The Many Facets of Lung Adenocarcinoma– A Disease Comprising Distinct Molecular Subgroups.

Anna Gleinig[1,2], Sabine Huth[3], Uta Dahmen[2], Olaf Dirsch[1]

[1] Institute of Pathology, Klinikum Chemnitz gGmbH, Chemnitz, Germany

[2] Research Group Experimental Transplantation Surgery, Department of General, Visceral and Transplantation Surgery, University Hospital Jena, Jena, Germany

[3] Internal Medicine Clinic IV, Klinikum Chemnitz gGmbH, Chemnitz, Germany

Contact:
Anna Gleinig: annagleinig@yahoo.com
Sabine Huth: s.huth@skc.de
Uta Dahmen: uta.dahmen@med.uni-jena.de
Olaf Dirsch: o.dirsch@skc.de

Abstract:
Lung cancer is one of the most prevalent types of cancer throughout the world and a leading cause of cancer-related deaths. Lung cancer comprises small cell and non-small cell lung cancer. The most prevalent type of non-small cell lung cancer is adenocarcinoma (AdC). 5-year survival rates of lung AdC range between 50 and 2 % for stage Ia and IV, respectively.
Novel strategies to treat lung cancer focus on personalized medicine. Personalized medicine is based on a thorough molecular characterization of tumors to tailor therapy specifically to each individual patient. This principle was developed following recent advances in molecular tumor biology that lead to the detection of oncogenic driver mutations. Oncogenic driver mutations confer cancer-properties to the cell and the cancer cell depends on the oncogenic driver mutation to sustain its phenotype (oncogene addiction).
Oncogenic driver mutations can be exploited in the treatment of cancer by targeted therapies. Targeted therapies specifically attack cancer cells while causing less side effects than conventional chemotherapy.
In lung AdC, oncogenic driver mutations define distinct molecular subgroups. This review aims at presenting the 9 best characterized molecular subgroups of lung AdC. We want to illustrate 1) the respective oncogenic mechanism, 2) patient characteristics, 3) prognostic significance and 4) targeted therapy options for each subgroup.

Key words: lung adenocarcinoma, oncogenic driver mutations, targeted therapy

1. Introduction

Lung cancer is one of the leading types of cancer in the world. In 2012 there were about 1.8 million estimated new cases worldwide (American Cancer Society, 2013), with a proportion of approximately 410.000 cases in Europe (Ferlay et al., 2013). In the developed countries, lung cancer is the leading cause of cancer-related death in men and women, responsible for about 354.000 deaths in Europe in 2012 (Ferlay et al., 2013). The survival rates depend largely on the stage of lung cancer. The 5-year survival rate of patients with stage Ia tumors is about 50 % and the rate for patients with stage IV tumors is as low as 2 % (Gibson et al., 2013).

Lung cancer can be divided by morphology into small-cell and non-small-cell lung cancer (NSCLC). NSCLC comprises adenocarcinoma (AdC), squamous cell carcinoma (SqC) and large cell carcinoma, with AdC being the most prevalent type. The histologic differentiation between especially AdC and SqC is very important, as research has shown that the histologic type of NSCLC is correlated with distinct molecular features. For instance, mutations within the epidermal growth factor receptor (EGFR) gene occur much less frequently in SqC compared to AdC (Kosaka et al., 2004) and these mutations render AdC, but not SqC, sensitive to EGFR tyrosine kinase inhibitor (TKI) therapy (Chou et al., 2005).

The main determinant in treatment of lung AdC is the stage of disease. Surgical resection with curative intent is the preferred therapy for the lower stages, I to IIIa (Al-Shahrabani et al., 2014). 70 % of patients are diagnosed with unresectable advanced disease (Howlader et al., 2015), though. Another determinant is the patient's lung function, which can be impaired due to old age or comorbidities, rendering the patient functionally unresectable. Classical non-invasive therapeutic approaches for advanced lung AdC or inoperable patients include radio- and chemotherapy. Despite the development of a huge number of chemotherapy agents and combination treatments, it seems that therapeutic advances are exhausted. Even when combining different drugs, the median survival in stage IIIb/IV is approximately 10 months and the 1-year-survival rate ranges between 30 and 40 % (Manegold and Thatcher, 2012). Another major disadvantage of chemotherapy are potential severe side effects and a higher strain on patients due to intravenous hospital application. Another therapy option is immunotherapy, aiming at activating the immune system to attack cancer cells.

A novel strategy to treat lung cancer is personalized medicine. Personalized medicine aims at tailoring therapy specifically to the individual patient. To this end, tumors are subjected to thorough molecular characterization to search for 'targetable' alterations. Such alterations were uncovered by research on molecular tumor biology and are called oncogenic driver mutations. Oncogenic driver mutations confer oncogenic properties to the cell and are vital to sustain this cancer phenotype. This dependency is described as 'oncogene addiction' (Weinstein, 2002).

'Oncogene addiction' is exploited by targeted therapies that inhibit oncogenic driver mutation products and thus specifically attack cancer cells. Thus targeted therapy can confer survival advantages to patients harboring the respective oncogenic driver mutation, while causing less side effects compared to conventional chemotherapy. In 2005 erlotinib, a tyrosine kinase inhibitor (TKI)

targeting EGFR, was the first targeted drug approved for the therapy of NSCLC in Europe (European Medicines Agency, 2015).

Today it is accepted that lung AdC is a disease comprising distinct molecular subgroups classified by oncogenic driver mutations. Many different oncogenic driver mutations have already been detected in lung AdC, and the list is growing. This diversity in oncogenic driver mutations is mirrored by distinct consequences on the prognosis and treatment of lung AdC patients.

