DNA repair protein expression in resected NSCLC: a different predictive value for platinum benefit in adenocarcinoma versus squamous-cell carcinoma?

Lung cancer remains the leading cause of cancer-related mortality worldwide, with non-small-cell lung cancer (NSCLC) accounting for >80% of all newly diagnosed cases of lung cancer. For patients with early-stage disease, from clinical stage IA through IIB, surgical resection represents the standard of care. However, ~50% of those patients undergoing surgical resection will relapse and die of recurrent disease within 5 years [1].

In order to improve survival in patients with early-stage NSCLC, efforts have been focused on the use of chemotherapy before or after surgery with the aim of reducing the risk of relapse. In 1995, the meta-analysis carried out by the Non-Small Cell Lung Cancer Collaborative Group was published; this included data from 14 studies (4357 patients and 2574 deaths) that compared surgery alone with surgery followed by adjuvant chemotherapy [2]. Results from eight studies using cisplatin-based combinations (n = 1394) showed an absolute survival benefit of 5% at 5 years with the use of adjuvant chemotherapy, although these findings were not statistically significant (HR = 0.87, P = 0.08). Results from this meta-analysis prompted additional evaluation of platinum-based regimens in resectable NSCLC, resulting in further prospective randomized phase III trials. The major adjuvant studies conducted in this regard were the International Adjuvant Lung Cancer Trial (IALT) [1], the Adjuvant Lung Project Italy (ALPI) [3], the Big Lung Trial (BLT) [4], the National Cancer Institute of Canada Clinical Trials Group (NCIC-BR10) [5] trial, the Adjuvant Navelbine International Trialist Association (ANITA) [6] study, and the Cancer and Leukemia Group B (CALGB 9633) [7] study. Information from these adjuvant studies conducted after 1995 yielded conflicting results regarding the benefit of adjuvant treatment. For that reason, The Lung Adjuvant Cisplatin Evaluation (LACE) pooled individual data of 4854 patients from five randomized adjuvant trials using adjuvant cisplatin combinations, the ALPI, the ANITA, the BLT, the IALT, and the NCIC-BR10 studies [8].

Adjuvant chemotherapy was associated with an improvement in overall survival with an 11% relative reduction in the risk of death (HR = 0.89, CI 0.82–0.96, P = 0.04). However, the degree of benefit varied depending on stage. In stages II and III, overall survival benefit was 5.3% at 5 years (HR = 0.83, CI 0.73–0.95, and HR = 0.83, CI 0.72–0.94, respectively), whereas a small and not statistically significant benefit was observed in stage IB (HR = 0.93, CI 0.78–1.10) and a detrimental effect in stage IA (HR = 1.40, CI 0.95–2.06). At present, the benefit of adjuvant platinum-based chemotherapy is widely accepted for patients with resected stage II–IIIA [8].

Overall, adjuvant chemotherapy in NSCLC improves survival at 5 years between 4% and 15%. However, adjuvant chemotherapy is associated with a potentially significant toxicity and not all patients treated with adjuvant chemotherapy benefit from it. Furthermore, recovery from thoracic surgery may compromise the administration of adjuvant treatment. A real challenge, therefore, is to find a way to identify which patients are most likely to obtain clinical benefit from a given chemotherapy treatment. In surgically resected patients, identifying prognostic factors that help predict patients at risk of recurrence and defining predictive factors for treatment benefit in this group are essential if we are to successfully optimize treatment.

To better define those patients that may benefit from adjuvant platinum-based chemotherapy, the study published by Pierceall et al. [9] analyzed prognostic and predictive markers in tumor samples from patients in the IALT study. In this paper, the authors carried out an immunohistochemistry (IHC) evaluation to determine the prognostic and predictive value of levels of seven key proteins involved in DNA damage response and/or DNA repair pathways [ERCC1, XPF, MSH2, BRCA1, p53, PARP1 (PARP, poly(ADP-ribose) polymerase), ATM (ataxia-telangiectasia mutated)] in 769 samples from patients included in the IALT trial. The findings detailed in the present manuscript are clinically relevant; the IALT trial has been the largest randomized trial in the adjuvant setting comparing cisplatin-based chemotherapy with no treatment and it is one of the most relevant studies that led to adjuvant chemotherapy being considered standard of care.

