Published in final edited form as:

Cancer Res. 2013 December 1; 73(23): . doi:10.1158/0008-5472.CAN-13-1989.

Benefits of vascular normalization are dose- and time-dependent

Yuhui Huang¹, Triantafyllos Stylianopoulos¹, Dan G. Duda¹, Dai Fukumura¹, and Rakesh K. Jain¹

¹Steele Laboratory for Tumor Biology, Department of Radiation Oncology, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114

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 existing literature (2-4). Vascular normalization after antiangiogenic therapy could explain the seemingly paradoxical synergism between antiangiogenic agents and concurrent systemic anti-cancer 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 et al. 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 anti-angiogenic therapy impair the delivery of antibodies? The answer is likely yes, as the normalization time-window may narrow or even disappear when anti-angiogenic agents are dosed to potentiate anti-vascular effects. Anti-vascular 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 et al. (1), i.e., a predominant anti-vascular rather than vascular normalizing effect of bevacizumab in their experimental setting. The potential of antiangiogenics to produce both vascular normalizing and anti-vascular effects is of great clinical relevance for how these drugs should be combined with other therapeutics. Indeed, clinical studies have shown that 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 anti-angiogenic therapy in the clinic. Our recent work suggests that anti-VEGFR2 antibody (DC101) treatment can normalize orthotopic tumor vasculature and enhance the delivery of anti-cancer agents in a dose- and time-dependent manner (Fig. 2) (3, 4). It also shows that these benefits may be lost when using high-dose antiangiogenic therapy due to pronounced anti-vascular effects. Thus, optimizing combinations of anti-angiogenic agents with anti-cancer 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 anti-vascular effects.

References

1. Arjaans M, Oude Munnink TH, Oosting SF, Terwisscha van Scheltinga AG, Gietema JA, Garbaci ET, et al. Bevacizumab-induced normalization of blood vessels in tumors hampers antibody uptake. *Cancer Res.* 2013; 73:3347–55. [PubMed: 23580572]
2. Jain RK. Normalizing tumor microenvironment to treat cancer: bench to bedside to biomarkers. *J Clin Oncol.* 2013; 31:2205–18. [PubMed: 23669226]
3. Huang Y, Yuan J, Righi E, Kamoun WS, Ancukiewicz M, Nezivar J, et al. Vascular normalizing doses of antiangiogenic treatment reprogram the immunosuppressive tumor microenvironment and enhance immunotherapy. *Proceedings of the National Academy of Sciences of the United States of America.* 2012; 109:17561–6. [PubMed: 23045683]
4. Chauhan VP, Stylianopoulos T, Martin JD, Popovic Z, Chen O, Kamoun WS, et al. Normalization of tumour blood vessels improves the delivery of nanomedicines in a size-dependent manner. *Nature nanotechnology.* 2012; 7:383–8.
5. Pastuskovas CV, Mundo EE, Williams SP, Nayak TK, Ho J, Ulufatu S, et al. Effects of anti-VEGF on pharmacokinetics, biodistribution, and tumor penetration of trastuzumab in a preclinical breast cancer model. *Mol Cancer Ther.* 2012; 11:752–62. [PubMed: 22222630]

Normalization window is dose- and time-dependent

Figure 1. Vascular normalization hypothesis

Due to imbalance between pro- and anti-angiogenic 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 anti-angiogenic 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 immune therapy. If the anti-angiogenic 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, “inadequate vasculature”, and reduced delivery of oxygen and concurrently administered therapeutics. Higher doses can also lead to adverse effects in normal tissues. Reproduced from (2).

Figure 2. Dose and time-dependence of vascular normalization in solid tumors

A and B: Dose-dependent effects of anti-angiogenic treatment on vascular morphology and function in orthotopic breast tumors. When MCA-P0008 tumors reached 4-5 mm in diameter, mice were treated with DC101 (10, 20 or 40 mg/kg bw) or rat IgG as control (40 mg/kg bw), 4 doses, every three 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 are 1000 μm . **B.** The fraction of Hoechst 33342 positive area in whole tumor area ($n=10-14$ mice/group). Only low doses of DC101 improved perfusion but not the highest dose. Adapted from Huang et al, PNAS, 2012 (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 5mg/kg DC101 or non-specific 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's t-test) and 5 ($P = 0.017$, Student's 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 5mg/kg DC101.

Normalization improved the permeability of 12nm particles on day 2 by a factor of 2.7 in EO771 ($P = 0.049$, Student's t-test), while not improving delivery for larger nanoparticles (60 and 125 nm). Animal number $n = 5$ for all groups. **C**, **D** and **E** were adapted from Chauhan et al, Nature Nanotechnology, 2012(4).