This review aims at presenting currently recognized molecular subtypes of lung AdC, focusing on the 9 best characterized oncogenic driver mutations. We want to 1) present the underlying oncogenic mechanisms, 2) provide epidemiologic characteristics and 3) summarize information about prognostic value and treatment for each molecular subgroup. Limitations of currently employed methods to detect oncogenic driver mutations will be pointed out and a direction for future diagnostic / therapeutic concepts will be given.

2. Methods

Relevant publications for preparing this systematic review (Liberati et al., 2009) were extracted from PubMed using the following search items: "*respective oncogenic driver mutation*" + "lung cancer", "non-small cell lung cancer", "NSCLC", "lung adenocarcinoma", "characteristics","prognosis", "therapy". Publications were restricted to full text research articles, clinical studies and reviews authored in English language. There were no restrictions regarding the year of publication. Relevant publications were identified by scanning titles and reading abstracts. In studies covering the same patient population, more recent or more informative publications were preferred. Information on each driver mutation was extracted by AG grouped under the following topics: type of molecular alteration, oncogenic mechanism, frequency in lung AdC, clinico-pathologic characteristics, prognostic significance, targeted therapies. For a brief summary on each molecular subtype see Table 1. Literature search was performed between June and August 2015.

3. Oncogenic driver mutations in lung AdC

We want to describe the 9 the best characterized oncogenic driver mutations in lung AdC. They are grouped by frequency into common, intermediate-frequency and rare oncogenic driver mutations.

3.1. Common oncogenic driver mutations

Common oncogenic driver mutations appear in a frequency of 10 – 30 % in lung AdC and thus together affect the majority of these tumors. They include mutations in the genes for EGFR and KRAS.

3.1.1 EGFR

3.1.1.1. Definition and oncogenic mechanism:

The EGFR is a receptor tyrosine kinase (RTK) transmitting signals of proliferation and cell survival following EGF binding. Mutations in the ATP binding pocket of the tyrosine kinase domain stabilize the binding of ATP (Lynch et al., 2004). The stabilized binding of ATP leads to prolonged activation of EGFR and enhanced downstream signaling. These activating somatic mutations mainly target exon 19 and 21. Mutations within the EGFR gene are often associated with copy number gain (Hirsch et al., 2006).

3.1.1.2. Epidemiology:

The prevalence of EGFR mutations within the lung AdC population varies

between 10 – 30 % (Travis et al., 2011). Activating EGFR mutations appear mutually exclusive with other oncogenic driver mutations. It has been observed that EGFR activating mutations are significantly associated with female gender, non-smoking status, Asian ethnicity and non-mucinous tumors (Finberg et al., 2007; Shigematsu, Lin, et al., 2005).

3.1.1.3. Prognosis and therapy:
Activating EGFR mutations were found to have no prognostic significance when treating patients with resection or conventional chemotherapy (Q. Zhang et al., 2014; Z. Zhang et al., 2014). The mutated EGFR is an oncogenic driver mutation that can be effectively targeted with approved TKIs such as erlotinib, gefitinib and afatinib (Melosky, 2014). Since the mutation leads to prolonged activation of the EGFR upon ATP-binding, it also renders the mutated EGFR more sensitive to inhibition with ATP-competitive TKIs than wild-type EGFR (Lynch et al., 2004). That is why patients with oncogenic EGFR mutations can profit enormously from EGFR TKI. A recent meta-analysis of published literature has revealed that EGFR TKIs can significantly prolong progression-free survival (PFS) in patients with activating EGFR mutations (W. Q. Zhang, Li and Li, 2014). The meta-analysis also confirmed a better quality of life for patients receiving EGFR TKI compared to chemotherapy, as indicated by the significant difference in the incidence of severe side effects. A major problem emerging under EGFR TKI therapy is the development of resistance via different mechanisms. Novel compounds and treatment strategies are under development to overcome this problem, but to go into more detail is beyond the scope of this review.

3.1.2. KRAS

3.1.2.1. Definition and oncogenic mechanism:
KRAS is a GTPase belonging to the RAS gene family and involved in many signaling pathways such as the mitogen-activated protein kinase – extracellular signal-regulated kinase (MAPK-ERK) pathway. After activation by GTP binding it can initiate cell proliferation via the RAS-dependent kinase cascade. Mutations affecting codons 12, 13 and 61 (Rodenhuis and Slebos, 1990) lead to constitutive activation of KRAS by disrupting GTPase activity (Tong et al., 1989). Due to the loss of GTPase activity, RAS stays in the GTP-bound, active state and constitutively transmits signals.

3.1.2.2. Epidemiology:
Activating KRAS mutations affect between 10 – 30 % of lung AdCs (Travis et al., 2011) and are mutually exclusive with other oncogenic driver mutations. It could be shown that KRAS mutations occur significantly more often in male gender, current or former smokers, non-Asian ethnicity and invasive mucinous AdCs (Ahrendt et al., 2001; Finberg et al., 2007; Shigematsu, Lin, et al., 2005).

3.1.2.3. Prognosis and therapy:
A meta-analysis of published studies proved that activating KRAS mutations are an unfavorable prognostic factor in lung AdC (Mascaux et al., 2005). Since mutated KRAS is the driving oncogene in a significant proportion of lung AdCs there is great demand for targeted therapies. Unfortunately, despite considerable efforts in research, such therapies are currently not available. It has been tried to directly target mutated KRAS with farnesyl transferase inhibitors, but this approach showed little clinical efficacy (Adjei et al., 2003). Instead the focus shifted to indirect inhibition by blocking

downstream targets, such as MEK1/MEK2 (Janne et al., 2013). Many other approaches are currently being investigated, including direct targeting of codon 12-mutated KRAS (Rothschild, 2015).

