A Systematic Review and Canadian Consensus Recommendations on the Use of Biomarkers in the Treatment of Non-small Cell Lung Cancer

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Introduction: Greater understanding of molecular pathways important in cell growth and proliferation of thoracic malignancies, particularly non-small cell lung cancer (NSCLC), has resulted in intense clinical and translational research. There is now considerable interest in personalizing treatment based on an understanding of tumor histology and molecular abnormalities. However, there is a multiplicity of data, often with discordant results resulting in confusion and uncertainty among clinicians.

Methods: We conducted a systematic review and a consensus meeting of Canadian lung cancer oncologists and pathologists to make recommendations on the use of biomarkers in NSCLC. PubMed covering 2005 to March 2010 was searched using MESH terms for NSCLC and randomized trials, plus text words for the biomarkers of interest. Conference proceedings from 2005 to 2009 ASCO, ESMO, IASLC, and USCAP were also searched. The articles were reviewed by pairs of oncologists and pathologists to determine eligibility for inclusion.

Results: Ten oncologists and pathologists reviewed and summarized the literature at a meeting attended by 37 individuals. Draft recommendations were formulated and agreed upon by consensus process. There is some evidence that histology is prognostic for survival. There is evidence from multiple randomized clinical trials to recommend the following: histologic subtype is predictive of treatment efficacy and for some agents toxicity. Immunohistochemistry testing should be performed on NSCLC specimens that cannot be classified accurately with conventional H&E staining. As *EGFR* mutations are predictive of benefit from tyrosine kinase inhibitors, diagnostic NSCLC samples should be routinely tested for *EGFR*-activating mutations. Clinical data on *K-RAS* mutations are inconsistent, therefore testing is not recommended. There is insufficient evidence to recommend other biomarker testing. No biomarkers to date reliably predict improved efficacy for anti-VEGF therapy. Routine assessment for *EML4/ALK* mutations is not recommended at present, although emerging data suggest that it may become valuable in the near future.

Conclusions: Assessment of NSCLC biomarkers is becoming increasingly important. Therefore, adequate diagnostic material must be obtained for accurate histologic subtyping and relevant molecular biology assays.

Key Words: Non-small cell lung cancer, Systematic review, Biomarker.

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Significant advances in the treatment of non-small cell lung cancer (NSCLC) have been made over the past 25 years (Figure 1). We have evolved from a state of relative nihilism about the role of systemic therapy for this malignancy to one in which there is high quality evidence supporting the use of first,[1–3] second,[4–6] and even third-line systemic therapies.[7] The trials have not only demonstrated improved survival of patients with advanced NSCLC but also shown improvements in patient symptoms and quality of life.[8] Until recently, it was assumed that these treatments should be applied irrespective of histologic subtype of NSCLC.

Nevertheless, the improved outcomes from current treatment options are modest. More recently, research has

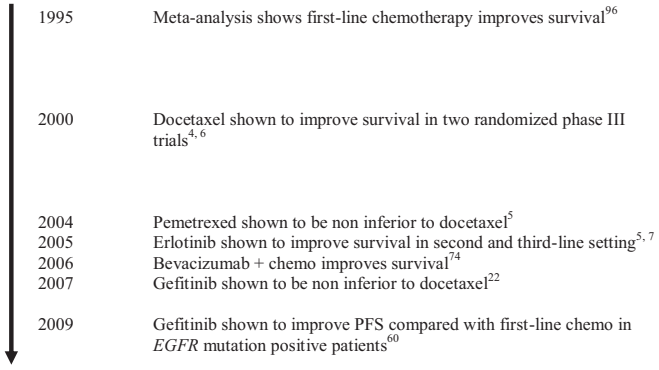

FIGURE 1. Time line for major advances in the treatment of non-small cell lung cancer.

focused on understanding the molecular abnormalities associated with NSCLC cell growth and proliferation and their impact on response to treatment and survival. These may result in altered function or overexpression of a number of cell surface receptors and downstream intracellular pathways which are thought to be clinically important determinants of cell growth. However, it is important to separate those markers which inform the natural history of the disease (prognostic marker) from those markers which identify a subgroup of patients likely to benefit more or less from a specific treatment (predictive marker).

Common molecular abnormalities in NSCLC involve the epidermal growth factor receptor (EGFR), the *KRAS* oncogene, and the vascular endothelial growth factor and its receptors (VEGFR). Activating mutations of *EGFR*,[9,10] upregulation through increased gene copy number, or protein overexpression occur frequently.[11,12] *KRAS* mutations occur in 20 to 25% of patients[13] and have been reported to affect prognosis negatively.[14] In addition, less common genetic mutations, such as *EML4/ALK* translocations, may also be clinically important in NSCLC pathogenesis.[15] Other molecular abnormalities involving genes such as excision repair cross-complement 1 (*ERCC1*), *p53*, *BRCA*, and *β-tubulin* may also be important predictors of sensitivity to systemic treatment options.

The availability of newer treatment options targeting specific molecular abnormalities in NSCLC has resulted in considerable biomarker research aimed at identifying predictive biomarkers of efficacy. As a result of these changes, there has been a shift from a treatment algorithm applicable to the majority of patients to a more complicated situation in which treatment decisions are influenced by a variety of factors including histologic subtype and molecular phenotype. The rapid expansion of knowledge has resulted in considerable uncertainty about treatment choices in NSCLC. Furthermore, biomarker research findings are variable between studies, and variable technologies may be difficult to implement or standardize in the diagnostics laboratory.

Molecular abnormalities may be measured using protein expression through immunohistochemistry (IHC), gene expression or copy number using fluorescence in situ hybridization (FISH) or quantitative polymerase chain reaction (PCR), or gene mutations using direct sequencing or PCR techniques. Questions also exist about the appropriate cut point to distinguish between "positive" and "negative" test results. Therefore, confusion and uncertainty exist among clinicians with regard to what biomarkers to routinely incorporate into treatment algorithms for patients with NSCLC. There is a need to provide guidance in this area. This article reports the results of a systematic review and recommendations from a consensus meeting of Canadian oncologists and pathologists involved in the diagnosis and treatment of lung cancer.

MATERIALS AND METHODS

A representative sample of 37 medical oncologists and pathologists from across Canada specializing in thoracic oncology participated in this consensus meeting. The specific objectives were the following:

- To review the literature and make recommendations concerning the prognostic or predictive value of histologic classification of NSCLC.
- To review the literature and make recommendations concerning the prognostic and predictive value of molecular biomarkers in NSCLC.

