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Evaluation of *RAS* mutational status through BEAMing assay to monitor disease progression of metastatic colorectal cancer: a case report

Elena Lastraioli[a], Daniele Lavacchi[b], Valeria Emma Palmieri[b], Francesca Castiglione[c], Luca Messerini[a], Francesco Di Costanzo[b] and Lorenzo Antonuzzo[b,*]

Since the introduction of antiepidermal growth factor receptor (anti-EGFR) monoclonal antibodies (moAbs), the treatment of metastatic colorectal cancer (mCRC) has become crucially dependent on the mutation profile of the tumour over the last two decades. Recently, rechallenge strategy with cetuximab-based chemotherapy has demonstrated to be active in a subgroup of patients whose tumour maintained wild-type *RAS* and *RAF* status. In this setting, liquid biopsy may replace tissue sample for the identification of specific subgroups of pretreated patients that may benefit from the reintroduction of anti-EGFR moAbs. In November 2014, a 64-year-old man with IVB stage *BRAF*, *KRAS* and *NRAS* wild-type mCRC was admitted in our hospital. He received FOLFIRI cetuximab as first-line treatment with deep and long-lasting partial response (PR), followed by cetuximab maintenance therapy until January 2016. At the time of disease progression, FOLFIRI cetuximab regimen was reintroduced resulting in stabilization of disease and he continued with capecitabine cetuximab therapy until disease progression in October 2016. Then, the patient consecutively received FOLFOX bevacizumab, TAS-102, regorafenib and FOLFIRI followed by de Gramont maintenance treatment. Finally, he was retreated with FOLFIRI cetuximab with disease progression within 3 months and died in May 2019. During his clinical course, liquid biopsy detected two mutations: one in KRAS Cd.12 and one in NRAS Cd. 61. The longitudinal assessment of *RAS* status offers considerable advantages in order to avoid side effects and economic costs for ineffective treatment choices. Liquid biopsy could help better monitor the disease and provide molecularly guided treatments.

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[a]Department of Experimental and Clinical Medicine, University of Florence, [b]Medical Oncology, Azienda Ospedaliero-Universitaria Careggi, Florence and [c]Section of Pathological Anatomy, Department of Health Sciences, University of Florence, Azienda Ospedaliero-Universitaria Careggi, Florence, Italy

Correspondence to Lorenzo Antonuzzo, MD, PhD, Medical Oncology, Azienda Ospedaliero-Universitaria Careggi, Largo GA Brambilla 3, 50134 Florence, Italy
Tel: +39 055 7948406; fax: +39 055 7947908;
e-mail: lorenzo.antonuzzo@gmail.com

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Introduction

Standard approaches for tumour bearing patients strongly rely on histology to confirm clinical suggestions and provide an accurate diagnosis. Histology is also the main technique used to define the most appropriate therapy as well as for disease monitoring. In recent years, molecular techniques aimed at evaluating alterations in DNA that might help in clinical management of neoplastic patients have been developed. The main source of DNA for molecular analyses is represented by tissue, obtained either from biopsies or surgical samples. In both cases, only a single snap-shot of the genetic features (and alterations) of the tumour can be derived. Therefore, using this kind of samples might not be representative of the whole tumour due to its intrinsic heterogeneity. Moreover, when dealing with biopsies, only limited amount of DNA can be extracted and it may give not conclusive results.

Standard biopsies might also be used for disease monitoring during treatment but especially in metastatic patients characterized by poor healthy conditions, performing multiple biopsies should be avoided. Based on these premises, the concept of liquid biopsy as a surrogate of tissue sample was proposed [1–7]. In recent years, technical improvements allowed to study circulating tumour cells (CTC) and circulating tumour DNA (ctDNA) in patients with advanced disease [8,9]. ctDNA has been shown to be associated with histotype, stage and tumour burden among other clinical features [4,10–12].

A high proportion (86–100%) of metastatic colorectal cancer (mCRC) patients show detectable ctDNA in plasma and 1.9–27% have mutations [11].

mCRC patients are characterized by a high frequency of *KRAS* mutations that are the main determinants of the failure of anti-EGFR-based therapy. For this reason, the

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guidelines of clinical practice have been modified and currently mCRC patients are screened for both *KRAS* and *NRAS* to select the most appropriate patients to be treated with anti-EGFR [13]. *RAS* status is currently determined in tissue samples, either primary tumour or metastasis obtained by biopsies or surgery. Recently, several methods aimed at evaluating *RAS* mutational status in plasma have been developed and optimized.

