



# The role of Sym004, a novel EGFR antibody mixture, in patients with refractory colorectal cancer

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Colorectal cancer is the fourth most frequently diagnosed cancer in the United States and a major cause of mortality throughout the world. It is estimated that in 2015, 132,700 new patients have been diagnosed with colorectal cancer in the United States and 49,700 have died from the disease (1). Even though recent advances in the treatment of metastatic colorectal cancer have significantly prolonged clinical outcome, long-term survival does not exceed 10%. Ongoing research has focused on targeted therapies in order to further increase survival rates.

The epidermal growth factor receptor (EGFR) is one of the key molecules frequently disrupted in colorectal cancer. Activation of the receptor through homo- or hetero-dimerization with other members of the ErbB family results in autophosphorylation and initiation of a signaling cascade leading to increased cell proliferation, survival and angiogenesis. Deregulated activity of the EGFR signaling pathway has been associated with disease progression and poor survival in patients with colorectal cancer (2). Mechanisms include overexpression of the ligands or the receptor itself, receptor mutations and activation of neighboring receptors that promote dimerization. Therefore, EGFR constitutes an important therapeutic target and several efforts have been made to successfully inhibit its activity.

Cetuximab (Erbbitux<sup>®</sup>), a chimeric mouse-human IgG1 antibody and panitumumab (Vectibix<sup>®</sup>), a fully human monoclonal IgG2 antibody, have been developed to target EGFR. These antibodies mainly act by blocking EGFR ligands from binding to the extracellular domain III of the

receptor, thus preventing its dimerization and signaling. To a lesser extent, they induce receptor internalization and degradation and activate antibody-dependent cellular cytotoxicity (with the exception of panitumumab). Both monoclonal antibodies (mAbs) have been approved for the treatment of KRAS and NRAS wild-type metastatic colorectal cancer in combination with chemotherapy (first to third line) or as single agents (in later lines of therapy). Studies have shown that they prolong progression-free survival (PFS), overall survival (OS) and improve objective response rate and quality of life (3). However, due to acquired mechanisms of resistance, most patients usually develop progressive disease. Studies have presented several molecular mechanisms implicated in secondary resistance to anti-EGFR antibodies; upregulation of EGFR ligands competing with the antibodies, acquired mutations of the extracellular domain of the receptor preventing binding of mAbs, overexpression of downstream molecules bypassing EGFR blockade and tyrosine kinase amplification leading to activation of parallel pathways (4). Several investigators have focused on uncovering ways to overcome these mechanisms of resistance and ultimately improve clinical benefit.

Previous studies have shown that 15–20% of patients treated with anti-EGFR antibodies develop resistance due to acquired mutations in the extracellular domain of the EGFR. These mutations often inhibit the binding of cetuximab and panitumumab to the receptor. In the process of exploring ways to overcome this mechanism of resistance, investigators identified a promising novel synergistic anti-EGFR antibody mixture (5). Sym004 is a combination

of two distinct antibodies targeting non-overlapping epitopes of the extracellular domain of EGFR. The authors demonstrated that Sym004 was not only able to bind to the mutated EGFR, but also to induce receptor internalization followed by degradation. This provided a Sym004 advantage, compared to cetuximab and panitumumab which mainly block the binding of the ligands to the receptor. Resistance to these mAbs is frequently mediated by upregulation of the ligands or EGFR mutation and reinstatement of EGFR signaling. Sym004 overcomes these mechanisms of resistance by binding mutated and wild type EGFR and inducing rapid internalization of the receptor. Preclinical *in vitro* studies have established the efficacy of the antibody mixture in cetuximab-resistant cell lines (6), even in the presence of high-affinity EGFR ligands (7). Sym004 has also shown *in vivo* activity in xenografts (5,6), leading to significant tumor growth inhibition.