Literature Search

A literature search from 2005 to March 2010 was conducted using PubMed and the following search terms: "nonsmall cell lung cancer/drug therapy" and "randomized" or "randomised" or "randomly" or "trial" or "trials" or "study," plus text words for specific biomarkers. A second search was conducted to identify articles concerning histology using the following terms: "nonsmall cell lung cancer" and "subtyping" or "subtype" or "histologic" or "classification" or "classifying." Conference proceedings for American Society of Clinical Oncology (ASCO), European Society of Medical Oncology (ESMO), and the International Society for the Study of Lung Cancer (IASLC) World Lung Congresses were searched from 2005 to 2009.

Study Selection Criteria

Articles eligible for inclusion included full-text articles and abstracts of randomized clinical trials involving patients with NSCLC, in which data on prognostic and predictive impact of histology or biomarker evaluation were reported. Single-arm phase II trials were only included if there were no data from randomized trials. Only studies published in English were considered. Outcomes of interest included objective response rate (ORR), time to progression (TTP), progression-free survival (PFS), and overall survival (OS). A total of 5439 articles were identified, and 89 articles were included in this systematic review.

Consensus Process

This is a Canadian consensus developed after a systematic review and interdisciplinary evaluation. The articles were reviewed by five pairs of oncologists and pathologists to determine studies for inclusion. Data were summarized and presented at the consensus meeting. Draft recommendations were formulated at this meeting and agreed upon by consen-

sus. The consensus recommendations were sent to all meeting attendees for review and approval. The final manuscript was also sent to meeting attendees for review before submission.

RESULTS

1a: In Patients with NSCLC, is There Sufficient Evidence to Recommend That Histologic Subtype Should Influence the Choice of Systemic Therapy?

Seven trials ($n = 5408$) provide information about the prognostic and predictive value of histology for patients with NSCLC (Table 1).[7,16–21] Several, but not all, trials show worse OS for patients with squamous cell histology, suggesting that this subtype is a negative prognostic factor.[20,22,23]

There is consistent information from three randomized trials that histology is predictive of benefit from pemetrexed.[5,17,20,21] A retrospective analysis of the JMEI trial of second-line pemetrexed versus docetaxel demonstrated that patients with nonsquamous histology treated with pemetrexed had significantly longer survival (9.3 versus 8.0 months, HR 0.82 versus 1.40, interaction test [int] $p = 0.04$).[20,23] That trial mostly showed that squamous patients treated with pemetrexed had an inferior outcome. Similar results were demonstrated in the JMDB first-line trial comparing cisplatin and pemetrexed versus cisplatin and gemcitabine.[21] Survival was significantly better in patients with nonsquamous histology who were treated with cisplatin plus pemetrexed (11.8 versus 10.4 months, HR 0.84 versus 1.23, int $p = 0.002$). However, survival favored the combination of cisplatin plus gemcitabine among patients with squamous cell histology (OS 10.8 versus 9.4 months, $p = 0.05$). The same observation was observed in the JMEN trial of maintenance pemetrexed.[17] The improvement in PFS from maintenance pemetrexed was observed in patients with nonsquamous histology (PFS, HR 0.44, 95% CI, 0.36–0.55; OS, HR 0.70, 95% CI, 0.56–0.88; $p = 0.002$). A significant treatment-by-histology interaction with both PFS ($p = 0.036$) and OS ($p = 0.033$) was noted. A treatment-by-interaction effect for histology was not observed in one other trial comparing carboplatin with either pemetrexed or gemcitabine, although the primary outcome of this trial was quality of life.[24]

A differential effect of treatment according to histology has also been observed in trials of some molecularly targeted agents. In both the BR.21 trial of erlotinib versus best supportive care[7] and the ISEL trial of gefitinib versus best supportive care,[25] a higher ORR was observed in patients with adenocarcinoma (14% versus 4%, $p = 0.001$, 12% versus 5%). However, in BR.21, there was no significant interaction according to histology for OS according to histology (HR 0.8 versus 0.7, int $p > 0.05$). Similarly, an analysis of the FLEX trial evaluating the addition of the EGFR monoclonal antibody cetuximab to cisplatin and vinorelbine demonstrated a significant improvement in survival across all histological subtypes among patients receiving cetuximab.[26]

Trials evaluating therapies directed against VEGF have also demonstrated a treatment versus histology effect. A randomized phase II trial of carboplatin and paclitaxel ± bevacizumab showed an increased risk of fatal hemoptysis in patients randomized to chemotherapy plus bevacizumab.[18] This appeared to be associated with squamous histology and so patients of this histologic subtype subsequently were excluded from phase III trials of bevacizumab. Subsequent analysis of trials of bevacizumab suggests that the risk of

TABLE 1. Summary of Predictive Biomarkers for Histology in NSCLC

Marker	Line of Therapy	Patients (N)	Study	Clinical Results
Histologic subtype	First-line NSCLC	878	E4599	Adenocarcinoma subtype associated with increased PFS (6.6 vs. 5.0 mo, HR 0.65, 0.54–0.78) and OS (14.2 vs 10.3 mo, HR 0.69, 0.58–0.83) with Bev + CT as compared with CT alone. OS data for remaining histology subtypes inconclusive.[19]
	First-line NSCLC	99	Phase II	Apparent improvement in ORR (50% vs. 20%), TTP (7.1 vs. 4.0 mo, $p = 0.01$) and median survival (17.8 vs. 12.2 mo, $p = 0.57$) in nonsquamous histologic subtype. Squamous subtype associated with pulmonary hemorrhage and bleeding with Bev treatment.[18]
	Second-, third-line NSCLC	731	BR.21	Adenocarcinoma subtype associated with significantly higher ORR (14% vs. 4%, $p = 0.001$) to erlotinib but not predictive for OS (HR 0.8 vs 0.7, int $p = 0.05$).[7]
	NSCLC	741	S9806, S0003, S9308	Histologic subtype not associated with OS and PFS with antimicrotubule-platinum therapy.[16]
	Second-line NSCLC	571	JMEI	Nonsquamous histology associated with significantly longer PFS (0.82 vs. 1.40, int $p = 0.04$) and OS for patients treated with pemetrexed versus docetaxel (HR 0.78 vs. 1.56, int $p = 0.001$).[20]
	First-line NSCLC	1725	JMDB	Treatment-by-histology interaction analysis predictive of improved PFS (0.95 vs. 1.36, int $p = 0.02$) and OS (0.84 vs. 1.23, int $p = 0.002$) for patients with nonsquamous histology with Cis + Pem.[21]
	Maintenance NSCLC	663	JMEN	Significant treatment-by-histology interaction predictive of improved PFS (HR 0.44 vs. 0.69, int $p = 0.036$) and OS (HR 0.70 vs. 1.07, int $p = 0.033$) for patients with nonsquamous histology with Pem.[17]

