Pharmacology basis of targeted therapy: EGFR and HER2 inhibitors

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Second University of Naples, Italy
EGFR Blockade as Cancer Therapy: J. Mendelsohn’s Hypothesis (Early 1980s)

- The blockade of EGFR activation may inhibit cancer cell proliferation.

- Cancer cells may be selectively sensitive to EGFR inhibition as compared to normal cells.

- Selective anti-EGFR agents may be developed.
Growth Inhibition of Human Tumor Cells in Athymic Mice by Anti-Epidermal Growth Factor Receptor Monoclonal Antibodies

Hideo Masui, Tomoyuki Kawamoto, J. Denry Sato, Bonnie Wolf, Gordon Sato, and John Mendelsohn

Cancer Center, Q-058, University of California, San Diego, La Jolla, California 92093

ABSTRACT

Monoclonal antibodies (MoAbs) were raised against epidermal growth factor (EGF) receptors on a human epidermoid carcinoma cell line, A431. Administration of anti-EGF receptor MoAbs inhibited tumor formation in athymic mice by A431 cells and by another epidermal carcinoma cell line, T222. When one of the same MoAbs was used in therapy against Li-7 (a human hepatoma) and HeLa cells (a cervical carcinoma), tumor growth was not affected. The number of EGF receptors on A431 cells was about 100-fold higher than on T222, Li-7, and HeLa cells, suggesting that the number of EGF receptors may not be an important determinant in suppressing tumor growth. Three anti-EGF receptor MoAbs were used in the present studies. MoAbs 528 (immunoglobulin G2a) and 225 (immunoglobulin G1) are capable of competing with EGF for receptor binding and inhibit proliferation of A431 cells in culture. The other MoAb, 455 (immunoglobulin G1), is incapable of blocking the binding of EGF to its receptors and has no effect on the proliferation of cultured A431 cells. All three MoAbs inhibited A431 tumor growth in athymic mice, indicating that the antibody isotype and the site of binding on the EGF receptor are not the determinants of antiproliferative activity in vivo. The observation that MoAb against the receptor for EGF is cytostatic rather than cytotoxic in vitro against A431 cells, yet completely prevents tumor growth in vivo, suggests that some host animal responses also may be involved in the antitumor effect. MoAbs against growth factor receptors could provide useful immunotherapeutic agents.

the growth of human tumor xenografts in athymic mice (8, 9, 16, 27). Furthermore, the results of several clinical studies on the treatment of leukemia and lymphoma using MoAbs have been published, but in these cases, clinically significant results have not been obtained (17, 18, 20, 23–25), with one exception. This was a report on the treatment of a human B-cell lymphoma with anti-idiotypic antibody, in which the patient has been free from disease for more than 1.5 years (19).

In most of these studies, MoAbs against tumor-associated antigens have been used for treatment. Another type of MoAb which might be used for cancer immunotherapy is an antibody against plasma membrane receptors for growth factors. It is well known that the proliferation of tumor cells in culture is controlled by various growth factors, and a similar control mechanism is postulated for the control of tumor cell growth in vivo (2). Therefore, MoAbs against growth factor receptors, which could block access of growth factors to their receptors, may provide useful therapeutic agents. Recently, Trowbridge and Domingo (29) reported that treatment with anti-transferrin receptor MoAb can inhibit tumor formation by a human melanoma cell line in athymic mice.

A431 cells, a human epidermoid carcinoma cell line, express an unusually large number of EGF receptors on the cell surface membrane (1 to 3 × 10⁴/cell) (4, 7), and addition of EGF to the culture medium inhibits the proliferation of these cells in culture (1, 6). We have developed MoAbs against the EGF receptor using partially purified EGF receptors from A431 cells as antigen (13). In this paper, we report that the administration of anti-EGF
Inhibition of: Cancer cell proliferation, invasion, metastasis and tumor-induced neo-angiogenesis

Induction of: Cancer cell cycle arrest, potentiation of cytotoxic drugs and of radiotherapy antitumor activity
A

