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Parameters for individualizing systemic therapy in non-small cell lung cancer

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Abstract

Rational drug design based on molecular targets is starting to revolutionize cancer care. To maximize its potential for patients, a concomitant leveraging of molecular knowledge for selection of patients to future and current therapeutic options is paramount. The terms “individualized”, “personalized”, or “precision therapy” are currently used to describe these efforts. Here, we summarize current knowledge for selection of systemic targeted and cytotoxic therapy for patients with non-small-cell lung cancer. Based on this knowledge, we present a potential decision algorithm to best select patients for currently available therapies, which include the treatment options single-agent erlotinib or gefitinib, the ALK inhibitor crizotinib, double agent gemcitabine and platinum, double agent platinum and pemetrexed, and as a default option a taxane combined with a non-platinum drug, for instance a vinca alkaloid. The addition of bevacizumab to double-agent chemotherapy is also discussed. Currently available data on predictive biomarkers are largely based on subgroup or companion biomarker analyses of patient cohorts or clinical trials. Current and emerging markers must be incorporated prospectively into the design of clinical trials that test novel and established agents to better understand their clinical utility and to refine selection parameters and marker interactions. Future development will lead to increasing complexity in clinical decision making with substantial anticipated benefits to patients including increased therapeutic efficacy, reduced toxicity, and better quality of life.

Keywords

Lung cancer; ERCC1; RRM1; TS; EGFR; EML4-ALK; Crizotinib; Bevacizumab

1. Introduction

Variability in patients’ responses to therapeutic agents in cancers originating from different organ sites, including lung cancer, is well recognized. Molecular investigations have demonstrated that this is primarily a result of differential expression and altered function of key molecules involved in drug efficacy among tumors. Clinical research is now increasingly focused on translating these molecular advances to patient care with the expectation that the utilization of parameters predictive of drug efficacy will result in a speedier clinical drug development, reduction of development costs, improved therapeutic

efficacy, reduction of unwanted side-effects, and ultimately longer survival and better quality of life for patients. Terms frequently used to describe these efforts are “individualized”, “personalized”, or “precision” therapy. One of the first examples of individualized therapy in medical oncology was the use of tamoxifen in estrogen receptor (ER) positive breast cancer.

In lung cancer management, tumor histopathology has long been used as a parameter for treatment selection. In fact, the classification of lung cancers into small-cell (SCLC) and non-small-cell lung cancers (NSCLC) was based on the clinical observation of differential responsiveness to chemotherapy and radiation; however, until very recently apart from this distinction, lung cancer therapy had not been based on molecular or histopathological parameters. In the last several years, new parameters have emerged that can guide therapeutic decisions. This review discusses predictive markers and their roles in individualizing therapy of lung cancer patients today and in the future. For the purpose of distinction, a *predictive* parameter is defined as a characteristic that is associated with therapeutic outcome from a specific agent or regimen, while a *prognostic* factor is defined as a characteristic that is associated with outcome regardless of the chosen therapy. For instance, ER expression in breast cancer is both predictive and prognostic (Dunnwald et al., 2007; Payne et al., 2008). Tumors with ER expression are significantly more likely to respond to tamoxifen than tumors without ER expression; i.e., ER expression is predictive of therapeutic benefit from tamoxifen. Also, patients with ER positive tumors have a better outcome than patients with ER negative tumors regardless of therapy; i.e., ER expression is prognostic of better survival. Only appropriately designed trials can distinguish between predictive and prognostic effects.

2. Predictive markers for outcome of chemotherapy in NSCLC

2.1. Histology and thymidylate synthase (TS) expression

For many years the efficacy of various chemotherapeutic agents utilized for the treatment of NSCLC was similar among the different histologic subtypes. Recently, three randomized clinical trials have shown that the efficacy of pemetrexed is superior in non-squamous subtypes compared to the squamous subtype of NSCLC when given as mono-therapy or in combination with cisplatin (Ciuleanu et al., 2009; Hanna et al., 2004; Scagliotti et al., 2008; Scagliotti et al., 2009). These trials also showed that the efficacy of pemetrexed was inferior to other treatments. As a result, pemetrexed has received approval by regulatory agencies in the U.S. and in Europe in non-squamous NSCLC patients for front-line therapy in advanced stage disease in combination with cisplatin, as maintenance therapy following front-line therapy and in patients with recurrent disease. Retrospective assessments of other phase III studies have failed to show such a differential activity with any other chemotherapeutic agent (Chansky et al., 2009).

