Nimotuzumab, an Antitumor Antibody that Targets the Epidermal Growth Factor Receptor, Blocks Ligand Binding while Permitting the Active Receptor Conformation

Ariel Talavera,1,2 Rosmarie Friemann,2,3 Silvia Gómez-Puerta,1 Carlos Martinez-Fleites,4 Greta Garrido,1 Aleem Rabasa,1 Alejandro López-Requena,1 Amaury Pupo,1 Rune F. Johansen,1 Oliberto Sánchez,5 Ute Krengel,1 and Ernesto Moreno1

1Center of Molecular Immunology, Havana, Cuba; 2Department of Chemistry, University of Oslo, Oslo, Norway; and 3Center for Molecular and Behavioral Neuroscience, Institute of Medical Microbiology, University of Oslo, Rikshospitalet HF, Oslo, Norway; 4Department of Chemistry, University of York, Heslington, York, United Kingdom; and 5Center for Genetic Engineering and Biotechnology, Havana, Cuba

Abstract

Overexpression of the epidermal growth factor (EGF) receptor (EGFR) in cancer cells correlates with tumor malignancy and poor prognosis for cancer patients (1). Therefore, different strategies have been developed to inhibit the aberrant EGFR-associated signal transduction cascade. The most important therapeutic approaches include small-molecule tyrosine kinase inhibitors (2), which act by interfering with ATP binding to the receptor, and monoclonal antibodies (3), which bind specifically to the extracellular region of the EGFR (eEGFR), leaving the dimerization "arm" in domain II ready for binding a second monomer (4, 5). It has been shown that the eEGFR adopts a "tethered" or inactive conformation in the absence of EGF (6). In this characteristic conformation, the dimerization arm is hidden by interactions with domain IV, whereas domains I and III remain separated. Thus, to adopt the "extended" or active conformation observed in the crystal structure of the complex with EGF (4), the receptor must undergo a major conformational change that brings together domains I and III (6). On the cell surface, the tethered and extended conformations are in equilibrium, and it has been estimated that >85% of the EGFR molecules adopt the more energetically favorable tethered or inactive conformation (7, 8). EGF as well as other EGFR ligands shift this equilibrium in favor of the active conformation.

In the latest years, new structural data have shown how different antibodies inhibit the signal transduction via EGF by different mechanisms. Cetuximab, IMC-11F8, and Zalutumumab bind to domain III and act by blocking the binding of EGF and by sterically interfering with the active conformation (9–11). Matuzumab, which also binds to domain III, does not block EGF binding, but it does sterically interfere with the domain rearrangement needed for receptor dimerization (12). Monoclonal antibody (mAb) 806 follows a different inhibition mechanism, binding directly to the dimerization domain (13).

Nimotuzumab is a humanized mAb that binds to the extracellular domain of the EGFR and inhibits EGF binding (14). Nimotuzumab has been approved in several countries for the treatment of head and neck tumors (15) and glioma (16), and is in clinical trials for various tumor types including colorectal, glioma (pediatric and adult), pancreatic, prostate, non–small cell lung, esophageal, cervical, and breast cancer (17). One striking outcome from the use of Nimotuzumab in the clinic is the absence of severe adverse effects. In particular, no serious skin rash has been reported (18). This is in contrast to most of the other drugs targeting the EGFR, either monoclonal antibodies or small molecules, for which the severe skin rash they provoke has been assumed as an unavoidable negative side effect and, moreover, as a surrogate marker of antitumor effect (19, 20). The reason for the low toxicity of Nimotuzumab might lie in its intermediate affinity, as proposed in ref. 15, but it might as well result from a different inhibition mechanism, determined by the way in which the antibody binds to the eEGFR.

