Advanced non-squamous non-small-cell lung cancer: who and when should be biologically screened today? Tomorrow?

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Abstract There is a need for identification of prognostic and/or predictive biomarkers to improve the choice of therapeutic strategies for patients diagnosed with advanced non-squamous NSCLC. Only two predictive biomarkers have been validated: *EGFR* mutations and *EML4-ALK* translocations. These markers can be used to predict the response to targeted therapy (erlotinib and gefitinib or crizotinib). Several emerging biomarkers are being studied in ongoing clinical trials to predict the response of new targeted therapy or resistance to *EGFR*-TKI or *ALK* inhibitors. Every patient diagnosed with advanced NSCLC should be screened for prognostic and predictive biomarkers. There is no longer any place for clinical screening of patients potentially eligible for biological analysis. The ASCO recommends analysis of biomarkers at the time of diagnosis. However, there is evidence supporting the need for serial analysis. The purpose of these data and recommendations is to individualize therapeutic strategies for patients with advanced NSCLC and to improve their survival.

Keywords Non-small-cell lung cancer · Non-squamous · Advanced · Biomarkers · Biological screening · *EGFR* · *EML4-ALK*

Introduction

Lung cancer is still the leading cause of death by cancer worldwide, with more than 951,000 deaths recorded in 2008 [1]. Approximately 50 % of NSCLC are advanced at the time of diagnosis. Prognosis of advanced non-small-cell lung cancer (NSCLC) has been improved over the last two decades with the development of third-generation cytotoxic chemotherapy. However, patients with advanced NSCLC still have a median survival of only 8 to 12 months and one-year survival was 30 to 40 % in 2002 [2]. These data highlight the need for prognostic and predictive biomarkers to improve clinical outcome for patients with NSCLC and to individualize therapeutic strategies.

The National Cancer Institute defines a biomarker as a biological molecule found in blood, other fluids, or tissues that is a sign of a normal or abnormal process or of a condition or disease. A predictive biomarker is used to predict response to treatment [3].

Approximately 50 % of advanced NSCLC are adenocarcinomas and their incidence is increasing. Consequently, this histological type was the most studied for assessment of biomarkers. First, studies focused on the search for predictive biomarkers for response to cytotoxic chemotherapy. *ERCC1* (excision repair cross-complementation group 1) is involved in DNA repair and can repair bulky DNA adducts induced by platinum chemotherapy. Recently, a meta-analysis showed that high *ERCC1* expression was associated with reduced response to platinum-based chemotherapy (average RR=0.80; 95 % CI 0.64–0.99) [4]. However, this biomarker is not used in routine practice because there are many polymorphisms that cannot predict response to chemotherapy and because molecular analysis of *ERCC1* expression is not sufficiently reproducible [5]. Other biomarkers, for example *RRM1* and *BRCA1*, were studied in this field but none is validated for clinical practice [6].

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Clinical trials are of individualized chemotherapy based on BRCA1 and RRM1 mRNA are ongoing (NCT 01424709 and NCT 00705549) [7].

Recently, our knowledge of the mechanisms of carcinogenesis has increased [8••]. Moreover, Ding et al. studied the most common mutations in advanced lung adenocarcinomas and shed a new light on several key pathways involved in carcinogenesis [9]. These data enable the identification of potential targets for treatment of NSCLC and the development of targeted therapy, which are drugs or other substances that block the growth and spread of cancer by interfering with specific molecules involved in tumor growth and progression. Biomarkers were then developed to predict response to targeted therapy and to individualize patients with potential good response to specific therapeutic strategies.

The purpose of this review is to identify which biomarkers should be screened today and tomorrow, which patients with advanced non-squamous NSCLC are involved, and when is the best time for biological screening.

Which biomarker should be screened?

Today

The only biomarkers approved for clinical practice for advanced non-squamous NSCLC are *EGFR* (epidermal growth factor receptor) mutations, predictive for the response to EGFR-tyrosine kinase inhibitors (EGFR-TKI), for example erlotinib and gefitinib, and *EML4-ALK* translocations, predictive for the response to crizotinib, which is another TKI.

