Individualized therapy in the adjuvant setting for non-small cell lung cancer (NSCLC)

Celine Mascaux · Simon Ekman · Christophe Doods ·
Frances A. Shepherd

Published online: 30 December 2012
© Springer Science+Business Media New York 2012

Abstract Based on meta-analyses and the results of several trials, adjuvant chemotherapy is currently recommended for stage II and stage III non-small cell lung cancer (NSCLC) patients after complete tumor resection, and may be considered for patients with stage IB NSCLC ≥ 4 cm. As individualized therapy becomes integrated into the care of advanced NSCLC, its role in early stage NSCLC and, in particular, in the adjuvant setting, also will become of greater importance. In the adjuvant setting, prognostic and predictive biomarkers might identify which patients have a worse prognosis and, among them, those who could benefit from customized chemotherapy. In the present review, we summarized the available data and ongoing trials testing the role of individualized adjuvant therapy (chemotherapy, targeted therapy and immunotherapy) in NSCLC.

Keywords Non-small cell lung cancer · Adjuvant · Individualized · Customized · Biomarkers · Prognostic · Predictive

Introduction

Lung cancer remains the leading cause of cancer death worldwide, with 226,000 new cases and 160,000 deaths in the US in 2012 and more than 1.2 millions deaths in the world yearly. Globally, 5-year survival remains around 16 %. Until recently, surgery alone was the standard care for patients with stage I–IIIA non small cell lung cancer (NSCLC), resulting in survival rates from 23 % in stage IIIA, 33 % in stage IIB and up to 89 % in stages IA [1]. In 1995, a large meta-analysis, pooling individualized data from randomized trials of adjuvant chemotherapy vs. best supportive care (BSC), showed a trend for prolonged overall survival (OS) with adjuvant chemotherapy, especially with platinum based regimens, but the differences were not statistically significant. [2]. Thus, the evidence was not felt to be adequate to recommend adjuvant chemotherapy after complete surgical resection. However, the results of this meta-analysis prompted several randomized trials testing adjuvant chemotherapy in completely resected NSCLC, using newer and more active chemotherapeutic agents combined with a platinum analogue. Several large international trials demonstrated statistically significant survival benefits with adjuvant chemotherapy, including the International Adjuvant Lung Cancer Trial (IALT) [3], the National Cancer Institute of Canada Clinical Trials Group JBR.10 (NCIC-CTG JBR.10) trial [4], the Adjuvant Navelbine International Trial Association (ANITA) study [5], and the Cancer and Leukemia Group B (CALBG) 9633 trial [6]. The IALT trial was the first to show a benefit of postoperative cisplatin-based adjuvant chemotherapy [3]. Subsequently, both JBR.10 and ANITA confirmed reported significant prolongation of survival with cisplatin/vinorelbine [4, 5]. As three large randomized trials with similar design showed consistent results, adjuvant chemotherapy based on a cisplatin doublet is recommended in stage II–IIIA NSCLC with a level 1 of evidence [7–9]. In addition,

C. Mascaux (✉)
Division of Computational Biomedicine, Department of Medicine,
Boston University School of Medicine,
72 East Concord Street, E631,
Boston, MA 02118, USA
e-mail: celinejmmascaux@gmail.com

S. Ekman
Section of Oncology, Department of Radiology, Oncology
and Radiation Science, Uppsala University, Uppsala, Sweden

C. Doods
Respiratory Oncology Unit (Pulmonology) and Leuven Lung
Cancer Group, University of Gasthuisberg, Leuven, Belgium

F. A. Shepherd
University of Health Network, Princess Margaret Hospital
and the University of Toronto, Toronto, Ontario, Canada

the LACE (Lung Adjuvant Cisplatin Evaluation) meta-analysis pooling individual patient data from five trials (IALT, JBR.10, ANITA, CALGB-9633 and the Spanish NATCH trials) [10••], as well as an update of the 1995 meta-analysis [11], confirmed that adjuvant chemotherapy increases survival in stage II and stage IIIA. The LACE meta-analyses demonstrated an absolute decrease of 6.9 % for lung cancer death and an absolute increase of 5.4 % for overall survival at 5 years in patients with completely resected NSCLC [10••]. The updated meta-analyses continued to confirm a 5 % increase of survival at 5 years [11]. The LACE meta-analysis also showed a nonsignificant survival benefit in stage IIB [10••], but a potentially detrimental effect in stage IA [10••]. CALGB-9633, which was limited to stage IB, demonstrated a significant global benefit in disease-free survival (DFS), as well as overall survival and DFS in an unplanned subset analysis of patients with tumors of 4 cm or more [6]. Based on these meta-analyses and the results of several individual trials, adjuvant chemotherapy now is recommended to fit patients with stage II and stage III NSCLC after complete tumor resection, and may be considered for patients with stage IB NSCLC ≥ 4 cm [7–9].

Despite improvements in survival with adjuvant chemotherapy after surgical resection of localized NSCLC, giving a survival advantage of approximately 5 % at 5 years, there is still a relatively high risk of relapse, even for early-stage NSCLC. Up to 40 % of all stage IB, and 60 % of stage II patients relapse and die from lung cancer despite receiving adjuvant chemotherapy [6]. This suggests that more sensitive biomarkers are needed to identify the subgroups of patients at extremely high risk, and to develop strategies to enhance their treatment. Initially tested and integrated into the treatment selection for advanced NSCLC, individualized therapy may also play a role in the adjuvant setting. The integration of prognostic and predictive biomarkers has the potential to identify which patients are cured with surgery alone and who do not need further therapy; and conversely, which patients may derive the greatest benefit from chemotherapy, and in particular, customized treatment based on individual tumor profiling. The present review aims to analyze the available published data and the ongoing trials testing the role of individualized therapy in the adjuvant setting of NSCLC.

Individualized therapy in adjuvant therapy

Here we review the current status of prognostic biomarkers for completely resected NSCLC and predictive biomarkers for adjuvant chemotherapy, targeted therapy and immunotherapy of patients who underwent a complete resection for an early stage NSCLC. Translational research projects for JBR.10, the International Adjuvant Lung Cancer Trial

Biologic Program (IALT-Bio), and the Lung Adjuvant Cisplatin Evaluation Biologic Program (LACE-Bio) attempted to characterize and/or validate these biomarkers for the selection of those patients who and do not benefit from adjuvant chemotherapy. Only few data are available about targeted therapy, mainly one study (BR.19) about Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) and one trial for immunotherapy. The results of the concluded studies of this section are shown in Table 1. The ongoing trials are described in Table 2.

