Chemotherapy Treatment Options for Non-small-cell Lung Cancer

a report by

Drs Rafael Rosell,[1] **Miguel Taron**,[1] **Giuseppe Altavilla**[2] *and*
Mariacarmela Santarpia[1,2]

1. Catalan Institute of Oncology, Hospital Germans Trias i Pujol, Spain; 2. Medical Oncology
Department, University of Messina, Italy

∑Dr Rafael Rosell is Scientific Director at the Institut Català d'Oncologia, Hospital Germans Trias i Pujol. He is also Chief of the Medical Oncology Service, Hospital Germans Trias i Pujol, and a professor at the University of Barcelona. He is Past President of the Asociación Española de Investigación sobre el Cáncer (ASEICA), Chairman of the Spanish Lung Cancer Group (SLCG) and Past Editor of *Revista de Oncología*, the official publication of five Spanish societies of oncology (1998 to 2001). He is on the editorial board of *Lung Cancer* and *Annals of Oncology* and *Journal of Clinical Oncology*.

Dr Miguel Taron is Head of the Cancer Molecular Biology Laboratory and Coordinator of the Oncohematology Research Laboratory ICO-Hospital Germans Trias i Pujol. Badalona. From 2002 to 2004 he was Head of the Laboratory Cancer Molecular Biology Laboratory Hospital Germans Trias i Pujol Badalona. He is a member of the International Association for the Study of Lung Cancer (IASLC) and is on the Scientific Committee for the 2005 World Conference on Lung Cancer (IASLC), to be held in Barcelona, Spain.

Dr Giuseppe Altavilla is Associate Professor of Medical Oncology, University of Messina. He is also Director of Medical Oncology and Innovative Therapies Unit, University Hospital, Policlinico G Martino Messina. He is a member of the American Society of Clinical Oncology (ASCO) and Coordinator of the Sicily Section Associazione Italiana Oncologia Medica (AIOM). Dr Altavilla is the author of more than 100 papers published in peer-reviewed medical and scientific journals.

Dr Mariacarmela Santarpia works in the department of Clinical Oncology and Innovative Therapy at the University of Messina School of Medicine, specialising in clinical oncology. Since 2004, she has also been working in the Laboratory of Molecular Biology of the Catalan Institute of Oncology (ICO) Hospital Germans Trias i Pujol Badalona.

Cisplatin-based chemotherapy trials demonstrate a benefit for cisplatin over supportive care alone with a hazard ratio (HR) of 0.73, which translates to a 27% reduction in the risk of death and a 10% improvement in survival at one year.[1] Between 1993 and 1999, 1,436 patients with stage IV or IIIB non-small-cell lung cancer (NSCLC) with effusion were treated with platinum-based doublets (involving either paclitaxel, docetaxel, vinorelbine or gemcitabine). The response rates and median survival times were 20% and 8.2 months. One- and two-year survivals were 33% and 11%, respectively.[2–4] In a multivariate analysis, lower performance status (PS) – PS 1 versus 0 – was identified as one prognostic factor.[2] The salient finding of the Eastern Cooperative Oncology Group (ECOG) trial[4] was that no survival differences were observed between any of the different platinum-based doublets, with a modest benefit for chemotherapy in NSCLC. However, reality shows that survival can vary significantly between individual patients with some surviving years and others succumbing to their disease within a few months.[4] Patients with lower DNA repair capacity are more chemosensitive than those who carry a proficient DNA repair system.[5–7] More recent results with docetaxel 75mg/m^2 and cisplatin 75mg/m^2 yielded a response rate of 31%, with a median survival of 11 months.[8] A meta-analysis of randomised clinical trials comparing cisplatin with carboplatin disclosed that cisplatin doublets yielded 11% longer survival than carboplatin doublets (HR=1.1; p=0.03).[9] The European Organization for Research and Treatment of Cancer (EORTC) compared cisplatin plus teniposide versus cisplatin plus paclitaxel; response rates were significantly in favour of the paclitaxel combination although there were no differences in median survival.[10] Based on this study, the EORTC compared paclitaxel plus cisplatin versus paclitaxel plus gemcitabine versus gemcitabine plus cisplatin. Although there were no differences in outcomes, there was a tendency towards lower survival in the non-cisplatin arm.[11] A Greek study compared docetaxel plus cisplatin versus docetaxel plus gemcitabine; both arms were supported with the use of recombinant human granulocyte colony-stimulating factor, because the dose of docetaxel was 100mg/m^2. No differences in response or survival were observed.[12] Recent studies of gemcitabine plus docetaxel versus vinorelbine plus cisplatin also show similar outcomes for the two regimens in terms of response and median survival.[13,14] Along the same lines, a new meta-analysis has found that there are no differences in survival between platinum regimens and third-generation-based non-platinum regimens. One-year survival was 36% for platinum regimens and 35% for non-platinum regimens.[15] At the 2005 ASCO meeting, a phase III trial comparing gemcitabine plus carboplatin versus gemcitabine plus paclitaxel versus paclitaxel plus carboplatin showed similar outcomes for the three regimens, with an overall time to progression of five months, a median survival of eight months, a one-year survival of 33% and a two-year survival of 10%.[16]