Immune effector cell

Anti-EGFR Mab

EGFR

Activation of antibody-dependent cell-mediated cytotoxicity

Tumor-cell lysis

B

Endocytosis

EGFR degradation
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Blocking Monoclonal Antibodies</th>
<th>Small-Molecule Tyrosine Kinase Inhibitors</th>
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<tr>
<td>Route of administration</td>
<td>Intravenous (generally once a week or every 2 wk)</td>
<td>Oral (generally daily continuous dosing)</td>
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<td>Structure</td>
<td>Recombinant immunoglobulins (150–180 kD)</td>
<td>Low-molecular-weight compounds (400–600 kD)</td>
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<td>Target selectivity</td>
<td>Exclusively specific for EGFR</td>
<td>Relatively specific for EGFR; may inhibit only one or all EGFR family receptors; some EGFR tyrosine kinase inhibitors also inhibit other growth factor receptors (e.g., dual inhibitors of EGFR and VEGFR)</td>
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<tr>
<td>Mechanism of interference with EGFR activation</td>
<td>Bind extracellular portion of receptor, preventing ligand binding and receptor dimerization by occluding ligand region (cetuximab)</td>
<td>Bind intracellular portion of receptor within tyrosine kinase domain, generally by competing with ATP and inhibiting receptor autophosphorylation; most are reversible; irreversible EGFR tyrosine kinase inhibitors are in clinical development</td>
</tr>
<tr>
<td>Cellular effects of EGFR inhibition</td>
<td>Inhibit cancer-cell proliferation (G1 phase arrest), angiogenic growth factor production (VEGF) and tumor-induced angiogenesis, and cancer-cell invasion; potentiate antitumor activity of cytotoxic drugs and radiotherapy</td>
<td>Inhibit cancer-cell proliferation (G0–G1 phase arrest), angiogenic growth factor production (VEGF) and tumor-induced angiogenesis, and cancer-cell invasion; potentiate antitumor activity of cytotoxic drugs and radiotherapy</td>
</tr>
<tr>
<td>Induction of EGFR internalization, down-regulation, and degradation</td>
<td>Yes</td>
<td>No (although irreversible EGFR tyrosine kinase inhibitors can cause EGFR degradation and subsequent EGFR down-regulation)</td>
</tr>
<tr>
<td>Inhibition of EGFR-dependent intracellular signaling</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>Activity against mutant EGFR proteins</td>
<td>Probably yes, for mutations of EGFR tyrosine kinase domain, since anti-EGFR monoclonal antibodies bind to EGFR extracellular domain; not completely known for mutations of EGFR extracellular domain</td>
<td>Yes, for most mutations of EGFR tyrosine kinase domain (mutation in codons 746–750 in exon 19 and L858R in exon 21), since these EGFR mutant proteins bind with higher-affinity small-molecule EGFR tyrosine kinase inhibitors, such as erlotinib or gefitinib; no, for gefitinib- or erlotinib-acquired EGFR-resistance mutation (T790M in exon 20), although several new-generation EGFR tyrosine kinase inhibitors that are active against mutant EGFR proteins are in early clinical development</td>
</tr>
<tr>
<td>Activation of host immune response</td>
<td>Yes — antibody-dependent cytotoxicity may significantly contribute to anticancer activity of some anti-EGFR monoclonal antibodies, such as cetuximab; however, no antibody-dependent cytotoxicity has been reported for panitumumab</td>
<td>No</td>
</tr>
</tbody>
</table>

* EGFR denotes epidermal growth factor receptor, VEGF vascular endothelial growth factor, and VEGFR VEGF receptor.
Antibodies are Immunoglobulin Molecules (IgG)

Antigen binding

Mediates biologic activity

Fc

Heavy chain

Light chain

Hypervariable regions

Hinge

Complement binding region

Binds to Fc receptor

Fc

Carbohydrates

V_H

C_H1

C_K

C_H2

C_H3

V_K

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Monoclonal Antibodies

- Humanized Ab (90% human)
- Chimeric Ab (60-70% human)
- Mouse MoAb (0% human)
- Fab-fragment
- scFv (single chain Fragment variable)
# From Murine to Reshaped MoAbs