In this work, we aimed at identifying the epitope recognized by Nimotuzumab on EGF, by combining X-ray crystallography, binding experiments, and molecular modeling. We report here the crystal structure of the unliganded Fab fragment of Nimotuzumab at...
2.5 Å resolution, which shows some unique features, untypical for antibody structures. This structure, together with key binding data, provided the starting point to construct a model of the Nimotuzumab-EGFR complex, supported by mutagenesis studies. The obtained model indicates that Nimotuzumab has a very peculiar way of inhibiting EGFR-mediated signaling, which may explain the unique clinical profile of this molecule.

### Materials and Methods

**Purification of Nimotuzumab (h-R3) Fab fragments**

Fab fragments were prepared from purified mAb (produced at the Center of Molecular Immunology) by papain digestion (21). Further purification of the isoforms was performed as described by Krengel and colleagues (21). The pH gradient was created in this case by mixing 50 mmol/L DEAA (pH 9.6) and 50 mmol/L DETA (pH 8.6). The largest peak was collected and the buffer exchanged by dialysis against 25 mmol/L Tris (pH 7.0). Finally, the protein was concentrated to 12 mg/mL.

**Crystallization and data collection**

Initial crystallization screening was carried out with sparse matrix screens using the hanging drop vapor diffusion technique at room temperature. Typically, 1 to 2 μL of the precipitant solution were pipetted onto 1 to 2 μL of the protein solution (12 mg/mL) and equilibrated against 0.7 to 1 mL of the precipitant solution. Highest diffracting crystals were obtained at 20°C in 25% polyethylene glycol (PEG) 1.000 and 0.1M MES (pH 6.75), within 10 d. The square crystals (0.1 × 0.1 × 0.1 mm³) belong to space group P4₃2₁2, contain one molecule per asymmetric unit and have cell axes of a = b = 84.2 Å and c = 138.5 Å. The Matthews parameter is 2.5 Å³/Da, which corresponds to a solvent content of 50% (22).

Diffraction data to 2.5 Å resolution were collected at cryogenic temperature (100K) at beamline 7111 at MAXII, Lund, Sweden, using a MAR CCD detector (180 frames, at 1°/frame). The solvent content of 50% (23) was added at a dilution of 1:200 and incubated for 1 h at 37°C. The concentration of Nimotuzumab was kept constant at 5 μg/mL whereas the concentration of Cetuximab was gradually lowered to Nimotuzumab/Cetuximab concentration ratios ranging from 10⁻¹ to 10⁴. Alkaline phosphatase-conjugated streptavidin (Serotec) was added at a dilution of 1:200 and incubated for 1 h at 37°C. The reaction was developed by adding 1 mg/mL of p-nitrophenyl phosphate (Sigma-Aldrich). The absorbance (at 405 nm) was determined with a microplate reader (Organon Teknika).

**Surface plasmon resonance (SPR/Biacore).** Experiments were performed using a Biacore 3000 instrument (GE Healthcare) at room temperature (25°C) with HBS EP (GE Healthcare) as running buffer. eEGFR was coupled to a Biacore CM5 sensor chip by routine amine coupling. eEGFR was diluted to 5 μg/mL in 10 mmol/L sodium acetate (pH 5.0) and flowed over the chip for 144 s at a flow rate of 5 μL/min obtaining an immobilization level of 400 RU. Different concentrations of antibody Fab fragments were injected at 50 μL/min for 4.5 min. After each point measurement, the surface was regenerated by washing with 2 × 30 s injections of 1 mol/L glycine (pH 2.5) and 1 mol/L NaCl. The affinity values were calculated from the kₐ/kₑₐ ratios, which were directly obtained from the kinetics experiments. The data were evaluated with the Langmuir 1:1 binding model. The EGF binding inhibition experiment for Nimotuzumab and eEGFR was carried out following the protocol described by Li and colleagues (9).

