How close are we to customizing chemotherapy in early non-small cell lung cancer?

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Abstract: Although surgery is the only potentially curative treatment for early-stage non-small cell lung cancer (NSCLC), 5-year survival rates range from 77% for stage IA tumors to 23% in stage IIIA disease. Adjuvant chemotherapy has recently been established as a standard of care for resected stage II-III NSCLC, on the basis of large-scale clinical trials employing third-generation platinum-based regimens. As the overall absolute 5-year survival benefit from this approach does not exceed 5% and potential long-term complications are an issue of concern, the aim of customized adjuvant systemic treatment is to optimize the toxicity/benefit ratio, so that low-risk individuals are spared from unnecessary intervention, while avoiding undertreatment of high-risk patients, including those with stage I disease. Therefore, the application of reliable prognostic and predictive biomarkers would enable to identify appropriate patients for the most effective treatment.

This is an overview of the data available on the most promising clinicopathological and molecular biomarkers that could affect adjuvant and neoadjuvant chemotherapy decisions for operable NSCLC in routine practice. Among the numerous candidate molecular biomarkers, only few gene-expression profiling signatures provide clinically relevant information warranting further validation. On the other hand, real-time quantitative polymerase-chain reaction strategy involving relatively small number of genes offers a practical alternative, with high cross-platform performance. Although data extrapolation from the metastatic setting should be cautious, the concept of personalized, pharmacogenomics-guided chemotherapy for early NSCLC seems feasible, and is currently being evaluated in randomized phase 2 and 3 trials. The mRNA and/or protein expression levels of excision repair cross-complementation group 1, ribonucleotide reductase M1 and breast cancer susceptibility gene 1 are among the most potential biomarkers for early disease, with stage-independent prognostic and predictive values, the clinical utility of which is being validated prospectively. Inter-assay discordance in determining the biomarker status and association with clinical outcomes is noteworthy.

Keywords: non-small cell lung cancer, adjuvant therapy, neoadjuvant therapy, biomarkers, individualized therapy

Where do we stand? Where should we go?

Although one third of non-small cell lung cancer (NSCLC) cases are detected at an early resectable stage, the overall long-term outlook remains disappointing because of a high rate of distant relapse. Of note, 23% of patients with pathological stage T1aN0M0 according to the revised Union for International Cancer Control (UICC)/American Joint Committee on Cancer (AJCC) classification are expected to die within 5 years from intent-to-cure surgery, implying that

NSCLC can be micrometastatic even at early diagnosis [Rami-Porta *et al.* 2009]. This is supported by previous studies revealing occult tumor-cell spread to bone marrow at the time of thoracotomy, associated with disease recurrence and inferior survival [Coello *et al.* 2004].

Only recently has the concept of adjuvant systemic treatment for NSCLC been endorsed in clinical practice, based on evidence from large-scale clinical trials that employed third-generation

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platinum-based chemotherapy. According to the most recent individual-patient-data meta-analysis report, the absolute 5-year overall survival (OS) benefit does not exceed 5% [Arriagada *et al.* 2010a]. Furthermore, based on previous *pre hoc* subgroup analyses and a phase III study focused on stage IB, the improvement in OS seems to be limited to stages II and III [Pignon *et al.* 2008; Strauss *et al.* 2008].

Compared with the analogous experience with other solid tumors, data supporting adjuvant chemotherapy in NSCLC derive from a relatively small number of patients with a short median follow up, and hardly apply to the average patient. In the lack of robust evidence to justify non-cisplatin-based regimens, an ideal candidate for postoperative therapy would be a relatively young patient with a good performance status and no significant, smoking-related or other comorbidities, who duly recovers from a lobectomy [Crinò *et al.* 2010; Ettinger *et al.* 2010]. A recent concern addressed by the updated data of the phase III International Adjuvant Lung Cancer Trial (IALT), the largest-to-date adjuvant trial, is the potential long-term contribution of platinum chemotherapy to noncancer mortality via cardiopulmonary events [Arriagada *et al.* 2010b].

We can readily assume that under the current standard clinical practice, there are subsets of patients within stage II and, less probably, stage IIIA, who have excellent prognosis and could be spared the toxicity of unnecessary therapy, and others, most notably the elderly or less fit, as well as those with stage I disease, who remain undertreated despite potential benefit from adjuvant chemotherapy. As reflected by the modest magnitude of OS improvement, early NSCLC comprises a heterogeneous group of diseases, with diverse innate aggressiveness and responsiveness to cytotoxic agents. This underscores the need for a customized approach to tailor adjuvant chemotherapy according to patient characteristics and tumor features. Identification and application of the appropriate biomarkers would enable the selection of only high-risk patients to receive the most effective treatment.

As a brief reminder, biomarkers are objectively measurable characteristics that can serve as indicators of normal biological or pathogenic processes, as well as pharmacological responses to therapeutic interventions. Their clinical utility

relies on the ability to improve either patient outcome, quality of life or care costs, and ultimately, to affect treatment decision making [Atkinson *et al.* 2001; Hayes *et al.* 1996]. While prognostic biomarkers indicate the natural course of disease, irrespective of the treatment offered, those defined as predictive can foresee differential therapeutic outcomes, and some combine both functions. To establish their clinical value, large randomized clinical trials or meta-analyses are required for candidate predictive biomarkers, whereas retrospective studies, followed by independent cross validation, may be sufficient for candidate prognostic biomarkers [Buyse *et al.* 2010; Freidlin *et al.* 2010]. The adjuvant setting can facilitate the identification of prognostic tumor biomarkers by observational studies, as well as the assessment of their time-dependent impact, but may be challenging for the design and interpretation of predictive biomarker studies.

Clinicopathological prognostic and predictive factors

By consensus, pathological stage, as defined by tumor size and nodal status, is the most important factor in determining adjuvant therapy decisions for early-stage NSCLC, and the only prospectively validated clinicopathological biomarker with both prognostic and predictive value. Although it is often emphasized that *pre hoc* subgroup analyses showed no benefit for patients with pathological stage IB disease (UICC/AJCC 6th edition), the interpretation of these results should be cautious, as the test for interaction between treatment effect and stage was not significant in any of the phase III trials employing cisplatin-based regimens [Douillard *et al.* 2006; Winton *et al.* 2005; Arriagada *et al.* 2004; Pocock *et al.* 2002]. Only one of the available meta-analyses did demonstrate a significant differential treatment effect, largely driven by the stage IA subgroup, suggesting that patients with stages II and IIIA garner the most benefit [Pignon *et al.* 2008]. The reported positive results of adjuvant tegafur-uracil (UFT) in stage I NSCLC cannot be extrapolated to non-Asian patients [Hamada *et al.* 2005]. Also, it should be stressed that, although biologically plausible, the assumption of therapeutic benefit for stage IB disease with tumor size larger than 4 cm is based on an unplanned subgroup analysis [Strauss *et al.* 2008]; the revised classification of T1bN0 disease as stage II may resolve this controversy. Overall, in an alternative statistical

approach, a Bayesian meta-analysis determined the probability of each survival–benefit level given the results from three adjuvant trials, thus offering more practical information for clinical decision making. It was suggested that the 5-year absolute OS benefit for stage II–III NSCLC may be as high as, but not likely to exceed 7%, while the probability of a clinically meaningful benefit for stage I is low [Miksad *et al.* 2009]. Relevant to the importance of accurate staging, a recent retrospective analysis of lymph node dissection in more than 20,000 patients with pathological stage I NSCLC implied that the number of recovered lymph nodes might be predictive for survival outcomes, although this could well be attributed to a direct therapeutic effect [Varlotto *et al.* 2009].