NSCLC, non-small cell lung cancer; CT, chemotherapy; HR, hazard ratio; PFS, progression-free survival; OS, overall survival.

severe pulmonary hemorrhage was associated with the presence of baseline cavitation in lung lesions.[27] In the ECOG 4599 trial of carboplatin and paclitaxel ± bevacizumab, the largest survival benefit was observed in patients with adenocarcinoma histology treated with bevacizumab (14.2 versus 10.3 months, HR 0.69, 95% CI, 0.58–0.83).[19] Similar data are not available from the AVAiL trial of cisplatin and gemcitabine ± bevacizumab.[28,29] In addition, differential outcomes have been observed according to histology. The ESCAPE trial of carboplatin and paclitaxel ± sorafenib permitted entry of patients with squamous histology and showed no difference in the incidence of fatal hemoptysis according to histology.[30] However, there was a trend toward worse OS for patients with squamous carcinoma randomized to chemotherapy plus sorafenib. In contrast, a phase III trial of vandetanib, a tyrosine kinase inhibitor (TKI) active against VEGFR and EGFR, compared with erlotinib, did not show any increased risk of hemoptysis or a differential effect in any histologic subgroups.[31]

1b: In Patients with Newly Diagnosed NSCLC, What Immunohistochemical Testing Should be Performed Routinely to Classify All Tumors Including NSCLC-Not Otherwise Specified into Specific Histologic Types?

A majority of patients with NSCLC present with unresectable disease and frequently the diagnosis is made on small biopsies or cytology specimens. Light microscopy and routine H&E staining may be sufficient to distinguish NSCLC histologic subtypes in a subset of small biopsies or cytology specimens, but in some instances, histologic subtyping is not possible and a diagnosis of NSCLC-not otherwise specified (NOS) is applied to cases that cannot be classified further.

Retrospective studies on resected, cytologic, biopsy, and tissue microarray specimens have investigated the utility of histochemical stains including mucicarmin, PAS/PASD, Alcian blue (AB), and antibodies against p63,[32–37] cytokeratin (CK 5/6),[32,34–36,38,39] high-molecular-weight cytokeratins (34βE12),[40,41] thyroid transcription factor-1 (TTF-1),[37,39,41] CK7,[39–41] and more recently desmocollin-3[42] and napsin A[43–45] in distinguishing squamous cell carcinoma from adenocarcinoma. Different methodologies, comparison of different tests, as well as differences in the antibody clones used, or the interpretation and scoring systems for individual tests make comparison of data from these studies difficult.

Two recent publications have investigated using a panel of IHC markers in small biopsies for greater diagnostic accuracy.[46,47] Loo et al.[46] observed that using AB/periodic acid Schiff for mucin in combination with IHC staining for p63 and TTF-1 in undifferentiated NSCLC samples was able to predict the histologic subtype of NSCLC, as diagnosed on resection specimens and classified on morphology alone in concordance with the latest World Health Organization Classification of Lung Cancer (4th edition). Nicholson et al.[47] found that markers against cytokeratin 5/6, P63, TTF-1, and a D-PAS stain for mucin increased the diagnostic ability to classify NSCLC-NOS tumors into squamous cell carcinoma or adenocarcinoma.

Consensus Recommendation

There is some evidence that histology is prognostic for survival. There is strong evidence that histologic subtype is predictive of treatment efficacy and/or toxicity. Therefore, every effort to accurately subclassify all NSCLC specimens should be undertaken and could be performed on H&E-stained slides based on the tumor morphology. In cases with equivocal morphologic features, routine stains for mucin (such as AB/PAS) and IHC stains including TTF-1, p63, and CK5/6 should be performed and their interpretation stated in the pathology report. The staining pattern should be used to favor adenocarcinoma or squamous cell carcinoma or to report the tumor as NSCLC-NOS in cases with equivocal staining patterns.

Assessment of biomarkers in patients with NSCLC is likely to become increasingly important. Therefore, it is recommended that adequate diagnostic material be obtained to perform appropriate testing for both histologic subtyping and biomarker assessment. Wherever possible, it is recommended that a biopsy from a primary (lung) or metastatic site should be obtained rather than a cytology specimen only. For cytology specimens, it is strongly recommended that a cell block be prepared in all cases with sufficient material.

2: In Patients with Newly Diagnosed NSCLC, Should Diagnostic Material be Evaluated Routinely for Mutations of the Epidermal Growth Factor Receptor? Should Such Testing be Performed in all Patients or in a Subset of Patients Selected on Pathological and/or Clinical Characteristics?

EGFR Protein Expression (IHC)

Information is available from six trials (Table 2, $n = 2691$).[11,12,48–51] These trials did not examine the prognostic value of EGFR protein expression. Clinical data on the utility of EGFR protein expression as a predictive biomarker have been inconsistent. An analysis from the BR.21[7,12] and ISEL[11,25] studies showed that patients with EGFR expression treated with an EGFR-TKI had a higher ORR than those with EGFR IHC-negative tumors (BR.21: 11% versus 4%; $p = 0.03$; ISEL: 8.2% versus 1.5%; p value not reported). However, a differential survival benefit was not demonstrated in BR.21 (HR 0.68 versus 0.93, int $p > 0.05$), whereas there was a qualitative interaction based on EGFR expression in ISEL that was of borderline significance (HR 0.77 versus 1.57, $p = 0.049$). The SATURN trial of maintenance erlotinib versus placebo after platinum-based chemotherapy also showed no significant interaction for survival between EGFR IHC status and treatment effect (HR 0.69 versus 0.77, int $p = 0.63$).[48]

In the INTEREST trial comparing gefitinib to docetaxel, no differences in ORR, PFS, or OS were observed according to EGFR protein expression.[49] Similar results were obtained in an analysis of the BMS099 trial comparing carboplatin and paclitaxel ± cetuximab.[52] Demonstrating a treatment interaction based on biomarker status in trials