3.2. Oncogenic driver mutations with intermediate frequency

Oncogenic driver mutations with intermediate frequency appear in approximately 5 % of lung AdC and comprise translocations involving anaplastic lymphoma kinase (ALK).

3.2.1. ALK

3.2.1.1. Definition and oncogenic mechanism:

ALK is a member of the insulin family of RTKs along with PDGF receptors, EGF receptor or insulin receptor. Constitutive ALK signaling increases cell proliferation and survival via different pathways, including PI3K/AKT/mTOR, JAK/STAT and RAS/MEK/ERK. In NSCLC the carboxyterminal portion of ALK is connected to the aminoterminal portion of echinoderm microtubule-associated protein-like 4 (EML4) (Soda et al., 2007). In these oncogenic translocation products the kinase domain of ALK remains intact. Both EML4 and ALK are located on the short arm of chromosome 2 in opposite directions, so the fusion results from inversion. There are different variants of EML4-ALK fusion proteins with varying break- and fusion points. The breakpoints within the ALK locus map to intron 19, suggesting a common fragile locus (Mano, 2008). The oncogenic potential of EML4-ALK fusion proteins results from constitutive dimerization.

Dimerization is conferred by an oligomerization motif within the EML4 portion (coiled-coil domains) (Mano, 2008; Soda et al., 2007).

3.2.1.2. Epidemiology:

EML4-ALK fusion can be detected in approximately 5 % of NSCLC patients (Fan et al., 2014; Kwak et al., 2010). EML4-ALK fusions describe a distinct subtype of lung AdC as they appear mutually exclusive with other oncogenic driver mutations. Patients with EML4-ALK fusion harbor distinct characteristics. They are usually younger than average lung AdC patients and significantly more often never- or former light smokers (Blackhall et al., 2014; Kwak et al., 2010). Some studies furthermore identified a higher prevalence of females (Fan et al., 2014; Shaw et al., 2009).

3.2.1.3. Prognosis and therapy:

Most studies assessing the prognostic relevance of EML4-ALK fusions in patients treated with resection or conventional chemotherapy found no correlation (J. H. Paik et al., 2012; Shaw et al., 2009; Takeda et al., 2012). Other studies found conflicting results, though (Blackhall et al., 2014; Yang et al., 2012). All in all, a meta-analysis of conducted studies would be of great help to define the prognostic significance of EML4-ALK rearrangements. EML4-ALK-driven lung AdC can be treated with specific, targeted drugs inhibiting ALK kinase. Crizotinib, ceritinib and alectinib are approved compounds (Iragavarapu et al., 2015). Recently a systematic literature review with meta-analysis investigating the efficacy and safety of crizotinib in the treatment of locally advanced or metastatic ALK-rearrangement-positive NSCLC has been conducted (Qian et al., 2014). It found evidence that crizotinib treatment generally extended survival and had higher response rates compared to chemotherapy. Thus, crizotinib represents the favorable treatment option for patients with EML4-ALK rearrangements in advanced stages. The

problem of resistance development under TKI therapy also exists in patients with ALK rearrangements. Clinical and pre-clinical research addresses this problem with different novel drugs and treatment approaches, a description of which is beyond the scope of this review.

3.3. Rare oncogenic driver mutations

Rare oncogenic driver mutations appear in less than 5 % of lung AdC cases. What seems like a negligible proportion still accounts for considerable numbers of cases, given the high prevalence of lung AdC throughout the world. Most of the recently detected driver mutations can be placed in this group. We want to focus on ROS1 rearrangements and mutations of MET, BRAF, HER2, PIK3CA and NRAS in order of prevalence.

3.3.1. MET

3.3.1.1. Definition and oncogenic mechanism:

MET is a RTK and the receptor of hepatocyte growth factor (HGF). MET receptor signaling activates different downstream targets promoting tumor growth, angiogenesis and invasive behavior. MET can be rendered oncogenic by mutation or amplification/protein over-expression. Mutations of MET in lung AdC mostly lead to loss of exon 14 within the juxtamembrane domain, where the Cbl binding site is located. The loss of Cbl binding to MET results in decreased ubiquitination and downregulation. Due to this blockade of negative regulation there is sustained activation of MET promoting oncogenesis (Kong-Beltran et al., 2006). Another oncogenic mechanism is triggered by MET gene amplification resulting in protein over-expression. It has been shown that amplification leads to increased basal phosphorylation of MET. It is proposed that this activation of MET receptors could be caused by receptor clustering independent of ligand binding (Lutterbach et al., 2007).

3.3.1.2. Epidemiology:

MET mutations and amplifications appear in a mutually exclusive way (Onozato et al., 2009) and occur in 3 % (Onozato et al., 2009) and 2 – 20 % (Beau-Faller et al., 2008; Schildhaus et al., 2015) of lung AdC, respectively. Overexpression of MET protein is reported in 13 – 69 % of lung AdC (Guo et al., 2014). MET amplification, but not mutation, frequently co-exist with other oncogenic driver mutations. Clinico-pathologic characteristics associated with higher MET mutation or amplification frequencies have not been found (Beau-Faller et al., 2008; Onozato et al., 2009).

