For reprint orders, please contact: reprints@futuremedicine.com

Setting future standards for *KRAS* testing in colorectal cancer

"The most relevant and recent development in this field is the discovery that in patients with metastasized colorectal cancer EGFR targeting treatment is only effective in those patients that have wild-type KRAS, a discovery that has attracted wide publicity."

Selection of patients with metastasized colorectal cancer, who do not benefit from treatment directed against the epidermal growth factor receptor (EGFR) is now feasible. Since the start of the new millennium, the outlook for patients with metastasized colorectal cancer has improved significantly, mainly thanks to the introduction of new, biological treatments that targets the vascular endothelial growth factor receptor (VEGFR) or the EGFR. However, not all patients benefit from these treatments and much effort is undertaken to identify predictive markers that can be used to select patients most likely to benefit from these expensive and potentially toxic agents. The most relevant and recent development in this field is the discovery that in patients with metastasized colorectal cancer EGFR targeting treatment is only effective in those patients that have wild-type *KRAS* [1,2], a discovery that has attracted wide publicity.

"KRAS testing needs to be performed before EGFR targeting therapy is administered in patients with metastasized colorectal cancer"

KRAS mutational analysis has become part of the standard care in patients treated with EGFR targeting therapy. A variety of studies have shown that in patients with metastasized colorectal cancer the addition of either cetuximab (a chimeric IgG1 monoclonal antibody against the EGFR) or panitumumab (a fully human IgG2 EGFR antibody) as monotherapy or when added to chemotherapy increases the progression free survival [3–5]. However, only patients without a mutation in the *KRAS* oncogene benefit from this regimen [1,6,7]. By contrast to the situation in lung cancer, where mutation in the *EGFR* gene can serve as an additional predictive factor [8], so far in patients with colorectal cancer *KRAS* mutational status is the only

predictive biomarker that is available, and it is now widely accepted that *KRAS* testing needs to be performed before EGFR targeting therapy is administered in patients with metastasized colorectal cancer [2].

Research into the EGF pathway has gone from bench to bedside. The EGFR receptor is a growth receptor expressed on many cells and overexpression occurs in many different forms of cancer, especially carcinomas. Overexpression can be due to amplification of the gene, mutations in the gene or post-translational factors. Binding of a ligand (EGF, TGF- α , epiregulin) to the receptor leads to dimerization of EGFR and stimulation of intracellular signal transduction pathways ultimately resulting in the stimulation of cell growth and proliferation and the inhibition of apoptosis. Therefore, EGFR is an attractive target for therapeutic strategies. Several approaches using either small molecules or monoclonal antibodies have become available and have shown effect in a variety of tumor types, including cancer of the epithelia of the oropharynx and larynx [9], lung cancer [8] and colorectal cancer [3,4]. Since not all patients respond to this treatment studies into predictive factors were initiated, comparing responding to nonresponding patients. An obvious target for study was the EGFR itself but the response to EGFR targeting agents appeared not to be limited to patients expressing EGFR on their tumors [10] and also, many patients with EGFR expression on the tumor cells did not respond. An increased *EGFR* gene copy-number, however, is associated with increased responsiveness to cetuximab in patients with advanced colorectal cancer [11], but is an uncommon event and many patients without *EGFR* gene amplification do respond. Mutations in the intracellular domain of the EGFR are observed in non-small-cell lung cancer and associated with response to the EGFR small molecules gefitinib and erlotinib [8] but in colorectal cancer these mutations are only very

Han van Krieken*

*Author for correspondence:
 Radboud University Medical
 Center, Nijmegen,
 The Netherlands
 Tel.: +31 243 614 352
 Fax: +31 243 668 750
j.vankrieken@pathol.umcn.nl

Jolien Tol

Radboud University Medical
 Center, Nijmegen,
 The Netherlands

future
 medicine part of fsg

rarely observed [12]. Therefore, other predictive markers are needed, especially in patients with colorectal cancer.

The *KRAS* oncogene is a key molecule in the effects of EGFR targeted therapy as it is involved in an important downstream signaling cascade of EGFR. Stimulation of EGFR leads to activation of *KRAS* resulting in the stimulation of cell growth. In normal states the *KRAS* protein is continuously degraded but when a mutation in the *KRAS* gene is present this degradation does not occur and activated *KRAS* accumulates. This disturbed balance for *KRAS* activation and degradation leads to autonomous *KRAS* activation and resistance to EGFR blockade. Several studies, both *in vitro* and on tumor tissues from patients treated in clinical trials have now proven that EGFR blocking agents have an effect only in patients with colorectal cancer that have wild-type *KRAS* [2], which is the case in about 60% of the cases [13]. In Europe panitumumab and cetuximab are therefore only approved in patients with a *KRAS* wild-type tumor.

“Quality assurance is crucial for reliable implementation of *KRAS* mutation testing for predicting unresponsiveness for EGFR targeted therapy.”

Testing for *KRAS* mutations is not standardized. A total of 7 different mutations in codon 12 and 13 of the *KRAS* gene account for more than 95% of the activating *KRAS* mutations [101]. There is a multitude of techniques available that can detect these *KRAS* mutations. Most techniques are developed and used in a limited number of laboratories, but some tests are commercially available and have been CE marked. There is presently very little data available on which test is the most reliable. Since it is known that Her2 testing in breast cancer, another predictive biomarker, has a relatively poor reliability (about 75% according to recent data), the European Society of Pathology (ESP) has initiated a quality assurance (QA)-program for *KRAS* testing in Europe, based on experiences of and in collaboration with national programs [14]. This is important since *KRAS* testing is presently available in only a limited number of laboratories, but it is expected that large numbers of tumors need to be tested in the near future.