Chemotherapy

DNA repair and DNA replication

Resected early stage lung tumors, compared with corresponding normal lung tissue, frequently exhibit significant transcript overexpression of genes belonging to double strand DNA break repair (e.g. *BRCA1*, *XRCC2*) pathways and DNA replication (e.g. *TS*, *RRM1*) pathways [12]. Transcriptional overexpression of some of these genes has shown strong correlation with more aggressive clinical behavior, and potentially may explain reduced sensitivity to chemotherapy. To date, there is preliminary evidence from studies in advanced NSCLC to suggest that both excision repair cross-complementing rodent repair deficiency, complementation group 1 (ERCC1), ribonucleotide reductase M1 (RRM1) and Breast Cancer 1 (BRCA1) may be prognostic and predictive, and that they may play a role in selecting tailored chemotherapy in advanced stage NSCLC. Prospectively accrued patients treated with a ‘customized therapy’ approach based on RMM1 and ERCC1 mRNA tumor expression levels were compared to ‘standard, non-customized therapy’ approaches [13]. ERCC1/RMM1 customized chemotherapy appeared to be a predictor of improved overall survival in the multivariable Cox proportional hazards model analysis. Another prospective non-randomized customized chemotherapy trial based on BRCA1 expression levels was associated with good overall survival for both cisplatin plus gemcitabine in patients with low levels of BRCA1 mRNA and taxanes in patients with high levels of BRCA1 [14].

ERCC1 Cisplatin establishes covalent cross-links between DNA strands, and thereby inhibits DNA replication. This cisplatin-induced DNA damage can be repaired by several DNA repair mechanisms, including nucleotide excision repair (NER). ERCC1 is a key protein involved in the NER complex. High DNA repair capacity eliminates cisplatin-induced DNA adducts and thus leads to platinum resistance. Within IALT-Bio, ERCC1 expression was assessed retrospectively by immunohistochemistry (IHC) on tumor tissue [15]. High ERCC1 expression was shown to be a favorable prognostic

Table 1 Results of the concluded customized adjuvant therapy clinical trials in non-small cell lung cancer

Study	Biomarker (assay)	Marker low or absent HR (95 % CI); <i>p</i> value	Marker high or present HR (95 % CI); <i>p</i> value	Interaction test <i>p</i> value
BR.19 [41•]	<i>EGFR</i> , mutation	1.21 (0.84–1.73); 0.301	1.58 (0.83–3.00); 0.16	
JBR.10 [40]	<i>EGFR</i> , mutation	0.78 (0.57–1.06); 0.11	0.44 (0.11–1.70); 0.22	0.50
IALT [15]	ERCC1, IHC	0.65 (0.50–0.86); 0.002	1.14 (0.84–1.55); 0.04	0.009
IALT [17]	MSH2, IHC	0.76 (0.59–0.97); 0.03	1.12 (0.81–0.97); 0.48	0.06
IALT [24•]	p27, IHC	0.66 (0.50–0.88); 0.006	1.09 (.82–1.45); 0.54	0.02
LACE [25•]	p27, IHC	0.87 (0.64–1.18); 0.26	0.83 (0.59–1.15); 0.37	0.83
JBR.10 [26]	p53, IHC	1.40 (0.78–2.52); 0.26	0.54 (0.32–0.92); 0.02	0.02
LACE [27]	p53, IHC	0.82 (NR); 0.03	0.87 (NR); 0.22	0.64
JBR.10 [26]	<i>p53</i> , mutation	0.67 (0.46–0.98); 0.04	0.78 (0.46–1.32); 0.35	0.65
LACE [27]	<i>p53</i> , mutation	0.79 (0.63–0.98); 0.03	1.03 (0.80–1.34); 0.80	0.12
IALT,JBR.10 [28]	Bax, IHC	1.13 (0.89–1.45); 0.31	0.72 (0.56–0.91); 0.007	0.009
JBR.10 [30]	TUBB3, IHC	1.00 (0.57–1.75); 0.99	0.64 (0.39–1.04); 0.07	0.25
LACE [31]	TUBB3, IHC	1.03 (0.81–1.30); 0.82	0.83 (0.66–1.04); 0.11	0.81
JBR.10 [26]	<i>KRAS</i> , mutation	0.69 (0.49–0.97); 0.03	0.95 (0.53–1.71); 0.87	0.29
LACE [32]	<i>KRAS</i> , mutation	0.89 (0.76–1.06); 0.20	1.02 (0.73–1.41); 0.91	0.50
[60]	MAGE-A3, RT-PCR	NA	0.66 (0.36–1.20); 0.088	NA
[66]	15-gene signature	3.67 (1.22–11.06); 0.0133	0.33 (0.17–0.63); 0.0005	0.0001

factor in patients not receiving adjuvant chemotherapy. On the other hand, adjuvant cisplatin-based chemotherapy was associated with longer overall survival compared to observation alone in ERCC1 negative tumors (HR=0.65, *p*=0.002), but not in ERCC1 positive tumors (HR=1.14, *p*=0.40). This qualitative interaction was highly significant (interaction *p*=0.009) [15]. Recent analyses of ERCC1 expression in various histologic subtypes suggest that this cisplatin efficacy might be limited to squamous cell carcinoma with low ERCC1 expression [16]. Although these results are encouraging, validation is required before ERCC-1 expression (IHC or RT-PCR) can be recommended for clinical use, and currently, LACE-Bio is performing retrospective validation of this ERCC1 protein expression. Several prospective randomized

trials, such as the Tailored Post-Surgical Therapy in Early Stage NSCLC (TASTE), Southwest Oncology Group SWOG-0720 and the International Tailored Chemotherapy Adjuvant (ITACA) trials, are testing the feasibility and use of ERCC1 as a predictive biomarker in the adjuvant setting (Table 1). TASTE is a Phase II/III trial comparing adjuvant chemotherapy (cisplatin/pemetrexed) with customized adjuvant treatment, based on *EGFR* mutation status and ERCC1 IHC expression in stage II–IIIA non-squamous NSCLC. SWOG-0720 is a Phase II feasibility trial based on ERCC1/RMM1 expression in stage I NSCLC. ITACA is a Phase III trial comparing customized adjuvant chemotherapy based on ERCC1 and thymidylate synthase (TS) gene expression in stage II–IIIA NSCLC to standard chemotherapy.

