Targeting of a Conformationally Exposed, Tumor-Specific Epitope of EGFR as a Strategy for Cancer Therapy

Hui K. Gan\textsuperscript{1,2}, Antony W. Burgess\textsuperscript{2}, Andrew H. A. Clayton\textsuperscript{3}, and Andrew M. Scott\textsuperscript{2}

Abstract

Epidermal growth factor receptor (EGFR) and its most common extracellular mutant, EGFRvIII, are important therapeutic targets in multiple cancer types. A number of monoclonal antibodies and small-molecule inhibitors against these receptors are now used for anticancer treatments. New insights into the structure and function of these receptors illustrate how they can be targeted in novel ways, with expected improvements in the therapeutic efficacy. Monoclonal antibody 806 (mAb806) is an antibody that targets a conformationally exposed epitope of wild-type EGFR when it is overexpressed on tumor cells or in the presence of oncogenic mutations such as EGFRvIII. The mechanism of action of mAb806, which allows for EGFR inhibition without normal tissue toxicity, creates opportunities for combination therapy and strongly suggests mAb806 will be a superior targeted delivery system for antitumor agents. Targeting of the epitope for mAb806 also appears to be an improved strategy to inhibit tumors that express EGFRvIII. This concept of conformational epitope targeting by antibodies reflects an underlying interplay between the structure and biology of different conformational forms of the EGFR family. Cancer Res; 72(12): 2924–2930. ©2012 AACR.

Introduction

Epidermal growth factor receptor (EGFR), which is one of 4 members of the ErbB family, is a cell surface receptor with an oncogenic role in many tumors, and its inhibition has been used to improve treatment in several tumor types (Table 1). The basic structure and conformation states (Fig. 1) of wild-type EGFR (wtEGFR) have been elucidated (1–3). It is also clear that ligand binding promotes the formation of a back-to-back dimer (Fig. 1B), wherein dimerization occurs primarily through interactions between the dimerization arms of the CR1 domains, with a subsequent increase in kinase autophosphorylation and activation. The resulting phosphorylases recruit myriad downstream effector proteins that are involved in signal transduction pathways, such as mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K). Despite substantial research, the mechanisms by which ligand binding promotes receptor dimerization/oligomerization and activation have yet to be fully elucidated (2, 3).

Therapeutic EGFR inhibition mainly employs monoclonal antibodies that bind to the EGFR extracellular domain (ECD) or tyrosine kinase inhibitors (TKI) that block kinase activation directly (4). Such therapies have had some clinical success, but resistance develops in nearly all patients (4). They are also associated with characteristic side effects (especially rash) that affect patients' quality of life (4). All of the antibodies currently in routine clinical use (cetuximab, panitumumab, and nimotuzumab) bind to the L2 domain of EGFR, preventing ligand binding and/or sterically inhibiting the subsequent adoption of the extended conformation that is necessary for dimerization (Fig. 1B; refs. 2, 5).

There is increasing recognition that the EGFR truncation mutant, EGFRvIII, is an important and druggable target for cancer therapy. EGFRvIII, which is almost always associated with EGFR gene amplification, is the most common ECD mutant of EGFR (6). EGFRvIII comprises an in-frame deletion of 267 amino acids from the EGFR ECD, with a novel glycine residue at the deletion site (Fig. 1A). Although it was initially thought to be relevant only in high-grade gliomas, it is now clear that EGFRvIII is relevant in a number of cancer types (Table 1). EGFRvIII is tumor specific, and there is no evidence that it occurs in normal tissues. Although it is unable to bind ligand, EGFRvIII has low-level constitutive kinase activity and impaired endocytosis and degradation (6). A substantial body of work shows that EGFRvIII is highly tumorigenic (6). Its introduction into cells, even those that already express wtEGFR, results in a more aggressive tumor phenotype, with increased invasion, proliferation, angiogenesis, and evasion of apoptosis (6). EGFRvIII also confers radioresistance (7–10) and chemoresistance (11–14). Therefore, targeting of EGFRvIII is highly attractive and is much less likely to show nonspecific binding to normal tissues. This has been a significant problem for other agents that target wtEGFR, especially for the delivery of toxic payloads. However, EGFRvIII is relatively resistant to conventional anti-EGFR therapeutics such as gefitinib (15–17), erlotinib (18), and cetuximab (11, 19–21), all of which also target...
wtEGFR on normal cells. Cetuximab is unable to directly inhibit the growth of EGFRvIII-expressing cells in vitro (20, 21) and in vivo (11), or to fully reverse the radioresistance mediated by EGFRvIII (21).

