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Stratified Treatment in Lung Cancer

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Summary
Even though great efforts have been made to improve chemotherapy-based treatment approaches for lung cancer, the prognosis of patients with advanced and metastasized disease remains particularly poor. In recent years, a growing number of genetic aberrations driving lung cancer have been identified. Targeted inhibition of some of these aberrations, most prominently mutated EGFR and ALK, by tyrosine kinase inhibitors has dramatically increased efficacy and tolerability of systemic lung cancer treatment in subsets of patients. However, the duration of response is limited due to the acquisition of molecular mechanisms of resistance to targeted treatment. Modern next-generation inhibitors aim to break resistance. A deep understanding of the mechanisms of treatment failure is imperative to the development of new approaches. In this review, we focus on the current status of stratified therapy in lung cancer and highlight new, potentially promising treatment approaches.

Principles of Stratified Medicine

While for decades lung cancer subtype classification and therapeutic strategies were restricted to histology and morphology only, the disease now has turned out to be highly heterogeneous on the genomic level. This development does not only help us to understand the clinical heterogeneity of lung cancer, but also enables us to achieve therapeutic breakthrough advances by developing biological-rational treatment approaches based on a precise understanding of the molecular mechanisms underlying malignant transformation and its inhibition.

First-line treatment with cisplatin and etoposide has been standard of care for metastasized small cell (SCLC) and non-small cell lung cancer (NSCLC) for many years. This paradigm changed with the introduction of second-generation chemotherapeutic agents such as paclitaxel, docetaxel, and gemcitabine. Platinum-combinations with these drugs were shown to be superior to cisplatin and etoposide in NSCLC patients and defined new standards [1–4]. Newer chemotherapeutic agents, such as pemetrexed and antiangiogenic drugs, have moderately increased efficacy and tolerability of conventional treatment predominantly in adenocarcinoma [5–9]. However, response rates and overall survival have only marginally improved with non-stratified treatment approaches. With a range of 1 year, median overall survival remains almost unchanged and it becomes evident that the limits of conventional systemic approaches have been reached.

Since the discovery of oncogenic *EGFR* mutations in a distinct subgroup of NSCLC patients and the first evidence of the high efficacy of EGFR inhibition in these, a big step forward has been made for an increasing number of genetically defined NSCLC sub-entities. More and more molecularly stratified treatment strategies were successfully tested and implemented into clinical routine in the recent years [10–18]. Broad comprehensive analyses of patients' characteristics and genetic tumor profiles were undertaken by several groups in order to better understand the natural history of lung cancer and the characteristics of the different genetic subgroups [19–21]. Large molecular screening and treatment networks have been established in different countries to implement comprehensive genetic testing and stratified treatment into clinical routine. Through these efforts the number of patients receiving stratified treatment increased steadily to about 20% of all lung cancer patients nowadays. However, for the majority of patients no promising targeted treatment approaches could be established so far, including tumors with complex genetic profiles like aberrations in tumor suppressor genes or G-proteins, which are mostly found in tobacco-triggered disease [22]. For these patients, immune checkpoint inhibitors are now a new and promising option. This review will focus on stratified therapies directed against transforming genetic aberrations.

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Stratified treatment follows a recurrent principle, which should not be confounded with targeted treatment in unselected patients, e.g. antiangiogenic treatment. The difference lies in the identification of molecular targets triggering oncogenic growth (dependency) and thus predicting efficacy through specific inhibition (vulnerability).

Resistance and New Generation TKIs

Like other malignancies, lung cancer is characterized by a high genomic instability and increased mutation rate [23]. The constant acquisition of genetic aberrations and treatment pressure by targeted antineoplastic drugs result in the selection of desensitized tumor cells following Darwinian rules [24]. Following stratified tyrosine kinase inhibitor (TKI) treatment, resistance is often mediated through secondary mutations in the target gene preventing the enzymatic inhibition [25]. Substitution of the 'gatekeeper' threonine for example is a recurrent pattern in different cancer entities after treatment with targeted drugs [25].