Table 2 Ongoing customized adjuvant therapy clinical trials in non-small cell lung cancer

Trial	Phase	Trial identifier	Treatment	Biomarker
GECP-SCAT	III	NCT00478699	Standard vs. Customized therapy	BRCA1, mRNA
SWOG0720	II	NCT00792701	Observation vs. Intervention	ERCC1/RMM1, mRNA
TASTE	II/III	NCT00775385	Standard vs. Customized therapy	ERCC1 (IHC)/ <i>EGFR</i> (mutation)
ITACA	III	NA	Standard vs. Customized therapy	ERCC1/TS, mRNA
—	III	C-TONG 1104, NCT01405079	gefitinib vs. vinorelbine/platinum	<i>EGFR</i> /sensitizing mutations
RADIANT	III	NCT00373425	erlotinib vs. placebo	<i>EGFR</i> /EGFR+by IHC and/or FISH
SELECT	II	NCT00567359	erlotinib	<i>EGFR</i> /sensitizing mutations
TASTE	II/III	NCT00775385	standard CT vs. customized treatment	<i>EGFR</i> /sensitizing mutations, ERCC1
E1505	III	NCI-2009-00509, NCT00324805	Platinum doublet +/- bevacizumab	VEGF/unselected
MAGRIT	III	NCT00480025	MAGE-A3 ASCI or placebo	MAGE-A3/PCR+
START	III	NCT00409188	L-BLP25 vs. placebo	MUC1/unselected

MSH2 MutS homologue 2 (MSH2) is another protein that has a role in the DNA repair of cisplatin-induced DNA damages. During the repair process, MSH2 cooperates with other DNA repair mechanisms such as ERCC1. IALT-Bio assessed MSH2 expression by IHC [17] and reported that adjuvant cisplatin-based chemotherapy was associated with a trend towards improved OS compared to observation in patients with tumors with low MSH2 expression (HR=0.76, $p=0.03$), while no difference in OS was observed in the high MSH2 group (HR=1.12, $p=0.48$, interaction $p=0.06$).

BRCA1 BRCA1 plays a central role in DNA repair and also is involved in mitosis and cell division. BRCA1 mRNA level is has been reported to be prognostic in patients who underwent resection for early stage NSCLC, with high expression associated with shorter overall survival compared to low expression (HR=1.98, $p=0.02$) [18]. In the Phase II nonrandomized pilot Spanish Customized Adjuvant Treatment (pilot SCAT) trial, treating with single-agent docetaxel without a platinum analogue in BRCA1 high expression had no detrimental effect on survival, compared to cisplatin/gemcitabine in BRCA1 low expression [19]. These observations form the basis of the validation randomized Phase III Spanish Customized Adjuvant Treatment (validation SCAT) trial in resected stage II–III NSCLC, testing standard adjuvant chemotherapy (cisplatin/docetaxel) vs. customized adjuvant chemotherapy based on BRCA1 mRNA expression as previously done in the pilot SCAT.

RRM1 Ribonucleotide reductase is important for DNA synthesis. High RRM1 expression has been reported to be a good prognostic factor for overall survival in patients who underwent a resection for early stage NSCLC [20]. RRM1 may be a biomarker for gemcitabine chemotherapy. Whether RRM1 is predictive in the adjuvant chemotherapy setting is currently being evaluated in SWOG-0720.

TS TS is an important enzyme in the DNA replication and repair mechanisms. Low TS is a prognostic marker for longer survival in resected NSCLC [21]. TS is also a target of newer antifolate drugs such as pemetrexed [22]. High TS expression is associated with reduced efficacy of pemetrexed in vitro [23]. The predictive value of combined ERCC1 and TS profiles in resected NSCLC is being studied prospectively in the randomized Phase III ITACA trial.

Regulation of cell cycle, apoptosis and mitosis

p27 The p27 protein is a member of the cyclin-dependent kinase inhibitory family of proteins that prevent transition from the G1-phase to the S-phase of the cell cycle. Overexpression of p27 may confer de novo resistance to cisplatin by giving necessary time to repair cisplatin-induced DNA

damage. Within the IALT-Bio program, patients with p27 IHC negative tumors had a longer survival after chemotherapy compared with surgery alone (HR=0.66, $p=0.006$), but this was not shown (HR=1.09, $p=0.54$) for patients with p27 IHC positive tumors (interaction test $p=0.02$) [24]. However, a cross-validation and pooled analysis within the LACE-Bio program could not confirm this predictive value of p27 (HR=0.87, $p=0.26$ and HR=0.83, $p=0.37$, for p27 negative and positive patients, respectively, interaction $p=0.83$) [25].

p53 The protein encoded by the tumor suppressor gene p53 binds to DNA and plays a role in cell cycle regulation, induction of apoptosis and stabilization of the genome. The role of p53 has been studied both at the protein and DNA level. In JBR.10, p53 protein expression (> 15 %) was a poor prognostic factor (HR=1.89, $p=0.03$) and also a predictor of outcome with cisplatin/vinorelbine adjuvant chemotherapy. Patients with p53 IHC positive tumors derived significant benefit from adjuvant therapy (HR 0.54, $p=0.02$), but patients with p53 negative tumors did not (HR=1.4, $p=0.26$, interaction $p=0.02$) [26]. However, validation studies from LACE-Bio failed to confirm significant interaction for p53 protein expression (HR=0.82, $p=0.03$ and HR=0.87, $p=0.22$, for p53 negative and positive patients, respectively, interaction $p=0.64$). The p53 mutation was not a prognostic factor (HR=1.15, $p=0.45$) in the JBR.10 and not predictive for response to adjuvant chemotherapy (HR=0.67, $p=0.04$ and HR=0.78, $p=0.35$, for patients with p53 wild type and mutated, respectively, interaction $p=0.65$). The absence of predictive role for p53 mutation was confirmed in the LACE-Bio (HR=0.79, $p=0.03$ and HR=1.03, $p=0.80$, for patients with p53 wild type and mutated, respectively, interaction $p=0.12$) and adjuvant chemotherapy [27].

