
DOI: 10.1097/00000478-200309000-00003

236. M. Blaukovitsch, I. Halbwedl, H. Kothmaier, M. Gogg-Kammerer and H. H. Popper: Sarcomatoid carcinomas of the lung--are these histogenetically heterogeneous tumors? Virchows Arch 449, 455-461 (2006)
DOI: 10.1007/s00428-006-0256-8

237. A. Italiano, A. B. Cortot, M. Ilie, G. Martel-Planche, T. Fabas, D. Pop, J. Mouroux, V. Hofman, P. Hofman and F. Pedeutour: EGFR and KRAS status of primary sarcomatoid carcinomas of the lung: implications for anti-EGFR treatment of a rare lung malignancy. Int J Cancer 125, 2479-2482 (2009)
DOI: 10.1002/ijc.24610

238. T. W. Fan, A. N. Lane, R. M. Higashi, M. A. Farag, H. Gao, M. Bousamra and D. M. Miller: Altered regulation of metabolic

pathways in human lung cancer discerned by C stable isotope-resolved metabolomics (SIRM) Mol Cancer 8, 41 (2009)
DOI: 10.1186/1476-4598-8-41

239. P. P. Massion, P. M. Taflan, S. M. Jamshedur Rahman, P. Yildiz, Y. Shyr, M. E. Edgerton, M. D. Westfall, J. R. Roberts, J. A. Pietenpol, D. P. Carbone and A. L. Gonzalez: Significance of p63 amplification and overexpression in lung cancer development and prognosis. Cancer Res 63, 7113-7121 (2003)

240. P. P. Massion, P. M. Taflan, Y. Shyr, S. M. Rahman, P. Yildiz, B. Shakthour, M. E. Edgerton, M. Ninan, J. J. Andersen and A. L. Gonzalez: Early involvement of the phosphatidylinositol 3-kinase/Akt pathway in lung cancer progression. Am J Respir Crit Care Med 170, 1088-1094 (2004)
DOI: 10.1164/rccm.200404-487OC

241. X. Tang, D. Liu, S. Shishodia, N. Ozburn, C. Behrens, J. J. Lee, W. K. Hong, B. B. Aggarwal and Wistuba, II: Nuclear factor-kappaB (NF-kappaB) is frequently expressed in lung cancer and preneoplastic lesions. Cancer 107, 2637-2646 (2006)
DOI: 10.1002/cncr.22315

242. J. Shen, C. Behrens, Wistuba, II, L. Feng, J. J. Lee, W. K. Hong and R. Lotan: Identification and validation of differences in protein levels in normal, premalignant, and malignant lung cells and tissues using high-throughput Western Array and immunohistochemistry. Cancer Res 66, 11194-11206 (2006)
DOI: 10.1158/0008-5472.CAN-04-1444

243. R. Ullmann, M. Bongiovanni, I. Halbwedl, A. E. Fraire, P. T. Cagle, M. Mori, M. Papotti and H. H. Popper: Is high-grade adenomatous hyperplasia an early bronchioloalveolar adenocarcinoma? J Pathol 201, 371-376 (2003)
DOI: 10.1002/path.1460

244. M. Mori, S. K. Rao, H. H. Popper, P. T. Cagle and A. E. Fraire: Atypical adenomatous hyperplasia of the lung: a probable forerunner in the development of adenocarcinoma of the lung. Mod Pathol 14, 72-84 (2001)
DOI: 10.1038/modpathol.3880259

245. W. H. Westra, I. O. Baas, R. H. Hruban, F. B. Askin, K. Wilson, G. J. Offerhaus and R. J. Slebos: K-ras oncogene activation in atypical alveolar hyperplasias of the human lung. Cancer Res 56, 2224-2228 (1996)

246. K. Suzuki, T. Ogura, T. Yokose, K. Nagai, K. Mukai, T. Kodama, Y. Nishiwaki and H. Esumi: Loss of heterozygosity in the tuberous sclerosis gene associated regions in adenocarcinoma of the lung accompanied by multiple atypical adenomatous hyperplasia. Int J Cancer 79, 384-389 (1998)
DOI:10.1002/(SICI)1097-0215(19980821)79:4<384::AID-IJC 13>3.3.CO;2-O
DOI:10.1002/(SICI)1097-0215(19980821)79:4<384::AID-IJC13>3.0.C O;2-5

247. K. Takamochi, T. Ogura, K. Suzuki, H. Kawasaki, Y. Kurashima, T. Yokose, A. Ochiai, K. Nagai, Y. Nishiwaki and H. Esumi: Loss of heterozygosity on chromosomes 9q and 16p in atypical adenomatous hyperplasia concomitant with adenocarcinoma of the lung. Am J Pathol 159, 1941-1948 (2001)
DOI: 10.1016/S0002-9440(10)63041-6

248. H. Awaya, Y. Takeshima, O. Furonaka, N. Kohno and K. Inai: Gene amplification and protein expression of EGFR and HER2 by chromogenic in situ hybridisation and immunohistochemistry in atypical adenomatous hyperplasia and adenocarcinoma of the lung. J Clin Pathol 58, 1076-1080 (2005)
DOI: 10.1136/jcp.2004.025585

249. H. Sakamoto, J. Shimizu, Y. Horio, R. Ueda, T. Takahashi, T. Mitsudomi and Y. Yatabe: Disproportionate representation of KRAS gene mutation in atypical adenomatous hyperplasia, but even

distribution of EGFR gene mutation from preinvasive to invasive adenocarcinomas. J Pathol 212, 287-294 (2007) DOI: 10.1002/path.2165