TABLE 2. Summary of the Predictive Biomarkers for EGFR in NSCLC

EGFR protein expression	Second-, third-line	325	BR.21	EGFR IHC positivity associated with significantly higher response to Erl (11% vs. 4%, $p = 0.03$) but not OS (HR 0.68 vs. 0.93, int $p > 0.05$)[12]
	Second-, third-line NSCLC	379	ISEL	EGFR IHC positivity associated with significantly better survival with Gef (HR 0.77 vs. 1.57, int $p = 0.049$)[11]
	First-line NSCLC	365	IPASS	EGFR IHC not predictive of improved PFS (HR 0.73 vs. 0.97, int $p = 0.21$) with Gef[50]
	Second-, third-line NSCLC	380	INTEREST	EGFR IHC not predictive of improved ORR, PFS (HR 1.29 vs. 0.90) or OS (HR 1.0 vs. 1.0, int $p = 0.87$) with Gef[49]
	Maintenance NSCLC	742	SATURN	EGFR IHC not predictive of improved PFS (HR 0.69 vs. 0.77, int $p = 0.63$) with Erl[48]
	First-line NSCLC	500	TALENT	EGFR IHC not predictive of improved OS (HR 1.0 vs. 1.02) with Erl[51]
EGFR copy number	Second-, third-line NSCLC	159	BR.21	High *EGFR* copy number associated with significantly higher response (21% vs. 55%, $p = 0.02$) and increased OS (HR 0.43 vs. 0.80, int $p = 0.33$) with Erl[55]
	Second-, third-line NSCLC	370	ISEL	High *EGFR* gene copy number associated with significantly longer median survival with Gef (HR 0.61 vs. 1.16, int $p = 0.045$)[11]
	First-line NSCLC	406	IPASS	High *EGFR* copy number not predictive of PFS (HR 0.66 vs. 1.24, int $p = 0.044$) independent of EGFR mutation status ($FISH^+/Mut^+$ HR 0.48, $FISH^+/Mut^-$ HR 3.85) with Gef[50]
	Second-, third-line NSCLC	374	INTEREST	High *EGFR* copy number not predictive of improved PFS (HR 0.84 vs. 1.30, int $p = 0.112$) or OS (HR 1.09 vs. 0.93, int $p = 0.52$) with Gef[49]
	Maintenance NSCLC	488	SATURN	FISH not predictive of improved PFS (HR 0.68 vs. 0.81, int $p = 0.35$) with Erl[48]
	First-line NSCLC	500	TALENT	No improvement in ORR or OS with Erl in patients with *EGFR* gene amplification[51]
	First-line NSCLC	245	TRIBUTE	No improvement in ORR (12% vs. 22%, $p > 0.05$) or OS with Erl in patients with *EGFR* gene amplification[57]
	First-line NSCLC	453	INTACT I/II	No improvement in OS with Gef in patients with *EGFR* gene amplification (HR 2.03 vs. 1.01, $p > 0.05$)[53]
EGFR mutations	Second-, third-line NSCLC	204	BR.21	*EGFR* mutations predictive of response to Erl (27% vs. 7%, $p = 0.35$) but not predictive of improved OS (HR 0.55 vs. 0.74, int $p = 0.47$)[12,55]
	Second-, third-line NSCLC	215	ISEL	*EGFR* mutations associated with higher response rates with Gef (37.5% vs. 2.6%); insufficient data for survival analysis[11]
	Maintenance NSCLC	437	SATURN	*EGFR* mutations predictive of improved PFS (HR 0.16 vs. 0.78, int $p < 0.001$) with Erl but not predictive for OS (HR 0.83 vs. 0.77)[48]
	First-line NSCLC	202	BMS099	*EGFR* mutations not predictive of improved PFS (HR 1.17 vs. 0.95) or OS (HR 1.62 vs. 0.91) from cetuximab[52]
				EGFR mutations associated with significantly increased:
	First-line NSCLC	437	IPASS	ORR and PFS (HR 0.48 vs. 2.85, int $p < 0.0001$) with Gef but not OS (data not mature)[60]
	First-line NSCLC	309	FIRST-SIGNAL	ORR (OR 9.2 vs. 0.32) and PFS (HR 0.61 vs. 1.51) with Gef but not OS (HR 0.82 vs. 1.20, data not mature)[61]
	First-line NSCLC§	172	WJTOG3405	ORR (62% vs. 32%, $p < 0.0001$) and PFS (HR 0.49, 0.37–0.71) with Gef; data still immature for OS[59]
	Second-, third-line NSCLC	297	INTEREST	PFS (HR 0.16 vs. 1.24) and OR (42% vs. 21%, $p = 0.04$) with Gef but not predictive for OS (HR 0.83 vs. 1.02, int $p = 059$)[49]
	First-line NSCLC§	230	NEJ002	ORR (74% vs. 31%, $p < 0.0001$) and PFS (HR 0.30, 0.22–0.41) with Gef but not OS[58]
	Second-/third-line NSCLC	79	IDEAL I/II	Predictive of increased ORR to Gef[53]
	First-line NSCLC	312	INTACT I/II	Not predictive of OS with Gef (HR 1.77 vs. 0.91, $p > 0.05$)[53]
	First-line NSCLC	293	TALENT	Not predictive of ORR, PFS (HR 0.59 vs. 0.95), or OS (HR 0.95 vs. 1.15) with Erl[51]
	First-line NSCLC	274	TRIBUTE	Predictive of ORR (53% vs. 18%, $p < 0.01$) but not predictive of TTP (12.5 vs. 6.6 mo, $p = 0.092$) or OS with Erl[57]

NSCLC, non-small cell lung cancer; NR, not reported; Erl, erlotinib; Gef, gefitinib; ORR, overall response rate; PFS, progression-free survival; TTP, time to progression.

comparing one active treatment to another, or the addition of a targeted agent to standard chemotherapy, is likely to be more difficult than in placebo-controlled trials, as the magnitude of any treatment difference is likely to be smaller, and factors predicting better outcomes from the targeted agent might also predict better outcomes from chemotherapy.

EGFR Copy Number (FISH or qPCR)

There are data on 2994 patients from nine trials assessing EGFR gene copy number (Table 2).[11,48–51,53–55] Data from the BR.21 trial suggest that increased *EGFR* gene copy number is associated with a worse OS (HR 1.93, 95% CI, 1.09–3.44, Table 5).[55]

Conflicting results have been observed regarding the predictive value of *EGFR* gene copy number and the efficacy of EGFR-TKIs or EGFR monoclonal antibodies (Table 2). No differential improvement in OS was observed in patients with *EGFR* amplification assessed by PCR in the TALENT,[51] TRIBUTE,[54] and INTACT I/II[53] trials of platinum-based chemotherapy ± an EGFR-TKI. Similarly, FISH status was not associated with a significant difference in OS in the INTEREST trial (HR 1.09 versus 0.93, int $p = 0.52$), although there was a trend toward better PFS in FISH-positive patients receiving gefitinib (HR 0.84 versus 1.3, $p = 0.112$).[49] FISH status was not predictive for survival in the SATURN trial (HR 0.68 versus 0.81, int $p = 0.35$)[48] or the trials evaluating chemotherapy ± cetuximab.[52,56] The only trials demonstrating some predictive value from FISH are trials comparing an EGFR-TKI to placebo. The second-/third-line trials of gefitinib (HR 0.61 versus 1.16, int $p = 0.45$) or erlotinib (HR 0.43 versus 0.80, int $p = 0.33$) found a correlation between increased *EGFR* gene copy (FISH) and OS.[7,11,12,55]