**Docking simulations of the Nimotuzumab/eEGFR-dIII complex**

Several docking simulations involving the variable region (Fv) of Nimotuzumab and a fragment of eEGFR, composed of amino acids 310 to 501, were run using the program RosettaDock (36). The structure of the eEGFR fragment was extracted from PDB entry 1IVO (4). Each run (in perturbation mode) started from a different orientation of the Fv fragment with respect to eEGFR-dIII. A grid of 28 points was created over a selected region of eEGFR-dIII to anchor each of these orientations. In addition to the

### Table 1. Data collection and refinement statistics

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*Numbers in parentheses refer to high-resolution shell.

1, Rmerge = ∑₀∑₀|I₀−I₀|/∑₀|I₀| where I₀ is the average intensity over symmetry-related measurements.

2, Rfactor = ∑₀|F₀−F₀|/∑₀|F₀| where F₀ and F₀ are the observed and calculated structure factors, respectively.

3, r.s.c.c., real space correlation coefficient.

4, RMSD, root mean square deviation from ideal geometry.

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crystal geometry, two alternative conformations of the flexible CDR H3, obtained from a 10-ns molecular dynamics (MD) simulation performed for the Nimotuzumab Fv fragment, were used in the docking runs. More than 300,000 decoys were produced in total, in several docking rounds. Finally, we obtained a small funnel (in the energy versus RMSD landscape) of Fv orientations, which is a sign of a possible true solution (36).

MD simulations for the Fv/eEGFR-dIII complex
Aiming to improve the stereochemistry of the final docking decoys, we ran a 10-ns MD simulation, taking the structure of the lowest energy complex as starting geometry. The simulations were performed with the complex embedded in a water box, using the program NAMD (37). Approximately after the first nanosecond, the Nimotuzumab Fv fragment was accommodated in a position slightly displaced from the starting geometry, where it remained for the rest of the simulation. The fluctuations of the whole Fv fragment around the average binding position were very small, with RMSDs for the Cα carbons in the order of 1.5 to 3.0 Å. More significantly, CDR H3, which plays the most important role in the interaction with eEGFR-dIII (as detailed below), remained practically fixed after the first nanosecond, with deviations in the order of 1 Å. Overall, the whole binding interface between the two molecules showed a remarkable stability. As the final model, we selected a representative frame from the MD trajectory.

Construction, expression, and analysis of EGFR mutants
The genes coding for EGFR mutants were chemically synthesized by the company Geneart (Geneart AG). The gene called chEGFR-III is a chimeric version of the murine EGFR (Genbank accession code: AK004944.1), in which the nucleotide sequence coding for domain III was replaced by the corresponding sequence from the human EGFR (Genbank: AK290352.1). A few substitutions were introduced to keep five murine amino acids (at positions 324, 337, 340, 369, and 390). As a result, 16 amino acids were mutated to their human counterparts (at positions 353, 359, 366, 388, 418, 443, 460, 461, 467, 468, 471, 473, 474, and 478–480).

A second synthetic gene, called chEGFR-dIII-siteC, is a mutated version of the dIII segment of the murine EGFR, in which seven codons corresponding to amino acids located within the epitope recognized by Cetuximab were replaced by their human counterparts. This gene was used to substitute the dIII in the chEGFR-III construction, obtaining the chimera named chEGFR-siteC.

Both the chEGFR-III and chEGFR-siteC chimera were cloned into the expression vector pcDNA3 (Invitrogen). BHK-21 cells (ATCC CCL-10) were transfected using polyethylenimine. Transfected cells were stained with Nimotuzumab and binding was revealed with a FITC-goat anti-human IgG antibody (Sigma-Aldrich). The analysis was performed using a fluorescence-activated cell sorting (FACS) scan flow cytometer and the CellQuest program (Becton Dickinson). CT-26 cells (ATCC CRL-2638) were used as a control for the negative binding of Nimotuzumab to the murine EGFR. A polyclonal anti-mouse EGFR serum (produced at CIM) and a FITC-goat anti-mouse immunoglobulin antibody (Jackson) were used to confirm the presence of the murine EGFR on the surface of these cells.