It has been postulated that the lack of activity of pemetrexed in squamous cell patients may be related to high thymidylate synthase (TS) levels in these tumors (Nakagawa et al., 2004; Hashimoto et al., 2006; Ceppi et al., 2006; Zheng et al., 2008). TS is a folate pathway enzyme involved in DNA synthesis and it is the principal intracellular putative target of pemetrexed (Adjei, 2002; Bepler et al., 2008; Mendelsohn et al., 1999; Tonkinson et al., 1999), although secondary targets including dihydrofolate reductase (Mendelsohn et al., 1999) and the *de novo* purine biosynthetic enzymes, β -glycinamide ribonucleotide formyltransferase (Mendelsohn et al., 1999) and 5-amino-4-imidazolecarboxamide ribonucleotide formyltransferase (Racanelli et al., 2009), have also been implicated. Pre-clinical studies (Adjei, 2002; Tonkinson et al., 1999) and one prospective clinical trial (Bepler et al., 2008) have shown that high TS expression is associated with resistance to pemetrexed. Analyses of clinical specimens have shown that the median TS mRNA

expression levels are significantly higher in squamous cell carcinomas than in adenocarcinomas, which may contribute to pemetrexed resistance (Ceppi et al., 2006). However, it is important to recognize that there is variability in the expression levels of TS within each histologic category. It is unclear if the assessment of this marker will improve upon the current strategy of limiting the use of pemetrexed to non-squamous NSCLC patients.

2.2. Excision repair cross-complementation group 1 (ERCC1) expression

Platinum-based therapy has been the primary treatment of NSCLC for many years. Platinum agents form bulky adducts with DNA, which results in helix distortion, interference with replication and transcription, and ultimately cell death. The nucleotide excision repair (NER) pathway is crucial in repairing this type of DNA damage. Components of the NER pathway have been assessed in various cancers including lung cancer as potential markers of benefit from or resistance to platinum agents, and ERCC1 has emerged as a crucial molecule (Gazdar, 2007). In addition, ERCC1 is also involved in the function of other types of DNA damage repair mechanisms.

The largest clinical data set regarding this marker in lung cancer patients is from the International Adjuvant Lung Cancer Biology Trial (IALT-Bio). The IALT is the largest adjuvant trial conducted in NSCLC patients, and showed that survival was improved among 1867 patients by 4.1% at 5 years with platinum-based chemotherapy (Arriagada et al., 2004). The IALT-Bio study was conducted to assess the predictive value of various tumor-related markers for benefit from adjuvant therapy. Investigators analyzed ERCC1 expression by immunohistochemistry (IHC) in paraffin-embedded tumors of 761 patients from the IALT clinical trial (Olaussen et al., 2006). Among patients with tumors that were deemed ERCC1 negative (defined as ERCC1 expression below the cohort median), the 5-year survival rates were 47% in the adjuvant chemotherapy group compared to 39% in the control group (HR 0.65, $p = 0.002$). Among patients with tumors that were ERCC1 positive (defined as ERCC1 expression above the cohort median), the 5-year survival rates were 40% in the adjuvant chemotherapy group compared to 46% in the control group (HR 1.14, $p = 0.4$). These data suggest that the benefit from adjuvant platinum-based chemotherapy was restricted to patients whose tumors were ERCC1 negative. The above data also show that in the patients who did not receive adjuvant chemotherapy the 5-year survival rate among ERCC1 positive patients (46%) was higher than in ERCC1 negative patients (39%), (HR 0.66, $p = 0.009$). Thus the IALT-Bio data show that tumoral ERCC1 expression can be not only predictive of benefit from platinum-based chemotherapy (only patients with low tumoral ERCC1 expression derive benefit from platinum-based therapy) but also appears to have prognostic value (patients with high tumoral ERCC1 expression have a better prognosis). These data have recently been confirmed on tissue microarrays with an automated quantitative image analysis system using an immunofluorescent-labeled monoclonal ERCC1 antibody (Bepler et al., in press). An attempt to further validate these results with specimens from other completed adjuvant trials is under way. In addition, prospective studies that assess the utility of using ERCC1 expression levels for therapeutic decisions on platinum use are ongoing.

In metastatic NSCLC patients, a prospective study conducted by Cobo et al. assessed the response rate to platinum-based therapy compared to non-platinum therapy in a randomized trial (Cobo et al., 2007). Stage IV NSCLC patients were randomized in a 2:1 ratio to therapy based on ERCC1 mRNA expression or to the control arm of cisplatin and docetaxel. In the experimental arm, patients with low tumoral ERCC1 levels received cisplatin and docetaxel and patients with high ERCC1 levels received docetaxel and gemcitabine. Among 346 eligible patients, the response rate in patients who received therapy based on ERCC1

expression was 51% compared to 39% in the control arm ($p = 0.02$). The progression free and overall survival rates were not significantly different. This lack of difference in survival may have been a result of the small number of patients included in this trial. A reasonable conclusion from this study is that tumoral ERCC1 expression can be used to enhance the clinical benefit derived from platinum-based chemotherapy in patients with advanced NSCLC.

2.3. Ribonucleotide reductase subunit 1 (RRM1) expression

RRM1 is a component of ribonucleotide reductase, a key enzyme in the synthesis of deoxynucleotides, which are required for DNA synthesis and repair. Another function of RRM1 is suppression of cell migration and metastasis formation, which is at least partially mediated through induction of PTEN (Gautam et al., 2003; Gautam and Bepler, 2006). The gene for RRM1 is located on chromosome segment 11p15.5, a region with a frequent loss of heterozygosity (LOH) in NSCLC (Bepler et al., 1999; Pitterle et al., 1999). LOH at this locus was found to be a significant adverse prognostic factor in NSCLC patients (Bepler et al., 2002).

