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CORRESPONDENCE

Prognostic and Predictive Roles of Thymidylate Synthase Expression in Lung Cancer: The Debate Is Still Open

TO THE EDITOR: Sun et al[1] report data from an observational phase II study in which they prospectively investigated the correlation between tumor baseline expression of thymidylate synthase (TS) and outcome in patients with nonsquamous non–small-cell lung cancer (NSCLC) who were randomly assigned to receive either pemetrexed and cisplatin or gemcitabine and cisplatin treatment. Low TS protein levels were predictive of significantly increased response rates and prolonged progression-free survival in patients treated with pemetrexed and cisplatin compared with the gemcitabine-and-cisplatin group. Regarding the same treatment allocation, high TS expression was not correlated with the outcome.

In our opinion, some key points should be discussed in more detail. Without a consideration of treatment arms, low TS levels were associated with significantly prolonged overall survival and emerged as a good prognostic factor in the multivariable analysis (hazard ratio, 0.60; 95% CI, 0.42 to 0.84). However, the potential prognostic role of TS has not been clarified. TS levels may be associated with the stage of disease, lymph node metastasis, tumor differentiation, and cellular proliferation. However, several studies showed controversial results in terms of their clinical relevance, as reviewed in the article by Galvani et al.[2] In particular, automated in situ protein quantification of cytoplasmic TS, which demonstrated improved survival for patients with NSCLC and high TS expression,[3] appears to contradict the results of the current study. These discrepancies might be the result of different methods, treatment heterogeneity, and relatively small and/or heterogeneous samples. Therefore, a power analysis regarding the number of patients appears to be crucial to obtain reliable prognostic results, to enhance data interpretation, and to plan future studies.

The technique to evaluate TS status remains a critical point. Immunohistochemistry is a sensitive method widely used for the detection of specific proteins in tissue preparations. However, it is largely empirical and depends on the antibody and on several physicochemical properties of the tissue, such as fixation, as well as on the pathologist's expertise. The TS-specific mouse monoclonal antibody clone-106 has been used in studies that showed a partial relationship between TS mRNA, protein expression, activity, and sensitivity to TS inhibitors.[4,5] Despite this, a consensus has not been reached yet with regard to scoring, and detailed procedures are urgently needed to improve experimental reproducibility and to correct the interpretation of results. Appropriate controls should be included to be used for intralaboratory and interlaboratory validation. Optimization and standardization of technical procedures is indeed crucial to validate the best biomarkers for personalized chemotherapy.

Sun and colleagues claimed that, because of the key role of TS in DNA replication and subsequent cellular proliferation, low TS expression is correlated with well-differentiated tumors and a low proliferation index. Therefore, additional information about tumor grading, as well as on markers of proliferation such as Ki-67, are warranted. Ki-67 is a bona fide cell-cycle marker that has been linked to proliferation. However, TS levels are not exclusively associated with the S phase because their variation between exponentially growing and confluent cell populations appears to be the results of differences between G0 and G1 cells.[6] Moreover, TS expression is regulated by multiple mechanisms occurring at transcriptional, posttranscriptional, and translational levels.[2,7] These physiologic mechanisms are associated with cellular proliferation and apoptosis, which are often altered in tumors. Therefore, we believe that functional studies to evaluate key factors that modulate TS status are necessary before larger prospective pharmacogenetic trials are launched. TS may indeed have a mixed role as a prognostic and a predictive biomarker, one that possibly depends on the drug and the disease.

Because the response rate to gemcitabine and cisplatin was unexpectedly low in the group of patients with low TS, Sun and collaborators also hypothesized that these patients can benefit more from pemetrexed-based therapy than from other chemotherapeutic regimens whose antitumoral targets are not associated with TS. However, we recently demonstrated that gemcitabine can inhibit TS, presumably by means of the phosphorylated metabolite difluorodeoxyuridine monophosphate, which enhances the misincorporation of 2'-deoxyuridine into DNA, hence causing indirect damage.[8] We would, therefore, suggest an assessment to check for whether an increased proliferation rate could account for higher sensitivity to cell cycle–specific drugs, such as gemcitabine.

Last but definitely not least, although overexpression of TS is the most studied drug-resistance mechanism involved in pemetrexed chemoresistance, multiple mechanisms of antifolate resistance might affect pemetrexed activity. This supports further evaluation of the expression and/or mutational status of emerging determinants, such as the proton-coupled folate transporter PCFT/SLC46A1, which displays an exceptionally high affinity for pemetrexed.[9,10]

In conclusion, we congratulate Sun et al for their study, but we look forward to additional preclinical and clinical investigations that may strengthen these results regarding the role of TS for predicting the outcome of patients with NSCLC and mesothelioma who are treated with pemetrexed-based regimens, as well as for prognostic purposes.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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