So Much Effort, So Little Progress?

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In colorectal cancer (CRC), the treatment guidelines differ for Union International Contre Cancer (UICC) stage II and III colon cancer patients. In patients with stage II colon cancer without risk factors such as T4 tumor, tumor perforation, emergency surgical procedure, or less than 12 removed lymph nodes, the low risk of recurrence is justifying no role of adjuvant chemotherapy. It would be critical to identify patients with stage II colon cancer who have higher risk of recurrence based on molecular testing and would benefit from adjuvant chemotherapy. On the other hand, for UICC stage III patients, 5-fluorouracil (5-FU)–based adjuvant chemotherapy has increased the absolute five-year survival rate by 10% to 15%, and the addition of oxaliplatin in the adjuvant setting further increased the absolute five-year disease free survival rate by 5% to 6% to 71% to 73% (1,2). The combination of a fluoropyrimidine in combination with oxaliplatin for a period of six months is recommended for those patients (1,2). Without the identification of high risk factors of recurrence and markers of efficacy of FOLFOX, a large proportion of patients will receive chemotherapy without any benefit. The future challenge is to define the patient population that benefits from adjuvant chemotherapy in stage II and III based on a biomarker test that helps to guide this critical decision.

During the last decade, several molecular signatures/tests have been introduced, however, the predictive value, despite huge efforts, is only marginally better than the assessment of clinical risk factors such as obstruction, T4 tumor, perforation, the presence of lymphovascular or perineural invasion, and a high carcinoembryonic antigen.

Di Narzo and colleagues (3) tested the predictive value of four (Genomic Health, Veridex, ALMAC, and an MD Anderson score) previously published and in part validated colon cancer recurrence risk scores using microarray gene expression data from FFPE (formalin-fixed paraffin-embedded [tissue]) material from patients enrolled in PETACC3 (4). With the exception of the ALMAC score, which also used microarray data from FFPE samples, all other scores either used different material (fresh frozen), a different expression assay (Q-RT-PCR), or both to calculate the risk of recurrence-free survival (RFS), survival after relapse (SAR), or overall survival (OS). Therefore, the data coming from PETACC3 microarrays had to be adjusted to the respective score. Using multivariate analysis, RFS was predicted by two tests (Genomic Health and Veridex), SAR by one score (MD Anderson), and OS by two (Genomic Health and MD Anderson) tests. The ALMAC test showed predictive value only in univariate testing for RFS and OS. None of the scoring systems was able to predict all three endpoints, and concordance between the tests was poor. The highest correlation was seen between the Genomic Health System and MD Anderson scores agreeing to the classification into high- and low-risk patients in 70.3% of the cases.

Why are those sophisticated gene expression signatures not able to predict RFS, OS, and SAR? It is important to understand the patient cohorts in which these recurrence risk signatures were developed and validated. For example, the PETACC3 study tested 5-FU vs 5-FU plus irinotecan in the adjuvant setting of UICC stage II-III tumors. The primary endpoint, a difference in five-year disease-free survival, was not met (4), and today’s standard of care chemotherapy in stage III disease is fluoropyrimidine (5-FU or capecitabine) and oxaliplatin (1,2). The question is whether PETACC3 is the ideal patient cohort to test the predictive value of cancer risk scores. Furthermore, most of the scores were calculated using “adjusted” gene expression data. As discussed by the authors, it cannot be excluded that minor errors may occur caused by this adjustment. Tissue microarray (TMA) samples contain a certain amount of stromal cells and normal tissue contamination, which can impact the gene expression signatures and therefore the results of the Di Nazaro study.

Our molecular understanding of colorectal cancer carcinogenesis has expanded over the last decade. We have learned that gene expression signatures most likely won’t reflect the tumor subclones, which might be critical for prediction and prognosis in these patients. Colorectal cancer is a heterogenic disease with multiple subclones of cancer cells that can be identified by a distinguished mutational (5) and gene expression status (6). Gene expression analysis will not capture those subclones that might be ultimately responsible for drug resistance and progression of the disease. Another challenge is the fact that the investigated tumor is the result of a clonal evolution during carcinogenesis, and the chronology of events is not caught by a single time-point analysis. Early aberrations or mutations may appear in most of the cells, but later occurring changes are only seen in a subpopulation. As shown for the subgroup of tumors resulting from serrated adenomas, a preexisting BRAF mutation is marginal but gains prognostic value when p16 expression is lost at a later time-point (7). So expression data alone would not be helpful, but the combination with mutational analysis and histological morphology should be further evaluated.

Tumor heterogeneity may explain why the predictive values of these tests don’t correlate with all clinical endpoints (DFS, OS, and SAR). Cell populations that are predictive for a longer disease-free interval may either be successfully treated by adjuvant chemotherapy or do not have the ability to metastasize and are treated adequately by surgery alone. The surviving subclones that are responsible for recurrence may not be identified by the expression profile. But those subclones and their ability
to metastasize and sustain adjuvant chemotherapy are responsible for survival after recurrence. It would be of high clinical significance to use the same expression platform for recurrent tumor tissue to identify the gene expression signature of the recurrent subclone(s). This is supported by the fact that expression profiles change at the time of recurrence (8). Gene expression signatures will allow insights into the activation of pathways, however, it might be important to include the DNA mutational status to better understand the critical signaling in the tumor, in order to design the best and most effective treatment options. With the completion of the The Cancer Genome Atlas Network in CRC (9), we will get a better understanding of the complexity of the mutational make up and the activation of pathways involved in CRC carcinogenesis and progression. Combining RNA signatures with DNA mutational status might be powerful to predict outcomes. Overall survival is a compound of DFS and SAR and may be dependent on multiple factors, such as performance status, the administered chemotherapy, and rat-sarcoma proto-oncogene (RAS)-mutational status.

How can we move forward with more and more complex genomic data sets facing the challenges of tumor heterogeneity and the dynamics of molecular alterations during tumor progression and under the selection pressure of chemotherapy? The goal of adjuvant chemotherapy for UICC stage II /III patients is to prevent cancer recurrence. A meaningful endpoint for cancer recurrence risk scores/test should be disease-free survival. Tests should be powered for DFS instead of OS. Understanding tumor heterogeneity, cancer stemness and resistant subclones will be critical to better understanding the molecular mechanisms of recurrence. Combining gene expression with mutational analysis and the assessment of particularly conspicuous cell clones might get us further to predicting individual cancer recurrence. Utilizing liquid biopsies might be an important tool in the identification of the driving molecular pathways, leading to tumor recurrence. These methodological advances and technologies should be able to improve the limited predictive value of gene expression signatures!

References


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