Next-generation TKIs are designed to overcome resistance mediated by secondary mutations and have shown to be highly successful in NSCLC [25–28].

Owed to intra-tumor heterogeneity, resistance may alternatively be mediated by aberrations independent of the target gene or by more than one mechanism simultaneously [29, 30]. This complexity represents a growing challenge and combination therapies are tested to overcome resistance through multiple factors [29, 31, 32]. The heterogeneous nature of resistance makes tissue analysis by thorough genetic profiling indispensable to treatment decision-making.

EGFR

Mutations in *EGFR* have been the first targetable aberrations to be identified in lung cancer [33, 34]. Activating *EGFR* mutations are detected in approximately 10 % of Caucasian NSCLC patients and the most common aberrations include the substitution L858R in exon 21 and a variety of small deletions in exon 19 [19–21, 33, 34]. With the majority of *EGFR*-mutant NSCLC patients being non-smokers and female, this subgroup is characterized by distinct clinico-pathological features [19–21, 35]. Superiority in terms of response rates and progression-free survival (PFS) of the first-generation TKIs gefitinib and erlotinib in first-line compared to chemotherapy was proven in several trials [10–13, 15, 36]. However, due to cross-over of chemotherapy patients to TKI therapy after progression, overall survival (OS) was not significantly improved with either gefitinib or erlotinib. In a cohort analysis of the LUX-Lung 3 and 6 trials, the second-generation inhibitor afatinib was shown to prolong OS in *EGFR* del19-positive NSCLC patients [37]. Although an OS benefit could not be demonstrated in randomized trials, there is no doubt that treatment with any EGFR TKI significantly prolongs OS in *EGFR*-mutated patients compared to chemotherapy. In a retrospective analysis of the German Network Genomic Medicine, the median OS with chemotherapy treatment was significantly longer with EGFR TKI treatment as compared to chemotherapy (31.5 vs. 9.6 months, HR 0.169) [21]. A comparable OS benefit was shown in analyses by the US Lung Cancer Mutational Consortium and the French Cooperative Thoracic Intergroup [19, 20].

Even though big advances were made with the clinical use of EGFR TKIs, median PFS does not exceed 12–14 months and treatment inevitably fails due to the acquisition of molecular resistance. In approximately 60 % of cases a secondary mutation of the 'gatekeeper' threonine in exon 20 of *EGFR*, *EGFR* T790M, is responsible for the desensitization to EGFR TKIs [25, 38, 39]. Modern third-generation TKIs are able to overcome resistance in patients with *EGFR* T790M-positive NSCLC [27, 28]. Osimertinib, so far the only drug approved in this setting by the Federal Drug and Food Administration (FDA) and the European Medicines Agency (EMA), showed a response rate (RR) of 61 % and a PFS of 9.6 months in a phase I trial [40]. Other third-generation EGFR TKIs such as EGF816 and olmutinib (BI 1482694/HM61713) are in clinical development and show similar results regarding efficacy and safety [41–43].

Resistance to EGFR TKI may not be restricted to the acquisition of a single mechanism. Co-occurrance of *EGFR* T790M and other mechanisms such as amplification of *MET* and *HER2* or the activation of the MEK/ERK pathway were found in subsets of patients [29–32, 44]. Single agent treatment with third-generation EGFR TKIs seems to be insufficient in this setting [29, 30, 32, 44, 45]. Trials combining TKIs that target EGFR as well as MET or MEK are currently enrolling patients (NCT02143466; NCT02335944).

Several mechanisms of acquired resistance to third-generation TKIs were recently identified, including the same EGFR-independent mechanisms that mediate resistance to first- or second-generation EGFR TKIs [29, 44]. The substitution mutation *EGFR* C797S inhibits third-generation EGFR TKIs to bind to the protein and block the enzymatic activity, thus mediating resistance to the treatment [46, 47]. Currently, no drug is in clinical testing to break this mechanism of acquired resistance.

