PostScript

LETTERS

Authors' reply

In my commentary (*Gut* 2006;55:759-61), that accompanied Jover *et al*'s paper (*Gut* 2006;55:848-55), we indicated several important issues regarding colon cancer microsatellite instability-high (MSI-H) and the influence of fluorouracil-based (FU) chemotherapy on patient survival that Iacopetta and Watanabe (*Gut* 2006;55:1671-2) raise in their letter, some of which need to be clarified. Most important is the separation of improved survival due to a patient's tumour microsatellite instability status from improved survival due to FU treatment. A comparison of patients with MSI-H colon tumours who received chemotherapy and patients with MSI-H tumours who did not receive chemotherapy is the only way to separate out the influence of FU on survival in this group. This is because patients with MSI-H tumours already have an improved survival over those with microsatellite stable (MSS) tumours,¹ and most earlier publications compared MSI-H/FU+ with MSS/FU- or MSS/FU+ patients. In the papers that Iacopetta and Watanabe mention,^{2,7} a direct comparison of MSI-H/FU- to MSI-H/FU+ is lacking. In patients with stage IV colon cancer,³ the authors never compared patients with MSI-H/FU- with those with MSI-H/FU+ statistically, but the Kaplan-Meier curves completely overlap between these two groups, indicating no benefit of 5-FU therapy for patients with stage IV MSI-H (who normally have poor survival). However, in two retrospective studies^{8,9} and two recent prospective studies,¹⁰ direct comparisons were made between patients with MSI-H/FU- and those with MSI-H/FU+, all with the same results indicating no survival advantage for patients with stage II or III MSI-H receiving FU-based chemotherapy. Indeed, in one of the prospective articles indicated by Iacopetta and Watanabe,¹⁰ the survival difference in patients with stage II or stage III MSI-H receiving FU chemotherapy compared with those who did not receive FU was not significant ($p>0.15$), indicating no benefit from FU in this group. This is again highlighted in Jover *et al*'s paper, for which the probability of survival of patients with MSI-H tumours was not statistically different between those who received FU and those who did not ($p>0.40$).

Iacopetta and Watanabe raise an additional interesting point. The influence of other molecular defects, such as hypermethylation of *hMLH1*, or the presence of *BRAF* mutations, both of which are present in patients with sporadic MSI-H colorectal cancers but are absent in patients with Lynch syndrome, may be important in determining FU response. This is intriguing, but is largely untested scientifically. If we view colorectal cancers as developing and propagating from different forms of genomic instability, sporadic MSI-H tumours are probably a subset of the CpG island methylator phenotype (CIMP) form as *hMLH1* is one targeted gene for methylation and silencing of its promoter, and there may be broader consequences that extend beyond loss of DNA mismatch repair

deficiency with the CIMP phenotype. However, a retrospective analysis of 92 patients with Lynch syndrome failed to show a survival advantage with FU compared with those who did not receive FU,¹¹ exactly the findings from the four articles on sporadic colorectal cancer in which comparisons were made between patients with MSI-H/FU- and those with MSI-H/FU+.⁸⁻¹⁰ Additionally, there is now evidence from two independent laboratories that DNA mismatch repair proteins themselves can bind FU-altered DNA to potentially trigger cell death, suggesting a direct interaction.^{12,13} Thus, loss of mismatch repair function, typically indicated by the presence of MSI-H in colorectal tumours, could be a direct mechanism to circumvent the intended cancer cell killing by FU, regardless of how mismatch repair function is lost.

These data form the basis for our recommendation that there is a need to re-evaluate the current chemotherapeutic approach to patients with MSI-H tumours. In addition to a patient's tumour stage, the biology and genomic instability present in the tumour may influence the outcome of patients and their response to certain chemotherapies.