EGFR is the most studied of all lung cancer predictive biomarkers. EGFR is a trans-membrane tyrosine kinase receptor and belongs to the HER family. The binding of the ligand to the EGFR extracellular domain leads to dimerization of the receptor and to phosphorylation of its intracellular domain. Several intra-cytoplasm pathways are then activated: the KRAS, BRAF, MEK pathway, the PI3K, AKT, mTOR pathway, and the STAT pathway. All these pathways lead to several mechanisms of lung carcinogenesis (cellular proliferation, survival, and inhibition of apoptosis) [10]. *EGFR* gene mutations were first described in 2004. They are found in approximately 12 % of lung adenocarcinomas and are located in exons 18 to 21 [11]. Two small-molecule EGFR-TKI were developed (erlotinib and gefitinib). A Spanish team showed the feasibility of routine screening for *EGFR* mutations and the importance of these mutations as a predictive marker of response to EGFR-TKI [12]. In the same way, the French National Cancer Institute organized systematic screening of seven biomarkers for all patients with advanced non-squamous NSCLC everywhere

in France. More than 20000 analyses were performed in 2011 [13]. Moreover, gefitinib and erlotinib improve progression-free survival (PFS) of patients with EGFR-mutant NSCLC in comparison with first-line chemotherapy: hazard ratio for progression or death was 0.48 (95 % CI 0.36–0.64; $p<0.001$) for gefitinib and 0.37 (95 % CI 0.25–0.54; $p<0.0001$) for erlotinib [14, 15]. EGFR was then the first predictive biomarker approved for management of advanced NSCLC.

EML4-ALK translocations were first described in 2007 [16] and arise because of an inversion within chromosome 2p, leading to a fusion transcript which is another trans-membrane tyrosine kinase protein. EML4-ALK is involved in carcinogenesis for only 5 % of NSCLC patients. Shaw et al. studied clinical features and outcomes for patients with NSCLC and EML4-ALK translocation in comparison with EGFR-mutant and wild-type NSCLC. Patients with EML4-ALK were significantly younger ($p<0.001$), were more likely to be men ($p=0.03$), and were never or light smokers ($p<0.001$). Eighteen of the 19 EML4-ALK tumors were adenocarcinomas, predominantly the signet ring cell subtype [17]. EML4-ALK patients seem to be less sensitive to EGFR-TKI and more sensitive to pemetrexed. Crizotinib is a TKI targeting EML4-ALK translocation. The PROFILE phase II and III trials studied the efficacy and tolerance of crizotinib in second and third-line treatment of advanced NSCLC with EML4-ALK translocation. Preliminary results of the 2007 study were discussed at the ESMO congress. Crizotinib improved progression-free survival in comparison with pemetrexed or docetaxel (7.7 versus 4.2 and 2.4 months, respectively) [18]. This study is still in progress. Crizotinib already has the FDA (Food and Drug Administration) and EMEA approval and EML4-ALK translocations are a new predictive biomarker routinely used for advanced NSCLC.

Tomorrow: emerging biomarkers

Many molecular biomarkers are being investigated for advanced non-squamous NSCLC, for instance molecular markers of resistance to EGFR-TKI. Indeed, even if *EGFR*-mutant NSCLC usually respond well to EGFR-TKI, every patient finally progresses, and median progression-free survival does not exceed 10 to 18 months [19]. Acquired resistance is defined by systemic progression of the disease in accordance with RECIST (response evaluation criteria in solid tumors) or WHO (world health organization) criteria appearing while on continuous treatment with erlotinib or gefitinib for patients with *EGFR*-mutant NSCLC or after a previous clinical benefit for more than 12 weeks [20]. Sequist et al. studied 37 patients with *EGFR*-mutant NSCLC who acquired resistance to EGFR-TKI. The most common molecular markers of resistance found in this study were

T790M *EGFR* mutations, *MET* amplifications, and *PI3K* mutations, which is consistent with literature [21]. More recently, *EML4-ALK* translocations were also described as predictive markers of acquired resistance to EGFR-TKI [17]. Primary resistance is defined as the lack of response to EGFR-TKI since first tumor assessment. The most studied biomarkers of primary resistance to EGFR-TKI are *KRAS* mutations [22] and *HER2* mutations [23]. These mutations can activate downstream signaling pathways independently of EGFR and induce survival and proliferation even in cases of EGFR inhibition. Analysis of these biomarkers of resistance to EGFR-TKI may refine prediction of sensitivity to EGFR-TKI for first-line treatment of *EGFR*-mutant NSCLC.