Case report

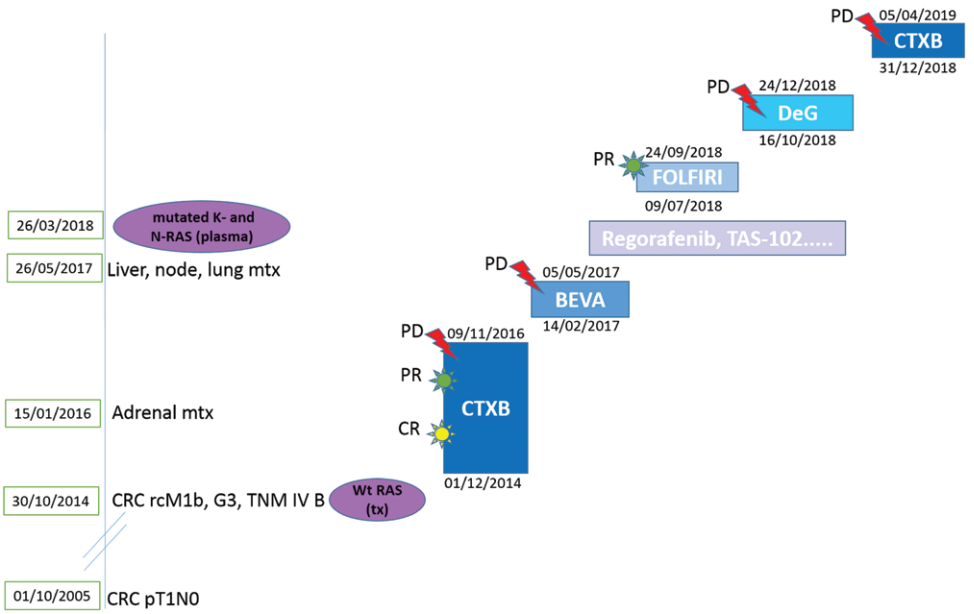
AQ7 In November 2014, a 64-year-old man who had undergone surgical resection for a pT1N0M0 G2 colorectal cancer (CRC) in 2005 was admitted in our hospital. In October 2014, he presented with multiple thyroid nodules and enlarged lymph nodes in the neck. In addition, a computed tomography (CT) scan showed multiple lung, mediastinal and lateral cervical nodal, adrenal, thyroid and hepatic metastases. Both thyroid and lung biopsies revealed the presence of a metastatic dissemination of CRC G3, CK20+, TTF1-. *BRAF*, *KRAS* and *NRAS* status were determined on the metastatic tissue by Maldi TOF mass spectrometry associated to Single Base Extension technology with CE-IVD Myriapod COLON status kit, validated on MassARRAY Analyzer 4 System (Sequenom, San Diego, California, USA). Being *RAS* wild-type, the patient received FOLFIRI cetuximab from December 2014 to May 2015. Initially, he obtained a partial response (PR) and he continued cetuximab maintenance therapy until lung and adrenal disease progression in January 2016. From January 2016 to July 2016, FOLFIRI cetuximab regimen was reintroduced resulting in stabilization of disease and he continued with capecitabine cetuximab therapy until lung, hepatic and adrenal disease progression in October 2016. From November 2016 to May 2017, the patient consecutively received FOLFOX bevacizumab for 6 months TAS-102 for 8 months, and regorafenib for 4 months. All these treatments were discontinued due to PD. In May 2018, a CT scan showed a lung, hepatic and nodal PD with development of vertebral metastases. A palliative radiotherapy for bone metastases was performed with clinical benefit in June 2018. In addition, the patient received FOLFIRI for five courses with PR, followed by de Gramont maintenance treatment from July 2018 to December 2018, until a CT scan demonstrated disease progression. In December 2018, given the deep and long-lasting response with previous cetuximab-containing treatments, he was retreated with FOLFIRI cetuximab but rapidly progressed within 3 months and died in May 2019. Interestingly, in March 2018, 8 mL of peripheral blood were collected and plasma was used for the determination of *KRAS* and *NRAS* status with OncoBEAM RAS CRC assay (Sysmex Inostics, Hamburg, Germany). When plasma was analysed, two mutations were detected as follows: one in *KRAS* Cd.12 and another one in *NRAS* Cd. 61 (Fig. 1). F1

A schematic representation of the clinical history of the patient is reported in Fig. 2. F2