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Authors' reply

We thank Iacopetta and Watanabe for their interest in our paper (*Gut* 2006;55:1671-2). We do not agree, however, with their conclusions. There is important evidence in the recent literature that supports a relative lack of efficacy for 5-fluorouracil (5-FU) in patients with mismatch repair (MMR)-deficient tumours, with several studies reaching similar conclusions.¹⁻⁴ In our study, we did not find major differences in patients with MMR-deficient tumours in survival or disease-free survival regarding adjuvant treatment, especially when we compared those results with the ones obtained in patients with MMR-proficient tumours. Although we acknowledge that we had only 53 patients with MMR-deficient tumours, our results are in line with findings from at least four different groups,¹⁻⁴ and we believe that there is enough evidence to consider a revision in the treatment of colorectal cancer in this subgroup of patients. We are not saying that 5-FU should not be used as an adjuvant treatment in patients with stage III MMR-deficient tumours, but that there is enough evidence to consider the use of other chemotherapeutic agents (such as capecitabine or oxaliplatin) in the treatment of these patients. We believe that randomised clinical trials stratified in function of MMR status comparing 5-FU and other chemotherapeutic drugs should be carried out.

The well-established good prognosis of MMR-deficient tumours⁵ could be a confounding factor in studies that evaluate the efficacy of adjuvant chemotherapy, especially if the efficacy of 5-FU in MMR-deficient tumours is not compared with a non-treated group. As we pointed out in our discussion, the studies that initially found a benefit from adjuvant 5-FU in tumours with microsatellite instability (MSI) did not take into account the prognosis of patients who did not receive adjuvant chemotherapy. Therefore, the apparent good response could have been due to the intrinsic good prognosis of MMR-deficient tumours. To reach a consistent conclusion about the efficacy of this treatment, we have to compare the prognosis of MSI tumours in patients who are treated and not treated. Every single study that used this approach found a clear benefit from adjuvant 5-FU treatment in patients with stable tumours but not in patients with MMR-defective tumours.

We agree with Iacopetta and Watanabe about the possible molecular heterogeneity of

MSI tumours and also think that we should spare no efforts in trying to understand the molecular basis of the response to chemotherapy in that kind of tumour. The importance of DNA methylation in prognosis and chemotherapy response is not completely known, and, in our opinion, research in that area is scarce and inconclusive. In our own and other published studies, most of the tumours that show a lack of efficacy of 5-FU in MMR-deficient tumours are sporadic (and therefore with MSI caused by hypermethylation of the promoter region of *MLH1*), suggesting that the lack of efficacy of adjuvant 5-FU on MSI tumours could happen in both hereditary and sporadic MSI tumours. Further studies on the role of hypermethylation in prognosis and chemotherapy response in colorectal cancer are under way in our series. Soon, we will be able to report the 5-year follow-up analysis in these series, which will bring forth new and interesting data on this topic.

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Paraoxonase 1, 2 and 3 DNA variants and susceptibility to childhood inflammatory bowel disease

Inflammatory bowel disease (IBD) is characterised by an imbalance between pro-oxidant and anti-oxidant mechanisms. Cellular detoxification systems in IBD seem unable to adequately control the amplified generation of reactive oxygen species, put a stop to free radical injury and repair damaged cellular elements, suggesting that an increase in oxidative stress might be involved in the pathogenesis of IBD,^{1,2} although the underlying molecular mechanisms remain largely undefined. Human paraoxonase family members (PON1, PON2, PON3) possess anti-oxidant and anti-inflammatory properties.^{3–5} Despite the large impact of genetic variation on PON activity, its important role in controlling oxidative stress and inflammation,^{5–8} as well as its association with several pathophysiological conditions,⁴ no

information is available on the relationship between PON genetic variants and IBD.

