GOOD SCIENCE
BETTER MEDICINE
BEST PRACTICE

Pharmacology basis of targeted therapy: EGFR and HER2 inhibitors

Fortunato Ciardiello
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.

[CANCER RESEARCH 44, 1002–1007, March 1984]

Growth Inhibition of Human Tumor Cells in Athymic Mice by Anti-Epidermal Growth Factor Receptor Monoclonal Antibodies[1]

Hideo Masui,[2] Tomoyuki Kawamoto, J. Denry Sato,[3] Bonnie Wolf, Gordon Sato,[4] and John Mendelsohn[5]

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

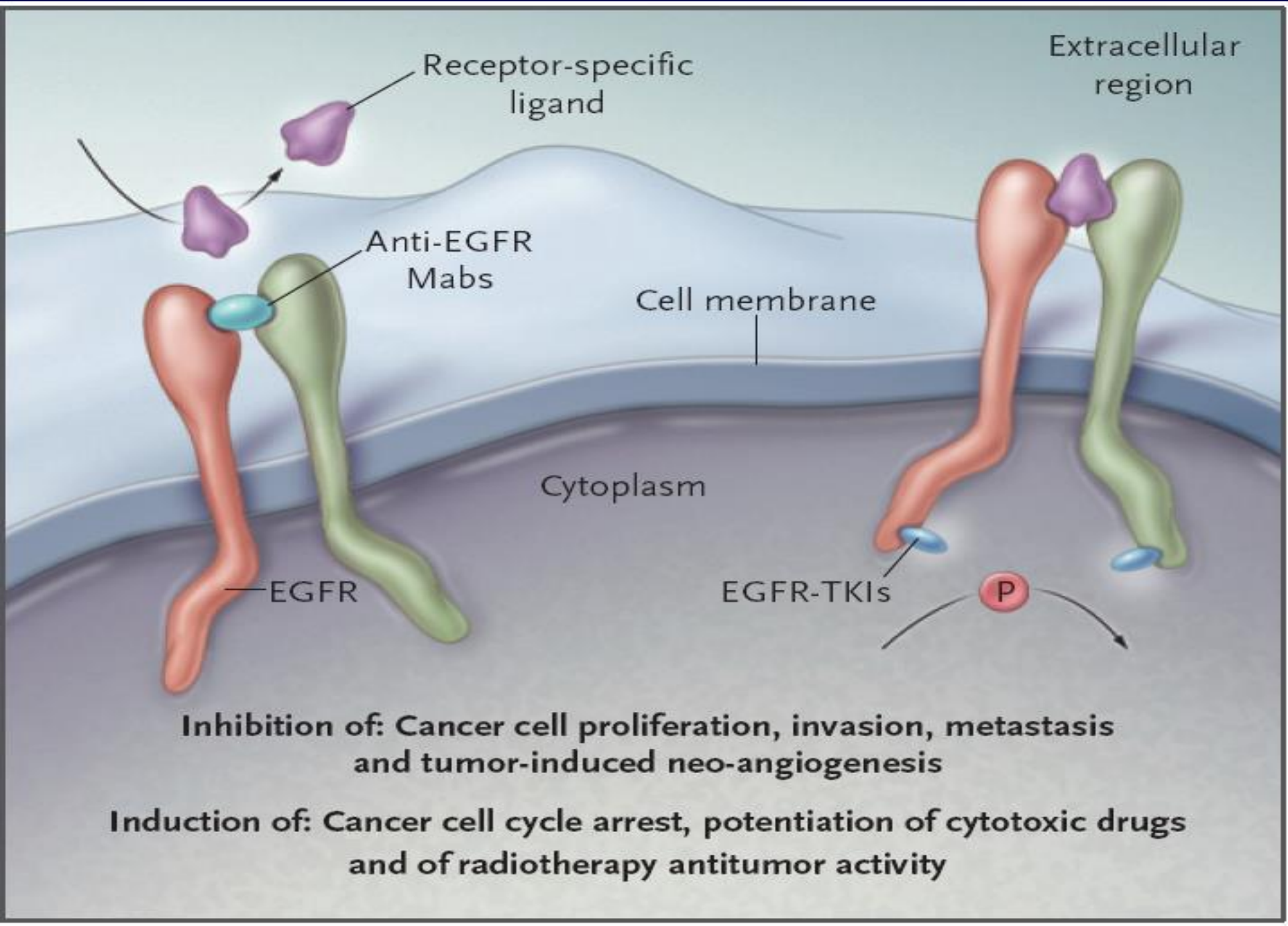
ABSTRACT

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 cytocidal *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.

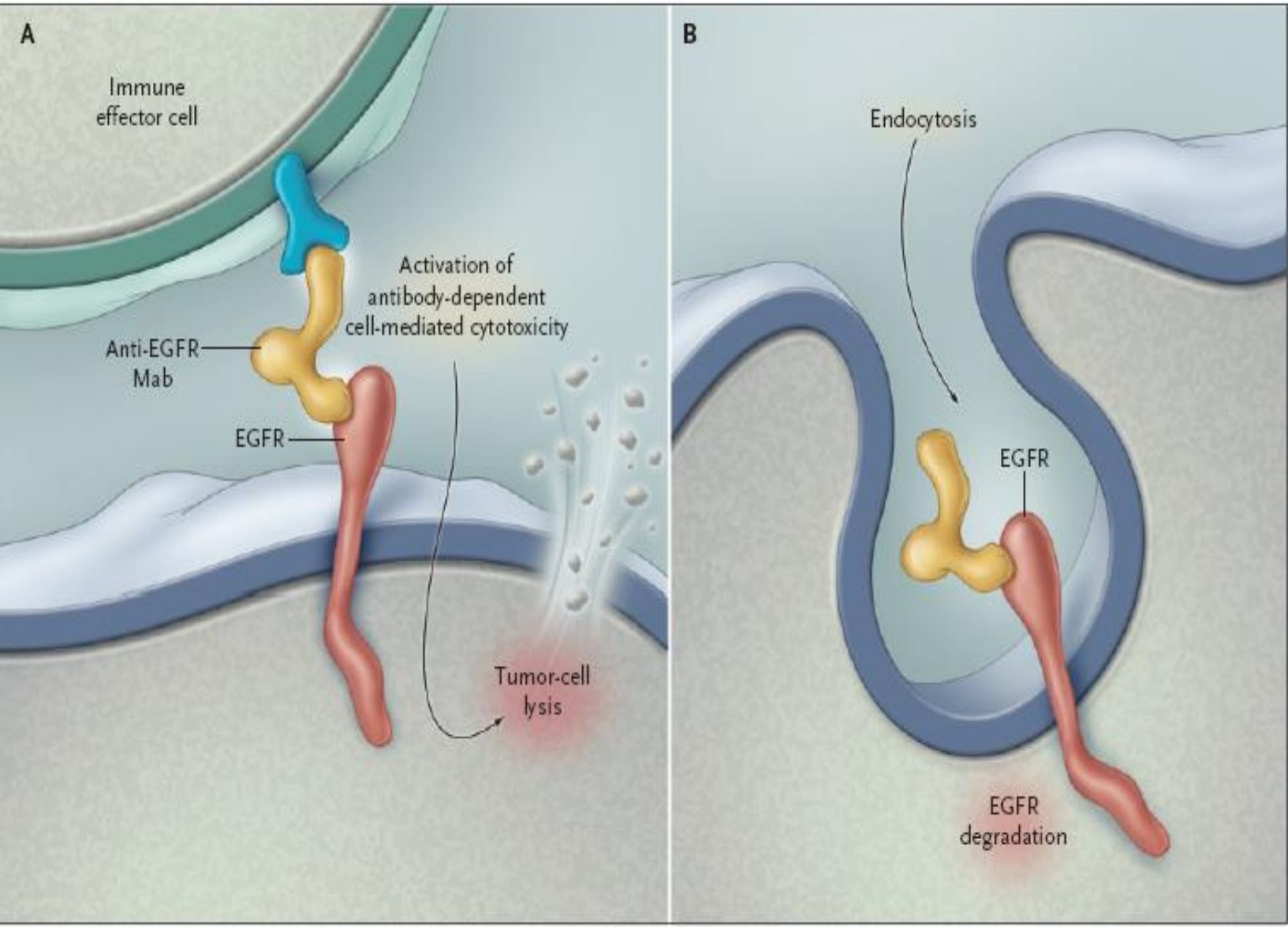
the growth of human tumor xenografts in athymic mice (8, 9, 16, 27). Furthermore, the results of several clinical studies on the treatment of leukemia and lymphoma using MoAbs have been published, but in these cases, clinically significant results have not been obtained (17, 18, 20, 23–25), with one exception. This was a report on the treatment of a human B-cell lymphoma with anti-idiotype 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 × 10^6/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



Ciardiello F and Tortora G. New Engl J Med 2008;358:1160–74.



