Commentary

Is the ColDx assay a valid prognostic marker for stage II colon cancer?

Jessica Felton[1], Jean-Pierre Raufman[2]

[1]Department of Surgery, [2]Department of Medicine, University of Maryland School of Medicine and Veterans Affairs Maryland Health Care System, Baltimore, Maryland, USA

Correspondence to: Jessica Felton, MD. Department of Surgery, University of Maryland School of Medicine, 22 S. Greene Street, S8B02, Baltimore, Maryland, USA. Email: jfelton@som.umaryland.edu.

Comment on: Niedzwiecki D, Frankel WL, Venook AP, *et al.* Association Between Results of a Gene Expression Signature Assay and Recurrence-Free Interval in Patients With Stage II Colon Cancer in Cancer and Leukemia Group B 9581 (Alliance). J Clin Oncol 2016;34:3047-53.

Submitted Oct 05, 2016. Accepted for publication Oct 31, 2016.
doi: 10.21037/tcr.2016.11.64
View this article at: http://dx.doi.org/10.21037/tcr.2016.11.64

Introduction

In the United States, colorectal cancer is the fourth most prevalent neoplasm and the second most frequent cause of cancer-related death (1). Surgery alone is standard-of-care for stage I cancer, whereas adjuvant chemotherapy is recommended for stage III. Surprisingly, despite its prevalence, the most appropriate management for stage II colon cancer remains uncertain. Currently-accepted guidelines recommend adjuvant chemotherapy for those considered 'high-risk' for recurrence, defined as stage T4 cancer, poorly-differentiated histology, inadequate lymph node sampling, positive or unknown margins, lympho-vascular or perineural invasion, bowel obstruction, or perforation (2).

Over the past decade, several studies have suggested that these clinicopathological risk markers for cancer recurrence are unreliable and that molecular markers may offer better predictive value. Several gene expression profiles have been developed, tested, and validated. The present report seeks to provide further validation for ColDx, a gene expression microarray-based assay previously associated with recurrence-free interval and overall survival in colon cancer (3).

Summary of the study

Niedzwiecki *et al*. prospectively validated the ColDx assay as a prognostic marker in stage II colon cancer. The investigators used formalin-fixed, paraffin-embedded (FFPE) specimens collected as part of the Alliance for Clinical Trials in Oncology (Alliance), formerly the Cancer and Leukemia Group B (CALGB), phase II clinical trial (C9581) (3). Using this dataset, the authors analyzed a total of 393 study subjects using a case-cohort sampling design with 360 subjects from a randomly selected sub-cohort with 91 recurrence-free interval (RFI) events and 33 subjects with RFI events outside of the random sub-cohort. The primary end-point was RFI, measured from study entry to distant recurrence or colon cancer-related death.

Subjects were categorized as low- or high-risk, based on a ColDx-fixed prognostic score; 45% were classified as low-risk and 55% high-risk. Univariate analyses revealed the ColDx score was significantly associated with recurrence-free interval after adjustment for other prognostic factors. Analyses also demonstrated that age and DNA mismatch repair status were only borderline significant. Compared to ColDx low-risk subjects, ColDx high-risk subjects had significantly shorter RFI and the recurrence-free probability at five years for those at high-risk was 82% compared to 91% for those at low-risk (3).

Problems and limitations

While the investigators reported a 'positive' primary outcome—statistical significance for the ColDx prediction of RFI in stage II colon cancer—the study has potentially important clinical implications, hence their study design and data warrant careful scrutiny and interpretation. Key questions must be addressed before this positive finding

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can be deemed sufficient for commercialization of the test and its use to modify clinical practice (4). The investigators deserve credit for employing a large sample size and testing a clinically-important primary outcome; ColDx has promise as an assay whose results can be used to withhold chemotherapy from those with stage II colon cancer who are likely to derive harm but limited benefit.

Several important limitations of this study should be noted. First, this is a retrospective study. Given the prior retrospective validation of the ColDx assay, a prospective study would better gauge ColDx's clinical potential. Second, since FFPE is the current standard of tumor tissue preservation, the ColDx assay used by Niedzwiecki *et al*. was developed for use in tissue preserved in FFPE rather than frozen (3). However, during the initial analysis, technical failure led the investigators to repeat the study using new reagents. The advanced age of tissue samples likely contributed to the high proportion of assay failures; the average age of FFPE tissue was 13.2 years (3). While this technical issue was recognized and rectified, it raises concerns regarding the use of RNA extracted from FFPE tissue. Several factors can influence the stability of mRNA derived from FFPE tissue, including variability in tissue processing, tissue sources, and RNA extraction methods. Notably, formalin degrades and fragments RNA (5,6). Abdueva *et al*. reported that, regardless of RNA fragmentation in FFPE tissue compared to fresh-frozen specimens, RNA extracted from FFPE could be used in functional gene array profiles; however, this remains controversial (5).

