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Microsatellite Instability, Prognosis and Drug Sensitivity of Stage II and III Colorectal Cancer: More Complexity to the Puzzle

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Microsatellite instability (MSI), the abnormal shortening or lengthening of DNA by 1–6 repeating base pair units, is the result of inactivation of the DNA mismatch repair (MMR) system and characterizes approximately 15% of colorectal cancers (CRCs) (1). Two distinct mechanisms can cause MMR deficiency (dMMR): 1) germline mutational inactivation of the MMR genes, present in CRC patients with Lynch Syndrome and 2) epigenetic silencing by hypermethylation of CpG islands in the MMR gene promoters, present in the vast majority of sporadic MSI CRCs (2,3). It has long been recognized that MSI CRCs form a unique subset of CRC patients with different clinical behavior compared with patients with proficient DNA MMR (pMMR) cancers, whose tumors are characterized as microsatellite stable (MSS). A number of retrospective studies and a meta-analysis (4) have supported the favorable stage-adjusted prognosis of MSI CRC patients compared with MSS CRC patients. However, a putative predictive role of MSI for response to 5-fluorouracil (5-FU) adjuvant chemotherapy has been proven to be a contentious issue and was the topic of two recent articles (5,6) and a 2009 American Society of Clinical Oncology (ASCO) abstract (7). The article by Ribic et al. (5) found that untreated CRC patients with MSI tumors have a modestly better prognosis than those with MSS tumors, but did not seem to benefit from the 5-FU-based adjuvant chemotherapy. There was even a suggestion of harm in terms of overall survival (OS) in treated CRC patients with MSI tumors. These findings were confirmed 5 years later, when a pooled analysis was performed by Sargent et al. (6) that included 457 new CRC patients and the previously published 570 CRC patients (5). Of the total 1027 patients analyzed, 515

untreated stage II and stage III CRC patients with MSI tumors showed a clearly improved rate of 5-year disease-free survival (DFS) compared with MSS tumors, whereas no such difference in 5-year DFS rate was observed in the other 512 treated stage II and stage III patients (6), which suggested that the survival benefit of patients with MSI tumors was abrogated by 5-FU treatment. However, in more than 600 stage II and stage III patients under treatment with 5-FU in the control arm of the PETACC3 study (7), we observed a statistically significant difference in the rate of 5-year DFS in patients with MSI tumors compared with MSS tumors ($P = .0077$) (7), suggesting that the improved prognosis in patients with MSI tumors was maintained under 5-FU treatment. Furthermore, we showed in another 2009 ASCO abstract (8) that the incidence of MSI differed between stage II and stage III colon cancer and the prognostic impact of MSI was substantially stronger in stage II colon cancer patients compared with stage III patients. These results suggested that prognosis should be stratified not only by treatment, but also by stage, if the current controversial data on prognosis are to be resolved. Patient series that are large enough to stratify per stage and randomized for treatment or no treatment are necessary to study prognostic vs predictive effects of MSI. Unfortunately, to date, the available datasets have not been sufficiently informative to provide unambiguous answers.

In this issue of the Journal, Sinicrope et al. (9) present a new effort at getting sufficient samples from internationally diverse adjuvant clinical trials in which CRC patients were randomized between 5-FU and no treatment or no 5-FU treatment to further explore the impact of dMMR on clinical outcomes. They elaborated the association of MMR status with CRC recurrence variables such as rate, time to recurrence (TTR), and site of recurrence (local, intra-abdominal, or distant), as well as with survival (DFS and OS). Furthermore, they examined the impact of 5-FU-based adjuvant chemotherapy on the above-mentioned recurrence variables in an attempt to provide further insights into the clinical behavior of MSI vs MSS CRC tumors that could be useful in patient management and clinical decision making.

