Editorial

On Receptor Inhibitors and Chemotherapy

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In the search for rational targets for drug development in cancer, receptor tyrosine kinases have become a favorite. The rationale for directing efforts to developing inhibitors of these receptors is compelling. Mutated or overexpressed growth factor receptors play a pivotal role in malignant transformation and in maintaining the growth of cancers in model systems. Strategies that inactivate growth factor receptors or their downstream pathways halt tumor growth. Of particular recent interest are members of the EGFR[2] family including the EGFR (c-erbB1/HER1) and Her2 (c-erbB2/HER2) molecules. Both receptors are overexpressed in a broad range of human cancers of epidermal origin, and their presence has been associated with a poor prognosis and resistance to cytotoxic chemotherapy (1–4). These receptors are composed of an external domain responsible for ligand binding. Binding in turn stimulates dimerization and activates an internal domain that has tyrosine kinase activity. Both the receptor ligand binding function and the kinase domain present potential targets for inhibition of receptor function. In recent years, molecules of both types (external domain antagonists and kinase inhibitors) have reached the clinic and shown evidence of modest activity in breast cancer [Herceptin (5)] and non-small cell lung cancer [EGFR antibody C225 and Iressa (6, 7)].

Although these effects are important in demonstrating the potential of such agents, the ultimate value of the antireceptor approach may lie elsewhere, namely, in the combined use of these inhibitors with cytotoxic drugs or irradiation, a subject addressed in several recent articles in this journal (8–11) and in related publications (12–14).

The mechanism whereby tyrosine kinase inhibitors enhance the activity of cytotoxic drugs is complex and incompletely understood. Growth factor signaling pathways provide a stimulus for proliferation through a complex receptor-to-nucleus cascade. In addition, they up-regulate the expression of genes that antagonize apoptosis, or programmed cell death, such as *Bcl-2* and *Bcl-XL* and thereby raise the threshold for cell death in response to DNA damage (15). Finally, these pathways stimulate the secretion of angiogenic factors such as VEGF (16). All of these actions confer a growth or survival advantage, but the apoptotic effects seem most important in combination with drugs.

When combined with drugs, inhibitors of growth factor receptors enhance the efficacy of conventional cytotoxic agents. This has been demonstrated convincingly with Herceptin and paclitaxel or Herceptin and doxorubicin in xenograft experiments (17) and in patients with metastatic breast cancer (12), for whom the combination has resulted in a significant increase in response rates and improved survival. Promising preclinical experiments with C225 (18, 19), an anti-EGFR monoclonal antibody, have led to clinical trials of the antibody combined with radiotherapy in head and neck cancer (13, 14). Of nine patients with head and neck cancers treated with C225 and cisplatin, six had major responses, and three of these six had previously failed cisplatin-based chemotherapy (13). Fourteen of 15 patients achieved complete responses after treatment with C225 and once- or twice-daily irradiation for locally advanced head and neck malignancies. In these patients, a complete response rate of 50% or less would have been expected with radiation alone (14).

The explanation for these effects may lie in the effects of receptor inhibition on the threshold for apoptosis. In the case of paclitaxel interaction with Her2, paclitaxel disrupts microtubule integrity, leading to cell cycle arrest and apoptosis (20). Overexpression of Her2 confers resistance to paclitaxel by increasing levels of p21, a cyclin-dependent kinase inhibitor, which delays entrance into mitosis and prevents mitotic arrest (20). Resistance to paclitaxel can be overcome by blocking the Her2 receptor-mediated signaling, and the consequent increase in p21. A second antiapoptotic signal emanates from the EGFR, which, on ligand binding, activates a number of downstream signals (21–24) including protein kinase A, which, in turn, phosphorylates bcl-2 (22). This interaction provided the rationale for combined therapy with C225, antisense oligonucleotides targeting the RIα regulatory subunit of protein kinase A, and docetaxel in the human breast cancer cell line ZR-75-1 (24). These three agents, used in combination at suboptimal doses, led to a greater degree of Bcl-2 phosphorylation and apoptosis than seen with any single agent alone in this cell line (24).

