BJC

British Journal of Cancer (2018) 118, e3 | doi: 10.1038/bjc.2017.343

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

Sandra Tapial[1], Daniel Rueda[1,2], María Arriba[3], Juan Luis García[4], Lorena Brandáriz[5], Jessica Pérez[4], Yolanda Rodríguez[6], Damián García-Olmo[5], Rogelio González-Sarmiento[4], Miguel Urioste[7,8] and José Perea*[,5]

[1]Digestive Cancer Research Group, 12 de Octubre Research Institute, Madrid 28041, Spain; [2]Molecular Biology Laboratory, Department of Biochemistry, 12 de Octubre University Hospital, Madrid 28041, Spain; [3]Department of Biochemistry, Gregorio Marañón University Hospital, Madrid 28009, Spain; [4]Molecular Medicine Unit, Biomedical Research Institute of Salamanca (IBSAL), Institute of Molecular and Cellular Biology of Cancer (IBMCC), University of Salamanca-USAL-CSIC, Salamanca 37007, Spain; [5]Department of Surgery, Fundación Jiménez Díaz University Hospital, Madrid 28040, Spain; [6]Department of Pathology, 12 de Octubre University Hospital, Madrid 28041, Spain; [7]Familial Cancer Clinical Unit, Spanish National Cancer Centre (CNIO), Madrid 28029, Spain and [8]Center for Biomedical Network Research on Rare Diseases (CIBERER), Institute of Health Carlos III, Madrid 28029, Spain

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).

*Correspondence: J Perea; E-mail: josepereag@hotmail.com
Published online 2 January 2018

