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# Reply to Jaskowski *et al*

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Jaskowski *et al*[1] have confirmed our previous paper[2] reporting an association between *BAT26* stability and germline *MSH2* deletion. Among seven highly unstable tumours with *MSH2* deletion spanning exon 5 they found normal *BAT26* sequences in 4. Although their analysis was based on a small number of tumours, they observed this phenomenon in 57% of cases, a percentage very close to the 68% we obtained in the analysis of 19 tumour DNAs.[2] It is reasonable to suppose that the molecular mechanism leading to somatic loss of *BAT26* sequences, as we described previously,[2] is responsible for the observed *BAT26* stability in both subsets of *MSH2* deleted tumours.

In our current dataset including 29 *MLH1*- and 49 *MSH2*-mutated tumours (27 of which retaining exon 5), only two additional cases displayed *BAT26* stability; these were from two patients who were heterozygous for point mutations in *MSH2* and *MLH1*, respectively. On the contrary, in the study by Jaskowski *et al*[1] 6/48 (12.5%) samples from patients with *MSH2* germline mutations with exon 5 retention and only one out of 53 (1.8%) samples from *MLH1* mutation carriers were found to be stable at *BAT26*. A different prevalence of *BAT26* instability was also detected in a series of moderate–high-risk tumours, for which distinction between *MLH1*- and *MSH2*-related cases was based on immunohistochemistry alone. In the hereditary tumours such differential *BAT26* stability was associated with an overall lower degree of instability in *MSH2* compared to *MLH1*-related cancers (69.9 and 81.5% unstable markers, respectively).

On the other hand, extrapolating data from some previous studies on different series of unstable tumours with *MLH1* and *MSH2* defects and considering only the markers belonging to the reference NCI panel, the average percentage of instability was 92 and 79.4%,[3] 92.3 and 86.8%[4], 80 and 72%,[5] for *MLH1* and *MSH2* tumours, respectively. Nonetheless, despite the constant slight instability excess of *MLH1* tumours, statistically significant differences could not be highlighted, even when these data were pooled (89.8 and 80.1%; $P=0.08$, Fisher's exact test).

We also analyzed in more detail our MSI data on a total of 78 unstable tumours with unequivocal test results (among which 61 were colorectal adenocarcinomas); in this series, we could not find a higher instability in the *MLH1* group. In fact, despite the inclusion of 14 samples with germline *MSH2* intragenic deletion and wild-type

*BAT26* sequences, the average number of unstable markers per tumour was 79.6% in 29 *MLH1*-deficient tumours and 83.6% in 49 *MSH2*-deficient tumours ($P=0.38$, Fisher's exact test). Similar figures were obtained when the analysis was restricted to the 23 *MLH1*- and 38 *MSH2*-mutated unstable colorectal carcinomas only (78.3 and 86.8%; $P=0.1$, Fisher's exact test).

Histopathological features and mutational spectra of the tumours analyzed could account for the discrepancies between ours and Jaskowski *et al*'s[1] data.

In conclusion, we welcomed additional evidences of the limited usefulness of *BAT26* alone for the detection of MMR deficiency, and we read with interest this letter suggesting that *MSH2* mutation carriers have increased *BAT26* stability overall, compared to *MLH1* mutation carriers. It is noteworthy that while our study evidenced a strong association between *BAT26* stability and exon 5 *MSH2* loss, Jaskowski *et al*'s[1] data showed that the absence of *BAT26* instability is indicative of a generic *MSH2* mutation. If further confirmed, this could represent an additional element to properly address mutational analyses. It is unclear whether the observed *BAT26* stability is a consequence of a lower overall degree of instability in the *MSH2*-mutated tumours or it is a locus-specific molecular phenomenon connected with the presence of *BAT26* in the *MSH2* gene. It will be very interesting to investigate this issue in more detail, for instance by analyzing several mono- and di-nucleotides on additional larger series, to verify the proportion of markers that escape instability in *MSH2*-deficient tumours.

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