Bax Bax is a pro-apoptotic factor that is activated by p53. In a pooled analysis of IALT-Bio and JBR.10 specimens, Bax protein expression by IHC was significantly predictive for benefit from adjuvant chemotherapy on overall survival (HR=1.13, $p=0.31$ and HR=0.72, $p=0.007$, for Bax negative and positive patients, respectively, interaction $p=0.009$) [28].

TUBB3 Class III beta-tubulin (TUBB3) is a building block for microtubules. Tubulin-targeting agents (such as taxanes and vinca alkaloids) disrupt normal microtubule function and cause mitosis arrest. High TUBB3 protein expression is associated with non-squamous cell lung carcinoma and KRAS mutation [29]. In JBR.10, high TUBB3 expression was associated a borderline better prognostic in the observation arm ($p=0.08$), but did not predict response to adjuvant chemotherapy (interaction test $p=0.25$) [30]. The LACE-Bio cross-validation

study confirmed the absence of predictive effect for high TUBB3 (interaction test $p=0.81$) [31].

KRAS *KRAS*, a member of the *RAS* family of oncogenes, encodes a protein that is involved in signal transduction modulating the cell cycle and resulting in cell proliferation and survival. *KRAS* mutations in codons 12 and 13 have been reported in up to 25 % of lung adenocarcinoma. In JBR.10, *KRAS* mutation was not a significant prognostic marker for survival [26]. Wild-type *KRAS* status was associated with a survival benefit from adjuvant chemotherapy compared to observation only ($HR=0.69$, $p=0.03$), whereas patients with a *KRAS* mutation did not appear to benefit from adjuvant chemotherapy ($HR=0.95$, $p=0.87$). However, the interaction was not significant (interaction $p=0.29$) [26]. In the LACE-Bio pooled validation study, neither a prognostic nor a predictive effect of *KRAS* mutation status could be derived ($HR=0.89$, $p=0.20$ and $HR=1.02$, $p=0.91$, for *KRAS* wild type or mutated, respectively, interaction $p=0.50$) [32].

Targeted therapy

Novel targeted therapies, such as inhibitors of epidermal growth factor receptor (EGFR), angiogenesis inhibitors, and immunotherapy, have demonstrated effect in advanced NSCLC and are now the focus of studies in the early disease setting as adjuvant therapy.

EGFR

EGFR TKIs were the first molecularly targeted therapies approved to treat NSCLC and were initially studied in unselected patients as second-line and third-line treatment; the ISEL trial [33] evaluated the effect of gefitinib vs. best supportive care (BSC) and the BR.21 trial [34] erlotinib vs. BSC. Patients with certain clinical characteristics, such as adenocarcinoma histology, never-smokers, female sex, and Asian ethnicity, displayed increased sensitivity to EGFR TKIs [35]. Although clinical characteristics may help identify patients who are more likely to benefit from an EGFR TKI, it is crucial that patients are selected for this type of therapy based on measurable biologic characteristics. In the search for molecular mechanisms explaining the better response in certain clinical subgroups, tumor-associated mutations in the tyrosine kinase domain of *EGFR* were found to be associated with response to EGFR TKIs [36–38]. The most common *EGFR*-sensitizing mutations encompass deletions in exon 19 and a point mutation at L858R in exon 21; together, they account for approximately 85 % of *EGFR* mutations in NSCLC [35]. Other studies have indicated that increased *EGFR* copy number and EGFR protein expression may also be predictive of response to EGFR TKIs [39].

In the adjuvant setting, the patients with *EGFR* mutations in the JBR.10 trial had a potential but not significant benefit to chemotherapy ($HR=0.44$, $p=0.22$, interaction $p=0.50$). The nonsignificant difference can be explained by the low number of patients with *EGFR* mutations in the JBR.10. However, these results indicate that patients with EGFR mutations should not be treated with EGFR TKIs alone at this time and should be included in clinical trials [40].

In the BR.19 Phase III randomized trial of the epidermal growth factor receptor inhibitor gefitinib in completely resected stage IB–IIIA NSCLC, no survival benefit over placebo was observed in unselected patients ($HR=1.24$, $p=0.14$ and 1.22 , $p=0.15$ for OS and DFS, respectively). Although not powered to draw any conclusions, even the subgroup of patients with *EGFR*-mutant tumors did not show any benefit from gefitinib treatment (HR for OS 1.58 , $p=0.16$) [41]. The trial was closed prematurely on the basis of the negative results of the ISEL and SWOG-0023 trials. Contrary to this, a retrospective study by Janjigian et al. observed a trend, although not statistically significant, toward improved disease-free survival (DFS) among patients with resected stages I–III lung adenocarcinomas harboring *EGFR*-mutations who received EGFR tyrosine kinase inhibitors (TKIs) as adjuvant therapy compared with patients who did not receive adjuvant TKI. The 2-year DFS was 89 % for patients treated with adjuvant TKI compared with 72 % in the control group ($HR=0.53$, $p=0.06$). The 2-year overall survival was 96 % with adjuvant EGFR TKI and 90 % in the control group ($HR=0.62$; $p=0.296$) [42]. A Phase III trial is currently evaluating gefitinib vs. vinorelbine/platinum as adjuvant treatment in stage II–IIIA (N1–N2) NSCLC with *EGFR* mutation.

Erlotinib is another EGFR TKI in under evaluation in the ongoing Phase III Randomized Double-Blind Trial in Adjuvant NSCLC with erlotinib (RADIANT) study. Patients with resected stage IB–IIIA NSCLC who are EGFR-positive by IHC and/or fluorescent in situ hybridization (FISH) are randomized to erlotinib or placebo for 2 years after surgical resection and optional adjuvant chemotherapy. Accrual is complete and final results are awaited. Another ongoing study, the Phase II SELECT trial, is evaluating adjuvant erlotinib for two years in patients with resected, early-stage NSCLC (stage IA–IIIA) and confirmed mutations in *EGFR* [43].

