Analysis of the role of RAS family mutations in metastatic colorectal cancer (mCRC): current treatment practice

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Abstract: A major challenge in metastatic colorectal cancer (mCRC) is the identification of specific biomarkers that are likely to predict which patients will benefit from a specific treatment. To this date, a number of studies have shown that a tumour’s mutational profile influences treatment outcome in patients with mCRC and should, therefore, be used to guide treatment decision. For more than 5 years now, we know that patients with tumours harbouring mutations in exon 2, codons 12 and 13 of the *KRAS* oncogene gain no benefit from the administration of anti-epidermal growth factor receptor (EGFR) monoclonal antibodies (moAbs), Cetuximab and Panitumumab. It has just recently been elucidated that *KRAS* mutations outside codons 12 and 13 and mutations in the *NRAS* gene confer resistance to Cetuximab and Panitumumab, as well. Thus, since June 2013, the analysis of exons 2, 3 and 4 of *KRAS* and *NRAS* has been incorporated in daily clinical practice to improve patients’ selection for anti-EGFR moAbs treatment. Nevertheless, even if patients’ outcome under anti-EGFR moAbs therapy is improved with better selection based on Ras mutational status, more research is needed in this field; the matter is far from being resolved, since there are still a minority of wt *RAS* patients who do not respond upfront to such a treatment.

Keywords: *RAS • Family mutations • Metastatic • Colorectal • Cancer*

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Introduction

Mounting evidence behind colorectal cancer’s (CRC’s) molecular genetics has substantially changed the way we understand the disease today.[1] Especially in the metastatic setting, as it will be elaborated further, our approach towards treatment options has become more rational and reasonable. We have used clinical and pathologic parameters, in the past years, to base our treatment choices; nevertheless, they seemed and still are inadequate to guide patients’ selection towards specific treatment strategies. As it is generally true for

modern oncology, in CRC as well, a major challenge is the identification of specific biomarkers that are likely to predict which patients will benefit from a specific treatment, illustrating the concept of “Personalized Medicine”. Up to this date, a number of studies have shown that the mutational profile of the tumours influences treatment outcome in patients with metastatic CRC (mCRC) and should therefore guide treatment decision.

KRAS mutational status determination represents a paradigm for biomarkers development in the era of molecular targeted therapies. For more than 5 years now, it has been proven that patients with mutations in

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exon 2, codons 12 and 13 of the *KRAS* oncogene gain no benefit from anti-epidermal growth factor receptor (EGFR) treatment, specifically from the two monoclonal antibodies (moAbs) against EGFR, Cetuximab and Panitumumab.[2,3] and that eventually a detrimental effect in *Kras* mutant occurred when Oxaliplatin was part of the chemotherapy backbone. Nevertheless, it soon became evident that this approach was not enough to explain the full picture, since several patients with *KRAS* wild-type (wt) tumours did not respond to anti-EGFR inhibition.[2] Beyond *KRAS* exon 2 mutations, the clinical relevance of other proposed mechanism of resistance to anti-EGFR moAbs, such as *KRAS* mutations outside codons 12 and 13, and mutations in other *RAS* family members, such as *NRAS*, is just recently being elucidated, with the aim to better select patients who have the highest probability to respond to this kind of treatment.[4,5]

KRAS mutations in exon 2 and codons 12 and 13

Although the anti-EGFR moAbs Cetuximab and Panitumumab were initially registered for patients whose tumours were found to immunohistochemically express the EGFR protein, it soon became clear that this methodology was neither enough nor adequate to predict treatment efficacy. The *KRAS* proto-oncogene encodes K-ras, a G protein that has a key role in the Ras/MAPK signalling pathway. K-ras is located downstream of many growth factor receptors, including EGFR, that are involved in CRC carcinogenesis. The activation of a growth factor receptor induces the recruitment of K-ras, which initiates the activation of a cascade of serine-threonine kinases leading to the signal transduction from the cell surface to the nucleus.[6] *KRAS* mutations are present in more than one-third of CRCs and in approximately 90% of the cases they are located in exon 2 and codons 12 and 13.[2,3] *KRAS* mutations lead to the activation of one of the most important pathways for cell proliferation, the Ras/MAPK pathway, by inducing cyclin D1 synthesis. Consequently, in the presence of a *KRAS* mutation this pathway activation cannot be significantly inhibited by an anti-EGFR moAb (Cetuximab or Panitumumab), which acts upstream the K-ras protein.[7]