While Niedzwiecki *et al*. report the utility of the ColDx assay in predicting recurrence, the overall importance of their findings are uncertain. In an unadjusted analysis, the authors show that those classified high-risk by ColDx had a significantly shorter RFI than those at low-risk (hazard ratio, 2.03; P<0.01). However, a more clinically-important end-point, the overall survival difference between the two groups was only 'marginally' significant (hazard ratio, 1.74; P=0.06). On the other hand, while survival may be not be meaningfully altered by using this prognostic microarray, it may save patients at low risk of recurrence from the toxicity and potential deleterious side effects of adjuvant therapy.

Whereas this work was published recently, the results are not new and perhaps not newsworthy. The current work provides a modest expansion on a previous study by Kennedy *et al*. published in 2011, wherein the authors reported development of the DNA microarray-based assay for FFPE tissue that identified those with stage II colon cancer at high risk for recurrence after surgery (6). That study, which used a separate cohort, was the first to suggest that the ColDx assay might serve as an independent prognosticator of recurrence-free interval for stage II colon cancer (7). Hence, the current study by Niedzwiecki *et al*., using the CALGB patient cohort, represents the second such retrospective validation of the ColDx assay (3). The authors claim their work provides "an external validation of the prognostic value" of the assay (3), but a prospective validation would have been more informative.

It is not surprising that a second study was performed to validate the ColDx assay; the assay's strongest competitor, the 12-gene Oncotype DX colon cancer assay, was independently validated in three separate large studies (8). In fact, an extensive review of this field in 2015 identified 12 studies describing the development, validation, clinical and economic utility of this assay (8). Moreover, in addition to the Oncotype DX and ColDx assays, at least two other gene expression signatures have been developed for the same purposes in colon cancer, ColoPrint and Veridex (9).

Expanding interest in developing, testing, producing, and marketing prognostic gene assays falls within the larger context of precision oncology which promises to tailor diagnostic and therapeutic approaches for individuals by enhancing available technology (10). Perhaps the best example of this approach is in breast cancer, where receptor status helps determine prognosis and guide therapy. Similar to the colon cancer gene assays discussed above, several prognostic gene assays are used for breast cancer, including Oncotype DX and MammaPrint. Recently, the results of the MINDACT trial, a randomized, phase 3 study designed to assess five-year survival in subjects with early-stage breast cancer, high-risk clinical features, and a low-risk gene-expression profile (MammaPrint) who did not receive chemotherapy, were published (11). MammaPrint, the 70-gene signature used to determine genomic risk, was developed to provide valuable information in addition to traditional clinical and pathological factors in considering who would benefit from adjuvant chemotherapy (11). Similar to the studies of colon cancer gene assays, these studies play a valuable role in the ongoing development of precision oncology.

Conclusions

ColDx offers promise as a novel prognostic assay for stage II colon cancer and may help determine the need for adjuvant chemotherapy; it has been validated twice as a tool

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for prognosticating recurrence-free interval. Nonetheless, several limitations must be addressed before this assay can be deemed ready for clinical use. First, it should be tested prospectively. Second, it should be considered whether measuring gene expression in fresh-frozen cancer tissue might be preferable to using FFPE tissue. As precision oncology becomes the wave of the present, rather than the future, it will be interesting to see if and how the ColDx assay, and its peers, succeed in improving patient care and outcomes.

Acknowledgments

Funding: Dr. Jessica Felton was supported by T32 training grant DK067872. This work was supported in part by Merit Review Award # BX002129 from the United States (U.S.) Department of Veterans Affairs Biomedical Laboratory Research and Development Program. The contents do not represent the views of the U.S. Department of Veterans Affairs or the United States Government.

Footnote

Provenance and Peer Review: This article was commissioned and reviewed by the Section Editor Gang Wang (Department of Pancreatic and Biliary Surgery, The First Affiliated Hospital of Harbin Medical University, Harbin, China).

Conflicts of Interest: Both authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr.2016.11.64). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Cite this article as: Felton J, Raufman JP. Is the ColDx assay a valid prognostic marker for stage II colon cancer? Transl Cancer Res 2016;5(Suppl 6):S1157-S1159. doi: 10.21037/tcr.2016.11.64