### Table 1. EGFR inhibition as a therapeutic modality in selected cancer types

<table>
<thead>
<tr>
<th>Tumor</th>
<th>EGFR expression (%)</th>
<th>EGFRvIII expression (%)</th>
<th>EGFR therapeutics in standard clinical use</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>GBM</td>
<td>40–63</td>
<td>24–64</td>
<td>Nimotuzumab: approved in some countries.</td>
<td>Jungbluth et al. (22); Moscatello et al. (54); Shinojima et al. (55); Feldkamp et al. (56); Saikali et al. (57); Mellinghoff et al. (58); Heimberger et al. (59)</td>
</tr>
<tr>
<td>Head and neck</td>
<td>43–100</td>
<td>42–48&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Cetuximab: with radiotherapy in locally advanced disease; as monotherapy in metastatic disease after failure of platinum-based therapy Nimotuzumab: approved in some countries for head-and-neck and nasopharyngeal carcinoma</td>
<td>Sok et al. (11); Pectasides et al. (60)</td>
</tr>
<tr>
<td>Non–small cell lung cancer</td>
<td>32–84</td>
<td>0–5</td>
<td>Gefitinib: restricted indication for patients who are known to be benefiting based on prior exposure to gefitinib</td>
<td>Ji et al. (17)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Erlotinib: treatment of locally advanced/metastatic disease after chemotherapy failure</td>
<td>Sihto et al. (62)</td>
</tr>
<tr>
<td>Breast</td>
<td>14–91</td>
<td>27–36</td>
<td>Not applicable</td>
<td>Ohtsuka et al. (63); Sasaki et al. (64); Wikstrander et al. (65); Ge et al. (66)</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>25–77</td>
<td>0</td>
<td>Cetuximab: treatment of EGFR-expressing metastatic disease (± irinotecan) progressing on or refractory to irinotecan Panitumumab: treatment of EGFR-expressing metastatic disease progressing on or following fluoropyrimidine-, oxaliplatin-, and irinotecan-containing regimens</td>
<td>Spindler et al. (67); Azuma et al. (68)</td>
</tr>
<tr>
<td>Pancreas</td>
<td>30–95</td>
<td>Not available</td>
<td>Erlotinib: treatment of advanced, unresectable, or metastatic disease in combination with gemcitabine</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Adapted from Dei Tos and Ellis (69).

<sup>b</sup>This figure may be substantially lower in Japanese patients; one study showed no evidence of EGFRvIII in a cohort of 82 patients (70).

mAb806, a Novel Antibody That Specifically Targets Tumor-Associated EGFR

Jungbluth and colleagues (22) generated monoclonal antibody 806 [mAb806(IgG2b)] by immunizing mice with murine
NR6 fibroblasts expressing human EGFRvIII but not wtEGFR. Characterization of mAb806 confirmed binding to EGFRvIII, but, unexpectedly, mAb806 also bound to tumor cells overexpressing wtEGFR at a level in excess of $1 \times 10^6$ receptors per cell (23–26). In vivo studies confirmed that mAb806 bound to and reproducibly inhibited xenograft tumors that expressed EGFRvIII or overexpressed wtEGFR (19, 27–30). Of interest, no mAb806 binding was detected in normal tissues such as the liver (22).

The mechanism of mAb806’s tumor specificity was elucidated with the discovery that the epitope is located between amino acids 287 and 302 in the CR1 domain of the EGFR ECD (31, 32). This epitope is masked in the inactive monomer state (Fig. 1A) or the fully liganded back-to-back dimer state (Fig. 1B), which explains the lack of mAb806 binding to normal tissues in which EGFR is quiescent. However, the conditions that allow for the exposure of this epitope preferentially occur under tumor-specific conditions. Dysregulated EGFR activity as a result of EGFR overexpression or autocrine loops enables mAb806 binding, probably through transient epitope exposure.

Epitope exposure also occurs as a result of ECD truncations, such as the EGFRvIII mutation, or by mutation of a disulphide bond flanking the epitope (32). Finally, changes in receptor conformation as a result of glycosylation changes or concurrent treatment with the EGFR TKI AG1478 also increase epitope exposure (33).

To confirm the tumor-specific properties of mAb806 in human subjects, Scott and colleagues (34) reported on a trial of a single dose of ch806 (the chimeric form of mAb806) in patients with a range of EGFR-positive tumors. Treatment was well tolerated, with no significant toxicity at doses up to 40 mg/m². The disease stabilization rate was 63%. Of importance, linear pharmacokinetics in blood were observed across all dose levels. Biodistribution studies with $^{111}$In-ch806 in the same patients showed excellent tumor uptake without associated normal tissue uptake, even in organs that express significant amounts of wtEGFR (e.g., the liver). Consistent with the latter finding, no patient experienced any rash. These results confirmed the tumor-specific properties of mAb806, and in particular the lack of typical toxicities associated with EGFR.

