Strategies to Enhance Epidermal Growth Factor Inhibition: Targeting the Mevalonate Pathway

Jim Dimitroulakos,1,3 Ian A. Lorimer,1,3 and Glenwood Goss2,3

Abstract Mevalonate metabolites play an essential role in transducing epidermal growth factor (EGF) receptor (EGFR)–mediated signaling, as several of these metabolites are required for the function of this receptor and the components of its signaling cascades. Thus, the depletion of mevalonate metabolites may have a significant effect on EGFR function. Lovastatin is a specific and potent inhibitor of 3-hydroxy-3-methylglutaryl CoA reductase, the rate-limiting enzyme of the mevalonate pathway. Targeting 3-hydroxy-3-methylglutaryl CoA reductase using lovastatin induces a potent tumor-specific apoptotic response in a variety of tumor types at therapeutically achievable levels of this drug. The effects of lovastatin on EGFR function and the potential combination effects with EGFR tyrosine kinase inhibitors, such as gefitinib, were evaluated. Lovastatin treatment inhibited EGF-induced EGFR autophosphorylation and its downstream signaling cascades by 24 hours. Combining lovastatin and gefitinib showed enhanced inhibition and cooperative cytotoxicity in a variety of cell lines that included all eight squamous cell carcinomas, four non–small cell lung carcinoma, and four colon carcinoma cell lines tested. Isobologram analyses confirmed that this combination was synergistic, inducing a potent apoptotic response. A phase I study has shown the safety and potential clinical benefit of high-dose lovastatin in patients with recurrent squamous cell carcinoma. The use of lovastatin, which is metabolized by CYP3A4, is contraindicated with drugs, such as gefitinib and erlotinib, which are also metabolized by CYP3A4 due to greatly enhanced toxicity. Rosuvastatin, a relatively new potent mevalonate pathway inhibitor that is not metabolized significantly by CYP3A4, is a more appropriate statin to combine with either erlotinib or gefitinib. The combination of erlotinib and rosuvastatin has been proposed for a phase I/II study in advanced non–small cell lung carcinoma.

The ErbB family of receptor proteins [ErbB1/epidermal growth factor (EGF) receptor (EGFR), ErbB2, ErbB3, and ErbB4] plays a key role in the growth, differentiation, migration, and cell survival of epithelial tissues (1, 2). These cell membrane receptors are functionally divided into three regions: an extracellular ligand-binding region, an intracellular region with tyrosine kinase activity and regulatory functions, and a region that spans the cell membrane and anchors the receptor to the cell (3). In the inactive state, each ErbB receptor exists as a monomer. Despite their high degree of structural homology, the ErbB receptors differ in their ligand specificities. EGFR, ErbB3, and ErbB4 are activated by a large family of ligands, with each receptor having differences in its ligand specificity (4). ErbB2 has no ligand but preexists in a conformation that allows it to heterodimerize with other ErbB family members (4). These ErbB2-containing heterodimers form the highest affinity binding sites for their respective ligands (4). Ligand binding to the EGFR promotes either homodimerization (EGFR/EGFR) or heterodimerization (EGFR/ErbB2), activating (via autophosphorylation of specific residues) the receptor tyrosine kinase (5). This biochemical trigger starts a series of downstream signaling cascades that regulates the effects of these receptors on cell proliferation and cell survival. These effects are mediated by a complex series of signaling mechanisms, such as engagement of the mitogen-activated protein kinase and phosphatidylinositol 3-kinase pathways (1, 6).

ErbB2 as a Therapeutic Target

As stated, the EGFR is a key regulator of growth, differentiation, and survival of epithelial cells, but it is also involved in the development and progression of cancers derived from these tissues (1, 6). In malignant cells, this receptor and its downstream signaling pathways often are deregulated, leading to cell hyperproliferation, enhanced cell survival, and increased metastatic potential (1, 6). High EGFR expression has been associated with advanced tumor stage, with resistance to standard therapies (chemotherapy and radiation) and, in some tumors, with poor patient prognosis (7). Consequently, EGFR has been proposed as a rational target for antitumor strategies. More than 200 studies have analyzed relapse-free-interval or...
survival data directly in relation to EGFR levels in >20,000 patients (reviewed in ref. 7). The EGFR was found to act as a strong prognostic indicator in head and neck, cervical, esophageal, bladder, and ovarian cancers. In colorectal, gastric, breast, and endometrial cancers, the EGFR provided more modest prognostic information, whereas in non–small cell lung cancer (NSCLC), EGFR expression levels only rarely related to patient outcome (7). In addition to considering EGFR, the expression of EGFR heterodimerization partners may also provide valuable prognostic information. Although EGFR expression alone was not a significant predictor of survival for NSCLC patients, the expression of both ErbB2 and EGFR identified patients with a poorer prognosis (7).

