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Clonal evolution and *KRAS-MET* coamplification during secondary resistance to EGFR-targeted therapy in metastatic colorectal cancer

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We report the clinical and molecular characteristics of a 69-year-old woman with metastatic colorectal cancer, treated with the epidermal growth factor receptor (EGFR)-targeted monoclonal antibody panitumumab, displaying peculiar molecular tumour heterogeneity at progression consisting of *KRAS* and *MET* amplification as distinct drivers associated with acquired resistance.

The patient had rectosigmoid junction adenocarcinoma, G3, *KRAS* (exon 2) wild type, pT3N2(5/14)M0 treated with surgery in March 2007 and then adjuvant capecitabine (Xeloda) and oxaliplatin (XELOX) chemotherapy. In April 2009 the patient had pelvic relapse and underwent presacral, paraortic and inferior mesenteric lymphadenectomy confirming metastatic colon adenocarcinoma, *KRAS* (exon 2), *BRAF* and *PIK3CA* wild type, human epidermal growth factor receptor 2 (HER2) 2+ without amplification by in situ hybridisation[1] and no amplification of *KRAS* or *MET*. The patient received subsequent chemotherapy for stage IV disease with XELOX with progression and subsequently FOLFIRI. At disease progression, based on the *RAS* wild type status, on August 2010 the patient started treatment with panitumumab, achieving partial response which was maintained for 1 year. At that time disease progression occurred in the retroperitoneum, abdominal lymph nodes, liver and lung. Since the lymph nodes involvement caused ureteral dilation and liver involvement was limited to a single lesion in segment VII, in September 2011 the patient underwent surgery for excision of retroperitoneum and parailiac lymph nodes and atypical liver resection of segment VII. The histological diagnosis was consistent with metastases of colon adenocarcinoma in all three tumour metastatic sites.

Molecular assessment was performed on the second metastasectomy and compared to data of the previous one. Interestingly, a peculiar intratumour heterogeneity was demonstrated, as the liver metastasis was found to be *MET* amplified while *KRAS* was negative (figure 1A and see online supplementary figure S1); conversely, in the retroperitoneum *KRAS* was amplified while *MET* was negative (figure 1C and see online supplementary figure S1). Finally, in the ureteral metastatic deposit an amplification of both oncogenes was concomitantly present (figure 1B and see online supplementary figure S1). Overexpression of HER2 was not detected in any of the metastatic sites analysed (data not shown). This molecular status was different from that demonstrated in the tumour specimens of previous metastasectomy performed before treatment with EGFR-targeted therapy, where no amplification of either *KRAS* or *MET* was detected (data not shown).

We and others previously reported *KRAS* and *MET* amplifications as *bona fide* secondary resistance mechanisms to pharmacological pressure exerted by cetuximab or panitumumab.[2–4] Here we show that these molecular abnormalities can simultaneously arise within the same patient after initial response to treatment. Further, data from this case study highlight how these distinct genetic alterations can coexist in the same tumour lesion but also might display substantial intrapatient heterogeneity. In conclusion, this molecular case study highlights how selective drug pressure can sustain tumour evolution consisting in the emergence of polyclonal mechanisms of resistance – within the same patient and even within the same metastatic lesion–that eventually drive cancer progression. The knowledge of these coexisting molecular abnormalities can inform targeted therapeutic strategies to overcome drug resistance.[5]