Ciardiello F and Tortora G. New Engl J Med 2008;358:1160–74.

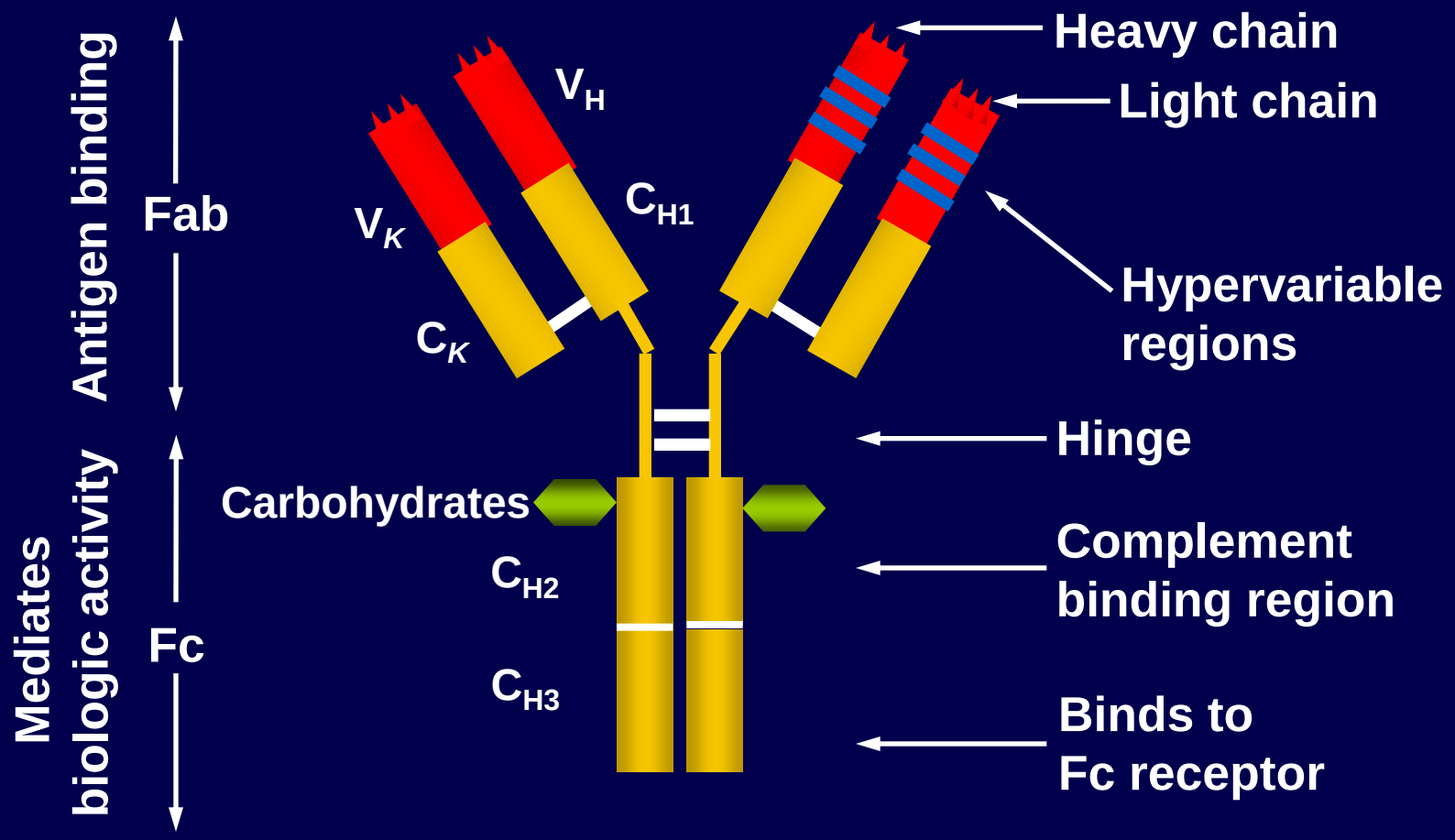
Table 1. Functional and Pharmacologic Characteristics of EGFR Inhibitors.*

Characteristic	Blocking Monoclonal Antibodies	Small-Molecule Tyrosine Kinase Inhibitors
Route of administration	Intravenous (generally once a week or every 2 wk)	Oral (generally daily continuous dosing)
Structure	Recombinant immunoglobulins (150–180 kD)	Low-molecular-weight compounds (400–600 kD)
Target selectivity	Exclusively specific for EGFR	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)
Mechanism of interference with EGFR activation	Bind extracellular portion of receptor, preventing ligand binding and receptor dimerization by occluding ligand region (cetuximab)	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
Cellular effects of EGFR inhibition	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	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
Induction of EGFR internalization, down-regulation, and degradation	Yes	No (although irreversible EGFR tyrosine kinase inhibitors can cause EGFR degradation and subsequent EGFR down-regulation)
Inhibition of EGFR-dependent intracellular signaling	Yes	Yes
Activity against mutant EGFR proteins	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	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
Activation of host immune response	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	No

* EGFR denotes epidermal growth factor receptor, VEGF vascular endothelial growth factor, and VEGFR VEGF receptor.

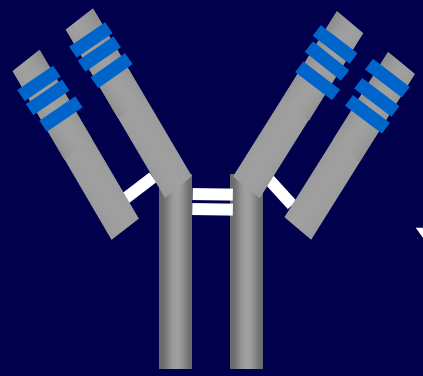
Ciardiello F and Tortora G. New Engl J Med 2008;358:1160–74.

Antibodies are Immunoglobulin Molecules (IgG)

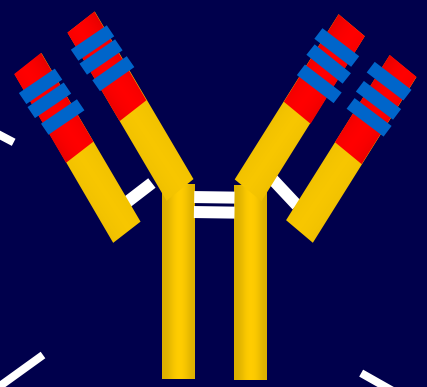


©TPWG Dec 2005

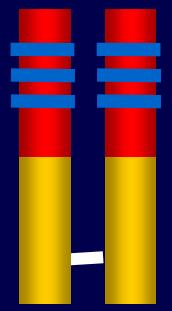
Monoclonal Antibodies



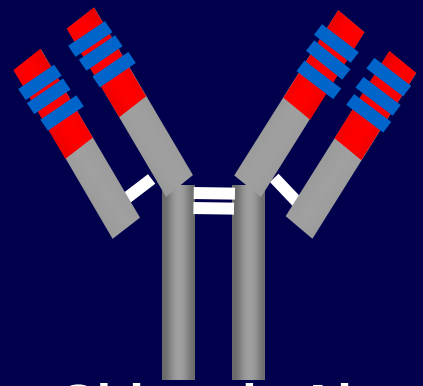
Humanized Ab
(90% human)



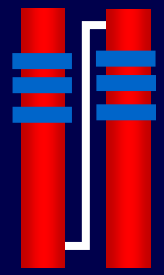
Mouse MoAb
(0% human)



Fab-fragment



Chimeric Ab
(60-70% human)