The Tailored Post-Surgical Therapy in Early Stage NSCLC (TASTE) trial is a Phase II/III study randomizing patients with stage II–IIIA (non-N2) resected non-squamous NSCLC to a non-customized standard adjuvant chemotherapy arm and a genotypic arm, in which patients with known *EGFR* activating mutations receive erlotinib for 1 year, while those who have wild-type *EGFR* receive chemotherapy based on the level of ERCC1 expression, with low levels of ERCC1 being treated with cisplatin/pemetrexed and high levels of ERCC1 with non-platinum based chemotherapy.

Angiogenesis inhibition

Vascular endothelial growth factor (VEGF) stimulates angiogenesis, which is essential for tumor growth and progression [44]. Overexpression of VEGF is common in NSCLC and is associated with adverse clinical outcomes. Bevacizumab is a recombinant humanized monoclonal antibody against VEGF that inhibits angiogenesis [45]. It has been evaluated in combination with chemotherapy in advanced NSCLC, and has demonstrated modest clinical benefit in two large trials [46, 47]. Based on these studies, the US Food and Drug Administration (FDA) and European Medicines Agency (EMA) approved bevacizumab in combination with platinum-based chemotherapy as a first-line treatment option for advanced non-squamous NSCLC. No prospectively useful predictive biomarkers for treatment response to bevacizumab have been identified so far. Existing data indicate a possible predictive role for single nucleotide polymorphisms (SNPs) in the VEGF signaling pathway, as well as treatment-emergent hypertension, but the clinical importance of this remains to be evaluated [48]. However, another VEGF receptor inhibitor, cediranib, showed no predictive association for hypertension in the NCIC Clinical Trials Group study BR24 [49].

The role of bevacizumab in the adjuvant setting of NSCLC currently is being evaluated in ECOG-1505. In this trial, patients with resected stage IB (≥ 4 cm), II, and IIIA NSCLC are randomized to four cycles of adjuvant platinum-based doublet chemotherapy with or without bevacizumab for one year. Patients with squamous histology are included in this trial because resection of the primary tumor eliminates the source for potential tumor-related hemorrhage. In the adjuvant setting, there may be concerns with bevacizumab treatment for wound healing, as well as toxicities such as hemorrhage, hypertension and thrombotic events. Interim safety data including 557 patients reported no unexpected toxicities with a significant increase in risk of grades 3/4 hypertension (0.7 % vs. 19.7 %, $p<0.001$), proteinuria (0.7 % vs. 3.4 %, $p=0.03$), abdominal pain (0.4 % vs. 4.6 %, $p=0.001$), and overall grade 3/4 toxicity (68.5 % vs. 83.4 %, $p<0.001$) associated with bevacizumab. One case each of fatal hemoptysis and nonfatal bronchopleural fistula were seen in patients receiving bevacizumab [50].

Novel targets

With the rapid development of molecular techniques to analyze genetic aberrations in tumors, a greater understanding of the molecular mechanisms behind tumorigenesis and the identification of new therapeutic targets has stimulated the development of novel agents with the potential to improve the standard chemotherapy regimens for NSCLC. So far, these novel targeted agents have been or are currently

evaluated in advanced disease, but it also will be of great interest to study these agents in the adjuvant setting once their efficacy in advanced NSCLC has been confirmed. In 2007, Soda and colleagues identified the echinoderm microtubule associated protein-like 4 (*EML4*)-anaplastic lymphoma kinase (*ALK*) fusion gene in a subset of patients with NSCLC [51]. This genetic alteration originates from a translocation within chromosome 2p that leads the intracellular tyrosine kinase domain of *ALK* to fuse with various (N-terminal) truncated portions of *EML4*, resulting in constitutively enhanced *ALK* activity, which stimulates tumor growth. Various reports indicate that 1.6–8.6 % of patients with NSCLC are *ALK*-positive [52]. In 2011, the FDA approved crizotinib, an *ALK* kinase inhibitor, for use in patients with *ALK*-positive NSCLC on FISH analysis. The approval was based on data from an early-stage single arm 82 patient trial that reported an overall response rate of 57 % to crizotinib, including one confirmed complete response and 33 % of patients with stable disease in patients with the mutation [53]. An updated analysis of this study showed that 87 of 143 patients had an objective response (60.8 %), including three complete responses and 84 partial responses. Median PFS was 9.7 months, whereas for median OS data are not yet mature, but estimated OS at 6 and 12 months was 87.9 % and 74.8 %, respectively [54].

Other novel targets of potential interest in the adjuvant setting include *ROS1*, which was identified as a transforming fusion gene with the 5' region of *SLC34A2* or the 5' region of *CD74* [55], and *RET* showing in-frame fusion transcripts with *KIF5B* (the kinesin family 5B gene) or *CCDC6* leading to constitutively active *RET* kinase [56, 57].

Individualized immunotherapy

Recently, promising results have been obtained with immunotherapy in the context of the adjuvant setting of NSCLC. A hypothetical explanation for the success of immunotherapy in the adjuvant setting as compared to advanced disease could be the lower tumor tolerance for immune response for smaller volume of tumor cells [58]. Cancer vaccines, including antigen-specific, tumor-cell specific and dendritic-cell-based vaccines, have been developed. In particular, two vaccines, the antigen-specific melanoma-associated antigen 3 (MAGE-A3) vaccine (MAGE-A3 ASCI) and the liposomal BLP25 vaccine (L-BLP25) targeting MUC1 are being tested in Phase III trials in patients with early NSCLC (Table 2).

MAGE-A3 is a tumor specific antigen expressed in 35–50 % of NSCLC [59, 60]. A total of 182 patients, all MAGE-A3 positive (assessed by RT-PCR expression on fresh tissue), stage IB/II NSCLC were treated postoperatively with either MAGE-A3 ASCI or placebo [60]. A trend toward longer

disease-free interval (HR=0.74, $p=0.107$), DFS (HR=0.73, $p=0.093$) and OS (HR=0.66, $p=0.088$) were observed in patients treated with MAGE-A3 ASCI. An ongoing large Phase III placebo controlled trial has completed accrual of more than 2,000 patients; MAGRIT (MAGE-A3 as Adjuvant non small cell lung cancer ImmunoTherapy) has completed accrual and results are awaited [61].

