Commentary on "Recombinant Humanized Anti-HER2 Antibody (Herceptin) Enhances the Antitumor Activity of Paclitaxel and Doxorubicin against HER2/*neu* Overexpressing Human Breast Cancer Xenografts" (A Follow Up)

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See related article by Baselga et al., Cancer Res 1998;58:2825–31.

It is an honor to have our publication reporting on combined therapy with a targeted antibody plus chemotherapy selected as a classic article from *Cancer Research*. For our research team at the University of California San Diego (San Diego, CA), this area of investigation began in 1980–1983, when we performed our studies reporting the production of mAbs against the EGF receptor, which blocked ligand binding, downregulated the receptor, prevented activation of the receptor tyrosine kinase, and inhibited the proliferation of tumor cells in culture and in xenografts (1–3). These reports were the first to demonstrate that a tyrosine kinase was a valid potential target for anticancer therapy. At the time, only three tyrosine kinases had been described (the EGF and PDGF receptors and C-SRC). The fact that C-SRC was the product of a known oncogene, the observation that EGF receptors were overexpressed on many cancers, and the evidence for autocrine stimulation of EGF receptors were critical in our decision to pursue this project. Our hypothesis that blocking ligand binding to EGF receptors might inhibit tyrosine kinase activation and cell proliferation was novel and unusual enough that, without any preliminary data, our grant proposal to the NCI was rejected. Fortunately, we found the needed funds, and after our first publication appeared, the project was well funded by grants.

In 1985, the University of California San Diego licensed our mAb 225 against the EGF receptor to Hybritech, the first start-up biotech company in San Diego, which began the necessary scale-up and preparations for regulatory approval to investigate the antibody in the clinic. The use of mAbs for therapy was not at all an accepted approach to cancer treatment at the time, although the laboratories of Ron Levy and Lee Nadler had reported clinical studies with antibodies against cell surface markers on malignant lymphocytes in the early 1980s.

In 1985 and 1989, antibodies were produced to the closely related HER-2 receptor, which blocked growth of tumor cells bearing high levels of these receptors (4, 5). I visited Genentech in the late 1980s to compare the preclinical data, justifying our plans to pursue a clinical trial with our anti-EGF receptor mAb 225 and their plans to move the recombinant human version of their anti-HER2 mAb 4D5 into the clinic. Our approaches were comparable, and the laboratory research results reinforced the validity of moving both projects forward.

In 1991, we published the results of our initial phase I clinical trial sponsored by Hybritech, the first clinical study with an anticancer agent targeting a signaling receptor and the first targeting a tyrosine kinase (6). We demonstrated the safety of this approach, which was critical, and by labeling murine mAb 225 with trace amounts of iridium^{III}, we were able to show preferential localization in squamous lung carcinomas and their metastases identified by CT scan. Our pharmacologic studies demonstrated that the concentrations of mAbs in the blood reached levels adequate to saturate EGF receptors. Two weeks after treatment, each patient developed human antibodies against the murine mAb, without adverse clinical sequelae. The NCI arranged for the conversion of murine IgG1 mAb 225 into a human:murine IgG2a chimera, called C225 (Chimeric 225) or cetuximab (trade name, Erbitux), which retained all properties of the original murine mAb.

A major concern was that in culture, cetuximab was only cytotoxic against a single colon adenocarcinoma cell line, DiFi, for which EGF receptor stimulation was an auxotrophic event. For other cell lines (all bearing EGF receptors), treatment of cultures with the mAb produced cell-cycle arrest in G_1 phase, or in some cases, only a modest retardation in cell proliferation. Likewise, tumor xenografts typically showed retardation of growth and prolonged survival of the mice, but, except for DiFi, tumors were not eliminated in most experiments. Therefore, we were pleased to see a 1988 publication from the laboratory of Michael Sela that reported experiments demonstrating additive inhibition of tumor xenograft growth by combination treatment with their anti-EGF receptor mAb and a chemotherapeutic agent, cisplatinum. We immediately began to explore combinations of our anti-EGF receptor mAbs with doxorubicin (7), cisplatinum, and paclitaxel, with excellent results against tumor xenografts. We observed elimination of the tumor in a substantial fraction of the mice. These results were published in 1993 and 1994 and formed the basis of subsequent clinical trials with cetuximab carried out by ImClone, which obtained the license from the University of California. However, there were major delays in moving these trials forward. Phase I trials with cetuximab began in 1996 and the results were published in 2000 (8).

