

Featured Publication Note

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Sym004: A Novel Anti-EGFR Antibody Mixture with Superior Anticancer Efficacy

The epidermal growth factor receptor (EGFR) signaling system is frequently unbalanced in human malignancies and is a validated target for anticancer therapy. Identifying EGFR antagonists with greater effectiveness than existing clinical agents is of interest to more effectively treat, and hopefully cure, more patients. Currently, two monoclonal antibodies (mAbs) which target EGFR, cetuximab (Erbix[®]) and panitumumab (Vectibix[®]), are approved for the treatment of metastatic colorectal cancer.

It has been shown that synergistic combinations of antibodies may be superior at controlling tumor growth. The researchers of this study have previously identified a potent antibody combination called Sym004, which contains an equal mixture of two anti-EGFR mAbs (992 and 1024), directed against distinct non-overlapping epitopes on EGFR. In this study, it was shown that Sym004, like other anti-EGFR mAbs in clinical use, synergistically inhibits cancer cell growth and survival.

As part of this study, the effect of Sym004 on EGFR internalization was investigated. Cancer cell lines were treated with different concentrations of Alexa Fluor[®] 488-labeled antibodies for varying periods of time and cells were examined using the **Opera[™] LX confocal high content screening system**. After a 15 min incubation, both Sym004 and cetuximab were located at the cell surface. After 4 h, most of the reference mAb was still located on the cell surface, whereas most of the antibody in Sym004-treated cells was located in visible intracellular antibody-containing vesicles. Internalization was quantified using **Acapella[™]** software to determine the number of intracellular vesicles (measured as the number of spots per cell). Sym004-treated cells had a significantly higher number of vesicles at different antibody concentrations, and they appeared much faster than in cells treated with mAbs.

Unlike other mAbs, Sym004 induces rapid and efficient removal of EGFR from the cancer cell surface by triggering EGFR internalization and degradation. This may result in a superior anti-tumor response and a reduced ability of tumor cells to acquire resistance to treatment. Sym004 also exhibited more pronounced growth inhibition *in vitro* and superior efficacy *in vivo* compared to the reference anti-EGFR mAbs. The findings of this study illustrate a strategy to target EGFR more effectively than existing clinical antibodies.

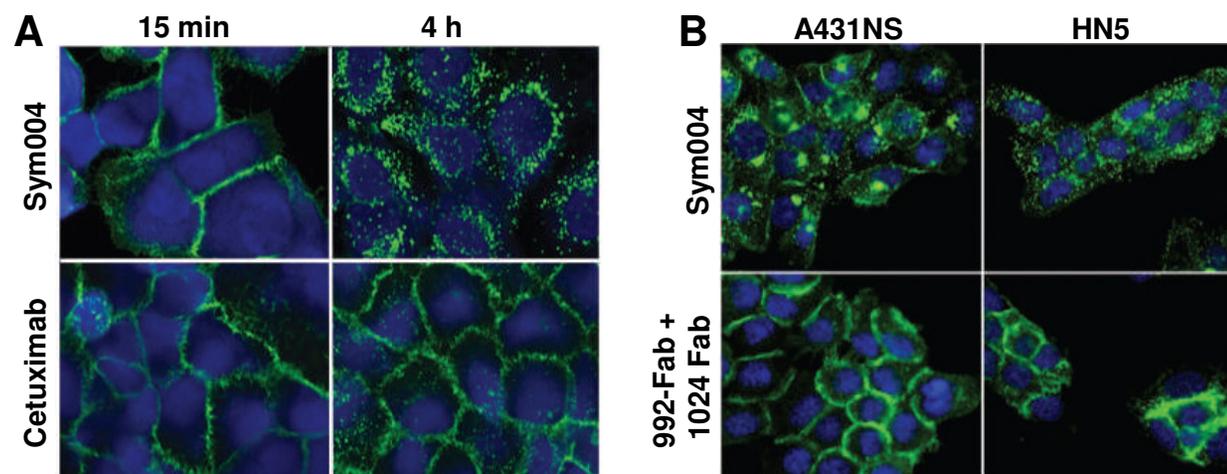


Figure: Sym004 induced internalization

A – Confocal images (400 x) of the location of Alexa Fluor[®] 488-labeled Sym004 and cetuximab in HN5 cancer cells after 15 min and 4 h of incubation.

B – Confocal images (400 x) of A431NS and HN5 cancer cells treated for 4 h with a mixture of either Alexa Fluor[®] 488-labeled 992 and 1024 (the two antibodies in Sym004), or Alexa Fluor[®] 488-labeled fragment antigen binding (Fab) fragments of 992 and 1024.

Cells were stained with 1 μ mol/l Hoechst and 2 μ g/ml CellMask[™] Blue. Images were acquired using the **Opera LX confocal high content screening system**.

Figure adapted with permission from the American Association for Cancer Research. Pedersen M W, Jacobsen H J, Koefoed K, Hey A, Pyke C, Haurum J S and Kragh M (2010). Sym004: A Novel Synergistic Anti-Epidermal Growth Factor Receptor Antibody Mixture with Superior Anticancer Efficacy (Figure 2). *Cancer Research*; 70 (2): 588–597.

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