EGFR FISH in colorectal cancer: what is the current reality?

To our knowledge, the first study to assess epidermal growth factor receptor (EGFR) gene copy number, and *KRAS* and *BRAF* mutations as biomarkers of response to EGFR-targeted monoclonal antibodies for metastatic colorectal cancer was published in *The Lancet Oncology* in 2005.[1] In this study, we reported that in patients treated with cetuximab or panitumumab, a correlation exists between clinical response and tumour *EGFR* copy number, and that *KRAS* or *BRAF* mutations occur mainly in patients with metastatic colorectal cancer resistant to treatment with these drugs.[1] These findings paved the way to subsequent studies that confirmed the association of *KRAS* mutations and resistance with such compelling evidence[2–7] that led to the approval of panitumumab in Europe for the treatment of KRAS wild-type only metastatic colorectal cancer. As for *EGFR* copy number, subsequent studies confirmed an association with clinical outcome.[4,6,8] After almost 3 years, some comments should be made to point out the existing knowledge about *EGFR* fluorescence in-situ hybridisation (FISH) analysis in patients with metastatic colorectal cancer.

Our first comment is that a biological phenomenon, underlying the association between *EGFR* copy number and clinical outcome, certainly does exist. In our first study,[1] we described the association between an increased *EGFR* copy number and tumour response in eight of nine responsive patients. Now, we know that most of the patients who respond have an increased *EGFR* copy number, but only a fraction of tumours with an increased gene copy number respond to treatment.[4,6,8]

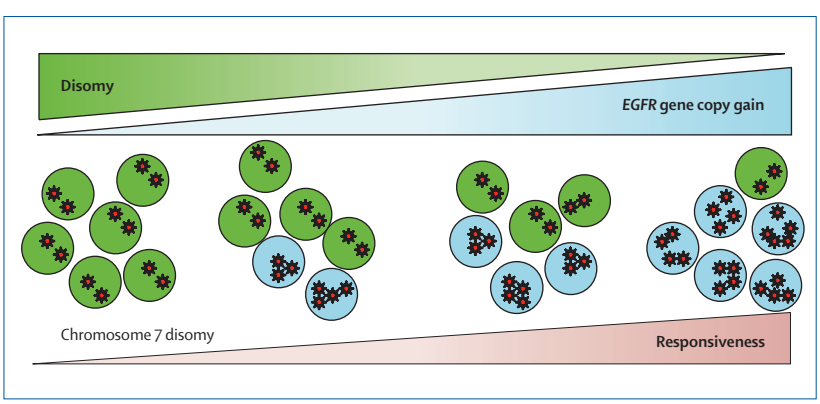

***Figure*: Representative model of responsiveness or refractoriness of colorectal cancer to *EGFR*-targeted monoclonal antibodies based on *EGFR* gene copy number assessed by FISH**
Stars represent *EGFR* signals detected by FISH.

Therefore, the high correlation between increased gene copy number and response to treatment was because of a high number of responsive patients selected in our first series in 2005. Actually, the crucial finding is that the non-increased, rather than the increased, *EGFR* copy number status is the most accurate predictive element for clinical outcome. Patients with low *EGFR* copy number are indeed unlikely to respond to treatment and have a worse time to progression and overall survival than patients with tumours with an increased gene copy number. In patients with tumours with an increased gene copy number, we described a 30% objective response, with six of 20 patients whose tumours had an increased *EGFR* copy number achieving an objective response compared with six of 58 patients in an unselected population.[8] In responsive patients, tumour growth is probably mainly driven by the EGFR pathway and this biological characteristic is evoked by an increase in *EGFR* copy number. In non-responsive tumours that harbour an increased *EGFR* copy number, the resistance to treatment is probably because of constitutive activation of signalling pathways downstream of the receptor by mutations of oncogenes such as *KRAS*, *BRAF*, or *PIK3C2A*,[2–7,9] or by the loss of a tumour suppressor gene such as *PTEN*.[4,9]

Our second comment is that, in all studies, assessment of *EGFR* copy number by quantitative PCR resulted in no association with clinical outcome.[1,5,10] In our experience, PCR analysis showed an increased *EGFR* copy number in a single patient with a high *EGFR* per cell count who had responsive disease, whereas detection of increased gene copy number in samples with a relatively low *EGFR* to chromosome enumeration probe 7 (CEP7) ratio was inconclusive.[1] PCR inefficacy is probably because of tumour DNA dilution by healthy cells during DNA extraction. Colorectal cancer rarely presents with high polysomy or amplification of *EGFR* and, even in patients with increased gene copy number, the *EGFR* per cell count is not so much higher than that detected in healthy tissue.

Our third comment is that *EGFR* copy number has more predictive rather than prognostic usefulness in patients with metastatic colorectal cancer. In 2006, Lenz and colleagues,[10] postulated higher prognostic than predictive usefulness, based on a significant

association of *EGFR* copy number (assessed by PCR not by FISH) with overall survival, but not with objective response or progression-free survival. To address this issue, in our 2007 series,[8] we assessed patients treated with panitumumab compared with patients who received best supportive care only, and we detected an association between *EGFR* copy number and progression-free survival only in patients treated with panitumumab, thus suggesting predictive rather than prognostic usefulness of *EGFR* copy number.[8]

Our final comment is that in metastatic colorectal cancer, *EGFR* FISH pattern is often not homogeneous, and has variable ratios of disomy versus polysomy or amplification. In these situations, scoring of *EGFR* signals and defining the *EGFR* pattern by FISH is sometimes difficult. To overcome this difficulty, we also assessed data as percentage of cells showing chromosome 7 polysomy (*EGFR* per nucleus ≥3) or *EGFR* amplification (*EGFR* to CEP7 ≥2).[8] Applying this criteria, an increased *EGFR* copy number was significantly associated with better clinical outcome (figure).[8] However, an exact definition of *EGFR* patterns and the reproducibility of data remain the largest obstacle for clinical applicability of the test; furthermore, although four studies[1,4,6,8] have confirmed its predictive usefulness, methods of tissue processing and *EGFR* scoring systems were not standardised between these studies.

In conclusion, together with *KRAS* and *BRAF* mutations,[2–7] the non-increased *EGFR* copy number detected by FISH is a predictive factor of resistance.[4,6,8] Therefore, effort should be made to better define the low copy-number pattern, which is frequently homogeneous. In our series,[8] chromosome 7 homogeneous disomy was described in 26 of 58 patients and was the most frequent pattern in tumours with non-increased *EGFR* copy number. Chromosome 7 disomy is easier to detect than an increase in *EGFR* copy number, and therefore, might enable a more reproducible FISH assay. However, further standardisation of methods is needed to reach better reproducibility and optimum sensitivity. From a clinical point of view, we can risk treating a non-responsive patient, but we cannot risk not treating a potentially responsive one.

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