Associations between TS, TTF-1, FR-α, FPGS, and Overall Survival in Patients with Advanced Non–Small-Cell Lung Cancer Receiving Pemetrexed Plus Carboplatin or Gemcitabine Plus Carboplatin as First-Line Chemotherapy

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Introduction: Pemetrexed is effective in the treatment of non–small-cell lung cancer, mainly in nonsquamous cell carcinomas. Inhibition of thymidylate synthase (TS) is considered the key mechanism of action. Folate receptor-α facilitates uptake of pemetrexed. Polyglutamation by folylpolyglutamate synthetase enhances activity and prolongs cellular retention of pemetrexed. Thyroid transcription factor-1 (TTF-1) is mainly positive in nonsquamous cell carcinoma and has been proposed as a marker for sensitivity to pemetrexed. The aim was to investigate associations between these biomarkers and survival in patients who participated in a phase III trial comparing pemetrexed plus carboplatin with gemcitabine plus carboplatin as first-line chemotherapy in advanced non–small-cell lung cancer (n = 436). In this study, there was no difference in overall survival between the two regimens.

Methods: Formalin-fixed, paraffin-embedded biopsies were collected. Percentages of tumor cells positive and highly positive for the biomarkers were assessed using immunohistochemistry (IHC) and an IHC score was calculated (range, 0–200).

Results: Two hundred thirty-six biopsies were analyzed (pemetrexed plus carboplatin: n = 114, gemcitabine plus carboplatin: n = 122). There was a significant difference in overall survival between those with TTF-1–positive and –negative tumors (10.4 versus 6.0 months; $p < 0.001$) and those with a low and a high TS IHC score (9.7 versus 6.2 months; $p < 0.001$). Folate receptor-α and folylpolyglutamate synthetase were not significant prognostic factors. In multivariate analyses adjusting for established prognostic characteristics, TS ($p = 0.002$) and TTF-1 ($p = 0.003$) remained significant. There were no differences in survival between the treatment arms depending on biomarker scores.

Conclusions: TTF-1 positivity and low TS level were associated with prolonged survival. The associations between the biomarkers and overall survival were similar for both chemotherapy regimens.

Key Words: Non-small-cell lung cancer, Biomarkers, Pemetrexed, Gemcitabine, Survival, Thyroid transcription factor-1, Thymidylate synthase, Folylpolyglutamate synthetase, Folate receptor.

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Platinum-doublet chemotherapy remains the standard treatment for most patients with advanced non–small-cell lung cancer (NSCLC).[1] However, the survival benefit is limited, and many experience severe side effects.[2] Identifying biomarkers that are associated with outcomes of specific regimens would help improve efficacy and avoid ineffective, potentially harmful therapy.

Pemetrexed is a multitargeted antifolate that inhibits three enzymes in the folate pathway involved in nucleotide synthesis; thymidylate synthase (TS), dihydrofolate reductase, and glycinamide ribonucleotide formyl transferase. Studies have demonstrated that pemetrexed is effective as first-line,[3,4] second-line,[5] and maintenance therapy of NSCLC.[6,7] Subgroup analyses have revealed that the agent is mainly effective and superior to other regimens in nonsquamous cell carcinomas (non-SCCs).[6,8]

Inhibition of TS is thought to be the main mechanism of action.[9] In cell lines from colon and lung cancer, resistance to pemetrexed was associated with TS overexpression.[10–13] In patients with malignant mesothelioma,[14,15] breast cancer,[16] and NSCLC,[17–20] low TS levels were associated with a better response to pemetrexed. Others have found that SCCs have higher TS levels than non-SCCs and that TS levels in small-cell lung cancer are higher than in NSCLC.[21–23] It has been

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hypothesized that this explains why non-SCCs are more sensitive to pemetrexed than SCCs,[21,22] and why most small-cell lung cancers are resistant to pemetrexed.[24,25]

Folate receptor-α (FR-α) mediates cellular uptake of folate essential for synthesis of RNA and DNA, may facilitate transport of pemetrexed into cells, and has been proposed as a biomarker for antifolate therapy.[26,27] Folylpolyglutamate synthetase (FPGS) activates pemetrexed via polyglutamation and prolongs its cellular retention. Low levels of FPGS may thereby decrease the antitumor activity. A study of leukemia cells suggested that low FPGS level was associated with resistance to pemetrexed.[28]

Thyroid transcription factor-1 (TTF-1) is mainly expressed in non-SCC and is an important marker for subclassification of NSCLC when no clear morphologic features can be found.[29] In one study, TTF-1 positive tumors had higher response rates to pemetrexed than TTF-1 negative,[18] and it has been proposed that the biomarker can explain why pemetrexed is mainly active in non-SCC.