Whether third-generation EGFR-TKIs will be used in first-line, is a topic of ongoing discussion. Preliminary results of osimertinib in first-line treatment, showing a PFS of about 20 months are promising [48].

ALK

Rearrangements of the *ALK* gene predominantly affect never-smokers of younger age and are found in approximately 5% of NSCLC patients [19–21, 49–51]. Upon the results of superiority in efficacy (RR, 74% vs. 45% and PFS, 10.9 vs. 7.0 months; HR, 0.45) and safety of first-line crizotinib compared to platinum-based chemotherapy, the drug recently received approval by the EMA and the FDA for expanded use in *ALK*-positive patients [16, 52].

Acquisition of secondary mutations in *ALK* is a common mechanism of resistance to crizotinib [26, 53, 54]. However, resistance factors independent of *ALK* have been described [53]. The development and approval of next-generation ALK inhibitors, that break resistance to crizotinib, has been remarkably fast. With re-

sponse rates around 50% the second-generation TKIs ceritinib and alectinib, have shown high efficacy in the setting of crizotinib resistance [17, 18, 55].

Up to 30% of patients with *ALK*-rearranged NSCLC have brain metastases at baseline and a large part of patients treated with crizotinib will experience isolated progression of central nervous system (CNS) metastases [56–58]. Intracranial RR of crizotinib in patients with untreated CNS metastases is 18% and thus much lower than the extracranial RR [57]. A possible explanation for the recurrent CNS failure of crizotinib may lie in the low blood-brain-barrier penetration of the drug [56, 57]. Second-generation ALK inhibitors exhibit a higher CNS penetration and subgroup analyses of study data suggest a higher activity in brain metastases than crizotinib [58, 59]. Still, the question will need further clinical investigation and low patient numbers as well as heterogeneous patient selection make comparison between the different trials difficult.

At this time, 2 potential treatment alternatives remain in patients with isolated CNS progression – treatment beyond progression with crizotinib and local treatment of CNS metastases or switch to a CNS penetrable ALK TKI [55]. Recently, the next-generation ALK inhibitors lorlatinib and brigatinib have been brought into clinical evaluation (NCT01970865; NCT01449461; NCT02737501). First results of lorlatinib showed RR of 42 % in patients who exhibit progression on 2 or more ALK TKIs. In patients who have received treatment with 1 TKI, lorlatinib and brigatinib show similar RR around 60% [58, 60].

ROS1

Like aberrations in *EGFR* and *ALK* oncogenic rearrangements of *ROS1* predominantly affect young never-smokers and are associated with a favorable prognosis. Prevalence is low, ranging between 1 and 2% of the overall NSCLC population [45, 49, 61, 62]. The ALK/ROS1/MET inhibitor crizotinib is the first drug to show efficacy in this patient subgroup. The phase I trial by Shaw and colleagues found a RR of 72% with a median PFS of 19.2 months and retrospective analyses showed similar efficacy [63]. Further prospective trials, including an international European trial (EUCROSS; NCT02183870), are testing crizotinib in this patient subgroup and are likely to confirm this data. Recently, the EMA and the FDA granted conditional approval for crizotinib treatment in *ROS1* rearranged NSCLC, setting a new standard of care for these patients.

Following the same biological rules, targeted therapy in *ROS1*-positive patients results in the acquisition of resistance [64–68]. Unlike EGFR and ALK TKI resistance, resistance to crizotinib in *ROS1*-positive patients is not well understood. Only a few secondary mutations in *ROS1* have been described so far and *ROS1*-independent mechanisms of resistance may be an important factor [69, 70]. The multi-target inhibitor cabozantinib showed efficacy in pre-clinical models and single patient cases with acquired resistance to crizotinib and the resistance mutations G2032R, D2033N and L2026M [65, 67, 68]. More selective drugs such as the next-generation ALK/ROS1 TKI lorlatinib are in clinical investigation and showed promising efficacy in this setting [71].