Here, we wanted to assess whether the genotype or haplotype distribution of PON polymorphisms was different in patients with IBD during childhood compared with healthy controls. Eight common variants in PON1, PON2 and PON3 were genotyped by polymerase chain reaction allele-specific oligonucleotide hybridisation assays in 192 incident cases of childhood IBD (mean SD age of onset 11.03 (3.9) years) diagnosed and treated at Ste-Justine Hospital (Montreal, Ontario, Canada) and in 92 controls, all of French-Canadian origin. In PON1, three promoter polymorphisms (–107 C→T, –824 A→G and –907 C→G) and two coding polymorphisms (L55M and Q192R) were genotyped. Although their distribution did not differ significantly between patients with IBD and controls, overall the frequency of the 192R (ex6 +78G) allele that seems to alter the structure of PON1 and influence enzyme activity in a substrate-dependent manner⁹ was consistently higher in patients with Crohn's disease (table 1). The structures of 11 haplotypes in French-Canadians, including seven with a frequency >7%, were inferred from the genotypic frequencies of the five PON1 variants (table 2). No significant differences were observed in the distribution of common haplotypes between patients with IBD and controls. The assessment of two linked PON2 polymorphisms (A148G and S311C) showed that people homozygous for 148GG and 311CC were less frequently found in patients with IBD than in controls (52% v 69%) suggesting a protective effect (odds ratio 0.5, 95% confidence interval 0.3 to 0.8, p = 0.011) against childhood IBD (table 3). No difference was observed in the frequency of PON2 variants in relation to the patient's characteristics (data not shown). The physiological role of PON2 is still unknown, but it was suggested that it may exert its anti-oxidant role by decreasing intracellular oxidative stress.³ Hence, it is plausible that subsequent to exposure to toxic

Table 1 Genotype distribution of paraoxonase 1 polymorphisms

Position	Controls (%)	IBD (%)	OR (95% CI)	CD (%)	OR (95% CI)	UC (%)	OR (95% CI)	IC (%)	OR (95% CI)
–907 G→C									
GG	19 (21)	37 (20)	0.97 (0.5 to 1.8)	26 (20)	0.93 (0.4 to 1.8)	6 (40)	2.4 (0.7 to 7.7)	5 (16)	0.7 (0.2 to 2.0)
GC	45 (50)	104 (59)		76 (59)		5 (33)		22 (71)	
CC	25 (28)	36 (20)		27 (20)		4 (27)		4 (13)	
–824 A→G									
AA	5 (6)	13 (7)	1.3 (0.4 to 3.8)	8 (6)	1.0 (0.3 to 3.5)	2 (13)	2.5 (0.4 to 14)	3 (10)	1.8 (0.4 to 8.0)
AG	42 (47)	83 (47)		64 (50)		4 (27)		15 (48)	
GG	42 (47)	80 (45)		56 (44)		9 (60)		13 (42)	
–107 C→T									
CC	22 (25)	44 (25)	1.0 (0.5 to 1.8)	33 (26)	1.0 (0.5 to 1.9)	6 (40)	2.0 (0.6 to 6.3)	5 (16)	0.5 (0.2 to 1.7)
CT	45 (50)	101 (57)		71 (55)		7 (47)		22 (71)	
TT	22 (25)	32 (18)		25 (19)		2 (13)		4 (13)	
Ex3+18T→A (L55M)									
TT	34 (38)	70 (38)	0.9 (0.4 to 2.0)	53 (39)	0.8 (0.3 to 1.9)	8 (53)	1.7 (0.4 to 7.3)	9 (29)	0.7 (0.2 to 3.0)
TA	45 (50)	93 (50)		69 (51)		4 (27)		19 (61)	
AA	11 (12)	22 (12)		14 (10)		3 (20)		3 (10)	
Ex6+78 A→G (Q192R)									
AA	46 (52)	81 (46)	1.1 (0.4–2.6)	51 (40)	1.3 (0.6–3.1)	10 (67)	0.6 (0.07–5.3)	20 (65)	0.6 (0.1 to 2.9)
AG	33 (38)	74 (42)		60 (47)		4 (27)		9 (29)	
GG	9 (10)	20 (11)		17 (13)		1 (7)		2 (6)	

CD, Crohn's disease; IBD, inflammatory bowel disease; IC, indeterminate colitis; UC, ulcerative colitis. The oligonucleotides and polymerase chain reaction assay conditions are available on request.