Antibody numbering scheme
We have adopted the Kabat numbering scheme for the antibody variable regions, which for this particular antibody introduced insertion letters after positions 52 (52A), 82 (82A–82C), and 100 (100A–100F) in the heavy chain sequence, and after position 27 (27A–27E) in the light chain sequence.

Results
Crystal structure of the Nimotuzumab Fab. The crystal structure of the Nimotuzumab Fab fragment was determined at 2.5 Å resolution and refined to crystallographic R/Rfree factors of 21.5% and 28.8%, respectively, with good stereochemistry and no outliers in the Ramachandran plot. For the Fv region of the Fab, the
electron density is very well-defined for a structure determined to 2.5 Å resolution. In the constant domains, close to the C-terminal regions of both the heavy and light chains, three loops were difficult to trace due to discontinuities in the electron density map. The variable domains are involved in a number of crystal contacts, whereas only few symmetry contacts were found for the constant domains. Two PEG fragments from the crystallization solution were found to be bound to the Nimotuzumab Fab in the crystal structure (data not shown).

**Distinct structural features of Nimotuzumab.** The overall crystal structure of the Nimotuzumab Fab is similar to the structures of other Fab fragments, except for two distinct features, both in the variable domain of the heavy chain (VH). An evident peculiarity concerns CDR H1, which was expected to adopt one of the canonical conformations described for this loop (38), but exhibits a more unusual conformation involving amino acids H25-H32 (see Fig. 1A). A search among all the immunoglobulin VH domain structures deposited in the Protein Data Bank yielded only two antibodies having a similar helix at the base of CDR H1 [PDB entries 1B2W (39) and 2CMR (40)].

The second unusual feature of the Nimotuzumab structure concerns Trp H103, a residue that plays an important structural role in antibodies. This residue is shifted by three amino acids toward the C-terminus of the VH domain (Fig. 1B), whereas its structurally conserved position is instead occupied by Phe H100F. This change has several important consequences for the structure of the entire variable region. First, the CDR H3 loop is “pulled down” from its C-terminal end, so that its length in effect is shortened by three amino acid residues. This displacement is compensated for by an unusual distorted helix involving amino acids H101-H105, which allows the last seven residues of VH (H107-H113) to keep their conserved positions in the structure. As shown in Fig. 1B, Trp H103 in Nimotuzumab (colored red in the figure) is situated in the middle of this helix, with the tip of its side chain pointing toward the interface between the variable and the constant domains. As a consequence of the distorted helix created around Trp H103, the conformation of the VH framework 1 is severely affected. Specifically, the side chain of residue Gln H6 is pushed away from its conserved position, whereas the five N-terminal residues are not visible in the electron density map.

Superimposition of all the crystal structures of the VH domains found in the PDB (984, from 680 entries) revealed that only one antibody [PDB code 1FH5 (41)] has the Trp H103 residue placed similarly toward the interior of the VH domain (see Fig. 1B; single side chain colored slate blue). Also in this case, the conformation of framework 1 is disrupted and the structural role of Trp H103 is taken over by Tyr H102.

**Binding studies.** In a previous report (14), we showed that Nimotuzumab competes with EGF for binding to the EGFR. More recently, the crystal structure of Cetuximab in complex with the eEGFR (9) revealed that this antibody, which also blocks EGF binding, interacts exclusively with domain III. We therefore decided to test whether Nimotuzumab competes with Cetuximab for binding to eEGFR, as a first step in locating its binding epitope. The first test was carried out by ELISA, coating the plate with eEGFR and adding different dilutions of the Cetuximab Fab fragment while keeping constant the concentration of the Nimotuzumab Fab. Figure 2A shows that Nimotuzumab binding decreases as the concentration of the Cetuximab Fab increases.