EGFR Mutations

Activating mutations of the *EGFR* gene were first reported in 2005. Deletions in exon 19 and L858R point mutations in exon 21 were reported to be associated with increased sensitivity to treatment with EGFR-TKIs.[9,10] Available data suggest that the incidence of such mutations is greatest among people of Asian ethnicity, adenocarcinoma, females, and never smokers.[53]

The presence of an *EGFR* activating mutation is generally associated with improved prognosis (Table 5).[49,55,57–60] Data from BR.21 did not show a prognostic effect for *EGFR* mutations.[55] However, data from both the INTEREST and TRIBUTE trials demonstrate that the patients with *EGFR* mutations have a longer PFS and OS irrespective of the treatment.[49,57] Data from the first-line trials comparing an EGFR-TKI to platinum-based chemotherapy[58–60] all show survival estimates considerably longer than the expected 8 to 10 months in patients with metastatic NSCLC.[3]

Fourteen trials ($n = 3259$, Table 2) have examined the predictive value of activating mutations of the *EGFR* gene.[11,12,48,49,51,53,55,57–61] Three trials compared an EGFR-TKI with placebo. In patients previously treated with chemotherapy in the BR.21 and ISEL trials,[11,12,55] the presence of *EGFR* mutations was associated with significantly increased ORR (BR.21: 27% versus 7%; $p = 0{\cdot}04$; ISEL: 37.5% versus 2.6%; p value not reported). However, the BR.21 trial did not demonstrate significantly improved survival for mutation-positive patients compared with *EGFR* wild type (HR 0·55 versus 0·74; int $p > 0.05$). Interestingly, biomarker analysis for the SATURN trial of maintenance erlotinib versus placebo demonstrated a large benefit in PFS for patients with an *EGFR* mutation (HR 0.10, 95% CI, 0.04–0.25; $p < 0.0001$), but there was no evidence of a greater survival benefit for *EGFR* mutation-positive patients (HR 0.83 versus 0.77).[62] One potential explanation for this is the effect of crossover among mutation-positive patients.

In the TALENT, TRIBUTE, IDEAL I/II, and INTACT I/II trials, the presence of an *EGFR* mutation was not predictive of improved survival from therapy with erlotinib or gefitinib.[51,53,57] However, trials comparing first-line EGFR-TKI versus platinum-based chemotherapy have shown a large benefit in PFS for patients with activating *EGFR* mutations. In the IPASS study comparing gefitinib to carboplatin and paclitaxel, PFS in *EGFR* mutation positive patients was significantly longer among patients who received gefitinib (HR 0.48; 95% CI, 0.36–0.64; $p < 0.001$).[60] However, there was a significant qualitative interaction in PFS for *EGFR*-negative patients receiving gefitinib (HR 2.85, 95% CI, 2.05–3.98; $p < 0.001$). No significant differences in OS were reported. Similar findings were observed in the First Signal trial of gefitinib versus cisplatin and gemcitabine (mutation positive, HR 0.394; 95% CI, 0.22–0.7; $p = 0.0006$, mutation negative, HR 0.86; 95% CI, 0.45–1.65; $p = 0.319$).[61] In trials limited to *EGFR* mutation positive patients, substantial improvements in PFS have also been observed, although no trial to date has demonstrated improved OS.[58,59] Similarly, the INTEREST trial of second-line gefitinib versus docetaxel demonstrated increased ORR and PFS (HR 0.16 versus 1.24) for *EGFR* mutation positive patients receiving gefitinib but no differential effect on OS (HR 0.83 versus 1.02, int $p = 0.59$).[49] Unlike results obtained from studies with the EGFR-TKIs, *EGFR* mutation status was not found to be a predictive biomarker for cetuximab efficacy in the BMS099 or FLEX trials.

Consensus Recommendation

There is sufficient evidence from randomized trials demonstrating that activating mutations of the epidermal growth factor receptor are predictive of greater benefit from treatment with TKIs of the epidermal growth factor receptor (EGFR-TKIs). It is recommended that diagnostic lung cancer samples of patients with NSCLC be tested routinely for activating mutations of the *EGFR*. Given the available clinical data, this testing should be limited to patients with advanced NSCLC and nonsquamous histology. Testing should be completed in a licensed clinical molecular genetics laboratory. Mutation testing is most relevant to treatment decisions in the first-line therapy setting. Clinical data for EGFR protein expression using IHC or gene copy using FISH testing are inconsistent. Therefore, testing using IHC or FISH is not recommended routinely.

3: Potential Markers of Chemotherapy Sensitivity: In patients with Newly Diagnosed NSCLC, is There a Role for the Routine Assessment of Molecular Markers Such as *K-RAS*, Ki-67, p27, p16, Cyclin-Dependent Kinases, *ERCC1*, *BRCA*, β-tubulin III, RRM1, P-53?

K-RAS

Data from four trials on the prognostic effect of *K-RAS* are summarized in Table 5.[48,55,63,64] These trials show no

clear effect of *K-RAS* on OS. However, a 2005 meta-analysis of 23 trials involving 2631 patients suggests that mutations of the *RAS* oncogene are a weak negative prognostic factor (HR 1.41, 95% CI 1.18–1.65).[65]

Information about the predictive value from eight trials ($n = 2442$, Table 3) of *K-RAS* mutations is somewhat conflicting.[48,49,51,52,55–57,63,64] In the BR.10 trial of adjuvant chemotherapy in completely resected stage IB and II NSCLC, patients with wild-type *K-RAS* had a significant improvement in OS from adjuvant chemotherapy (HR 0.69, 95% CI 0.49–0.95, $p = 0.03$), whereas patients with *K-RAS* mutations did not appear to benefit (HR 0.95, 95% CI 0.53–1.71, $p = 0.87$).[64] However, the p value for treatment interaction was not significant (int $p = 0.29$). In the E4592 trial of adjuvant radiation ± chemotherapy, *K-RAS* was not correlated with PFS or OS.[63]

There is also a lack of consistent information about the predictive value of *K-RAS* mutations for EGFR therapy. In the TRIBUTE trial, patients with *K-RAS* mutations who received chemotherapy plus erlotinib appeared to have the lowest ORR and the shortest PFS and OS.[57] Similarly, data from the BR.21 trial suggested that the patients with mutations of the *K-RAS* gene receiving erlotinib had a worse OS than *RAS* wild type patients (HR 1.67 versus 0.69), although a statistical test for treatment interaction was not significant (int $p = 0.09$).[12,55]

In contrast, several trials evaluating an EGFR-TKIs have not shown any differential treatment effect of *K-RAS* status. The SATURN trial of maintenance erlotinib demonstrated improved PFS in patients with both *K-RAS* mutations and wild type (HR 0.77 versus 0.70, int $p = 0.95$).[48] In the INTEREST trial comparing gefitinib to docetaxel, *K-RAS* mutations appeared predictive of ORR for both gefitinib (0% versus 9%) and docetaxel (3.7% versus 11.9%).[49] However, *K-RAS* did not predict either PFS (HR 1.16 versus 1.23) or OS (HR 0.81 versus 1.03, int $p = 0.51$). *RAS* status was not predictive of PFS or OS in either the BMS099[52] or FLEX[56] trials evaluating the EGFR monoclonal antibody, cetuximab.