3.3.1.3. Prognosis and therapy:

The prognostic value of MET gene amplification and protein over-expression has been evaluated in systematic literature reviews with meta-analyses. It was found that both the presence of MET amplifications and MET protein over-expression are poor prognostic factors (Dimou et al., 2014; Guo et al., 2014). The prognostic relevance of MET mutations has not been assessed to date. The targeted treatment of lung AdC with MET alterations is pursued in many clinical studies. One possibility is to apply MET antibodies that block HGF binding. An example for MET antibodies is onartuzumab, which is a monovalent form and thus also inhibits receptor dimerization. This approach has shown improved PFS and overall survival (OS) in combination with erlotinib in patients with high MET expression (Spigel et al., 2013). Another approach is the design of TKIs targeting MET and a number of clinical trials on different substances is currently being conducted (Sadiq and

Salgia, 2013)

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3.3.2. BRAF

3.3.2.1. Definition and oncogenic mechanism:

The serine/threonine kinase BRAF is a member of the MAPK signaling pathway that becomes activated by RAS and phosphorylates MEK to transmit signals of cell proliferation and survival. In lung AdC many different types of BRAF mutation are described. The predominant and best characterized type is the BRAF V600E mutation. This mutation is characterized by a strongly elevated basal kinase activity due to the disruption of the inactive conformation of the kinase domain. The substitution of glutamic acid for valine leads to a conformational change similar to that following phosphorylation, so that constitutive activation is triggered (Wan et al., 2004).

3.3.2.2. Epidemiology:

The prevalence of BRAF mutations in lung AdC is approximately 3 % (D. Chen et al., 2014). BRAF mutations generally appear mutually exclusive with other oncogenic driver mutations. The association of BRAF mutations and smoking status is currently unclear, since different results are reported (D. Chen et al., 2014; Luk et al., 2015; Villaruz et al., 2015). Characteristics like gender and tumor stage were found to have no associations with BRAF mutations (D. Chen et al., 2014). Only in the subgroup of V600E mutations female gender and never-smoking status could be found significantly more often. (D. Chen et al., 2014)

3.3.2.3. Prognosis and therapy:

When pooling all types of BRAF mutations there are no significant differences in survival in comparison to BRAF wild type patients (Cardarella et al., 2013; Litvak et al., 2014; Marchetti et al., 2011; P. K. Paik et al., 2011; Villaruz et al., 2015). A study stratifying by the type of BRAF mutation found V600E mutations as factor for worse prognosis when treating patients with radical resection or conventional chemotherapy (Marchetti et al., 2011). Targeting BRAF mutant lung AdC with specific inhibitors is an active field of research. BRAF V600E inhibitors (dabrafenib, vemurafenib) are already approved for the treatment of advanced, V600E BRAF mutant melanoma and are currently tested in clinical trials for the treatment of V600E BRAF mutant NSCLC (Sanchez-Torres et al., 2013). The strong potential of these drugs is demonstrated by promising case reports (Gautschi et al., 2012; Peters, Michielin and Zimmermann, 2013) and improved OS in a study including V600E BRAF mutant patients treated with targeted therapy (Litvak et al., 2014).

3.3.3. HER2

3.3.3.1. Definition and oncogenic mechanism:

The human epidermal growth factor 2 (HER2) is a member of the ErbB receptor tyrosine kinase family, along with EGFR, HER3 and HER4. In contrast to the other ErbB receptors, HER2 has no identified ligand but becomes activated via homo- or heterodimerization with other ErbB receptors. Activation of HER2 results in signaling via downstream pathways involved in cellular proliferation, differentiation, migration and apoptosis. The main oncogenic driver alteration of HER2 in lung cancer is in-frame duplication/insertion of 4 amino acids in exon 20 at codon 776 (YVMA776–779ins). Also amplification and overexpression of wild type HER2 are often reported. In vitro studies have proven significant transforming activity of mutant HER2, concluding that exon 20 insertions confer gain-of-function (S. E. Wang et al., 2006). The mechanism underlying

the oncogenic potential of exon 20 insertions is assumed in the perturbation of the interaction with Hsp90 (Telesco et al., 2011). The attachment of Hsp90 is thought to prevent HER2 dimerization in the inactive state. When Hsp90 binding is impeded HER2 can become constitutively active.

3.3.3.2. Epidemiology:
Activating insertion mutations in HER2 appear in 2 – 4 % (Arcila et al., 2012; Li et al., 2012; Suzuki et al., 2015) of lung AdC cases, with higher prevalence reported in Asian cohorts. HER2 mutations do not usually co-exist with other oncogenic driver mutations. There are many reports investigating clinico-pathologic features associated with HER2-mutated lung AdC. The only characteristic all reports agree on is never or former very light smoking status (Arcila et al., 2012; Li et al., 2012; Mazieres et al., 2013; Shigematsu, Takahashi, et al., 2005; Suzuki et al., 2015; Tomizawa et al., 2011). There is also a trend for younger patient age (Arcila et al., 2012; Suzuki et al., 2015; Tomizawa et al., 2011). Conflicting reports exist on other features, such as gender, ethnicity and tumor stage.

3.3.3.3. Prognosis and therapy:
So far, three large studies comparing the survival of patients with HER2 insertion mutations to patients with wild type HER2 were conducted. Two of them found no difference in overall survival (Arcila et al., 2012; Tomizawa et al., 2011), while the third reported reduced overall survival for patients with HER2 insertion mutations (Suzuki et al., 2015). In smaller studies, different prognostic properties of HER2 insertion mutations were indicated, illustrating the need for a comprehensive meta-analysis of published studies. There is not yet an approved therapy for lung AdC with HER2 insertion mutations, but different approaches are being pursued fostered by the success of targeted therapies in HER2 over-expressing breast cancer (Peters and Zimmermann, 2014). One class of drugs under investigation in NSCLC are irreversible TKIs targeting HER2/3 and EGFR, such as afatinib, neratinib, and dacomitinib. They delivered encouraging results in phase I/II clinical trials. Other therapeutics that could be applied, but so far did not demonstrate clinical benefit, comprise HER monoclonal antibodies (trastuzumab, pertuzumab) and TKIs dually inhibiting HER2 and EGFR (lapatinib). A case report of a lung AdC patient with a HER2 exon 20 insertion mutation treated with a combination of lapatinib, trastuzumab and the VEGF monoclonal antibody bevacizumab described impressive response (Falchook et al., 2013).