Other clinicopathological features prospectively shown to be independent, unfavorable prognostic factors in early NSCLC include older age, male sex and non-squamous-cell histology. Only performance status was likely to predict therapeutic effect [Pignon *et al.* 2008; Douillard *et al.* 2006; Winton *et al.* 2005]. High tumor grade (poor differentiation), vascular invasion and visceral pleural infiltration have long been recognized as poor prognostic determinants, based on mostly retrospective cohort studies, and are commonly recommended as adjunct selection criteria for patients who are borderline candidates for adjuvant chemotherapy [Sun *et al.* 2006; Khan *et al.* 2004; Thomas *et al.* 2002; Suzuki *et al.* 1999; Ichinose *et al.* 1995]. Likewise, certain histological subtypes, such as large-cell neuroendocrine carcinoma and pure bronchioloalveolar carcinoma, that confer, respectively, a worse and better outcome, could also guide treatment strategy [Iyoda *et al.* 2007; Travis *et al.* 2006]. Interestingly, the histological phenotype seems to highly correlate with many of the gene-expression profile signatures developed for early NSCLC. It was shown that incorporating the subtype and grade into conventional clinical models could provide predictive accuracy similar to that of well validated gene panels [Sun and Yang, 2006].

Biomolecular prognostic and predictive factors

Recent biotechnology advances have enhanced the quest for reliable molecular biomarkers in NSCLC via genomic, transcriptomic and proteomic high-throughput assays. Prognostic models based on DNA, mRNA, microRNA or protein

expression profiles appear particularly promising for patients with early-stage NSCLC who are borderline candidates for adjuvant treatment, most notably those classified as stage T2N0.

Gene-expression profiling signatures

While earlier microarray studies had focused on molecular characterization and subclassification of NSCLC, the primary objective of subsequent development was to identify tumor signatures that would predict patient survival by using global gene-expression profiling (GEP) of surgical biopsy specimens or human lung cancer cell lines. Although numerous GEP signatures have been reported as outperforming conventional clinical models in terms of predictive accuracy, there are several issues of concern when interpreting these results. Since the survival outcome of patients with NSCLC is significantly affected by other comorbidities, such as those linked to smoking, it is unlikely for a tumor-based biomarker to completely substitute for clinical covariates, thereby questioning whether OS is the most appropriate endpoint to use in these studies [Shedden *et al.* 2008]. Moreover, the commonly used statistical methods, such as regression analysis, are actually inadequate to test whether a new gene profile is a significantly better prognostic tool than a combination of standard risk factors [Pepe *et al.* 2004]. Finally, as mentioned previously, a considerable number of gene signatures are histology type specific and cannot apply to all patients.

The striking lack of overlap among the various gene sets proposed for NSCLC resembles that observed in breast cancer, and is attributed to the biological heterogeneity among training datasets, but also within tumors, combined with the diversity of bioinformatics (statistical methods) applied in model development. It is also possible that, despite differences in composition, gene signatures are connected at the protein level by sharing common signaling networks [Zhu *et al.* 2010]. The application of microarrays in routine clinical practice is restricted by the requirement for fresh-frozen tissue, the complexity of workup procedures, the lack of quantitative accuracy, the limited cross-platform reproducibility and the likelihood of spurious results due to multiple testing. In contrast, real-time quantitative polymerase chain reaction (RT-qPCR) offers a more practical alternative because it allows for accurate quantification of RNA expression with

high cross-platform performance, using small amounts of archival, paraffin-embedded specimens. Nevertheless, its analyzing capability is limited to relatively small numbers of genes.

A recent, elegant review of 16 published studies reporting the development of GEP signatures prognostic for NSCLC disclosed serious methodological flaws in the design and analysis, including inappropriate patient dataset selection, lack of independent validation, biased reporting of resubstitution statistics, incomplete protocol specification, and use of statistical methods. Most importantly, the authors highlighted the need for focused study planning in order to provide clinically relevant information that might potentially affect current management practice [Subramanian and Simon, 2010]. Indeed, only a handful of gene signature studies have yielded data specifically referring to NSCLC stages IA, IB, or II that would warrant further prospective validation [Kadara *et al.* 2011; Zhu *et al.* 2010; Boutros *et al.* 2009; Broet *et al.* 2009; Roepman *et al.* 2009; Bianchi *et al.* 2007; Lu *et al.* 2006]. Among these prognostic models, two are worthy of particular mention.

A prognostic 15-gene signature for early NSCLC was recently reported as the first deriving from prospectively collected tumor samples of patients enrolled in a phase III adjuvant trial. Specimens from the observation group of the National Cancer Institute of Canada Clinical Trials Group (NCIC CTG)-JBR.10 trial were used as the training set to develop a microarray signature that separated patients into good and poor prognosis subgroups, independent of stage and other clinical, as well as biomolecular variables [Zhu *et al.* 2010]. This was consistently validated in different datasets of patients with stage I–II disease and in a cross-platform setting, along with verification by RT-qPCR. Interestingly, the signature was shown to interact significantly with the effect of cisplatin plus vinorelbine chemotherapy, with high-risk patients benefiting the most, although its potential predictive role requires independent validation. Also clinically relevant to the adjuvant strategy, this signature was able to assign, separately, stage IB and II patients into high- and low-risk subgroups with significantly different OS. On replicating eight published GEP signatures in the NCIC CTG-JBR.10 trial set, only a six-gene model proved to be both significantly prognostic and predictive [Boutros *et al.* 2009].

The case of Lung Metagene Score (LMS) illustrates the importance of prudent, large-scale validation and robust interlaboratory reproducibility of candidate prognostic signatures before being evaluated in prospective, randomized biomarker studies. The phase III Cancer and Leukemia Group B (CALGB) 30506 trial was originally designed to validate the potential utility of LMS in selecting patients with pT₁–T₂N₀ tumors for adjuvant chemotherapy, but was recently amended as the authors of the initial research study failed to replicate their results (ClinicalTrials.gov identifier: NCT00863512; last updated on 25 March 2011) [Potti *et al.* 2011].

Individual and combinations of potential biomarkers

Tumor samples from almost half of the patients that participated in the landmark IALT study have been analyzed retrospectively by immunohistochemistry (IHC) for 19 candidate molecular biomarkers representing a wide spectrum of cell processes, such as DNA repair, drug transportation, signal transduction, apoptosis and cell-cycle regulation. Below are discussed the most clinically relevant biomarkers revealed in the ongoing IALT-bio project, combined with the results of other groups' research work. Tables 1 and 2 summarize, respectively, their prognostic and predictive value, along with references.

Excision repair cross-complementation group 1

As platinum compounds remain the cornerstone of standard adjuvant treatment for NSCLC, efforts are made to elucidate the role of factors that would potentially affect the therapeutic outcome. As a reminder, cisplatin reacts with DNA to form interstrand and intrastrand cross links that are the critical cytotoxic lesions. Reduced drug accumulation, increased drug detoxification, altered expression of regulatory genes, tolerance to DNA damage and enhanced activity of DNA repair are the main mechanisms of cancer cell resistance to cisplatin. Excision repair cross-complementation group 1 (ERCC1) is a rate-limiting enzyme in the nucleotide excision repair (NER) and interstrand cross-link repair pathways, which recognize and repair platinum-induced adducts. Cancer cells overexpressing ERCC1 are more likely to have *de novo* resistance to cisplatin. In the IALT-bio study, patients on the observation arm whose tumors were immunohistochemically positive for ERCC1 survived significantly longer than those with

Table 1. Prognostic biomarkers in early-stage non-small cell lung cancer.

