Growth and Molecular Interactions between Tamoxifen and Trastuzumab

To the editor: In the February 15, 2004 issue of Clinical Cancer Research, Argiris et al. (1) have presented some studies of the interaction between tamoxifen and trastuzumab on breast cancer cells. They evaluated cell growth, cell cycle, human epidermal growth factor receptor 2 (HER2) expression, and HER2 phosphorylation levels. Their article concludes that there is a synergism between tamoxifen and trastuzumab, which occurs without any effect on HER2/neu levels or signaling activity.

We have recently analyzed in detail the interactions between tamoxifen and trastuzumab on the breast cancer cell lines BT-474 and T47D, which are HER2 positive and hormone receptor positive (2). We believe that the experimental details of the study of Argiris et al. deserve some discussion; these include the interaction analytic approach, the drug administration schedule, and the molecular analysis.

It is important that preclinical studies of drug combinations present in full detail the interaction analytic approach (3). Argiris et al. analyzed the cell growth interactions of tamoxifen and trastuzumab using the Chou-Talalay method, although only in part. This is a well-known test that evaluates the coefficient interactions against a range of cell death proportions. The Chou-Talalay method requires that a constant molar ratio of the drug analyzed is set (4). Argiris et al. show the interactions result in 80% to 90% of cell fractions being affected, using four concentrations of trastuzumab and five concentrations of tamoxifen. None of these combinations seems to maintain a constant molar ratio. Due to this, no graphical representation of the interaction can be shown by the authors. Although the software program that they (and we) have used can actually calculate an individual combination index for a particular cell fraction affected, it warns that the classic isobologram cannot be constructed for the nonconstant ratio. To obtain an appropriate evaluation of two-drug interactions, a graph with all fractions is always preferred.

It is agreed that when researchers do not use a constant molar ratio, the analytic test of Berenbaum (5) is preferred. We have analyzed the interactions of tamoxifen and trastuzumab using constant and nonconstant molar ratios employing the Chou-Talalay and the Berenbaum methods, respectively. Using the Chou-Talalay test, we have reported that at the higher proportions of cell kill (e.g., 80%), an additive-synergistic effect is seen. At all other fractions, additive or frankly antagonistic effects are observed. When we used the Berenbaum method to analyze the interaction of tamoxifen and trastuzumab, a clear pattern of antagonism emerged, highlighting the need for a complete analytic evaluation and presentation of the data.

Argiris et al. tested the simultaneous use of tamoxifen and trastuzumab. However, no analysis of the sequential schedules of exposure was done. This is a very important issue because there are schedule-dependent interactions reported (6, 7). We have tested three different drug administration schedules of tamoxifen and trastuzumab in our analysis of the interaction of these two drugs. Our results show that the nature of the interaction changed among the different administration schedules. Whereas trastuzumab followed by tamoxifen showed antagonism at all effect levels, the effects of the other two schedules of exposure were additive or antagonistic.

A third point to discuss is the molecular mechanisms of the interactions between tamoxifen and trastuzumab. The molecular approach done by Argiris et al. tested the changes on the levels of expression and phosphorylation state of HER2, as well as the induction of apoptosis by the combined treatments. Unfortunately, no analysis was done to test the effects of tamoxifen and trastuzumab on the levels of expression of the estrogen receptor, the molecular target involved in the mechanism of action of tamoxifen (8). In our article, we tested at protein and mRNA levels the effects of the combination of tamoxifen and trastuzumab on both estrogen receptor and HER2/neu proteins, providing a possible mechanism to explain the nature of the interaction of these two drugs on breast cancer cell lines.

Finally, because the experiments of Argiris and ours used the same cell line (BT-474), it might be important to know whether there are differences between the BT-474 cancer cell lines used. In this sense, differences in the levels of expression of estrogen receptor and HER2 protein or in the relationship between its signaling pathways may affect the observed results and it might be interesting to know the level of expression of the estrogen receptor.

Given these considerations, we believe that, although the evaluation of Argiris et al. is to be praised, a more accurate analysis of the interaction of tamoxifen and trastuzumab on breast cancer cells is necessary to give a full view of the combination results.

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In Response: Ropero et al. (1) conducted an evaluation of the combination of Herceptin and tamoxifen with analyses of drug interaction, the conclusions of which partially contradict our findings previously reported in this journal (3). Similar to

our results, they found the drug combination to give a greater growth-inhibitory effect than either agent alone using breast cancer cells in culture. However, using similar pharmacologic analyses of drug interactions, we found the combination to give synergistic growth inhibition, whereas Ropero et al. found the combination to have an antagonistic interaction at doses corresponding to the IC_{30}. Both studies used estrogen receptor–positive, HER2-overexpressing BT-474 human breast carcinoma cells, and it seems that biological differences in the cells used, as well as differences in some of the experimental details, likely account for the disparate outcomes of drug interaction analysis.