Receptor blockade with ZD1839, a low molecular weight inhibitor of EGFR, exerts synergistic effects with paclitaxel or other chemotherapy in xenograft experiments (25). ZD1839, an anilinoquinazoline, is a potent and selective inhibitor of EGFR receptor kinase *in vitro* and *in vivo*. It has strong antiproliferative effects against the growth of the A431 tumor, which has high levels of expression of EGFR (26). Sirotnak *et al.* (8) report in this issue of *Clinical Cancer Research* that the combination of ZD1839 with taxanes, platinums, or the folate antagonist edatrexate markedly enhances antitumor activity. The combination of ZD1839 with all cytotoxic agents required a 2-fold or greater attenuation of the ZD1839 dose below its single-agent maximum dose of 150 mg/kg. Interestingly, the degree of en-

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[2] The abbreviations used are: EGFR, epidermal growth factor receptor; VEGF, vascular endothelial growth factor; bFGF, basic fibroblast growth factor; IL-8, interleukin 8; TCC, transitional cell carcinoma; PCNA, proliferating cell nuclear antigen.

hancement of cytotoxic activity was not dependent on high levels of EGFR expression. As a single agent at the maximum tolerated dose, ZD1839 induced partial regression of A431, a vulvar tumor expressing high levels of EGFR. ZD1839 also demonstrated 50–55% growth inhibition against the LX-1 lung tumor, which expresses very low levels of EGFR. A related compound, CP-358774, inhibited epidermal growth factor-dependent cell proliferation in a head and neck squamous cell carcinoma model with drug concentrations in the nanomolar range and eliminated *in vivo* epidermal growth factor-induced autophosphorylation of tumor (27). As receptor inhibitors, small molecules have important potential advantages compared with monoclonal antibodies; they should have better tissue penetration and potential oral bioavailability and, finally, an ability to inhibit the intracellular receptor tyrosine kinase activity directly, rather than as a secondary effect of interaction with the external receptor domain. They may have the important limitations of potential pharmacokinetic interaction with other agents, susceptibility to drug transporters such as MRP and MDR, and promiscuous interaction with related tyrosine kinases. Like the monoclonal antibodies, the small molecule receptor inhibitors may be tumoristatic rather than tumoricidal; it may be difficult to appreciate their primary activity as single agents. Imaging for metabolic effects or repetitive tumor biopsies may be helpful in establishing activity in humans.

Studies by Inoue *et al.* (9) and Perrotte *et al.* (16) also address the effects of receptor inhibitors on angiogenesis, a process that may also contribute to the synergy between receptor inhibitors and chemotherapy. Perrotte *et al.* (16) reported that *in vitro* treatment with C225 inhibited mRNA and protein production of VEGF, IL-8, and bFGF by the highly metastatic human TCC 253JB-V cells in a dose-dependent manner. C225 therapy of nude mice with orthotopically implanted TCCs resulted in inhibition of growth and metastases compared with controls. The expression of protein VEGF, IL-8, and bFGF as determined by immunohistochemical staining was significantly lower in treated tumors as compared with the expression of these proteins in controls. Microvessel density was significantly lower in tumors treated for 5 weeks with C225 as compared with the vessel density in control tumors. Furthermore, the down-regulation of VEGF, IL-8, and bFGF mRNA and protein preceded the reduction in microvessel density. Their data suggest that a mechanism that contributes, at least in part, to the antitumor effect of EGFR blockade therapy with C225 is inhibition of angiogenesis. C225 has also been shown to down-regulate VEGF expression in A431 cells (28) and in renal cell carcinoma cell lines (29). In human pancreatic carcinoma growing orthotopically in nude mice, systemic therapy with C225 alone and with C225 in combination with gemcitabine resulted in growth inhibition, tumor regression, and abrogation of metastases (10). This was associated with a significant reduction in PCNA-positive cells, production of VEGF and IL-8, and microvessel density.