A large body of experimental and clinical work thus supports the view that the EGFR is a relevant target for cancer therapy. Two therapeutic approaches have shown promise and are currently being evaluated in clinical studies: monoclonal antibodies and small-molecule inhibitors of the EGFR tyrosine kinase (1, 8, 9). Monoclonal antibodies are generally directed at the external domain of the EGFR to block ligand binding and receptor activation (1). The most clinically advanced EGFR-blocking antibody is cetuximab (Erbitux, IMC-C225; ref. 1). Tyrosine kinase inhibitors prevent the autophosphorylation of the intracellular tyrosine kinase domain of the EGFR (8, 9). These molecules are generally reversible competitors with ATP for binding to the intracellular catalytic domain of the tyrosine kinase. The most promising small-molecule EGFR tyrosine kinase inhibitors include gefitinib (Iressa, ZD1839) and erlotinib (Tarceva, OSI-774; refs. 8, 9). These EGFR inhibitors have shown efficacy in relevant preclinical models, such as human cancer cell lines and human tumors xenografted to immunodeficient mice in vitro (10). These EGFR tyrosine kinase inhibitors efficiently inhibit the autophosphorylation of multiple tyrosine residues in the kinase domain of the EGFR on ligand activation (10).

Several phase I and II clinical trials of gefitinib or erlotinib showed clinical activity of these agents in NSCLC patients (8, 9, 11). Phase III evaluations of combinations of gefitinib or erlotinib and chemotherapy in NSCLC patients failed to show increased response rates and survival when compared with chemotherapy alone (12–14). However, a trial comparing erlotinib to placebo in NSCLC patients who had failed previous chemotherapy showed a survival benefit with erlotinib treatment (15). EGFR inhibitors therefore seem very promising, but there are still a large number of questions about how to maximize their potential in cancer therapy. We have shown in preclinical studies that combining EGFR inhibitors with inhibitors of the mevalonate pathway may enhance their efficacy (16, 17). This review describes the rationale and potential of using these inhibitors in combination to treat cancer.

The Mevalonate Pathway

The mevalonate pathway produces various end products that are critical for functioning in both normal and cancerous cells. These products include cholesterol, dolichol, ubiquinone, isopentenyladenine, geranylgeranyl pyrophosphate, and farnesyl pyrophosphate (18). Cholesterol is essential in maintaining cellular membrane structure and integrity. It also serves as a precursor for the synthesis of steroid hormones and bile acid (18). Dolichol works as a carrier molecule of oligosaccharides in N-linked protein glycosylation for the production of glycoproteins (18). Ubiquinone is involved in mitochondrial respiration, whereas isopentenyladenine is an essential substrate for the modification of certain tRNAs (18). Geranylgeranyl transferase and farnesyl transferase use geranylgeranyl pyrophosphate and farnesyl pyrophosphate, respectively, for post-translational modifications of a wide variety of cellular proteins. These include Ras, nuclear lamins, and many small GTP-binding proteins, such as members of the Rab, Rac, and Rho families (19, 20). These proteins regulate cell proliferation, intracellular trafficking, and cell motility, and this post-translational modification functions as a membrane anchor critical for their activity (19, 20). The rate-limiting step of the mevalonate pathway is the conversion of 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) to mevalonate, which is catalyzed by HMG-CoA reductase (18). Blockade of this pathway by HMG-CoA reductase inhibitors results in decreased levels of mevalonate and its downstream products and, thus, may have significant influences on many critical cellular functions.

Malignant cells seem highly dependent on the sustained availability of the end products of the mevalonate pathway (21). Deregulated or elevated activity of HMG-CoA reductase has been shown in a range of different tumors (21). The statin family of drugs, widely used as hypercholesterolemia treatments, is a potent inhibitor of HMG-CoA reductase (22, 23). Statins are molecular mimics of the intermediate formed in the conversion of HMG-CoA to mevalonate. They bind to the active site of HMG-CoA reductase with up to a 1,000 times higher affinity than its natural substrate (22, 23). Recent analyses have shown that statin treatment can directly block tumor cell growth, invasion, and metastatic potential both in vitro and in vivo (21, 24, 25). Because HMG-CoA reductase inhibitors exhibit such diverse effects on various aspects of carcinogenesis, several clinical trials have been undertaken to assess potential clinical benefit.