MET

The oncogenic potential of the receptor tyrosine kinase (RTK) MET may be triggered by different mechanisms of activation in multiple tumor entities [72–75]. In NSCLC, activation of MET was reported by overexpression in 22–25%, by high-level amplification in 3% and most recently by exon 14 skipping mutations in approximately 3% of patients [73, 76, 77]. Amplification of *MET* is associated with worse survival after resection and in patients with advanced cancer stage [73, 78].

Various MET inhibitors or monoclonal antibodies have been in clinical development for many years now and most have failed to prove convincing efficacy. The most prominent example is the MET antibody onartuzumab [79]. Based on the promising phase II results of onartuzumab in combination with erlotinib in patients with high MET expression, a phase III trial was launched to test this combination in this patient subgroup. The trial was stopped for futility, and a debate emerged on the possible reasons, focusing on the misleading phase II results and the insufficient molecular selection of patients due to unsuccessful definition of biomarkers.

Modern, more potent MET inhibitors such as crizotinib and capmatinib have shown high efficacy in patients with high-level amplification or expression of *MET* as well as *MET* splice site mutations in clinical trials, retrospective analyses, and case reports [77, 80–83]. However, further comprehensive analyses are needed to define reliable cut-offs for true *MET* positivity predicting response to MET inhibition.

BRAF

Mutations in *BRAF* may have different effects on the encoded RTK. Mutations involving codon V600 are activating, whereas mutations involving other bases may be inactivating. However, both kinds of mutations harbor oncogenic potential and were described in 2–4% of NSCLCs [19–21, 84–86]. *BRAF* mutations are predominantly found in adenocarcinomas and patients share similar epidemiological characteristics with the overall NSCLC population. Inhibitors of BRAF have long been used in patients with *BRAF* V600-mutated melanoma [87, 88]. Recently, clinical trials have shown moderate activity of the BRAF inhibitors dabrafenib and vemurafenib in V600-mutated NSCLC as monotherapy [89, 90]. RR are considerably increased to 63 % when dabrafenib is combined with the MEK inhibitor trametinib, whereas, toxicity profiles are similar. In patients with *BRAF* V600E-positive lung cancer suffering from relapse after standard chemotherapy, treatment with this combination should be considered either in clinical trials or off-label.

New Targets in SCLC and NSCLC

The number of biomarkers accessible to targeted treatments is continuously increasing in adenocarcinomas of the lungs. In squamous cell lung cancer and SCLC major screening efforts have long failed to identify targetable aberrations.

Most recently, a promising targeted approach was brought to clinical development in SCLC. A major fraction of 72–85% of SCLC shows an overexpression of the delta-like 3 surface protein (DLL3), a marker for neuroendocrine phenotype [91]. These findings led to the development of the antibody-drug conjugate (ADC) rovalpituzumab teserine (Rova-T) targeting DLL3. An early clinical trial presented at ASCO 2016 showed a RR of 30% in patients with SCLC harboring DLL3 expression of ≥50% independent of the number of prior lines of therapy [92]. Trials further investigating the drug in DLL3-positive SCLC patients in different lines of therapy have been launched (NCT02674568, NCT02819999).

The discovery of amplifications of *FGFR1* in SCLC has led to the investigation of several FGFR inhibitors in these tumors [93–95]. However, the results of early clinical trials with lucitinib, BGJ398, dovitinib or ADZ4547 in *FGFR1*-amplified NSCLC have been disappointing [96–98]. Nevertheless, long-lasting responses are proof-of-principle for the activity in patients with *FGFR1*-amplified NSCLC [98]. Yet, it is unclear what co-occurring factors influence response to FGFR inhibition or whether amplification assessed by FISH is the right biomarker for prediction of response.

Rearrangements of *NTRK1*, encoding the RTK TRKA have recently been found in 0.1% of all NSCLC patients and in 3% of driver-oncogene negative adenocarcinoma patients [99–101]. Patients with *NTRK1*-rearranged NSCLC are particularly young and predominantly never-smokers. The pan-TRK inhibitors entrectinib and LOXO-101 are subject to clinical development and show promising activity in pre-clinical models and early clinical setting [68, 99, 102–104].