Figure 1. A, the structure of wtEGFR. The ECD has 2 cysteine-poor, homologous large domains (L1 and L2) and 2 cysteine-rich domains (CR1 and CR2). The EGFR ECD of EGFRvIII contains a deletion affecting L1 and CR1 from aa 6–273, with insertion of a novel glycine residue. The epitope of mAb806 is located between aa 287 and 302. B, the ECD is shown as a tethered or untethered monomer. The transition from tethered monomer to untethered monomer requires a 130% rotation of 2 domains (L1 and CR1) around a pivot point at the junction of the CR1-L2 domains, resulting in exposure of the dimerization arm (shown as the blue ovoid in CR1). C, the ligated back-to-back dimer comprises 2 extended monomers that are able to interact through their respective dimerization arms. Of importance, the epitope of mAb806 is not available for binding in either the tethered monomer (B) or the ligated dimer (C); instead, exposure of the epitope only occurs in certain truncation mutants of EGFR, such as EGFRvIII, or in certain conformations, such as the extended untethered monomer (B) or some form of an alternative, extended dimer (D). Adapted from Kozer et al. (45) and Gan et al. (33). aa, amino acids.
antibodies that target the L2 domain (4). Currently, a humanized version of mAb806 (ABT-806) is being developed as a therapeutic agent for patients with EGFR-positive tumors (ClinicalTrials.gov identifiers: NCT01255657 and NCT01406119).

Overall, mAb806 is an antibody that targets a conformationally exposed epitope of wtEGFR when EGFR is overexpressed, truncated, or involved in other tumor-associated abnormalities. mAb806 has significant antitumor activity without nonspecific binding to normal tissues. In addition to its use as a direct therapeutic antibody, the specificity and internalization properties of this antibody suggest a potential role as a diagnostic radioligand (34, 35) or as an immunonuc conjugate for the delivery of drugs, toxins, or radioisotopes to tumors. The benefits of targeting conformationally sensitive epitopes are also being pursued by a handful of other groups working with EGFR (36), HER2 (37, 38), and solid tumors such as prostate cancer (39), colorectal cancer (40), and melanoma (41). MDX-1097, an antibody against a conformation epitope in myeloma, recently completed phase I testing (42, 43). Targeting of the conformational epitope has been more actively pursued in immunologic circles. In a recent study, Kelker and colleagues (44) showed that careful immunogen selection resulted in the generation of antibodies with promising therapeutic potential. By targeting cryptic, usually conserved epitopes in the gp120 protein of HIV, they also showed strong therapeutic potential. By targeting cryptic, usually conserved epitopes in the gp120 protein of HIV, they also showed strong therapeutic potential.

**Conformationally Exposed Epitopes as a Target for Antibody Therapy**

The specificity of mAb806 for restricted conformations of wtEGFR and EGFRvIII provides new insights into the structure-function relationships of wtEGFR and EGFRvIII dimers, which are now increasingly thought to occur spontaneously and to potentially represent the preactivation state of these receptors (3). Kozer and colleagues (45) examined the native conformation and oligomerization status of EGFR and EGFRvIII using image correlation spectroscopy and homo-Forster resonance energy transfer in BaF3 cells transfected with wtEGFR and EGFRvIII, a cellular system devoid of secreted ligands or other ErbB receptor members. They found that 80% to 95% of wtEGFR or EGFRvIII exist as preformed (unliganded) dimers or even higher-order oligomers. Because some of the wtEGFR dimers are reactive with mAb806, which is unable to bind the back-to-back dimer, they must exist as some alternative untethered/dimer conformation (46). Furthermore, mAb806 was shown to have a different effect on EGFR activation, clustering, and downstream signaling compared with mAb528, a mAb that binds the L2 domain of EGFR in a manner similar to that observed for cetuximab (47). mAb528 increased the clustering of inactive wtEGFR dimers but had no effect on the oligomerization or constitutive signaling of EGFRVIII. The latter observation is consistent with previous studies in which treatment of cell lines expressing EGFRVIII alone (i.e., lacking concomitant wtEGFR expression) with cetuximab showed no reduction in EGFRVIII phosphorylation in vitro and no inhibition of tumor growth in vivo (21, 45, 48). In one study, enhancement of EGFRVIII phosphorylation with cetuximab treatment was observed (21). Similarly, other studies have shown that cetuximab treatment of EGFRVIII-expressing cell lines is insufficient to completely downregulate key signaling effectors such as Akt (20, 21) and MAPK (20, 21). Overall, these data raise the possibility that the antitumor effects of cetuximab in most EGFRVIII-expressing tumors are relatively weak and rely on inhibition of wtEGFR or wtEGFR/EGFRVIII interactions, or possibly an immunologic mechanism such as antibody-dependent, cell-mediated cytotoxicity (20).