Customised Chemotherapy Based on Expression of DNA Repair Genes

In 1995, it was shown that elevated DNA repair capacity is associated with drug resistance in lung cancer cell lines, and it was suggested that modulation of DNA repair mechanisms, such as the incorporation of specific DNA repair inhibitors in therapeutic regimens, could help to improve therapeutic strategies. The overall DNA repair capacity was estimated by the ability of cells to reactivate a plasmid damaged by cisplatin (host cell reactivation assay).[17] Cytotoxicity from cisplatin and other platinum-containing drugs results from the formation of platinum DNA adducts, and clinical outcome is better in patients with higher levels of these adducts, indicating that these patients have lower DNA repair capacity. Nucleotide excision repair (NER) is the major mechanism for repairing platinum DNA adducts,[5–7] involving the co-ordinated activity of more than 20 enzymes that remove a segment of DNA containing a bulky adduct, and then restore that segment by replicating the intact complementary strand. The predictive potential of cisplatin adducts in buccal cells was demonstrated in NSCLC patients receiving daily

combined treatment with concomitant cisplatin and radiotherapy. Patients who had higher adduct levels by immunocytochemistry had a median survival of 30 months, in comparison with only five months for patients with low adduct levels.[18] It was hypothesised that patients with genetically determined effective DNA repair activity would be more likely to effectively repair DNA adducts in tumour tissue than patients with genetically determined suboptimal DNA repair. To test this hypothesis, a functional assay of DNA repair capacity – the ability to repair benzo[a]pyrene diol epoxide (BPDE)-induced DNA adducts – in peripheral lymphocytes, rather than in tumours, was used. Median survival was 8.9 months for patients whose DNA repair capacity was in the top quartile, compared with 15.8 months for patients whose DNA repair capacity was in the bottom quartile (p=0.04).[19]

Cisplatin resistance is associated with increased expression of the excision repair cross–complementing 1 (ERCC1) gene.[20] Cancer tissues from ovarian cancer patients whose tumours were clinically resistant to therapy showed greater levels of ERCC1 messenger RNA (mRNA).[21] complementary DNA (cDNA) derived from primary gastric tumours before chemotherapy was used to determine ERCC1 mRNA levels expressed as the ratio of the polymerase chain reaction (PCR) product of the ERCC1 gene and the β-actin gene. Response and survival with cisplatin plus fluorouracil were significantly associated with levels of ERCC1 ≤5.8. More modern studies have used cDNA derived from paraffin-embedded tumour specimens to determine ERCC1 mRNA expression relative to the internal reference gene β-actin, using fluorescence-based, realtime reverse transcriptase PCR. Colorectal cancer patients treated with oxaliplatin plus fluorouracil with low ERCC1 expression had a significantly longer survival compared with patients with high intratumoural ERCC1 mRNA levels.[23] The authors carried out a study to examine the role of ERCC1 mRNA levels in advanced NSCLC patients treated with gemcitabine plus cisplatin. Patients with low ERCC1 mRNA levels attained a response rate of 52%, while in those with high levels, the response rate was 36%. This difference was not significant; however, when a cut-off of 5.8 was used for ERCC1 expression, median survival was 15 months for patients with low levels and only five months for those with high levels (p<0.001).[24]

Based on these findings, an ERCC1 mRNA customised chemotherapy trial was carried out. More than 400 patients have been included and randomised to the control or the experimental arm. The control arm received docetaxel plus cisplatin, and patients in the experimental arm received either