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<th>Murine</th>
<th>Chimeric</th>
<th>Humanized</th>
<th>Human</th>
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<tr>
<td>% Murine Protein</td>
<td>100%</td>
<td>≈25%</td>
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<tr>
<td>HAMA Induction</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Half-Life</td>
<td>short</td>
<td>long</td>
<td>long</td>
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<tr>
<td>Effectiveness in ADCC</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>ND</td>
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Monoclonal Antibodies Nomenclature (Suffix)

Mouse MoAb
„momab“

Chimeric Ab
„ximab“

Humanized Ab
„zumab“
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<tr>
<td><strong>Trastuzumab</strong></td>
<td>Humanized anti-HER2 MAb</td>
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<tr>
<td><strong>Cetuximab</strong></td>
<td>Chimeric anti-EGFR MAb</td>
</tr>
<tr>
<td><strong>Panitumumab</strong></td>
<td>Fully human anti-EGFR MAb</td>
</tr>
<tr>
<td><strong>Pertuzumab</strong></td>
<td>Humanized anti-HER2 MAb</td>
</tr>
<tr>
<td><strong>Sym004</strong></td>
<td>1:1 mixture of two chimeric anti-EGFR MAb</td>
</tr>
<tr>
<td><strong>T-DM1</strong></td>
<td>Trastuzumab linked to microtubule inhibitor emtansine</td>
</tr>
<tr>
<td><strong>GA201</strong></td>
<td>Glycositination modified humanized anti-EGFR MAb</td>
</tr>
</tbody>
</table>
Cetuximab

- IgG1 (chimerized antibody)
- 150 kDa
- Exclusive for EGFR and its heterodimers
- Prevents ligand binding to EGFR
- High affinity. $K_d = 0.39 \text{ nM}$
  
  \[
  (M-225 K_d = 1 \text{ nM})
  \]
- 1 log > natural ligand
- Stimulates receptor internalization
- Blocks receptor dimerization, tyrosine kinase phosphorylation, signal transduction
Sym004: A Novel Synergistic Anti–Epidermal Growth Factor Receptor Antibody Mixture with Superior Anticancer Efficacy

Mikkel Wandahl Pedersen, Helle Jane Jacobsen, Klaus Koefoed, Adam Hey, Charles Pyke, John Sørensen Haurum, and Michael Kragh

Abstract

Epidermal growth factor receptor (EGFR) is a validated therapeutic target in cancer and EGFR antagonists with greater effectiveness than existing clinical agents remain of interest. Here, we report a novel approach based on Sym004, a mixture of two anti-EGFR monoclonal antibodies directed against distinct nonoverlapping epitopes in EGFR extracellular domain III. Like anti-EGFR monoclonal antibodies in current clinical use, Sym004 inhibits cancer cell growth and survival by blocking ligand-binding receptor activation and phosphorylation and downstream receptor signaling. However, unlike the other antibodies, Sym004 induces rapid and efficient removal of the receptor from the cancer cell surface by triggering EGFR internalization and degradation. Compared with reference anti-EGFR monoclonal antibodies, Sym004 exhibited more pronounced growth inhibition in vitro and superior efficacy in vivo. Together, these findings illustrate a strategy to target EGFR more effectively than existing clinical antibodies. Cancer Res; 70(2); 588–97. ©2010 AACR.
Figure 3. Sym004-induced EGFR degradation in vitro. A, immunoblot analyses of EGFR levels in HN5, A431NS, MDA-MB-468, and GEO cell lines after treatment with 10 µg/mL of the indicated antibodies. B, quantification of EGFR levels in cell lines after 24 h of antibody treatment relative to untreated control cells measured in arbitrary units from Western blots as in A. Columns, mean; bars, SD. *, $P < 0.05$. Student’s t test, Sym004 versus cetuximab. C, quantification of the level of mAb 1030 binding to HN5 cells from images taken at 400x after different treatment periods with either Sym004 or cetuximab. Points, mean; bars, SE. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Student’s t test, Sym004 versus cetuximab. D, proposed model for the Sym004 mechanism of action.
Phase I Pharmacokinetic and Pharmacodynamic Dose-Escalation Study of RG7160 (GA201), the First Glycoengineered Monoclonal Antibody Against the Epidermal Growth Factor Receptor, in Patients With Advanced Solid Tumors