Futhermore, Inoue *et al.* (9) evaluated the efficacy of using the combination of C225 and paclitaxel. Paclitaxel has known antitumor effects against TCC of the bladder, as a single agent and in combination with other cytotoxic agents. The combination of C225 (at the IC_{50} dose of 100 μg/ml) and paclitaxel (at the IC_{50} dose of 10 μg/ml) resulted in increased apoptosis *in vitro* as compared with either agent alone. *In vivo*, treatment with paclitaxel, followed by C225, led to significantly greater regression of tumors compared with treatment with either agent alone. When measured by immunohistochemistry for PCNA and terminal deoxynucleotidyl transferase-mediated nick end labeling, the number of PCNA-positive cancer cells was significantly lower in tumors from mice treated with the combination of C225 and paclitaxel, compared with either agent alone. Therapy with initial paclitaxel followed by C225 significantly increased the number of apoptotic cancer cells; this treatment also resulted in an increase in the apoptotic index for endothelial cells. Paclitaxel did not enhance the reduction of microvessel density seen after C225 therapy alone. The authors speculate that the increased antitumor activity with combined activity is the result of the summation of effects on tumor cell pathways regulating apoptosis.

The combination of conventional chemotherapy agents with inhibitors of the receptor tyrosine kinase pathways has exciting potential for clinical use. Clinical trials combining C225 with chemotherapy or radiation for head and neck cancers are ongoing. Clinical testing with ZD1839 is also currently under way in patients with metastatic lung and breast cancer. Because the EGFR is frequently overexpressed in epithelial tumors and has been correlated with tumor resistance to cytotoxic agents and chemotherapy, the emerging preclinical data are particularly encouraging and warrant further study. Clinical investigators must appreciate that the value of these new, targeted drugs may not be obvious in single-agent studies but may only be realized in combination trials with chemotherapy. The early initiation of combination studies is an important strategy in the age of "molecularly targeted" drug discovery and development.

References

1. Fox, S. B., Smith, K., Hollyer, J., Greenall, M., Hastrich, D., and Harris, A. L. The epidermal growth factor receptor as a prognostic marker: results of 370 patients and review of 3009 patients. Breast Cancer Res. Treat., *29:* 41–49, 1994.

2. Grandis, J. R., Melham, M. F., Gooding, W. E., Day, R., Holst, V. A., Wagener, M. M., Drenning, S. D., and Tweardy, D. J. Levels of TGF-α and EGFR protein in head and neck squamous cell carcinoma and patient survival. J. Natl. Cancer Inst. (Bethesda), *90:* 824–832, 1998.

3. Uhlman, D. L., Nguyen, P., Manivel, J. C., Zhang, G., Hagan, K., Farley, E., Aeppli, D., and Niehans, G. Epidermal growth factor receptor and transforming growth factor α expression in papillary and nonpapillary renal cell carcinoma: correlation with metastatic behavior and progress. Clin. Cancer Res., *1:* 913–920, 1995.

4. Neal, D. E., Sharples, L., Smith, K., Fennelly, J., Hall, R. R., and Harris, A. The epidermal growth factor receptor and the prognosis of bladder cancer. Cancer (Phila.), *65:* 1619–1625, 1990.

5. Cobleigh, M. A., Vogel, C. L., Tripathy, D., Robert, N. J., Scholl, S., Fehrenbacher, L., Paton, V., Shak, S., Lieberman, G., and Slamon, D. Efficacy and safety of Herceptin (humanized anti-HER2 antibody) as a single agent in 222 women with HER2 overexpression who relapsed following chemotherapy for metastatic breast cancer. Proc. Am. Soc. Clin. Oncol., *17:* 97a, 1998.

6. Baselga, J., Pfister, D., Cooper, M. R., Cohen, R., Burtness, B., Bos, M., D'Andrea, G., Seidman, A., Norton, L., Gunnett, K., Falcey, J., Anderson, V., Waksal, H., and Mendelsohn, J. Phase I studies of anti-epidermal growth factor receptor chimeric antibody C225 alone and in combination with cisplatin. J. Clin. Oncol., *18:* 904–914, 2000.

7. Ferry, D., Hammond, L., Ranson, M., Kris, M. G., Miller, V., Murray, P., Tullo, A., Feyereislova, A., Averbuch, S., and Rowinsky, E. Intermittent oral ZD1839 (Iressa), a novel epidermal growth factor

receptor tyrosine kinase inhibitor (egfr-tki), shows evidence of good tolerability and activity: final results from a Phase I study. Proc. Am. Soc. Clin. Oncol., *19:* 5E, 2000.