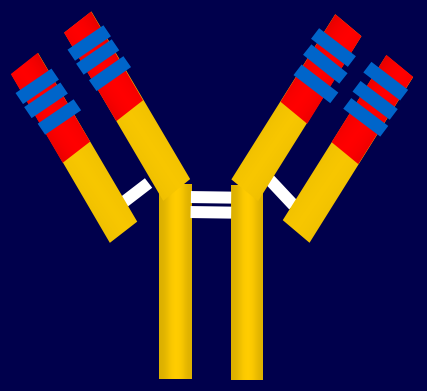
scFv
(single chain
Fragment variable)

©TPWG Dec 2005

From Murine to Reshaped MoAbs

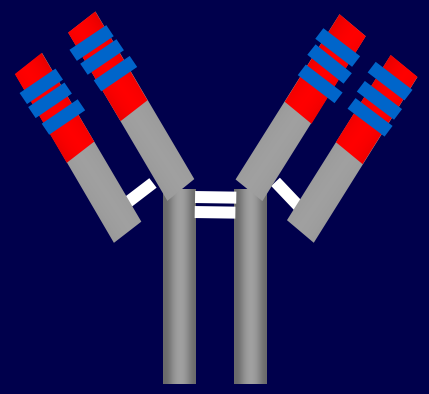
	Murine	Chimeric	Humanized	Human
% Murine Protein	100%	≈25%	<5%	0%
HAMA Induction	+++	+	+	-
Half-Life	short	long	long	long
Effectiveness in ADCC	++	+++	+++	ND

Monoclonal Antibodies Nomenclature (Suffix)



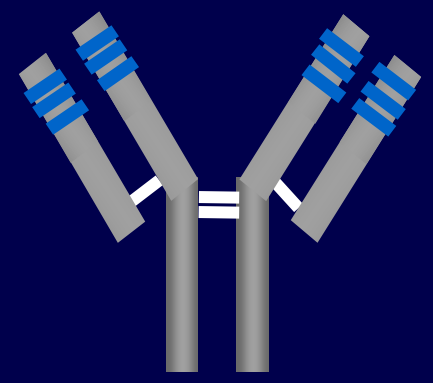
Mouse MoAb

„momab“



Chimeric Ab

„ximab“



Humanized Ab

„zumab“

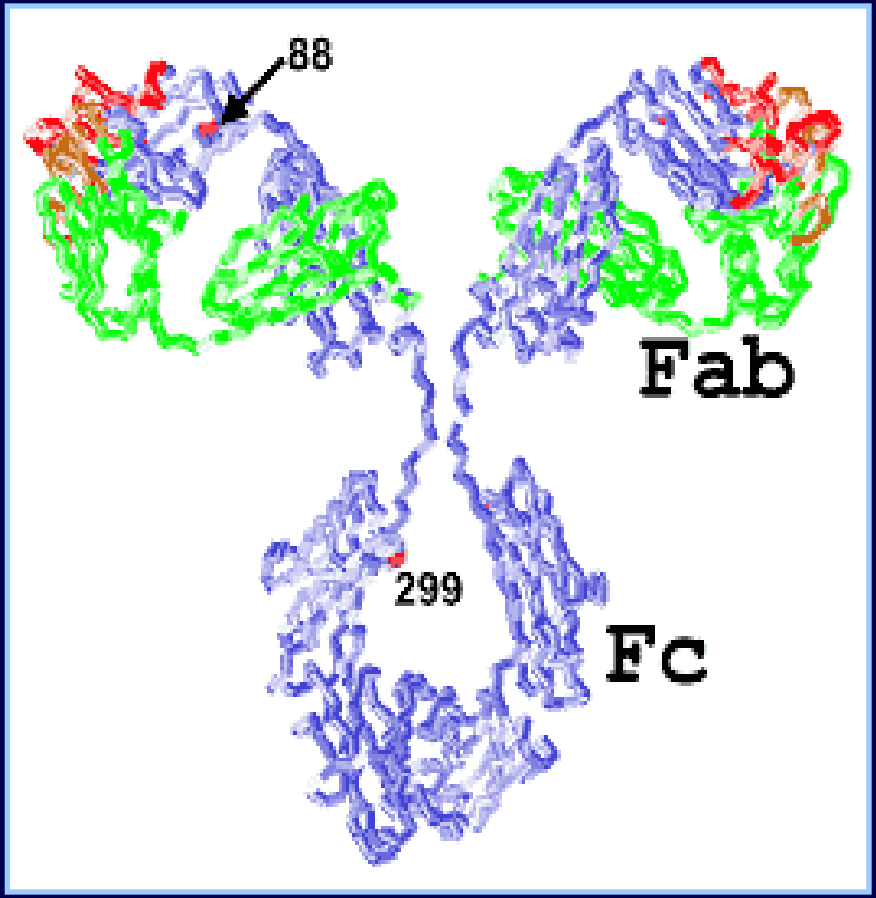
©TPWG Dec 2005

Monoclonal antibodies

Trastuzumab	Humanized anti-HER2 MAb
Cetuximab	Chimeric anti-EGFR MAb
Panitumumab	Fully human anti-EGFR MAb
Pertuzumab	Humanized anti-HER2 MAb
Sym004	1:1 mixture of two chimeric anti-EGFR MAb
T-DM1	Trastuzumab linked to microtubule inhibitor emtansine
GA201	Glycositation modified humanized anti-EGFR MAb

Cetuximab

- IgG1 (chimerized antibody)
- 150 kDa
- Exclusive for EGFR and its heterodimers
- Prevents ligand binding to EGFR
- High affinity. K_d = 0.39 nM (M-225 K_d = 1 nM)
- 1 log > natural ligand
- Stimulates receptor internalization
- Blocks receptor dimerization, tyrosine kinase phosphorylation, signal transduction