In a number of trials and studies, it has been shown that specific *KRAS* mutations in the hotspots of codons 12 and 13 were predictors of resistance to anti-EGFR moAbs therapy and were associated with reduced efficacy and shorter progression-free survival (PFS) and overall survival (OS).[2,3,8-11] Thus, in June

2009, mutational analysis of *KRAS* codons 12 and 13 in the tumours of CRC patients became mandatory at the presentation of metastatic disease before the administration of anti-EGFR moAbs.

Despite that, as previously already noted, not all patients with *KRAS* tumours benefit from the administration of Cetuximab or Panitumumab. In fact, the response rate of anti-EGFR moAbs is less than 25% when they are administered as salvage monotherapy, clearly indicating that other mechanism of resistance must be implicated in this process.[12-15]

KRAS mutations outside hotspots in codons 12 and 13

Besides the majority of *KRAS* mutations that are located in exon 2 (codons 12 and 13), others exist, accounting approximately for 10% of the *KRAS* mutated cases and found mainly in exon 3 (codon 61) and 4 (codons 117 and 146).[4,5,16] Preclinical data indicate that the above-mentioned mutations behave similar to the hotspots codon 12 and 13 mutations, leading to RAS-RAF-MAPK kinase pathway activation. In contrast, other *KRAS* mutations such as those found in codons 19 and 164 are phenotypically equivalent to wt *KRAS*. [17,18] The clinical relevance of codons 61 and 146 *KRAS* mutations has been retrospectively investigated in small patients' cohorts and lack of response to anti-EGFR moAbs treatment has been documented. [16,17] In addition, in the retrospective analysis of the Panitumumab registration trial, it was shown that the addition of codon 61 *KRAS* mutation alongside the already established ones slightly improved patients' selection for anti-EGFR moAbs treatment administration. [5] Furthermore, in the large PRIME study,[4] as well as in PEAK[19] when patients were considered as *RAS* wt if their tumours were wt for *KRAS* codons, 12, 13, 61 and 146 (thus, exons 2, 3 and 4), a significant improvement in response rate, PFS and OS was documented. In line with this mounting evidence and in accordance with the strong biologic rationale, from June 2013, the analysis of exons 2, 3 and 4 of *KRAS* and their wt characterization is now mandatory before the administration of anti-EGFR moAbs treatment in daily clinical practice.

NRAS mutation status

NRAS encodes for a protein – a member of the *RAS* superfamily of GTPases, and like the rest of them, plays a key role in the critical MAPK signalling pathway. Besides CRC, *NRAS* mutations are found in other solid

tumours as well, like melanomas and thyroid carcinomas. [20,21] Approximately 80% of the reported *NRAS* gene mutations are located in codon 61.[20] *NRAS* mutation status has been shown to putative predict response to BRAF inhibition in metastatic melanoma patients [22,23] and anti-EGFR treatment in mCRC.[5]

Retrospectively, it was shown that patients with *KRAS* and *BRAF*wt but *NRAS* mutated tumours had significantly reduced response rates when treated with Cetuximab in the salvage treatment setting.[18] Furthermore, in the retrospective analysis of the Panitumumab registration trial, it was shown that Panitumumab had no effect in *KRAS* wt but in *NRAS* mutated patients.[5] As already previously mentioned for *KRAS* mutational analysis of exons 3 and 4 besides exon 2, in the large PRIME study,[4] as well as in PEAK[19] when patients were considered as RAS wt if their tumours were wt for *KRAS* codons, 12, 13, 61 and 146 (thus, exons 2, 3 and 4) and *NRAS* exons 2 (codons 12 and 13), 3 (codon 61) and 4 (codons 117 and 146), a significant improvement in response rate, PFS and OS was documented. In line with this mounting evidence and in accordance with the strong biologic rationale, from June 2013, the analysis of exons 2, 3 and 4 of *NRAS* is also incorporated in daily clinical practice in the pre-treatment analytical panel, in addition to *KRAS*, to improve patients' selection who are candidates for anti-EGFR mAbs treatment.

