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Redefining the Target Again: Chemotherapeutics as Vascular Disrupting Agents?

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In this issue of *Cancer Cell*, Shaked et al. (2008) provide novel mechanistic evidence that some chemotherapeutics induce circulating endothelial progenitor (CEP) mobilization with subsequent homing to tumor vasculature. Addition of an anti-VEGFR2 antibody increased antitumor activity only in combination with CEP-mobilizing chemotherapeutics. Here we discuss the implications of this work.

How does chemotherapy work? Every practicing oncologist has struggled to answer this seemingly simple question. Initially thought to simply inhibit tumor cell proliferation, additional mechanisms of action for chemotherapy continue to emerge. In this issue of *Cancer Cell*, Shaked et al. (2008) add vascular disrupting agents (VDAs) to the list. VDAs are distinct from antiangiogenic agents, causing rapid shutdown of the established tumor vasculature, leading to intratumoral necrosis, and leaving a viable tumor rim. In earlier work, Shaked et al. (2006) reported that treatment of tumor-bearing mice with VDAs leads to acute mobilization of circulating endothelial progenitors (CEPs), which home to the viable tumor rim. Furthermore, blockage of the VDA-induced CEP spike by an antiangiogenic drug reduces the tumor rim size and blood flow, thereby enhancing antitumor activity. Here Shaked et al. demonstrate that some, but importantly not all, chemotherapeutics induce acute CEP mobilization when administered near the maximum tolerated dose (MTD). As in the investigators' previous studies, the mobilized CEPs subsequently homed to the tumor vasculature and contributed to tumor recovery. CEP mobilization was at least partly mediated by SDF-1α and inhibited by anti-VEGFR2 antibodies. Notably, the addition of the anti-VEGFR2 antibody only augmented the effect of chemotherapy agents that induced CEP mobilization.

So what are CEPs? Confusion and controversy abound. The field has been hampered by differing methodologies and conflicting nomenclature, a problem compounded by the antigenic promiscuity of related cell populations including CEPs (called EPCs by some investigators), circulating endothelial cells (CECs), hematopoietic progenitor cells (HPCs), hemangiocytes, Tie2-expressing monocytes (TEMs), and others. Using in vitro cell culture and in vivo functional assays, Yoder et al. (2007) redefined two distinct populations of bone marrow-derived progenitor cells. "Endothelial colony-forming cells" (ECFCs, also known as late-outgrowth EPCs) are the true endothelial progenitor cells, with high proliferative potential and vessel-forming activity in vivo. These rare ECFCs are distinct from the more common "colony-forming unit-endothelial cells" (CFU-ECs, also known as early-outgrowth EPCs), which facilitate angiogenesis but do not form secondary endothelial cell colonies or perfused vessels in vivo. Alternatively, CEPs can be defined based on specific cell surface antigen expression and enumerated by flow cytometry. Though flow cytometry ensures enumeration of a homogeneous population and is more easily applied to the clinic, the definition of CEPs by this method is complicated by the absence of unique CEP markers and the uncertainty of cell lineage and function of the defined cell populations. For example, $CD34^+AC133^+VEGFR2^+$ cells, identified as CEPs in the majority of studies, do not form colonies in endothelial clonogenic assays and are devoid of vessel-forming activity in vivo but do form colonies using hematopoietic assays and express the hematopoietic lineage-specific antigen CD45 (Case et al., 2007). Though protocols for enumerating distinct EPC populations by multiparameter flow cytometry have been proposed, the true origin and function of these subpopulations have not been assessed by either endothelial or hematopoietic clonogenic assays. Cross-study comparisons and our understanding of the functional role of these cell populations will remain limited until agreement on common nomenclature and methodology is reached.