Luis G. Paz-Ares, Carlos Gomez-Roca, Jean-Pierre Delord, Andres Cervantes, Ben Markman, Jesus Corral, Jean-Charles Soria, Yann Bergé, Desamparados Roda, Fiona Russell-Yarde, Simon Hollingsworth, José Baselga, Pablo Umana, Luigi Manenti, and Josep Tabernero

Abstract

Purpose
We conducted a phase I dose-escalation study to characterize the safety, efficacy, pharmacokinetic (PK), and pharmacodynamic properties of RG7160 (GA201), a humanized and glycoengineered immunoglobulin G1 anti–epidermal growth factor receptor (EGFR) monoclonal antibody with enhanced antibody-dependent cell-mediated cytotoxicity.

Patients and Methods
Seventy-five patients with advanced EGFR-positive solid tumors received RG7160 (50 to 1,400 mg) administered every week, every 2 weeks, or every 3 weeks. Dose escalation followed a three-plus-three trial design.

Results
No maximum-tolerated dose was reached for any dosing schedule. Common adverse events (AEs) included rash (80% of patients), infusion-related reactions (77%), and hypomagnesemia (56%). Grades 3 and 4 AEs were rash (grade 3, 25%), infusion-related reaction (grade 3, 7%; grade 4, 1%), paronychia (grade 3, 3%), and hypomagnesemia (grade 3, 1%; grade 4, 1%). RG7160 exposure increased greater than proportionally over the 50- to 400-mg dose range (with greater than proportional decline in clearance) and approximately dose proportionally above 400 mg (where clearance plateaued). A marked reduction in circulating natural killer cells and increased infiltration of immune effector cells into skin rash were seen. Clinical efficacy included one complete response and two partial responses in patients with colorectal cancer (including one with KRAS mutation) and disease stabilization in 27 patients.

Conclusion
RG7160 had an acceptable safety profile with manageable AEs and demonstrated promising efficacy in this heavily pretreated patient cohort. On the basis of modeling of available PK parameters, the RG7160 dose selected for part two of this study is 1,400 mg on days 1 and 8 followed by 1,400 mg every 2 weeks.

J Clin Oncol 29:3783-3790. © 2011 by American Society of Clinical Oncology
Figure 1. Structure of T-DM1 and mechanisms of action. After T-DM1 binds HER2, the HER2/T-DM1 complex undergoes internalization, followed by lysosomal degradation. This process results in the intracellular release of DM1-containing catabolites that bind to tubulin and prevent microtubule polymerization as well as suppress microtubule dynamic instability. T-DM1 has also been shown to retain mechanisms of action of trastuzumab, including disruption of the HER3/PI3K/AKT signaling pathway and Fcγ receptor-mediated engagement of immune effector cells, which leads to antibody-dependent cellular cytotoxicity.
Pertuzumab and Trastuzumab Possess Distinct Epitope Specificity for HER2

Pertuzumab and Trastuzumab Binding on HER2 receptors with intact ECD

Baselga J. Cancer Cell 2002;2:93–95
# Small Molecule Tyrosine Kinase inhibitors

<table>
<thead>
<tr>
<th>Drug</th>
<th>Description</th>
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<tbody>
<tr>
<td>Gefitinib</td>
<td>Reversible anti-EGFR TKI</td>
</tr>
<tr>
<td>Erlotinib</td>
<td>Reversible anti-EGFR TKI</td>
</tr>
<tr>
<td>Lapatinib</td>
<td>Reversible dual anti-EGFR/HER2 TKI</td>
</tr>
<tr>
<td>Vandetanib</td>
<td>Reversible multi-TKI (EGFR, VEGFRs, RET)</td>
</tr>
<tr>
<td>Afatinib</td>
<td>Irreversible dual EGFR/HER2 TKI</td>
</tr>
</tbody>
</table>
EGFR Selective Small Molecule Tyrosine Kinase Inhibitors

- Gefitinib and Erlotinib compete for ATP binding
- Reversible inhibitors
- Orally bioavailable small molecules

EGFR tyrosine kinase activity requires ATP
**Vandetanib: targeting key signalling pathways in cancer**