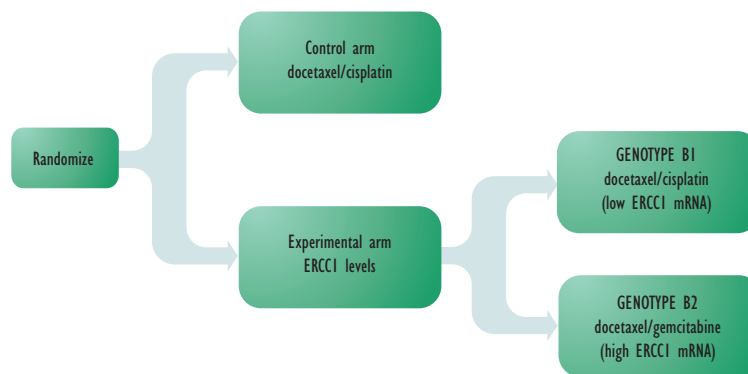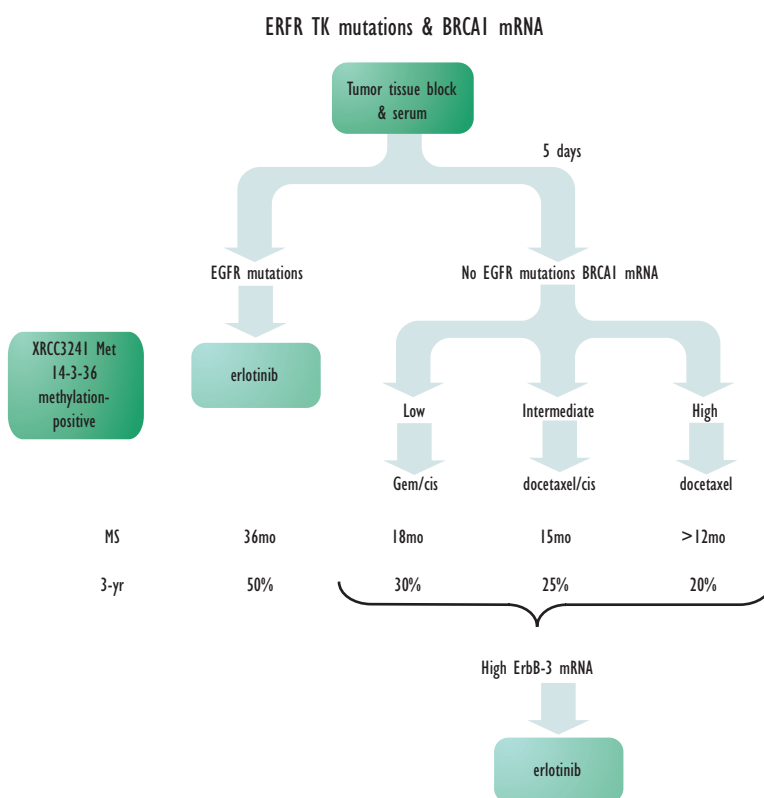Figure 1: ERCC1-Customised Chemotherapy in Advanced NSCLC

Figure 2: Adenocarcinoma

the same combination of docetaxel plus cisplatin if their ERCC1 mRNA levels were low, or docetaxel plus gemcitabine if their levels were high (see *Figure 1*). The preliminary results on 264 patients, presented at ASCO 2005,[25] showed that the response rate for patients with low ERCC1 levels was 56.6% while for patients in the control arm it was 40.4% (p=0.02). When patients in the control arm were split according to the ERCC1 levels, those with low levels had a response rate of 47.3%, while those with high levels had a response rate of 26.1%. The logistic regression model for tumour progression indicated a significant improvement for patients randomised to docetaxel plus cisplatin based on low ERCC1 levels. Although the results are still preliminary, time to progression and survival adjusted for age are significantly in favour of the group with low ERCC1 levels.