*

Therapeutics, Targets, and Chemical Biology

Cancer Research

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.*

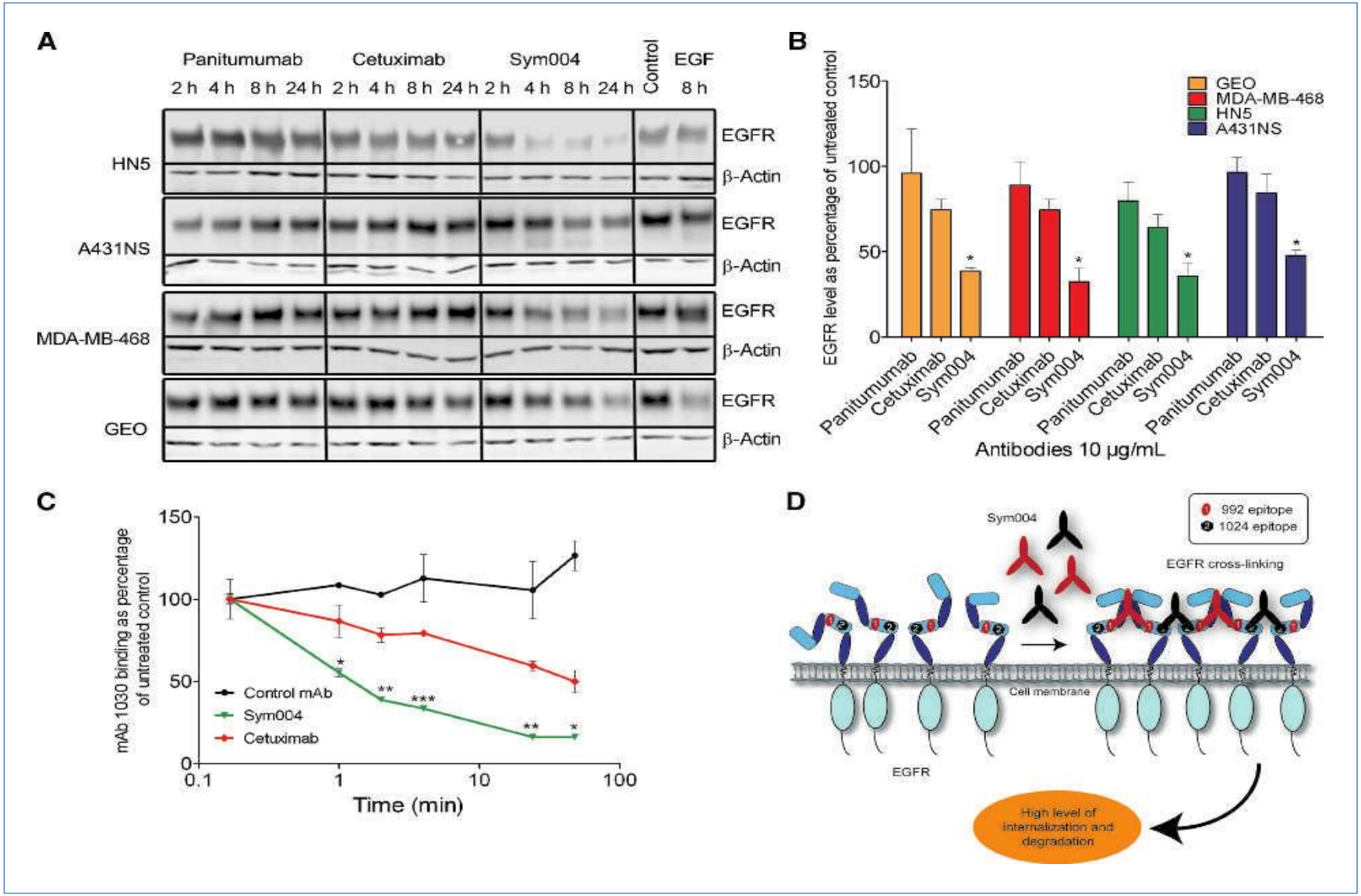


Figure 3. Sym004-induced EGFR degradation *in vitro. A,* immunoblot analyses of EGFR levels in HN5, A431NS, MDA-MB-468, and GEO cell lines after treatment with 10 µg/mL of the indicated antibodies. *B,* quantification of EGFR levels in cell lines after 24 h of antibody treatment relative to untreated 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 400× 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

A B S T R A C T

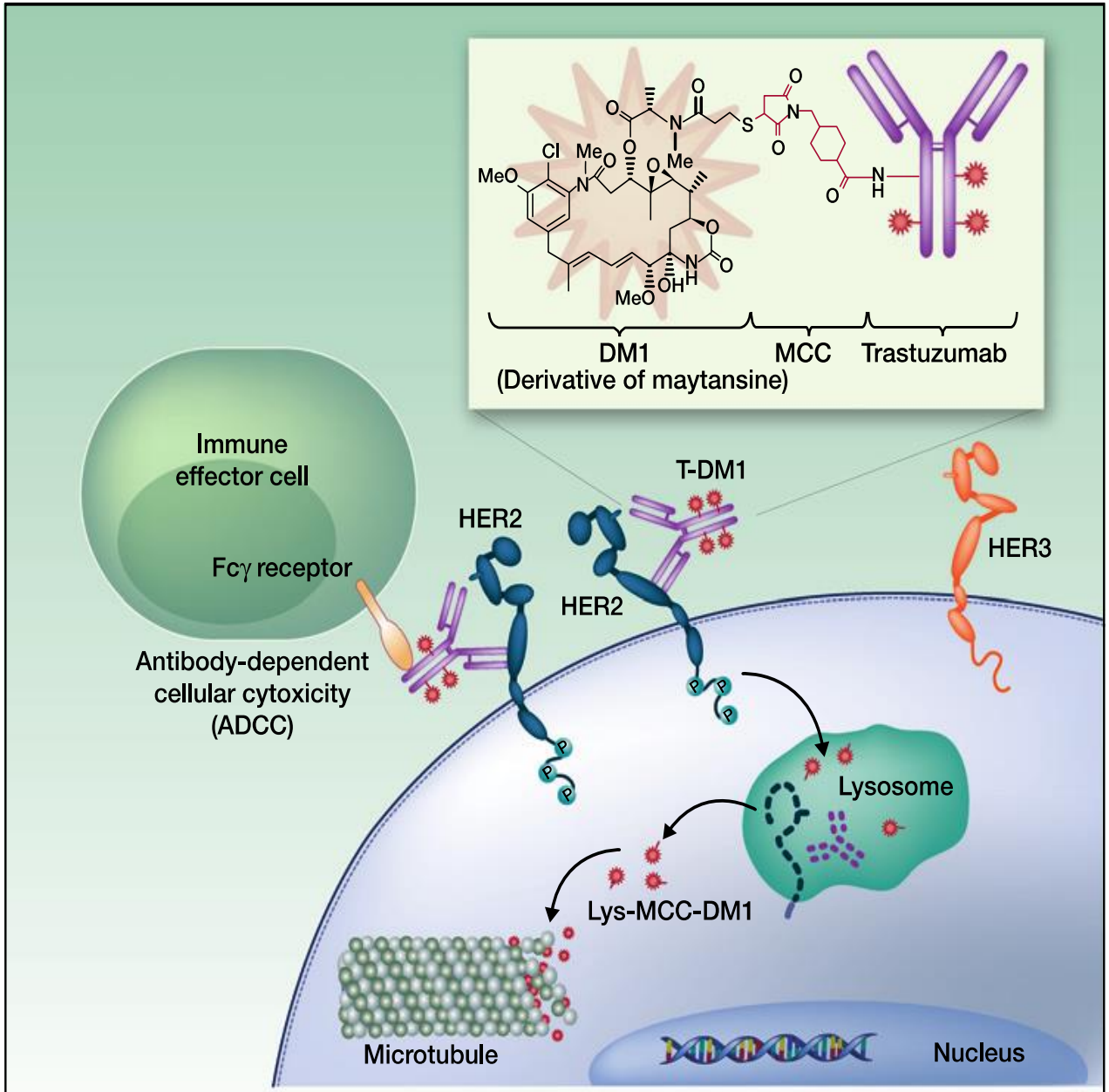
Purpose
We conducted a phase I dose-escalation study to characterize the safety, efficacy, pharmacokinetic (PK), and pharmacodynamic properties of RG7160 (GA201), a humanized and glycoengineered immunoglobulin G_1 anti–epidermal growth factor receptor (EGFR) monoclonal antibody with enhanced antibody-dependent 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.

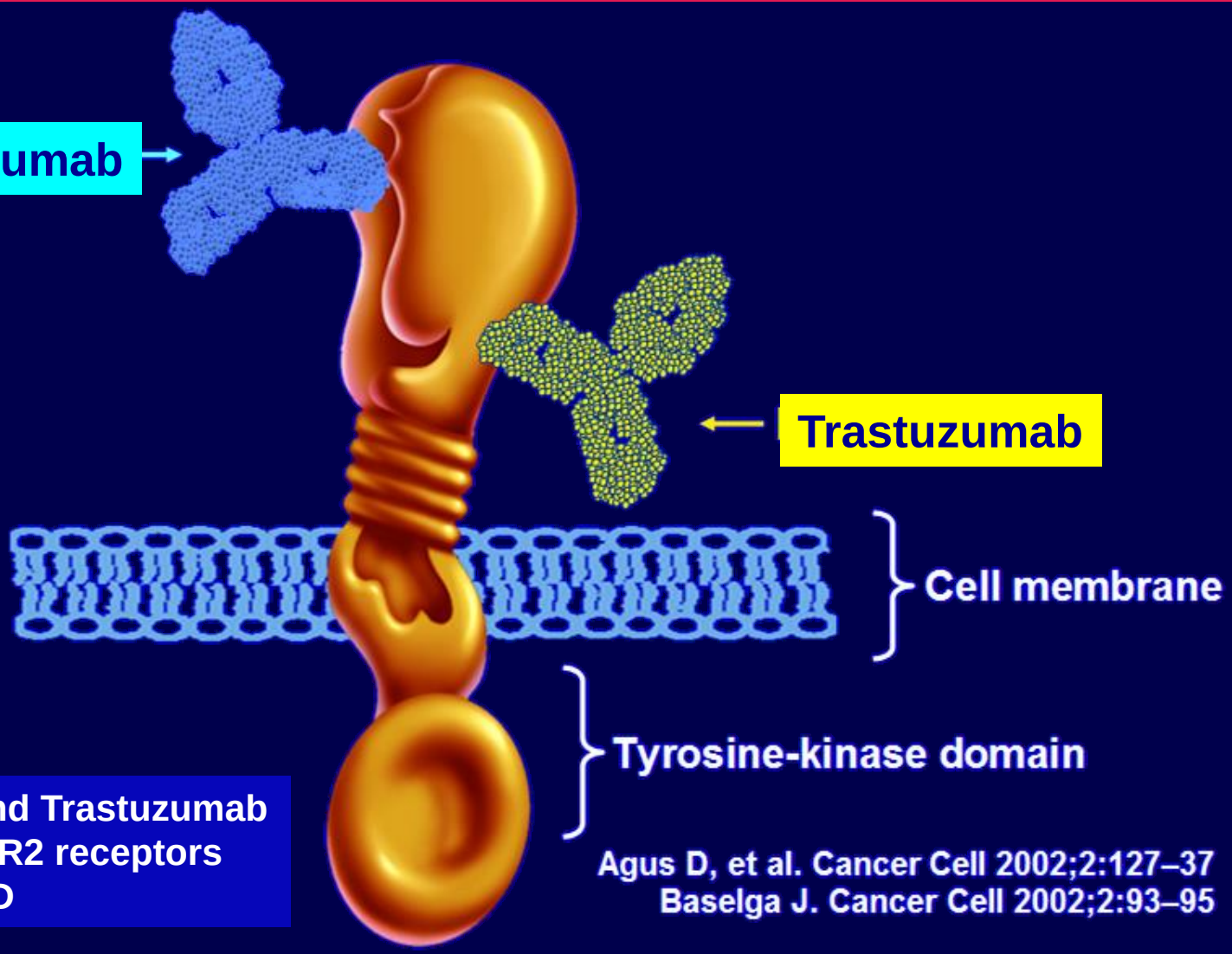
J Clin Oncol 29:3783-3790. *© 2011 by American Society of Clinical Oncology*