8. Sirotnak, F. M., Zakowski, M. F., Miller, V. A., Scher, H. I., and Kris, M. G. Efficacy of cytotoxic agents against human tumor xenografts is markedly enhanced by co-administration of ZD1839 (IRESSA™), an inhibitor of EGFR tyrosine kinase. Clin. Cancer Res., *6:* 4885–4892, 2000.

9. Inoue, K., Slaton, J. W., Perrotte, P., Davis, D. W., Bruns, C. J., Hicklin, D. J., McConkey, D. J., Radinsky, R., and Dinney, C. P. N. Paclitaxel enhances the effects of the anti-epidermal growth factor receptor monoclonal antibody ImClone C225 in mice with metastatic human bladder transitional cell carcinoma. Clin. Cancer Res., *6:* 4874–4884, 2000.

10. Bruns, C. J., Harbison, M. T., Davis, D. W., Portera, C. A., Tsan, R., McConkey, D. J., Evans, D. B., Abbruzzese, J. L., Hicklin, D. J., and Radinsky, R. Epidermal growth factor receptor blockade with C225 plus gemcitabine results in regression of human pancreatic carcinoma growing orthotopically in nude mice by antiangiogenic mechanisms. Clin. Cancer Res., *6:* 1936–1948, 2000.

11. Ciardiello, F., Bianco, R., Damiano, V., De Lorenzo, S., Pepe, S., De Placido, S., Fan, Z., Mendelsohn, J., Bianco, A. R., and Tortora, G. Antitumor activity of sequential treatment with topotecan and anti-epidermal growth factor receptor monoclonal antibody C225. Clin. Cancer Res., *5:* 909–916, 1999.

12. Slamon, D., Leyland-Jones, B., Shak, S., Paton, V., Bajamonde, A., Fleming, T., Eirmann, W., Wolter, J., Baselga, J., and Norton, L. Addition of Herceptin (humanized anti-HER2 antibody) to first line chemotherapy for HER2 overexpressing metastatic breast cancer markedly increases anticancer activity: a randomized, multinational controlled Phase III trial. Proc. Am. Soc. Clin. Oncol., *17:* 98a, 1998.

13. Mendelsohn, J., Shin, D. M., Donato, N., Khuri, F., Radinsky, R., Glisson, B. S., Shin, H. J., Metz, E., Pfister, D., Perez-Soler, R., Lawhorn, K., Matsumoto, T., Gunnertt, K., Falcey, J., Waksal, H., and Hong, W. K. A Phase I study of chimerized anti-epidermal growth factor (EGFr) monoclonal antibody, C225, in combination with cisplatin (CDDP) in patients (pts) with recurrent head and neck squamous cell carcinoma (SCC). Proc. Am. Soc. Clin. Oncol., *18:* 389a, 1999.

14. Ezekial, M. P., Bonner, J. A., Robert, F., Meredith, R. F., Spencer, S. A., LoBuglio, A. F., and Waksal, H. W. Phase I trial of chimerized anti-epidermal growth factor receptor (anti-EGFr) antibody in combination with either once-daily or twice-daily irradiation for locally advanced head and neck malignancies. Proc. Am. Soc. Clin. Oncol., *18:* 389a, 1999.

15. Page, C., Lin, H. J., Jin, Y., Castle, V. P., Nunez, G., Huang, M., and Lin, J. Overexpression of Akt/AKT can modulate chemotherapy-induced apoptosis. Anticancer Res., *20:* 407–416, 2000.

16. Perrotte, P., Matsumoto, T., Inoue, K., Kuniyasu, H., Eve, B. Y., Hicklin, D. J., Radinsky, R., and Dinney, C. P. N. Anti-epidermal growth factor receptor antibody C225 inhibits angiogenesis in human transitional cell carcinoma growing orthotopically in nude mice. Clin. Cancer Res., *5:* 257–264, 1999.

17. Baselga, J., Norton, L., Albanell, J., Kim, Y. M., and Mendelsohn, J. Recombinant humanized anti-HER2 antibody (Herceptin) enhances the antitumor activity of paclitaxel and doxorubicin against HER2/neu-overexpressing human breast cancer xenografts. Cancer Res., *58:* 2825–2831, 1998.