BRCA1 Levels and Cisplatin Resistance

BRCA1 was overexpressed in the cisplatin–resistant breast cancer cell line MCF-726. BRCA1 is a component of multiple DNA repair pathways and functions as a molecular determinant of response to a range of cytotoxic chemotherapeutic agents. It has been demonstrated that BRCA1 abrogates the apoptotic phenotype induced by a range of DNA-damaging agents, including cisplatin, etoposide and bleomycin, and induces dramatic responses to a range of antimicrotubule agents, including paclitaxel and vinorelbine. These landmark findings indicate that BRCA1 functions as a differential regulator of chemotherapy-induced apoptosis.[27–29] Sporadic cancers, such as breast, ovarian and NSCLC, can have the BRCA1 function abrogated by methylation or other mechanisms. In addition, methylation of FANCF has been observed in these tumours. These characteristics, known as 'BRCAness', increase sensitivity to cisplatin and related DNA cross-linking agents and may increase resistance to antimicrotubule drugs.[30] Recently, it has been shown that BRCA1 or BRCA1 dysfunction profoundly sensitises cells to the inhibition of poly(ADP-ribose) polymerase (PARP).[31] It has been observed that BRCA1 mRNA expression closely correlates with ERCC1 mRNA expression, and that BRCA1 mRNA expression predicts outcome in locally advanced NSCLC patients treated with neoadjuvant gemcitabine plus cisplatin followed by surgery. Median survival has not been reached in patients with the lowest BRCA1 mRNA levels, while survival was very poor in patients with the highest levels.[32] These findings are along the same lines as pre-clinical data, indicating that patients with high BRCA1 levels could respond more favourably, not to non–cisplatin regimens but to antimicrotubule drugs. Although gemcitabine is a neutral drug for the BRCA1 mRNA effect,[27] it has been observed that elevation of ERCC1 and BRCA1 is closely related to high levels of ribonucleotide reductase, which is one of the principal mechanisms of resistance to gemcitabine.[33]

Maintenance Chemotherapy

To date, there has been no clear evidence that maintenance chemotherapy after partial remission or stabilisation can prolong survival[34–36] although the Belani et al. study[35] suggests that paclitaxel maintenance may have some effect on survival. Intriguingly, the Southwest Oncology Group (SWOG) trial S9504 in stage IIIB showed that the consolidation of three cycles of docetaxel after completion of chemoradiotherapy resulted in an impressive median survival of 26 months.[37,38]

Interestingly, a large study in stage IV disease is being carried out by Schiller et al.,[39] where more than 400 patients have received four cycles of gemcitabine plus carboplatin; patients with response or stable disease are then randomised to receive maintenance docetaxel or docetaxel at the time of progression. This study will shed light on the role of tumour DNA repair. It can be speculated that patients with low DNA repair capacity (low ERCC1 or BRCA1 mRNA levels) will obtain better response from gemcitabine plus carboplatin, while they could be potentially resistant to up-front docetaxel. However, these patients could derive the maximum benefit from maintenance docetaxel. It has been observed that cisplatin induces ERCC1 mRNA levels both in pre-clinical models[40] and in the clinical setting.[41,42] When pre- and post-treatment ERCC1 mRNA levels are looked at, a great variation is observed because cisplatin and other chemotherapeutic agents can induce ERCC1 mRNA expression, which may be a major reason for short-lived response to platinum doublets. This elevation of ERCC1 or associated DNA repair genes can be used to advantage in the later administration of antimicrotubule agents like docetaxel. This hypothesis merits being tested in translational research studies.

EGFR Mutations and DNA Repair Capacity

Recently, EGFR mutations have been found to occur at a significantly higher frequency in hereditary breast cancer than in sporadic breast cancer.[43] As the authors state, this finding may not be surprising given the functional effect of BRCA1 and BRCA2 mutations. Defects in BRCA1 and BRCA2 have been shown to disrupt the DNA repair mechanism, which leads to genomic instability. Along with others, it has been observed that EGFR mutations are highly predictive of response and are a prognostic marker for survival in lung adenocarcinoma patients.[44–48] Dramatic responses have been observed, including brain metastases, with only treatment with tyrosine kinase inhibitors, without the need for radiotherapy or other therapeutic interventions. So far, the data has been collected retrospectively on gefitinib-treated patients after second- or third-line chemotherapy failure. In the authors' experience, the preliminary evidence of EGFR mutations in lung adenocarcinoma patients in Spain[47] indicates a frequency of 20%. The authors have undertaken a prospective study of EGFR mutation assessment in advanced lung adenocarcinomas. The EGFR assay is performed rapidly with an average wait of five-days for results. Patients without EGFR mutations can be examined to determine their BRCA1 mRNA levels for customised chemotherapy (see *Figure 2*). ■

References

1. 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: pp. 899–909.

