



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

Andrea Sartore-Bianchi,¹ Emanuele Valtorta,¹ Alessio Amatu,¹ Silvio Veronese,¹ Calogero Lauricella,¹ Erica Bonazzina,¹ Giulia Siravegna,^{2,3} Mauro Truini,¹ Alberto Bardelli,^{2,3} Salvatore Siena^{1,4}

To cite: Sartore-Bianchi A, Valtorta E, Amatu A, *et al*. Clonal evolution and *KRAS*-*MET* coamplification during secondary resistance to EGFR-targeted therapy in metastatic colorectal cancer. *ESMO Open* 2016;1:e000079. doi:10.1136/esmoopen-2016-000079

► Prepublication history and additional material is available. To view please visit the journal (<http://dx.doi.org/10.1136/esmoopen-2016-000079>).

Received 25 May 2016
Accepted 13 June 2016

¹Niguarda Cancer Center, Grande Ospedale Metropolitano Niguarda, Milano, Italy

²Istituto di Candiolo, Fondazione del Piemonte per l'Oncologia-IRCCS, Candiolo, Italy

³Dipartimento di Oncologia, Università di Torino, Torino, Italy

⁴Dipartimento di Oncologia e Emato-Oncologia, Università degli Studi di Milano, Milano, Italy

Correspondence to

Professor Salvatore Siena;
salvatore.siena@ospedaleniguarda.it

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* hybridisation¹ 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

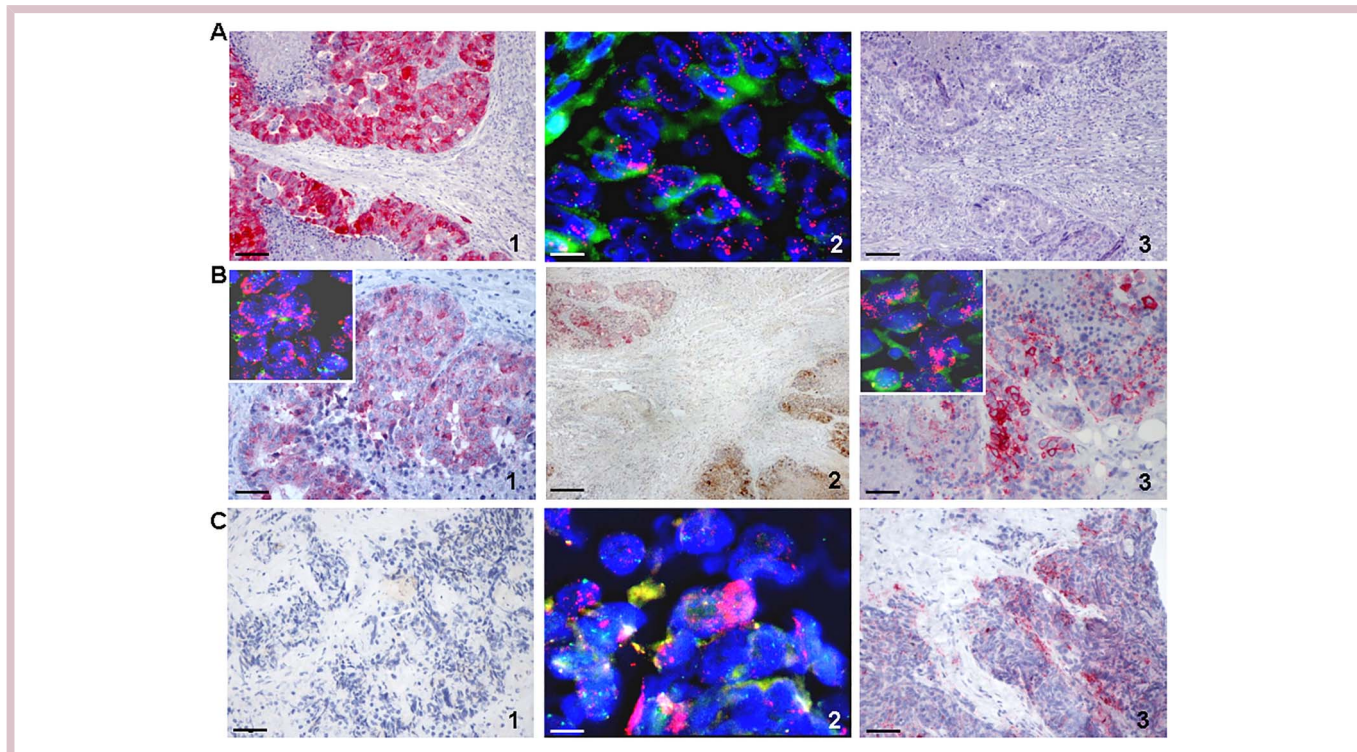


Figure 1 Immunohistochemistry (IHC) and fluorescence in situ hybridisation (FISH) analysis of *MET* and *KRAS*. For IHC, the specific *MET* antibody (Met (D1C2) XP Rabbit mAb, Cell Signaling Technology, Inc.; dilution 1:1000) and *KRAS* (F234) antibody (SC-30, mouse monoclonal IgG_{2a} Santa Cruz Biotechnology; dilution 1:100) have been used. For FISH analysis the *c-MET* amplification probe (cytowell) and *KRAS/CEN12q* FISH probe (Abnova) have been used. Magnification for IHC pictures is $\times 200$ (scale bar: 100 μm), except for B2 that have $\times 40$ (scale bar: 500 μm). Magnification for FISH pictures is $\times 630$ (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.

Funding Partly supported by AIRC, Associazione Italiana Ricerca Cancro—2010 Special Programme Molecular Clinical Oncology 5 \times 1000, project 9970. Investigators at Niguarda Cancer Center were partly supported by grant Terapia Molecolare Tumori provided by Fondazione Oncologia Niguarda.

Competing interests None declared.

Provenance and peer review Not commissioned; internally peer reviewed.

Open Access This is an Open Access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

REFERENCES

1. Valtorta E, Martino C, Sartore-Bianchi A, *et al.* Assessment of a HER2 scoring system for colorectal cancer: results from a validation study. *Mod Pathol* 2015;28:1481–91.
2. Siravegna G, Mussolin B, Buscarino M, *et al.* Clonal evolution and resistance to EGFR blockade in the blood of colorectal cancer patients. *Nat Med* 2015;21:795–801.
3. Valtorta E, Misale S, Sartore-Bianchi A, *et al.* *KRAS* gene amplification in colorectal cancer and impact on response to EGFR-targeted therapy. *Int J Cancer* 2013;133:1259–65.
4. Bardelli A, Corso S, Bertotti A, *et al.* Amplification of the *MET* receptor drives resistance to anti-EGFR therapies in colorectal cancer. *Cancer Discov* 2013;3:658–73.
5. Russo M, Siravegna G, Blaszkowsky LS, *et al.* Tumor, heterogeneity and lesion-specific response to targeted therapy in colorectal cancer. *Cancer Discov* 2016;6:147–53.