A communal sense of déjà vu is justified here. Previous studies have suggested that some, though not all, conventional cytotoxics have distinct antiangiogenic activity (reviewed in Miller et al., 2001). Most convincingly shown for the taxanes, cyclophosphamide, and vinblastine, antiangiogenic activity requires lower doses but more consistent administration—so-called "metronomic" therapy. In contrast to the MTD schedules used by Shaked et al. (2008), metronomic therapy decreases CEP mobilization (Bertolini et al., 2003). Despite the wealth of compelling laboratory data, direct clinical evidence for either the vascular-damaging or antiangiogenic activity of paclitaxel is limited. Consistent with a vascular-damaging effect, CEPs and SDF-1α levels increased acutely in patients treated with paclitaxel-based regimens but did not change in patients treated with other agents (Shaked et al., 2008). Consistent with an antiangiogenic effect, Taghian et al. (2005) reported that paclitaxel but not doxorubicin significantly decreases tumor interstitial fluid pressure and increases tumor oxygenation independent of tumor size and response. So which schedule is optimal? Both increasing the frequency of MTD paclitaxel (presumably increasing the vascular-damaging effect; Citron et al., 2003) and weekly administration of lower doses (presumably increasing the antiangiogenic effect; Sparano et al., 2007) are superior to every-3-weeks administration. Direct

Table 1. Bevacizumab-Based Regimens as Initial Chemotherapy for Metastatic Breast Cancer

	E2100 (Miller et al., 2007)		AVADO (Miles et al., 2008)			XCALIBr (Sledge et al., 2007)
Chemotherapy	paclitaxel 90 mg/m2 d1, 8, 15 q28d		docetaxel 100 mg/m2 q21d			capecitabine 1000 mg/m2 bid d1–14 q21d
Bevacizumab	10 mg/kg q2wks	none	15 mg/kg q3wks	7.5 mg/kg q3wks	placebo q3wks	15 mg/kg q3wks
Patients enrolled	347	326	247	248	241	106
Prior adjuvant chemotherapy (%)	56.5	55.5	68	65	65	65
Prior adjuvant taxane (%)	17.3	14.7	17	15	15	26.2
ER+ (%)	59.9	62.9	76[a]	78[a]	78[a]	54
HER2+ (%)	1.4	0.9	0	0	0	0
ORR (%)	36.9	21.2	63[b]	55[b]	44[b]	38[b]
PFS (months)	11.8	5.9	8.8	8.7	8.0	5.7

ER+, estrogen receptor-positive; HER2+, human epidermal growth factor receptor 2-positive; ORR, overall response rate; PFS, progression-free survival.

[a] Includes patients with ER+ and/or progesterone receptor-positive (PR+) disease.

[b] ORR only reported in patients with measurable disease at study entry.

comparisons of these strategies have not been reported.

This study challenges our assumptions, and in doing so should both inform future trial design and enrich our interpretation of unexpected results. Many have assumed that the addition of an antiangiogenic to any chemotherapeutic (at any dose and schedule) would result in a similar improvement in outcome. Consequently, selection of combinations for clinical investigation has all too frequently been guided by regulatory rather than biologic considerations. Major differences in progression-free survival (PFS) in three clinical trials incorporating bevacizumab into the initial chemotherapy of women with metastatic breast cancer have been largely attributed to relatively minor differences in the patient population and the duration of chemotherapy (Table 1). The work of Shaked et al. (2008) offers another plausible explanation, namely that some chemotherapy agents offer potential mechanistic synergy that others lack. Similarly, correlative efforts in previous trials, largely focused on changes in circulating angiogenic peptides and endothelial surface antigens after weeks of therapy, have failed to identify reliable surrogates. Here, again, the work of Shaked et al. is instructive: perhaps our search has been both in the wrong place and at the wrong time. While it took decades to redefine the target of chemotherapy, first as antiangiogenic and now as VDA, Shaked et al. force us to redefine the target of antiangiogenic therapy as well, from simply inhibiting endothelial proliferation to blocking the host response to vascular damage. We dare not assume that all antiangiogenic agents will share this activity. Perhaps most importantly, Shaked et al. remind us that the way forward continues to lie in increased knowledge of fundamental biology. Francis Bacon's words still ring true: "Nature to be commanded must first be obeyed."

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