Zheng et al. investigated the expression levels of RRM1, ERCC1, and PTEN in tumors from 187 patients with resected stage I NSCLC (Zheng et al., 2007). They analyzed these markers utilizing immunofluorescence combined with automated quantitative analysis (AQUA), a methodology that is objective, reliable and reproducible. Median disease-free-survival (DFS) and overall survival (OS) of patients with tumors that had low RRM1 expression (lower than the cohort median) was 54.5 months and 60.2 months respectively, compared to over 120 months for both parameters in patients with high RRM1 expressing tumors (DFS: HR 2.2, $p = 0.004$; OS: HR 1.6, $p = 0.02$). In a multivariate model RRM1 expression was the only factor that was significantly associated with DFS. However, in this model RRM1 expression did not correlate with OS. They also found that AQUA scores of RRM1 significantly correlated with ERCC1 scores but not with PTEN scores. In addition, utilizing the expression levels of both RRM1 and ERCC1, they identified a group of patients whose tumors had high expression of both markers (55 of 187 patients) and these patients had significantly longer median DFS and OS compared to the other groups of patients. A conclusion from this investigation is that RRM1 expression alone, and in particular in combination with ERCC1 expression, has prognostic significance in resected early-stage NSCLC patients.

Gemcitabine is known to interfere with the activity of ribonucleotide reductase. Pre-clinical models have shown that tumors that overexpress ribonucleotide reductase are resistant to gemcitabine (Bepler et al., 2006; Bergman et al., 2005; Davidson et al., 2004). Rosell et al. retrospectively analyzed the expression of *RRM1* mRNA in tumors of 100 patients treated on a phase III trial evaluating different chemotherapy regimens including cisplatin/gemcitabine (Rosell et al., 2004). The median survival was significantly longer in the gemcitabine/cisplatin arm among patients with low RRM1 levels (13.7 months) compared to patients with high levels (3.6 months; $p = 0.009$). There was also a statistically insignificant trend towards longer median survival in the gemcitabine/cisplatin arm among patients with low ERCC1 levels. Similar differences in outcomes based on expression of these markers were not found in patients treated with other regimens. However, there was no significant association between RRM1 levels and response to therapy.

Bepler et al. evaluated the correlation of tumoral expression of *RRM1* and *ERCC1* and tumor response to combination carboplatin/gemcitabine in a prospective clinical trial and found that both markers correlated inversely with response (Bepler et al., 2006). This was recently confirmed in a prospective multi-institutional, community-based, randomized phase III trial in patients with poor performance status, advanced stage NSCLC (Reynolds et al., 2009).

These results prompted a phase II study to evaluate the ability of these molecular markers to guide chemotherapy in patients with advanced NSCLC (Simon et al., 2007). The study required collection of fresh-frozen tumor specimens before therapy and *RRM1* and *ERCC1* were analyzed utilizing real-time quantitative reverse transcriptase polymerase chain reaction (RT-PCR). Based on previous experience of the group, they defined low *RRM1* as values of ≤ 16.5 and low *ERCC1* as values of ≤ 8.7 . Patients with low *RRM1* and low *ERCC1* received carboplatin and gemcitabine; patients with low *RRM1* and high *ERCC1* received gemcitabine and docetaxel; patients with high *RRM1* and low *ERCC1* received carboplatin and docetaxel; patients with high *RRM1* and high *ERCC1* received docetaxel and vinorelbine. Among the 53 patients treated on the trial, the disease control rate (PR and stable disease) was 88.5%. The median OS was 13.3 months and the median progression free survival (PFS) was 6.6 months. These results strongly suggest that selection of chemotherapy based on gene expression of *RRM1* and *ERCC1* is feasible in advanced NSCLC patients and can lead to improved outcomes. Based on these results a phase III study is currently being conducted to confirm the utility of this strategy.

2.4. BRCA1 (breast cancer 1) expression

The *BRCA1* gene was originally described as a tumor suppressor gene in breast and ovarian cancer (Miki et al., 1994). It is involved in DNA damage response pathways, in particular those that involve DNA double-strand break repair. Preclinical studies have suggested that elevated *BRCA1* expression levels may predict for resistance to platinum compounds while simultaneously sensitizing cells to apoptosis induced by the taxane-class of anti-microtubulin agents (Scully et al., 2004; Mullan et al., 2001; Quinn et al., 2003; Kennedy et al., 2004).