Gemcitabine is one of the standard therapies of NSCLC.[30] It is a pyrimidine-analogue that inhibits DNA synthesis by inducing depletion of cellular deoxynucleotides and through incorporation into DNA.[31]

Our study group conducted a phase III trial comparing pemetrexed plus carboplatin (PC) with gemcitabine plus carboplatin (GC) as first-line chemotherapy in advanced NSCLC.[3] The aim of this study was to investigate associations between TTF-1, TS, FR-α, or FPGS and overall survival in participants of this trial, and to determine whether there were different associations between these biomarkers and overall survival between the two treatment arms.

MATERIALS AND METHODS

The main eligibility criteria for the phase III trial were stage IIIB (ineligible for curative radiotherapy) or stage IV NSCLC; no previous chemotherapy; age ≥18 years; and World Health Organization performance status (PS) 0 to 2. Patients received up to four cycles of carboplatin area under the curve = 5 (Calvert's formula) plus pemetrexed 500 mg/m^2 day 1 (PC) or carboplatin area under the curve = 5 day 1 plus gemcitabine 1000 mg/m^2 days 1 and 8 (GC) every 3 weeks. Four hundred thirty-six eligible patients were enrolled from May 2005 until July 2006 at 35 hospitals in Norway. The survival analyses were finalized in July 2007 after a median observation time of 19 months. The main conclusions were that there were no differences in health-related quality of life or overall survival between the arms. More hematological toxicity was observed on the gemcitabine arm.[3]

Patients were included in the present study if we were able to collect formalin-fixed, paraffin-embedded tumor tissue for immunohistochemicial analyses.

Design and Approval

This retrospective biomarker study was approved by the Regional Committee for Medical Research Ethics, Central Norway; the Norwegian Social Science Data Services; and the Norwegian Directorate for Health and Social Affairs.

Immunohistochemical Assays

Tissue micro arrays were built of one to three cores (1-mm diameter) from the tumor samples when possible. Otherwise, sections were cut from the whole remaining tissue blocks. Sections, cut at 4 µm, were positioned on Superfrost Plus slides (Menzel-Glaser, Braunschweig, Germany) and

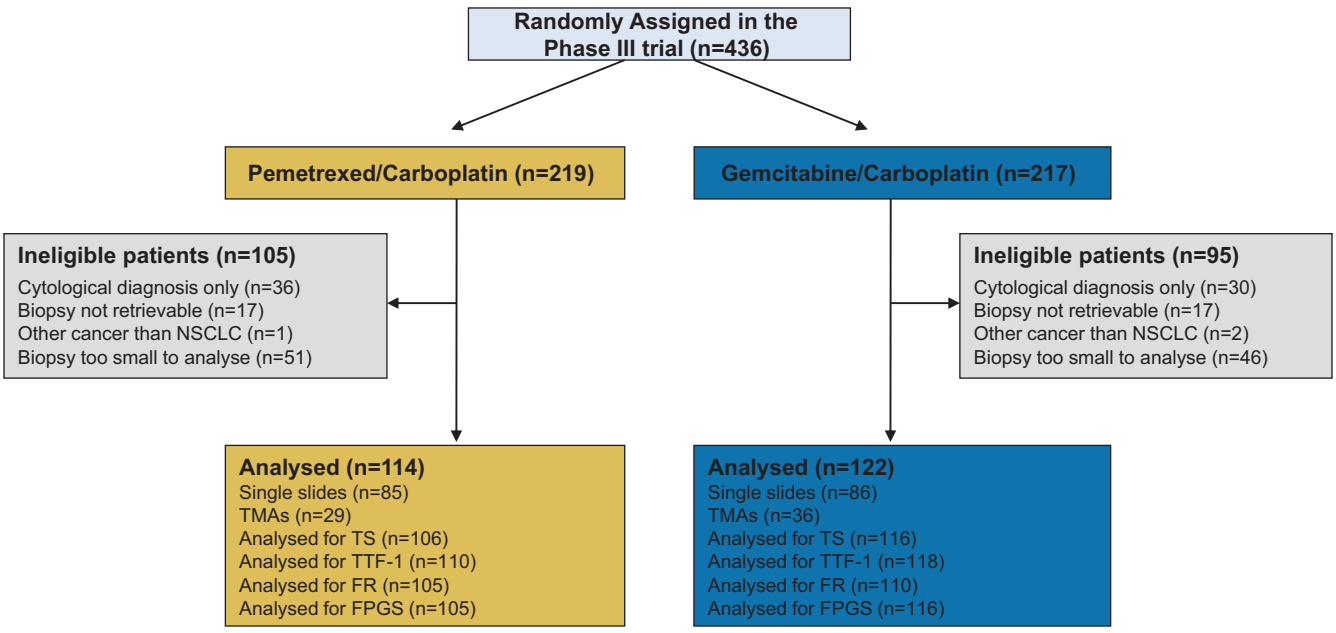

FIGURE 1. Patient selection for the biomarker study. TS, thymidylate synthase; TTF-1, thyroid transcription factor-1; FR, folate receptor; FPGS, folylpolyglutamate synthetase; NSCLC, non–small-cell lung cancer; TMAs, tissue micro arrays.