MUC1 is a cell surface protein glycoprotein that is over-expressed in several cancers, including NSCLC [62]. The L-BLP25 vaccine is a liposomal molecule targeting MUC1. A Phase II trial compared L-BLP25 plus best supportive care (BSC) to BSC alone after chemotherapy in patients with stage IIIB/IV NSCLC. This study showed a trend for longer median OS (HR=0.55, $p=0.16$) in the subgroup of patients with loco-regional stage IIIB treated with L-BLP25 [63, 64]. The Phase III placebo controlled trial, the Stimulating Targeted Angiogenic Responses to NSCLC Trial (START), evaluates L-BLP25 in patients with unresectable stage III with an objective response or a stable disease after a first line of chemotherapy [65]. START has completed accrual and results are awaited.

A role for panel of biomarkers

Panels of biomarkers or signatures may be used to predict prognosis or response to therapy. However, to date all signatures lack sufficient validation to be implemented in clinical practice.

In early NSCLC, Zhu et al. analyzed the role of a prognostic 15-gene signature derived from patients participating in JBR.10. This signature identified patients at high and low risk within the observation arm (HR=13.32 (2.86–62.11), $p<0.0001$ and HR=13.47 (3.00–60.43), $p<0.0001$, for stage IB and stage II patients, respectively). Furthermore, high-risk patients derived significant benefit from chemotherapy (HR=0.33, CI 0.17–0.63, $p=0.0005$), but not in those at low-risk where chemotherapy was detrimental as compared to observation (HR=3.67, CI 1.22–11.06, $p=0.0133$; interaction $p=0.0001$) [66]. These results require validation in other studies, but few randomized trials have frozen sample tumor banks to perform the necessary microarray studies. Attempts to translate the signature to paraffin-embedded samples are ongoing.

Conclusions

Despite improvements in survival with adjuvant chemotherapy after complete resection of localized NSCLC, giving a survival advantage of approximately 5 % at 5 years, there is still a relatively high risk of relapse even for early-stage NSCLC. Therefore, efforts are ongoing to identify new therapies in the adjuvant setting and in the new era of personalized medicine to select patients for individualized

treatment on the basis of biomarkers. In the heterogeneous patient population of surgically resected patients, it would be of great value to identify those patients who are most likely to benefit from adjuvant therapy and to match these patients with a biomarker-driven targeted therapy.

Acknowledgments CM is supported by grant from the Louisiana Chapter of the National Lung Cancer Partnership (NLCP).

Disclosure No potential conflicts of interest relevant to this article were reported.

References

Papers of particular interest, published recently, have been highlighted as:

- Of importance
- Of major importance

1. Goldstraw P, Crowley J, Chansky K, et al. The IASLC Lung Cancer Staging Project: proposals for the revision of the TNM stage groupings in the forthcoming (seventh) edition of the TNM Classification of malignant tumours. *J Thorac Oncol*. 2007;2:706–14.
2. Non-small Cell Lung Cancer Collaborative Group. Chemotherapy in non-small cell lung cancer: a meta-analysis using updated data on individual patients from 52 randomised clinical trials. *BMJ*. 1995;311:899–909.
3. Arriagada R, Bergman B, Dunant A, et al. Cisplatin-based adjuvant chemotherapy in patients with completely resected non-small-cell lung cancer. *N Engl J Med*. 2004;350:351–60.
4. Winton T, Livingston R, Johnson D, et al. Vinorelbine plus cisplatin vs. observation in resected non-small-cell lung cancer. *N Engl J Med*. 2005;352:2589–97.
5. Douillard JY, Rosell R, De Lena M, et al. Adjuvant vinorelbine plus cisplatin versus observation in patients with completely resected stage IB–IIIA non-small-cell lung cancer (Adjuvant Navelbine International Trialist Association [ANITA]): a randomised controlled trial. *Lancet Oncol*. 2006;7:719–27.
6. Strauss GM, Herndon 2nd JE, Maddaus MA, et al. Adjuvant paclitaxel plus carboplatin compared with observation in stage IB non-small-cell lung cancer: CALGB 9633 with the Cancer and Leukemia Group B, Radiation Therapy Oncology Group, and North Central Cancer Treatment Group Study Groups. *J Clin Oncol*. 2008;26:5043–51.
7. Pisters KM, Evans WK, Azzoli CG, et al. Cancer Care Ontario and American Society of Clinical Oncology adjuvant chemotherapy and adjuvant radiation therapy for stages I–IIIA resectable non small-cell lung cancer guideline. *J Clin Oncol*. 2007;25:5506–18.
8. Brodowicz T, Ciuleanu T, Crawford J, et al. Third CECOG consensus on the systemic treatment of non-small-cell lung cancer. *Ann Oncol*. 2012;23:1223–9.
9. Crino L, Weder W, van Meerbeeck J, Felip E. Early stage and locally advanced (non-metastatic) non-small-cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*. 2010;21 Suppl 5:v103–15.
10. •• Pignon JP, Tribodet H, Scagliotti GV, et al. Lung adjuvant cisplatin evaluation: a pooled analysis by the LACE Collaborative Group. *J Clin Oncol*. 2008;26:3552–9. *This meta-analysis pooling individual patients data from five trials confirmed that adjuvant*

chemotherapy increases survival in stage II and stage IIIA NSCLC after surgical resection.