2. Hoang T, Xu Ronghui, Schiller J H, et al., "Clinical model to predict survival in chemonaive patients with advanced non-small-cell lung cancer treated with third-generation chemotherapy regimens based on Eastern Cooperative Oncology Group data", J. Clin. Oncol. (2005);23: pp. 175–183.

3. Kelly K, Crowley J, Bunn P, et al., "Randomized Phase III trial of paclitaxel plus carboplatin versus vinorelbine plus cisplatin in the treatment of patients with advanced non-small-cell lung cancer: A Southwest Oncology Group trial", J. Clin. Oncol. (2001);19: pp. 3,210–3,218.

4. Schiller J, Harrington D, Belani C, et al., "Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer", N. Engl. J. Med. (2002);346: pp. 92–98.

5. Rosell R, Taron M, Camps C, et al., "Influence of genetic markers on survival in non-small cell lung cancer", Drugs of Today (2003);39: pp. 775–786.

6. Rosell R, Taron M, Barnadas A, et al., "Nucleotide excision repair pathways involved in cisplatin resistance in non-small-cell lung cancer", Cancer Control (2003);10: pp. 297–305.

7. Rosell R, Taron M, Alberola V, et al., "Genetic testing for chemotherapy in non-small cell lung cancer", Lung Cancer (2003);41: pp. S97–S102.

8. Fossella F, Pereira J, von Pawel J, et al., "Randomized, multinational, Phase III study of docetaxel plus platinum combinations versus vinorelbine plus cisplatin for advanced non-small-cell lung cancer: The TAX 326 study group", J. Clin. Oncol. (2003);21: pp. 3,016–3,024.

9. Hotta K, Matsuo K, Ueoka H, et al., "Meta-analysis of randomized clinical trials comparing cisplatin to carboplatin in patients with advanced non-small-cell lung cancer", J. Clin. Oncol. (2004);22: pp. 3,852–3,859.

10. Giaccone G, Splinter T, Debruyne C, et al., "Randomized study of paclitaxel-cisplatin versus cisplatin-teniposide in patients with advanced non-small-cell lung cancer", J. Clin. Oncol. (1998);16: pp. 2,133–2,141.

11. Smit E F, van Meerbeeck J, Lianes P, et al., "Three-arm randomized study of two cisplatin-based regimens and paclitaxel plus gemcitabine in advanced non-small-cell lung cancer: A phase III trial of the European Organization for Research and Treatment of Cancer Lung Cancer Group- EORTC 08975", J. Clin. Oncol. (2003);21: pp. 3,909–3,917.

12. Georgoulias V, Papadakis E, Alexopoulos, et al., "Platinum-based and non-platinum-based chemotherapy in advanced non-small-cell lung cancer: a randomized multicentrre trial", Lancet (2001);357: pp. 1,478–1,483.

13. Georgoulias V, Ardavanis A, Tsiafaki X, et al., "Vinorelbine plus cisplatin versus docetaxel plus gemcitabine in advanced non-small-cell lung cancer: A phase III randomized trial", J. Clin. Oncol. (2005);23: pp. 2,937–2,945.

14. Pujol J L, Breton J L, Gervais R, et al., "Gemcitabine-docetaxel versus cisplatin-vinorelbine in advanced or metastatic non-small-cell lung cancer: a phase III study addressing the case for cisplatin", Ann. Oncol. (2005);16: pp. 602–610.

15. D'Addario G, Pintilie M, Leighl N, et al., "Platinum-based versus non-platinum-based chemotherapy in advanced non-small-cell lung cancer: A meta-analysis of the published literature", J. Clin. Oncol. (2005);23: pp. 2,926–2,936.

16. Treat J, Belani C, Edelman M, et al., "A randomized phase III trial of gemcitabine (G) in combination with carboplatin (C) or paclitaxel (P) versus paclitaxel plus carboplatin in advanced (Stage IIIB, IV) non-small-cell lung cancer (NSCLC): Update of the Alpha Oncology trial", J. Clin. Oncol. (2005);ASCO Annual Meeting Proceedings; (abstr LBA7025): 627s.

17. Zeng-Rong N, Paterson J, Alpert L, et al., "Elevated DNA repair capacity is associated with intrinsic resistance of lung cancer to chemotherapy", Cancer Res. (1995);55: pp. 4,760–4,764.