Cisplatin is the backbone of chemotherapy regimens used to treat NSCLC. Therefore, to molecularly define those patients with more possibilities to benefit from this drug would be of great relevance. The main cytotoxic activity of cisplatin is based on the formation of mono-/bifunctional adducts in the DNA, which cause inter-/intrastrand cross-linking. Proposed mechanisms of resistance include decreased intracellular accumulation, increased detoxification through its conjugation with glutathione, and increased tolerance to DNA damage resulting from a highly efficient DNA repair capacity. In this line of research, the study published by Pierceall et al. [9] has a clear relevance, in which the authors carried out an IHC evaluation of seven key proteins (ERCC1, XPF, MSH2, BRCA1, p53, PARP1, ATM) involved in DNA damage response and/or DNA repair pathways among 769 samples from patients included in the IALT trial.
Analyzing the relationship between DNA repair protein levels and the benefit of cisplatin combinations has a clear rationale. There are four basic DNA repair pathways: the nucleotide excision repair (NER), the base excision repair, the mismatch repair, and the double-strand break repair [10, 11]. The removal of the cross-linked DNA is carried out by the NER system. That is to say, DNA cross-linking by cisplatin activates the NER mechanism, which includes a large complex consisting of at least 30 proteins, including ERCC1, XPA, XPF/ERCC5, XPC, XPD/ERCC2, XPF/ERCC4, and others [12–14]. A few of these proteins cut out the damaged DNA strand, the gap is filled by polymerases, and the ends are connected by ligases. These events restore the configuration of the damaged DNA strand. But if repair proves inadequate, NER triggers apoptosis. Thus, it is the balance between DNA damage and repair that determines the fate of cancer cells exposed to platinum. A limiting step in the activation of the NER system is the formation of the heterodimer formed between ERCC1 and XPF. As a unit, they execute the 5′ incision into the DNA strand, relative to the site of DNA damage, in the NER process, which will be involved in platinum resistance [10, 11, 14]. Some studies support the hypothesis that in tumor cells treated with cisplatin, the decrease in NER system function leads to upper cell damage and consequently higher levels of cell death [15].

In view of these preclinical data, ERCC1 (repair gene located on chromosome 19) has emerged as one of the single-gene targets in biomarker development for platinum-based chemotherapy. Several studies have analyzed the potential prognostic and predictive role of ERCC1. Initially, in advanced NSCLC patients, Lord et al. [16] suggested that ERCC1 expression determined by real-time (RT)-PCR could be a predictive factor for survival after cisplatin/gemcitabine therapy. Subsequently, the Genomic International Lung Trial (GILT) of customized chemotherapy carried out by the Spanish Lung Cancer Group tested prospectively the predictive value of ERCC1 for platinum-based chemotherapy in a randomized trial in patients with advanced NSCLC [17]. The hypothesis was that the individualized treatment based on ERCC1 mRNA levels in a pretreatment sample could favorably affect a patient’s outcome. In this prospective phase III trial of individualized chemotherapy, the control arm consisted of cisplatin/docetaxel, and the genotypic arm was based on the level of ERCC1 mRNA expression in the tumor sample (patients with low ERCC1 mRNA levels received cisplatin/docetaxel, whereas those with higher levels received docetaxel/gemcitabine). Radiological response was observed in 39.3% of patients in the control arm and in 50.7% in the genotypic arm (P = 0.024). However, no differences in progression-free survival or overall survival between the two arms were found.