Study	Biomarker (assay)	Study design (LOE)	Number of patients/specimens	Stage of disease	Biomarker status	HR for overall survival (<i>p</i> value)
[Kamal <i>et al.</i> 2010]	MSH2 (IHC)	Retrospective analysis within IALT study (II)	1867/673	I–III	High expression (H-score = 3)	0.66 (0.01)
[Olaussen <i>et al.</i> 2006]	ERCC1 (IHC)	Retrospective analysis within IALT study (II)	1867/761	I–III	Positive expression (H-score > median value)	0.66 (0.009)
[Filipits <i>et al.</i> 2007a]	MRP2 (IHC)	Retrospective analysis within IALT study (II)	1867/782	I–III	Positive expression (>0% positive tumor cells)	1.37 (0.007)
[Tsao <i>et al.</i> 2007]	p53 (IHC)	Retrospective analysis within NCIC CTG-JBR.10 (II)	482/253	IB–II	Positive expression (staining score ≥ 15%)	1.89 (0.03)
[Graziano <i>et al.</i> 2010]	p53 (IHC)	Retrospective analysis within CALBG 9633 (II)	344/250	IB	Positive expression	2.30 (0.0005)
[Seve <i>et al.</i> 2007]	βTUBIII (IHC)	Retrospective analysis within NCIC CTG-JBR.10 (II)	482/265	IB–II	High expression (H-score > median value)	1.72 (0.04)
[Graziano <i>et al.</i> 2010]	Mucin (IHC)	Retrospective analysis within CALBG-9633 (II)	344/250	IB	Positive expression	2.03 (0.004)
[Cappuzzo <i>et al.</i> 2009]	MET (FISH)	Retrospective analysis of cohort data (III)	447/435	I–II	Negative (mean gene copy number <5 copies/cell)	0.66 (0.04)
[Rosell <i>et al.</i> 2007]	BRCA1 (RT-qPCR)	Retrospective analysis of cohort data (III)	126; 58 (validation cohort)	I–IIIA; IB–IIB	High expression (relative gene expression > median value)	1.98 (0.02); 2.4 (0.04)

BRCA1, breast cancer susceptibility gene 1; βTUBIII, class III beta-tubulin; CALBG, Cancer and Leukemia Group B; ERCC1, endonuclease excision repair cross-complementing 1; FISH, fluorescent *in situ* hybridization; HR, hazard ratio; IALT, International Adjuvant Lung Cancer Trial; IHC, immunohistochemistry; LOE, level of evidence for grading clinical utility of tumor markers [Hayes *et al.* 1996]; MET, hepatocyte growth factor receptor; MRP2, multidrug resistance protein 2 MSH2; MutS homolog 2; NCIC CTG, National Cancer Institute of Canada Clinical Trials Group; p53, tumor protein 53; RT-qPCR, real-time quantitative polymerase-chain reaction.

ERCC1-negative disease [Olaussen *et al.* 2006]. Interestingly, a multivariate analysis showed that ERCC1 expression was significantly correlated with older age, squamous cell histology and pleural invasion; that is, three clinicopathological biomarkers previously reported as determinants of poor survival. The favorable prognostic value of ERCC1 was also supported in a cohort of patients with completely resected NSCLC, who did not receive perioperative chemotherapy or radiation, by using RT-qPCR. High *ERCC1* mRNA expression, defined as levels above the

median value, was significantly associated with longer OS [Simon, 2005].

In a pharmacogenomic trial for advanced NSCLC, conducted on a biomarker-strategy design, overall response rate was significantly higher in the customized arm, where chemotherapy regimen was tailored by *ERCC1* mRNA expression. Patients in the control arm were not evaluated for the biomarker and received standard platinum-based combination. Within the genotypic arm, patients with low *ERCC1* levels

Table 2. Predictive biomarkers in early-stage non-small cell lung cancer.

Study	Biomarker (assay)	Study (LOE)	design	Number of patients/specimens	Stage of disease	Biomarker status	HR for overall survival (<i>p</i> value); <i>p</i> value for interaction test
[Kamal <i>et al.</i> 2010]	MSH2 (IHC)	Retrospective analysis within IALT study (II)		1867/673	I–III	Low expression vs high expression (H-score = 3)	0.76 (0.03) vs 1.12 (0.48); 0.06
[Olaussen <i>et al.</i> 2006]	ERCC1 (IHC)	Retrospective analysis within IALT study (II)		1867/761	I–III	Negative expression vs positive expression (H-score > median value)	0.65 (0.002) vs 1.14 (0.40); 0.009
[Filipits <i>et al.</i> 2007b]	p27 ^{Kip1} (IHC)	Retrospective analysis within IALT study (II)		1867/778	I–III	Negative expression vs positive expression (H-score > median value)	0.66 (0.006) vs 1.09 (0.54); 0.02
[Tsao <i>et al.</i> 2007]	p53 (IHC)	Retrospective analysis within NCIC CTG-JBR.10 (II)		482/253	IB–II	Positive expression (staining score ≥ 15%) vs negative	0.54 (0.02) vs 1.40 (0.26); 0.02
[Pirker <i>et al.</i> 2007]	ERCC1/p27 ^{Kip1} (IHC)	Retrospective analysis within IALT study (II)		1867/778	I–III	Both negative vs both positive	0.52 (95% CI: 0.36–0.74) vs 1.27 (95% CI: 0.87–1.84); not specified
[Kamal <i>et al.</i> 2010]	MSH2/ERCC1 (IHC)	Retrospective analysis within IALT study (II)		1867/658	I–III	Both low vs both high	0.65 (0.01) vs 1.32 (0.19); 0.01
[Kamal <i>et al.</i> 2010]	MSH2/p27 ^{Kip1} (IHC)	Retrospective analysis within IALT study (II)		1867/not defined	I–III	Both low vs both high	0.65 (0.01) vs 1.31 (0.22); 0.01

ERCC1, endonuclease excision repair cross-complementing 1; HR, hazard ratio; IALT, International Adjuvant Lung Cancer Trial; IHC, immunohistochemistry; LOE, level of evidence for grading clinical utility of tumor markers [Hayes *et al.* 1996]; MSH2, MutS homolog 2; NCIC CTG, National Cancer Institute of Canada Clinical Trials Group; p27^{Kip1}, cyclin-dependent kinase inhibitor 1B; p53, tumor protein 53; vs, versus.

were treated with the same regimen as the control arm, whereas those with high levels received a nonplatinum regimen [Cobo *et al.* 2007]. Regarding the biomarker's predictive ability in the adjuvant setting, the IALT-bio study showed that patients with ERCC1-positive tumors had no survival benefit from the addition of chemotherapy, in contrast to the overall study population. In this subgroup analysis, ERCC1 expression interacted significantly with the treatment activity. This paradox of favorable long-term outcome despite cisplatin chemoresistance probably indicates that intact DNA repair mechanisms prevent the accumulation of genetic aberrations that confer a high malignant potential [Gazdar, 2007]. A recent meta-analysis failed to establish an association of two common *ERCC1* gene polymorphisms (C118T and C8092A) with clinical outcomes in patients with NSCLC

treated with platinum-based chemotherapy [Yin *et al.* 2010].

Ribonucleotide reductase M1 and M2 subunits

Ribonucleotide reductase catalyses the *de novo* synthesis of deoxyribonucleotides, and is a key enzyme for DNA synthesis and repair. It consists of two dimerized subunits, ribonucleotide reductase RRM1 and RRM2, and is the major target of the nucleoside analogue gemcitabine, a commonly used agent in the treatment of NSCLC. RRM1 encodes the regulatory subunit of ribonucleotide reductase, which is considered a predominant determinant of cancer cell resistance to gemcitabine. Although relevant data for the adjuvant setting are lacking, correlative studies within randomized clinical trials in advanced NSCLC have shown that RRM1 overexpression, either at the mRNA or protein level, predicts a

poor response to gemcitabine-based chemotherapy [Reynolds *et al.* 2009; Boukovinas *et al.* 2008; Rosell *et al.* 2004a].

