Molecular Diagnostics in Advanced NSCLC: Trying to Maximize a Non-Ideal Situation

To the Editor:

I refer to the Editorial in the May 2007 issue of the JTO, entitled *Should Mutational Analyses of Tumor Samples Bypass Histopathology?*

We are glad that our work has helped to highlight a clinical management issue concerning patients with advanced non-small cell lung cancer (NSCLC), namely the lack of sufficient tissue/cells for mutational analysis. Although efforts to better detect and manage early disease continue, it is a fact that most NSCLC cases are in the advanced stage at presentation. Therefore, where improvement of clinical management is concerned, patients with advanced-stage NSCLC pose immediate and substantial challenges.

By incorporating molecular knowledge into clinical practice, we had hoped to further the translation of bench findings into patient benefit. This is of particular relevance to East Asian populations, in whom the frequency of EGFR mutations is high. However, health systems need to contend with economic feasibility issues, e.g., availability of resources and expertise, impact on hospital workflows, and assay reliability.

Analyzing tumor cells from patients with advanced-stage NSCLC is complicated by the low tumor cell numbers in biopsies and our general reluctance, for compassionate reasons, to subject patients to multiple biopsy procedures. Under such constraints, it would be reasonable to tackle the problem by developing more sensitive detection methods and/or by maximizing the diagnostic procedure. We attempted to determine the limits of the latter, and in so doing, address some of the economic feasibility issues.

We do not "regard split samples obtained from a single biopsy as equivalent," precisely because one is histologically validated and the other is not. In fact, we stated in the *Discussion* that it was "less than ideal" that the low-volume samples for mutational analysis were without histological validation. We were not addressing the question of "whether histology-bypassed biopsy samples (could) provide results equivalent to analyses performed on FFPE samples that were processed primarily for histologic diagnosis." We were seeking the maximal yield of mutation information from low-volume biopsies using standard DNA sequencing techniques.

To clarify, we did not take low-volume biopsies from surgically resected tumors for mutational analysis. The copious amounts of DNA from surgically resected tissue were used to demonstrate the reliability of our sequencing technique and validity of the sequence quality. Now that the feasibility of obtaining sequence data from low-volume samples has been demonstrated, the concordance of sequence data from the low-volume biopsy (at diagnosis) and surgical specimen from the same patient may be examined.

The analysis of the surgically resected tissue showed EGFR mutation rates (our original Table 4) in the subgroups of adenocarcinoma (44%), female (67%), and non-smoker (73%) as being consistent with rates previously reported. The difference in distribution of patient and tumor characteristics (our original Table 1) between the surgical and non-surgical groups was addressed in the *Discussion*, where the consent-giving process may have introduced a bias, giving rise to different patient profiles in which EGFR mutations most frequently occurred.

The L833V mutations could not be confirmed. This was because of the rationing of sample DNA to interrogate as many different exons as possible, instead of confirming mutations by separate independent polymerase chain reaction. This is indeed a limitation of low tumor cell numbers and reiterates the need for the development of alternative genotyping platforms that are more sensitive and still affordable for clinical use.

Overall, we did not set out to prove that histopathology should be bypassed in mutational analysis.[1] In an ideal situation, we are able to interpret mutational results in the context of histopathologic and cytologic findings. The nub is in figuring out how to maximize non-ideal situations. We do not presume to have found the ideal solution, but we wanted to share with our colleagues our attempt at having a stab at the problem.

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REFERENCES

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