Based on these data, Rosell et al. initiated a study to assess the feasibility and efficacy of customizing therapy based on *EGFR* mutation status and *BRCA1* expression (Rosell et al., 2009a). Patients with tumoral *EGFR* mutations were treated with erlotinib. Patients without such mutations received chemotherapy based on *BRCA1* expression. Patients with low tumoral levels of *BRCA1* were treated with cisplatin and gemcitabine, those with intermediate levels were treated with cisplatin and docetaxel, and those with high levels received docetaxel alone. Among the patients treated with chemotherapy, the response rates were 21% in the low *BRCA1* group, 40% in the intermediate group, and 39.4% in the high group. The median survivals for the three groups were 11 months, 9 months, and 11 months, respectively. The authors concluded that it was feasible to customize therapy based on *BRCA1* expression levels with promising results. Su et al recently assessed the correlation of the expression of *ERCC1*, *RRM1*, and *BRCA1* mRNA levels in tumors and outcomes of advanced NSCLC patients treated with different platinum based chemotherapy regimens (Su et al., 2010). The investigators found that the outcomes of patients with low *ERCC1* expressing tumors were better than those with high *ERCC1* tumors. Patients with high *BRCA1* expressing tumor had better outcomes if treated with an anti-tubulin agent and patients with low *RRM1* expressing tumors did better with gemcitabine. These data raise the possibility that incorporating the expression levels of these markers may enhance the ability to select chemotherapy for advanced NSCLC patients and could lead to better outcomes compared to those observed with unselected therapy. However, formal proof of this concept in large randomized clinical trials is outstanding.

2.5. Beta-tubulin expression

The targets of taxane compounds, microtubules, are complex polymers consisting of α -tubulin and β -tubulin dimers. Microtubules form the cellular cytoskeleton and are critical for cell growth and division, maintenance of cell shape, transport of intracellular vesicles and organelles and cellular signaling. The tubulins can exist in various isotypes and the

expression of different iso-types can vary in normal and tumor tissues. Antitubulin agents bind to specific regions of β -tubulin. β III tubulin expression levels appear to have prognostic significance in advanced NSCLC (Seve and Dumontet, 2008). In addition, β III tubulin expression levels may predict for clinical benefit from taxanes (Seve and Dumontet, 2008). Apart from expression levels, β III tubulin mutations may occur and presence of these mutations may predict for taxane-resistance in patients with advanced NSCLC (Monzo et al., 1999). In a study of 49 patients with NSCLC, the median survival was 3 months for those patients ($n = 16$) exhibiting a mutation in the β III tubulin gene compared with 10 months for patients ($n = 33$) without a mutation ($p = 0.0001$). Though there is promise about the predictive utility of this marker, the currently available data are too preliminary to allow for inclusion of this marker into therapeutic decision making.

2.6. EGFR mutations

One of the major predictive factors in NSCLC therapeutic decision-making are somatic mutations in the epidermal growth factor receptor (*EGFR*) gene. About 10–15% of NSCLCs in a non-Asian population of typical patients have these mutations (Lynch et al., 2004; Paez et al., 2004) and the presence of exon 19 and exon 21 mutations in the *EGFR* gene predict for a high likelihood of benefit from the *EGFR* tyrosine kinase inhibitors (TKIs) gefitinib and erlotinib (Sholl et al., 2010).

Recently in a study by Mok et al., it was demonstrated that the PFS with gefitinib as front-line therapy for advanced disease in NSCLC patients whose tumors harbor *EGFR* mutations was better than the PFS of patients treated with carboplatin/paclitaxel (Mok et al., 2009). However, this study also showed that the response rate with chemotherapy (43%) was better among patients with the *EGFR* mutations than in patients with wild-type *EGFR* (27%). These data suggest that the presence of *EGFR* mutations may lead to improved efficacy with platinum/taxane therapy. In a recent analysis of *ERCC1* expression in NSCLC tumor specimens, Gandara et al. showed that the *ERCC1* gene expression was lower among *EGFR* mutant tumors than tumors with wild-type *EGFR* (Gandara et al., 2010). This difference in *ERCC1* expression may explain the greater efficacy of the chosen double-agent chemotherapy in *EGFR* mutant tumors in the above referenced trial.

2.7. K-ras mutations

K-ras a member of the Ras family of oncogenes and is involved in signal transduction. *K-ras* mutations usually occur in codons 12 and 13 and have been reported in about 20% of the patients (Massarelli et al., 2007; Zhu et al., 2008). Presence of *K-ras* mutations may have prognostic significance. In a meta-analysis of 881 NSCLC patients presence of *K-ras* mutation was associated with worse outcome (Huncharek et al., 1999). However in a separate analysis by Schiller et al. *K-ras* was only weakly prognostic and the results did not reach statistical significance (Schiller et al., 2001).

In an adjuvant trial (JBR-10), the survival was significantly improved with adjuvant chemotherapy in patients with *K-ras* wild-type tumors (HR -0.69 ; $p = 0.03$) but there was benefit in patients with *K-ras* mutated tumors (HR -0.95 ; $p = 0.87$). However in the COX model the test for interaction between *K-ras* status and benefit from chemotherapy was not significant ($p = 0.29$) (Tsao et al., 2007). Also in this study *K-ras* status did not have prognostic significance.

2.8. Gene expression profiles as predictors of response to chemotherapy

Investigators have developed gene expression profiles from cell lines that predict for sensitivity to various chemotherapeutic agents (Potti et al., 2006). They first established gene signatures from cell lines and validated the signature in independent cell lines, as well

as in clinical samples. One particular advantage of this approach is that the tumor sample is analyzed simultaneously for most expressed genes thus providing a snapshot of the molecular state of the tumor and its environment in one analysis. The clinical utility of this type of signature has not yet been evaluated in prospective clinical trials.