In contrast with mAb528, mAb806 alters the conformational state of EGFRVIII oligomers (45), which was associated with a reorientation of its intracellular domains and a reduction in EGFRVIII phosphorylation with ongoing therapy in animal models (19). Although mAb806 monotherapy does not change the oligomerization status of EGFRVIII, coadministration of mAb528 with mAb806 results in increased cross-linking of EGFRVIII (45).

Taken together, the biophysical and preclinical data suggest that conformational epitopes are not restricted to tethered and extended monomeric conformations, but also apply to preformed oligomeric receptor states.

**Mechanism of Action and Therapeutic Strategies**

*In vivo* studies support the above findings that mAb806 and mAb528 have different effects on EGFR activation and clustering. mAb806 clearly and reproducibly inhibits the growth of wtEGFR-overexpressing tumors in vivo (29, 48, 49) with associated antiangiogenic effects and vascular normalizing effects, and reduced tumor hypoxia (49). These results are quite different from those seen with mAb528 treatment, and appear to require a functional NF-κB pathway (49). The ability of mAb806 to foster vascular normalization and reduce tumor hypoxia suggests a role as a radiosensitizing agent in EGFRVIII-expressing tumors, which is important because cetuximab is not radiosensitizing in these tumors (21). This was confirmed in experiments with EGFRVIII xenografts, in which the combination of mAb806 with radiotherapy resulted in schedule-dependent radiosensitization (50). This combination inhibited both tumor angiogenesis and growth. mAb806 treatment appears to achieve radiosensitization by selectively blocking the phosphorylation of Akt.

The ability of mAb806 to partner with other therapeutic modalities is not restricted to radiotherapy. Preclinical data suggest that combinations with small-molecule inhibitors such as the EGFR TKI AG1478 or Src inhibitors such as dasatinib (28, 29, 48) can be advantageous. mAb806 can also be effectively combined with mAb528 in vivo, resulting in increased inhibition of receptor phosphorylation and increased antitumor efficacy (29). Furthermore, mAb806’s specificity for tumors and its rapid internalization into tumor cells provide a compelling rationale for using mAb806 to deliver toxins or payloads to tumors in patients (51).
The potential benefits of dual targeting of EGFR, either by using 2 ECD-targeting antibodies or by combining an ECD-targeting antibody with a kinase or other signaling inhibitor, are clearly of interest to clinicians who treat cancers that express EGFRvIII or overexpress wtEGFR. The feasibility of this approach was recently shown in a trial of cetuximab and erlotinib, in which both high response rates and high toxicity were observed (52). In this regard, mAb806’s low toxicity, along with the above data showing the benefits of combining mAb806 with other cancer therapies, makes it of particular interest. Further work is required to determine the mechanisms by which mAb806 exerts its effect. One limitation has been the fact that the antitumor activity of mAb806, which has been clearly and reproducibly shown in experiments using xenograft tumors (19, 27–30), appears to use mechanisms that are difficult to replicate in vitro (28, 48). It will also be important to explore the effects of mAb806 in models in which other ErbB family members are coexpressed, because the concurrent expression of other ErbB receptors (e.g., ErbB2/HER2) can modulate the behavior of EGFR (53). Resistance to mAb806, and to EGFR therapeutic agents in general, is also another area of major interest.

Conclusions

More than 50 years ago, EGFR became the first cell membrane protein to be linked directly to cancer. However, only recently has knowledge about its structure and biology allowed us to exploit the inhibition of EGFR for therapeutic purposes. mAb806 illustrates the unique approach of targeting a conformationally exposed epitope in the CRI domain of EGFR that is tumor specific, without associated normal tissue binding. This approach not only reduces toxicity but also appears to provide superior inhibition of tumors that express EGFRVIII. Ongoing improvements in our understanding of EGFR structure and biology are vital if we are to continue developing better therapies and rational strategies for combing both targeted agents and conventional treatments such as chemotherapy and radiotherapy. Other challenges include the complexity of combining individual anti-EGFR agents with each other and with agents that target other members of the ErbB family, collateral signaling pathways, and downstream effector molecules.

Disclosure of Potential Conflicts of Interest

A.W. Burgess and A.M. Scott are inventors on mAb806 patents. A.M. Scott has received funding from the Ludwig Institute for Cancer Research and from Abbott Pharmaceuticals and has served as a consultant for Life Science Pharmaceuticals. No potential conflicts of interest were reported by the other authors.

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Targeting Conformationally Exposed Epitope of EGFR

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