

## Predicting Progression of Oral Dysplasia—Response

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We thank Drs. Gomes, Fonseca-Silva, and Gomez for their comments on our recently published article (1). That article validated a LOH risk model for use in differentiating between high-risk and low-risk oral dysplasias—a critical barrier in selecting patients for advanced oral cancer preventive intervention. We agree with Gomes and colleagues that there is a need to build on this finding and to develop a clinical tool that is accessible to a broad range of users. However, we stress caution in the way in which this evolution in technology occurs and the need to ensure that changes in protocol result in a new technology that has similar (or improved) capacity to predict outcome for such lesions.

The data shown by Gomes and colleagues illustrate some of the inherent difficulties that can occur when making a transition between different platforms as biomarkers evolve. Our protocol used a "radiation-labeling visual inspection" of PCR products separated on polyacrylamide gels. Gomes and colleagues propose the evolution to a "dye-labeling intensity comparison" with samples separated by capillary electrophoresis. It is important to note that the interpretation of data can be subjective in both systems and each will have its own limitations and biases, with experience of the user being important. For example, the primer set used in Fig. 1A resulted in the 2 bands differing widely in intensity on polyacrylamide gels; in our opinion, not ideal for gel electrophoresis. It was scored by Gomes and colleagues as negative by visual inspection, but we suggest that there seems to be some imbalance in the allele patterns. With an increase in exposure time, the imbalance may become more apparent and be scored as a loss. Figure 1B points to a sample showing somewhat weaker intensity of the upper band compared with the lower band in the gel analysis. This might qualify for LOH if the upper band was better resolved. The authors score this sample as negative for

LOH in the capillary electrophoresis system based on deviation from a chosen cutoff value for differences in intensities of the 2 alleles in control and test samples. The choice of cutoff values for different primer sets affects the sensitivity of the gene scan to distinguish low levels of change in LOH. These levels cannot be arbitrarily chosen but need to be set and then validated clinically. The authors also consider capillary electrophoresis to be more sensitive than gel electrophoresis. It is important to consider the possibility that higher sensitivity of detection may not always result in an improvement in prediction of outcome. In this case, increasing sensitivity might detect smaller numbers of cells with LOH rather than a clone (many cells with LOH). The capacity to clonally expand could matter.

There are other possibilities that could be considered as next steps, many of them cutting edge, such as the development of a "single molecule-based" quantitative analysis using next sequencing technologies such as the Ion Torrent Platform, which would produce a call out of number of gene copies (2). We suggest that the choice for technologic change requires a careful comparative study between platforms to look for similarities and differences in the LOH calls, to determine the relative ability to detect LOH and its clinical relevance. To that end, the procedure used in our article represents the only system to be validated prospectively for association with oral cancer risk. As such it represents the validated standard by which other systems in the future could be compared for association with outcome. Perhaps such an approach should be accepted to determine the next evolution of a device—much like drug studies are run—with a validated drug shown to affect outcome being used as the comparative arm in trials using new drugs, to determine if outcome is improved.

The best situation would be for the development of such formal studies with new technologies to be collaborative efforts that would lead to a universal tool that would be broadly adopted instead of a divergence into many "tools" without appropriate validation. The latter course, with multiple tools, means losing the ability to directly compare data from different populations and in different settings between laboratories. The time to develop that universal tool is now, before we lose both the opportunity and the momentum.

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doi: 10.1158/1940-6207.CAPR-13-0111

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## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Received March 28, 2013; accepted March 29, 2013; published OnlineFirst April 16, 2013.

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# Cancer Prevention Research

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*Cancer Prev Res* 2013;6:616. Published OnlineFirst April 16, 2013.

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