Tsao et al. assessed the utility of gene expression profiling to predict benefit from adjuvant chemotherapy (Tsao et al., 2008). They conducted an analysis on 133 tumor samples of patients enrolled on JBR-10, a study that documented the benefits of adjuvant cisplatin/vinorelbine in stage IB/II NSCLC patients. A 15 gene signature was developed that correlated with survival and was independent of stage, histology, age, and sex. This signature divided the 62 patients with analyzable tumor specimens in the observation group into high and low risk groups (HR 60.1, $p < 0.0001$). Adjuvant chemotherapy significantly reduced the risk of death in the high risk group both in stage IB and stage II patients (HR 0.33, $p = 0.0005$). Conversely, in patients with the low risk signature, survival was actually inferior with chemotherapy compared to observation. A prospective validation of this signature has not been reported.

2.9. Chemotherapy sensitivity and resistance assays (CSRAs)

The concept of *in vitro* chemotherapy sensitivity analysis of patients' tumors similar to an antibiotic sensitivity analysis for microorganisms has existed for many decades. Multiple assays have been developed in the past including the soft agarose human tumor stem cell assay (Salmon et al., 1978). There is an obvious appeal to the concept of utilizing CSRAs to guide chemotherapy for cancer patients. CSRAs could potentially assist in avoiding toxic chemotherapy that is not likely to be effective and allow treatment with drugs that are most likely to be effective. Many different assays have been utilized in this regard. The assays involve growing a portion of the tumor or some cells either *in vitro* or in an *in vivo* model and utilizing proliferation or metabolism to assess the sensitivity/resistance of the tumor to various chemotherapy drugs. The American Society of Clinical Oncology (ASCO) came out with a policy statement regarding these tests in 2004. In this statement, ASCO concluded that the data regarding CSRAs did not justify their use in routine clinical care (Schrug et al., 2004). In fact, a prospective trial of chemotherapy selection for patients with NSCLC and SCLC utilizing a CSRA did not show a significant benefit (Shaw et al., 1996). Some experts differ with this opinion. However, evidence from prospective, controlled, clinical trials validating their benefit in the management of NSCLC or SCLC is lacking.

2.10. Perspective on predictive markers for chemotherapy

Combination chemotherapy has been used to treat NSCLC patients for many years. Despite the availability of many drugs with different mechanisms of action and the recognition that these drugs are effective only in a proportion of patients, there continues to be *random* selection of chemotherapy drugs in the treatment of NSCLC. Data generated over the last few years suggest that this practice is likely to change and that chemotherapy drugs should only be used in patients with tumors that are likely to respond to the agents selected. At the present time the only criteria being utilized in standard practice to individualize chemotherapy is tumor histology, as a result of the approval of pemetrexed from regulatory agencies in patients with non-squamous cell carcinomas. None of the other molecular parameters discussed are currently being used routinely in the clinical care of NSCLC patients. This is partially a result of lack of unequivocal prospective data from randomized trials, but also a result of past approval of agents without a link to specific molecular parameters of efficacy. Another important factor that has limited the use of these markers in clinical practice is the variability in the methodologies used in the reported studies. Standardization of assessment procedures for the different markers is necessary for their applicability in the management of patients. The available data strongly suggest that

assessment of ERCC1, RRM1, and potentially also TS expression in tumors will be used in the near future to individualize therapy in NSCLC patients. However, this area of research is in evolution, and predicting the future use of specific molecular markers is extremely challenging.

3. Predictive markers for EGFR Tyrosine Kinase Inhibitors (TKIs)

3.1. EGFR mutations

Early during the clinical development of EGFR-TKIs for lung cancer therapy it was recognized that the clinical benefit was highly variable in different groups of patients (Miller et al., 2004). Patients with adenocarcinomas, those who had never smoked, Asians, and women were much more likely to derive benefit from EGFR-TKIs. The differential activities of these agents in NSCLC patients prompted a search for tumor markers that could predict benefit from or resistance to these agents. Lynch et al. and Paez et al. identified somatic mutations in the tyrosine kinase domain of the *EGFR* gene and the presence of these mutations in tumors correlated with increased likelihood of therapeutic benefit (Lynch et al., 2004; Paez et al., 2004). Among the various *EGFR* mutations found in NSCLCs, the two mutations that predicted for benefit were exon 19 deletions and exon 21 point mutations. Pre-clinical studies suggested that tumors with these mutations had increased dependency on EGFR signaling and inhibition of signal transduction induced growth suppression and apoptosis at much lower drug concentrations than in tumors without these mutations (Sordella et al., 2004). These preclinical observations have now been confirmed in clinical settings. In a clinical trial, conducted in East Asian countries and restricted to patients with advanced adenocarcinoma of the lung that were never or light smokers, treatment-naïve patients were randomized to gefitinib or carboplatin/paclitaxel (Mok et al., 2009). The primary objective was to document non-inferiority of gefitinib in terms of PFS. Gefitinib demonstrated superior PFS in the 1,217 patients enrolled on the study compared to chemotherapy. The investigators determined the *EGFR* mutation status in 261 trial participants (21%). Patients with tumoral EGFR mutations had a significantly greater PFS with gefitinib than chemotherapy (HR 0.48; $p < 0.001$), and in patients with wild-type EGFR the PFS was significantly worse with gefitinib (HR 2.85; $p < 0.001$) compared to chemotherapy. Other studies evaluating front-line therapy with EGFR-TKIs have observed the similar differential clinical efficacy (Maemondo et al., 2010; Mitsudomi et al., 2010).