Discussion

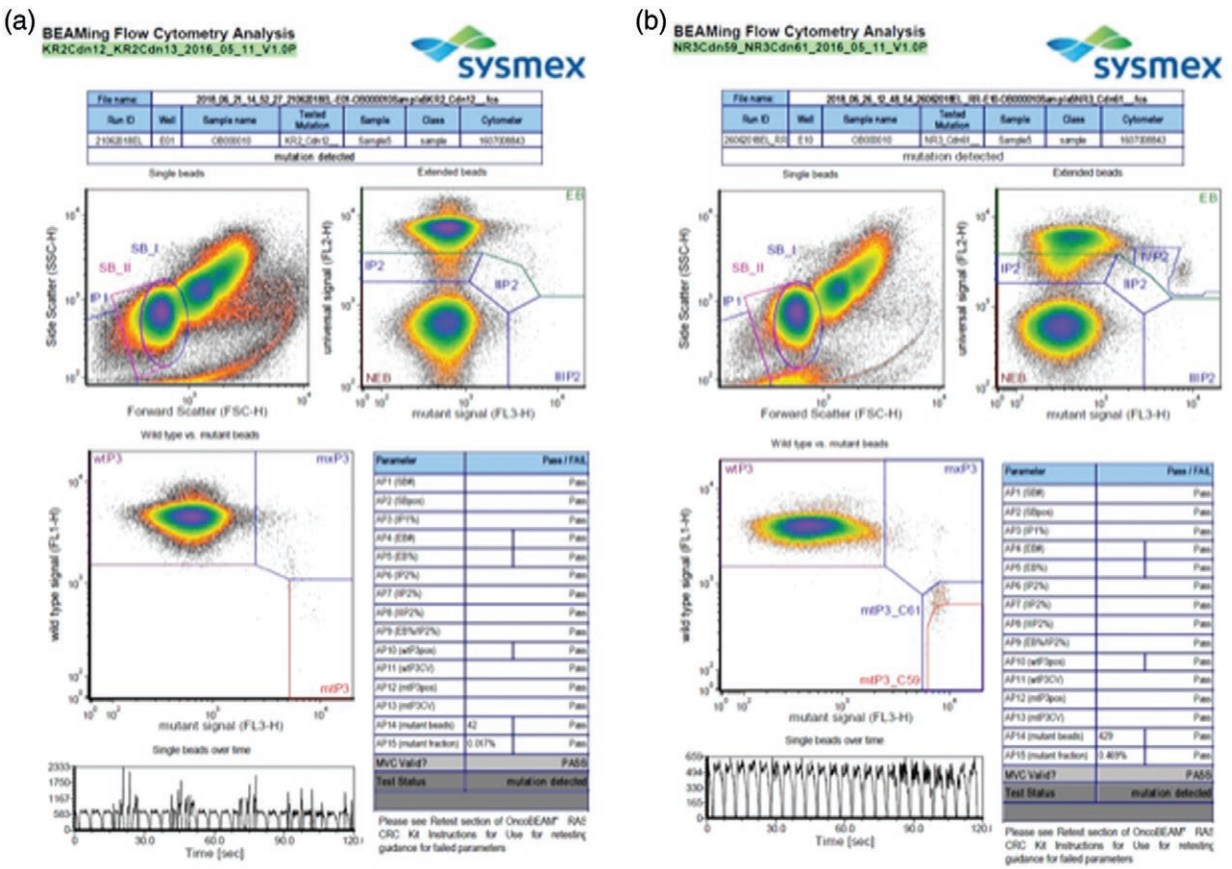
The treatment of mCRC has undergone remarkable changes over the last two decades, at first with the use of anti-VEGF/VEGFR and anti-EGFR monoclonal

Fig. 1



KRAS and *NRAS* mutations detected by OncoBEAM RAS CRC assay in *KRAS* codon 12 (panel A) and *NRAS* codon 61 (panel B). CRC, colorectal cancer; PR, partial response.

Fig. 2



AQ9 Key points of the clinical history of the patient.

antibodies (moAbs) in combination with standard chemotherapy and recently with the introduction of regorafenib and trifluridine/tipiracil [13]. Several studies have shown that the rechallenge with an anti-EGFR moAb could offer a benefit for chemotherapy-refractory patients. However, this benefit was observed in only a limited subgroup of molecularly selected patients, albeit heavily pretreated [14]. In a multicenter phase 2 trial, 28 patients with *RAS* and *BRAF* wild-type mCRC, who responded to irinotecan- and cetuximab-containing first-line therapy and progressed to oxaliplatin- and bevacizumab-containing second-line therapy, received cetuximab plus irinotecan as third-line treatment. Overall, PRs were observed in 21% of the patients. In the preplanned exploratory analysis, *RAS* status in ctDNA was determined at the time of rechallenge. Notably, the frequency of *RAS* mutations in liquid biopsies was 48% and no PR was obtained in patients with *RAS* mutated ctDNA [15]. We described the case of a patient whose tumour developed a change in *RAS* status during chemotherapy lines, going from wild-type *RAS* to *RAS*-mutant tumour (*KRAS* codon 12 and *NRAS* codon 13 mutations). This event led to the failure of cetuximab-containing chemotherapy at the time of retreatment. Since the switch in *RAS* status occurs in about half of the patients treated for mCRC [16], the longitudinal assessment of *RAS* status offers considerable advantages in order to avoid unnecessary toxic effects and economic costs for ineffective treatment choices.

AQ8

Treatment of mCRC has become deeply dependent on the molecular profile of the tumour. The development of real-time molecular monitoring of tumour characteristics during sequential therapies is a successful strategy in the direction of molecularly guided precision therapy.

Our data suggest that *RAS* mutations should be performed in both tissue and plasma to better monitor the disease and define the best treatment options for each patient. As in this case, liquid biopsy could help identify patients who may benefit from a rechallenge strategy.

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