Letter to the Editor

Cancer Research

Benefits of Vascular Normalization Are Dose and Time Dependent—Letter

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Arjaans and colleagues reported that treatment with the anti-VEGF antibody bevacizumab hampers antibody uptake in an ectopic xenograft model of human ovarian cancer in mice (1). They found that bevacizumab decreased vessel number and increased pericyte coverage and concluded that treatment-induced normalization is detrimental for antibody delivery. This conclusion is not supported by their data and is in disagreement with the existing literature (2–4).

Vascular normalization after antiangiogenic therapy could explain the seemingly paradoxical synergism between antiangiogenic agents and concurrent systemic anticancer therapies (2). The vascular normalization paradigm was originally described to have four key elements: structural normalization, functional normalization, the transient nature of the effects, and the dose dependence (Fig. 1), with functional normalization being the most important element (2).

Arjaans and colleagues used a relatively high dose of bevacizumab and detected structural changes consistent with structural vascular normalization. However, they present no evidence that bevacizumab functionally normalized tumor blood vessels, nor do they mention when antibody treatment was delivered with respect to the vascular normalization time window. These deficiencies notwithstanding, the study raises an important question: Could antiangiogenic therapy impair the delivery of antibodies? The answer is likely yes, as the normalization time window may narrow or even disappear when antiangiogenic agents are dosed to potentiate antivascular effects. Antivascular effects could lead to a transient delay in tumor growth. The remaining vessels may be pericyte-covered, but because these treatments greatly reduce the number of functional vessels, they could potentially hinder the delivery of drugs at that time—a status referred to as "inadequate" rather than "normalized" vasculature (2, 5). This scenario is more consistent with the report by Arjaans and colleagues (1), that is, a predominant antivascular rather than vascular normalizing effect of bevacizumab in their experimental setting. The potential of antiangiogenics to produce both vascular normalizing and antivascular effects is of great clinical relevance for how these drugs should be combined with other therapeutics. Indeed, clinical studies have shown that the addition of high-dose bevacizumab does not improve overall survival when combined with chemotherapeutics in some cancers, such as ovarian or breast cancers. Therefore, it is high time we design better ways for using antiangiogenic therapy in the clinic. Our recent work suggests that anti-VEGFR2 antibody (DC101) treatment can normalize orthotopic tumor vasculature and enhance the delivery of anticancer agents in a dose- and time-dependent manner (Fig. 2; refs. 3, 4). It also shows that these benefits may be lost when using high-dose antiangiogenic therapy due to pronounced antivascular effects. Thus, optimizing combinations of antiangiogenic agents with anticancer treatments will require imaging and/or circulating biomarkers based on an in-depth understanding of the dynamics of the tumor vascular response, in particular the balance between vascular normalizing and antivascular effects.

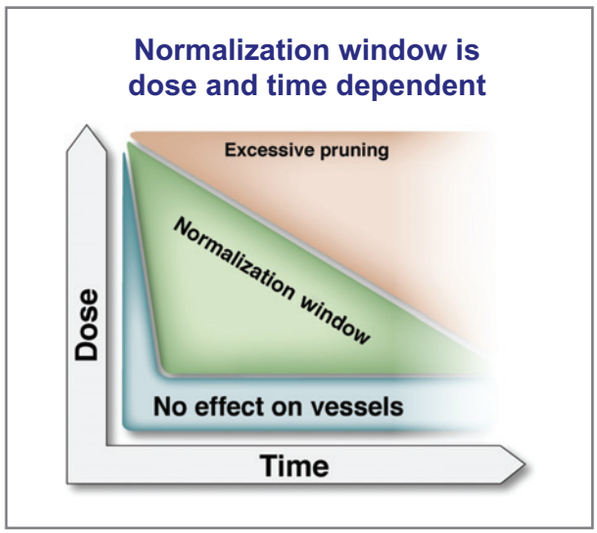

Figure 1. Vascular normalization hypothesis. Because of imbalance between pro- and antiangiogenic signaling, tumor vessels are highly abnormal both structurally and functionally. This creates a hostile microenvironment in tumors—characterized by hypoxia, low pH, and elevated fluid pressure—which fuels tumor progression via genetic instability, angiogenesis, endothelial–mesenchymal transition, immunosuppression, inflammation, resistance to apoptosis/autophagy, etc. Anti-VEGF treatment, using a judicious dose of bevacizumab (or another antiangiogenic agent), can prune some abnormal vessels and remodel the rest, resulting in a "normalized vasculature." In turn, this can reduce tumor hypoxia, acidity, and fluid pressure, improving the outcome of chemo-, radio-, and immunotherapy. If the antiangiogenic agent is too potent or the dose is too high, the balance can tip in the other direction, resulting in the pruning of an excessive number of vessels, leading to a shorter window of normalization or "inadequate vasculature" and reduced delivery of oxygen and concurrently administered therapeutics. Higher doses can also lead to adverse effects in normal tissues. Reproduced from the work of Jain (2). Reprinted with permission. © 2013 American Society of Clinical Oncology. All rights reserved.