The frequency of EGFR-TKI therapy sensitizing mutations varies among different populations. In a recent study from Spain, the rate of sensitizing mutations among adenocarcinoma patients was 17% (Rosell et al., 2009b). In the United States the mutation rate is similar to the rate reported from Spain (Sequist et al., 2008). Since the clinical benefit from EGFR-TKIs among these patients is substantial, *EGFR* mutation testing of tumors in patients with adenocarcinomas of the lung is becoming increasingly popular. Presently, the test is not FDA (Federal Drug Administration) approved, and none of the EGFR-TKIs have received approval for front line treatment of NSCLC in the United States.

3.2. Factors predictive of resistance to EGFR-TKIs

Molecular alterations other than the sensitizing *EGFR* mutations can confer resistance to EGFR-TKIs. For instance, point mutations in exon 20 of *EGFR* predict for resistance to gefitinib and erlotinib (Wu et al., 2008). These mutations represent less than 5% of all *EGFR* mutations. Preclinical data suggest that these mutations lead to activated EGFR signaling and can result in transformation. *In vitro* studies have shown that EGFR-TKIs are ineffective in inhibiting EGFR signaling in tumors with these mutations. In addition, Her-2 mutations are also associated with resistance to these agents (Shigematsu et al., 2005; Wang et al., 2006).

3.3. K-ras mutations

K-ras mutations encountered in lung cancers result in constitutive activation of downstream signaling pathways such as the PI3K and MAPK pathways, rendering them independent of EGFR signaling. *K-ras* mutations are present in 15–20% of NSCLCs (Massarelli et al., 2007; Zhu et al., 2008) and are more common among smokers than among never smokers. Clinical data suggest that patients with *K-ras* mutations rarely respond to EGFR-TKIs (Massarelli et al., 2007). For instance, in the BR21 trial, a randomized study that evaluated erlotinib versus placebo in patients with recurrent NSCLC, the interaction between *K-ras* mutation status and treatment was marginally significant ($p = 0.09$), suggesting lack of benefit from erlotinib in *K-ras* mutant patients (Zhu et al., 2008). In a study comparing gefitinib to docetaxel in a similar population of patients, no difference in outcome of patients with *K-ras* mutations between the two agents was observed (Douillard et al., 2010). As a result, *K-ras* mutant tumors may be less likely to respond to EGFR-TKIs but the clinical benefit from these agents is no worse than that observed with single-agent docetaxel. It is important to interpret these data cautiously, since only a fraction of trial participants had tumor material available for molecular analysis. In addition, there are case reports of lung cancer patients with *K-ras* mutations responding to EGFR-TKIs (Pesek et al., 2009; Lee et al., 2010). However, the cumulative evidence suggests that clinical benefit from EGFR-TKIs in NSCLC patients with *K-ras* mutations is extremely low.

3.4. Proteomic analysis

Matrix-assisted laser desorption/ionization (MALDI) time-of-flight mass spectrometry (MS) is an evolving technique for biomarker discovery and analysis in a wide spectrum of samples, such as serum, urine, and tissue. Peaks in the mass spectrum correspond to ions formed predominantly by peptides and proteins. Taguchi et al. reported the ability of this technique to classify patients into good, poor, or indeterminate responders to EGFR-TKIs (Taguchi et al., 2007). The investigators developed a proteomic signature based on MALDI analysis of blood collected from patients treated with gefitinib or erlotinib. The proteomic signature was validated in separate cohorts of patients treated with these drugs. In addition the proteomic signature was predictive and not prognostic; i.e., the signature failed to categorize patients who were not treated with EGFR TKIs into distinct prognostic groups. These encouraging preliminary results were followed-up by a much larger analysis. Carbone et al. presented the MALDI-MS results on baseline plasma samples from 441 patients enrolled in the BR21 trial (Carbone et al., 2010). Of these, 266 patients (60%) had a ‘good’ proteomic profile and 170 (38%) had a ‘poor’ proteomic profile. The patients with the ‘good’ profile had a significant improvement in survival with erlotinib (10.5 months) compared to placebo (6.6 months, $p = 0.002$). This was not true for patients with the ‘poor’ profile (4.0 vs. 3.1 months; $p = 0.11$). However, the interaction test to assess the predictive utility of this marker was not statistically significant. These results may be related to low numbers of patients in each category and also raise the possibility that this proteomic profile may have prognostic utility since there was a difference with the median survival of placebo patients (6.6 vs. 3.1 months).