Other genetic aberrations such as mutations in *HER2* or rearrangements of *RET* have been reported in subsets of patients with NSCLC. TKIs and monoclonal antibodies that potentially inhibit the two proteins do exist, but clinical evaluation is still ongoing [49, 105–107].

How and What to Test?

One of the most crucial challenges of precision medicine lies in the detection of the increasing number of genetic aberrations that are subject to targeted drugs. Currently, conventional approaches by sequential single gene testing are widely applied. Tissue from biopsies of lung cancer is often restricted and requires reasonable processing. The amount of DNA needed for single gene based molecular pathologic characterization of a tumor increases with every sequential analysis. Starting with the most likely aberration, it may be possible that rare oncogenic events are not identified for the lack of further tumor tissue. The main hurdle of modern diagnostics are therefore the need to test an increasing number of genetic aberrations in a limited amount of tumor tissue.

Next generation sequencing (NGS) approaches are able to overcome these difficulties. Targeted massively parallel (MPS) or hybrid-capture based DNA-sequencing allow the analysis of multiple amplicons of a pre-defined subset of relevant genes of a large number of individuals in a single run [19–21, 108, 109]. Aberrations can be detected on single-nucleotide level, such as point mutations in *EGFR* or *KRAS*, or at a larger scale, including deletions, amplifications, or rearrangements. In clinical routine, however, amplifications and rearrangements are often detected by fluorescence in-situ hybridization, which is the most evaluated method and still the gold-standard in most cases.

Modern approaches to detect and sequence cell-free tumor DNA (cfDNA) have been developed to detect genetic aberrations on nucleotide level [110, 111]. The third-generation EGFR inhibitor osimertinib has been approved for the treatment of patients with *EGFR* T790M detected by cfDNA analyses. However, tumor heterogeneity responsible for more complex resistance profiles, such as *MET* or *HER2* amplification, may not be detected by conventional single gene sequencing technologies [44]. The value of liquid biopsies in the treatment of lung cancer, especially in the resistance setting needs further evaluation.

Recommendations on what genes to test in metastasized NSCLC are inconsistent and underlie constant change. A recently published consensus report from the Molecular Analysis for Personalised Therapy Conference (MAP) recommends testing for mutations in *EGFR, ALK,* and *ROS1* in daily routine but also recommends testing of further 20 genes within screening programs to allow patients the participation in clinical trials or off-label treatment [112]. With an increasing number of potential treatment options as outlined above, determination of EGFR, ALK, and ROS1 status is insufficient nowadays. Large European screening platforms like the Network Genomic Medicine or the French Cooperative Thoracic Intergroup have proven to efficiently perform screening for a large subset of relevant genes in huge numbers of NSCLC patients [19, 21].

Conclusions

An increased understanding of the molecular mechanisms underlying malignancy has led to a new perception of lung cancer as a disease of many genetically defined subgroups. This has enabled the development of biologically rational stratified treatment strategies against molecular alterations defining these subgroups. Today, about 20% of lung cancer patients benefit from such approaches either with approved drugs or in clinical trials. For patients with oncogenic aberrations in *EGFR*, *ALK,* and *ROS1* specific kinase inhibitor therapy has already become standard of care. Genetic aberrations in *BRAF*, *HER2*, *RET*, *MET*, *NTRK,* and others are in clinical evaluation. More and more the biological mechanisms underlying the resistance to TKI therapy are understood and enable the successful therapy with next-generation inhibitors, in particular in *EGFR*-mutated and *ALK*-positive patients. The application of these stratified therapies requires broad molecular testing of tumor biopsies and, in the near future, possibly also blood, at first diagnosis as well as in relapse. In view of the enormous dynamics in the field a particular challenge of this development is the reorganization of the collaboration between highly specialized academic centers, community hospitals, and private practice-based physicians to enable access of all lung cancer patients to these therapeutic options.

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