© 2011 American Association for Cancer Research

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

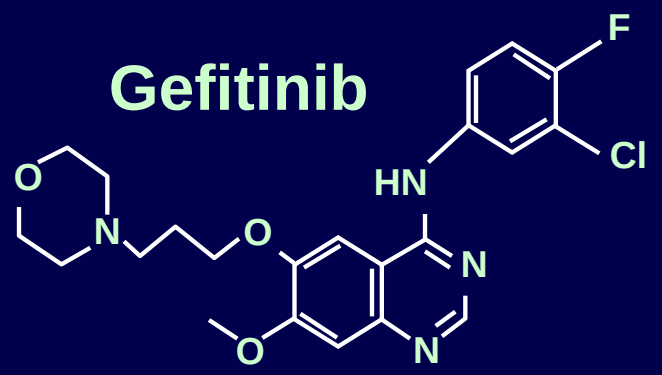
Agus D, et al. Cancer Cell 2002;2:127–37
Baselga J. Cancer Cell 2002;2:93–95

Small Molecule Tyrosine Kinase inhibitors

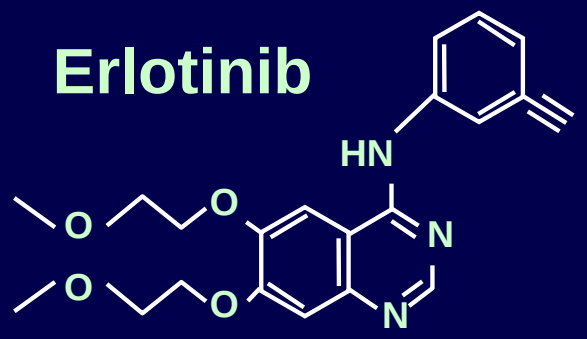
Drug	
Gefitinib	Reversible anti-EGFR TKI
Erlotinib	Reversible anti-EGFR TKI
Lapatinib	Reversible dual anti-EGFR/HER2 TKI
Vandetanib	Reversible multi-TKI (EGFR, VEGFRs, RET)
Afatinib	Irreversible dual EGFR/HER2 TKI

EGFR Selective Small Molecule Tyrosine Kinase Inhibitors

Gefitinib

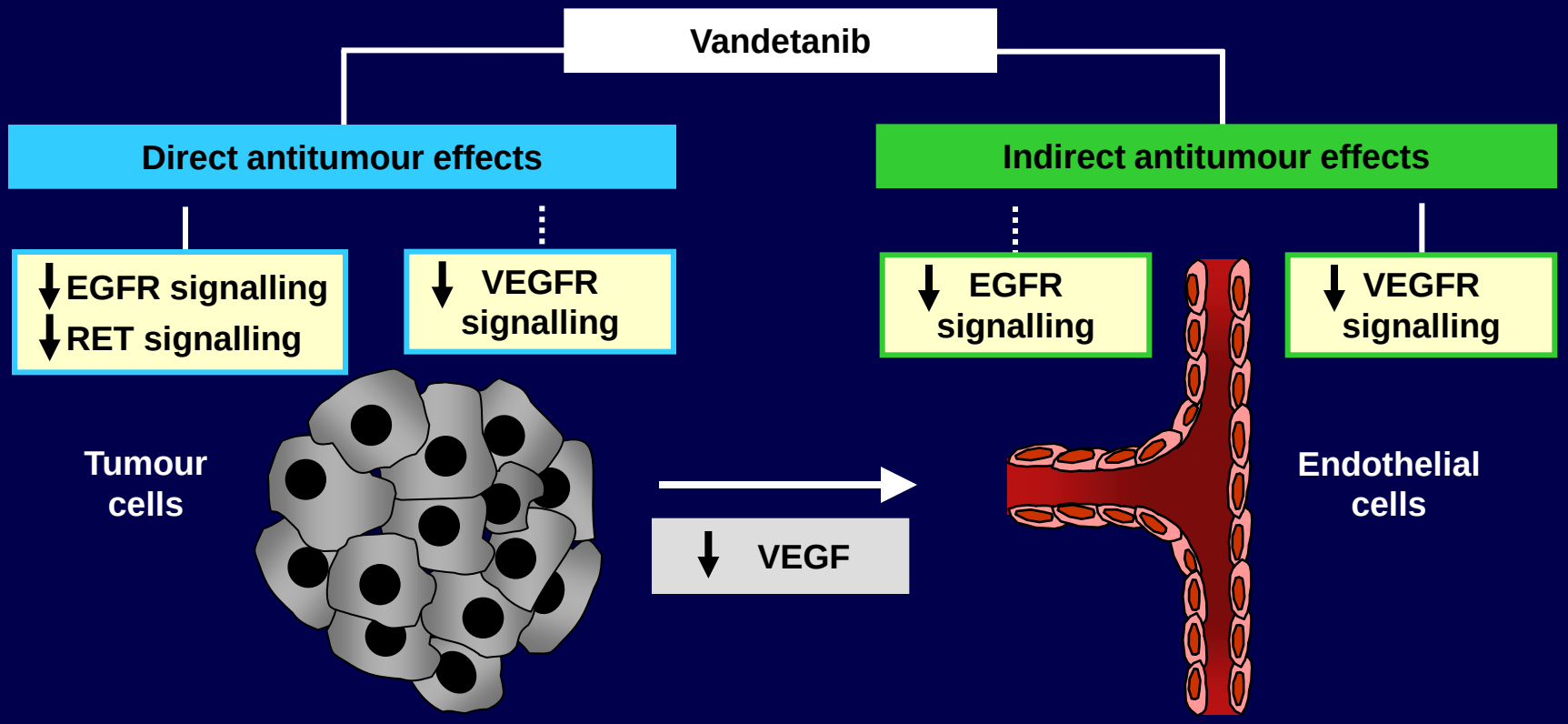


Erlotinib



- EGFR tyrosine kinase activity requires ATP
- Gefitinib and Erlotinib compete for ATP binding
- Reversible inhibitors
- Orally bioavailable small molecules