18. Van de Vaart P J M, Belderbos J, De Jong D, et al., "DNA-adduct levels as a predictor of outcome for NSCLC patients receiving daily cisplatin and radiotherapy", Int. J. Cancer (2000);89: pp. 160–166.

19. Bosken C, Wei Q, Amos C, et al., "An analysis of DNA repair as a determinant of survival in patients with non-small-cell lung cancer", J. Natl Cancer Inst. (2002);94: pp. 1,091–1,099.

20. Ferry K V, Hamilton T C and Johnson S W, "Increased nucleotide excision repair in cisplatin resistant ovarian cancer cells", Biochem. Pharmacol. (2000);60: pp. 1,305–1,313.

21. Dabholkar M, Vionnet J, Bostick-Bruton F, et al., "Messenger RNA levels of XPAC and ERCC1 in ovarian cancer tissue correlate with response to platinum-based chemotherapy", J. Clin. Invest. (1994);94: pp. 703–708.

22. Metzger R, Leichman C G, Danenberg K, et al., "ERCC1 mRNA levels complement thymidylate synthase mRNA levels in predicting response and survival for gastric cancer patients receiving combination cisplatin and fluorouracil chemotherapy", J. Clin. Oncol. (1998);16: pp. 309–316.

23. Shirota Y, Stoehlmacher J, Brabender J, et al., "ERCC1 and thymidylate synthase mRNA levels predict survival for colorectal cancer patients receiving combination oxaliplatin and fluorouracil chemotherapy", J. Clin. Oncol. (2001);19: pp. 4,298–4,304.

24. Lord R V N, Brabender J, Gandara D, et al., "Low ERCC1 expression correlates with prolonged survival after cisplatin plus gemcitabine chemotherapy in non-small-cell lung cancer", Clin. Cancer Res. (2002);8: pp. 2,286–2,291.

25. Rosell R, Cobo M, Isla D, et al., "ERCC1 mRNA-based randomized phase III trial of docetaxel (doc) doublets with

cisplatin (cis) or gemcitabine (gem) in stage IV non-small-cell lung cancer (NSCLC) patients (p)", J. Clin. Oncol. *(2005), ASCO Annual Meeting Proceedings (abstr 7002): 621s.*

26. *Husain A, He G, Venkatraman E S, et al., "BRCA1 up-regulation is associated with repair-mediated resistance to cis-diamminedichloroplatinum (II)"*, Cancer Res. *(1998);58: pp. 1,120–1,123.*

27. *Quinn J E, Kennedy R D, Mullan P B, et al., "BRCA1 functions as a differential modulator of chemotherapy-induced apoptosis"*, Cancer Res. *(2003);63: pp. 6,221–6,228.*

28. *Tassone P, Tagliaferri P, Perricelli A, et al., "BRCA1 expression modulates chemosensitivity of BRCA1-defective HCC1937 human breast cancer cells"*, BJC *(2003);88: pp. 1,285–1,291.*

29. *Fedier A, Steiner R A, Schwarz V A, et al., "The effect of loss of Brca1 on the sensitivity to anticancer agents in p53-deficient cells"*, Int. J. Oncol. *(2003);22: pp. 1,169–1,173.*

30. *Turner N, Tutt A and Ashworth A, "Hallmarks of "BRCAness" in sporadic cancers"*, Nat. Rev. Cancer *(2004);4: pp. 1–6.*

31. *Farmer H, McCabe N, Lord C J, et al., "Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy"*, Nature *(2005);434: pp. 917–921.*

32. *Taron M, Rosell R, Felip E, et al., "BRCA mRNA expression levels as an indicator of chemoresistance in lung cancer"*, Hum. Mol. Genet. *(2004);13: pp. 2,443–2,449.*

33. *Rosell R, Danenberg K D, Alberola V, et al., "Ribonucleotide reductase messenger RNA expression and survival in gemcitabine/cisplatin-treated advanced non-small-cell lung cancer patients"*, Clin. Cancer Res. *(2004);10: pp. 1,318–1,325.*

34. *Smith I E, O'Brien M, Talbot D E, et al., "Duration of chemotherapy in advanced non-small-cell lung cancer: a randomized trial of three versus six courses of mitomycin, vinblastine and cisplatin"*, J. Clin. Oncol. *(2001);19: pp. 1,336–1,343.*