The better selection of the target patient population increases the benefit/risk ratio of the use of anti-EGFR MoAbs in all lines. Depending on the techniques used to extend the mutation analysis, the percentage of patients with Wild-type Kras Exon 2 harbouring less frequent mutations in other codons or exons in K and N ras varies between 15% and 25%. The population enrichment based on Kras was demonstrated mostly on retrospective or prospective/retrospective analysis, starting from no Kras

testing, followed by Kras Exon 2 testing and more recently Kras and Nras exons 2, 3 and 4 testing chronologically over a period of 10 years or more.

Application and future considerations

Important technical application considerations regarding all the above-mentioned mutational analysis are the validation of the assay for testing *KRAS/NRAS* mutation in exons 2 (codons 12, 13) – 3 (codon 61) – 4 (codons 117 and 146), the evaluation of the sensitivity of the methodology for each exon, the comparison of sensitivity and specificity of the methodology when surgical or biopsy specimens are analysed and the development of a multiplex assay (compared with the standard techniques). Furthermore, hugely important aspects are the correlations of clinical and pathological parameters to the specific *KRAS* and *NRAS* mutations in mCRC patients, as well as the willingness of the clinicians, the medical oncologists, to assess all *KRAS/NRAS* mutations in exons 2 (codons 12 and 13), 3 (codon 61) and 4 (codons 117 and 146) in the tumours of their mCRC patients to choose the best possible treatment algorithm.

Even if outcomes in terms of response rate, PFS and OS are improved with better selection on the Ras mutational status, there are still a minority of patients not responding upfront to combined modality treatments with anti-EGFR MoAbs. Further research should be performed to look at other pre-existing mechanisms of resistance. Finally, most patients under treatment will also develop tumour progression reflecting the possibility of tumour escape and acquisition of resistance under therapeutic pressure that will need to be better understood to be appropriately treated.

References

- [1] (2012) Comprehensive molecular characterization of human colon and rectal cancer. *Nature* 487: 330-337.
- [2] Amado RG, Wolf M, Peeters M, Van Cutsem E, Siena S, et al. (2008) Wild-type *KRAS* is required for panitumumab efficacy in patients with metastatic colorectal cancer. *J Clin Oncol* 26: 1626-1634.
- [3] Karapetis CS, Khambata-Ford S, Jonker DJ, O'Callaghan CJ, Tu D, et al. (2008) K-ras mutations and benefit from cetuximab in advanced colorectal cancer. *N Engl J Med* 359: 1757-1765.
- [4] Douillard JY, Oliner KS, Siena S, Tabernero J, Burkes R, et al. (2013) Panitumumab-FOLFOX4 treatment and RAS mutations in colorectal cancer. *N Engl J Med* 369: 1023-1034.
- [5] Peeters M, Oliner KS, Parker A, Siena S, Van Cutsem E, et al. (2013) Massively parallel tumor multigene sequencing to evaluate response to panitumumab in a randomized phase III study of metastatic colorectal cancer. *Clin Cancer Res* 19: 1902-1912.
- [6] Olayioye MA, Neve RM, Lane HA, Hynes NE (2000) The ErbB signaling network: receptor heterodimerization in development and cancer. *EMBO J* 19: 3159-3167.
- [7] Lievre A, Bachet JB, Le Corre D, Boige V, Landi B, et al. (2006) *KRAS* mutation status is predictive of response to cetuximab therapy in colorectal cancer. *Cancer Res* 66: 3992-3995.

