Figure 1 Assessment of tumour response to imatinib. (A) Initial evaluation by endorectal ultrasonography: four masses in the anterior and left wall of the rectum, located just above the anal sphincter (sum of the two major lesions 11.1 cm²); hypoechoic, solid, and with regular contour. (B) Evaluation after nine months of treatment: achievement of maximal response to treatment. The four masses are still identifiable but are considerably smaller (sum of the same two lesions 1.8 cm²). On the left side (3 o'clock) is the dominant, still hypoechoic, one, and the others, keeping the solid appearance, are now hyperechoic. All have regular and well defined margins. (C) Morphology of gastrointestinal stromal tumour from the rectum before treatment with imatinib. Haematoxylin-eosin stain of local recurrence (biopsy); spindle cell neoplasm with high cellularity, no necrosis, and 5 mitosis/10 high power fields. (D) CD117 positive immunostaining (biopsy) before treatment with imatinib. (E) Haematoxylin-eosin stain (surgical specimen) after treatment with imatinib: four adjacent nodules entirely composed of hyaline connective tissue, with no identifiable tumour cells. CD117 and CD34 were negative.

GIST, first operated on 13 years earlier, and who now presented with four adjacent masses located in the rectovaginal septum and no distant metastasis. Molecular analysis identified a 6 bp deletion in exon 11 of the *c-kit* proto-oncogene. As curative surgery would have implied abdominoperineal resection with permanent colostomy, treatment with imatinib at a standard dose (400 mg/day) was started. Response to treatment was evaluated using endorectal ultrasound and magnetic resonance imaging every three months. Quantification of tumour mass was obtained by adding the products of two perpendicular axes of the two major lesions. Maximal response was obtained at nine months, which was maintained after 12 months of treatment. At that time, total tumour mass reduction was 83%, which was enough to allow rectal resection with a coloanal anastomosis (fig 1A, B). Pathological analysis of the surgical specimen identified four white firm masses with diameters ranging from 1 to 2 cm. These lesions were composed of hyaline connective tissue, which did not stain with either CD34 or CD117, and no viable tumour cells were identified in the whole specimens (fig 1C–E). Imatinib was suspended and after 24 months of follow up there is no evidence of local or distant recurrence. This is, to our knowledge, the first report of a complete pathological response of a GIST treated with imatinib.

Most GISTs have been shown to have activating mutations of the *c-KIT* or PDGF receptor alpha (PDGFRA) proto-oncogenes which result in constitutive activation of a tyrosine kinase.^{2,3} The main treatment for GIST is, whenever possible, based on surgical resection, as these tumours are resistant to chemotherapy and radiation therapy. For tumours which are not amenable to surgical resection, a selective tyrosine kinase inhibitor, imatinib (Glivec; Novartis, Basel, Switzerland), is actually the firstline treatment as it specifically inhibits most *c-KIT* and PDGFRA activated proteins. Preliminary results using imatinib treatment were very satisfactory, with greater than 50% reduction of tumour mass reported in 54% of cases.⁴

Although to date no data support the use of imatinib as neoadjuvant therapy, the last consensus meeting for the management of GISTs⁵ suggests that this form of therapy may be used for tumours with certain locations (for example, rectum, or oesophagus), when function sparing surgery is the goal.

Our case report is consistent with a recently published multicentre randomised trial involving 946 patients who received imatinib for local, advanced, or metastatic GIST.⁶ In this study, the authors observed that the time needed to achieve maximal response was 4–6 months, thereby recommending that studies on neoadjuvant therapy should be designed with a duration of treatment ranging from 4 to 6 months, which could be extended up to one year. The present report further supports these recommendations, mainly in cases with typical activating mutations and in those in whom surgery would lead to a marked loss of organ function.