Other Potential Markers of Chemosensitivity

A variety of other molecular markers have been postulated to predict sensitivity to chemotherapy including *RRM1*, ERCC1, BRCA, class III β-tubulin, *p-53*, plus Ki-67, p27, p16, and cyclin-dependent kinases (Table 4). Most of these analyses either have been done in limited patient populations or lack validation studies that prevent firm recommendations.

An analysis of the IALT trial of adjuvant chemotherapy demonstrated that high *ERCC1* expression may be prognostic for improved OS (Table 5; HR 0.88, 0.71–1.10, $p = 0.26$) and predictive for lack of benefit from chemotherapy (HR 1.14 versus 0.65, int $p = 0.009$).[66] Data from three other randomized trials suggests the *ERCC1* mRNA did not predict for ORR or OS.[67–69]

Data from the BR.10 trial suggested that the high levels of class III β-tubulin were prognostic for worse OS (Table 5; HR 1.72, 1.02–2.88, $p = 0.04$) and predictive of benefit from adjuvant chemotherapy (HR 0.64 versus 1.0, int $p = 0.25$).[70] However, the results of the LACE Bio meta-analysis found that β-tubulin was not predictive of benefit from adjuvant chemotherapy.[71] Data on other markers such as RRM1,[69,72] BRCA,[72] *p-53*,[64] Ki-67, p27, p16, and cyclin-dependent kinases[73] are all insufficient to make any recommendations supporting routine assessment.

Consensus Recommendation

Mutations of the *KRAS* gene are weakly prognostic for poorer survival in NSCLC patients. Clinical data concerning the predictive value of *KRAS* for chemotherapy or EGFR therapy are inconsistent. Therefore, there is currently insufficient evidence to recommend testing of *KRAS*.

There is currently insufficient evidence that testing for ERCC1, BRCA, β-tubulin III, *RRM1*, and *P-53* influences clinical treatment decisions. Therefore, such testing is currently not recommended. There are no data to support testing of Ki-67, p27, p16, and cyclin-dependent kinases.

TABLE 3. Summary of the Predictive Biomarkers for *K-RAS* in NSCLC

KRAS mutations	Adjuvant NSCLC	450	JBR.10	Suggests lack of survival benefit with Adj CT for *KRAS* mutations (HR 0.69 vs. 0.95, int $p = 0.29$), but test for statistical interaction negative[64]
	First-line NSCLC	274	TRIBUTE	*KRAS* mutations associated with significantly decreased TTP (1.1 vs. 3.8 mo) and median survival with Erl + CT (4.4 vs. 13.5 mo, $p = 0.019$)[57]
	First-line NSCLC	163	TALENT	*KRAS* mutations not predictive of PFS (HR 0.79 vs. 0.88) or OS (HR 1.66 vs. 1.07) with Erl[51]
	Second-, third-line NSCLC	206	BR.21	Trend to worse OS in patients with *KRAS* mutations receiving Erl (HR 1.67 vs. 0.69, int $p = 0.09$)[55]
	Maintenance NSCLC	493	SATURN	*KRAS* mutations not predictive of PFS (HR 0.77 vs. 0.70, int $p = 0.95$) or OS benefit with erlotinib[48]
	Second, third line NSCLC	275	INTEREST	*KRAS* mutations not predictive of differential ORR, PFS (HR 1.16 vs. 1.23) and OS (HR 0.81 vs. 1.03, int $p = 0.51$) between Gef and Doc[49]
	First-line NSCLC	379	FLEX	*KRAS* mutations not predictive of differential ORR (37% vs. 37%), PFS (HR 0.84 vs. 0.97), or OS (HR 1.00 vs. 0.96) benefit with Cet + CT vs. CT[56]
	First-line NSCLC	202	BMS099	*KRAS* mutations not predictive of differential PFS (HR 0.64 vs. 1.07) and OS (HR 0.97 vs. 0.93) benefit with Cet + CT vs. CT[52]

NSCLC, non-small cell lung cancer; Adj, adjuvant; NR, not reported; Erl, erlotinib; Gef, gefitinib; Car, carboplatin; Cis, cisplatin; CT, chemotherapy; Doc, docetaxel; Gem, gemcitabine; ORR, overall response rate; Pem, pemetrexed; Tax, taxane; PFS, progression-free survival; TTP, time to progression.

TABLE 4. Summary of the Predictive Biomarkers for Other Markers of Chemosensitivity for NSCLC