We also performed kinetic measurements of the binding of Nimotuzumab and Cetuximab Fab fragments to the immobilized eEGFR by surface plasmon resonance (SPR). The values obtained for the dissociation constant (k_{off}) and for the association and dissociation rate constants (k_{on} and k_{off}, respectively) for Nimotuzumab were as follows: k_{on} = 2.1 \times 10^{10} \text{mol/L s}^{-1}, k_{off} = 1.1 \times 10^{-3} \text{s}^{-1}; whereas for Cetuximab: k_{on} = 1.8 \times 10^{10} \text{mol/L}, k_{off} = 3.1 \times 10^{6} \text{mol/L}^{-1}, k_{off} = 5.8 \times 10^{-3} \text{s}^{-1}. The K_D value obtained for the Cetuximab Fab is practically the same as the value reported previously (2.3 \times 10^{-9} \text{mol/L}, ref. 9). The K_D of Nimotuzumab is one order of magnitude higher due mainly to its much lower k_{on}, whereas its k_{off} is only slightly lower.

In addition, we performed SPR/Biacore experiments in which EGF was immobilized on a CM5 chip and different concentrations of either Nimotuzumab or Cetuximab were applied together with a constant concentration of eEGFR. Figure 2B shows the inhibition curves obtained for both antibodies, revealing a difference in their inhibition properties that is in agreement with their different dissociation constants.
The model of the Nimotuzumab-EGFR complex: correspondence with the experimental data. We decided to construct a computer model of the Nimotuzumab-eEGFR-dIII complex, in parallel to ongoing efforts to obtain the crystal structure of this molecular complex. According to the obtained model, and in agreement with our experimental data, the Nimotuzumab binding epitope strongly overlaps with the binding surface recognized by Cetuximab, as shown in Fig. 3. The model shows a remarkable geometric and chemical complementarity between Nimotuzumab and the EGFR, with a high number of hydrogen bonds, as

Figure 3. Structure of the predicted Nimotuzumab/eEGFR complex. A, overall view of the model, showing also the strong overlap in binding positions between Nimotuzumab and Cetuximab. The figure was made by superimposing the EGFR-dIII fragment of the computer model onto the eEGFR-dIII domain of the Cetuximab/eEGFR complex (PDB entry 1YY9), in which the eEGFR adopts an inactive conformation. Nimotuzumab is shown in a cartoon representation with transparent molecular surface (VL in wheat and VH in green), whereas Cetuximab is represented as a red cartoon. B, detailed representation of the molecular interface between Nimotuzumab and eEGFR. The antibody is positioned in the front, with VH (CDRs H1 and H3) in green and VL (CDRs L1, L2) in wheat. eEGFR is in the back, in light blue. The side chains at the interface are shown as sticks. Labels in black capital letters correspond to Nimotuzumab residues, whereas those in magenta concern eEGFR. eEGFR residues that differ between the human and murine versions of the EGF receptor are marked in orange. Black dashed lines, direct intermolecular hydrogen bonds; pink dashed lines, water-mediated hydrogen bonds. C, blocking of EGF binding to EGFR by Nimotuzumab, while permitting the active receptor conformation. Our computer model was overlaid onto the EGF/eEGFR complex (PDB entry 1IVO) by superimposing the eEGFR-dIII fragments of the two structures. eEGFR in its active conformation is shown in blue, with its domains marked by roman numerals. EGF is in red and Nimotuzumab’s VH and VL domains are in green and wheat, respectively. The figure shows the C-terminus of EGF bumping into the light chain of Nimotuzumab, whereas domain I of the EGFR approaches the antibody, without clashing. D, Cetuximab (light blue and yellow) blocks the binding of EGF (red) and clashes with domain I, preventing the EGFR (blue) from adopting its active conformation. The figure was prepared based on PDB entries 1YY9 and 1IVO.
A total area of 1,584 Å² becomes buried upon Nimotuzumab binding to eEGFR, slightly less than the area buried in the Cetuximab-EGFR complex (1,770 Å²; ref. 9), which fits well with the higher affinity of Cetuximab. The overall shape complementarity parameter (42) calculated for our model, however, is better than the one reported for the Cetuximab-EGFR complex (0.78 versus 0.71; ref. 9).

