Several recent studies, including that of Magnifico and coworkers (1) in this issue of Clinical Cancer Research, suggest that the remarkable clinical efficacy of trastuzumab may relate to its ability to target breast cancer stem cells. These studies have important clinical implications for patient selection as well as for the development of new therapeutic strategies to target the cancer stem cell population.

The HER2 gene is amplified in approximately 20% of human breast cancers in which it is associated with aggressive disease and early development of metastasis (2). One of the greatest advancements in the treatment of breast cancer has been the development of trastuzumab to target HER2-positive disease. When added to chemotherapy, trastuzumab significantly increases both disease-free survival as well as overall survival in women with metastatic breast cancer. The clinical utility of trastuzumab has been even more remarkable in the adjuvant setting. The addition of trastuzumab to cytotoxic chemotherapy has reduced the recurrence rate by ~50% in women whose breast cancers display HER2 amplification. Furthermore, the natural history of HER2-amplified breast cancer as well as the flattening survival curves suggest that a considerable proportion of these patients may be cured of their disease. Although it is clear that HER2 plays an important role in breast tumorigenesis, the molecular mechanisms which account for the clinical benefit of HER2 inhibition remain unknown.

There is emerging evidence that many tumors, including breast cancer, may be initiated and maintained by a subpopulation of cells that maintain or acquire stem cell characteristics (3). These tumor stem cells may also contribute to tumor metastasis as well as treatment resistance. Indeed, both in vitro and mouse models have provided evidence for the relative resistance of breast cancer stem cells to chemotherapy and radiation therapy (4, 5). Recent neoadjuvant chemotherapy trials showing an increase in cells expressing stem cell markers following neoadjuvant chemotherapy support this concept (6).

Magnifico and colleagues used several HER2-overexpressing breast cancer cell lines to show an important role for HER2 in maintaining the cancer stem cell population. They showed that within each cell line, cells displaying stem cell properties such as sphere formation (1) or increased aldehyde dehydrogenase expression also have increased HER2 expression compared with the bulk cell population (1). This suggests that in addition to genetic alterations such as amplification, expression of HER2 is also regulated by epigenetic factors independent of gene amplification. Furthermore, they show that the HER2 inhibitor trastuzumab or the combined HER2 and epidermal growth factor inhibitor lapatinib are able to specifically target this HER2-overexpressing cancer stem cell population (1). This was shown in vitro as well as by transplantation into nude mice. These studies are consistent with and extend our recent report that HER2 amplification increases the cancer stem cell population driving tumorigenesis as well as invasion and metastasis (7).

It has previously been shown that the Notch pathway plays an important role in the function of normal and malignant breast cancer stem cells (8). A relationship between Notch and HER2 signaling has been suggested by the demonstration that the HER2 promoter contains Notch-binding sequences (9). Magnifico and coworkers (1) further demonstrate a relationship between Notch and HER2 expression by showing that HER2-overexpressing cells display activated Notch signaling. In contrast, inhibition of Notch signaling using a small interfering RNA or a γ-secretase inhibitor results in down-regulation of HER2 expression resulting in decreased sphere formation. These studies show important interactions between the Notch and HER2 pathways, both of which are involved in the regulation of cancer stem cells. The interaction of these pathways is illustrated in Fig. 1.

The study of Magnifico and colleagues (1) and others relating to the regulation of breast cancer stem cells have important clinical implications. These studies provide a biological explanation for the observation that lapatinib was able to reduce the cancer stem cell population following neoadjuvant chemotherapy (6). An important question raised by these studies is the clinical significance...
of HER2 overexpression in the absence of gene amplification. Tumors with this phenotype may be heterogeneous for HER2 protein expression as detected by immunohistochemistry (10). More importantly, the ability of HER2 targeting agents such as trastuzumab to target cancer stem cells in tumors without HER2 amplification suggests that such agents could potentially have clinical benefit in patients with breast cancer with non–HER2-amplified tumors. Indeed, clinical discrepancies in the utility of HER2 inhibitors when used in the metastatic or adjuvant setting may reflect targeting of the cancer stem cell versus bulk cell populations. There is evidence in a number of studies that clinical benefit from trastuzumab or lapatinib is directly related to the levels of HER2 expression and these benefits are found primarily in women with HER2-amplified tumors (11). These studies, which combine chemotherapy with HER2 inhibition, usually measure tumor shrinkage as assessed by Response Evaluation Criteria in Solid Tumors (RECIST) guidelines or relapse-free survival. These results have led to the widespread use of HER2 testing with the use of immunohistochemistry and fluorescence in situ hybridization to select patients for anti-HER2 therapy. In the adjuvant setting, the use of trastuzumab and lapatinib has largely been limited to women whose tumors display HER2 amplification. Recent reports, however, suggest that women with HER2-negative breast cancer who did not meet established criteria for HER2-positive disease on central review received as much clinical benefit from adjuvant trastuzumab as those women with HER2-amplified tumors (6). Although these discrepancies have been attributed to HER2 testing errors (11), consideration of the role of HER2 in regulating breast cancer stem cells provides an alternative biological explanation. In metastatic disease, the clinical end points of tumor regression or time to tumor progression may reflect changes in bulk cell populations. The efficacy of trastuzumab or lapatinib in this setting may reflect the overexpression of HER2 in both cancer stem cells and bulk cell populations. In contrast, in the adjuvant setting, tumor recurrence may be driven by the cancer stem cell compartment. This compartment in turn may be driven by pathways such as Notch that do not depend on HER2 amplification. This could explain the benefit of HER2 inhibition in the adjuvant setting in patients whose tumors do not display HER2 amplification. It would be interesting to determine whether these tumors display Notch activation, which has been reported to occur in as many as 40% of human breast cancers (12). In these patients, inhibition of Notch signaling in addition to HER2 blockade represents a rational therapeutic strategy. These concepts may be tested in future trials as γ-secretase inhibitors that inhibit Notch signaling are currently in clinical development. These and other strategies designed to target cancer stem cells will determine whether the elimination of these cells improves patient outcomes.

Disclosure of Potential Conflicts of Interest

M.S. Wicha holds equity in and is a consultant for OncoMed Pharmaceuticals.
References