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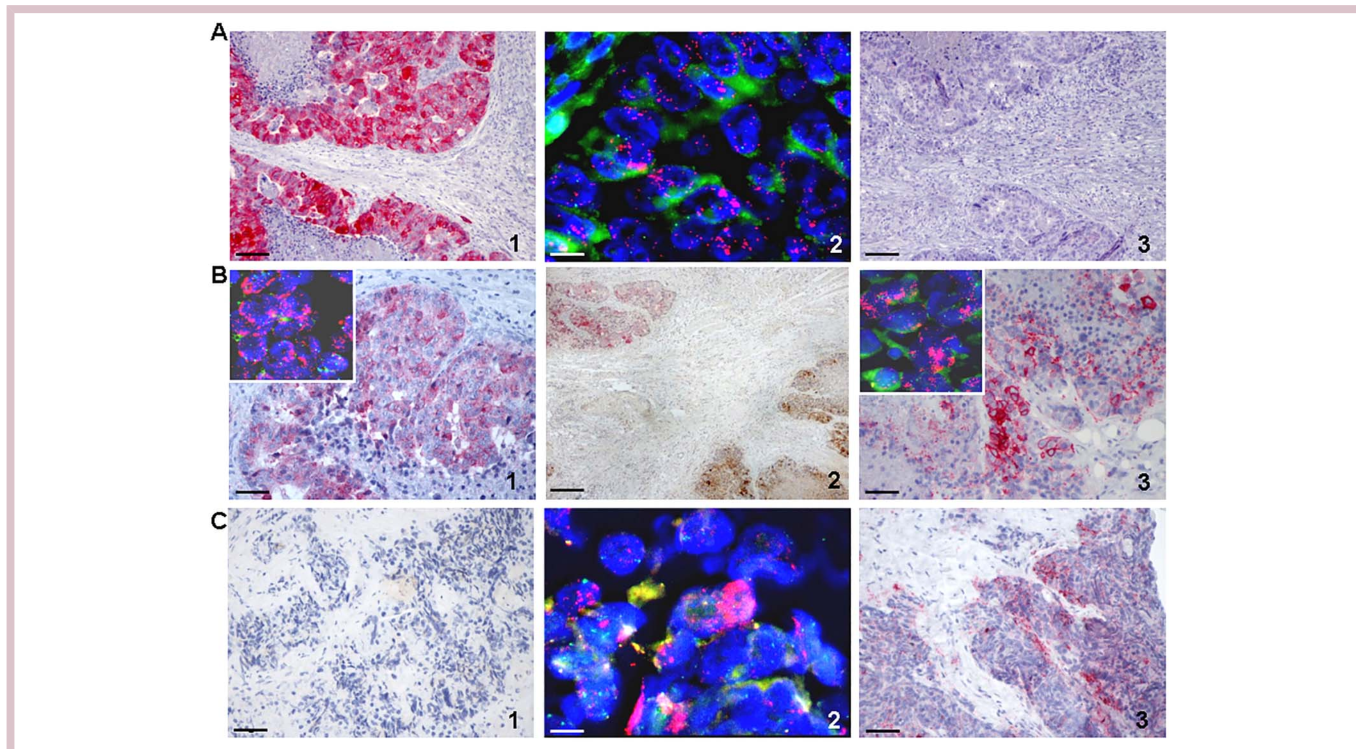

Figure 1 Immunohistochemistry (IHC) and fluorescence in situ hybridisation (FISH) analysis of *MET* and *KRAS*. For IHC, the specific *MET* antibody (Met (D1C2) XP Rabbit mAB, Cell Signaling Technology, Inc.; dilution 1:1000) and *KRAS* (F234) antibody (SC-30, mouse monoclonal IgG_{2a} Santa Cruz Biotechnology; dilution 1:100) have been used. For FISH analysis the c-*MET* amplification probe (cytocell) and KRAS/CEN12q FISH probe (Abnova) have been used. Magnification for IHC pictures is ×200 (scale bar: 100 µm), except for B2 that have ×40 (scale bar: 500 µm). Magnification for FISH pictures is ×630 (scale bar: 10 µm). (A) Liver metastasis. (1) IHC showing cytoplasmic *MET* overexpression (red staining); (2) *MET* gene amplification (red dots) by FISH in tumour nuclei; (3) IHC negative staining for *KRAS* protein expression in the same tumour area where *MET* protein is overexpressed. (B) Ureteral metastasis. (1) IHC showing cytoplasmic *MET* overexpression (red staining) and FISH analysis (inset) showing *MET* gene amplification; (2) dual-IHC assay showing overexpression of *MET* (red staining, upper left) and *KRAS* (brown staining, bottom right) proteins in two different areas of the same specimen; (3) showing cytoplasmic and membrane *KRAS* overexpression (red staining) and FISH analysis (inset) showing *KRAS* gene amplification in the tumour nuclei. (C) Retroperitoneal metastasis. (1) IHC analysis showing negative staining for *MET* protein; (2) FISH analysis showing *KRAS* gene amplification (red dots) in tumour nuclei; (3) IHC showing cytoplasmic and membrane *KRAS* overexpression (red staining) in the same area where *MET* staining was negative.

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