In contradiction to predicting chemoresistance, *RRM1* overexpression seems to confer a favorable outcome by suppressing tumor metastatic potential through various mechanisms, such as induction of the phosphatase and tensin homologue (*PTEN*) [Gautam *et al.* 2003]. Loss of heterozygosity at chromosome segment 11p15.5 that includes *RRM1* gene was an independent determinant of poor survival in a large cohort of patients with stage I and II NSCLC [Bepler *et al.* 2002]. In addition, high *RRM1* transcriptional expression, defined as mRNA levels above the median value, was favorably prognostic of survival in two independent cohorts of patients with resected NSCLC, most of whom were diagnosed at early stage and treated only with surgery. In this study, *RRM1* overexpression was a stage-independent predictor of survival, albeit highly correlated with *PTEN* expression [Bepler, 2004]. The prognostic role of *RRM1* was also confirmed in a large cohort of chemonaïve patients with stage I disease by using an automated quantitative method (AQUA) to assess *in situ* protein expression [Zheng *et al.* 2007]. In this report, *RRM1* was not associated with clinicopathological variables, and was an independent predictor only for improved disease-free survival (DFS). In contrast to the previous study, there was no correlation with *PTEN* expression at the protein level. Interestingly, the concomitant high expression of *RRM1* and *ERCC1* delineated a subgroup of patients with excellent survival outcomes, accounting for 30% of the cohort. The conduct of comparative analyses between gene and IHC/AQUA protein expression of *ERCC1* and *RRM1* might be useful to better define the biomarker status and interpret study results. In the previous report, there was a significant, moderate correlation between the two assays only for *RRM1*.

Breast cancer susceptibility gene 1

Breast cancer susceptibility gene 1 (*BRCA1*) has recently emerged as one of the most appealing biomarkers for personalized chemotherapy in NSCLC. The protein encoded by *BRCA1* has a crucial role in DNA repair as a component of the transcription-coupled NER and the homologous recombinant repair pathways, but is also involved in other important biological processes, such as cell-cycle checkpoints and mitotic spindle

assembly [Deng, 2006]. For its localization to sites of DNA double-strand breaks, the upstream activity of the receptor-associated protein 80 (*RAP-80*) is required. Preclinical and clinical evidence has shown that, through the functions mentioned above, *BRCA1* sensitizes cancer cells to apoptosis induced by antimicrotubule drugs, such as taxanes and vinca alkaloids, while conferring resistance to DNA-damaging agents, most notably platinum compounds. In a cohort of patients with predominantly locally advanced NSCLC, treated with neoadjuvant cisplatin plus gemcitabine chemotherapy followed by surgery, those with the lowest levels of *BRCA1* mRNA expression (that is, within the lowest quartile of values) had significantly greater benefit from chemotherapy in terms of clinical and pathological downsizing, as well as OS [Taron *et al.* 2004]. Similarly, in a phase II study of customized first-line treatment in advanced NSCLC, patients with the lowest expression of both *BRCA1* and *RAP-80* (defined as the lowest tercile of values), receiving cisplatin plus gemcitabine chemotherapy, achieved outcomes similar to patients with epidermal growth factor receptor (*EGFR*) mutation treated with erlotinib. In addition to a close correlation with *BRCA1*, *RAP-80* expression was identified as an independent predictor for OS. Although, qualitatively, the two biomarkers share the same predictive value for differential tumor sensitivity to taxane- and platinum-based chemotherapy, it was shown that *RAP-80* can modulate the effect of *BRCA1* [Rosell *et al.* 2009]. There are no data for this interaction in the early-stage setting. In a feasibility phase II study of customized adjuvant chemotherapy for stage II–IIIA NSCLC, patients with the higher *BRCA1* transcriptional levels (that is, within the higher tercile of values) were treated with single-agent docetaxel, whereas cisplatin-based doublets were reserved for those with intermediate and low *BRCA1* expression [Cobo *et al.* 2008]. Interestingly, the customized approach was not detrimental in terms of survival for patients receiving monotherapy compared with the other two groups.

The potential prognostic role of *BRCA1* was investigated in two independent cohorts of chemonaïve patients with early-stage NSCLC, analyzed by RT-qPCR. Among a panel of nine candidate biomarkers, including *ERCC1* and *RRM1*, *BRCA1* was found to be the only independent factor affecting OS, with overexpression (defined as mRNA levels above the cohort

median) predicting a poor outcome [Rosell *et al.* 2007]. The striking lack of prognostic significance of the other biomarkers included in this study may be partially due to the observed strong intergene coexpression, such as that between *BRCA1* and *ERCC1*. The independent adverse prognostic effect of high *BRCA1* expression was confirmed in another cohort of patients with early-stage NSCLC, also being evaluated for *ERCC1* and *RRM1* mRNA levels. In this study, Xeroderma pigmentosum complementation group G (*XPG*), a key gene for the NER system, was identified as an independent favorable predictor of survival outcomes, as well as a potential modulator of recurrence risk among patients with *BRCA1* overexpression [Bartolucci *et al.* 2009].

Kirsten rat sarcoma viral oncogene homolog

RAS-encoded protein has GTPase-enzyme activity and is a downstream component of signal-transduction pathways of growth factor receptors. In its mutated form, it is constitutively active, thus promoting uncontrolled cell proliferation and survival. Among three highly homologous human *RAS* genes, mutations of kirsten rat sarcoma viral oncogene homolog (*KRAS*) are the most prominent, being detected in around 20% of NSCLC cases and mostly affecting codon 12. *KRAS* mutation status is associated with cigarette smoking and adenocarcinoma histology, and, according to several published studies, it is prognostic of poor survival in early NSCLC [Mascaux *et al.* 2005; Grossi *et al.* 2003; Mitsudomi *et al.* 1991; Slebos *et al.* 1990]. In the phase III NCIC CTG-JBR.10 adjuvant trial, where patients with stage IB–II disease were prospectively stratified by the presence of mutation in any of the *RAS* genes, this did not prove an independent predictor of OS. In the same study, the effect of *RAS* mutation status on the treatment outcome was not significant. Nevertheless, the lack of benefit from the cisplatin plus vinorelbine combination in patients with *RAS* mutation, in contrast to the total study population, may suggest a negative predictive role for *RAS* mutations [Tsao *et al.* 2007]. Similarly, a retrospective analysis of patients with stage IB disease enrolled in the phase III CALGB-9633 study indicated that, among those with tumors larger than 4 cm, *KRAS* mutations may predict less OS benefit from the carboplatin plus paclitaxel combination. Again, it should be stressed that the formal test for interaction between the biomarker and treatment

effect did not achieve statistical significance [Capelletti *et al.* 2010]. Finally, although no safe conclusions can be drawn by extrapolating from the metastatic setting, retrospective biomarker analyses of a phase III trial suggested that the addition of EGFR tyrosine kinase inhibitors (TKIs) to platinum-based regimens may adversely interfere with chemotherapeutic efficacy in patients with *KRAS* mutations [Eberhard *et al.* 2005].

Epidermal growth factor receptor

EGFR is a transmembrane receptor with intrinsic tyrosine kinase activity that, upon binding to its specific extracellular growth factor ligands, becomes activated to transmit signals promoting cell proliferation and survival. EGFR status in NSCLC is currently determined either by the level of protein expression, the gene copy number or the presence of mutations. While no correlation exists between the three methods, it has become clear that the latter better reflects the dependency on the EGFR pathway, thus predicting sensitivity to EGFR-TKIs [Sholl *et al.* 2010]. The prevalence of sensitizing *EGFR* mutations in NSCLC ranges from approximately 10–15% in white patients to 30–40% in Asian patients, and is higher in women, non-smokers or former smokers, and those with adenocarcinoma. It also appears to be related to the disease stage [Goss *et al.* 2010; Richardson *et al.* 2009]. Patients with *exon 19* deletions are reported to have better clinical outcomes following treatment with EGFR-TKIs compared with those harboring *exon 21* mutations [Zhou *et al.* 2010; Jackman *et al.* 2009]. In the majority of NSCLC cases, *EGFR* and *KRAS* mutations are mutually exclusive. Their rate may be related to the disease stage.