3.3.4. ROS1
3.3.4.1. Definition and oncogenic mechanism:
ROS1 is a RTK of the insulin receptor family. The natural ligand is currently unknown and the function of the wild type protein is still not completely resolved. The translocation of ROS1 involves different fusion partners. The breakpoint in the ROS1 gene lies at the 5′ end of exons 32, 34, 35, or 36 and the kinase domain remains intact in all fusion constructs (Davies and Doebele, 2013). The exact mechanism of constitutive activation of ROS1 via the rearrangement is unknown. A possibility is that the fusion partner provides a dimerization domain, but many of the fusion partners do not possess such domains and whether dimerization is involved in wild type ROS1 signaling is currently unclear. Also the cellular localization of the fusion proteins varies. ROS1 fusion proteins probably activate similar

pathways as other RTKs (Davies and Doebele, 2013).

3.3.4.2. Epidemiology:
The frequency of ROS1 translocations in lung AdC approximates 2.5 % (Zhu et al., 2015). Translocations involving ROS1 appear mutually exclusive with other oncogenic driver mutations in lung AdC. Patient characteristics associated with increased prevalence of ROS1 translocations resemble those of ALK rearrangements. Patients are mainly never-smokers, females, have tumors with advanced stage (Zhu et al., 2015) and are younger than average lung AdC patients (Bergethon et al., 2012; Y. F. Chen et al., 2014).

3.3.4.3. Prognosis and therapy:
In a recent study investigating the prognostic significance of ROS1 rearrangements it was found that pretreated patients with ROS1 translocations receiving conventional chemotherapy or crizotinib had an improved OS compared to other NSCLC patients (Scheffler, Schultheis, et al., 2015). Another study, however, found no significant difference in survival between previously untreated patients
with ROS1 translocation receiving chemotherapy and patients with wild type ROS1 (Y. F. Chen et al., 2014). Therapies targeting the ROS1 fusion kinase are currently tested in clinical trials. Crizotinib, which is also effective against the ALK fusion kinase, has shown clinical efficacy in ROS1 fusion positive patients (Shaw et al., 2014), probably due to the fact that the kinase domains of ROS1 and ALK share 77% sequence identity within the ATP binding sites. Further clinical trials are planned or ongoing, involving different ROS1 TKIs or inhibitors of Hsp90, that have demonstrated efficacy in ROS1 rearranged cells in preclinical studies (Davies and Doebele, 2013).

3.3.5. PIK3CA
3.3.5.1. Definition and oncogenic mechanism:
PIK3CA encodes the p110α catalytic subunit of phosphatidylinositol 3-kinase (PI3K). PI3K is a lipid kinase that is involved in the PI3K/AKT/mTOR pathway. Activation of this pathway, for example by growth factor receptors, induces cell proliferation, survival, motility and invasion. Mutations in PIK3CA occur in various cancer types and target hot spot areas. The main types of mutation in lung AdC are located in the helical (exon 9) and kinase (exon 20) domains. Exon 9 mutations (mainly E545K) lead to elevated lipid kinase activity (Kang, Bader and Vogt, 2005) because of the release of auto-inhibition (Gabelli et al., 2014). The mutations weaken inhibitory interactions within the enzyme, so that physiological activation is mimicked. Exon 20 mutations (mainly H1047R) also confer increased lipid kinase activity (Samuels and Velculescu, 2004), but via a different mechanism. They lead to increased interaction of PI3K with the cell membrane, thus increasing the access to PI3K's substrate PIP2, which is a membrane component (Gabelli et al., 2014). Due to facilitated substrate access the catalytic activity of PI3K is increased.

3.3.5.2. Epidemiology:
Mutations in PIK3CA are generally detected in approximately 2 -3 % of lung AdC (Chaft et al., 2012; Scheffler, Bos, et al., 2015; L. Wang et al., 2014). It has to be said that up to 70 % of PIK3CA mutations co-exist with further genetic alterations, such as EGFR or KRAS mutations (Chaft et al., 2012). This raises the question whether PIK3CA mutations can be regarded as genuine driver mutations and whether they truly define a distinct subset of lung AdC. Several studies investigated

clinico-pathologic characteristics of lung AdC patients harboring PIK3CA mutations. They found no association with age, gender, smoking history or staging and grading of the tumors (Chaft et al., 2012; L. Wang et al., 2014; L. Zhang et al., 2013). Certain characteristics were sporadically reported, such as a higher occurrence of lymph node metastasis (L. Zhang et al., 2013), a higher incidence of mutations in the kinase domain in never-smokers (Chaft et al., 2012), a higher prevalence of smoking and a cancer history (NSCLC as second malignancy) (Scheffler, Bos, et al., 2015).

3.3.5.3. Prognosis and therapy: It has been reported that there is no difference in survival between patients whose tumors harbor any PIK3CA mutation and those with wild type PIK3CA (Scheffler, Bos, et al., 2015; L. Wang et al., 2014; L. Zhang et al., 2013). Some reports compared survival between patients with single PIK3CA mutations and those with co-existing mutations, but published conflicting results (Chaft et al., 2012; L. Wang et al., 2014). A stratification according to mutations in exon 9 and 20 also gave rise to conflicting results (L. Wang et al., 2014; L. Zhang et al., 2013). There is currently no approved therapy for PIK3CA-mutated lung AdC. Therapeutic inhibition of PI3K signaling could be applied in many different cancer types, and consequently many compounds are tested in clinical and pre-clinical trials. Different therapeutic strategies are tested, such as direct PI3K inhibition, inhibition of the PI3K signaling pathway at different positions or the co-inhibition of other crucial pathways (Heavey, O'Byrne and Gately, 2014). The latter two approaches are especially aimed at overcoming or avoiding resistance development that usually evolves when applying TKIs. A recent phase II clinical trial investigated the effectiveness of PX-866, a pan class I specific inhibitor of PI3K, in combination with chemotherapy in patients with relapsed or metastatic NSCLC (Levy et al., 2014). The trial failed to demonstrate improved survival. A major limitation, though, was that patients were not selected according to their mutation status and only very few harbored mutations in PI3K pathway genes.