Furthermore, these biomarkers are potential targets for new treatments in development [24]. Indeed, T790M *EGFR* mutation changes the three-dimensional conformation of EGFR and reduces its affinity for erlotinib or gefitinib [25]. This de-novo mutation of *EGFR* is responsible for 50 % of acquired resistance to EGFR-TKI and can be targeted by EGFR irreversible inhibitors, for example afatinib, which was studied in the LUX-Lung clinical trial program [26] or PF-299804, for which results were promising in comparison with erlotinib in second or third-line treatment of NSCLC [27]. In the same way, *MET* is a transmembrane tyrosine kinase receptor and *MET* amplification is responsible for EGFR-TKI resistance because of the persistent activation of the PI3K/AKT/mTOR pathway, despite EGFR inhibition [28]. *MET* is a target for a TKI (tivantinib) and a monoclonal antibody. Both were studied in second or third-line treatment of NSCLC in association with erlotinib in comparison with erlotinib alone, and promising results were obtained for both in phase 2 studies [29, 30]. Phase 3 studies are ongoing (NCT 01456325 and NCT 01244191) [7]. PI3K are cytoplasmic molecules conducting proliferation and survival signals to nuclear effectors. *PI3KCA* gene mutations are found in 2 % of NSCLC and activate a downstream pathway independently of EGFR activation [31]. PI3K is a target for several new molecules undergoing investigation in phase 1 clinical trials [32]. *HER2* is another tyrosine-kinase receptor known to be the target of trastuzumab, validated in the treatment of breast cancer and investigated in NSCLC. *HER2* mutations seem to be a predictive biomarker for the response of *HER2* inhibitors, for example trastuzumab or afatinib, for treatment of NSCLC [33, 34]. *KRAS* is the most frequent mutated oncogene in non-squamous NSCLC and is found mutated in approximately 30 % of patients. Many strategies have been developed to target *KRAS* or other molecules of the *KRAS/BRAF/MEK/ERK/MAP-kinases* pathway. Several new treatments are still being investigated in phase 1 studies [35].

Other biomarkers, for example *BRAF* mutations, are being investigated as potential targets for new treatments.

BRAF is a cytoplasmic protein and is part of the *KRAS* signaling pathway. *BRAF* mutations are found in approximately 1 to 3 % of lung adenocarcinomas. *BRAF* is targeted by GSK-2118436, investigated for treatment of advanced NSCLC patients with the V600E *BRAF* mutation in a phase 2 study (NCT01336634) [32].

Analysis of these emerging biomarkers may improve prediction of sensitivity to EGFR-TKI and enable screening of patients potentially eligible for clinical trials investigating new bio-guided therapy.

Who should be biologically screened?

The clinical features associated with *EGFR* mutations or *EML4-ALK* translocations are already known. Shigematsu et al. studied DNA samples isolated from 617 NSCLC patients. *EGFR* mutations were statistically more frequent in never smokers (51 % versus 10 % in ever smokers), in adenocarcinomas (40 versus 3 % for other histology), for East Asian ethnicity (30 versus 8 % for other ethnicity), and for females (42 versus 14 % in males) [36]. In the same way, *EML4-ALK* translocations are known to be found more often in never smokers diagnosed with an adenocarcinoma of the lung, especially the signet ring cell subtype [17].