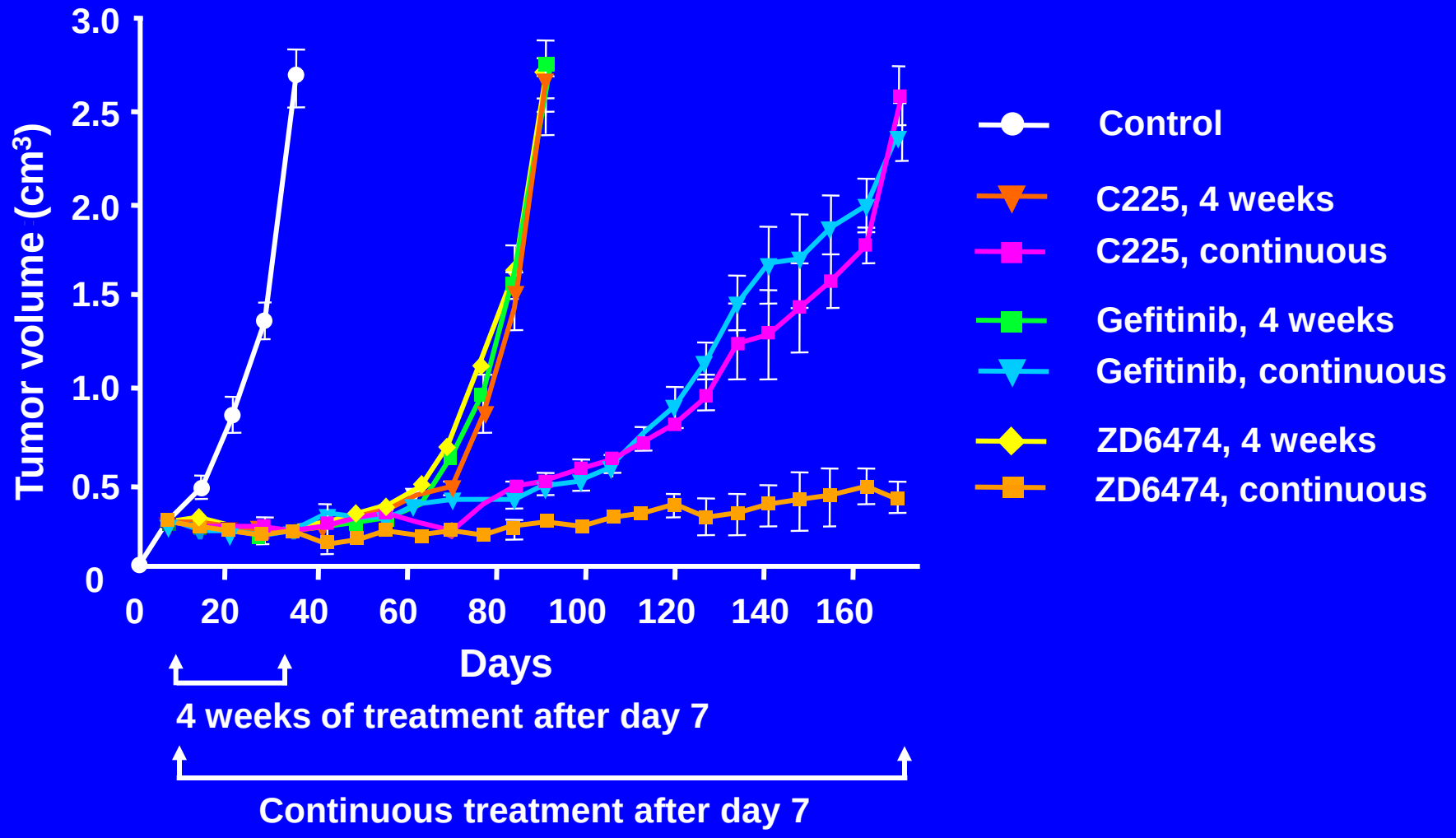
Vandetanib: targeting key signalling pathways in cancer



- 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

- 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

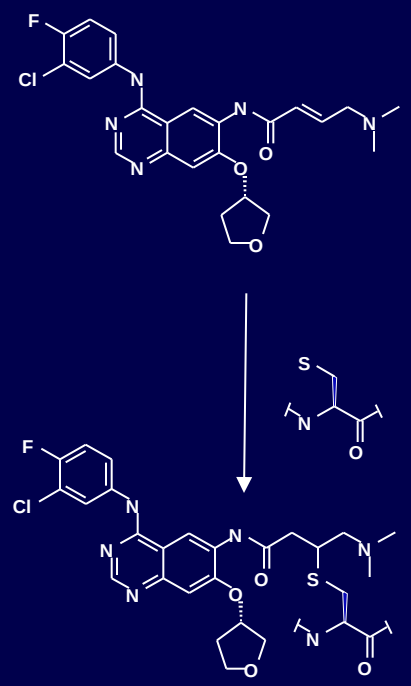


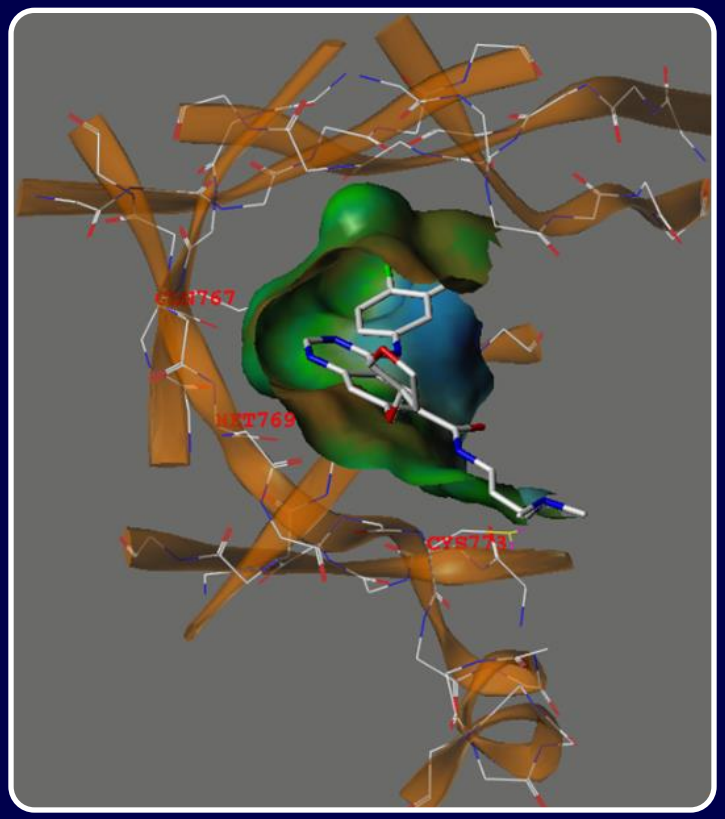
Ciardiello F et al. Clin Cancer Res 2004;10:784–793

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





Solca F et al. Presented at AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics, Philadelphia, 14–18 Nov 2005

Afatinib maintains inhibitory activity in EGFR mutants resistant to erlotinib/gefitinib