<table>
<thead>
<tr>
<th>Direct antitumour effects</th>
<th>Indirect antitumour effects</th>
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</thead>
<tbody>
<tr>
<td>↓ EGFR signalling</td>
<td>↓ EGFR signalling</td>
</tr>
<tr>
<td>↓ RET signalling</td>
<td>↓ VEGFR signalling</td>
</tr>
<tr>
<td>↓ VEGFR signalling</td>
<td>↓ VEGFR signalling</td>
</tr>
</tbody>
</table>

- **Tumour cells**
  - Inhibition of EGFR and RET signalling blocks tumour cell growth, proliferation and secretion of proangiogenic factors
  - Inhibition of VEGFR signalling may contribute to direct antitumour activity

- **Endothelial cells**
  - Inhibition of VEGFR-dependent endothelial cell proliferation, migration and survival, and vascular permeability
  - Inhibition of EGFR signalling may contribute to indirect antitumour activity
Vandetanib Chronic Treatment Does Not Result in Tumor Resistance in Established GEO Xenografts

Afatinib has been designed to irreversibly inhibit the EGFR and HER2 kinases.

**Structural model of afatinib in the EGFR kinase domain**

Afatinib was designed to covalently bind to EGFR and the related HER2 receptor.
Afatinib maintains inhibitory activity in EGFR mutants resistant to erlotinib/gefitinib

<table>
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<th>Mutation:</th>
<th>WT</th>
<th>Activated</th>
<th>Resistance</th>
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<tr>
<td>wild type</td>
<td>H1666</td>
<td>L858R</td>
<td>L858R+T790M</td>
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<tr>
<td>H3255</td>
<td>NCI1975</td>
<td>Target</td>
<td>Binding mode</td>
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<tr>
<td>Afatinib¹</td>
<td>60</td>
<td>0.7</td>
<td>99</td>
</tr>
<tr>
<td>Gefitinib¹</td>
<td>157</td>
<td>5</td>
<td>&gt;4000</td>
</tr>
<tr>
<td>Erlotinib¹</td>
<td>110</td>
<td>40</td>
<td>&gt;4000</td>
</tr>
<tr>
<td>Lapatinib¹</td>
<td>534</td>
<td>63</td>
<td>&gt;4000</td>
</tr>
<tr>
<td>CP 714,724²</td>
<td>&gt;4000</td>
<td>561</td>
<td>&gt;4000</td>
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</table>

EC50 values for the inhibitory activities of different compounds on the proliferation of NSCLC cells with EGFR mutations

WT, wild type
Effects of Anti-EGFR MoAb in Combination with Cisplatin on A431 Epidermoid Cancer Xenografts

ABSTRACT

Recombinant humanized anti-HER2 antibody, rhuMAb HER2, inhibits the growth of breast cancer cells overexpressing HER2 and has clinical activity. We explored in preclinical models its capacity to enhance the tumoricidal effects of paclitaxel and doxorubicin. In cultures of naturally HER2-overexpressing cancer cells, rhuMAb HER2 inhibited growth and enhanced the cytotoxic effects of paclitaxel. Treatment of well-established BT-474 breast cancer xenografts overexpressing HER2 in athymic mice with rhuMAb HER2 resulted in a dose-dependent antitumor activity. In combination studies, treatment with paclitaxel and rhuMAb HER2 or doxorubicin and rhuMAb HER2 resulted in greater inhibition of growth than that observed with any agent alone. The combination of paclitaxel and rhuMAb HER2 resulted in the highest tumor growth inhibition and had a significantly superior complete tumor regression rate when compared with either paclitaxel or rhuMAb HER2 alone. Clinical trials that are built on these results are under way.

INTRODUCTION

The HER2 gene (also known as neu and as c-erbB-2) encodes a 185-kDa transmembrane tyrosine/kinase receptor, designated p185HER2, that has partial homology with the other members of the EGFR4 family (1–3). HER2 is overexpressed in 25–30% of breast cancers and predicts for a worse prognosis as measured by lower overall survival and disease free survival (4–6). Antibodies directed at p185HER2 can inhibit the growth of tumor xenografts and transformed cells that express high levels of this receptor (7–10). The murine MAb 4D5, directed against the extracellular domain of patients with HER2-overexpressing metastatic breast cancer. Weekly administration of rhuMAb HER2 induced tumor responses and the combined rate of clinical response and disease stabilization was half of the evaluable patients (14).