- [8] Bokemeyer C, Bondarenko I, Hartmann JT, de Braud F, Schuch G, et al. (2011) Efficacy according to biomarker status of cetuximab plus FOLFOX-4 as first-line treatment for metastatic colorectal cancer: the OPUS study. *Ann Oncol* 22: 1535-1546.
- [9] Douillard JY, Siena S, Cassidy J, Tabernero J, Burkes R, et al. (2010) Randomized, phase III trial of panitumumab with infusional fluorouracil, leucovorin, and oxaliplatin (FOLFOX4) versus FOLFOX4 alone as first-line treatment in patients with previously untreated metastatic colorectal cancer: the PRIME study. *J Clin Oncol* 28: 4697-4705.
- [10] Van Cutsem E, Kohne CH, Lang I, Folprecht G, Nowacki MP, et al. (2011) Cetuximab plus irinotecan, fluorouracil, and leucovorin as first-line treatment for metastatic colorectal cancer: updated analysis of overall survival according to tumor KRAS and BRAF mutation status. *J Clin Oncol* 29: 2011-2019.
- [11] Kohne CH, Lenz HJ (2009) Chemotherapy with targeted agents for the treatment of metastatic colorectal cancer. *Oncologist* 14: 478-488.
- [12] Saridaki Z, Georgoulas V, Souglakos J (2010) Mechanisms of resistance to anti-EGFR monoclonal antibody treatment in metastatic colorectal cancer. *World J Gastroenterol* 16: 1177-1187.
- [13] Saridaki Z, Papadatos-Pastos D, Tzardi M, Mavroudis D, Bairaktari E, et al. (2010) BRAF mutations, microsatellite instability status and cyclin D1 expression predict metastatic colorectal patients' outcome. *Br J Cancer* 102: 1762-1768.
- [14] Saridaki Z, Tzardi M, Papadaki C, Sfakianaki M, Pega F, et al. (2011) Impact of KRAS, BRAF, PIK3CA mutations, PTEN, AREG, EREG expression and skin rash in ≥ 2 line cetuximab-based therapy of colorectal cancer patients. *PLoS One* 6: e15980.
- [15] Souglakos J, Philips J, Wang R, Marwah S, Silver M, et al. (2009) Prognostic and predictive value of common mutations for treatment response and survival in patients with metastatic colorectal cancer. *Br J Cancer* 101: 465-472.
- [16] De Roock W, Claes B, Bernasconi D, De Schutter J, Biesmans B, et al. (2010) Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. *Lancet Oncol* 11: 753-762.
- [17] Loupakis F, Ruzzo A, Cremolini C, Vincenzi B, Salvatore L, et al. (2009) KRAS codon 61, 146 and BRAF mutations predict resistance to cetuximab plus irinotecan in KRAS codon 12 and 13 wild-type metastatic colorectal cancer. *Br J Cancer* 101: 715-721.
- [18] Lurkin I, Stoeck R, Hurst CD, van Tilborg AA, Knowles MA, et al. (2010) Two multiplex assays that simultaneously identify 22 possible mutation sites in the KRAS, BRAF, NRAS and PIK3CA genes. *PLoS One* 5: e8802.
- [19] Schwartzberg LS, Rivera F, Karthaus M, Fasola G, Canon JL, et al. (2014) PEAK: A Randomized, Multicenter Phase II Study of Panitumumab Plus Modified Fluorouracil, Leucovorin, and Oxaliplatin (mFOLFOX6) or Bevacizumab Plus mFOLFOX6 in Patients With Previously Untreated, Unresectable, Wild-Type KRAS Exon 2 Metastatic Colorectal Cancer. *J Clin Oncol*.
- [20] Hacker E, Nagore E, Cerroni L, Woods SL, Hayward NK, et al. (2013) NRAS and BRAF mutations in cutaneous melanoma and the association with MC1R genotype: findings from Spanish and Austrian populations. *J Invest Dermatol* 133: 1027-1033.
- [21] Park SJ, Sun JY, Hong K, Kwak JY, Kim EK, et al. (2013) Application of BRAF, NRAS, KRAS mutations as markers for the detection of papillary thyroid cancer from FNAB specimens by pyrosequencing analysis. *Clin Chem Lab Med* 51: 1673-1680.
- [22] Banerji U, Affolter A, Judson I, Marais R, Workman P (2008) BRAF and NRAS mutations in melanoma: potential relationships to clinical response to HSP90 inhibitors. *Mol Cancer Ther* 7: 737-739.
- [23] Joseph RW, Sullivan RJ, Harrell R, Stemke-Hale K, Panka D, et al. (2012) Correlation of NRAS mutations with clinical response to high-dose IL-2 in patients with advanced melanoma. *J Immunother* 35: 66-72.