QA is crucial for reliable implementation of *KRAS* mutation testing for predicting unresponsiveness for EGFR targeted therapy. The ESP QA-program aims to look at the whole process of *KRAS* testing and several key issues will be

included [14]. For a reliable test, the minimum amount of invasive tumor cells needs to be determined, the quality of the DNA assessed, and the outcome of the test assured. The available data indicate that seven mutations in the *KRAS* gene need to be detected by a test, and thus these need to be included in the QA-program. The program will not evaluate methods for testing on reliability, but since the data from the different laboratories will be collected in a central database, information on the reliability of different tests will become available.

A coordinated QA-program will be available in Europe in 2009. Within the next few months a first series of core laboratories, in eight different European countries, will be involved in a test. Unstained slides of formalin-fixed paraffin embedded tissue of tumors with known mutations in the *KRAS* gene will be circulated. The participating laboratories will assess a slide after staining, indicate the areas of invasive tumor cells, microdissect the indicated area, extract the DNA, assess the quality of the DNA and perform the *KRAS* mutation analysis according to their local protocols. All these steps will be documented, but no guideline on the protocols to be used will be given. For each of the seven mutations a sample will be distributed as well as three wild-type samples. The final result on all ten cases needs to be correct to pass this QA-test. Subsequently, the core laboratories will organize a similar test in their region, so that in the first half of 2009 a QA-program is available in many areas in Europe.

This process will be amply documented and described on the website of the ESP [102] and on this website relevant literature, protocols and guidelines will also become available. By this approach the ESP hopes that standardization of *KRAS* testing will come along with the introduction of the EGFR blocking agents, rather than after widespread use. This approach can also serve as a future example for the introduction of predictive markers that are based on the evaluation of tumor tissues.

Financial & competing interests disclosure

JHJM van Krieken has received honoraria from Amgen and research grants from D&S and Merck, all dealing with *KRAS*-testing in colorectal cancer. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Bibliography

- 1 Amado RG, Wolf M, Peeters M *et al.*: Wild-type *KRAS* is required for panitumumab efficacy in patients with metastatic colorectal cancer. *J. Clin. Oncol.* 26(10), 1626–1634 (2008).
- 2 Linardou H, Dahabreh IJ, Kanakoupiti D *et al.*: Assessment of somatic *k-RAS* mutations as a mechanism associated with resistance to EGFR-targeted agents: a systematic review and meta-analysis of studies in advanced non-small-cell lung cancer and metastatic colorectal cancer. *Lancet Oncol.* (2008) (Epub ahead of print).
- 3 Cunningham D, Humblet Y, Siena S *et al.*: Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. *N. Engl. J. Med.* 351(4), 337–345 (2004).
- 4 Jonker DJ, O'Callaghan CJ, Karapetis CS *et al.*: Cetuximab for the treatment of colorectal cancer. *N. Engl. J. Med.* 357(20), 2040–2048 (2007).
- 5 van Cutsem E, Peeters M, Siena S *et al.*: Open-label Phase III trial of panitumumab plus best supportive care compared with best supportive care alone in patients with chemotherapy-refractory metastatic colorectal cancer. *J. Clin. Oncol.* 25(13), 1658–1664 (2007).
- 6 de Roock W, Piessevaux H, De Schutter J *et al.*: *KRAS* wild-type state predicts survival and is associated to early radiological response in metastatic colorectal cancer treated with cetuximab. *Ann. Oncol.* 19(3), 508–515 (2008).
- 7 Di Fiore F, Blanchard F, Charbonnier F *et al.*: Clinical relevance of *KRAS* mutation detection in metastatic colorectal cancer treated by Cetuximab plus chemotherapy. *Br. J. Cancer* 96(8), 1166–1169 (2007).
- 8 Riely GJ, Pao W, Pham D *et al.*: Clinical course of patients with non-small cell lung cancer and epidermal growth factor receptor exon 19 and exon 21 mutations treated with gefitinib or erlotinib. *Clin. Cancer Res.* 12(3 Pt 1), 839–844 (2006).
- 9 Bonner JA, Harari PM, Giralt J *et al.*: Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. *N. Engl. J. Med.* 354(6), 567–578 (2006).
- 10 Chung KY, Shia J, Kemeny NE *et al.*: Cetuximab shows activity in colorectal cancer patients with tumors that do not express the epidermal growth factor receptor by immunohistochemistry. *J. Clin. Oncol.* 23(9), 1803–1810 (2005).
- 11 Moroni M, Veronese S, Benvenuti S *et al.*: Gene copy number for epidermal growth factor receptor (EGFR) and clinical response to antiEGFR treatment in colorectal cancer: a cohort study. *Lancet Oncol.* 6(5), 279–286 (2005).
- 12 Ogino S, Meyerhardt JA, Cantor M *et al.*: Molecular alterations in tumors and response to combination chemotherapy with gefitinib for advanced colorectal cancer. *Clin. Cancer Res.* 11(18), 6650–6656 (2005).
- 13 Andreyev HJ, Norman AR, Cunningham D, Oates JR, Clarke PA: Kirsten *ras* mutations in patients with colorectal cancer: the multicenter 'RASCAL' study. *J. Natl Cancer Inst.* 90(9), 675–684 (1998).
- 14 van Krieken JH, Jung A, Kirchner T *et al.*: *KRAS* mutation testing for predicting response to anti-EGFR therapy for colorectal carcinoma: proposal for an European quality assurance program. *Virchows Arch.* 453(5), 417–431 (2008).

Websites

- 101 The Wellcome Trust Sanger Institute: Distribution of somatic mutations in *KRAS*. Accessed September 2008
www.sanger.ac.uk/perl/genetics/CGP/cosmic?action=bygene&ln=KRAS&start=1&end=189&coords=AA:AA
- 102 European Society of Pathology
www.esp-pathology.org