# Comment on 'Distinct clinical outcomes of two CIMP-positive colorectal cancer subtypes based on a revised CIMP classification system'

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#### Sir,

We read with interest the article by Bae *et al* (2017), where they proposed a revised CpG island methylator phenotype (CIMP) classification system for colorectal cancer (CRC) based on the methylation level, which was found to correlate with molecular alterations and the corresponding prognostic implications of CIMP-positive CRCs. They categorised CIMP-positive CRCs as CIMP-P1 or CIMP-P2 based on the number of methylated markers to better associate with clinicopathological and molecular features. Moreover, they have also underlined that CIMP-P1 CRCs should be more aggressive than CIMP-N and CIMP-P2 CRCs. They analysed a total of 1370 CRC patients for this new revised CIMP classification (Bae *et al*, 2017).

Since a few years ago, we are trying to characterise specific subgroups within CRC, some of them according to the age of onset of the disease, and others focusing on the development of multiple primary tumours. To date, these subset subgroups appear to be different in comparison with others CRCs (Kirzin *et al*, 2014; Lam, Chan and Leung, 2014; Pajares and Perea, 2015; Arriba *et al*, 2017). We have confirmed this finding, studying clinicopathological, familial and molecular points of view, and compared all the features found between those groups and with other sporadic CRCs subsets.

Taking as the starting point the work by Bae *et al* (2017), we have analysed the same aspects described in their work, but differentiating patients with early-onset CRC (EOCRC; younger than 45 years), patients with late-onset CRC (LOCRC; older than 70 years), and individuals diagnosed with synchronous CRC (SCRC), that did not encompass cases with familial adenomatous polyposis or Lynch syndrome (in order to study more strictly SCRC with still unknown molecular basis). The methods used to analyse the microsatellite instability (MSI) status, CIMP and the other molecular features as well as the clinicopathological variables described, have been published before (Perea *et al*, 2014; Arriba *et al*, 2017).

First, Bae et al (2017) linked the proportions of molecular alterations in relation to the number of methylated genes. The molecular characteristics of CRCs were not different between CRCs with no methylated genes and CRCs with four methylated genes. However, the frequency of alterations associated with the serrated neoplasia pathway, such an MSI-high status and MLH1 methylation, was moderately increased in CRCs with five or six methylated genes compared with CRCs with less than five methylated genes. CRCs with seven or eight methylated genes lacked KRAS mutations, had a high rate of BRAF mutations, and had MSI-high and positive MLH1 methylation statuses (Bae et al, 2017). Our results, particularly focused on MLH1 methylation, MSI status, and BRAF mutations are shown in Figure 1. Unexpectedly, only LOCRC cases showed the same increasingly high rates for the three features according to the number of methylated genes. Moreover, EOCRC did not show any similarity, and SCRC only did, in relation with MLH1 methylation status. Nonetheless, there were also SCRC cases with a low rate of methylated genes showing it, as well.

With their refined CIMP classification system, CRCs from 1287 (93.9%), 62 (4.6%) and 21 (1.5%) patients were classified as CIMP-N, CIMP-P1 and CIMP-P2, respectively. In our three CRC subsets, EOCRC appeared to have the most similar proportions: 84%, 10% and 6%, respectively. LOCRC showed slight differences, with 74%, 17% and 9%. Finally, on the contrary appeared SCRC cases, with 37%, 4% and 18%; the other 41% arose for the CIMP-MM (Mismatch), where the tumours within the same patient show different CIMP status, as previously described (Arriba et al, 2017). Bae et al (2017) also tested the correlation between this revised CIMP classification and the pathological and molecular features. They associated patients with CIMP-P1 CRCs with proximal location, more advanced stage, poorly differentiated tumour and greater mucin production, compared with CIMP-N. In addition, CIMP-P2 CRCs showed some of those differential features as well (e.g., mucin production and proximal colon location; Bae et al, 2017). Some conclusion should be given according to the clinical and molecular features associated with CIMP categories, when we analysed our different age-of-onset groups and SCRC. In relation with EOCRC, patients with CIMP-P1 and CIMP-P2 CRCs showed higher proportions of proximal location (P = 0.013). Rendering familial cancer history, while CIMP-P1 were mainly sporadic cases, CIMP-N showed some predisposition for familial cancer aggregation, and CIMP-P2 mainly fulfilled Amsterdam criteria for Lynch syndrome. MLH1 methylation was mainstream in this last category. In the case of LOCRC, patients with CIMP-P1 CRCs were associated with the same molecular features described by Bae et al (2017; BRAF mutations, MSI cases and high MLH1 methylation). Results for patients diagnosed with SCRC showed that CIMP positive groups (CIMP-P1, CIMP-P2 and CIMP-MM) showed more MLH1 methylation than CIMP-N (p < 0.0001).

The findings shown by Bae *et al* (2017) according to the prognostic results are confirmed in our subsets only partially for patients with SCRC, in which the worst prognosis subgroup was also CIMP-P1, but CIMP-P2 subtype showed worse prognosis than CIMP-MM and CIMP-N (p = 0.002). On the other hand, within EOCRC and LOCRC groups, CIMP-P2 showed the best prognostic results, being within EOCRC, CIMP-N the worst category, while within LOCRC CIMP-N and CIMP-P1 equivalent, although these results did not reach statistical significance.

Our results suggest again the importance of taking into account some criteria, as age-of-onset or multiple primary neoplasms, when analysing CRC. In this direction, LOCRC seems to be the most comparable subset to the results showed by Bae *et al* (2017). Maybe this could explain the difficulty of achieving a consensus about CIMP classification and the correlation with clinical and molecular phenotypes (Ogino *et al*, 2009; Lee *et al*, 2017).

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Figure 1. Molecular characteristics of colorectal cancers in relation to the number of methylated genes. Marked in blue, cases with 0-4 methylated genes; in green, cases with 5-6; in red, cases with 7-8 methylated genes. For the definition of MLH1 methylation cases within SCRC, tumours showing MLH1 methylation were counted individually, whether or not were in the same individual. MSI: Microsatellite Instability. EOCRC: Early-onset colorectal cancer. Abbreviations: LOCRC = Late-onset colorectal cancer; SCRC = Synchronous colorectal cancer. A full colour version of this figure is available at the *British Journal of Cancer* journal online.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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