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Editorial

EDITORIAL

# Use of Gene Expression Profiles to Distinguish Molecular Subtypes in Colorectal Cancer: Progression Toward Primetime

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Stratified medicine has already transformed the paradigm of drug therapy for some cancers and is set to underpin future developments across all of cancer therapeutics. Some stratified medicine approaches are specific: a single molecular biomarker corresponding to the molecular target of a specific treatment (such as vemurafenib in *BRAF* V600E-mutated melanoma). A key alternative concept in stratified medicine is that different patients' cancers, while individual and heterogeneous, may have disruptions to the same cellular pathways or functions, leading to similarities in tumor behavior and, crucially, similar response to specific treatments. So, while recognizing that no two patients' cancers are identical, we may still develop molecular classifications that identify quasi-consistent groups for whom distinct treatment strategies may be needed, or in whom different novel targeted therapies might logically be tested.

RNA expression arrays offer an important platform from which to develop disease stratification, and since 2012 several colorectal cancer schemes have been published based on Affymetrix or Agilent arrays, each resulting in three to six patient strata, but with wide differences between the contributing genes (1–6). In an important collaboration in 2015, data sets from six published algorithms were then co-analyzed to generate a single Consensus Molecular Subtype (CMS) scheme (7). CMS classification using a 273-gene expression classifier defines four groups (CMS1-CMS4), each with a set of pathway alterations and clinicopathological features. For example, CMS4 or "mesenchymal-type" tumors are characterized by upregulation of genes implicated in epithelial-to-mesenchymal transition, TGF$\beta$ signaling, angiogenesis, and matrix proteins and are rich in stromal cells such as fibroblasts.

Perhaps the most immediate potential clinical application of CMS status is where it can be shown to correlate with prognosis and so contribute to decisions about existing standard treatments. In the consensus study data set, patients with CMS4 tumors had statistically significantly inferior postoperative relapse-free and overall survival compared with CMS1-3 (7). Evaluation of CMS4 status alongside other known prognostic factors (TNM stage, vascular invasion, primary tumor location, etc.) is needed to evaluate whether it adds to these factors in decision-making for adjuvant chemotherapy.

Second and perhaps more importantly, CMS stratification, by identifying tumors with a degree of biological commonality, has the potential to be used to select groups of patients for evaluation of novel targeted therapies, where the known biological characteristics of the CMS group provide a rationale for a specific treatment approach. Currently no targeted systemic therapies are used as standard in the treatment of early-stage colorectal cancer. For example, CMS1 is characterized by genes associated with T-cell infiltration and immune evasion pathways, with high rates of microsatellite instability and CIMP, making this a promising subpopulation for evaluation of immunotherapy.

However, prospective use of CMS status to stratify our treatment decisions, whether in research or for future standard practice, requires that patients be assessed rapidly, reliably, cost-effectively, and preferably without the need for additional biopsies, none of which can be listed as defining features of array-based RNA assays. There is therefore a strong rationale to develop rapid, low-cost, reliable techniques that correlate with CMS strata and are better suited for the routine laboratory.

In this issue of the Journal, Ubink and colleagues propose a method to identify one of the CMS types, CMS4, using a four-gene reverse transcription polymerase chain reaction (RT-PCR) assay (8). Their approach was to select five candidate genes

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based on their overexpression in mesenchymal type cancers, their known contribution to colorectal cancer progression and potential as drug targets, then to assess the ability of that restricted five-gene panel to predict CMS4 designation from the full 273-gene classifier. This was done first in a 566-patient in silico training series, then (after excluding one gene as noncontributory) validated in further in silico data sets. Finally, they transferred assessment of the four-gene panel to a reverse transcription quantitative polymerase chain reaction (RT-qPCR) assay, a technology commonly in use in routine molecular pathology labs, and used this assay to assess intratumoral heterogeneity in 20 further tumors. They demonstrate that the four-gene RT-qPCR correlates well with array-based identification of CMS4 tumors and have used the new assay to assess intratumoral heterogeneity for this subtype, consistent with recent studies using single-cell whole-exome sequencing (9).

This is a welcome step toward translating array-derived research findings into a deliverable clinical tool. The approach combines both statistical and financial parsimony and is intrinsically preferable to black-box molecular stratification assays (8). The authors recognize that many more steps are needed for clinical application. For example, the approach needs to be extended to distinguish all four CMS subtypes in order to open up a greater range of clinical applications, and validation in formalin-fixed preserved tissue would greatly improve widespread uptake and allow analysis of retrospective series.

The greatest attraction of CMS is its potential as a predictive tool, but at this point predictive associations are speculative. The CMS4 profile includes high TGFβ expression with activation of cancer-associated fibroblasts, and Ubink and colleagues propose, reasonably, that patients with CMS4 tumors would be a rational group in whom to evaluate drugs targeting the PDGF/KIT pathway, such as imatinib or dasatinib (8). Recent umbrella clinical trial designs, such as the ongoing MRC FOCUS4 trial in the United Kingdom, are demonstrating that it is both feasible to prospectively select patient subgroups across a whole national clinical trials network and to evaluate hypothesis-based novel therapies in suites of parallel randomized trials (10). Rapid, reliable assays, such as those now being developed by Ubink and colleagues, may provide the necessary tools to progress the field more rapidly.

## Note

The authors have no conflicts of interest to disclose.

## References


1. Budinska E, Popovici V, Tejpar S, et al. Gene expression patterns unveil a new level of molecular heterogeneity in colorectal cancer. *J Pathol*. 2013;231(1):63–76.
2. Marisa L, de Reynies A, Duval A, et al. Gene expression classification of colon cancer into molecular subtypes: Characterization, validation and prognostic value. *PLoS Med*. 2013;10(5):e1001453.
3. Roepman P, Schlicker A, Tabernero J, et al. Colorectal cancer intrinsic subtypes predict chemotherapy benefit, deficient mismatch repair and epithelial-to-mesenchymal transition. *Int J Cancer*. 2014;134(3):552–562.
4. De Sousa E Melo F, Wang X, Jansen M, et al. Poor-prognosis colon cancer is defined by a molecularly distinct subtype and develops from serrated precursor lesions. *Nat Med*. 2013;19(5):614–618.
5. Sadanandam A, Lyssiotis C, Homicsko K, et al. A colorectal cancer classification system that associates cellular phenotype and responses to therapy. *Nat Med*. 2013;19(5):619–625.
6. Schlicker A, Beran G, Chresta C, et al. Subtypes of primary colorectal tumors correlate with response to targeted treatment in colorectal cell lines. *BMC Med Genomics*. 2012;5:66.
7. Guinney J, Dienstmann R, Wang X, et al. The consensus molecular subtypes of colorectal cancer. *Nat Med*. 2015;21(11):1350–1356.
8. Ubink I, Elias S, Moelans S, et al. A novel diagnostic tool for selecting patients with mesenchymal-type colon cancer reveals intratumor subtype heterogeneity. *J Nat Cancer Inst*. 2017;109(7):djw303.
9. Wu H, Zhang X, Hu Z, et al. Evolution and heterogeneity of non-hereditary colorectal cancer revealed by single-cell exome sequencing. *Oncogene*. 2016; in press.
10. Kaplan R, Maughan T, Crook A, et al. Evaluating many treatments and biomarkers in oncology: A new design. *J Clin Oncol*. 2013;31(36):4562–4568.