Further preclinical studies assessing the safety and toxicity of Sym004 in non-human subjects, reported side effects, which were expected based on the experience with the commonly used anti-EGFR mAbs (8). The first phase I study provided promising data for patients with refractory tumors (7). In this study, the majority of patients with metastatic colorectal cancer had previously received at least one anti-EGFR mAb treatment. All patients had initially responded to treatment but eventually progressed. Commonly observed adverse events with Sym004 were skin rash, hypomagnesemia, pruritus and diarrhea. Serious drug-related adverse events were reported in 30% of the patients. Interestingly, this study reported an overall disease control rate of 67% and a median PFS of 3.3 months, showing that monotherapy with anti-EGFR monoclonal antibodies remains an option in a highly selected EGFR-addicted population.

In the study published in *Clinical Cancer Research* in February 2016, Sánchez-Martín and colleagues further explore the efficacy of Sym004 in cetuximab-resistant colorectal tumors, harboring extracellular domain EGFR mutations (9). They demonstrate that Sym004 is active both *in vitro* and *in vivo* in cetuximab-resistant tumors. Using cellular models, they initially confirm that, unlike cetuximab and panitumumab, Sym004 is able to bind to EGFR harboring ectodomain mutations. Additionally, the investigators demonstrate that Sym004 effectively prevents the phosphorylation of all EGFR mutants, even in the presence of high concentrations of EGF ligands. These *in vitro* models verify the high efficacy of Sym004

in cetuximab-resistant cell lines harboring mutations in EGFR extracellular domains. The mutations reported (S492R, R451C, K467T, and G465R) have been identified in patients following treatment with anti-EGFR monoclonal antibodies.

Sym004 was also tested in tumors derived from cetuximab-resistant, EGFR-mutant cell lines injected to mouse xenografts. Investigators report profound inhibition of tumors harboring the S492R *EGFR* mutation and significant growth delay of tumors harboring the G465R *EGFR* mutation. Cetuximab did not provide similar results in EGFR mutant tumors, and was only able to inhibit tumor growth from the parental EGFR wild-type cell lines. Using genomic data from 13 patients treated in the phase I study (7), the authors highlight the efficacy of Sym004 in a patient who had progressed after treatment with cetuximab. Tumor biopsy, obtained after disease progression, revealed the presence of the extracellular G465 *EGFR* mutation. The authors acknowledge that the response to Sym004 is used as a proof of principle and that prospective studies are mandatory to validate such results. The findings of this study are promising for patients who become resistant after treatment with cetuximab or panitumumab due to acquired mutations, however, these are responsible for secondary resistance in only a fraction of cases. It is equally important to globally explore alternative mechanisms that confer resistance to such treatments and identify ways to overcome them.

Preclinical models provide useful insights about the different mechanisms of acquired resistance to anti-EGFR therapies (10-13). Investigators have identified mutations in *KRAS* (4,14,15), *NRAS*, *BRAF* (14) and in the kinase domain of EGFR in liquid biopsies (16); these mutations were not identified in the primary tumors or in the plasma of patients prior to treatment with anti-EGFR mAbs. *KRAS* (11,17,18), *MET* (17) and *HER2* (18) amplification have also been implicated in resistance in patients with refractory colorectal cancer. Investigators are already assessing combinations of targeted compounds, based on the genomic variation identified in each tumor after treatment with anti-EGFR monoclonal antibodies (19). Liquid biopsies and sensitive molecular methods are likely to provide a non-invasive opportunity for monitoring tumor genetic and epigenetic changes that arise from the more virulent malignant clone over time and consequently an opportunity to tailor therapies for each patient accordingly.

To conclude, Sym004 is a new weapon targeting EGFR

in the arsenal against colorectal cancer. Studies demonstrate similar efficacy and safety profile with the previously approved anti-EGFR mAbs. The advantage of this new agent lies upon its ability to overcome resistance mediated by acquired mutations in the extracellular domain of EGFR. Subsequent steps will be important and are mandatory: it is important to accurately define the population who will benefit from this and the line of therapy administration (early versus late), to expand safety and activity data, to explore its combination with chemotherapy backbones and investigate biomarkers of efficacy/resistance, in order to obtain the maximum clinical benefit.

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