11. Arriagada R, Auperin A, Burdett S, et al. Adjuvant chemotherapy, with or without postoperative radiotherapy, in operable non-small-cell lung cancer: two meta-analyses of individual patient data. *Lancet*. 2010;375:1267–77.
12. Saviozzi S, Ceppi P, Novello S, et al. Non-small cell lung cancer exhibits transcript overexpression of genes associated with homologous recombination and DNA replication pathways. *Cancer Res*. 2009;69:3390–6.
13. Simon GR, Schell MJ, Begum M, et al. Preliminary indication of survival benefit from ERCC1 and RRM1-tailored chemotherapy in patients with advanced nonsmall cell lung cancer: evidence from an individual patient analysis. *Cancer*. 2012;118:2525–31.
14. Rosell R, Perez-Roca L, Sanchez JJ, et al. Customized treatment in non-small-cell lung cancer based on EGFR mutations and BRCA1 mRNA expression. *PLoS One*. 2009;4:e1533.
15. Olaussen KA, Dunant A, Fouret P, et al. DNA repair by ERCC1 in non-small-cell lung cancer and cisplatin-based adjuvant chemotherapy. *N Engl J Med*. 2006;355:983–91.
16. Pierceall WE, Olaussen KA, Rousseau V, et al. Cisplatin benefit is predicted by immunohistochemical analysis of DNA repair proteins in squamous cell carcinoma but not adenocarcinoma: therapeutic modeling by NSCLC constituent histological subclasses. *Ann Oncol*. 2012;23:2245–52.
17. Kamal NS, Soria JC, Mendiboure J, et al. MutS homologue 2 and the long-term benefit of adjuvant chemotherapy in lung cancer. *Clin Cancer Res*. 2010;16:1206–15.
18. Rosell R, Skrzypski M, Jassem E, et al. BRCA1: a novel prognostic factor in resected non-small-cell lung cancer. *PLoS One*. 2007;2:e1129.
19. Cobo M, Massuti B, Moran T, et al. Spanish customized adjuvant trial (SCAT) based on BRCA1 mRNA levels. *J Clin Oncol*. 2008;26:7533.
20. Bepler G, Sharma S, Cantor A, et al. RRM1 and PTEN as prognostic parameters for overall and disease-free survival in patients with non-small-cell lung cancer. *J Clin Oncol*. 2004;22:1878–85.
21. Grimminger PP, Schneider PM, Metzger R, et al. Low thymidylate synthase, thymidine phosphorylase, and dihydropyrimidine dehydrogenase mRNA expression correlate with prolonged survival in resected non-small-cell lung cancer. *Clin Lung Cancer*. 2010;11:328–34.
22. Bertino JR, Banerjee D. Thymidylate synthase as an oncogene? *Cancer Cell*. 2004;5:301–2.
23. Giovannetti E, Mey V, Nannizzi S, et al. Cellular and pharmacogenetics foundation of synergistic interaction of pemetrexed and gemcitabine in human non-small-cell lung cancer cells. *Mol Pharmacol*. 2005;68:110–8.
24. • Filipits M, Pirker R, Dunant A, et al. Cell cycle regulators and outcome of adjuvant cisplatin-based chemotherapy in completely resected non-small-cell lung cancer: the International Adjuvant Lung Cancer Trial Biologic Program. *J Clin Oncol*. 2007;25:2735–40. *The IALT-Bio attempts to find biomarkers for adjuvant therapy.*
25. • Pirker R, Rousseau V, Paris E, et al. LACE-Bio: Cross-validation and pooled analyses of the putative prognostic/predictive biomarkers p27, p16 and cyclin E in IALT, ANITA, JBR10 and CALBG 9633. *J Thorac Oncol*. 2010;5:S503. *The LACE-Bio is an international group trying to identify and validate biomarkers for the adjuvant therapy. They study several markers including those reported in this publication.*
26. Tsao MS, Aviel-Ronen S, Ding K, et al. Prognostic and predictive importance of p53 and RAS for adjuvant chemotherapy in non-small-cell lung cancer. *J Clin Oncol*. 2007;25:5240–7.
27. Graziano SL, Paris E, Ma X. LACE-Bio pooled analysis of the prognostic and predictive value of p53 mutations and expression by immunohistochemistry (IHC) in patients with resected non-small cell lung cancer (NSCLC). *Ann Oncol*. 2010;21:Viii130.
28. Brambilla E, Bourredjem A, Lantuejoul S, et al. Bax expression as a predictive marker of survival benefit in non-small cell lung carcinoma treated by adjuvant cisplatin-based chemotherapy. *J Thorac Oncol*. 2010;5:S503–4.
29. Levallet G, Bergot E, Antoine M, et al. High TUBB3 expression, an independent prognostic marker in patients with early non-small cell lung cancer treated by preoperative chemotherapy, is regulated by K-Ras signaling pathway. *Mol Cancer Ther*. 2012;11:1203–13.
30. Seve P, Lai R, Ding K, et al. Class III beta-tubulin expression and benefit from adjuvant cisplatin/vinorelbine chemotherapy in operable non-small cell lung cancer: analysis of NCIC JBR.10. *Clin Cancer Res*. 2007;13:994–9.
31. Reiman T, Lai R, Veillard AS, et al. Cross-validation study of class III beta-tubulin as a predictive marker for benefit from adjuvant chemotherapy in resected non-small-cell lung cancer: analysis of four randomized trials. *Ann Oncol*. 2012;23:86–93.
32. Tsao MS, Hainaut P, Bourredjem A. LACE-Bio pooled analysis of the prognostic and predictive value of KRAS mutation in completely resected non-small cell lung cancer. *Ann Oncol*. 2010;21:viii63–77.
33. Thatcher N, Chang A, Parikh P, et al. Gefitinib plus best supportive care in previously treated patients with refractory advanced non-small-cell lung cancer: results from a randomised, placebo-controlled, multicentre study (Iressa Survival Evaluation in Lung Cancer). *Lancet*. 2005;366:1527–37.
34. Shepherd FA, Rodrigues Pereira J, Ciuleanu T, et al. Erlotinib in previously treated non-small-cell lung cancer. *N Engl J Med*. 2005;353:123–32.
35. Miller VA, Kris MG, Shah N, et al. Bronchioloalveolar pathologic subtype and smoking history predict sensitivity to gefitinib in advanced non-small-cell lung cancer. *J Clin Oncol*. 2004;22:1103–9.
36. Toschi L, Cappuzzo F. Understanding the new genetics of responsiveness to epidermal growth factor receptor tyrosine kinase inhibitors. *Oncologist*. 2007;12:211–20.
37. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med*. 2004;350:2129–39.
38. Paez JG, Janne PA, Lee JC, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science*. 2004;304:1497–500.
39. Cappuzzo F, Hirsch FR, Rossi E, et al. Epidermal growth factor receptor gene and protein and gefitinib sensitivity in non-small-cell lung cancer. *J Natl Cancer Inst*. 2005;97:643–55.
40. Tsao MS, Sakurada A, Ding K, et al. Prognostic and predictive value of epidermal growth factor receptor tyrosine kinase domain mutation status and gene copy number for adjuvant chemotherapy in non-small cell lung cancer. *J Thorac Oncol*. 2011;6:139–47.
41. • Goss GD, Tsao MS, O'Callagan CJ, et al. A phase III randomized, double-blind, placebo-controlled trial of the epidermal growth factor receptor inhibitor gefitinib in completely resected stage IB-IIIA non-small cell lung cancer (NSCLC): NCIC CTG BR.19. *J Clin Oncol*. 2010;28:18s. LBA7005. *The BR19 is pioneer in addressing the role of biomarkers (EGFR mutation) for predicting response to gefitinib in adjuvant setting.*
42. Janjigian YY, Park BJ, Zakowski MF, et al. Impact on disease-free survival of adjuvant erlotinib or gefitinib in patients with resected lung adenocarcinomas that harbor EGFR mutations. *J Thorac Oncol*. 2011;6:569–75.
43. Pennell NA, Neal JW, Govindan R, Janne PA, Evans TL, Costa DB, Rosovsky RPG, Lanuti M, Azzoli CG, Sequist LV. The SELECT trial: A multicenter phase II trial of adjuvant erlotinib (E) in patients with resected, early-stage non-small cell lung cancer