At least two phase I clinical studies have evaluated the maximum tolerable dose and toxicity of lovastatin, the most widely studied and used statin, in advanced malignancies. Thibault et al. (26) conducted a phase I trial to characterize the safety and tolerability of lovastatin administered at progressively higher doses in 88 cancer patients with advanced solid tumors. Most patients had prostate cancer or central nervous system tumors. Peak drug concentrations of 0.1 to 3.9 μmol/L were reached; however, no significant antitumor responses were observed. Larner et al. (27) conducted a phase I/II trial of lovastatin in 18 patients with either anaplastic astrocytoma or glioblastoma multiforme. Similar to the study by Thibault et al., no significant antitumor responses were observed. About the clinical efficacy of other statins, Kawata et al. (28) reported encouraging results of a randomized trial of pravastatin in patients with advanced hepatocellular carcinoma. Pravastatin–treated patients showed significantly increased survival in combination with 5-fluorouracil than patients treated with 5-fluorouracil alone. In all three trials, statins were well tolerated and the main toxicity observed was rhabdomyolysis (muscle wasting) that resolved with discontinued use and ubiquinone supplementation. The results reported by Kawata et al. suggest the potential of statins in combined modality approaches.

The induction of programmed cell death or apoptosis is the major mechanism of tumor targeting by chemotherapy and
radiation-based therapies. Elevated and prolonged exposure of lovastatin can induce apoptosis. However, the concentration of lovastatin required to achieve apoptosis was between 30 and 100 μmol/L for various tumor-derived cell lines, including breast, colon, and prostate cancers (24, 25). Such high concentrations are not achievable in animal models or in the phase I clinical trials discussed. New optimism about the use of HMG-CoA reductase inhibitors as antineoplastic agents has emerged from recent studies. We identified HMG-CoA reductase as a biological mediator of the effects of retinoids (29). Retinoids are potent growth inhibitors and differentiation inducers of specific tumor types in vitro, including pediatric malignancies and myeloid leukemias as well as squamous cell carcinomas, including head and neck squamous cell carcinoma (HNSCC; ref. 29). Retinoids repressed the expression of HMG-CoA reductase in a variety of retinoid-responsive tumor-derived cell lines (29). We then showed that various retinoid-responsive cancers, including neuroblastoma, acute myelogenous leukemia, juvenile monomyelocytic leukemia, pediatric solid malignancies, and squamous cell carcinoma of the cervix and of the head and neck, are susceptible to lovastatin-induced apoptosis within therapeutically achievable levels (<5 μmol/L; refs. 29–31). Similar results were shown by others in medulloblastoma, mesothelioma, and glioblastoma (32, 33). These results rekindled the interest of HMG-CoA reductase inhibitors as anticancer compounds.

The identification of specific tumor types that are susceptible to lovastatin-induced apoptosis spurred further clinical evaluation by the group at the Princess Margaret Hospital (Toronto, Ontario, Canada). Based on the cytotoxic effects induced by lovastatin, clinical evaluation of lovastatin in a phase I trial in recurrent HNSCC and cervical carcinomas was undertaken. Although no tumor regressions were observed, 23% of patients exhibited stable disease longer than 3 months in a group of patients with rapidly progressive and resistant tumors, further supporting the feasibility of this approach (34).

**Open Discussion**

**Dr. Thomas Lynch:** As a clinician, I am thinking, here is Dr. Goss with the NCI Canada, sitting on an experiment that has already been done. There must be at least 100 or 200 people on the BR.21 trial who are on statins for cholesterol. Any chance you can go back and mine those data to figure out...
who is on statins and to see if there is any difference in therapy outcome with erlotinib?

Dr. Goss: We are going to do that. Within the NCIC, it is not always that simple. You have to apply for permission. As you know, there has been a New England Journal of Medicine publication [N Engl J Med. 2005;352:2184-92] looking at the incidence of colorectal cancer in a huge cohort of patients with cardiovascular disease, and there did seem to be a lower incidence of cancer than in the general population.

Dr. John Heymach: Regarding the mechanism for this, there are data about tyrosine kinase signaling from a number of different receptors being dependent on lipid rafts and lipid composition of the membrane to get effective signaling and the clustering of different molecules. This is something that doesn’t show up if you are just looking at protein levels or phosphorylation. Are there any efforts to look at whether the statin is exerting membrane effects like that on raft formation, perhaps by measurements of flow across the membranes?