M Salazar

Department of Gastroenterology, Instituto Português de Oncologia Francisco Gentil, Lisbon, Portugal

A Barata

Department of Surgery, Instituto Português de Oncologia Francisco Gentil, Lisbon, Portugal

S André

Department of Pathology, Instituto Português de Oncologia Francisco Gentil, Lisbon, Portugal

J Venâncio

Department of Radiology, Instituto Português de Oncologia Francisco Gentil, Lisbon, Portugal

I Francisco

Department of Molecular Biology, Instituto Português de Oncologia Francisco Gentil, Lisbon, Portugal

M Cravo, C Nobre-Leitão

Departments of Gastroenterology and Molecular Biology, Instituto Português de Oncologia Francisco Gentil, Lisbon, Portugal

Correspondence to: Dr M Salazar, Serviço de Gastroenterologia, IPOFG-CROL, SA, R. Prof Lima Basto, 1099-023 Lisboa, Portugal; msalazar@netcabo.pt

doi: 10.1136/gut.2005.086744

Conflict of interest: None declared.

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Germline epimutations of APC are not associated with inherited colorectal polyposis

We have recently reported that germline epimutations of the mismatch repair gene, *MLH1*, produce a clinical phenotype consistent with hereditary non-polyposis colorectal cancer (HNPPC).¹ *MLH1* germline epimutations have now been reported in eight individuals clinically classified as having HNPPC and in whom no sequence mutations of the mismatch repair genes were identified.^{1–4} These manifest as soma-wide mono-allelic hypermethylation of the promoter (in the absence of a sequence mutation within the gene) and cause transcriptional silencing of the epimutant allele.¹ These cases provide evidence that germline epimutations represent a novel mechanism for disease, which can mimic that of a genetic mutation by conferring a similar phenotype. We hypothesised that germline epimutations may occur in genes other than *MLH1* to cause other cancer prone syndromes apart from HNPPC. The *APC* gene represented a likely candidate as heterozygous inactivating mutations of *APC* are strongly associated with colorectal polyposis and cancer, but not all individuals harbour germline sequence mutations. Autosomal dominant familial adenomatous polyposis (FAP) is caused by heterozygous sequence mutations of the *APC* gene,⁵ and a recessive form is associated with biallelic mutations of *MYH*.⁶ However, no mutations of either gene are identifiable in approximately 20% of cases. Furthermore, somatic methylation of the CpG island encompassing the *APC*-1A promoter is observed in colorectal carcinomas that do not express APC, indicating that this promoter is susceptible to epigenetic inactivation.^{7–9}

To determine whether germline epimutation of the *APC* gene contributes to the development of the polyposis syndromes, we screened probands with a clinical diagnosis of FAP, attenuated FAP, or hyperplastic polyposis (HPS), for methylation of the *APC*-1A promoter. Methylation of the major 1A promoter is observed in up to 20% of colorectal

Figure 1 Methylation analysis of the APC promoter. (A) Map of the APC-1A promoter. The transcription start site of APC exon 1 (grey box) is at nucleotide +1. The position of the primers used to amplify a 229 bp fragment of the promoter upstream of exon 1 is shown by arrows. Individual CpG dinucleotides are denoted by vertical black bars. The diagnostic restriction enzyme sites that cleave specific CpG sites when methylated are indicated. (B) Gel showing an example of combined bisulfite and restriction analysis of APC-1A promoter. L, pUC19/MspI DNA ladder; C+, in vitro methylated genomic DNA shows cleavage of the methylated amplicons with RsaI to give a digestion pattern of 162 and 67 bp (and 141, 60, and 28 bp for TaqI, not shown); C-, unmethylated genomic DNA from a normal control individual remains undigested. Wells labelled 1–9 contain peripheral blood DNA from nine familial adenomatous polyposis subjects, each negative for APC-1A methylation.

carcinomas, but no methylation changes have been detected at the 1B promoter from which a minor transcript is derived, and so this latter promoter was not analysed.^{7–9} With the approval of the Human Research and Ethics Committee of St Vincent's Hospital, this study enrolled 140 individuals with colonic polyps from family cancer clinics in Australia and the UK. None of the individuals harboured pathogenic germline sequence mutations in the APC gene, and biallelic MYH mutations had been excluded in all but 10 cases. Of the 140 individuals, 22 were diagnosed with HPS (mean age 60.5 (9.6) years), 29 with FAP (mean age 27.8 (10.5) years), and 89 with attenuated FAP (mean age 59 (18.6) years).