- (NSCLC) and confirmed mutations in the epidermal growth factor receptor (EGFR). *J Clin Oncol*. 29:2011 (suppl; abstr TPS209).
44. Rossi A, Maione P, Ferrara ML, et al. Angiogenesis inhibitors and vascular disrupting agents in non-small cell lung cancer. *Curr Med Chem*. 2009;16:3919–30.
 45. Ferrara N, Hillan KJ, Gerber HP, Novotny W. Discovery and development of bevacizumab, an anti-VEGF antibody for treating cancer. *Nat Rev Drug Discov*. 2004;3:391–400.
 46. Sandler A, Gray R, Perry MC, et al. Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. *N Engl J Med*. 2006;355:2542–50.
 47. Reck M, von Pawel J, Zatloukal P, et al. Phase III trial of cisplatin plus gemcitabine with either placebo or bevacizumab as first-line therapy for nonsquamous non-small-cell lung cancer: AVAIL. *J Clin Oncol*. 2009;27:1227–34.
 48. Das M, Wakelee H. Targeting VEGF in lung cancer. *Expert Opin Ther Targets*. 2012;16:395–406.
 49. Goodwin R, Ding K, Seymour L, et al. Treatment-emergent hypertension and outcomes in patients with advanced non-small-cell lung cancer receiving chemotherapy with or without the vascular endothelial growth factor receptor inhibitor cediranib: NCIC Clinical Trials Group Study BR24. *Ann Oncol*. 2010;21:2220–6.
 50. Wakelee HA, Dahlberg SE, Keller SM, Gandara DR, Graziano SL, Leighl NB, Adjei AA, Schiller JH. Interim report of on-study demographics and toxicity from E1505, a phase III randomized trial of adjuvant (adj) chemotherapy (chemo) with or without bevacizumab (B) for completely resected early-stage non-small cell lung cancer (NSCLC). *J Clin Oncol*. 2011; 29:(suppl; abstr 7013).
 51. Soda M, Choi YL, Enomoto M, et al. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. *Nature*. 2007;448:561–6.
 52. Scagliotti G, Stahel RA, Rosell R, et al. ALK translocation and crizotinib in non-small cell lung cancer: an evolving paradigm in oncology drug development. *Eur J Cancer*. 2012;48:961–73.
 53. Kwak EL, Bang YJ, Camidge DR, et al. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. *N Engl J Med*. 2010;363:1693–703.
 54. Camidge DR, Bang YJ, Kwak EL, et al. Activity and safety of crizotinib in patients with ALK-positive non-small-cell lung cancer: updated results from a phase 1 study. *Lancet Oncol*. 2012;13:1011–9.
 55. Rikova K, Guo A, Zeng Q, et al. Global survey of phosphotyrosine signaling identifies oncogenic kinases in lung cancer. *Cell*. 2007;131:1190–203.
 56. Kohno T, Ichikawa H, Totoki Y, et al. KIF5B-RET fusions in lung adenocarcinoma. *Nat Med*. 2012;18:375–7.
 57. Li F, Feng Y, Fang R, et al. Identification of RET gene fusion by exon array analyses in "pan-negative" lung cancer from never smokers. *Cell Res*. 2012;22:928–31.
 58. Gridelli C, Rossi A, Maione P, et al. Vaccines for the treatment of non-small cell lung cancer: a renewed anticancer strategy. *Oncologist*. 2009;14:909–20.
 59. Gure AO, Chua R, Williamson B, et al. Cancer-testis genes are coordinately expressed and are markers of poor outcome in non-small cell lung cancer. *Clin Cancer Res*. 2005;11:8055–62.
 60. Vansteenkiste J, Zielinski CC, Linder A. Final results of a multicenter, double-blind, randomized, placebo-controlled phase II study to assess the efficacy of MAGE-A3 immunotherapeutic as adjuvant therapy in stage IB/II non-small cell lung cancer (NSCLC). *J Clin Oncol*. 2007;25:7554.
 61. Tyagi P, Mirakhur B. MAGRIT: the largest-ever phase III lung cancer trial aims to establish a novel tumor-specific approach to therapy. *Clin Lung Cancer*. 2009;10:371–4.
 62. Ho SB, Niehans GA, Lyftogt C, et al. Heterogeneity of mucin gene expression in normal and neoplastic tissues. *Cancer Res*. 1993;53:641–51.
 63. Butts C, Murray N, Maksymiuk A, et al. Randomized phase IIB trial of BLP25 liposome vaccine in stage IIIB and IV non-small-cell lung cancer. *J Clin Oncol*. 2005;23:6674–81.
 64. Sangha R, Butts C. L-BLP25: a peptide vaccine strategy in non small cell lung cancer. *Clin Cancer Res*. 2007;13: s4652–4.
 65. Powell E, Chow LQ. BLP-25 liposomal vaccine: a promising potential therapy in non-small-cell lung cancer. *Expert Rev Respir Med*. 2008;2:37–45.
 66. Zhu CQ, Ding K, Strumpf D, et al. Prognostic and predictive gene signature for adjuvant chemotherapy in resected non-small-cell lung cancer. *J Clin Oncol*. 2010;28:4417–24.