Negative NKX2-1 (TTF-1) as Temporary Surrogate Marker for Treatment Selection During *EGFR*-Mutation Analysis in Patients with Non–Small-Cell Lung Cancer

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Introduction: In the past decade, major progress has been made toward personalized medical treatment of non–small-cell lung cancer (NSCLC) through the discovery of epithelial growth factor receptor (*EGFR*) mutations. However, mutation analysis takes extra time and additional costs in the diagnostic evaluation of lung cancer patients. It has been hypothesized that *EGFR* mutations are restricted to terminal respiratory unit -type adenocarcinoma expressing thyroid transcription factor-1 (official symbol NKX2-1) as determined by immunohistochemistry. The aim of the current study is to evaluate the potential of NKX2-1 immunohistochemistry as a prescreening test for *EGFR* mutation analysis.

Methods: From 2004 to December 2010, 810 consecutive NSCLC tumor specimens were tested for *EGFR* mutations in a routine diagnostic procedure. Immunohistochemistry for NKX2-1 was performed (clone 8G7G3/1 [Dako]) and the results were compared with tumor *EGFR*-mutation status and clinicopathological characteristics.

Results: *EGFR* mutations were detected in 114 specimens (14%). NKX2-1 expression was present in 68%. In the cases with *EGFR* mutation, NKX2-1 staining was positive in 92%. NKX2-1 immunohistochemical (IHC) staining was significantly associated with the presence of *EGFR* mutations ($p = 5.3 \times 10^{-10}$). NKX2-1 increased the negative predictive value in NSCLC to more than 95%.

Conclusions: In case of a negative NKX2-1 IHC staining, and only if clinically urgent, the high negative predictive value of more than 95% for *EGFR* mutations is a suitable temporary surrogate marker for the choice of starting with chemotherapy. In case of positive NKX2-1 IHC, the best strategy is to wait for the outcome of *EGFR*-mutation analysis and then choose the appropriate treatment.

Key Words: TTF-1, NKX2-1, Lung cancer, *EGFR*-mutation analysis, Treatment.

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In the past decade, major progress has been made toward personalized treatment of non–small-cell lung cancer (NSCLC). The first breakthrough was the discovery of epithelial growth factor receptor (*EGFR*) mutations. The second major improvement was the discovery of tumor reaction on anaplastic lymphoma kinase (ALK) inhibitors in patients with *ALK* rearrangements.[1]

The outcome of *EGFR*-mutation analysis has a predictive value for the treatment with *EGFR-TKI*s. The tumors with activating *EGFR* mutations react better and those without *EGFR* mutation react worse on *EGFR TKI* than conventional chemotherapy.[2–5] Mutation analysis takes time, varying in daily practice from a few days to weeks, partly depending on access to a reliable test in the direct environment. Postponing a decision on the choice of treatment often collides with clinical urgency. A fast prescreening test to select for cases to undergo mutation testing might solve the postponement dilemma. The clinical factors associated with the presence of *EGFR* mutations are: nonsmoking status, female sex, and adenocarcinoma subtype. If one or more of these clinical parameters is present, the chance of having an *EGFR* mutation is higher, but lacking these characteristics does not exclude the presence of an *EGFR* mutation. On the basis of the separate clinical criterion mentioned above, respectively 33%, 30%, and 19% of tumors with *EGFR* mutations would be missed in a white population.[6] Currently, the acceptable percentage of *EGFR* mutations that can be missed in the treatment of lung cancer is a matter of debate. If it is acceptable to miss 1% of tumors with *EGFR* mutation, then these parameters are clearly insufficient for the selection of patients with *EGFR* mutation.

EGFR-mutation specific antibodies have been developed against a 15 base pair deletion in exon 19 and a L858R point mutation in exon 21. Currently, they are not recommended for predictive testing, as the sensitivity is not high enough, taking the other relevant mutations into account, especially exon 19 deletions of other base pair lengths.[7–13]

Yatabe et al.[14] suggested that the presence of an *EGFR* mutation is specific for terminal respiratory unit -type adenocarcinoma, characterized by the expression of thyroid transcription factor-1 (TTF-1). TTF-1 is a homeodomain-containing protein closely related to members of the Drosophila NK-2 gene family and its official full name is NK-2 homeobox 1

(NKX2-1).[15,16] NKX2-1 is also known as NXK2A, TTF-1, TTF1, TITF1, thyroid-specific enhancer-binding protein, and benign chorea[17] The name TTF-1 is most commonly used, but it may be confused with the official symbol for transcription termination factor, RNA polymerase I (TTF1). NKX2-1 regulates transcription of genes specific for the thyroid, lung, and diencephalon.[15,17–25]

Expression of NKX2-1 is required for the proper development of the thyroid and lungs.[26,27] NKX2-1 is expressed in the lungs in type-II-pneumocytes and nonciliated bronchiolar epithelial cells and it has a binding site in the promoter region of surfactants and Clara cell secretory proteins.[28–32] NKX2-1 plays an active role in sustaining lung cancer.[33–36]