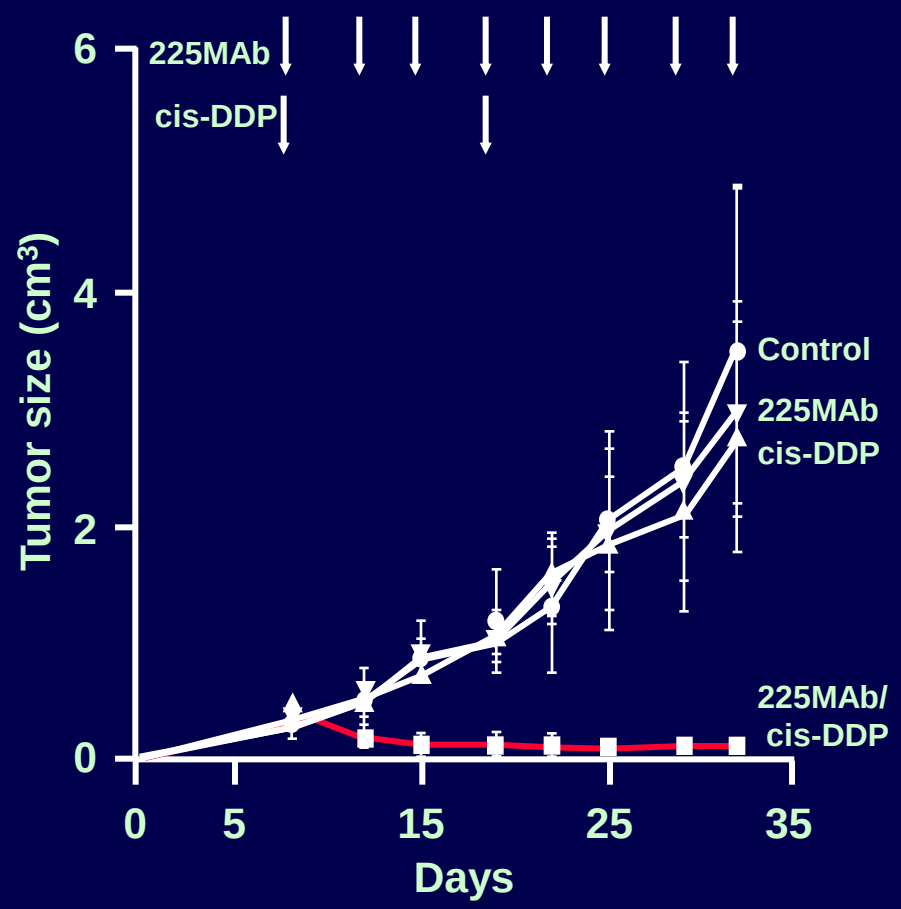
Mutation:	WT	Activated	Resistance		
	wild type H1666	L858R H3255	L858R+T790M NCI1975	Target	Binding mode
Afatinib[1]	60	0.7	99	EGFR/HER2	Irreversible
Gefitinib[1]	157	5	>4000	EGFR	Reversible
Erlotinib[1]	110	40	>4000	EGFR	Reversible
Lapatinib[1]	534	63	>4000	EGFR/HER2	Reversible
CP 714,724[2]	>4000	561	>4000	HER2	Reversible

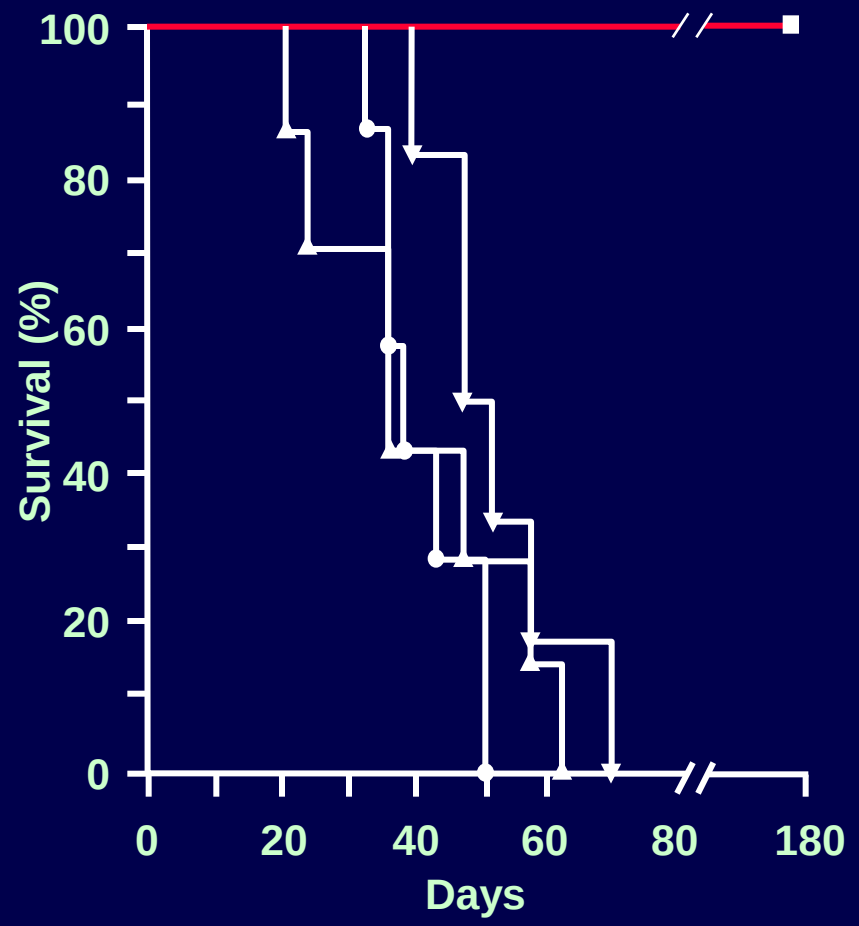
EC50 values for the inhibitory activities of different compounds on the proliferation of NSCLC cells with *EGFR* mutations

WT, wild type

1. Li D et al. Oncogene 2008; 27: 4702–4711; 2. Solca F et al. Proceedings, AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics. November 2005. 118 (Abstract A242)

Effects of Anti-EGFR MoAb in Combination with Cisplatin on A431 Epidermoid Cancer Xenografts





Fan Z et al. Cancer Res 1993; 53: 4637-4642.

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 $p185^{HER2}$, that has partial homology with the other members of the EGFR[4] 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 $p185^{HER2}$ 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 tients 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 Tabernero,[1] Marta Guzmán,[1] Sonia Rodriguez,[1] Joaquín Arribas,[1] José Palacios,[3] and José Baselga[1]

[1]Laboratory of Oncology Research, Medical Oncology Service, and [2]Pathology Service, Vall d'Hebron University Hospital, Barcelona, Spain; [3]Laboratory of Breast and Gynecological Cancer, Molecular Pathology Program, Centro Nacional de Investigaciones Oncológicas (CNIO), Madrid, Spain; and [4]Division 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**