The potential prognostic effect of *EGFR* amplification and the two most prominent mutations, *E19del* and *L858R*, remains elusive, as most relevant correlative studies are either retrospective or limited to patients with advanced disease receiving active treatment. Likewise, the potential predictive value of these biomarkers in the adjuvant setting with regard to the effect of chemotherapy is uncertain [Cappuzzo *et al.* 2009; Kim *et al.* 2008; Marks *et al.* 2008; Eberhard *et al.* 2005; Tsao *et al.* 2005]. *EGFR* status, defined by mutation analysis or as amplification by fluorescent *in situ* hybridization (FISH), was recently explored in correlation with the results of the phase III NCIC CTG-JBR.10 adjuvant trial.

Neither sensitizing mutations nor high gene copy were significantly prognostic in the observation arm. Similarly, although there was a trend toward a greater benefit from the cisplatin plus vinorelbine combination, the interaction between the biomarkers and treatment effect was not significant. The failure to disclose significant associations may be due to the relatively small number of *EGFR*-mutations identified in this particular study [Tsao *et al.* 2011]. Nevertheless, a recent large, prospective, cohort study of patients with stage I–III adenocarcinoma, 20% of whom had received perioperative chemotherapy, failed to show any significant association between OS and the mutation status of either *EGFR* or *KRAS*, after adjusting for covariates [D'Angelo *et al.* 2010].

β-tubulin

β-tubulin is an essential element of microtubules, which, in turn, serve as cellular structural components involved in vital processes, including mitosis. Class III β-tubulin (βTUBIII) corresponds to an isotype with an enhancing impact on microtubule dynamics, contributing to *de novo* cancer cell resistance to antimetabolic drugs. Evidence for the potential predictive value of βTUBIII expression at the transcript level in NSCLC derives from a retrospective analysis of patients with advanced disease who participated in a phase III trial comparing three platinum-based doublets, two of which contained antimicrotubule agents. In this study, high tumor expression of βTUBIII predicted poor therapeutic outcomes only for patients treated with the paclitaxel- and vinorelbine-containing regimens [Rosell *et al.* 2003]. Similar retrospective evidence for the predictive role of IHC expression of βTUBIII comes from a nonrandomized study in stage III and IV NSCLC, where the biomarker effect on survival was restricted to patients treated with paclitaxel-based chemotherapy, and was independent of clinicopathological variables [Seve *et al.* 2005]. However, these results were not confirmed in a retrospective evaluation of βTUBIII protein expression in tumor specimens from the phase III NCIC CTG-JBR.10 adjuvant trial, showing no significant interaction between the biomarker and the effect of cisplatin plus vinorelbine combination. Intriguingly, subgroup analysis suggested that high instead of low βTUBIII levels were predictive for benefit from chemotherapy, but, as previously said, such results should be interpreted with caution. However, high βTUBIII expression was shown

to be an independent adverse predictor of recurrence-free survival [Seve *et al.* 2007]. Its prognostic value was confirmed retrospectively in patients enrolled in the IALT study [Reiman *et al.* 2008]. The discordance of findings between the adjuvant and advanced-disease setting for the predictive value of βTUBIII underlines the potential pitfalls of data extrapolation.

Thymidylate synthase

Thymidylate synthase (TS) is a critical enzyme for maintenance of the cellular deoxythymidine monophosphate pool, which is important for DNA replication and repair. It is also the primary target of antimetabolite agents, including pemetrexed. Consistent findings across phase III trials in advanced NSCLC have established the favorable predictive effect of non-squamous-cell histology on treatment with pemetrexed [Scagliotti *et al.* 2009]. Differentially high TS expression in squamous cell NSCLC represents the main molecular basis underlying this treatment by histology interaction [Scagliotti *et al.* 2007]. Similar distinct expression patterns between NSCLC subtypes were observed at stages I–IIIA of disease, with a strong association between mRNA and IHC expression [Ceppi *et al.* 2006]. Nevertheless, no clinical data exist to confirm the predictive role of either histology or TS expression in the adjuvant setting. However, two different cohort studies of chemonaïve patients with resected early-stage NSCLC revealed an independent prognostic effect for TS, but with conflicting qualitative results. In the first report, high TS expression at the mRNA, but not the IHC level, was significantly associated with adverse DFS; in the second study, high TS expression as determined by AQUA, but not by RT-qPCR, predicted improved OS. In the latter study, TS protein levels did not correlate with those of ERCC1 and RRM1 [Zheng *et al.* 2008; Shintani *et al.* 2003].

Cyclin-dependent kinase inhibitor 1B

The cyclin-dependent kinase inhibitor 1B, p27^{Kip1}, is a tumor-suppressor protein that induces cell-cycle arrest in phase G1. Despite its antiproliferative properties, p27^{Kip1} upregulation leads to *de novo* resistance to platinum compounds by allowing cancer cells to repair DNA damage and avoid apoptosis. Among six cell-cycle regulators evaluated by IHC within the IALT-bio project, only p27^{Kip1} was identified to interact significantly with treatment effect. Its predictive ability was independent from ERCC1

expression, and as anticipated, survival benefit from cisplatin-based chemotherapy was solely evident in patients with p27^{Kip1}-negative tumors. On multivariate analysis, none of these candidate biomarkers were significantly associated with OS in the total study population. Furthermore, when combining the IHC features of ERCC1 and p27^{Kip1}, patients with tumors negative for both biomarkers seemed to benefit most from adjuvant chemotherapy. Although in an independent validation set double negativity was the commonest expression pattern of ERCC1 and p27^{Kip1}, discordant reactivity was observed in a considerable proportion of patients [Rekhtman *et al.* 2008; Filipits *et al.* 2007b; Pirker *et al.* 2007].

Tumor protein p53

The tumor suppressor protein, p53, has a wide range of functions most of which are mediated via regulation of gene transcription. Commonly described as ‘the genome guardian’, it is encoded by the *TP53* gene, and is involved in important cellular processes, such as stress response, cell-cycle control, DNA repair and apoptosis. *TP53* mutations are present in about 50% of NSCLC cases. Because missense mutant p53 protein has a longer half life than the wild-type counterpart, positive nuclear immunoreactivity for p53 has been considered a surrogate marker for the presence of *TP53* mutations. Nevertheless, the overall sensitivity and positive predictive value of p53 expression by IHC for *TP53* mutation status are estimated to be only 75% and around 65%, respectively [Greenblatt *et al.* 1994]. Among patients with NSCLC, *TP53* mutations and p53 protein expression are slightly more common in men and those with squamous cell histology. Although previous meta-analyses had indicated that *TP53* mutations and p53 expression are weak outcome predictors in NSCLC [Steels *et al.* 2001; Mitsudomi *et al.* 2000], a retrospective companion analysis of the phase III NCIC CTG-JBR.10 adjuvant trial showed p53 IHC overexpression to be an independent unfavorable prognostic factor among patients in the observation arm. In addition, there was a significant treatment by biomarker interaction, with only patients with p53-positive tumors deriving benefit from cisplatin plus vinorelbine combination. In contrast to p53 expression, *TP53* mutation status was neither prognostic for survival, nor predictive for efficacy of adjuvant chemotherapy [Tsao *et al.* 2007]. This discrepancy

suggests that the biological effects of *TP53* mutations and p53 protein aberrations are not equivalent, underlying their complex role in tumor aggressiveness and chemosensitivity. In a previous phase III trial of adjuvant radiotherapy with or without platinum-based chemotherapy for stage II–IIIA NSCLC, neither p53 expression nor *TP53* mutations were shown to have a predictive value, but this should be interpreted with consideration of the different study design and the use of an old-generation regimen [Schiller *et al.* 2001]. A recent ancillary biomarker study within the phase III CALGB-9633 adjuvant trial identified p53 and mucin overexpression as independent adverse prognostic factors for stage IB patients [Graziano *et al.* 2010].