On the basis of these data, several teams decided to screen only patients who had a high probability of harboring the *EGFR* mutation or *EML4-ALK* translocation. However, D'Angelo et al. studied the incidence of *EGFR* mutations for men or smokers and showed that if only women who were never smokers were tested, 57 % of *EGFR* mutations would be missed [37]. Biomarkers should, then, be screened for all patients with advanced adenocarcinoma of the lung. Indeed, the American Society of Clinical Oncology (ASCO) recommends *EGFR* mutations testing to predict the benefit of an EGFR-TKI for all patients with advanced NSCLC before first-line treatment [38]. In the same way, the European Society of Clinical Oncology (ESMO) recommends *EGFR* mutation testing for all patients with advanced NSCLC and the search for *EML4-ALK* translocations in countries where crizotinib is available [39].

When should patients be screened?

Because EGFR-TKIs are approved for first-line treatment of advanced NSCLC, the ASCO recommends screening for *EGFR* mutations at the time of diagnosis. Screening for EGFR-TKI-resistance factors, for example *KRAS*, *PI3K*, *HER2* mutations or *MET* amplifications should also be done at the time of diagnosis to refine the prediction of sensitivity to EGFR-TKI.

However, some resistance biomarkers, for example the T790M *EGFR* mutation, were initially described as appearing secondarily after EGFR-TKI treatment. In fact, sensitive

techniques are able to detect T790M mutations in EGFR-TKI-naïve tumors, and these resistant clones are probably selected after exposure to gefitinib or erlotinib, but most routinely used techniques are not able to detect T790M mutations at the time of diagnosis [40]. For this reason new biopsies should be conducted at the time of progression for patients with acquired resistance to EGFR-TKI. Indeed, these biomarkers of resistance to EGFR-TKI are targetable by new drugs investigated in ongoing clinical trials. In the same way, several emerging biomarkers are targetable by new drugs [24]. Identification of these biomarkers at the time of diagnosis or at the time of progression is the first step to screening patients for inclusion in these clinical trials. Sequist et al. reported several cases of patients with *EGFR*-mutant NSCLC who had serial biopsies at the time of each disease progression. Genotyping of several resistance or emerging biomarkers revealed a change in mutations with loss of some mutations that can reappear further, because of selective pressure of EGFR-TKI [21]. These data also emphasize the need for new biopsies at each time of progression.

Crizotinib, targeting *EML4-ALK* translocations, is approved by the FDA for second or third-line treatment of advanced NSCLC. However, it is still being investigated in ongoing clinical trials for first-line treatment in comparison with standard cytotoxic chemotherapy. The search for *EML4-ALK* translocations is, then, recommended at the time of diagnosis to screen patients eligible for crizotinib, whether in a clinical trial or not [38]. As for EGFR-TKI, some mechanisms of resistance to crizotinib of patients with the *ALK* gene rearranged have been described. Indeed, Doebele et al. studied tumor samples from 14 patients with *ALK* (+) NSCLC resistant to crizotinib. Several mechanisms of resistance were observed: novel *ALK* mutation (G1269A), *ALK* copy number gain, *EGFR* mutation, and *KRAS* mutation. For some patients *EML4-ALK* translocation has disappeared [41]. These resistance mutations should, then, be screened at the time of diagnosis and at the time of progression and genotype changes after targeted treatment exposure.

Conclusions

The need for identification of predictive biomarkers to improve the choice of therapeutic strategies for each patient diagnosed with advanced non-squamous NSCLC has been consensual for approximately ten years. Only two predictive biomarkers have been validated for NSCLC treatment: *EGFR* mutations and *EML4-ALK* translocations. These markers can be used to predict response to targeted therapy (EGFR-TKI, for example erlotinib, gefitinib, and crizotinib). In contrast, several emerging biomarkers are being studied in ongoing clinical trials to determine whether or

not they can be used predict response to new targeted therapy or resistance to EGFR-TKI. Every patient diagnosed with advanced non-squamous NSCLC should be screened for predictive biomarkers. There is no longer any place for clinical selection of patients potentially eligible for biological analysis. The ASCO recommends analysis for predictive biomarkers at the time of diagnosis. However, there is evidence supporting the need for serial analysis, especially for patients with *EGFR*-mutant NSCLC and acquired resistance to EGFR-TKI. The purpose of these data and recommendations is to individualize therapeutic strategies for patients with advanced NSCLC and to improve their survival.

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