In this issue of Clinical Cancer Research, Francia and colleagues explore pulsatile, maximally tolerated doses of cyclophosphamide chemotherapy or the same drug using a daily low-dose schedule combined with the human epidermal growth factor receptor 2 (HER2)-targeted antibody trastuzumab in a preclinical model of HER2-positive breast cancer (1). One of the most notable developments in breast cancer translational research has been the identification of the HER2-positive subset of human breast cancers and the subsequent successful development of HER2-targeted therapies. HER2 is a member of the epidermal growth factor receptor (EGFR) family of transmembrane glycoprotein receptor tyrosine kinases, which mediate cell growth, differentiation, and survival through complex, interconnected, and incompletely understood, signal transduction pathways (2). Amplification of HER2 or overexpression of its protein product is observed in approximately 20% of human breast cancers and has historically been associated with a poor prognosis (3). However, the negative prognostic impact of HER2 gene amplification and/or protein overexpression has largely been ameliorated by the development of HER2-targeted therapy. The first such agent was trastuzumab, a humanized, HER2-targeted monoclonal antibody, and it provides significant efficacy benefits in both the metastatic (4) and adjuvant settings (5, 6). However, a significant proportion of women with HER2-positive early stage breast cancer still experience distant relapses despite adjuvant trastuzumab therapy and the majority of patients with metastatic disease experience time-limited benefits (4–6). Furthermore, the majority of HER2-positive metastatic breast cancers are resistant to trastuzumab when used as a single agent (2). It is as yet poorly understood why more than 50% of HER2-positive metastatic breast cancers exhibit this intrinsic resistance to HER2-targeted monotherapy with trastuzumab and why tumors that initially exhibit sensitivity ultimately acquire apparent clinical resistance. On the other hand, it is difficult to explain why trastuzumab in combination with chemotherapy is active even after progression on trastuzumab (7). It is perhaps easier to explain activity in this treatment-refractory setting for a drug with a different mechanism of anti-HER2 activity, such as lapatinib, a HER1- and HER2-directed tyrosine kinase inhibitor (TKI), (the only other HER2-targeted agent approved by the United States Food and Drug Administration). This TKI was approved on the basis of a significant progression-free survival benefit in combination with capecitabine compared with capecitabine alone in women with HER2-positive, trastuzumab-refractory metastatic breast cancer (8). In contrast to the first-line trastuzumab study, in the pivotal lapatinib study the improvement in progression-free survival did not translate into a significant survival benefit.

The reasons for variable outcome improvements with different anti-HER2 agents and in different populations may include study design limitations and early closure (underpowering), inaccurate patient selection (testing), or suboptimal administration strategies (dose, schedule, and concurrent therapies). It is the least possibility that Francia and colleagues address in this issue of Clinical Cancer Research (1). Specifically, they explored a chemotherapeutic drug (cyclophosphamide) and schedule (low dose weekly) thought to act through inhibition of the enhanced angiogenic signal (vascular endothelial growth factor, VEGF) generated in response to HER2 signaling (2). In a preclinical model they combined trastuzumab with traditional, pulsatile, maximally tolerated doses of cyclophosphamide or the same drug using a daily low-dose (so-called metronomic) presumptively anti-angiogenic schedule in HER2-positive breast cancer.

Conventional chemotherapeutics typically exert their cytotoxic effects by damaging DNA or interfering with microtubules to inhibit or kill rapidly dividing cells (Fig. 1). Historically, cytotoxics have been administered at the highest possible, or maximum tolerated dose (MTD). However, because of the associated deleterious effects on rapidly dividing healthy tissues, most notably the infection-fighting white blood cell lines, successive cytotoxic administration requires scheduling that permits adequate recovery between cycles. This consideration has been overcome, in part, in the adjuvant breast cancer setting with the concomitant administration of
hematopoietic growth factor support. However, because chemotherapeutics target not only cancer cells but also endothelial cells in newly formed blood vessels (along with other stromal cells), and because cancer cells are dependent on neovascularization for growth (with VEGF production a specific consequence of HER2 signaling), it is postulated that there may be more (and less) anti-angiogenic effects when chemotherapy agents are given using different schedules. Previous work has shown that in contrast to conventionally derived MTD dosing using regularly intermittent dosing, low and minimally toxic doses of chemotherapy given more frequently can damage blood vessel endothelial cells thereby inhibiting tumor neovascularization and ultimately mediating tumor cell death (9). This strategy may be particularly relevant in HER2-positive breast cancers that generate VEGF via HER2-mediated signaling. If such tumors are treated with HER-family inhibitors but do not achieve full or adequate reductions in VEGF production, they could be particularly sensitive to the co-administration of anti-VEGF or anti-VEGF agents, and also to anti-angiogenic optimization of concurrently administered chemotherapy (2).

Consistent with results observed in human studies, minimal activity was observed in the study by Francia and colleagues with trastuzumab monotherapy in the metastatic model, but anti-tumor activity was more robust when chemotherapy was added. With regard to the schedule of administration of the chemotherapy, the benefits were similar for mice treated with trastuzumab and metronomic versus MTD cyclophosphamide, although greater toxicity was reported with the latter regimen. Again, these results are consistent with numerous adequately powered, randomized, adjuvant (10) and metastatic breast cancer studies (11), wherein cytotoxic agents administered on a frequent schedule and at a low dose are associated with greater tolerability and in some cases more activity than the same drug(s) administered at higher doses on a less frequent schedule. The lack of a clear efficacy benefit for the more frequent dosing is consistent with the clinical observation that trastuzumab may level the playing field, such that although an apoptotic signal is needed, how it is provided is not as critical as in settings in which this antibody is ineffective. For example, it is notable that consistent benefits were observed across the pivotal adjuvant trastuzumab trials, regardless of the chemotherapy foundation (5, 6, 12). Furthermore, in the metastatic setting, no difference was observed for weekly versus every 3-weekly paclitaxel chemotherapy when trastuzumab was administered in the HER2-positive subset (11). A critical and inadequately addressed issue is whether the anti-angiogenic activity of the more frequent dosing is a meaningful contribution to its activity because if not, that would explain the lack of a differential effect.

In summary, the study by Francia and colleagues highlights several critical issues about HER2-targeted therapy. First, it reminds us of the importance of HER2-targeted therapy as an adjunct to conventional cytotoxic strategies. Second, it suggests that further gains in the optimization of HER2-based treatment recommendations will likely depend on deeper and clearer elucidation of HER2-signaling pathways and the mechanism(s) of action of effective HER2-targeted agents such as trastuzumab. Third, it suggests that general chemotherapy principles may not apply evenly in all situations. In that regard, it is important to note that although the activity of conventional cytotoxics has...
been augmented by HER2-targeted therapies across preclinical and clinical (adjuvant and metastatic) models, there has been no study to date in which HER2-targeted therapy has supplanted conventional cytotoxic strategies. Consequently, investigations such as this one by Francia and colleagues remain important as they offer the opportunity to more fully deliver the promise of trastuzumab and perhaps other targeted therapies.

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**References**

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