MutS homologue 2

As previously described, aiming to enhance the predictive power of ERCC1, additional biomarkers related to the repair of cisplatin-induced DNA damage have been included in the IALT-bio project. MutS homolog 2 (MSH2) is a major active component of the mismatch repair machinery. Tumor sample analysis for IHC expression of MSH2 displayed a trend towards a significant interaction between the biomarker and cisplatin-based chemotherapy ($p=0.06$), with low MSH2 levels predicting a survival advantage with adjuvant treatment. When MSH2 and ERCC1 expression patterns were combined to form four phenotypes, the benefit from chemotherapy was significantly greater for patients with double-negative tumors. This was also noted when MSH2 expression was combined with that of p27^{Kip1}, suggesting that MSH2 immunostaining was a superior predictive biomarker when considered jointly with either of the two other variables. Similar to the prognostic role of other DNA-excision-repair proteins, high MSH2 levels predicted a significantly longer survival in patients on the observation arm [Kamal *et al.* 2010].

Multidrug resistance protein 2, Fas and Fas ligand

Multidrug resistance protein 2 (MRP2) is a transmembrane ATP-binding cassette transporter contributing to cancer cell resistance to various anticancer drugs. In the IALT-bio project, tumoral MRP2 overexpression was significantly associated with poorer prognosis, but without affecting benefit from cisplatin-based chemotherapy [Filipits *et al.* 2007a]. Within the same trial, a

retrospective analysis of apoptotic markers, including the death receptor Fas and its ligand FasL, concluded that a Fas:FasL ratio of 1 was a significant predictor for longer survival, but not for chemosensitivity at the level of significance $p < 0.01$ [Brambilla *et al.* 2007].

Insulin-like growth factor 1 receptor

While insulin and insulin-like growth factors are key regulators of normal cell metabolism and growth, insulin receptor (IR) and insulin-like growth factor receptor (IGF-1R) are implicated in the development and progression of NSCLC, either by interacting with the EGFR pathway or independently. In a cohort of patients with resected NSCLC, *IGF1R* amplification determined by FISH was an independent favorable prognostic factor, whereas IGF1R protein and gene expression did not significantly affect survival. It is noteworthy that *IGF1R* mRNA expression and IGF1R IHC score were significantly higher in squamous cell histology [Dzadzadzko *et al.* 2010]. A gene signature generated *in vitro* and reflecting IR and IGF-1R pathway activation was recently validated in two independent cohorts of patients with resected stage I–IIIA NSCLC, showing a significant association with poor survival. A parallel validation of the gene signature in NSCLC cell lines, along with other biomarkers, suggested that cellular invasiveness and metastatic potential underlie its adverse prognostic value [Bellil *et al.* 2010].

Hepatocyte growth factor receptor

Hepatocyte growth factor receptor (*c-MET*) is a proto-oncogene encoding hepatocyte growth factor receptor (HGFR), a transmembrane protein with tyrosine kinase activity that, upon activation by its exogenous specific ligand, induces several cellular responses that constitute a complex programme known as invasive growth. Upregulation of the *c-MET* signaling pathway occurs via different mechanisms, including HGFR overexpression and *c-MET* mutation or amplification. The latter is relatively uncommon in NSCLC, but occurs in up to 20% of cases with *EGFR* mutations and acquired resistance to EGFR-TKIs [Toschi and Cappuzzo, 2010]. In a cohort of patients with resected stage I–III disease, not treated with adjuvant chemotherapy, a high *MET* gene copy number, defined as a mean of at least 5 copies per cell, was an independent adverse prognostic factor. *c-MET*-positive status determined by FISH was associated with *EGFR* amplification, but no patient with

activating *EGFR* mutation was characterized as *c-MET* FISH-positive [Cappuzzo *et al.* 2009]. In a separate cohort, true *c-MET* amplification was more frequent in patients with squamous cell histology, rather than those with adenocarcinoma. In multivariate analysis, an increased *c-MET* gene copy number was significantly associated with shorter survival among patients with squamous NSCLC [Go *et al.* 2010].

Associations of clinicopathological features with molecular biomarkers

Gender-related disparity in outcomes of patients with NSCLC, even after adjustment for age, stage and treatment, has long been a subject of debate [Chansky *et al.* 2009; Cerfolio *et al.* 2006]. A consistent difference exists in the distribution of NSCLC histological types, with adenocarcinoma and squamous-cell carcinoma being the most common histology in women and men, respectively. Likewise, differential tumor expression of various prognostic and predictive biomarkers has been reported between sexes, implying distinct disease biology, with potential therapeutic implications [Planchard *et al.* 2009; Novello and Vavalà, 2008]. It is suggested that female hormones can partially explain these differences by influencing lung cancer biomolecular features. However, the results from two large datasets recently displayed that gender is not an independent variable for the expression or mutations of key biomarker genes. In contrast to general belief, an interim analysis of the largest to date prospective, observational study of early-stage NSCLC, exploring relationships between tumor biology with smoking and gender, concluded that only tobacco exposure and adenocarcinoma histology were independent variables for *KRAS* and *EGFR* mutations [Mack *et al.* 2010]. In the largest to date, retrospective database analysis of *ERCC1*, *RRM1* and *TS* mRNA expression, along with *EGFR* mutation status, there was no association of gene expression with either stage or gender. However, expression levels of all three biomarker genes were significantly higher in squamous cell lung carcinomas compared with adenocarcinomas, underscoring potential differences in chemosensitivity that might optimize treatment decision making. Intriguingly, *EGFR* mutations were associated with low *ERCC1* expression, even when adjusting for histology. This hypothesis-generating dataset also provided estimates of biomarker variance that could be useful in the design of future prospective trials

[Gandara *et al.* 2010]. Comparative studies specifically designed to investigate sex-associated differences are warranted to provide data clinically relevant for treatment customization.

Adjuvant EGFR-targeted therapy for early-stage NSCLC

Biomarker-driven integration of targeted agents in the management of NSCLC is an emerging paradigm of effective personalized cancer treatment. The low toxicity profile and oral availability of EGFR-TKIs, along with their promising efficacy in appropriately selected patients with advanced NSCLC, render their use as an attractive consideration for the adjuvant setting [Yang *et al.* 2010; Zhou *et al.* 2010; Mok *et al.* 2009].

According to the results of an Asian phase III trial and the interim analysis of a similar ongoing study in a Western population, erlotinib significantly prolongs progression-free survival compared with third-generation platinum-based regimens in chemo-naïve patients with advanced-stage disease carrying *EGFR*-activating mutations [Hoffmann-La Roche Ltd, 2011; Zhou *et al.* 2010]. Furthermore, mature data from the IPASS study, a phase III trial of first-line gefitinib *versus* carboplatin plus paclitaxel in patients with NSCLC selected by clinical criteria enriching for *EGFR*-sensitizing mutations demonstrated a survival advantage for patients with *EGFR* mutation-positive tumors over those with *EGFR*-wild-type tumors, regardless of the type of treatment. In addition to predicting significantly longer progression-free survival and better quality of life with the TKI therapy, positive *EGFR*-mutation status was associated with higher response to the platinum-based regimen. In support of the latter finding, a retrospective analysis of the same chemotherapy doublet with erlotinib *versus* placebo, as first-line treatment, had previously shown that *EGFR* mutations could predict significantly improved OS for unselected patients treated on both arms [Eberhard *et al.* 2005]. In the TORCH study, a phase III trial assessing the optimal sequential treatment strategy of erlotinib and chemotherapy in unselected patients with advanced NSCLC, the upfront chemotherapy approach conferred better OS outcome for those with *EGFR*-wild-type tumors [Gridelli *et al.* 2010]. Therefore, at least in the metastatic context, the therapeutic implications and clinical impact of *EGFR* status are likely more meaningful for patients without rather than with *EGFR* mutations.