One way to optimize the clinical role of anti-HER2 MAbs might be to administer them in combination with chemotherapy. Previous studies with anti-HER2 antibodies have shown enhancement of the antitumor activity of cisplatin (7, 15). It has been postulated that the mechanism for this interaction is the interference of anti-HER2 antibodies with repair of cisplatin-induced DNA-damage (15, 16). Paclitaxel and doxorubicin are two of the most active chemotherapeutic agents for the treatment of patients with breast cancer (17). Thus, finding enhanced antitumor activity of these drugs when combined with anti-HER2 MAbs would have distinct clinical implications for breast cancer therapy. We had previously observed that MAbs C225 and 528 directed at the EGFR, a member of the same tyrosine kinase receptor family, markedly enhanced the antitumor activity of doxorubicin and paclitaxel against cancer cells overexpressing the EGFR (18, 19). Taking these results into consideration, we decided to conduct the present studies with rhuMAb HER2 in combination with paclitaxel or doxorubicin. We have observed enhanced and concentration-dependent inhibition of growth in cultures of human cancer cell lines overexpressing HER2 treated with rhuMAb HER2 plus paclitaxel, and striking antitumor effects in breast carcinoma xenografts, resulting in the cure of well established tumors. RhuMAb HER2 also enhanced, but to a lesser extent, the in vivo antitumor effects of doxorubicin.
Featured Article

Combined Epidermal Growth Factor Receptor Targeting with the Tyrosine Kinase Inhibitor Gefitinib (ZD1839) and the Monoclonal Antibody Cetuximab (IMC-C225): Superiority Over Single-Agent Receptor Targeting

Pablo Matar,¹ Federico Rojo,² Raúl Cassia,³ Gema Moreno-Bueno,³ Serena Di Cosimo,⁴ José Taberner,¹ Marta Guzmán,¹ Sonia Rodriguez,¹ Joaquín Arribas,¹ José Palacios,³ and José Baselga¹

¹Laboratory of Oncology Research, Medical Oncology Service, and Pathology Service, Vall d’Hebron University Hospital, Barcelona, Spain; ²Laboratory of Breast and Gynecological Cancer, Molecular Pathology Program, Centro Nacional de Investigaciones Oncológicas (CNIO), Madrid, Spain; and ³Division of Medical Oncology “A”, Regina Elena Cancer Institute, Rome, Italy

Results: The combined treatment with gefitinib and cetuximab resulted in a synergistic effect on cell proliferation and in superior inhibition of EGFR-dependent signaling and induction of apoptosis. In a series of in vivo experiments, single-agent gefitinib or cetuximab resulted in transient complete tumor remission only at the highest doses. In contrast, suboptimal doses of gefitinib and cetuximab given together resulted in a complete and permanent regression of large tumors. In the combination-treated tumors, there was a superior inhibition of EGFR, mitogen-activated protein kinase, and Akt phosphorylation, as well as greater inhibition of cell proliferation and vascularization and enhanced
Fig. 7  Tissue-based studies of A431 tumor xenografts treated with gefitinib (50 mg/kg/d), cetuximab (1 mg per mouse twice a week) or the combination of both (from study design I). A, immunohistochemical analysis of tumor cells stained with hematoxylin and eosin (H&E), anti-phospho-EGFR, anti-phospho-MAPK, anti-phospho-Akt, anti-Ki67 nuclear antigen, and apoptosis by TUNEL (×400). B, quantification of proliferation index (Ki67), apoptosis index (TUNEL), and CD34 vessel staining (Chalkley Method). Results are expressed as percentage of positive cells for each marker (Mann–Whitney U test: *, P < 0.01).
A Phase I Pharmacokinetic (PK) and Molecular Pharmacodynamic Study (PD) of the Combination of Two Anti-EGFR Therapies, Cetuximab and Gefitinib, in Patients (pts) with Advanced Colorectal (CRC), Head and Neck (HNC) and Non-small Cell Lung Cancer (NSCLC)