18. Baselga, J., Norton, L., Masui, H., Pandiella, A., Coplan, K., Miller, W. H., Jr., and Mendelsohn, J. Antitumor effects of doxorubicin in combination with anti-epidermal growth factor receptor monoclonal antibodies. J. Natl. Cancer Inst. (Bethesda), *85:* 1327–1333, 1993.

19. Fan, Z., Baselga, J., Masui, H., and Mendelsohn, J. Antitumor effect of anti-epidermal growth factor receptor monoclonal antibodies plus *cis*-diamminedichloroplatinum on well-established A431 cell xenografts. Cancer Res., *53:* 4637–4642, 1993.

20. Yu, D., Jing, T., Liu, B., Yao, J., Tan, M., McDonnell, T. J., and Hung, M. C. Overexpression of erbB2 blocks Taxol-induced apoptosis by upregulation of $p21^{cip1}$, which inhibits $p34^{cdc2}$ kinase. Mol. Cell, *2:* 581–591, 1998.

21. Blagosklonny, M. V., Schulte, T., Nguyen, P., Trepel, J., and Neckers, L. M. Taxol-induced apoptosis and phosphorylation of Bcl-2 protein involves c-Raf-1 and represents a novel c-Raf-1 signal transduction pathway. Cancer Res., *56:* 1851–1854, 1996.

22. Srivastava, R. K., Srivastava, A. R., Korsmeyer, S. J., Nesterova, M., Cho-Chung, Y. S., and Longo, D. L. Involvement of microtubules in the regulation of Bcl2 phosphorylation and apoptosis through cyclic-AMP-dependent protein kinase. Mol. Cell. Biol., *18:* 3509–3517, 1998.

23. Ciardiello, F., and Tortora, G. Interactions between the epidermal growth factor receptor and type I protein kinase A: biological significance and therapeutic implications. Clin. Cancer Res., *4:* 821–828, 1998.

24. Tortora, G., Caputo, R., Pomatico, G., Pepe, S., Bianco, A. R., Agrawal, S. Mendelsohn, J., and Ciardiello, F. Cooperative inhibitory effect of novel mixed backbone oligonucleotide targeting protein kinase A in combination with docetaxel and anti-epidermal growth factor receptor antibody on human breast cancer cell growth. Clin. Cancer Res., *5:* 875–881, 1999.

25. Woodburn, J. R., Barker, A. J., Gibson, K. H., Ashton, S. E., Wakeling, A. E., Curry, B. J., Scarlett, L., and Henthorn, L. R. ZD1839, an epidermal growth factor receptor tyrosine kinase inhibitor selected for clinical development. Proc. Am. Assoc. Cancer Res., *38:* 4251, 1997.

26. Ciardiello, F., Caputo, R., Bianco, R., Damiano, V., Pomatico, G., DePlacido, S., Bianco, A. R., and Tortora, G. Antitumor effect and potentiation of cytotoxic drugs activity in human cancer cells by ZD-1839 (Iressa), an epidermal growth factor-selective tyrosine kinase inhibitor. Clin. Cancer Res., *6:* 2053–2063, 2000.

27. Iwata, K., Miller, P. E., and Barbacci, E. G. CP-358,774: a selective EGFR kinase inhibitor with potent antiproliferative activity against head and neck tumor cells. Proc. Am. Assoc. Cancer Res., *38:* 633, 1997.

28. Petit, A. M. V., Rak, J., Hung, M., Rockwell, P., Goldstein, N., Fendley, B., and Kerbel, R. S. Neutalizing antibodies to EGF and ErbB2/neu receptor tyrosine kinases down-regulate VEGF production in tumor cells *in vitro* and *in vivo*: angiogenic implications for transduction therapy of solid tumors. Am. J. Pathol., *151:* 1523–1530, 1997.

29. Prewett, M., Rothman, M., Waksal, H., Feldman, M., Bander, N. H., and Hicklin, D. J. Mouse-human chimeric anti-epidermal growth factor receptor antibody C225 inhibits the growth of human renal cell carcinoma xenografts in nude mice. Clin. Cancer Res., *4:* 2957–2966, 1998.

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