35. *Belani C P, Barstis J, Perry M C, et al., "Multicenter, randomized trial for stage IIIB or IV non-small-cell lung cancer using weekly paclitaxel and carboplatin followed by maintenance weekly paclitaxel or observation"*, J. Clin. Oncol. *(2003); 21: pp. 2,933–2,939.*

36. *Westeel V, Quoix E, Moro-Sibilot D, et al., "Randomized study of maintenance vinorelbine in responders with advanced non-small-cell lung cancer"*, J. Natl. Cancer Inst. *(2005);97: pp. 499–506.*

37. *Gandara D R, Chansky K, Albain K, et al., "Consolidation docetaxel after concurrent chemoradiotherapy in stage IIIB non-small-cell lung cancer: Phase II Southwest Oncology Group Study S9504"*, J. Clin. Oncol. *(2003);21: pp. 2,004–2,010.*

38. *Gandara D R, Chansky K, Gaspar L E, Albain K S, Lara P N, Crowley J, "Long term survival in stage IIIb non-small-cell lung cancer (NSCLC) treated with consolidation docetaxel following concurrent chemoradiotherapy (SWOG S9504)"*, J. Clin. Oncol. *(2005), ASCO Annual Meeting Proceedings (abstr 7059): 635s.*

39. *Schiller J H, Fidias P, Dakhil S R, et al., "A phase III study of induction therapy with gemcitabine + carboplatin (GC) followed by either delayed vs. immediate second-line therapy with docetaxel (D) in advanced non-small-cell lung cancer (NSCLC): a preliminary report of induction gemcitabine + carboplatin"*, J. Clin. Oncol. *(2005); ASCO Annual Meeting Proceedings (abstr 7142): 655s.*

40. *Li Q, Gardner K, Zhang L, et al., "Cisplatin induction of ERCC1 mRNA expression in A2780/CP70 human ovarian cancer cells"*, J. Biol. Chem. *(1998);273: pp. 23,419–23,425.*

41. *Schneider S, Uchida K, Brabender J, et al., "Downregulation of TS, DPD, ERCC1, GST-Pi, EGFR, and HER2 gne expression after neoadjuvant three-modality treatment in patients with esophageal cancer"*, J. Am. Coll. Surg. *(2005);200: pp. 336–344.*

42. *Rosell R, Felip E, Taron M, et al., "Gene expression as a predictive marker of outcome in stage IIIB-IIIA-IIIB non-small-cell lung cancer after induction gemcitabine-based chemotherapy followed by resectional surgery"*, Clin. Cancer Res. *(2004); (suppl); 10: pp. 4,215s–4,219s.*

43. *Weber F, Fukino K, Sawada T, et al., "Variability in organ-specific EGFR mutational spectra in tumour epithelium and stroma may be the biological basis for differential responses to tyrosine kinase inhibitors"*, BJC *(2005);92: pp. 1,992–1,926.*

44. *Tokumo M, Toyooka S, Kiura K, et al., "The relationship between epidermal growth factor receptor mutations and clinicopathologic features in non-small-cell lung cancers"*, Clin. Cancer Res. *(2005);11: pp. 1,167–1,173.*

45. *Mitsudomi T, Kosaka T, Endoh H, et al., "Mutations of the epidermal growth factor receptor gene predict prolonged survival after gefitinib treatment in patients with non-small-cell lung cancer with postoperative recurrence"*, J. Clin. Oncol. *(2005); 23: pp. 2,513–2,520.*

46. *Chou T Y, Chiu C-H, Li L-H, et al., "Mutation in the tyrosine kinase domain of epidermal growth factor receptor is a predictive and prognostic factor for gefitinib treatment in patients with non-small-cell lung cancer"*, Clin. Cancer Res. *(2005);11: pp. 3,750–3,757.*

47. *Taron M, Ichinose Y, Rosell R, et al., "Activating mutations in the tyrosine kinase domain of the epidermal growth factor receptor are associated with improved survival in gefitinib-treated chemorefractory lung adenocarcinomas"*, Clin. Cancer Res. *(2005);(in press).*

48. *Cortes-Funes H, Gomez C, Rosell R, et al., "Epidermal growth factor receptor activating mutations in Spanish gefitinib-treated non-small-cell lung cancer patients"*, Ann. Oncol. *(2005);(advance access published April 25), 16: pp. 1081–1,086.*

5