Her 2 in 1

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DOI 10.1016/j.ccr.2010.07.009

In this issue of *Cancer Cell*, Park et al. demonstrate that anti-erbB2 antibody primes adaptive immunity for increased tumor clearance and suggest that chemotherapy may in fact interfere with this process.

HER2 (also known as ErbB2 or neu) activates several pr-survival signaling pathways and is overexpressed in approximately 20%–30% of invasive breast cancers. Breast cancer patients who have $HER2^+$ breast cancer have a decreased overall survival compare to those who do not. Trastuzumab (Herceptin) is a humanized monoclonal antibody (mAb) that binds the extracellular juxtamembrane domain of HER2 and has proved to be an effective treatment of $HER2^+$ breast cancer. Blockade of HER2 oncogenic signaling and chemosensitization have been proposed as the central mechanisms responsible for the initial clinical response of $HER2^+$ tumors treated with Trastuzumab plus chemotherapy (Hudis, 2007; Moasser, 2007). Trastuzumab is also believed to mediate some of its therapeutic effect through the recruitment of Fc receptor (FcR)-expressing immune cells such as monocytes and natural killer (NK) cells (Clynes et al., 2000; Musolino et al., 2008).

Correlative evidence has additionally supported that antibody-dependent cellular cytotoxicity (ADCC) may play a major role in the anti-tumor effects of monoclonal antibodies such as Trastuzumab and Rituximab. Consistently, an increase of tumor-infiltrating leukocytes (TIL), especially FcR^+ cells such as NK cells, has been observed in tumor tissue after mAb treatment (Arnould et al., 2006; Varchetta et al., 2007), and patients responding to mAb treatment had higher in situ infiltration of leukocytes and an increased capacity to mediate in vitro ADCC activity (Gennari et al., 2004). While innate immune responses have been suggested to be instrumental to mAb cancer therapies, xenograft models used for preclinical evaluation of mAbs traditionally failed to account for adaptive immunity. Several ErbB2 transgenic mouse mammary tumor models have been developed based on expression of oncogenic rat ErbB2. Importantly, tumors can be harvested and transplanted back into immunocompetent hosts for therapeutic analysis.

Park et al. now extend previous findings in mice to demonstrate that the mechanisms of tumor regression by anti-ErbB2 mAb therapy also require host danger signals, Toll-like receptor signaling, and an adaptive immune response (Park et al., 2010). They showed that the mouse anti-rat ErbB2 mAb 7.16.4 required host FcR and, at least in part, HMGB-1, MyD88 signaling, $CD8\alpha^+$, cells and adaptive (RAG-dependent) immunity to mediate its optimal effect. HMGB-1 is an abundant nuclear DNA binding protein that acts as an immunogenic stimulator when released in the extracellular milieu by necrotic and inflammatory cells. Extracellular HMGB-1 binds its receptor RAGE on antigen-presenting cells (APC) and may signal the neighboring cells for tissue damage. Their data together suggested a pathway whereby FcR-dependent ADCC and/or ErbB2 signaling blockade causes tumor cell death and the release of danger signals, such as HMGB-1, that trigger an innate MyD88-dependent pathway in APC (Figure 1). Park et al. postulate that APC might also use FcR to internalize antigens based on the finding from several previous studies showing that anti-tumor antibody treatment enhances cross-priming of $CD8^+$ T cells through FcR-mediated phagocytosis and immune complex formation (Dhodapkar et al., 2002). However, the Park et al. study did not enumerate tumor-specific T cells or use an antigen or more specific deficiencies of $CD8\alpha^+$ DC or $CD8\alpha\beta^+$ T cells to fully explore the role of cross-priming in the generation of T cells specific to the tumor. It is also unclear what role ADCC plays and what might be the key signals from cells attacked in the initial assault that primes adaptive immunity. Although Park et al. show an increase in the tumor infiltration of $CD8^+$ cells and interferon-γ in lymphoid tissue of mice treated with anti-ErbB2 mAb, it remains unclear whether these events are linked and what the immune effector mechanisms that reduce the growth of the initial and rechallenged tumors are. These findings in mice were supported by increased TIL detected in a small number of $HER2^+$ breast cancer patients after treatment with chemotherapy plus Trastuzumab, but not in $HER2^-$ breast cancer patients treated with chemotherapy alone. However, further supporting clinical data are needed. The appropriate comparison here would have been among all $HER2^+$ breast cancers treated with or without Trastuzumab because the main issue here relates to Trastuzumab treatment, not $HER2^+$ versus $HER2^-$ breast cancer. Furthermore, $ER^+/HER2^-$ tumors are less likely to have TILs pre- and post-therapy. Since few, if any, patients are treated with Trastuzumab alone, the definitive role of adaptive immunity in the anticancer activity of Trastuzumab and similar mAbs in humans will require specialized controlled trials.