Meanwhile, Genentech was pushing forward with trials of their human version of mAb 4D5 against the HER-2 receptor, trastuzumab (trade name, Herceptin). We performed the first phase II trial with trastuzumab and demonstrated a 11.6% response rate in patients with metastatic breast cancer previously treated with

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chemotherapy (9). There were additional minor responses and disease stabilization in 37.2%, with a median time to progression of 5.1 months. Slamon and colleagues subsequently reported a phase II study of patients with advanced breast cancer treated with trastuzumab plus cisplatinum, showing a 24.3% response rate, more than double that which was observed with mAb alone (10). On the basis of these and other confirmatory trials, Genentech decided to move forward with a phase III registration trial of trastuzumab. The trial would involve first-line therapy for patients with metastatic breast cancer, treated with trastuzumab plus optimal chemotherapy compared with chemotherapy alone. The critical question was which chemotherapy to employ? While clinical trial data from Slamon and colleagues' study suggested the efficacy of combining trastuzumab with cisplatinum, it was our feeling that doxorubicin or paclitaxel would be better partners with trastuzumab, because they had greater antitumor activity than cisplatinum against breast cancer. Therefore, we performed preclinical experiments assessing the efficacy of trastuzumab plus doxorubicin or paclitaxel, compared with antibody or chemotherapy alone, against BT-474 HER-2–overexpressing breast cancer xenografts. The results showed a striking efficacy of both combinations, compared with either therapy alone. The results were published in 1998 in the article (11) that is the subject of this commentary. Combination therapy eliminated the tumor xenografts in 59% of mice treated with paclitaxel plus mAb, and in 33% treated with doxorubicin plus mAB, both highly significant improvements over treatment with chemotherapy or mAb alone. The phase III randomized clinical trial was launched by Genentech with two arms, one with trastuzumab plus doxorubicin and cyclophosphamide, and the other (for those who had been exposed to doxorubicin during previous adjuvant chemotherapy) with trastuzumab plus paclitaxel. In each case, a comparison was made with chemotherapy alone. The results of this trial, published by Slamon and Larry Norton with many colleagues, were positive for the combination therapies in both arms and resulted in regulatory approval of trastuzumab for this large group of patients (10). Subsequently, in a number of trials in patients with early breast cancer overexpressing HER-2, adjuvant administration of paclitaxel and trastuzumab-containing regimens resulted in major improvements in survival. These were pivotal trials, demonstrating efficacy of a gene-targeted therapy in patients with breast cancer.

The initial phase III trial was pivotal for another reason: it was the first clinical trial with a new targeted therapy in which a positive biomarker was a criterion for entry. The phase I and II clinical trials with trastuzumab had shown that only patients whose breast cancers expressed high levels of HER-2 responded to the mAb therapy, and only about 25% of breast cancers were found to express high levels. Furthermore, among these patients with high HER-2 expression, only one-third responded to the mAb therapy. Looking at the results of the initial phase III trial retrospectively, it is clear that if Genentech had not excluded low HER-2 expressers from enrolling, the positive response rate would have been less than 10% and it is likely that the therapy would not have received regulatory approval.

More than two decades later, the use of biomarkers in clinical trials with new targeted therapies has become standard of practice, when available, enabling the rapid regulatory approval of a number of mAbs that target, for example, the products of *ALK-EML4* rearrangements and mutated *B-RAF*. The case of crizotinib therapy for non–small cell lung cancers with fusions of the *ALK* gene emphasizes the importance of this principle. *ALK* rearrangements are found in only 4%–5% of lung cancers. If prescreening of a very large number of patients for the presence of the biomarker had not been a precondition for trial participation, the response rate would have been well under 5% and the drug would not likely have received regulatory approval.

While there are over 800 new targeted therapies in clinical development today, the biomarkers for determining their use are, in most cases, the demonstration of the aberrant gene whose product the new agent is targeting. It turns out that, unlike the situation with *BCR-ABL*, mutated *EGF* receptor genes, and the *ALK* rearrangement, this is usually not enough to accurately predict a substantial response rate for a targeted agent. Furthermore, most cancers, chronic myelogenous leukemia being the exception, are not driven by the abnormal functioning of just a single gene. There are two approaches to dealing with this challenge. One is to combine therapies against two or more different targets. Today, it is essential to more systematically analyze combination therapy with targeted agents, not only combinations with other targeted therapies, but also with conventional chemotherapies, immunotherapies, and radiation, with careful attention to optimal timing and dosing of multiple agents with different mechanisms of action and toxicities. The rationales and algorithms for selecting combined therapies are not adequate today, even with the use of systems biology algorithms to identify potentially interacting targets. Analysis of gene expression data in the cancer holds promise. Another approach is to invest far more energy and resources into developing functional murine models with single and multiple genetic aberrations, which can then be interrogated with multiple "omic" assays and therapies. Both approaches depend upon the heavy use of bioinformatics and computational analysis. Today, we have the techniques and analytic tools to accomplish these approaches and advance the efficacy of cancer therapy. It will entail a different kind of research, requiring far more interdisciplinary collaboration and real sharing of data. I believe that the biomedical research communities in academia, industry, and the government are ready to join in this effort.

Disclosure of Potential Conflicts of Interest

J. Mendelsohn has license income from the University of California and is a consultant/advisory board member for Merrimack Pharmacuticials, ZioPharm, and MedImmune.

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