In resected NSCLC, ERCC1 mRNA levels can be a prognostic factor in the absence of chemotherapy treatment. Simon et al. [18] evaluated the effect of the ERCC1 expression levels on survival by analyzing frozen tissue of patients with NSCLC who had undergone surgical intervention. In this study, increased ERCC1 expression was an independent predictor of survival improvement in surgically resected patients. In 2006, the prognostic and predictive value of ERCC1 protein expression, assessed by IHC, in 761 tumors from patients included in the IALT study was reported by the authors of the manuscript we are discussing here [19]. Among the 761 tumor samples examined, ERCC1 expression was considered positive in 335 (44%) and negative in 426 (56%). Among patients who did not receive adjuvant chemotherapy, those with ERCC1-positive tumors survived longer than those with ERCC1-negative tumors, further highlighting the prognostic significance of ERCC1. Adjuvant chemotherapy, when compared with observation, significantly prolonged survival among patients with ERCC1-negative tumors, but not among patients with ERCC1-positive tumors. In the group of patients with ERCC1-negative tumors who received cisplatin-based chemotherapy, the risk of death decreased by 35%, thereby indicating a potential predictive value of this biomarker. These findings raised the question of whether patients with ERCC1-positive tumors would benefit from alternative therapies, such as non platinum-based chemotherapy regimens, or whether they would be best served by receiving no chemotherapy at all. At present, a number of ongoing prospective trials in the adjuvant setting analyze the potential value of ERCC1 expression in selecting chemotherapy treatment. In the International Tailored Chemotherapy Adjuvant Trial (ITACA) study, patients with completely resected stage II–III NSCLC are treated with either a cisplatin-based doublet or a tailored treatment based on ERCC1 and TS levels [20]. In the experimental arm, patients receive cisplatin/pemetrexed (low ERCC1, low TS), cisplatin/gemcitabine (low ERCC1, high TS), pemetrexed (high ERCC1, low TS), or a taxane (high ERCC1, high TS). In the Tailored Post Surgical Therapy in Early-Stage NSCLC (TASTE) trial, patients with non-squamous stage II–IIIA NSCLC are randomized to standard cisplatin/pemetrexed chemotherapy or to a customized arm (erlotinib for EGFR mutated patients, cisplatin/pemetrexed in EGFR wild-type/low ERCC1 or observation in EGFR wild-type/high undetermined ERCC1). Finally, in the SWOG 0720 study, patients with resected T2N0M0 or tumors >2 cm are allocated to either adjuvant cisplatin/gemcitabine (low ERCC1 or low RRM1) or observation (high ERCC1 and high RRM1).

Another emerging factor in platinum resistance is BRCA1 (breast cancer gene 1). BRCA1 is a protein that belongs to the mismatch repair pathway, a mechanism of DNA distinct from NER which functions as a differential regulator of chemotherapy-induced apoptosis. In breast cancer cells, the absence of BRCA1 results in high sensitivity to cisplatin; conversely, BRCA1 expression increases sensitivity to antimitotic agents [21]. Based on these findings, Taron et al. [22] used quantitative RT-PCR (qRT-PCR) to measure BRCA1 expression in 55 surgically resected tumors of patients with NSCLC who had received neoadjuvant cisplatin/gemcitabine chemotherapy. In this study, patients with low BRCA1 expression levels had better outcomes than those with high levels. More recently, Rosell et al. [23] studied the prognostic impact of BRCA1 expression in 126 specimens of resected early-stage NSCLC. In this study, patients with overexpression of BRCA1 mRNA levels determined by qRT-PCR had significantly poorer survival. Based on these findings, the Spanish Lung Cancer Group is carrying out a prospective phase III randomized trial to evaluate BRCA1 levels, assessed
by qRT-PCR, as a predictive biomarker to optimize the use of adjuvant chemotherapy in completely resected stage II–III NSCLC patients. In the control arm, patients receive cisplatin/docetaxel; in the customized arm, patients with high BRCA1 expression receive adjuvant docetaxel alone, those with low expression receive cisplatin/gemcitabine, and those with intermediate expression receive cisplatin/docetaxel.

Other genetic markers that confer cisplatin resistance are related to defects in DNA mismatch repair, an important mechanism for maintaining fidelity of genomic DNA. The predictive value of MSH2 assessed by IHC with the use of adjuvant chemotherapy was studied within the IALT-Bio program [24]. In the low-MSH2 group, there was a trend toward improving overall survival with adjuvant chemotherapy when compared with observation (adjusted HR for death, 0.76; 95% CI 0.59–0.97; P = 0.03). In the high-MSH2 group, there was no difference in overall survival between the adjuvant chemotherapy arm and the observation arm.

Another relevant group which may help to identify those patients who will benefit from platinum combinations is the PARP family, a group of enzymes involved in a variety of cellular processes. PARP1 is a chromatin-associated enzyme involved in a number of distinct nuclear functions; its most relevant function is considered to be its role in several DNA repair processes. With low to moderate levels of DNA damage, PARP1 promotes cell cycle arrest and DNA repair. In the presence of extensive DNA damage, PARP1 mediates p53-regulated apoptosis and initiates cell death through necrosis [25, 26]. In the NCIC-BR10 trial, patients with p53 expression derived benefit from adjuvant chemotherapy (HR 0.54; 95% CI 0.32–0.92; P = 0.02), whereas patients without p53 expression did not. In contrast, p53 mutations were not a predictive factor for adjuvant chemotherapy benefit [27]. The predictive value of p53 expression and mutational status was also studied as part of the LACE-Bio program; no significant interaction between p53 expression or p53 mutations and treatment was found [28].