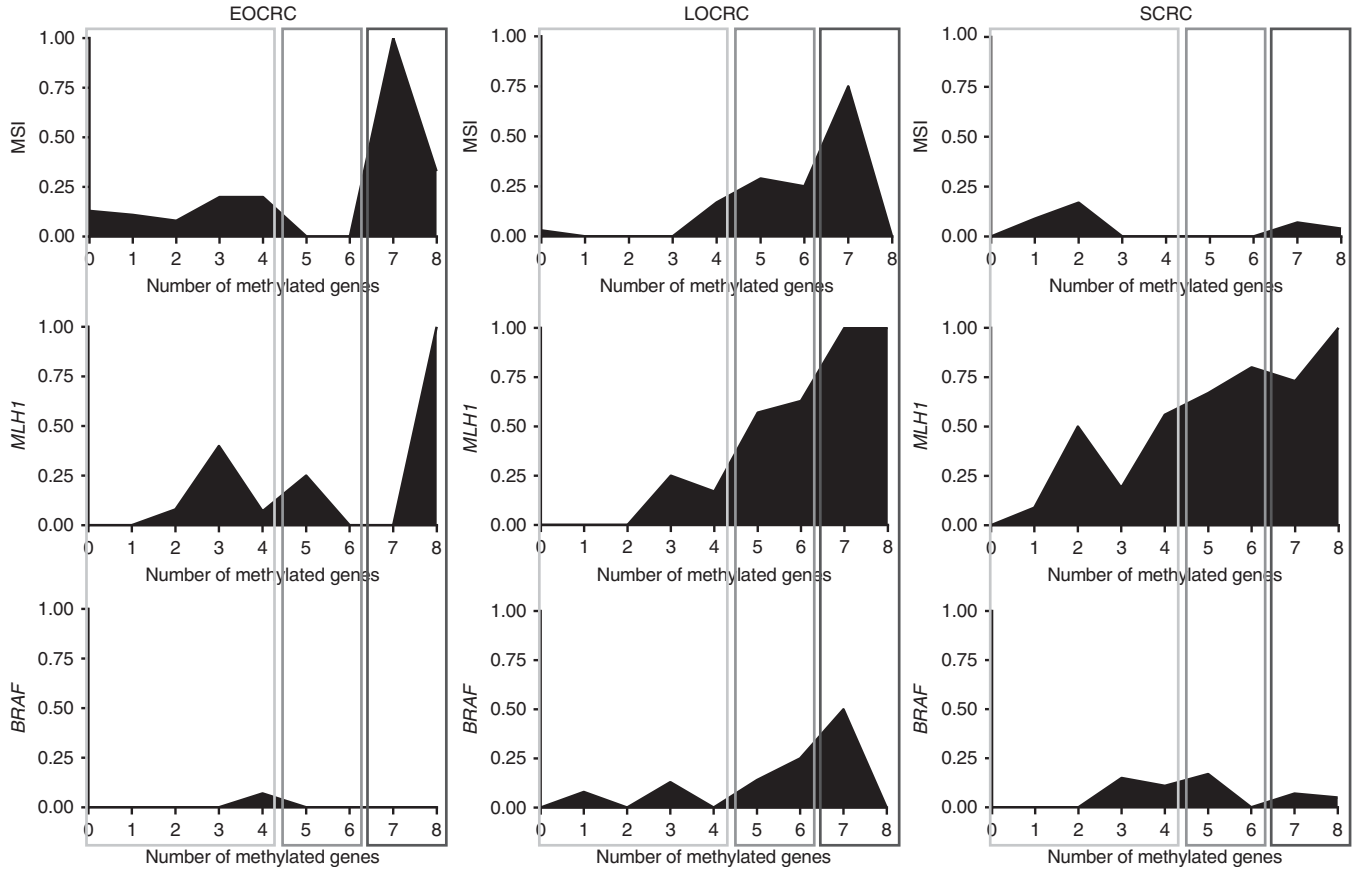

Figure 1. Molecular characteristics of colorectal cancers in relation to the number of methylated genes. Marked in blue, cases with 0-4 methylated genes; in green, cases with 5-6; in red, cases with 7-8 methylated genes. For the definition of MLH1 methylation cases within SCRC, tumours showing MLH1 methylation 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.

ACKNOWLEDGEMENTS

This work was funded by Projects PI13/01741, PI13/01273, PI16/01920 and PI16/01650 from the Spanish Ministry of Health and Consumer Affairs and FEDER, and Mutua Madrileña Foundation (2012-0036), and was approved by the Ethics Committee of our Institution. We thank the Tumor Registry of the Pathology Department of the 12 de Octubre University Hospital for providing us with paraffin-embedded tissues.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Arriba M, Sánchez R, Rueda D, Gómez L, García JL, Rodríguez Y, Pajares JA, Pérez J, Urioste M, Sarmiento RG, Perea J (2017) Toward a molecular classification of synchronous colorectal cancer: clinical and molecular characterization. *Clin Colorectal Cancer* **16**: 31–37.

Bae JM, Kim JH, Kwak Y, Lee DW, Cha Y, Wen X, Lee TH, Cho NY, Jeong SY, Park KJ, Han SW, Lee HS, Kim TY, Kang GH (2017) Distinct clinical outcomes of two CIMP-positive colorectal cancer subtypes based on a revised CIMP classification system. *Br J Cancer* **116**: 1012–1020.

Kirzin S, Marisa L, Guimbaud R, De Reynies A, Legrain M, Laurent-Puig P, Cordelier P, Pradère B, Bonnet D, Meggetto F, Portier G, Brousset P, Selves J (2014) Sporadic early-onset colorectal cancer is a specific sub-type of cancer: A morphological, molecular and genetics study. *PLoS One* **9**: e103159.

Lam AKY, Chan SSY, Leung M (2014) Synchronous colorectal cancer: Clinical, pathological and molecular implications. *World J Gastroenterol* **20**: 6815–6820.

Lee DW, Han SW, Cha Y, Bae JM, Kim HP, Lyu J, Han H, Kim H, Jang H, Bang D, Huh I, Park T, Won JK, Jeong SY, Park KJ, Kang GH, Kim TY (2017) Association between mutations of critical pathway genes and survival outcomes according to the tumor location in colorectal cancer. *Cancer* **123**: 3513–3523.

Ogino S, Nosho K, Kirkner G (2009) CpG island methylator phenotype, microsatellite instability, BRAF mutation and clinical outcome in colon cancer. *Gut* **58**: 90–96.

Pajares J, Perea J (2015) Multiple primary colorectal cancer: Individual or familial predisposition? *World J Gastrointest Oncol* **7**: 434–444.

Perea J, Rueda D, Canal A, Rodríguez Y, Álvaro E, Osorio I, Alegre C (2014) Age at onset should be a major criterion for subclassification of colorectal cancer. *J Mol Diagnostics* **16**: 116–126.