250. R. Ullmann, M. Bongiovanni, I. Halbwedl, S. Petzmann, M. Gogg-Kammerer, A. Sapino, M. Papotti, G. Bussolati and H. H. Popper: Bronchiolar columnar cell dysplasia--genetic analysis of a novel preneoplastic lesion of peripheral lung. Virchows Arch 442, 429-436 (2003)

251. W. Pankiewicz, A. Sulewska, W. Niklinska, W. Naumnik, J. Laudanski, J. Niklinski and L. Chyczewski: Immunoexpression of P16INK4a, Rb and TP53 proteins in bronchiolar columnar cell dysplasia (BCCD) in lungs resected due to primary non-small cell lung cancer. Folia Histochem Cytobiol 46, 89-96 (2008) DOI: 10.2478/v10042-008-0013-8

252. E. Stacher, R. Ullmann, I. Halbwedl, M. Gogg-Kammerer, L. Boccon-Gibod, A. G. Nicholson, M. N. Sheppard, L. Carvalho, M. T. Franca, F. Macsweeney, A. Morresi-Hauf and H. H. Popper: Atypical goblet cell hyperplasia in congenital cystic adenomatoid malformation as a possible preneoplasia for pulmonary adenocarcinoma in childhood: A genetic analysis. Hum Pathol 35, 565-570 (2004) DOI: 10.1016/j.humpath.2004.01.008

253. H. Sartelet, M. Decaussin, G. Devouassoux, B. Nawrocki-Raby, P. Y. Brichon, C. Brambilla and E. Brambilla: Expression of vascular endothelial growth factor (VEGF) and its receptors (VEGF-R1 (Flt-1) and VEGF-R2 (KDR/Flk-1)) in tumorlets and in neuroendocrine cell hyperplasia of the lung. Hum Pathol 35, 1210-1217 (2004) DOI: 10.1016/j.humpath.2004.07.009

254. D. L. Zynger, N. D. Dimov, L. C. Ho, W. B. Laskin and A. V. Yeldandi: Differential expression of neural-cadherin in pulmonary epithelial tumours. Histopathology 52, 348-354 (2008) DOI: 10.1111/j.1365-2559.2007.02952.x

255. L. P. Dehner: Pleuropulmonary blastoma is THE pulmonary blastoma of childhood. Semin Diagn Pathol 11, 144-151 (1994)

256. R. Novak, S. Dasu, D. Agamanolis, W. Herold, J. Malone and J. Waterson: Trisomy 8 is a characteristic finding in pleuropulmonary blastoma. Pediatr Pathol Lab Med 17, 99-103 (1997) DOI: 10.1080/713601257 DOI: 10.3109/15513819709168349 DOI: 10.1080/15513819709168349 DOI: 10.1080/107710497175056

257. M. Barnard, J. Bayani, R. Grant, I. Teshima, P. Thorner and J. Squire: Use of multicolor spectral karyotyping in genetic analysis of pleuropulmonary blastoma. Pediatr Dev Pathol 3, 479-486 (2000) DOI: 10.1007/s100240010094

258. L. Roque, R. Rodrigues, C. Martins, C. Ribeiro, M. J. Ribeiro, A. G. Martins, P. Oliveira and I. Fonseca: Comparative genomic hybridization analysis of a pleuropulmonary blastoma. Cancer Genet Cytogenet 149, 58-62 (2004) DOI: 10.1016/S0165-4608(03)00284-X

259. T. Kusafuka, S. Kuroda, M. Inoue, T. Ara, A. Yoneda, T. Oue, Y. Udatsu, Y. Osugi and A. Okada: P53 gene mutations in pleuropulmonary blastomas. Pediatr Hematol Oncol 19, 117-128 (2002) DOI: 10.1080/08880010252825704

260. I. Slade, C. Bacchelli, H. Davies, A. Murray, F. Abbaszadeh, S. Hanks, R. Barfoot, A. Burke, J. Chisholm, M. Hewitt, H. Jenkinson, D. King, B. Morland, B. Pizer, K. Prescott, A. Saggar, L. Side, H. Traunecker, S. Vaidya, P. Ward, P. A. Futreal, G. Vujanic, A. G. Nicholson, N. Sebire, C. Turnbull, J. R. Priest, K. Pritchard-Jones, R. Houlston, C. Stiller, M. R. Stratton, J. Douglas and N. Rahman: DICER1 syndrome: clarifying the diagnosis, clinical features and management implications of a pleiotropic tumour predisposition syndrome. J Med Genet 48, 273-278 (2011) DOI: 10.1136/jmg.2010.083790

261. M. McGinnis, G. Jacobs, A. el-Naggar and R. W. Redline: Congenital peribronchial myofibroblastic tumor (so-called "congenital leiomyosarcoma") A distinct neonatal lung lesion associated with nonimmune hydrops fetalis. Mod Pathol 6, 487-492 (1993)

262. B. F. Schneider, M. A. Lovell and W. L. Golden: Cytogenetic abnormalities in primary bronchopulmonary leiomyosarcoma of childhood. Cancer Genet Cytogenet 105, 145-151 (1998)
DOI: 10.1016/S0165-4608(98)00030-2

Key Words: Molecular techniques, Lung, Infection, Lung cancer, Targeted therapy, Review

Send correspondence to: Fiorella Calabrese, Department of Cardiac, Thoracic and Vascular Sciences, University of Padua Medical School. Via A. Gabelli 61, 35121 Padua, Italy, Tel: 39-39-049-8272268, Fax: 39-39-049-8272269, E-mail: fiorella.calabrese@unipd.it