It has long been suspected that chemotherapies may be immunosuppressive, but recently it has been shown that some chemotherapies can kill tumor cells in a manner that activates the NLRP3 inflammasome in APC, which primes tumor-specific adaptive immunity via IL-1β (Ghiringhelli et al., 2009). Park et al. describe results from mouse experiments that suggest that paclitaxel or cyclophosphamide administered shortly after anti-ErbB2 mAb may dramatically interfere with the tumor-specific memory generated by anti-ErbB2 mAbs (Figure 1). Collectively, this work makes it very clear

that the dose, timing, and effects of all first line therapeutics that directly or indirectly prime tumor-specific immunity need careful attention. The issue of what approach to take with trastuzumab refractory disease is complicated, however, so caution should be taken in not overinterpreting these findings. The data from Park et al. does not explain why Trastuzumab in combination with chemotherapy is active even after progression on Trastuzumab. Regardless, chemotherapy scheduling could have an impact in some people treated with Trastuzumab, but to optimize the clinical efficacy would require some major rethinking of clinical trial design in these patients. Clearly, TILs are an independent predictor of response to neoadjuvant chemotherapy in breast cancer (Denkert et al., 2010), so more large studies of effective breast cancer therapies need to more rigorously assess the importance of immune molecules and pathways in therapeutic outcomes.

The current work also raises the interesting possibility that alternative combination strategies may be used to capitalize on the adaptive tumor-specific immunity generated by anti-ErbB2 mAbs. Using intratumoral delivery of an adenovirus-LIGHT construct that reportedly increases adhesion and attraction of TIL, Park et al. observed dramatically improved anti-ErbB2 anti-tumor activity, but no clear mechanism studies were presented to support how best to use the strategy. Nevertheless, immunotherapeutic approaches in late-phase clinical trials that enhance $CD8^+$ T cell anti-tumor activity, such as anti-CTLA4 or anti-PD1 mAb, or a cancer vaccine, might be interesting in combination with Trastuzumab in these mouse models and eventually in the clinic. Although ErbB2-targeted therapy using Trastuzumab constitutes a major advance in the treatment of $ErbB2^+$ breast cancer, there remains an unmet medical need for patients who are on adjuvant Trastuzumab but develop metastatic disease. The Park et al. study illustrates one avenue to improve outcomes and importantly cautions rational combination therapies that are only based on the intrinsic activity of these agents upon the tumor and not the host immunity.

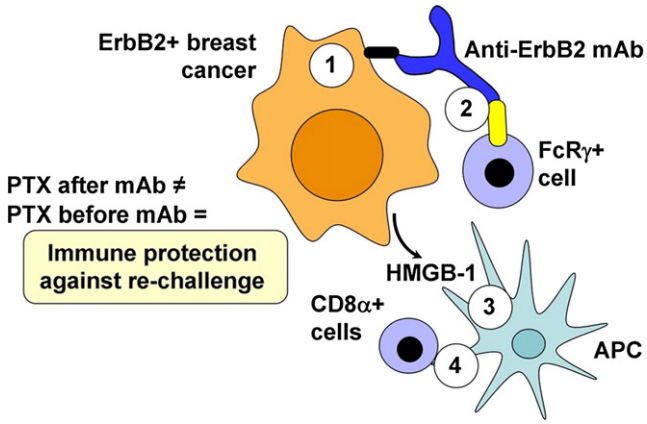

Figure 1. A Revised Mechanism of Action for Trastuzumab
The study by Park et al. proposes that tumor cell death induced by anti-ErbB2 monoclonal antibody (mAb) therapy causes the release of danger signals such as HMGB-1, triggers MyD88-dependent activation of antigen-presenting cells (APC), and generates CD8α-dependent adaptive anti-tumor immunity. Park et al. (2010) observed that paclitaxel (PTX) administration after anti-ErbB2 mAb abrogated secondary immune responses to tumor, while it preserved immune protection if administered 24 hr before anti-ErbB2 mAb. (1), Blocking of oncogenic signals; (2), FcRγ-dependent activation of innate immune cells; (3), HMGB-1-dependent and MyD88-dependent activation of APC; (4), Activation of CD8α-dependent adaptive anti-tumor immunity.

ACKNOWLEDGMENTS

These authors acknowledge funding support from the National Health and Medical Research Council of Australia, the Susan G. Komen Breast Cancer Foundation, and the Victorian Breast Cancer Research Consortium.

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