Most of the contacts of Nimotuzumab with eEGFR domain III are accounted for by the heavy chain. CDR H1 contributes to binding with only two residues: Tyr H27 and Tyr H32, the first of them being positioned in the helix turn at the base of CDR H1. It is CDR H3 which contributes the majority of the interactions, with seven consecutive residues (from H98 to H100D) being highly involved both in hydrophobic contacts and in hydrogen bonds with the receptor. Further contacts with the EGFR are provided by Asp H101 and Arg H94 (at the base of CDR H3; data not shown). CDR H2, in contrast, stays far from the EGFR surface. In the light chain, a total of seven residues interact with the EGFR, two from CDR L1 (Asn L28 and Tyr L32), four from CDR L2 (Tyr L49, Lys L50, Phe L55, and Ser L56) and Leu L46 at the base of CDR L2. In addition, there are three water molecules buried in the binding interface, mediating indirect hydrogen bonds between the antibody and the receptor.

The epitope recognized by Nimotuzumab on EGFR domain III, according to our model, involves 20 residues comprised within amino acids 441 and 477 (i.e., within the last 40 residues of domain III, which spans amino acids 310–481). All the amino acids within the sequence stretch from residues 462 to 474 are involved in contacts to Nimotuzumab, whereas the rest of the interacting residues correspond to sequence positions 441, 443, 449, 450, 453, 457, and 477. None of the saccharide chains in any of the reported EGFR crystal structures is close to this epitope.
One important experimental fact to take into account in analyzing the model is that Nimotuzumab practically does not recognize the murine EGFR (43), despite the high sequence similarity with the human receptor (88% sequence identity for domain III).

In our model, 5 of the 20 amino acids constituting the Nimotuzumab epitope correspond to sequence variations between the human and murine EGF receptors: I467M, S468N, G471A, N473K, and S474D (Fig. 3B). Notably, these five amino acids also belong to the epitope...
recognized by Cetuximab. The most critical mutations, according to the model, are the substitutions Ser 468→Asn (because there is no room for the slightly larger side chain of asparagine), and Gly 471→Ala (because the α carbon of glycine is situated within a very tight environment).

**Mutagenesis studies supporting the modeling results.** Two human/murine EGFR chimera were constructed, in which different groups of amino acids in domain III were mutated to their human counterparts (were "humanized"). In one of the chimera, called chEGFR-III, 16 of the 21 amino acids that differ between the murine and human versions of EGFR-dIII were humanized. The five unchanged residues were either located on a side opposite to the Cetuximab epitope, or shielded by the carbohydrate chains. In the second chimera, called chEGFR-siteC, only the epitope recognized by Cetuximab was humanized (mutated positions: 418, 443, 467, 468, 471, 473, and 474). From these seven amino acids, the last five belong to the Nimotuzumab epitope, according to our computer model (Fig. 3B).

The constructed EGFR chimera were cloned for transient expression in BHK-21 cells, and the transfected cells were then tested by FACS for recognition of Nimotuzumab. As shown in Fig. 4, cells expressing the chEGFR-III chimera were recognized by Nimotuzumab. The antibody also bound, with similar intensity, to the cells expressing the chEGFR-siteC mutant (Fig. 4C), in which the humanized surface patch allowing Nimotuzumab binding is more restricted.

**Nimotuzumab blocks EGF binding without inhibiting the active eEGFR conformation.** As shown in a previous report (14) and also in this work, Nimotuzumab and EGF compete for binding to the EGFR. To confirm that our computer model complies with this experimental fact, we superimposed the eEGFR structure in complex with EGF (PDB 1IVO; ref. 4) onto our model. The result is illustrated in Fig. 3C. Intriguingly, only the four C-terminal residues of EGF (of which the last two are not included in the crystal structure of the EGFR-EGF complex) overlap with the Nimotuzumab Fv fragment, bumping into CDR L2. Even more remarkable is the fact that, in contrast to Cetuximab (see Fig. 3D), the binding of Nimotuzumab is compatible with the extended, active conformation of EGF. As shown in Fig. 3C, the Nimotuzumab variable light chain (i.e., CDR L1) approaches domain I of the EGFR in the superimposed structures, without clashing into it.