Medical Oncology Department, Vall d’Hebron University Hospital, Barcelona, Spain; Department of General Medical Oncology, Gasthuisberg Hospital, Leuven, Belgium; Merck, Farma y Química SA, Barcelona, Spain; Astra Zeneca SA, Madrid, Spain

Presented at the ASCO Annual Meeting 2006
## Study Design

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<th>15</th>
<th>22</th>
<th>29</th>
<th>36</th>
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<td></td>
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### Biopsies (skin & tumor)

- Pharmacodynamics: EGFR, pEGFR, pMAPK, pAKT, Ki67, p27, TUNEL
- Pharmacogenomics

### PKs

### DLT period

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<th>0b</th>
<th>1</th>
<th>2a</th>
<th>2b</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>-1</th>
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<td>100</td>
<td>100</td>
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<td>250</td>
<td>500</td>
<td>500</td>
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<td>400</td>
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<td>RD</td>
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<td>3</td>
<td>5</td>
<td>Not done</td>
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<td>6</td>
<td>Not done</td>
<td>3+</td>
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# Tumor Response (All Tumor Types)

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<th>Dose level</th>
<th>All</th>
<th>0a C (400/250)</th>
<th>0b G (250)</th>
<th>1 G (100) C (320/200)</th>
<th>2a G (100) C (400/250)</th>
<th>3 G (250) C (400/250)</th>
<th>4 G (320/200) C (400/250)</th>
<th>5 G (500) C (400/250)</th>
<th>Total combo</th>
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<tr>
<td>N</td>
<td>40 ab</td>
<td>7 a</td>
<td>7 a</td>
<td>3</td>
<td>5</td>
<td>6 b</td>
<td>6</td>
<td>6</td>
<td>26 b</td>
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<tr>
<td>CR, n (%)</td>
<td>1 (3)</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1 (4)</td>
</tr>
<tr>
<td>PR, n (%)</td>
<td>7 (18)</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>6 (24)</td>
</tr>
<tr>
<td>SD, n (%)</td>
<td>11 (29)</td>
<td>-</td>
<td>3</td>
<td>-</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>8 (32)</td>
</tr>
<tr>
<td>PD, n (%)</td>
<td>19 (50)</td>
<td>6</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>10 (40)</td>
</tr>
<tr>
<td>ORR, n (%)</td>
<td>8 (21)</td>
<td>1</td>
<td>-</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>7 (28)</td>
</tr>
<tr>
<td>CB, n (%)</td>
<td>19 (50)</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>3</td>
<td>5</td>
<td>15 (60)</td>
</tr>
<tr>
<td>NE, n</td>
<td>2</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
</tbody>
</table>

C: cetuximab; G: gefitinib
CB: clinical benefit (CR + PR + SD), NE: non-evaluable

a One non-evaluable patient due to early discontinuation for ILD
b One non-evaluable patient due to early discontinuation for unexpected surgery
PD Results

**TUMOR**
- pEGFR: Cetuximab > Gefitinib, p > 0.05
- pMAPK: Cetuximab > Gefitinib, p > 0.05
- pAkt: Cetuximab > Gefitinib, p = 0.003
- p27: Cetuximab > Gefitinib, p = 0.016
- Ki67: Cetuximab > Gefitinib, p = 0.045
- TUNEL: Cetuximab > Gefitinib, p = 0.045

**SKIN**
- pEGFR: Cetuximab > Gefitinib, p = 0.001
- pMAPK: Cetuximab > Gefitinib, p = 0.047
- p27: Cetuximab > Gefitinib, p = 0.026
- Ki67: Cetuximab > Gefitinib, p = 0.123

*U Mann Whitney test: single administration vs combination*
Synergistic Antitumor Activity of Sorafenib in Combination with Epidermal Growth Factor Receptor Inhibitors in Colorectal and Lung Cancer Cells

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Abstract

**Purpose:** Cancer cell survival, invasion, and metastasis depend on cancer cell proliferation and on tumor-induced angiogenesis. We evaluated the efficacy of the combination of sorafenib and erlotinib or cetuximab.

**Experimental Design:** Sorafenib, erlotinib, and cetuximab, alone or in combination, were tested in vitro in a panel of non–small cell lung cancer (NSCLC) and colorectal cancer cell lines and in vivo in H1299 tumor xenografts.

