Editorial

Dual targeting of EGFR: ready for prime time?

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Monoclonal antibodies against the Epidermal Growth Factor Receptor (EGFR) are accepted therapies in number of cancer types including colorectal cancer (CRC), and head and neck cancer. Currently, cetuximab and panitumumab are approved as therapies for patients with *KRAS* wildtype CRC (1,2). However, as a result of mutations in *RAS* genes, collateral signaling through other members of the ErbB family or other receptor tyrosine kinases, and abnormalities of downstream signaling pathways such as the PI3K-Akt pathway, primary and secondary resistance are common (1). Recently, another mechanism of resistance has emerged, possibly more relevant to secondary resistance, which involves mutations in the extracellular domain (ECD) of EGFR. Montagut *et al.* initially described a point mutation in the ECD (S492R) of EGFR which resulted in impaired binding of cetuximab and panitumumab (3). Importantly, a patient with this mutant subsequently responded transiently to panitumumab (3). Subsequent work has confirmed that mutations in the EGFR-ECD contribute to cetuximab resistance mechanisms. Other EGFR resistance sites at R451C, K467T, S464L, G465R and I491M also mediate resistance to cetuximab and panitumumab (4). A recent study has shown that approximately 8% of patients treated with cetuximab were subsequently found to have S492R mediated resistance, and most of these patients also had concomitant *KRAS* mutations (5).

In a recent paper, Sánchez-Martín *et al.* (6) analyzed the impact of a 1:1 mixture of two recombinant, human–mouse chimeric monoclonal antibodies directed against non-overlapping EGFR epitopes (mAb992 and mAb1024), referred to as Sym004 (7,8), in pre-clinical and clinical cases of resistance to conventional anti-EGFR therapy mediated by EGFR ECD mutations. This study is latest in a series of studies with Sym004 exploring effects of a dual targeting EGFR strategy (7-9). Sym004 causes rapid EGFR internalization and subsequent degradation of the receptor, with concomitant inhibition of downstream signalling and significant anti-tumour activity (7-9). Preclinical studies with Sym004 showed superior antitumor activity as compared with other anti-EGFR antibodies such as cetuximab and in models of acquired cetuximab resistance (8,9). In the current study (6), Sánchez-Martín *et al.* showed that Sym004 was superior to cetuximab in binding to cells expressing a number of the EGFR ECD mutations (S492R, K467T, R451C and G465R). Although panitumumab also retained some ability to bind to these cells, Sym004 was also able to bind better than panitumumab to cells with the EGFR-ECD G465R mutation. Sánchez-Martín *et al.* (6) presented data that Sym004 was more effective than cetuximab and panitumumab for treating CRC, in abrogating ligand induced phosphorylation and inhibition of down-stream signaling and tumour growth. Interestingly, they also observed that a patient who had progressed on cetuximab and who had an EGFR-ECD G465R mutation had stabilization of disease for almost 4 months after treatment with Sym004.

Overall, the Sánchez-Martín *et al.* study (6) indicates that EGFR targeting using non-redundant anti-EGFR agents is more effective and single antibody treatments. Other approaches to multi-targeting of EGFR have also been

reported (10). MM-151 is a mixture of three antibodies against non-overlapping epitopes of EGFR and has been shown to inhibit EGFR signaling and cell growth in preclinical models where the EGFR has ECD mutations (10). At present, combination studies with the currently approved EGFR antibodies cetuximab and panitumumab have not been reported clinically or pre-clinically. Pre-clinical data exists to show that combining the murine version of ABT-806 with a murine anti-EGFR antibody equivalent to cetuximab results in superior inhibition of proliferation of EGFR driven tumor inhibition *in vivo* (11). ABT-806 binds to a tumor specific conformational epitope on EGFR which is distinct from that of cetuximab or panitumumab (12). Mechanistically, the binding of antibodies to distinctly different sites of the EGFR-ECD may result in improved kinase inhibition, in part due to altered oligomerization of the EGFR as a consequence of antibody: receptor interactions, and consequential inhibition of kinase activation (13). Other anti-EGFR antibodies which bind to EGFR-ECD epitopes distinct from cetuximab and panitumumab have also been reported, such as GC1118, so other antibody combination may also be possible (14).

Evidence of the safety and efficacy of combinations of antibodies to EGFR (e.g., Sym004 and MM-151) in clinical trials is emerging, some challenges remain. MM-151 is in phase 1 clinical testing (NCT01520389 and NCT02538627), and reported toxicity was frequent but manageable: grade 3/4 toxicities included infusion reactions (16%), rash and dermatological reactions (11%), hypomagnesemia (7%), hypophosphatemia (6%) and diarrhea (1%). The objective response rate for MM-151 treatment in CRC patients was 7% (15). The phase 1 study of Sym004 has also been reported, involving 62 patients with refractory CRC, which included expansion cohorts of patients who were previous responders to conventional anti-EGFR therapy but had since progressed (16). At the highest dose levels of Sym004 (9 and 12 mg/kg), the rates of grade 3+ skin toxicity and hypomagnesemia were 50% and 21% respectively. However, it was encouraging to see that an objective response rate of 13% and a disease control rate being of 67%. Interestingly, dual targeting of EGFR with cetuximab combined with erlotinib in 50 CRC patients has also been reported (17), with improved response rates compared to prior studies of either drug alone: 41% response rate in *KRAS WT* tumors for the dual drug treatment compared to historical data showing response rates of 7–20% for cetuximab alone (18-20), 17–22% for panitumumab alone (20,21), 0% for erlotinib alone (22) and 0% for gefitinib alone (23) in similar populations; but the toxicity observed for the cetuximab/erlotinib combination was greater than for cetuximab treatment.

There is a strong case that the concurrent targeting of EGFR with dual antibodies results in superior anti-tumor activity in CRC. Further exploration of the efficacy of the dual antibody treatment in Phase II trials in patients resistant to cetuximab is justified. Given that EGFR-ECD mutations are one of the resistance mechanisms to cetuximab, careful patient selection will be pivotal in study design. Morelli *et al.* (5) have shown it is possible to detect EGFR mutations and KRAS mutations non-invasively using circulating DNA. The data from the Phase 1 study of Sym004 also suggests that this molecular phenotyping may correlate with clinical outcomes (16). However, reducing the toxicity of dual EGFR targeting approaches and optimizing therapeutic dosing will be important to facilitate further clinical use of these agents. Towards this end, combination of tumor-specific EGFR antibodies may possibly reduce the toxicity of combination therapy. For example, antibody ABT-806 has none of the usual toxicities of other anti-EGFR antibodies: ABT-806 targets a unique conformational epitope of the EGFR-ECD which results in absence of normal tissue binding and minimal skin and gut toxicity in clinical trials (12,24). As such, a combination of cetuximab or panitumumab with ABT-806 may have the benefits of a combined EGFR blockade and improved response rates but less toxicity. The dual targeting of EGFR is likely to be relevant to other EGFR positive tumor types. These encouraging results for dual EGFR antibody therapy for CRC patients suggests that clinical trials should be initiated in patients with head and neck, brain and lung cancers. For *KRAS* wildtype CRC and all these other tumors types, dual treatment should result in reduced development of EGFR-ECD related resistance and these combinations should be compared to cetuximab alone.

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Footnote

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