**Figure 1.** A, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) analysis (cell viability assay) of 10 μmol/L lovastatin treatment for 72 hours, 10 μmol/L gefitinib for 48 hours, and pretreatment with lovastatin for 24 hours followed by the combination of agents for 48 hours. Cell lines examined include four HNSCCs, four cervical carcinomas, the epidermoid carcinoma cell line A431, and the breast adenocarcinoma MCF-7. Combination of treatments that displayed significant differences in MTT activity compared with either agent alone. *, P < 0.01, paired t test. B, isobologram analysis of the combination ofLovastatin and gefitinib. MTT50 (the concentration of agent that reduces MTT activity by 50%) values were determined for the SCC9 (HNSCC), SIHA (cervical carcinomas), and HCT116 (colon carcinoma) cell lines for 72-hour lovastatin and 48-hour gefitinib treatments. X axis, MTT50 values. The concentration of gefitinib that produced an MTT50 value in combination with 10 μmol/L lovastatin (24-hour pretreatment, 48 hours in combination) was identified and plotted. A combination index (CI) was determined based on the method of Chou et al. (44), where combination index = 1, additive; combination index > 1, antagonistic; and combination index < 1, synergistic. We also sequenced the EGFR ATP-binding domains in each of these three cell lines and no mutations were found. Figures were previously published in ref. (17).
Dr. Goss: Not specifically of flow across membranes, but Jim Dimitroulakos is working on the mechanisms, and his work has been published for publication.

Dr. Tim Eisen: Schering Plough did a study of carboplatin with or without SCH66336, which was a farnesyl transferase inhibitor. That was stopped for futility. Does that study have relevance to this, or do you think they were just inhibiting one of the possible mechanisms and there was an escape pathway?

Dr. Goss: It turns out that farnesylation is not as important as geranylation in the EGFR complex, so one would assume that it was just the wrong drug.

Dr. Jeffrey Settleman: I wonder a little about the concentration of lovastatin that you are using here. The IC50 is in the nanomolar range; you are using 50 micromolars. I wonder if you are hitting off-target.

Dr. Goss: The pharmacokinetic studies were done with lovastatin; we don’t know about rosuvastatin. We were well above the levels that inhibits lipid metabolism and within the therapeutic range. We could deliver enough drug to give us the ranges that we tested in the cell lines.

Dr. Settleman: So, in patients you can reach 50 micromolar?

Dr. Goss: Yes.

Dr. Lynch: Dr. Goss has discussed using mevalonate inhibitors and EGFR TKIs. Dr. Sandler’s presentation was on anti-VEGF agents plus EGFR TKIs. Are there other combinations that should be investigated? Dr. Bunn, if you could pick your first two that you want to see put together, what would they be?

Dr. Paul Bunn: Right now, I would have to say the anti-VEGFR with the small molecule TKIs because we know from Dr. Sandler’s trial that anti-angiogenic therapy can prolong survival. Down the road, EGFR TKIs need to be studied with signal transduction inhibitors. In vitro, we have some synergy with those of study.

Dr. Lynch: What about the mTOR inhibitors? Dr. Jänne, I know that Bruce Johnson is going to do a study with erlotinib and RAD001. Do you have thoughts on the potential there?

Dr. Pasi Jänne: The trial will initially be for patients with relapse from EGFR TKIs, so the question will be, is that combination going to have efficacy in this set of patients? We will wait to see.

Dr. Goss: Jose Baselga is doing a cetuximab plus EGFR-TKI study. There are some centers in the U.S. that are also looking at combining two EGFR inhibitors. Has anybody in the room seen any data from these studies?

Dr. Lynch: Your question brings up this concept of complete EGFR blockade, similar to the complete androgen blockade theory in prostate cancer. I know there are studies underway looking gefitinib/cetuximab, erlotinib/cetuximab, and erlotinib/pertuzumab, also looking at panitumumab, also combined with erlotinib. These are all in trial. I am not aware of any preliminary data.

Dr. Goss: People have been talking about trying higher doses of gefitinib. Did we get the dose wrong in the clinical trials? Should we have treated at 750 mg? Could we push the dose of erlotinib up? There are people doing high-dose erlotinib studies.

References


43. Mason RP, Walter MF, Day CA, Jacob RF. Inter-molecular differences of 3-hydroxy-3-methylglutaryl coenzyme a reductase inhibitors contribute to distinct pharmacologic and pleiotropic actions. Am J Cardiol 2005;96:11–23F.

Strategies to Enhance Epidermal Growth Factor Inhibition: Targeting the Mevalonate Pathway

Jim Dimitroulakos, Ian A. Lorimer and Glenwood Goss

*Clin Cancer Res* 2006;12:4426s-4431s.

**Updated version**
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/12/14/4426s

**Cited articles**
This article cites 44 articles, 17 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/12/14/4426s.full.html#ref-list-1

**Citing articles**
This article has been cited by 3 HighWire-hosted articles. Access the articles at:
/content/12/14/4426s.full.html#related-urls

**E-mail alerts**
Sign up to receive free email-alerts related to this article or journal.

**Reprints and Subscriptions**
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

**Permissions**
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.