Finally, the double-strand break repair capacity has been implicated in the survival of patients in several types of cancer. However, little is known about the prognostic importance of the key double-strand break repair gene, the ATM in NSCLC patients.

Pierceall et al. [9] report the evaluation of the expression levels of seven proteins (ERCC1, XPF, ATM, P53, PARP1, BRCA1, MSH2) involved in DNA damage response and/or DNA repair pathways in tumor samples from 769 patients with resected stage I–III who had been enrolled in the IALT study. The tumor samples analyzed included adenocarcinoma (ADC) histology (n = 248), squamous cell carcinoma (SCC) (n = 426), and a small percentage of other histological subtypes (n = 95). As mentioned earlier, the same group of investigators previously published the prognostic and predictive value of ERCC1 and MSH2 protein levels assessed by traditional IHC techniques in the samples from the IALT study and showed that the expression of these two markers may impact the benefit of adjuvant cisplatin treatment. They report their findings from the analyses of seven proteins (including ERCC1 and MSH2), using tissue microarray slides and employing a digital-based automated scoring system using the binary Q-score cut-off points that classified tumors into high/low expression. They also present the prognostic and predictive value of these markers for the overall population, as well as for the ADC and SCC subgroups. In patients from the control group (non-treated patients), none of the seven DNA repair biomarkers were found to have a prognostic effect either for overall survival or for disease-free survival in the whole patient population. Furthermore, no statistically significant prognostic impact of these seven markers was observed in the specific analyses carried out in the SCC or the ADC subgroups. When comparing disease-free survival in the chemotherapy group versus control groups according to DNA-repair marker status in the overall population, none of the markers was significantly predictive of the benefit of adjuvant chemotherapy. However, in the SCC subgroup, ATM, P53, PARP1, ERCC1, and MSH2 displayed predictive values, mainly in disease-free survival, with chemotherapy efficacy limited to low marker levels. In the ADC subgroup, however, results were not significant. In the present study, neither BRCA1 nor XPF displayed predictive value in either SCC or ADC patients.

At present, histologic subtype is a key factor when choosing the chemotherapy combination, and also in identifying the need for screening for certain genetic alterations. Interestingly, the results presented by Pierceall et al. [9] suggest that the DNA repair genes determine the clinical benefit of cisplatin only in the SCC subtype. The authors hypothesized in the discussion that one possible explanation for the results may be that DNA repair functionality plays a key role in chemosensitivity in SCC, whereas chemosensitivity in ADC may be less dependent on DNA repair. However, the authors also stated that the absence of significant results in the ADC group may be due to the small number of ADC patients (n = 248) analyzed.

Studies analyzing the influence of key elements of DNA repair pathways in predicting the benefit of cisplatin often use different methods. There are no studies comparing, for example, the value of IHC and qRT-PCR techniques for a given marker. In the present study, the authors use IHC for determining the marker levels and employ image digitalization for the interpretation of the levels. The optimal method for evaluating the levels of the DNA repair pathway markers is yet to be defined. Establishing the best approach to determining marker levels of the DNA repair pathway as well as standardizing measurement techniques should be carried out before the implementation of these markers in the clinical setting.

The identification of EGFR mutations and ALK translocations in NSCLC patients has defined particular populations with different treatment options. Whether a similar approach could be used at some future time for patients receiving cytotoxic chemotherapy remains unclear. At present, results of the analyses dealing with potential markers of chemotherapy benefit are not robust enough to recommend their determination in the clinical practice without further validation in clinical trials.

In summary, in the study published by Pierceall et al. [9], the authors indicate that the levels of some DNA repair genes assessed by IHC using a digital-based automated scoring system determine the clinical benefit of using adjuvant cisplatin only in the SCC subtype, not in the ADC subgroup.
Before clinical implementation of these markers, standardization of methods to determine levels of markers of DNA repair pathway is required, as is a clear definition of what is considered low/high levels. The results presented here are both relevant and hypothesis-generating and may well optimize the treatment choice in patients with SCC histology, who tend to have limited treatment possibilities.

E. Felip* & A. Martinez-Marti

Department of Medical Oncology, Vall d’Hebron University Hospital, Barcelona, Spain  (*E-mail: efelip@vhebron.net)

disclosure

The authors have declared no conflicts of interest.

references