**Discussion**

Nimotuzumab is one of the very few anti-EGFR monoclonal antibodies that have been approved for therapeutic use in cancer treatment. Clinical trials with this antibody, involving >2,000 patients worldwide, have shown that the classic side effects observed for antibodies and drugs that inhibit the EGFR signaling pathway (19) are negligible in the case of Nimotuzumab. This unique safety profile allows a longer term treatment and provides a better quality of life to patients. In this work, by combining several experimental and computational techniques, we have provided new mechanistic evidence that may explain the low toxicity observed for Nimotuzumab.

We have shown that Nimotuzumab binds to domain III of the extracellular region of the EGFR, as is the case for several other known anti-EGFR antibodies. Moreover, we have confirmed by site-directed mutagenesis that the Nimotuzumab epitope strongly overlaps with the Cetuximab binding site. According to our model, the epitope recognized by Nimotuzumab is displaced toward the C-terminus of eEGFR domain III, compared with the Cetuximab binding site (Fig. 3A). In this position, Nimotuzumab sterically interferes with EGF binding, while permitting eEGFR domain I to approach domain III and adopt the active conformation (Fig. 3C).

The prediction that Nimotuzumab may inhibit EGF binding without precluding the active conformation of the receptor introduces an important and unexpected element to the mechanistic study of this antibody, and provides a rationale for several results obtained in the clinics. As explained in the Introduction, the other anti-EGFR antibodies for which the molecular inhibition mechanisms are known create steric impediments, either to the formation of the active receptor conformation needed for dimerization [e.g., Cetuximab (9) and Matuzumab (12)] or directly for the dimerization event [e.g., mAb 806 (13)]. Nimotuzumab, on the other hand, is able to maintain the existing balance between the tethered and extended EGFR conformations.

As pointed out above, it has been estimated that without ligand-induced activation, only a very small fraction (~15%) of the EGFR molecules adopts the energetically less favorable active conformation (7, 8). On the other hand, there are several reports demonstrating the existence of a ligand-independent level of EGFR activation, both in tumor and normal cells (44,45). Nimotuzumab would not interfere with this basal level of EGFR signaling, which is needed for the survival of normal epithelial cells (46), because its binding is compatible with the active conformation of the receptor. A comparison between the inhibition mechanism followed by Cetuximab and the mechanism proposed here for Nimotuzumab is depicted in Fig. 5.

The low degree of adverse effects observed for Nimotuzumab in the clinics has been attributed to its intermediate affinity, compared with other anti-EGFR antibodies, e.g., Cetuximab (15). Another possible explanation given by our model is that Nimotuzumab is less toxic for normal epithelial cells because it does not disrupt the basal level of EGFR signaling. This reasoning further suggests that an antibody having a higher affinity than Nimotuzumab, but acting by the same mechanism, might also show a low toxicity. A simple inspection of the structure of the EGFR-EGF complex reveals that a similar mechanism of action could be exploited by binding to domain I of the EGFR.

Although the final validation of the model requires additional experiments, its remarkable geometric and chemical complementarity, the stability shown along extensive MD simulations and, more importantly, the correspondence with the available experimental data support its reliability. Most significantly, the proposed model has opened the door to new experiments, designed not only to validate its atomic details but foremost directed to corroborate the predicted mechanism of action of Nimotuzumab.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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References

**Correction: Article on Nimotuzumab Blocks the EGFR by a Novel Mechanism**

In the article on how Nimotuzumab blocks the EGFR by a novel mechanism in the July 15, 2009 issue of *Cancer Research* (1), the last footnote in Table 1 should read as follows: "RMSD, root mean square deviation from ideal geometry (47)."

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