p53 mutation	Adjuvant NSCLC	445	JBR.10	Suggests lack of survival benefit from Adj. CT in patients with *p53* mutations (HR 0.78, 0.46–1.32 vs. 0.67, 0.46–0.98, int $p = 0.65$)[64]
p53 expression			JBR.10	p53 expression associated with improved OS from Adj. CT (HR 0.54 vs. 1.40, int $p = 0.02$)[64]
ERCC1 expression	Adjuvant NSCLC	761	IALT-Bio	High ERCC1 expression (IHC) predictive of lack of survival benefit from Adj. CT (HR 1.14 vs. 0.65, int $p = 0.009$)[66]
	First-line NSCLC	66	BTOG1	ERCC1 mRNA expression not associated with response to (36% vs. 28%, $p = 0.79$), or survival (median OS 415 vs 327 d, $p = 0.81$) with first-line platinum-based CT[67]
	First-line NSCLC	366	ERCC1-based customized trial	Higher ORR in low ERCC1 mRNA expression (53% vs. 47%) to cisplatin-based chemotherapy but not predictive of TTP (HR 0.79, 0.61–1.03) or OS (median OS 10.4 vs. 9.5 mo)[68]
	First-line NSCLC	81	GEPC/98-02	Low ERCC1 mRNA levels trend for longer median survival (13.7 vs. 9.5 mo, $p = 0.19$) and TTP (8.4 vs. 5.1 mo, $p = 0.07$) with Gem + Cis‡. Longer median survival associated with low mRNA expression levels of both RRM1 and ERCC1 treated with Gem + Cis compared with high levels of RRM1 and ERCC1 (11 vs. 2.7 mo)[69]
RRM1 expression	First-line NSCLC	96	HORG	High *RRM1* mRNA expression associated with significantly increased risk of progression and death after first-line Gem + Doc (HR 1.02, 1.01–1.02)[72]
	First-line NSCLC	81	GEPC/98-02	Low RRM1 expression associated with significantly longer TTP (median PFS 8.4 vs. 2.7 mo, $p = 0.02$) and median survival after first-line Gem + Cis (median OS 13.7 vs. 3.6 mo, $p = 0.009$)[69]
BRCA1 expression	First-line NSCLC	96	HORG	High *BRCA1* mRNA levels associated with increased probability of response to first-line Gem + Doc (OR 1.09, 1.02–1.16)[72]
Tubulin expression	Adjuvant NSCLC	1149	LACE-Bio	Tubulin IHC positivity not associated with survival from Adj. chemotherapy (HR 1.03 vs. 0.83, int $p = 0.20$)[71]
	Early-stage NSCLC	412	IFCT-0002	Tubulin IHC negativity significantly associated with PFS (median PFS 31 vs. 60 mo, $p = 0.014$) and (OS 72 vs. >84 mo, $p = 0.013$) with neoadjuvant CT[97]
P27 expression	Adjuvant NSCLC	783	IALT-Bio	$p27^{Kip1}$ IHC negativity associated with significantly longer overall survival from Adj CT (HR 0.66 vs. 1.09, int $p = 0.02$)[73]
Biomarker for response to anti-VEGFR agents	First-line NSCLC	160	E4599	High baseline plasma VEGF levels significantly associated with ORR (33% vs. 8%, $p = 0.01$) and PFS (median 4.5 vs. 6 mo, $p = 0.04$) from Bev but not survival[75]
				Low baseline plasma ICAM levels significantly associated with ORR and (HR 1.0 vs. 2.14, int $p < 0.0001$) with CT ± Bev[75]
	First-line NSCLC	358	AVAiL	Low plasma ICAM1 levels associated with a trend toward improved PFS with Bev (HR 0.64 vs. 1.04, int $p < 0.15$)[76]
				High plasma bFGF levels associated with a trend toward improved PFS (HR 0.47 vs. 0.74, int $p < 0.15$) and OS (HR 0.52 vs. 1.13, int $p < 0.15$)[76]
	First-line NSCLC	123	Phase II	Increase in plasma ICAM1 levels after day 8 of vandetanib treatment significantly associated with improved PFS[77]

NSCLC, non-small cell lung cancer; Adj, adjuvant; NR, not reported; VEGF, vascular endothelial growth factor; ICAM, intercellular adhesion molecule; bFGF, basic fibroblast growth factor; Erl, erlotinib; Gef, gefitinib; Car, carboplatin; Cis, cisplatin; CT, chemotherapy; Doc, docetaxel; Gem, gemcitabine; ORR, overall response rate; Pem, pemetrexed; Tax, taxane; PFS, progression-free survival; TTP, time to progression.

4: Vascular Endothelial Growth Factor (VEGF). In Patients with NSCLC Who Are Candidates for Therapy with an Agent Active Against the VEGF Pathway, Are There Any Reliable Biomarkers That Can Be Recommended to Aid in the Selection of Patients for Anti-VEGF Therapy?

Two randomized trials (ECOG 4599 and AVAiL) evaluated the addition of bevacizumab to platinum-based chemotherapy.[28,29,74] Both trials demonstrated a higher ORR and improved PFS. However, only the ECOG 4599 trial demonstrated improved OS.[74] The addition of bevacizumab does result in additional toxicities. However, attempts to identify biomarkers to improve patient selection have been exploratory and so far unsuccessful.

Biomarker data from the ECOG 4599 trial showed that regardless of treatment arm, low baseline levels in intracellular adhesion molecule (ICAM) were prognostic for better OS ($p = 0.00005$) and 1-year survival (65% versus 25%, Table 5) and predictive of a higher ORR (32% versus 14%, $p = 0.02$), compared with high ICAM.[75] High serum VEGF levels were predictive of response to chemotherapy plus bevacizumab but not predictive of improved survival. In the AVAiL trial, high baseline ICAM, VCAM, βFGF, and VEGF were prognostic for shorter OS compared with lower levels.[76]

In a comprehensive biomarker study by Hanrahan et al.,[77] the authors explored a set of 35 plasma biomarkers in NSCLC patients at four time points after antiangiogenic therapy with (a) the VEGF receptor 2 TKI vandetanib alone, (b) chemotherapy alone, or (c) a combination of the two, in a

TABLE 5. Summary of Prognostic Biomarkers in NSCLC

Marker	Patients (*N*)	Chemotherapy	Study	Clinical Results
EGFR copy number	159	Second-, third-line NSCLC	BR.21	*EGFR* FISH-positive status prognostic for worse survival (HR 1.93, 1.09–3.44)[55]
EGFR mutation status	204	Second-, third-line NSCLC	BR.21	*EGFR* mutations not prognostic for survival ($p = 0.91$)[55]
	297	Second-, third-line NSCLC	INTEREST	*EGFR* mutations associated with improved OS irrespective of treatment with Gef or CT (14/17 mo vs. 7.6/8 mo)[49]
	274	First-line NSCLC	TRIBUTE	*EGFR* mutations associated with significantly better TTP (8 vs 5 mo, $p = 0.001$), and survival (10 mo vs. unable to calculate, $p < 0.001$) irrespective of treatment with Erl + CT or CT alone[57]
KRAS mutations status	445	Adjuvant NSCLC	JRB.10	*KRAS* mutations not prognostic for survival (HR 1.23, 0.76–1.71)[64]
	206	Second-, third-line NSCLC	BR.21	*KRAS* mutations not prognostic for survival ($p = 0.71$)[55]
	493	Maintenance NSCLC	SATURN	*KRAS* mutations associated with significantly shorter PFS[48]
	184	Adjuvant NSCLC	E3590	*KRAS* mutations not prognostic for survival (median OS 30 vs. 42 mo, $p = 0.38$)[63]
p53 expression status	445	Adjuvant NSCLC	JBR.10	p53 protein overexpression associated with worse OS (HR 1.89, 1.07–3.34)[64]
	180	Adjuvant NSCLC	E3590	p53 expression not prognostic for PFS and survival (1-yr OS 85% vs. 77%, $p = 0.93$)[63]
p53 mutation status	445	Adjuvant NSCLC	JBR.10	*p53* mutations not prognostic for survival (HR 1.15, 0.75–1.77)[64]
	183	Adjuvant NSCLC	E3590	*p53* mutations not prognostic for PFS and survival (median OS 38 vs. 52 mo, $p = 0.83$)[63]
ERCC1 expression	761	Adjuvant NSCLC	IALT-Bio	ERCC1 IHC positivity prognostic for survival (HR 0.88, 0.71–1.10, $p = 0.26$)[66]
	66	First-line NSCLC	BTOG1	Trend towards better survival in patients with high ERCC1 mRNA (HR 0.96, 0.92–1.004)[67]
Tubulin expression	1149	Adjuvant NSCLC	LACE-Bio	Tubulin IHC positivity prognostic for worse DFS (1.30, 1.11–1.53) and overall survival in early NSCLC (HR 1.27, 1.07–1.51)[71]
Antiangiogenic and other biomarkers	160	First-line NSCLC	E4599	Low baseline ICAM levels prognostic for overall survival (1-yr OS 65% vs. 25%, $p = 0.00005$)[75]
	358	First-line NSCLC	AVAiL	High baseline levels of VEGF, ICAM-1, VCAM-1, and bFGF associated with shorter overall survival[76]