3.5. Perspectives on predictors for EGFR-TKI inhibitors

EGFR-TKIs are being increasingly used in the management of NSCLC. Apart from utility in recurrent NSCLC, recent data from randomized trials show that these drugs also benefit patients with EGFR mutant tumors as front-line therapy and as maintenance therapy following the completion of 4 cycles of combination chemotherapy (Mok et al., 2009; Cappuzzo et al., 2010).

Many parameters have been assessed to predict benefit or lack of benefit from EGFR-TKIs in NSCLC patients. The only parameter consistently associated with benefit from these

agents is the presence of tumoral *EGFR* mutations. At the present time, the accepted consensus is to analyze *EGFR* mutation status in all patients with adenocarcinomas and in those who are never or light smokers. Patients found to have *EGFR* mutations in their tumors should be considered for EGFR-TKI therapy, even as front-line therapy. It is important to recognize that the lack of these mutations does not eliminate the possibility of clinical benefit from EGFR-TKIs; however, it substantially reduces the likelihood of benefit. The utility of other parameters remains less certain, and we do not currently recommend their use for clinical decision making.

4. Other therapies

4.1. ALK gene rearrangements

In 2007, EML4-ALK, a novel fusion gene arising as a result of an inversion in chromosome 2 that juxtaposed the 5' end of the EML4 gene with the 3' end of the ALK (anaplastic lymphoma kinase) gene, was reported in a subset of patients with NSCLC (Soda et al., 2007). Multiple variants of EML4-ALK, as well as other rare fusion partners, have since been reported (Solomon et al., 2009). In tumors with these rearrangements, the ALK protein is constitutively activated, and patients with these rearrangements live longer than those without (Shaw et al., 2010). Blocking ALK in these tumors both preclinically and clinically has produced dramatic benefits, similar to those seen with EGFR-TKIs in patients with EGFR mutations. In a recently reported clinical trial the ALK inhibitor crizotinib showed a clinical benefit rate of over 80% in EML4-ALK positive NSCLC patients, and the 6 month PFS rate was 72% (Bang et al., 2010).

ALK gene rearrangements are found in approximately 5% of NSCLC, and the likelihood is greater in patients with adenocarcinomas and those who are never smokers or light smokers. Current data also suggest that ALK gene rearrangements are mutually exclusive of EGFR mutations. Thus, lack of EGFR mutations may be utilized as a factor to consider testing for ALK gene rearrangements. Two ongoing clinical trials are further evaluating the benefits of crizotinib in ALK positive patients with advanced NSCLC. Results of these trials will define the true utility of this drug.

4.2. Parameters influencing the use of bevacizumab

The monoclonal antibody against vascular endothelial growth factor (VEGF), bevacizumab, is currently approved for the treatment of advanced NSCLC patients in combination with carboplatin and paclitaxel. The approval was based on a randomized trial, ECOG 4599, that demonstrated a survival advantage for patients who received bevacizumab in addition to chemotherapy (Sandler et al., 2006). However, the eligibility for this trial was highly restricted. One of the exclusion criteria was squamous cell histology. This restriction was based on the increased incidence of hemoptysis, including fatal events, among patients with squamous cell carcinomas, from an exploratory trial conducted by Johnson et al. (2004). Additional exclusion criteria among others included a history of hemoptysis, brain metastases, thromboembolic events, and the use of anticoagulants. However subsequent data, both prospective and retrospective, have not shown a significantly greater risk of cerebral hemorrhage in NSCLC patients with treated brain metastases who receive bevacizumab (Socinski et al., 2009; Besse et al., 2010). As a result, the FDA has approved the use of bevacizumab in patients with treated brain metastases.

An important concern with the use of bevacizumab in NSCLC patients is the development of pulmonary hemorrhage. In the ECOG 4599 trial, the incidence of severe pulmonary hemorrhage (grade 3) was 2.3%. In a retrospective analysis aimed at determining factors associated with pulmonary hemorrhage, baseline tumor cavitation emerged as the sole

predictive factor (Sandler et al., 2009). Based on these data, it has been suggested that patients with tumor cavitation should not be treated with bevacizumab. What is currently unclear is whether treatment of patients who develop tumor cavitation while on bevacizumab should be discontinued to reduce the risk of hemorrhage.