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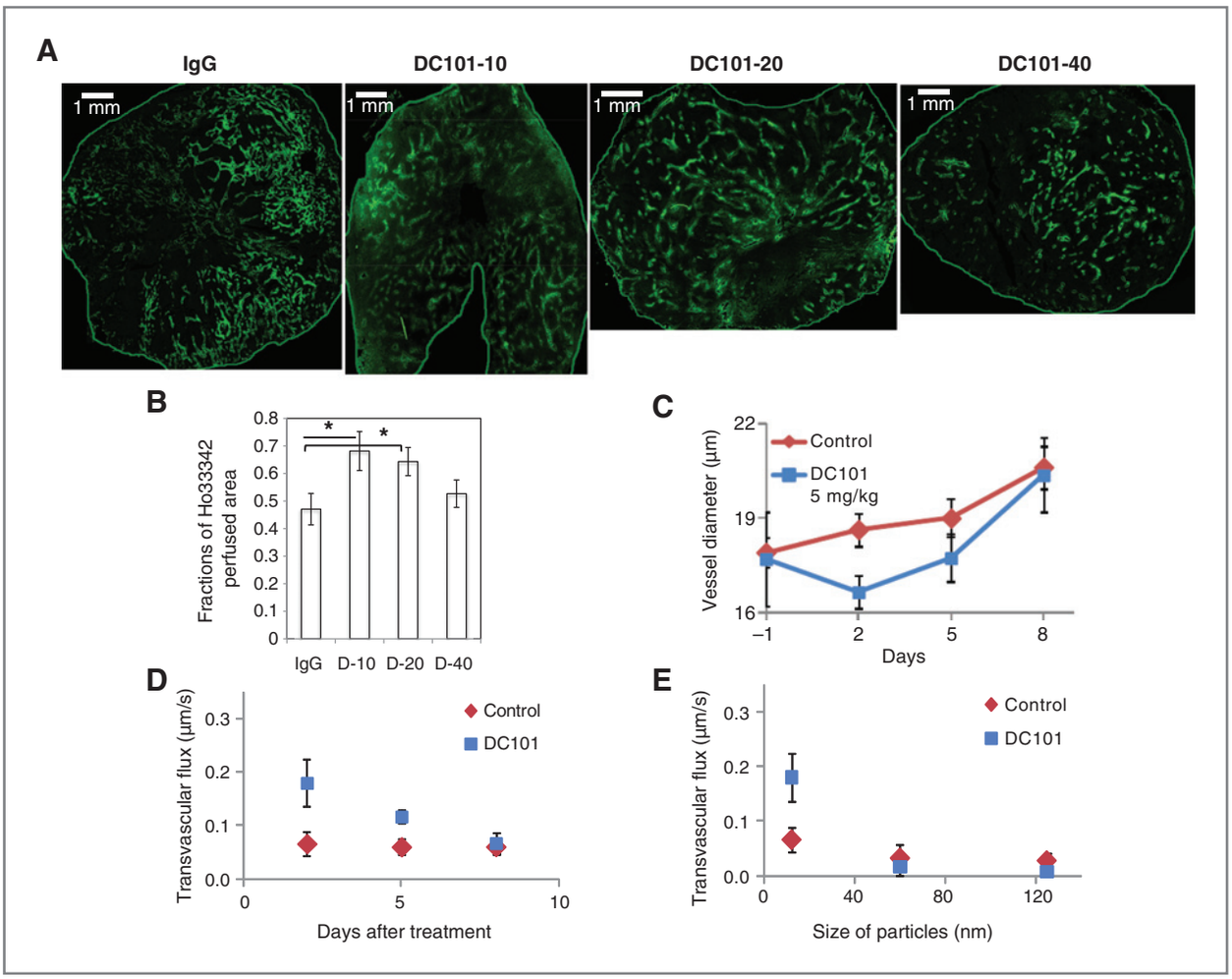

Figure 2. Dose and time dependence of vascular normalization in solid tumors. A and B, dose-dependent effects of antiangiogenic treatment on vascular morphology and function in orthotopic breast tumors. When MCaP0008 tumors reached 4 to 5 mm in diameter, mice were treated with DC101 (10, 20, or 40 mg/kg body weight) or rat IgG as control (40 mg/kg body weight), 4 doses, every 3 days starting on day 0. Mice were injected with 200 μg Hoechst 33342 i.v. before tumor harvest on day 11. Whole-tumor tissue perfusion images were taken by multispectral confocal microscopy. A, representative whole-tumor tissue perfusion images (from left to right: IgG, DC101-10, DC101-20, DC101-40). Green, Hoechst 33342. Scale bars, 1 mm. B, the fraction of Hoechst 33342–positive area in whole tumor area ($n = 10$–14 mice per group). Only low doses of DC101 improved perfusion but not the highest dose. Adapted from Huang and colleagues (3). C and D, time-dependent effects of vascular normalization on nanomedicine delivery in tumors. Measurements in orthotopic E0771 mammary tumors over an 8-day course of treatment with either 5 mg/kg DC101 or nonspecific rat IgG every 3 days starting on day 0. C, treatment with DC101 reduced vascular diameter on days 2 and 5, with no difference on day 8. D, treatment with DC101 enhanced effective vascular permeability (transvascular flux) on days 2 ($P = 0.049$; Student t test) and 5 ($P = 0.017$; Student t test), with no difference in the treatment groups by day 8. Animal number $n = 4$–5 for all groups. E, size-dependent effects of vascular normalization on nanoparticle delivery in tumors. Effective permeability for nanoparticles in orthotopic E0771 mammary tumors in mice treated with 5 mg/kg DC101. Normalization improved the permeability of 12-nm particles (which is in the size range of an antibody) on day 2 by a factor of 2.7 in E0771 ($P = 0.049$; Student t test) while not improving delivery for larger nanoparticles (60 and 125 nm). Animal number $n = 5$ for all groups. C–E were adapted from Chauhan and colleagues (4).

Disclosure of Potential Conflicts of Interest

D.G. Duda is a consultant/advisory board member for Hexal. R.K. Jain is on the board of Xtuit, H&Q Healthcare Investors, and H&Q Life Sciences Investors has grants from MedImmune, and Roche, has ownership interest (including patents) in Enlight, Xtuit, SynDevRx, and is a consultant/advisory board member for Noxxon Pharma, Zyngenia, WebMD, Enlight, and SynDevRx. No potential conflicts of interest were disclosed by the other authors.

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