NSCLC, non-small cell lung cancer; CT, chemotherapy; HR, hazard ratio; PFS, progression-free survival; OS, overall survival; TTP, time to progression.

randomized, three-arm trial. In this study, only lower levels of ICAM 1 at day 8 after treatment were found to be significantly associated with poorer treatment outcome in the groups of patients who received vandetanib.

Consensus Recommendation

No biomarkers to date reliably predict improved efficacy with VEGF or VEGFR inhibition. Clinical characteristics may be associated with toxicity, based on safety data from phase II trials. These include predominantly squamous histology (bevacizumab and other agents, but not all), significant hemoptysis at baseline, and baseline cavitation in a pulmonary lesion associated with an airway. Further molecular biomarker evaluation should be incorporated into clinical trials of these agents.

5. EML4/ALK: In Patients with Newly Diagnosed NSCLC, Should EML4/ALK Mutations be Assessed Routinely?

The fusion protein created by the translocation of the echinoderm microtubule-associated protein-like 4 (*EML4*) and the anaplastic lymphoma kinase (*ALK*) genes defines a new molecular subset of NSCLC with particular clinicopathologic features. Translocations of the *EML4*/*ALK* fusion gene are thought to represent 1.6 to 6.7% of all NSCLC.[78–84] Data would suggest that the ORR, TTP, and OS are similar among patients with wild-type and *EML4*/*ALK* translocations treated with platinum-based chemotherapy.[15] In contrast, patients with *ALK* mutations appear unlikely to benefit from therapy with an EGFR-TKI.

Recent data from a phase I trial demonstrate that the presence of the rearranged *EML4*/*ALK* gene is predictive of a high response rate to specific inhibitors of the *ALK* fusion protein.[85,86] Patients with a known translocation of the *ALK* gene treated with PF02341066 (crizotinib) demonstrated a high ORR (53%). Randomized trials of crizotinib compared with platinum-based chemotherapy or second-line docetaxel or pemetrexed are ongoing.

To date, no one method of reliably assessing *EML4*/*ALK* rearrangements has been determined. FISH,[15,87,88] IHC,[15,87] and PCR[81,89] techniques have all been assessed. Additional research is needed to determine the optimal method of assessment.

Consensus Recommendation

As no specific therapy is approved for *ALK*-associated NSCLC, routine assessment for this biomarker cannot be recommended at this time. Considering the emerging avail-

ability of specific targeted agents for these tumors, further studies aimed at standardizing assays, specifically comparing IHC to FISH to detect these cases seem urgently required.

DISCUSSION

The advances in understanding of the molecular abnormalities occurring in NSCLC have been met with much enthusiasm among clinicians involved in the treatment of this disease. This knowledge has generated a number of biological hypotheses about molecularly targeted agents and a corresponding large number of clinical trials evaluating such agents, either alone or in combination with standard systemic treatments. Many trials have failed to demonstrate improvements in survival and the observed benefits have generally been modest and have often resulted in additional and frequently unique toxicities. Therefore, there is a strong desire to be able to select patient subgroups that are more likely to benefit from these treatments.

To address these concerns, translational research has become a more important end point for clinical trials in recent years. However, the percentage of patients in whom tumor samples were available has varied considerably across clinical trials and there has been inconsistency observed in the results of such analyses. There is also inconsistency in the end points evaluated including tumor response rate, TTP, and OS. As a result, there is often conflicting evidence concerning the use of biomarkers and a lack of clarity about which biomarkers should be incorporated into routine practice.

Personalizing treatment approaches in NSCLC has become a frequent theme in international meetings, and a number of leading experts have put forward treatment algorithms based on a number of factors including histologic subtype, presence of activating mutations of the *EGFR*, *ALK* translocations, and expression of thymidalate synthase, ERCC1, or RRM1.[90]

However, it is clear from this systematic review that there is inadequate information about many of the molecular markers that have been proposed in NSCLC. The available data support the need for more accurate histologic subtyping of all NSCLC tumor samples when possible, as well as routine assessment for *EGFR* mutations. Knowledge in this area although continues to evolve rapidly. Recent publications have suggested algorithms to help in the classification of small tumor samples.[91] In addition, a recent publication from the International Association for the Study of Lung Cancer (IASLC) proposes a change in the classification of lung adenocarcinoma.[92] Such measures may help to implement changes in histologic reporting. Since the consensus meeting was held, impressive response rates obtained with crizotinib were published and suggest that *ALK* translocation detection may soon become an integral part of lung cancer diagnosis.[93] Furthermore, data from the LACE Bio meta-analysis presented at the 2010 ESMO meeting do not support the use of *K-RAS*[94] or *p53*[95] in selecting patients for adjuvant chemotherapy. For the majority of biomarkers, there is thus a need for prospective validation in clinical trials comparing empiric treatment selection versus biomarker-driven treatments.

The increasing use of biomarkers also highlights an issue for healthcare funders, particularly for publicly funded healthcare systems. There is often a desire to rapidly incorporate therapeutic advances into routine clinical practice. However, when the benefit of a new treatment, such as first line therapy with gefitinib, is limited to a population defined by a molecular abnormality, healthcare systems need to respond to the need for the molecular testing as well as the therapy itself. Well-structured and standardized laboratories with adequate quality control programs are necessary to realize the promise of appropriate patient selection.

With increasing data, biomarker use is likely to assume even greater importance for treatment decisions in lung cancer patients. This change will challenge the current diagnostic paradigm for NSCLC. Many patients are diagnosed from cytologic samples or small biopsies, where there may be inadequate amounts of tumor for such biomarker assessments. There is a strong need to obtain adequate diagnostic samples. Where possible, biopsy rather than cytologic material should be obtained. This will require education not only of oncologists but also of respirologists, thoracic surgeons, and pathologists involved in the diagnosis of patients with lung cancer.

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