Editorial

Clinical translation of predictive markers for anti-EGFR monoclonal antibody therapy in metastatic colorectal cancer

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Altered function of the epidermal growth factor receptor (EGFR) and associated mitogen-activated protein (MAP) kinase pathway plays a key role in disease progression in metastatic colorectal cancer (mCRC). Identification of EGFR as a therapeutic target has led to the development of anti-EGFR monoclonal antibodies (mAbs) cetuximab and panitumumab. However, the use of anti-EGFR mAbs in mCRC is associated with considerable treatment-related toxicity and a lack of response in a significant proportion of patients. Given the existence of alternate therapeutic options of comparable efficacy such as the anti-vascular endothelial growth factor receptor mAb bevacizumab (1), there is potential for improved outcomes through the identification of strategies to guide selection of the most appropriate first-line treatment.

Predictive markers can be used to identify patient sub-groups that are most likely to derive benefit from an intervention, facilitating selection of therapies with the greatest likelihood of success for an individual patient. By virtue of their capacity to influence treatment decisions, predictive markers are typically of greater clinical interest compared to prognostic markers (2,3). Assessment of a predictive marker requires the determination of a treatment effect (intervention versus control) in marker-positive and marker-negative cohorts (4). A formal statistical test for interaction between the marker and the treatment group is undertaken. In the oncology setting, the standard approach is to use a Cox proportional hazards model containing the treatment group, marker, and treatment-by-marker interaction term to model a time-to-outcome endpoint such as progression free survival (PFS) or overall survival (OS). As this analysis involves two or more comparison groups and evaluates the treatment effect of an intervention, data from randomised controlled trials (RCTs) are required. Due to the comparatively limited number of RCTs, particularly for targeted anticancer drugs, this requirement can limit the capacity to definitively validate these markers, compared to prognostic markers, which require only observational studies.

When considering the clinical translation of predictive markers for anti-EGFR mAb therapy in mCRC, sub-group analyses have been performed across a series of RCTs reporting outcomes for both cetuximab and panitumumab (*Table 1*). On the basis of these analyses, it has been well established that mutation of the downstream RAS oncogenes (collectively present in approximately 50% of mCRC tumors) is associated with a lack of treatment benefit (12). Accordingly, the use of cetuximab and panitumumab is limited to individuals with RAS wild-type (WT) tumors in treatment guidelines (13). While necessary to facilitate response to anti-EGFR mAb therapy, RAS WT status does not in itself ensure benefit, and there remains significant scope to identify additional predictive markers of treatment benefit. Highlighting the importance of consistency in the approach taken to assess predictive markers, due to differences in statistical interpretation, recent meta-analyses

Table 1 Randomised controlled trials reporting outcomes for anti-EGFR mAbs

Anti-EGFR agent *vs.* comparator	Trial (References)	Background therapy, lines of treatment
Cetuximab *vs.* no cetuximab	CO.17 (5)	BSC, ≥2nd line
	CRYSTAL and OPUS (6)	FOLFIRI (CRYSTAL) or FOLFOX-4 (OPUS), 1st line
	COIN (7)	Oxaliplatin and fluoropyrimidine chemotherapy, 1st line
Panitumumab *vs.* no panitumumab	20020408 (8)	BSC, ≥ 3rd line
	20050181 (9)	FOLFIRI, 2nd line
	PICCOLO (10)	Irinotecan, ≥ 2nd line
	PRIME (11)	FOLFOX-4, 1st line

considering the predictive value of BRAF mutation status in this setting have reported conflicting findings (14,15). This led to conjecture regarding the appropriate clinical translation of this marker (16). Similarly, despite pre-clinical and observational evidence for differential effects of individual RAS mutations on response to anti-EGFR mAb therapy, specifically that mCRC patients with KRAS G13D MT tumors may derive a benefit from treatment with anti-EGFR mAbs, a recent meta-analysis demonstrated no significant difference between KRAS G13D and other KRAS MT tumors in terms of benefit from anti-EGFR mAb therapy for mCRC (17).

Epiregulin (EREG) and amphiregulin (AREG) are ligands for EGFR that are overexpressed in mCRC (18), and as such are considered biologically plausible markers of EGFR pathway activity and inhibition (19). Consistent with this mechanistic insight, multiple observational studies (18,20-22) have reported positive correlations between AREG/EREG expression and anti-EGFR mAb efficacy in mCRC, whereby higher ligand expression is associated with improved survival (prognostic effect). More recently, sub-group analyses of two major RCTs (23,24) have reported AREG and EREG expression as a predictive marker of benefit for anti-EGFR mAb therapy in mCRC. It is important to note that assessment of EREG and AREG expression as a predictive marker of treatment benefit from anti-EFGR mAb therapy in mCRC is complicated as expression of these ligands is measured as continuous rather than discrete variables, and thus their analysis requires the determination of a threshold to discriminate marker-positive and marker-negative groups. In order to facilitate translation to clinical practice, both the predictivity of the marker and the robustness of the threshold determination require validation.

A sub-group analysis of the PICCOLO study reported in JAMA Oncology by Seligmann *et al.* (24) presents a novel dichotomous classification model to synthesize the combined effect of AREG/EREG expression as a predictive marker of treatment effect for panitumumab in mCRC. Using this model, the authors demonstrate that 'high' expression of AREG, EREG or both is predictive of benefit for panitumumab; hazard ratio for PFS in RAS WT patients of 0.38 (95% CI, 0.24 to 0.61) for 'high' expressors, compared to 0.93 (95% CI, 0.64 to 1.37) for 'low' expressors (test of interaction, P<0.001). In this analysis a 'pragmatically chosen' threshold was selected to define 'high' and 'low' ligand expression in order to give 'high' expressor (n=140/99 RAS WT) and 'low' expressor (n=183/120 RAS WT) groups of comparable size. While justified on the basis of maximising power within this analysis, the general applicability of this threshold to a broader patient cohort is unclear. Additionally, while partially inferable data are presented within the manuscript, no absolute criteria defining expression thresholds for either ligand are specified. Notably, in a secondary analysis considering AREG and EREG as independent, continuous markers, AREG (test for interaction, P=0.008) but not EREG (test for interaction, P=0.08) was demonstrated to predict PFS benefit from panitumumab. Trends toward superior OS were reported for 'high' expressors but were not statistically significant, although this may be anticipated given that the results of the PICCOLO trial were similarly negative for the primary outcome of OS (10). In a prior sub-group analysis of the CO.17 study (23), a threshold defining 'high' EREG expression based on a normalised delta cycle threshold (ΔCt) for EREG expression relative

to GAPDH expression (6.27) was reported to discriminate benefit from cetuximab in mCRC. Given the importance of reproducibility between cohorts and analyses required to facilitate clinical translation for predictive markers (25), the lack of absolute description of the criteria for assessment of AREG and EREG expression threshold presented by Seligmann *et al.* represents an important barrier to the clinical translation of AREG/EREG expression as a predictive marker of anti-EGFR mAb therapy in mCRC.

Given the emergence of multiple reports supporting the use of EREG/AREG as markers, both prognostic and predictive, for anti-EGFR mAb therapy in mCRC and the clinical imperative of selecting the most appropriate first-line intervention for these patients, clarification of robust and reproducible thresholds to facilitate clinical interpretation of an individual patient's level of expression independent of a study cohort is urgently required.

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Footnote

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