Probands were screened for hypermethylation of the APC-1A promoter in peripheral blood DNA using the method of combined bisulfite and restriction analysis. Genomic DNA was converted by standard sodium bisulfite treatment and subject to polymerase chain reaction (PCR) amplification using primers 5'-GGG TTA GGG TTA GGT AGG TTG-3' and 5'-ACA CCT CCA TTC TAT CTC CAA TAA C-3', which are specific to bisulfite converted DNA, and co-amplify both methylated and unmethylated alleles with equal efficiency (fig 1A). PCR amplification was performed using FastStart Taq Polymerase (Roche Diagnostics, New South Wales, Australia) with a final concentration of 3 mM MgCl₂, and annealing at 55°C. The amplification products were digested separately with the restriction enzymes TaqI and RsaI, which specifically cleave amplicons derived from templates that are methylated at the corresponding CpG dinucleotides, whereas the unmethylated promoter remains undigested (fig 1). To serve as a positive control, genomic DNA was methylated in vitro using CpG M.SssI methylase (New England Biolabs, Ipswich, Massachusetts, USA). Genomic DNA from a normal control subject was used as a negative control.

No methylation of the APC-1A promoter was identified in the constitutional DNA of any of the probands studied, indicating germline epimutations of APC do not underlie the phenotype in these individuals. Our results suggest that germline epimutation of

APC is not a significant factor in the polyposis syndromes studied, and does not account for cases with no detectable sequence mutation of the APC or MYH genes. This study adds further support to the concept that germline changes in APC are not responsible for HPS. However, germline epimutations remain a possibility in polyposis cases where there is a marked allelic imbalance in APC transcription. Yan *et al* reported an FAP family in which there was considerably reduced expression of one specific allele in each of the family members carrying this haplotype.¹⁰ No sequence mutation within the APC gene, including the promoter and 3' untranslated region, was identified despite intensive screening. However, the methylation status of the APC-1A promoter was not investigated in this family. It remains possible that some families and isolated cases such as this may have germline epimutations of APC, causing the affected allele to be silenced.

Although somatic APC promoter methylation occurs in a spectrum of cancers, unlike the association of microsatellite instability with MLH1 methylation, it does not give rise to an obvious phenotype. Furthermore, while germline APC mutations are well known as causing multiple adenomatous polyps and increased cancer risk, it is likely that germline defects in other genes can produce a similar phenotype. In APC mutation negative cases that produce a florid syndrome in the absence of a strong family history, it may still be worth investigating epigenetic mechanisms of gene inactivation as well as genetic causes.

M Hitchins

Medical Oncology Department, Faculty of Medicine, University of New South Wales, Sydney, Australia

C Suter

Victor Chang Cardiac Research Institute, Sydney, Australia

J Wong

Medical Oncology Department, Faculty of Medicine, University of New South Wales, Sydney, Australia

K Cheong

Medical Oncology Department, St Vincent's Hospital, Sydney, Australia

N Hawkins

Pathology Department, School of Biomedical Sciences, University of New South Wales, Sydney, Australia

B Leggett

University of Queensland, School of Medicine, Brisbane, Australia

R Scott, A Spigelman

University of Newcastle and the Hunter Medical Research Institute, Newcastle, Australia

I Tomlinson

Cancer Research UK, London Institute, UK

D Martin

Victor Chang Cardiac Research Institute, Sydney, Australia

R Ward

Medical Oncology Department, St Vincent's Hospital, Sydney, Australia, and Medical Oncology Department, Faculty of Medicine, University of New South Wales, Sydney, Australia

Correspondence to: Professor R Ward, Medical Oncology Department, St Vincent's Hospital Sydney, 384 Victoria Street, Darlinghurst, Sydney NSW 2010, Australia; r.ward@garvan.unsw.edu.au

doi: 10.1136/gut.2005.087486

This work was supported by a grant from the Australian National Health and Medical Research Council.

Conflict of interest: None declared.

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Tablet induced dysphagia in oesophageal cancer

We report a case of an unusual pattern of dysphagia in an 82 year old man. He first presented in January 2005 with a six month history of dysphagia and heartburn.