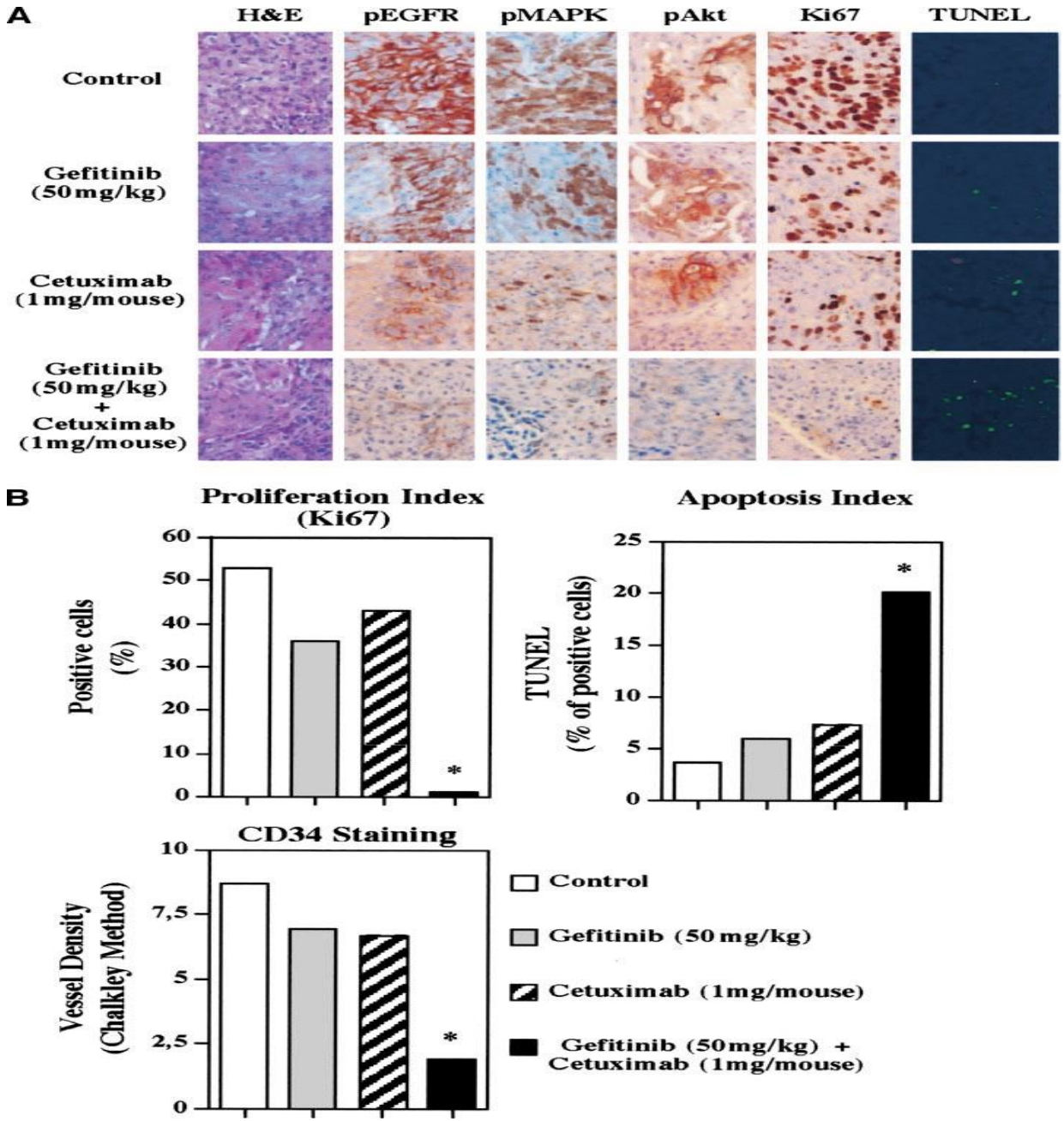


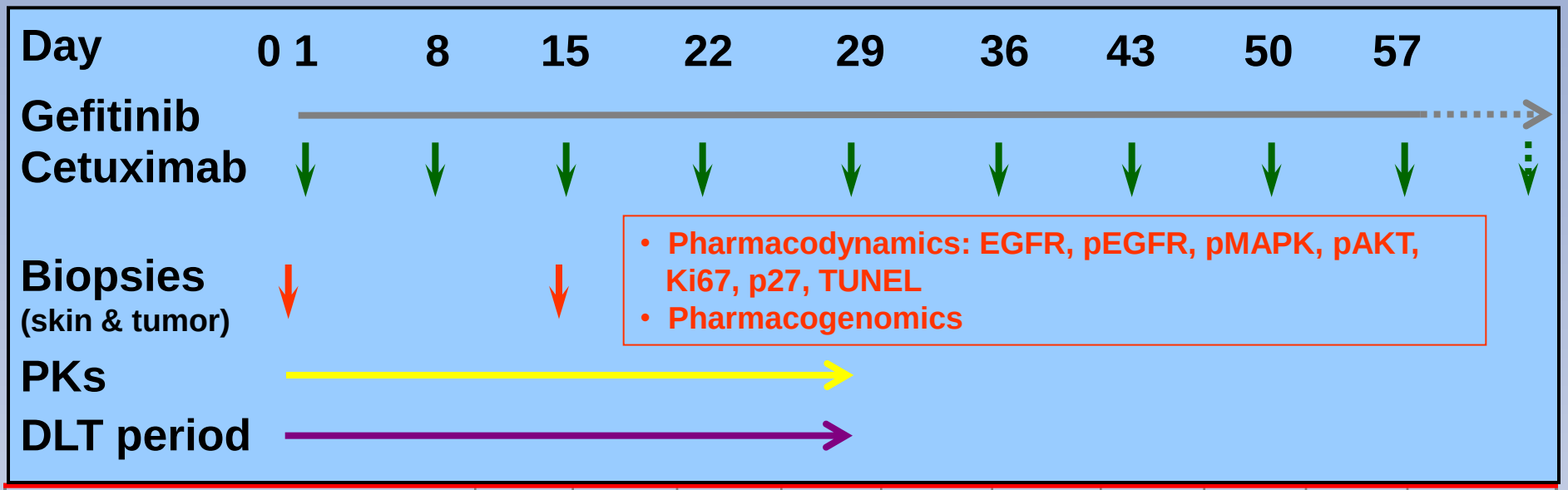
Fig. 7 Tissue-based studies of A431 tumor xenografts treated with gefitinib (50 mg/kg/d), cetuximab (1 mg per mouse twice a week) or the combination of both (from study design I), *A*, immunohistochemical analysis of tumor cells stained with hematoxylin and eosin (*H&E*), anti-phospho-EGFR, anti-phospho-MAPK, 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)

J. Baselga, P. Schöffski, F.Rojo, H. Dumez, F.J. Ramos, T. Macarulla, R. Cajal, E. Jiménez, E. Calvo, A. Van Oosterom, J. Tabernero

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



Group	0a	0b	1	2a	2b	3	4	5	-1	Part B
Daily Gefitinib (mg)	-	250	100	100	250	250	500	500	100	RD
Loading Cetuximab (mg/m^2)	400	-	320	400	320	400	320	400	250	RD
Weekly Cetuximab (mg/m^2)	250	-	200	250	200	250	200	250	160	RD
Patients planned	5	5	3-6	3-6	3-6	3-6	3-6	3-6	3-6	20
Patients included	7	7	3	5	Not done	6	6	6	Not done	3+

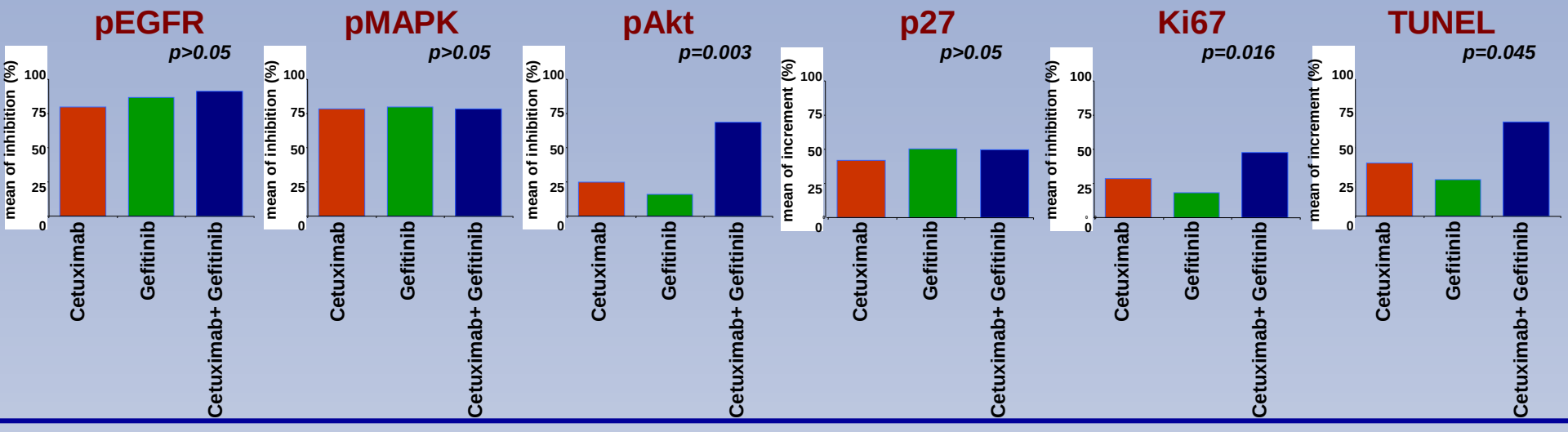
Tumor Response (All Tumor Types)

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

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



SKIN

U Mann Whitney test: single administration vs combination

Cancer Therapy: Preclinical

Clinical Cancer Research

Synergistic Antitumor Activity of Sorafenib in Combination with Epidermal Growth Factor Receptor Inhibitors in Colorectal and Lung Cancer Cells

Erika Martinelli[1], Teresa Troiani[1], Floriana Morgillo[1], Gabriella Rodolico[2], Donata Vitagliano[3], Maria Pia Morelli[1,4], Concetta Tuccillo[1], Loredana Vecchione[1], Anna Capasso[1], Michele Orditura[1], Ferdinando De Vita[1], S. Gail Eckhardt[4], Massimo Santoro[3], Liberato Berrino[2], and Fortunato Ciardiello[1]

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

Treatment	Average tumor volume (±SD) on day 35 after tumor cell injection, cm^3	Average survival (±SD), days
Control	2.5 (±0.2)	35 (±2)
Erlotinib	2.5 (±0.3)	37 (±4)
Sorafenib	0.56 (±0.01)	75 (±5)
Cetuximab	2.55 (±0.2)	34 (±5)
Erlotinib plus sorafenib	0.15 (±0.04)	145 (±5)
Cetuximab plus sorafenib	0.10 (±0.03)	165 (±10)

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) cm^3.
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 cm^3 (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.