In NSCLC, expression of NKX2-1 is retained, though it varies with the histological subtype. Results of immunohistochemistry also depend on the staining method used.[37] In adenocarcinoma (ACC), approximately 75% (range, 58–84%) of tumors are NKX2-1 positive.[38–52] Mucinous ACCs tend to show NKX2-1 expression less frequently (0–21%).[39,53–55] Most studies on squamous cell lung carcinomas show no expression of NXK2-1,[39,42,44,45,48,52] although others show expression in 5% to 38% of squamous lung carcinoma specimens.[40,47,51] Expression of NKX2-1 in pulmonary non-neuroendocrine large cell carcinoma is reported in 0 to 50%.[42,44,51,56] In pulmonary neuroendocrine large cell carcinoma expression in 57% to 75% of tumors is reported.[56,57]

NKX2-1 is determined by immunohistochemistry, a test much faster than mutation analysis. In several articles, NKX2-1 expression was found in 93% to 96% of tumors with *EGFR* mutations.[14,58,59] This may generate the hypothesis that NKX2-1 can be used to preselect patients for a temporary treatment decision. In case of negativity for NKX2-1 and a need for urgent treatment, start conventional chemotherapy, ahead of the outcome of *EGFR*-mutation analysis. In case of positivity for NKX2-1, wait for the results of EGFR-mutation analysis before starting treatment.

The aim of this study is to examine whether NKX2-1 immunohistochemical (IHC) staining is a useful temporary surrogate marker for choice of treatment guidance in metastatic NSCLC.

PATIENTS AND METHODS

A database was constructed of consecutive tumor specimens of NSCLC patients (n = 810) referred for *EGFR* mutation testing during the course of routine diagnostics from May 2004 to December 2010 at the VU University Medical Center in Amsterdam. The decision for referral (i.e., selection of patients for mutation analysis) was made by the treating local or referring pulmonologist/pathologist. The submitted histologic diagnosis was performed according to the World Health Organization classification.[60] All samples were used in compliance with the respective institutional ethical regulations.

Immunohistochemistry

NKX2-1IHC was carried out as described previously.[61] For NKX2-1IHC clone 8G7G3/1 (Dako) was used.[38] In every IHC staining procedure, a negative and an external positive control were used. Staining intensity was scored, using a method modified from a method described by Ruschoff et al.,[62] with intensity ranging from 0 to 3: 3 = strongly positive, with microscope objectives 2.5−4×; 2 = moderate with microscope objectives 10−20×; 0 = no nuclear staining, 1 = weak with microscope objective 40×. For each intensity, the

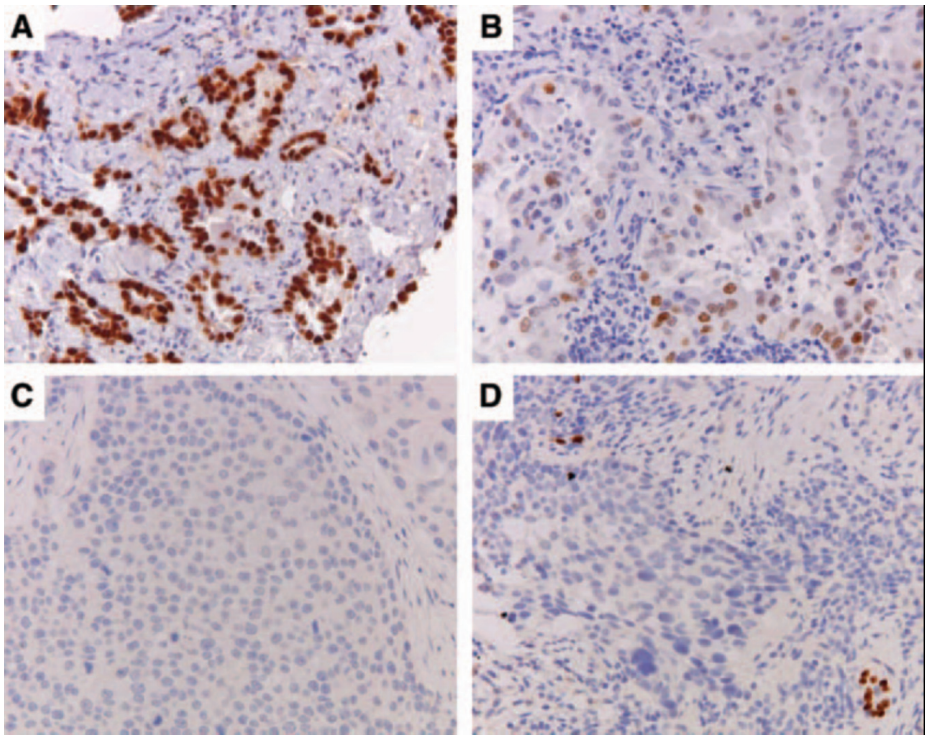

FIGURE 1. Examples of NKX2-1 immunohistochemistry. (*A*), Strong positivity for every tumor cell (intensity 3+, 100% of tumor cells); (*B*), heterogeneous staining; (*C*), no staining for NKX2-1, without internal control; and (*D*), with internal control; preexisting entrapped pneumocytes type II are positive (brown, smaller nuclei), all figures ×20 objective. NKX2-1, NK-2 homeobox 1.