Available data on the role of EGFR-TKIs and corresponding biomarkers in the adjuvant setting are currently limited to the results of the phase III NCIC CTG-CTG JBR.10 trial of gefitinib *versus* placebo for resected stage IB–IIIA NSCLC, the interpretation of which is confounded by several caveats. This study recruited clinically and molecularly unselected patients, the vast majority of whom were white and smokers. Despite a protocol amendment to permit adjuvant chemotherapy, the two arms were well balanced for prior therapeutic interventions. Given the negative interim results from the Southwest Oncology Group (SWOG)-0023 trial of maintenance gefitinib for locally advanced NSCLC, the study was prematurely closed. As a consequence, the final patient enrollment was considerably lower than the target accrual, while the median duration of active treatment was shorter than 5 months. Preplanned exploratory analyses included *KRAS* mutation status, *EGFR* gene copy number by FISH, as well as *EGFR*-sensitizing mutations, and the patients with available biomarker data were representative of the total study population. Given the baseline patient profile, the frequency of *EGFR* mutations was relatively high. Albeit underpowered, the study showed no improvement in OS or DFS with adjuvant gefitinib for either the total study cohort or any patient subset; in fact, by multivariate analysis, there was a trend of detrimental effect with the TKI use. Also disappointingly, neither of the evaluated biomarkers was prognostic among patients on the placebo arm, or predictive of the treatment effect on OS.

While, based on the current evidence, chemotherapy remains the only standard option of adjuvant treatment for patients with NSCLC with a good performance status, the upcoming results of studies incorporating EGFR-targeted therapy are eagerly anticipated. The phase III RADIANT (Randomized Double-blind Trial in Adjuvant NSCLC with Tarceva) trial is comparing adjuvant erlotinib with placebo in patients with stage IB–IIIA NSCLC that is EGFR-positive by either IHC or FISH, but not by mutation analysis, allowing for prior chemotherapy (ClinicalTrials.gov identifier: NCT00373425). Erlotinib is also being evaluated in two other adjuvant trials with a more targeted selection approach: as part of the customized treatment arm of the Tailored Post-Surgical Therapy in Early Stage NSCLC (TASTE) study, as described in the next section; and in a phase II

Table 3. Ongoing prospective biomarker studies in early-stage non-small cell lung cancer.

Trial	Stage/histology	Treatment arms	Study design (LOE)	Biomarker(s) (assay type)
SWOG-S0720	I (pT ≥ 2 cm) [AJCC 6th ed]	Cisplatin/gemcitabine (↓RRM1 and/or ERCC1) <i>versus</i> observation (↑RRM1 and ERCC1)	Phase II (feasibility) (I)	ERCC1, RRM1 (AQUA ± RT-qPCR ± protein polymorphisms)
ITACA	II–III [AJCC 6th ed]	Pharmacogenomic-guided ChT regimen <i>versus</i> control*	Phase III (I)	ERCC1, TS (RT-qPCR)
TASTE	II–IIIA (pN1) [AJCC 6th ed]	Customized treatment <i>versus</i> cisplatin/pemetrexed\$	Randomized phase II (feasibility)-3 (I)	ERCC1 (IHC), EGFR (mutations)
GECP-SCAT	/Non-SCC lung cancer pN1-N2 [AJCC 6th ed]	Customized ChT <i>versus</i> cisplatin/docetaxel‡ ± RT (pN2)	Phase III (I)	BRCA1 (RT-qPCR)/EGFR, KRAS (mutations)
RADIANT	IB–IIIA [AJCC 6th ed]	Erlotinib <i>versus</i> placebo ± adjuvant ChT (up to 4 cycles)	Phase III (I)	EGFR (IHC ± FISH)/EGFR, KRAS (mutations)§

AJCC, American Joint Committee on Cancer; AQUA, automated quantitative analysis; BRCA1, breast cancer susceptibility gene 1; ChT, chemotherapy; EGFR, epidermal growth factor receptor; ERCC1, endonuclease excision repair cross-complementing 1; FISH, fluorescent *in situ* hybridization; GECP-SCAT, Spanish Lung Cancer Group (Grupo Español de Cáncer de Pulmón)-Spanish Customized Adjuvant Trial; IHC, immunohistochemistry; ITACA, International Tailored Chemotherapy Adjuvant; KRAS, kirsten rat sarcoma viral oncogene homolog; LOE, level of evidence for grading clinical utility of tumor markers [Hayes *et al.* 1996]; Non-SCC, non-squamous-cell; RADIANT, Randomized Double-blind Trial in Adjuvant NSCLC with Tarceva; RRM1, ribonucleotide reductase M1; RT, radiotherapy; RT-qPCR, real-time quantitative polymerase-chain reaction; SCAT, Study of Customized Adjuvant Chemotherapy; SWOG, Southwest Oncology Group; TASTE, Tailored Post-Surgical Therapy in Early Stage NSCLC; TS, thymidylate synthase.

*Individualized chemotherapy according to the pharmacogenomic profile: taxane (↑ERCC1 and TS mRNA levels), or pemetrexed (↑ERCC1, ↓TS mRNA levels), or cisplatin/gemcitabine (↓ERCC1, ↑TS mRNA levels), or cisplatin/pemetrexed (↓ERCC1 and TS mRNA levels) *versus* standard chemotherapy with a platinum-based doublet chosen by the investigator.

\$Customized treatment according to EGFR and ERCC1 status: erlotinib (EGFR-mutant tumors) or observation (EGFR-wild type and ERCC1-positive tumors) or cisplatin/pemetrexed (EGFR-wild type and ERCC1-negative tumors).

‡Customized chemotherapy: docetaxel (high BRCA1 levels) or cisplatin/docetaxel (intermediate BRCA1 levels) or cisplatin/gemcitabine (low BRCA1 levels).

§Secondary exploratory analysis.

nonrandomized study for stage I–IIIA lung adenocarcinomas carrying an *EGFR*-sensitizing mutation, but not the *T790M* resistance mutation (ClinicalTrials.gov identifier: NCT00567359).

Looking towards the future

As already mentioned, the RT-qPCR strategy involving a relatively small number of gene biomarkers and the use of paraffin-embedded specimens seems to outperform wide-genome profiling, although cutoff point definition for continuous variables, such as transcript levels, is particularly challenging because of the great interindividual variation of gene expression. Another important point is the possible discordance of biomarker status between different types of assays, and the corresponding differences in association with clinical outcomes. As already discussed, mRNA expression of a biomarker gene does not necessarily correlate with the protein levels as determined by IHC or AQUA. Apart from multiple technical issues

that potentially affect the results of each method, biomarker expression at the protein level depends on additional translational factors, such as microRNA, posttranslational modifications and degradation.