In this study, Sinicrope et al. (9) included 1686 stage II and stage III CRC patients and their respective colorectal tissue specimens in addition to the previously published series of 457 patients (6) for analysis of an impressive total of 2141 CRC patients and specimens. This was accomplished by the inclusion of eight more adjuvant trials (10–13) and by increasing the number of CRC patients and tissue specimens from some of the previously included trials (6). Nevertheless, in spite of the impressive number of patients studied, it should be stressed that only patients with available tissue specimens from the adjuvant therapy trials were included in the analysis, thus representing a subset of the overall study cohorts. Of the 2141 CRC patients, 344 patients had MSI tumors (164 stage II and 180 stage III tumors). Although the adjuvant trials included in the analysis are rather old, run in several countries, have incomplete tissue availability, and some have comparative treatment groups, they have long periods of follow-up and meticulous data collection and reflect the international diverse adjuvant trial programs. Without any doubt, this is one of the strongest points of the current article (9).

The article (9) confirms the overall better prognosis of CRC patients harboring MSI tumors compared with MSS tumors, shown in previous studies (4–6). Indeed, more in line with what we have previously reported (7), treated and untreated patients with stage II and stage III MSI CRC tumors showed a statistically significant improvement in TTR ($P < .001$), DFS, and OS ($P = .004$) compared with patients with MSS tumors. However, when the data were analyzed by tumor stage in a univariate analysis, the association of MSI to improved outcome, although similar for stage II and stage III patients ($P_{\text{interactions}} \geq .641$), remained statistically significant only in stage III patients (TTR, $P = .016$; DFS, $P = .047$; OS, $P = .041$).

Unfortunately, Sinicrope et al. (9) did not formally compare the predictive value of MSI status for DFS or OS in patients treated in the adjuvant 5-FU-based clinical trials compared with untreated control groups in the available cohorts. The rationale for that decision, according to the authors, is that such an analysis had been performed in a previous article (6), which included a subset of the current study (9) population. In the previous article, Sargent et al. (6) observed a statistically significant improved DFS ($P = .001$) in patients with stage III MSS tumors receiving adjuvant 5-FU chemotherapy, but no treatment effect was observed in patients with stage III MSI tumors. A non-statistically significant benefit of adjuvant therapy was observed in patients with stage II MSS tumors, whereas a trend toward worse outcome was observed in patients with stage II MSI tumors (6). Given, the modest number of CRC patients with MSI tumors available for analysis, the authors were careful not to overstate the findings (6). Both accompanying editorials of this article (14,15) noted that the differences between MSS and MSI tumors and between stage II and stage III CRC patients were important and may have important implications for decisions on treatment, but the data produced did not justify exclusion of CRC patients with MSI tumors from the standard 5-FU-based adjuvant treatment. Although clinicians have been eager to implement this notion into clinical decision making, the results (6) were obviously in need of further validation by confirmation studies.

In the current article, Sinicrope et al. (9) report a benefit of 5-FU treatment for stage III CRC patients with MSI tumors, contrary to what was previously reported (5,6). However, the authors added an important new variable and studied the impact of MSI of presumed sporadic vs germline (ie, Lynch Syndrome) origin on the outcome after 5-FU-based chemotherapy. The beneficial treatment effect on TTR and DFS in stage III MSI CRC patients seemed to be restricted to tumors where MSI originated from a germline defect in one of the DNA MMR genes, whereas no benefit of 5-FU therapy was found in sporadic MSI tumors with an epigenetic origin. Unfortunately, no formal analysis of the treatment effect in stage II CRC patients with MSI tumors was performed. Furthermore, the article (9) did not provide any data on stage II MSI CRC patients, so the statistical significance of germline vs sporadic MSI in stage II colon cancer is not clear. Intuitively, this distinction makes perfect sense because of the different molecular mechanisms underpinning the MSI status of germline vs sporadic MSI tumors, and thus, if validated, would constitute a further subdivision of the MSI colon cancer patients. It should, however, be pointed out that unorthodox criteria were used to distinguish presumed hereditary vs sporadic MSI carcinomas. The used criteria were dMMR phenotype (either from MSI testing or

