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In Response:

We thank Dr. Toffalorio and coworkers for their comments and interest in our article. All their questions were valuable, and they reminded us of the potential problems of our study. As Dr. Toffalorio commented, standardization and optimization are big issues in immunohistochemistry (IHC). This is one of the major obstacles preventing generalization of IHC data. To maintain the quality of tests and standardization, we performed several pilot tests with different antibody concentrations in normal lung tissue and other cancer tissues. After this, we chose the optimal concentration level, and this was used in this study. To minimize the technical error, we made a tissue array and most of the procedures were performed by an automated method and not by the manual method. We initially tried to include a needle aspiration biopsy specimen, yet it was difficult to mount the small amount of tissue on the tissue array, so IHC was performed by the manual method; however, the quality of IHC staining was unstable. So, we discarded the data drawn from the manual method. It is our opinion that the method used in this study was quite reliable and reproducible.

Dr. Toffalorio and coworkers also questioned about clone 8F1 antibody. However, another research group in our institute and other researchers have already reported studies that were performed using the same antibody clone.[1–3] We think that this antibody is one of the reliable antibodies that can be used for IHC of ERCC1.

In terms of the correction of multiple variables, we did not perform multivariate analysis. Because we did not have a large number of cases in each stage, we thought it would be impractical to conduct multivariate analysis on such a small number of cases. Furthermore, the most important point that should be addressed was not the difference in survival but the difference of protein expression between the primary tumors and the metastatic lymph nodes, so we thought that multiple comparison correction was not a critical prerequisite for this study. However, we have a plan to include the results of multivariate analysis in a future study with a larger number of cases.

We had 30 N1 and 52 N2 patients in the study population. Because we selected non-small cell lung cancer patients with nodal metastasis, those patients are homogenous in terms of nodal metastasis in our opinion. We thought that the T stage was not an important factor because the purpose of this study was comparing between the primary tumor and the metastatic nodes, and the survival in this group of patients is usually determined by nodal metastasis rather than the status of the primary tumor. For the same reason, the treatment method (such as the extent of surgical resection) would not be an important factor for the difference in the protein expression levels. We thought that the treatment in this study was relatively homogenous in that all the patients received complete surgical resection and platinum-based chemotherapy.

Thanks again for your consideration.

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Serum Concentrations of Erlotinib at a Dose of 25 mg Daily

To the Editor:

In this journal, our group recently reported a retrospective review of the clinical efficacy of erlotinib at a dose of 25 mg/d for patients with metastatic non-small cell lung cancers (NSCLCs) with somatic mutations in the epidermal growth factor receptor (*EGFR*) gene.[1] The seven patients included in that study attained a response rate of 71.5% and a median progression-free survival of 17 months (95% CI, 6–35 months). We speculated that the serum concentrations achieved with erlotinib 25 mg/d were similar to the serum concentrations observed with gefitinib 250 mg/d. Based on the published phase I trials for these EGFR tyrosine kinase inhibitors, the mean serum trough concentration attained with gefitinib 250 mg/d was between 0.16 and 0.24 μg/mL or 0.35 and 0.53 μM,[2] whereas the mean serum concentration measured with erlotinib 25 mg/d was approximately 0.22 μg/mL or

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TABLE 1. Serum Concentration of Erlotinib in Patients With Stage IV *EGFR* Mutated NSCLC

Patient (ref.)	Clinical and Molecular Characteristics				Erlotinib Concentration			Efficacy		Toxicity (Grade–CTCAE)	
	Age (yr)	Sex	Ethnicity	*EGFR* Mutation	Daily Dose Erlotinib (mg)	Erlotinib Serum (μg/mL)	Erlotinib Serum (μM)	Response (RECIST)	PFS (mo)	Rash/ Pruritus	Diarrhea
1 (1)	64	F	Asian	delE746_A750	25	0.19	0.44	PR	9	None (0)	None (0)
2 (1)	46	M	Asian	L858R	25	0.38	0.88	SD	4	Yes (1)	None (0)
3 (4)	74	F	Caucasian	L858R-L747S	150	1.80	4.18	PR	6	Yes (3)	Yes (2)

EGFR, epidermal growth factor receptor; NSCLC, non-small cell lung cancer; PFS, progression-free survival; CTCAE, Common Terminology Criteria for Adverse Events v3.0; M, male; F, female; RECIST, Response Evaluation Criteria In Solid Tumors v1.0; PR, partial response; SD, stable disease; ref, reference.

0.51 μM.[3] However, at the time of our initial publication, we had not measured the concentrations of erlotinib in any of our studied patients.

We now report serum concentration measured with erlotinib for two of the seven patients,[1] initially described in our case series of a dose of erlotinib 25 mg/d and for an additional patient[4] treated with erlotinib 150 mg/d (Table 1). All patients had stage IV NSCLCs and received erlotinib orally. Erlotinib (molecular weight of 429.90) was measured using high-performance liquid chromatography with ultraviolet detection.[5] The measured serum concentration of erlotinib, by high-performance liquid chromatography, in both patients receiving 25 mg/d exceeded 0.4 μM (Table 1), whereas the concentration measured for the patient receiving 150 mg/d exceeded 4 μM. Skin and gastrointestinal toxicities correlated with the serum concentration of erlotinib.

These updated results further strengthen our clinical results and provide additional evidence that a dose of erlotinib 25 mg/d can lead to serum concentrations that are similar to those previously reported with gefitinib 250 mg/d. At doses as low as 0.1 μM of either gefitinib or erlotinib, NSCLC cell lines with sensitizing *EGFR* mutation are inhibited and undergo apoptosis.[6] Therefore, it is tempting to speculate that effective doses of gefitinib/erlotinib in NSCLC patients with sensitizing *EGFR* mutations are far below their maximum tolerated doses in humans. Prospective clinical trials of erlotinib at lower than approved doses are warranted and will help define less-toxic treatment strategies for NSCLC patients whose tumors harbor *EGFR* mutations.

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