3.3.6. NRAS

3.3.6.1. Definition and oncogenic mechanism:

The GTPase NRAS was originally identified in neuroblastoma cell lines and belongs to the RAS gene family. It shares conserved sequences with KRAS but the different RAS proteins have distinct signaling properties. The oncogenic mechanisms are similar, although NRAS mutations have a different nucleotide transversion profile compared to KRAS mutations. NRAS mutations in lung AdC mainly target codon Q61 in exon 3 (Ohashi et al., 2013), leading to loss of GTPase activity and constitutive signaling (Rodenhuis and Slebos, 1990).

3.3.6.2. Epidemiology:

Although NRAS mutations are much more frequent in melanoma or colorectal cancer, they are also found in approximately 1 % of lung AdC (Ohashi et al., 2013; Vujic et al., 2014). They appear mutually exclusive with other oncogenic driver mutations. To date there are not many publications addressing NRAS mutations in lung AdC. One study focused on the clinico-pathologic profile of NSCLC patients with NRAS mutations and found that they are correlated with active smoking status and possibly appear more frequently in African Americans (Ohashi et al., 2013).

3.3.6.3. Prognosis and therapy:

In the same study (Ohashi et al., 2013) it

was indicated that NRAS mutations may represent a factor for poor prognosis, at least in advanced stage disease (Ohashi et al., 2013). As with the therapy of KRAS-mutated lung AdC, there are no therapeutics available that directly inhibit mutant NRAS. It could be shown in cell culture models that NRAS-mutant cancer cells are sensitive to inhibition of downstream signaling molecules. MEK inhibitors (Ohashi et al., 2013) or dual-pathway inhibition via a combination of the MEK inhibitor trametinib with the PI3K/mTOR inhibitor GSK2126458 (Vujic et al., 2014) or the mTOR inhibiting, anti-diabetic metformin (Vujic et al., 2015) seem promising therapeutic approaches.

4. Limitations in oncogenic driver mutation detection

Lung AdC is nowadays recognized as a disease comprising distinct molecular subsets. About 60 % of lung AdC cases are driven by one of the oncogenic alterations presented in this review (Figure 1). It is striking, though, that the oncogenic driver mutation in approximately 40 % of lung AdC tumors is currently still unknown. One explanation for this large number may lay in the currently employed techniques for detection of oncogenic mutations. Most investigators focus on 'hotspot' locations within the respective gene, for example determined by the range amplified by PCR-based assay techniques. A recent study employed a next generation sequencing-method on lung AdC samples previously classified as negative for 11 oncogenic driver alterations (Drilon et al., 2015). They were able to identify mutations in a currently recognized oncogenic driver in 26 % of patients. In additional 39 % of patients an oncogenic mutation was detected that could be treated with compounds currently tested in clinical trials or in off-label use. In the remaining 35 % no mutation was detected or the detected mutations could not be treated with targeted therapy. It will be interesting to see how the application and implementation of novel, more in-depth technologies is going to close the gap for unknown driver mutations.

Another fact accounting for the 40 % of lung AdC without a known driver mutations is that the list of oncogenic driver mutations in lung AdC is continuously growing. Alterations in genes such as AKT1, MEK1, RET or RIT1 are currently considered as further discrete subtypes of lung AdC, and many more may follow.