4.3. Factors predictive of benefit from bevacizumab

Hypertension is a common adverse event with bevacizumab and other anti-VEGF agents. VEGF blockade decreases nitric oxide production, resulting in vasoconstriction and sodium retention leading to increased blood pressure. Several studies in different tumor types have suggested that patients who develop hypertension with anti-VEGF therapy have a better outcome than patients who do not develop hypertension (Bono et al., 2009; Dahlberg et al., 2010). The ECOG 4599 investigators conducted a retrospective analysis and found that patients who developed hypertension while on therapy with bevacizumab had a better clinical outcome than patients who did not (Dahlberg et al., 2010). The median survival of patients who developed hypertension was 15.9 months (95% CI, 13.4–20.3 months) compared to 11.5 months (95% CI, 10.4–13.4 months) for those not experiencing hypertension. As expected, between patients who did not get bevacizumab and developed hypertension vs. those that did not, the median survival was indistinguishable (10.3 months for those with hypertension, 10.1 months for those without hypertension). The utility of these data is limited by many factors, including the retrospective nature of the analysis. At the present time there is no factor identified that can predict for development of hypertension in a particular patient following therapy with bevacizumab. In addition, the current data do not suggest that clinical benefit from bevacizumab is exclusive to patients who develop hypertension. Multiple other markers have been tested for their ability to predict benefit from bevacizumab (Zhang et al., 2009; Dowlati et al., 2008; Vroling et al., 2010). Of interest, elevated levels of circulating VEGF are associated with a poor prognosis in NSCLC (Bernaards et al., 2010). Currently there is no marker with sufficient data for use in routine care of patients.

5. Utilization of biomarkers for individualizing therapy of NSCLC patients: current status

At the beginning of this century, the only option available for the systemic treatment of NSCLC patients was the unselected use of double-agent chemotherapy. Although the inclusion of a platinum-agent was considered desirable, overall survival did not differ significantly between groups of patients receiving platinum-containing doublets versus non-platinum doublets as first-line therapy (D'Addario et al., 2005). Systemic therapy of NSCLC has evolved substantially during the past decade and now involves the inclusion of various molecular, histological, and clinical parameters in treatment decision making for individual patients. The clinical utility of several prognostic and predictive parameters is summarized in Table 1. As emphasized in this review, data on many of these parameters are derived from subgroup analyses of clinical trials, and many of these did not *a priori* specify the respective biomarkers, analysis methods, or specimen procurement protocols.

A potential decision algorithm for selection of therapy in patients with advanced NSCLC is presented in Fig. 1. The first step is to consider if the tumor should be tested for EGFR mutations. If the tumor has exon 19 or 21 mutations, the patient should be offered treatment with single-agent erlotinib or gefitinib. In EGFR wild-type tumors, or in the event of lack of response, consideration should be given to testing for ALK rearrangements. If the tumor has ALK rearrangements, the patient should be offered treatment with single-agent crizotinib. It is only available on a clinical trial basis at the present time. In ALK wild-type tumors, or in the event of lack of response, consideration should be given to testing for ERCC1, RRM1,

and TS expression levels. If the levels for ERCC1 and RRM1 are low, the patient should be offered treatment with double-agent gemcitabine and platinum. If the levels for TS and ERCC1 are low, the patient should be offered treatment with double-agent pemetrexed and platinum. Similarly, patients with high tumoral ERCC1 levels (but low RRM1 and TS levels) should be treated with double-agent gemcitabine and pemetrexed. If ERCC1, RRM1, and TS levels are high, the patient should be offered treatment with a doublet that does not include a platinum agent or an anti-metabolite. A potential therapeutic option is the combination of a taxane and a vinca alkaloid. The addition of bevacizumab to double-agent chemotherapy should be based on histology, presence of tumoral cavitation, and the presence of untreated brain metastases among other considerations.

Undoubtedly, additional molecular markers predictive of efficacy of currently available and novel agents will emerge in increasing numbers. Prospective validation of current markers may lead to better refinement of selection parameters, acceptance of current knowledge, or abandonment of markers. Future development will lead to increasing complexity in clinical decision making with substantial anticipated benefits to patients including increased therapeutic efficacy, reduced toxicity, and better quality of life.

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Fig. 1.
An individualized algorithm for treatment selection in patients with advanced NSCLC.

Table 1

Prognostic and predictive value of molecular markers in non-small cell lung cancer.

Parameter	Prognostic value	Predictive value
ERCC1	Elevated levels of ERCC1 confer a better prognosis	Elevated ERCC1 levels confer resistance to platinum therapy
RRM1	Elevated levels of RRM1 confer a better prognosis	Elevated RRM1 levels confer resistance to gemcitabine therapy
TS	Elevated levels of TS confer a better prognosis	Elevated TS levels confer resistance to pemetrexed therapy
BRCA1	Elevated levels of BRCA1 confer a worse prognosis	Elevated BRCA1 levels confer resistance to platinum and sensitivity to taxane therapy
β -tubulin	Elevated β -tubulin levels confer a worse prognosis	Elevated β -tubulin levels are predictive of resistance to taxane therapy
Kras	Presence of Kras mutations confer a worse prognosis	Presence of Kras mutations is predictive of resistance to EGFR-TKI and possibly platinum-based therapy
EGFR	Presence of EGFR mutations confers a better prognosis	Presence of EGFR mutations is predictive of benefit from EGFR-TKI and platinum/paclitaxel therapy
ALK	Presence of ALK rearrangements confers a better prognosis	Presence of ALK rearrangements is predictive of benefit from crizotinib therapy
VEGF	Elevated levels of circulating VEGF confer a worse prognosis	Circulating VEGF levels are not predictive of bevacizumab efficacy