from MMR protein expression analyzed by immunohistochemistry), age at adjuvant study randomization (cutoff of 55 years from the modified Amsterdam criteria for Lynch Syndrome) (16,17), and BRAF mutation status (as it occurs only in sporadic dMMR CRCs) (18), but molecular genetic analysis of germline DNA (16,17) was not performed. This approach carries important potential bias, and thus the approach and its outcome have to be considered exploratory for the time being. Furthermore, the article reports a number of disturbingly quick recurrences before 6 months (and not after 1 year) in the presumed germline MSI CRC untreated patients, as shown in Figure 3, A (9), indicating a particularly bad prognosis and driving the observation of large benefit of 5-FU in this population. The worse prognosis of untreated CRC patients with presumed germline MSI tumors is unexpected and needs to be validated. On the reverse side, if indeed only the CRC patients with presumed germline MSI tumors benefit from 5-FU therapy, then CRC patients with presumed sporadic MSI tumors could be resistant to 5-FU therapy, possibly because of MMR gene hypermethylation, and this would affect the treatment paradigms for 10% of colon cancer patients. Furthermore, as noted by the authors (9), the possibility of a non–MMR-related mechanism being responsible for these findings cannot be excluded, which needs to be validated and confirmed.

This article (9) also demonstrates an association between the site of tumor recurrence, MMR status, and the outcome of adjuvant chemotherapy for the first time, and shows that CRC patients with MSI tumors had overall lower rates of tumor recurrence compared with MSS tumors, notably as regards recurrence at distant sites ($P < .001$). When analyzed by stage, the association between MMR status and site of recurrence was found in stage III and not in stage II patients. In addition, in patients with MSS stage III tumors, 5-FU-based adjuvant treatment vs observation (surgery alone) or no 5-FU therapy was associated with a lower number of distant recurrences for patients with MSI tumors ($P = .01$) and any recurrence ($P < .001$) (9).

This study (9) represents a valid opportunity to address the burning questions remaining after the study by Sargent et al. (6): 1) did lack of benefit of 5-FU therapy in stage III MSI CRC patients represent overtreatment and 2) was treatment of stage II MSI CRC patients with 5-FU harmful? Both issues have important clinical implications, and both tentative conclusions from the study by Sargent et al. (6) showing a lack of effect for stage III MSI CRC or harm for stage II MSI CRC patients could have represented spurious findings because of sample size (6), and urgently needed validation. In a multivariable analysis, Sinicrope et al. (9) showed MMR status to be a statistically significant independent prognostic variable in patients with MSI tumors, with an improved TTR ($P = .005$), DFS ($P = .035$), and OS ($P = .031$) compared with those with MSS tumors. However, despite analyzing a large number of new CRCs, the authors did not perform a stratified analysis of treatment effect by treatment group or by stage.

In conclusion, the results of this article by Sinicrope et al. (9) provide valuable information about the prognostic and certainly the predictive role of dMMR, including the intriguing difference between germline vs sporadic origin of dMMR, highlighting that sometimes the mechanism responsible for an event reflects more than the event itself. This hypothesis-generating information in the current analysis needs to be validated in an independent dataset. It is important to consider whether these results are sufficient to validate MMR assessment as a prerequisite for selecting CRC patients for adjuvant therapy.

From a practical point of view, on one hand, the data provided in this study (9) are important and reassuring to the clinicians because the lack of benefit of 5-FU therapy in stage III MSI tumors (5,6) could not be replicated. On the other hand, from an academic point of view, it is frustrating that even in studies with such large patient populations, questions such as the different impact of MSI status between stage II and stage III CRC patients and the potential harm in stage II CRC MSI patients after 5-FU treatment remain unanswered. Although hypothesis-generating data on the relationship between MMR status and relapse site, as well as data on the difference between germline and somatic MMR deficiencies are now available (9), the puzzle still has many missing pieces. In the future, other parameters, such as gene expression profiling, ploidy, methylation, and microRNA assessment, will probably have to be taken into account to robustly characterize clinically relevant biological subgroups. To get there, large datasets will be needed for prognostic and predictive studies. We may not have banked enough tissue samples in the past to ever get the answers that we need. Hopefully, in recent trials such as PETACC8 (19) and NCT00079274 (20–22), where the addition of cetuximab to the current standard adjuvant combination therapy of 5-FU, leucovorin, and oxaliplatin (FOLFOX) is elaborated in stage III CRC patients, the tissue banking performed will help us find the biological subgroups that will benefit from the standard and combination chemotherapy regimens.

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