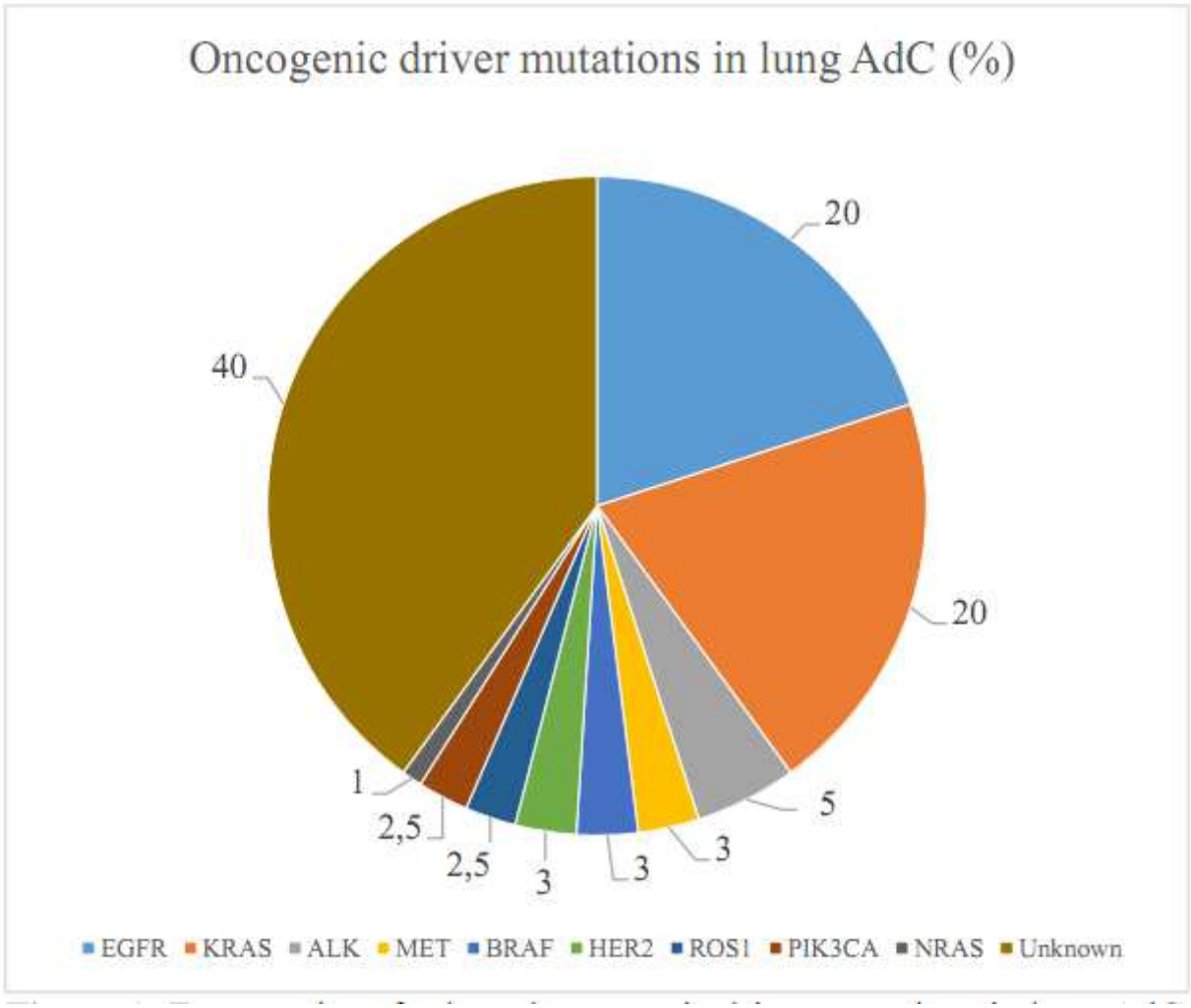

Figure 1: Frequencies of selected oncogenic driver mutations in lung AdC. For references see Table 1.

5. Conclusion

In summary, lung AdC is a disease comprising distinct molecular subtypes classified by oncogenic driver mutations. The type of mutation has implications on clinico-pathologic features and prognosis of patients. Several subtypes can be effectively treated with targeted therapy, yielding survival advantages compared to conventional therapy. However, despite considerable advances in our understanding of the underlying oncogenic mechanisms and effective targeted therapy options survival of patients could thus far not be sufficiently improved. TKI monotherapy has shown to frequently result in the development of resistance via different mechanisms.

Consequently there is a great need for novel diagnostic and therapeutic concepts. Novel diagnostic approaches could aim at analyzing the signaling pathways employed by tumors, rather than only focus on oncogenic driver mutations. This approach would deliver more detailed information on the molecular alterations of the tumor and thus detect more targets for interventions. Novel therapy options should also consider the blockage of multiple targets at the same time, rather than consecutively, to hamper resistance development. Also a combination of TKIs with immunotherapy might confer better disease control. Another aspect that should be translated into practice is that resistance development under targeted therapy should be monitored,

for example by repeated biopsies in regular intervals. This would allow quicker adaption of therapy to avoid disease relapse.

Overall, the concept of personalized medicine has largely entered the treatment algorithm in lung AdC. Ongoing implementation of molecular biological findings into clinical practice is warranted to further improve patient care.

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Table 1: Properties of selected oncogenic driver mutations in lung AdC

Affected gene	Type of alteration	Oncogenic mechanism	Frequency in lung AdC	Clinico-pathologic characteristics
Epidermal growth factor receptor (EGFR)	Mutation	Mutations alter structure of ATP binding pocket leading to prolonged activation upon ATP binding [1]	10 - 30 % [2]	Predominantly females, non-smokers, Asian ethnicity, non-mucinous tumors [3,4].
V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS)	Mutation	Mutations lead to loss of GTPase activity resulting in constitutive activation [1,2]	10 - 30 % [3]	Predominantly males, active or former smokers, non-Asian ethnicity, invasive mucinous adenocarcinomas [4,5,6].
Anaplastic lymphoma kinase (ALK)	Translocation	Cardoxyterminal portion of ALK is fused with aminoterminal portion of EML4; EML4 provides oligomerization motif, leading to constitutive dimerization and activation of fusion protein [1,2].	5 % [3,4]	Predomantly younger than average lung AdC population, never- or former light smokers, females [3,4,5,6].
Hepatocyte growth factor receptor (MET)	Mutation (M), amplification (A), overexpression (O)	Mutation: Erase cbl binding site; loss of cbl binding leads to decreased ubiquitination and downregulation [1]. Amplification/overexpression: High MET expression may cause receptor clustering and ligand-independend activation [2].	M: 3 % [3]; A: 2 - 20 % [4,5]; O: 13 - 69 % [6]	No correlations established [3,5].
V-raf murine sarcoma viral oncogene homolog B1 (BRAF)	Mutation	V600E predominant type in lung AdC; mutation disrupts inactive conformation of kinase domain leading to engangement of active conformation [1].	3% [2]	Association with smoking status is controversial [2,3,4]; no association with gender and stage [2]. In subgroup of V600E mutation more women and never-smokers [2].
Human epidermal growth factor receptor 2 (HER2)	Mutation	Main mutations are in-frame duplication/insertions leading to constitutive activation [1]; mutations disrupt binding of HSP90, that normally prevents dimerization [2].	2 - 4 % [3,4,5]	Predominantly never- or former very light smokers [3,4,5,6,7,8]; tendency to younger age [3,5,7].
V-Ros avian UR2 sarcoma virus oncogene homolog 1 (ROS1)	Translocation	Different fusion partners exist; exact oncogenic mechanism so far unknown and seems to depend on fusion partner [1].	2.5 % [2]	Predominantly younger than average lung AdC population [3,4], females, never-smokers and advances tumors [2].
Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit alpha isoform (PIK3CA)	Mutation	Mutation hot spots are located in helical domain and kinase domain. Helical domain: mutations increase lipid kinase activity [1] by disturbing autoinhibition via interaction with the inhibitory subunit p85 [2]. Kinase domain: mutations increase lipid kinase activity [3] by facilitating accessibility to substrate PIP2 [2].	2 - 3 % [4,5,6]	Most studies report no association with clinico-pathologic features [4,6,7].
Neuroblastoma ras viral oncogene homolog (NRAS)	Mutation	Mutations lead to loss of GTPase activity resulting in constitutive activation [1].	1% [2,3]	Predominantly current or former smokers and possibly higher prevalence in African americans [2].