**Results:** Epidermal growth factor receptor (EGFR) ligand mRNAs were expressed in all NSCLC and colorectal cancer cell lines with variable levels ranging from 0.4- to 8.1-fold as compared with GEO colorectal cancer cells. Lung cancer cells had the highest levels of vascular endothelial growth factors (VEGF) A, B, and C, and of VEGF receptors as compared with colorectal cancer cells.

Combined treatments of sorafenib with erlotinib or cetuximab produced combination index values between 0.02 and 0.5, suggesting a significant synergistic activity to inhibit soft agar colony formation in all cancer cell lines, which was accompanied by a marked blockade in mitogen-activated protein kinase and AKT signals. The in vitro migration of H1299 cells, which expressed high levels of both VEGF ligands and receptors, was inhibited by treatment with sorafenib, and this effect was significantly increased by the combination with anti-EGFR drugs. In nude mice bearing established human H1299 xenografts, treatment with the combination of sorafenib and erlotinib or cetuximab caused a significant tumor growth delay resulting in 70 to 90 days increase in mice median overall survival as compared with single-agent sorafenib treatment.

**Conclusions:** Combination treatment with sorafenib and erlotinib or cetuximab has synergistic antitumor effects in human colorectal and lung cancer cells. Clin Cancer Res; 16(20); 4990–5001. ©2010 AACR.
### Table 1. Antitumor activity of sorafenib in combination with erlotinib or cetuximab in H1299 human cancer xenografts

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Average tumor volume (±SD) on day 35 after tumor cell injection, cm³</th>
<th>Average survival (±SD), days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.5 (±0.2)</td>
<td>35 (±2)</td>
</tr>
<tr>
<td>Erlotinib</td>
<td>2.5 (±0.3)</td>
<td>37 (±4)</td>
</tr>
<tr>
<td>Sorafenib</td>
<td>0.56 (±0.01)</td>
<td>75 (±5)</td>
</tr>
<tr>
<td>Cetuximab</td>
<td>2.55 (±0.2)</td>
<td>34 (±5)</td>
</tr>
<tr>
<td>Erlotinib plus sorafenib</td>
<td>0.15 (±0.04)</td>
<td>145 (±5)</td>
</tr>
<tr>
<td>Cetuximab plus sorafenib</td>
<td>0.10 (±0.03)</td>
<td>165 (±10)</td>
</tr>
</tbody>
</table>

NOTE: Each treatment (erlotinib, sorafenib, cetuximab, erlotinib plus sorafenib, or cetuximab plus sorafenib) was started on day 7 following H1299 tumor cell s.c. injection when the average tumor volume was 0.30 (±0.05) cm³.

Erlotinib treatment: 75 mg/kg/dose orally 5 days/week starting on the day 7 following tumor cell injection.

Sorafenib treatment: 50 mg/kg/dose orally 5 days/week starting on the day 7 following tumor cell injection.

Cetuximab treatment: 1 mg/dose i.p. twice weekly starting on the day 7 following tumor cell injection.

Treatment was done for 4 weeks. Each group consisted of 10 mice.

Mice were sacrificed when tumor volume reached 2.5 cm³ (approximately 10% of a nude mouse body weight).
Open Clinical Issues for the Therapeutic Use of EGFR-Targeted Drugs

● Appropriate selection of potentially responding patients (are there differences between first generation reversible smTKIs, second generation irreversible smTKIs and different MAbs?):

  - EGFR expression is necessary. Is EGFR expression sufficient?
  - Role of EGFR gene amplification/increased gene copies (FISH).
  - “Gain of function” somatic EGFR gene mutations.
  - “Acquired resistance” somatic EGFR gene mutations.
  - Expression of ligands and receptors of the erbB family.
  - Downstream signaling molecules activation (PTEN; K-RAS; B-RAF; MEK; MAPK, AKT).
  - Activation of other growth factor receptors (IGF1-R; erbB2; erbB3; MET).

● Timing and schedule for the combination of cytotoxic treatments and EGFR-targeted agents (are there differences between MAbs and smTKIs?).

● Optimal combination with molecular targeted therapies.

● Overcoming cancer cell resistance to EGFR-targeted agents.