Vázquez-Martín et al. comment on our modeling of drug interactions. In analyzing our results, we employed the widely used Chou and Talalay method (4, 5) using commercially available software for the analysis (6). For this method to be applicable, each drug should show a dose-effect relationship, at least three data points are required for each single drug effect, and the linear correlation coefficient should be >0.90; in our study, all of these prerequisites were met. As noted, in our published study, we did not employ constant ratios of drugs in the combinations tested. Vázquez-Martín et al. are correct in stating that the method requires a constant molar ratio of drug combinations in order to graphically display certain relationships, in particular, the median-effect plot. However, the software program is nonetheless able to calculate the combination index when random ratios of drugs are employed, as in our published study, and this does not compromise the interpretation of the drug interaction or the validity of the conclusion (6).

In our study, we tested the dose-effect of the combination using various Herceptin concentrations ranging from 0.375 to 10 μg/mL, and tamoxifen from 0.5 to 8 μmol/L, and found that the combination index was <0.3 in all cases, signifying strong synergy. The concentrations employed are clinically relevant. In addition, subsequent to the publication of our results, we performed additional analyses using constant ratios (1 μg/mL Herceptin:1 μmol/L tamoxifen).[1] We used concentrations of Herceptin ranging from 0.2 to 10 μg/mL, giving effect levels (fraction affected) from 0.27 to 0.68, and tamoxifen from 0.2 to 10 μmol/L, giving effect levels from 0.20 to 0.64. The combination effects ranged from 0.39 to 0.90. These studies continued to indicate a synergistic interaction between the two drugs at all but the lowest combination tested (combination effect, 0.39, which was antagonistic). Vázquez-Martín et al. state in their correspondence that using the Chou-Talalay method, they do note additive-synergistic effects at higher proportions of cell kill, although additive to antagonistic interactions at lower levels.

What experimental details could contribute to the differences in the outcomes? In our work, insulin at 10 μg/mL was added to the culture medium, as suggested by the American Type Culture Collection, whereas Ropero et al. did not supplement with insulin. Because of the importance of insulin-like growth factor-1 and insulin signaling on properties being evaluated, and their potential interaction with HER2 and estrogen receptor signaling, this could influence the experimental results. Ropero et al. also analyzed proliferation at 72 hours; because BT-474 cells have a relatively slow doubling time, we prefer to analyze effects on proliferation at a later time point (5-6 days).

Most notably, Ropero et al. used relatively high doses of Herceptin (10-100 μg/mL) and tamoxifen (5-10 μmol/L), as the cells under their conditions were relatively resistant to the effects of these drugs, with an IC_{30} of 34 μg/mL for Herceptin (and "not dose-dependent" having a plateau effect at 10 μg/mL) and approximately 7 μmol/L for tamoxifen. In contrast, we noted IC_{50}'s of 0.4 μg/mL and 1 μmol/L, respectively. The individual dose-effect relationships noted in our work are consistent with literature reports (cited in our manuscript). Combining 10 μg/mL Herceptin with 7 μmol/L tamoxifen gave only 45% inhibition in the Ropero et al. studies, and this effect was antagonistic in their models. In contrast, using much lower drug concentrations that gave much greater degrees of growth inhibition, we noted strong synergy. It may be the inability to achieve greater effect levels that lead to the finding of an antagonistic interaction in the Ropero et al. study because we also noted antagonism at the lowest combination effect levels (above).

Ropero et al. have also evaluated the sequence of drug administration, and intriguingly found sequence-dependent effects in the analysis of interaction. Whereas sequencing of drugs, particularly cytotoxics or cytotoxic/targeted therapy combinations, could have dramatic effects on outcome, we chose only to analyze concurrent exposures because in patients treated with these drugs, continuous therapeutic drug levels are achieved.

We agree that what is most interesting from a biological view is the analysis of downstream signaling from the pertinent receptors when this drug combination is used. Ropero et al. noted an increase in HER2 mRNA (although with minor effects on protein levels), and surprisingly, an increase in activated/phosphorylated HER2 induced by either drug or the combination. In contrast, we found no effect on HER2 expression but a decrease in activated (phosphorylated) HER2 in response to Herceptin, with no additional effect of tamoxifen. Our results are consistent with others (cited in our manuscript), although some differences have been reported among other laboratories. At the time of our previous publication, we had not analyzed the effect of this drug combination on the expression or activity of estrogen receptor, or on signaling pathways downstream from HER2. Such analyses are the subject of a manuscript that is currently in press (2). We do confirm that our BT-474 cell line expresses readily detectable levels of estrogen receptor protein by immunoblotting (2).

Vázquez-Martín et al. call attention to important influences on the analysis of drug interactions that must be appreciated in conducting and interpreting such research. The differences in the effects of drugs on the BT-474 cells among the two laboratories may be due to differences in cell culture conditions, changes in the BT-474 cell lines themselves, or other experimental variables. Even with uniform experimental conditions, conclusions regarding drug interactions may differ depending on the level of effect, the ratio chosen for the two drugs, and the parameter being assayed. Our collective data and that of many others lead us to eagerly await the results of clinical trials of HER family–targeted therapeutics in combination with hormonal agents.

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[1] Koay et al., unpublished data.

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