Table 1: continued

Prognostic/predictive significance	Targeted therapy	References
When applying chemotherapy or resection no prognostic significance was found [5,6]; when applying EGFR TKIs progosis is improved [7].	Approved TKIs (erlotinib, gefitinib, afatinib) significantly improve survival and quality of life [7,8].	[1] Lynch et al., 2004; [2] Travis et al., 2011; [3] Shigematsu, Lin, et al., 2005; [4] Finberg et al., 2007; [5] Q. Zahng et al., 2014; [6] Z. Zhang et al., 2014; [7] W. Q. Zhang, Li and Li, 2014; [8] Melosky, 2014
Unfavorable factor for survival [7].	Direct inhibition showed little clinical efficacy, new approaches focus on indirect inhibition [8].	[1] Tong et al., 1989; [2] Rodenhuis and Slebos, 1990; [3] Travis et al., 2011; [4] Shigematsu, Lin, et al., 2005; [5] Finberg et al., 2007; [6] Ahrendt et al., 2001; [7] Mascaux et al., 2005; [8] Rothschild, 2015
When applying conventional therapy most studies report no influence on survival [6,7,8], but conflicting results have been published [5,9]. The application of ALK TKIs extends survival [10].	Approved TKIs (crizotinib, ceritinib, alectinib) extend survival and improve response rates [10,11].	[1] Soda et al., 2007; [2] Mano, 2008; [3] Kwak et al., 2010; [4] Fan et al., 2014; [5] Blackhall et al., 2014; [6] Shaw et al., 2009; [7] J. H. Paik et al., 2012; [8] Takeda et al., 2012; [9] Yang et al., 2012; [10] Qian et al., 2014; [11] Iragavarapu et al., 2015
Increased MET gene copy number and protein overexpression are associated with worse OS [6,7]. Currently no data on prognostic significance of MET mutations.	Different TKIs and antibodies are investigated in clinical trials [8].	[1] Kong-Beltran et al., 2006; [2] Lutterbach et al., 2007; [3] Onozato et al., 2009; [4] Schildhaus et al., 2015; [5] Beau-Faller et al., 2008; [6] Guo et al., 2014; [7] Dimou et al., 2014; [8] Sadiq and Salgia, 2013
In general no association with survival could be established [3,5,6,7,8]. One study reported V600E mutations as factor for worse prognosis [7].	Drugs targeting V600E BRAF already approved for melanoma and currently tested in NSCLC [9].	[1] Wan et al., 2004; [2] D. Chen et al., 2014; [3] Villaruz et al., 2015; [4] Luk et al., 2015; [5] P.K. Paik et al., 2011; [6] Litvak et al., 2014; [7] Marchetti et al., 2011; [8] Cardarella et al., 2013; [9] Sanchez-Torres et al., 2013
Contradictory results have been published [3,5,7].	Different TKIs and antibodies are investigated in clinical trials [9].	[1] S. E. Wang et al., 2006; [2] Telesco et al., 2011; [3] Arcila et al., 2012; [4] Li et al., 2012; [5] Suzuki et al., 2015; [6] Mazieres et al., 2013; [7] Tomizawa et al., 2011; [8] Shigematsu, Takahashi, et al., 2005; [9] Peters and Zimmermann, 2014
No difference in survival when applying conventional therapy [4], but survival advantage when treated with ROS1 TKI [5].	Ongoing clinical trials on different compounds [1].	[1] Davies and Doebele, 2013; [2] Zhu et al., 2015; [3] Bergethon et al., 2012; [4] Y. F. Chen et al., 2014; [5] Scheffler, Schultheis, et al., 2015
When regarding all types of PIK3CA mutations no difference in survival was found [5,6,7]. When subdividing by type of PIK3CA mutation or single- vs. multiple mutations, contradictory results have been published [4,6,7].	Different TKIs are investigated in clinical trials and further therapeutic strategies are pursued [8].	[1] Kang, Bader and Vogt, 2005; [2] Gabelli et al., 2014; [3] Samuels and Velculescu, 2004; [4] Chaft et al., 2012; [5] Scheffler, Bos, et al., 2015; [6] L. Wang et al., 2014; [7] L. Zhang et al., 2013; [8] Heavey, O'Byrne and Gately, 2014
Preliminary data suggest worse survival [2].	Currently no targeted therapy available, preclinical studies focus on inhibiting downstream molecules [2,3,4]	[1] Rodenhuis and Slebos, 1990; [2] Ohashi et al., 2013; [3] Vujic et al., 2014; [4] Vujic et al., 2015