Overall, the prognostic and/or predictive role of many of the aforementioned biomarkers has been strongly supported by a growing body of clinical evidence, mostly arising from retrospective translational studies. Although data extrapolation to the early-stage setting should be cautious, the concept of personalized, biomarker-driven treatment has been shown to be feasible and promising in terms of clinical outcomes in advanced NSCLC [Rosell *et al.* 2009; Simon *et al.* 2007]. Similar encouraging results have been produced from a pilot study of pharmacogenomics-guided chemotherapy in early NSCLC [Cobo *et al.* 2008]. Building on these findings, four prospective multicentre clinical trials of customized adjuvant strategy are currently underway (Table 3).

The SWOG-S0720 trial is a phase II feasibility study, accruing patients with pathological stage I NSCLC (≥ 2 cm in size), for whom adjuvant chemotherapy is currently not the standard of care. Based on ERCC1 and RRM1 levels assessed by AQUA, patients with tumors highly expressing both biomarkers are assigned to active observation, while those with tumors expressing low levels of either of the two biomarkers are offered adjuvant cisplatin plus gemcitabine combination. The rationale for the treatment algorithm is that high levels of both biomarkers suggest an excellent prognosis, along with cisplatin resistance, whereas low levels of ERCC1 and/or RRM1 predict a poor prognosis, along with tumor sensitivity to at least one of the two cytotoxic agents. If available, additional samples will be assessed by RT-qPCR (ClinicalTrials.gov identifier: NCT00792701). The International Tailored Chemotherapy Adjuvant (ITACA) trial is a phase III biomarker study utilizing an enrichment-design strategy that employs tumoral ERCC1 and TS mRNA expression levels as decision points to select regimen. Patients with pathological stages II and III are randomized to either a standard cisplatin-based doublet of the investigator's choice, or one of four prespecified regimens determined by the ERCC1/TS phenotype: taxane monotherapy for patients expressing high levels of both biomarkers; single-agent pemetrexed for those with high ERCC1 and low TS expression; cisplatin plus gemcitabine combination for those with low ERCC1 and high TS expression; and cisplatin plus pemetrexed doublet for those with low levels of both biomarkers. The rationale of treatment algorithm is based on the combined, anticipated predictive effects of the two biomarker genes [Vincent, 2010]. GECF-Study of Customized Adjuvant Chemotherapy (SCAT) is a phase III trial conducted on a biomarker-strategy design, in which patients with pN1–N2 disease are randomized to receive either cisplatin plus docetaxel combination as standard chemotherapy or to one of three prespecified regimens, driven by BRCA1 transcript levels: cisplatin plus gemcitabine combination for patients with tumors expressing low BRCA1 levels; single-agent docetaxel for those with high BRCA1 expression; and cisplatin plus docetaxel combination for those with intermediate BRCA1 expression (ClinicalTrials.gov identifier: NCT00478699). Lastly, the TASTE trial is a phase II/III study conducted on a combined biomarker design, and accruing patients with NSCLC with only non-squamous-cell histology

and pathological stages II–IIIA (non-pN2). Patients on the control arm are being treated with standard cisplatin plus pemetrexed combination, whereas those on the genotype arm are being assigned to either one of three modalities, according to EGFR mutation status and ERCC1 IHC expression levels: erlotinib for patients with EGFR-mutated tumors; cisplatin plus pemetrexed for those with EGFR-wild-type/ERCC1-negative tumors; and observation for those with EGFR-wild-type/ERCC1-positive tumors. The inclusion restriction to non-squamous-cell histology intends to enrich the EGFR-mutation rate and offer the optimal setting for use of cisplatin plus pemetrexed combination (ClinicalTrials.gov identifier: NCT00775385).

In the absence of robust supporting evidence from large-scale phase III trials and while awaiting the results of individual patient data meta-analyses, neoadjuvant platinum-based chemotherapy is at present considered an experimental treatment modality for early-stage NSCLC, and is probably reserved for patients expected to recover slowly from major surgery [Pisters *et al.* 2010; Song *et al.* 2010; Westeel *et al.* 2010; Scagliotti *et al.* 2008; Gilligan *et al.* 2007]. Although clinical staging is suboptimally accurate to guide treatment decision making, the neoadjuvant approach has several theoretical and practical advantages, including early control of potential micrometastases, tumor downstaging that facilitates surgery, and patient compliance that allows for optimal duration and dose intensity of chemotherapy. Concerns for disease progression during treatment and increased postoperative complications have not been confirmed. Most importantly, the neoadjuvant setting offers a unique opportunity for *in vivo* evaluation of tumor biology and the efficacy of customized chemotherapy. Given the availability of baseline and posttreatment tissue specimens, this approach allows the assessment of chemotherapy-induced changes in biomarker status, and the potential value of tumoral characteristics as pharmacodynamic markers of novel anticancer agents, or as surrogate markers for clinical endpoints. The latter is especially true in the early-stage setting, where prospective clinical trials require many years to complete. The available literature data on biomarker application in the neoadjuvant setting of early NSCLC do not offer important insights into improving personalized treatment, as most arise from retrospective analyses of small, noncontrolled studies,

in which biomarker evaluation on tumor samples was performed only prechemotherapy or post-chemotherapy [Kang *et al.* 2010; Bepler *et al.* 2008; Hwang *et al.* 2008; Fijolek *et al.* 2006; Rosell *et al.* 2004b]. The results are awaited with interest of expression analysis for promising biomarkers, including ERCC1, RRM and BRCA1, within the SLCG-NATCH trial. This is a phase III study, specifically designed to compare preoperative and postoperative platinum-based chemotherapy *versus* surgery alone in patients with clinical stage I (tumor size >2 cm) to IIIA (T3N1) NSCLC. Probably due to the predominance of stage I cases, this study failed to detect a significant benefit in DFS from the addition of either of the two modalities [Felip *et al.* 2010]. Another highly anticipated neoadjuvant trial is the phase II nonrandomized ECON study that incorporates erlotinib into a multimodality therapeutic approach, including chemotherapy and surgery, for resectable stage IB–IIIA NSCLC with documented *EGFR*-activating mutations (ClinicalTrials.gov identifier: NCT00577707). With pathological complete response rate as its primary endpoint, the rationale of this study is based on the strikingly high overall response rate achieved with gefitinib in advanced *EGFR*-mutation-positive disease. According to the protocol schema, patients are initially treated with preoperative erlotinib, followed by the addition of cisplatin plus pemetrexed combination, to further undergo surgical tumor resection and adjuvant erlotinib.

The discovery of molecular biomarkers with the potential of selecting high-risk patients and predicting drug efficacy is essential in the quest for personalized management of early-stage NSCLC. This is especially true for areas of controversy, such as treatment of elderly patients and those with stage I disease. Moreover, although cisplatin plus vinorelbine doublet is currently the standard option for adjuvant chemotherapy, the use of appropriate surrogate biomarkers would facilitate randomized clinical trials to establish alternative or superior regimens with smaller sample sizes and shorter follow-up time. ERCC1, RRM1 and BRCA1 are considered to be among the most promising biomarkers with stage-independent, combined prognostic and predictive value, the clinical utility of which is being validated in ongoing large-scale, randomized phase II and III trials. While the results are awaited with anticipation, none of these or other candidate biomarkers should be used in daily

clinical practice as decision-making criteria. Furthermore, because host–tumor interactions are expected to play a pivotal role in disease control, particularly in the adjuvant setting, it is unlikely for tumor-derived models to completely substitute for patient-related variables in reliably predicting clinical outcomes, such as survival, and to a lesser degree, treatment effects. Given the diversity of genetic aberrations underlying lung carcinogenesis, and the complexity of signaling networks governing the cellular phenotype, it would be unrealistic for single biomarker signatures to effectively define the disease profile for all patients with NSCLC.

Therefore, rather than conducting a lot more studies of candidate individual biomarkers, research interest should focus on the development and validation of clinically oriented, multivariate prognostic and predictive models, based on the most promising biomarker combinations and their potential interactions.

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