Supplementary Appendix

This appendix has been provided by the author to give readers additional information about his work.

Supplement to: Kerbel RS. Tumor angiogenesis. N Engl J Med 2008;358:2039-49.

Online Supplemental Section

Appendix 1: The Concept of "Vessel Normalization" and its Possible Impact of Antiangiogenic Drug Enhancement of Chemotherapy Efficacy

Vessel normalization in tumors induced by antiangiogenic drugs refers to the finding that many of the blood vessels of tumors are structurally chaotic and functionally abnormal, including having poor blood perfusion and flow as well. excessive leakiness, but that many of these abnormalities can be transiently reversed by treatment with some antiangiogenic drugs, so that the abnormal tumor vasculature becomes temporarily "normalized". The dysfunctional vasculature of tumors has been hypothesized to negatively impact on intrinsic chemotherapy sensitivity in several ways: by causing elevated interstitial fluid pressures through the leakage of plasma proteins and fluid, thus potentially impeding the delivery and diffusion of certain drugs into the through tumor tissue; and by contributing to elevated levels of tumor hypoxia as a result of suboptimal perfusion of blood, thus resulting in regions of reduced tumor cell proliferation. However, administration of an antiangiogenic drug such as an antibody to VEGF or VEGFR-2 can 'prune' a number of the more immature tumor capillaries – an antiangiogenic effect - leaving behind more mature functional vessels while some of the other remaining abnormal vessels can transiently acquire a more mature ("normalized") phenotype. During this

period of vessel normalization oxygen delivery is said to be increased, vessel leakiness and hence interstitial fluid pressures in tumors decreased, and tumor cell proliferation concurrently increased. Consequently the intratumoral delivery and diffusion of chemotherapy is transiently increased, and there are greater numbers of proliferating tumor cells for the chemotherapy to target – provided that the chemotherapy is administered during this "window" of tumor vessel normalization. The net result is increased chemotherapy anti-tumor efficacy. Similar to other theories about the ways in which an antiangiogenic drug may enhance the efficacy of chemotherapy, definitive clinical evidence for vessel normalization is currently lacking. Preclinically the impact of exploiting vessel normalization to improve chemotherapy has not yet been evaluated in the context of visceral metastatic disease in multiple organ sites.

For further information on the vessel normalization hypothesis, in addition to references 13, 16, and 88, some recent useful reviews include the following: DG Duda et al (RK Jain) "Antiangiogenics: the potential role of integrating this novel treatment modality with chemoradiation for solid cancers." *J Clin Oncol* 25: 4033-4042; RK Jain "Taming vessels to treat cancer" *Scientific American* January 2008. There are other alternative theories which may explain the ability of antiangiogenic drugs to enhance the efficacy of chemotherapy (e.g. see references 14 and 15), as also outlined in the review.

Appendix 2: The Concept and Application of "Metronomic" Low-Dose Chemotherapy

Metronomic chemotherapy is one of the author's major research interests. The term refers to the administration of conventional chemotherapy drugs in a somewhat unconventional manner, i.e., at very close, regular intervals – even daily – for extended periods, with no prolonged drug-free breaks, using relatively low, minimally or non-toxic doses. It is not usually designed to be "dose-intensive" over time, i.e., provide more total drug per unit time, and therefore does not generally require hematopoietic growth factor support. Chemotherapy administered in a metronomic low-dose manner appears to mediate anti-tumor effects which can sometimes be unexpectedly potent in preclinical models, especially when combined for long periods with a targeted antiangiogenic drug such as a VEGF pathway targeting drug or various other targeted therapies. The major mechanisms accounting for the anti-tumor effects of metronomic chemotherapy regimens appear to be the targeting of 'activated' endothelial cells in the growing tumor neovasculature, and circulating bone marrow-derived endothelial progenitor cells (CEPs), though other possible targets have been implicated as well. Key references include 69, 71, 72, 73, 74, 75, 99 and others, e.g. Bottini et al. "Randomized phase II trial of letrozole and letrozole plus low-dose metronomic oral cyclophosphamide as primary systemic treatment in elderly breast cancer patients." *J Clin Oncol* 24: 3623-3628, 2006; Colleoni et al. "Low-dose oral methotrexate and

cyclophosphamide in metastatic breast cancer: antitumor activity and correlation with vascular endothelial growth factor levels." *Ann Oncol* 13: 73-80, 2002; Colleoni et al. "Metronomic low-dose oral cyclophosphamide and methotrexate plus or minus thalidomide in metastatic breast cancer: anti-tumor activity and biological effects." *Ann Oncol* 17: 232-238, 2006. The clinical studies including reference 75 by Garcia et al., suggest that metronomic chemotherapy has activity in the clinic, most notably thus far in advanced breast or ovarian cancer, when combined with a targeted biologic agent. This remains to be validated in larger randomized clinical trials.

Appendix 3: Developing Better Preclinical Models to Study the Biology of Tumor Angiogenesis and Antiangiogenic Therapy

Similar to many other therapeutic modalities, antiangiogenic drugs frequently induce anti-tumor effects and benefits in mice the magnitude of which are rarely attained when subsequently evaluated in the clinical setting. A case in point: single agent bevacizumab usually prolongs survival of mice with localized primary human tumor xenografts of various and varied histologic organs, whereas in the clinic bevacizumab monotherapy in most cases does not; prolongation of overall survival of ovarian cancer patients with advanced disease may be an exception (Kaye SB "Bevacizumab for the treatment of epithelial ovarian cancer: will this be its finest hour?" *J Clin Oncol* 25: 5150-5152, 2007). This highlights the need for studying the biology of tumor angiogenesis and evaluating the preclinical efficacy of antiangiogenic drugs, or treatments, using models that are hopefully more reflective of human cancer. Some advances are being made in this regard, e.g. the use of spontaneous genetically engineered mouse models (GEMMs) of human cancer. With respect to transplanted human tumor xenografts, advances include the use of orthotopic tumor transplants and development of models of advanced metastatic disease, involving multiple organ sites. Such models may help bypass some of the factors responsible for obtaining overly optimistic/exaggerated outcomes in the more traditional models of cancer drug (including antiangiogenic drug) studies in mice, e.g. rapidly growing

subcutaneous (ectopic) tumor transplants. By way of example, advanced high volume metastatic disease, especially refractory to previously employed therapies, is usually less responsive to new therapies compared to therapy naïve disease. But such models have rarely been used for therapy testing in mice over the last 50 years of preclinical cancer research – even though most phase I and II clinical trials involve patients with advanced metastatic disease. In addition, metastases growing in a particular organ site may be responsive to an antiangiogenic drug but unresponsive in another site simply due to heterogeneity of drug target expression or other factors. So, development and use of models of advanced metastatic disease, one of the author's major current research interests, could be one of the ways of obtaining results in mice which have a better chance of predicting subsequent clinical outcome for a particular experimental therapy though this possibility still needs to be validated in prospective randomized clinical trials. For more discussion and information relevant to this topic some useful papers by the author include: RS Kerbel "Human tumor xenografts as predictive preclinical models for anticancer drug activity in humans. Better than commonly perceived – but they can be improved." *Cancer Biology and Therapy* 2: 108-113, 2003; Munoz et al. "Highly efficacious non-toxic preclinical treatment for advanced metastatic breast cancer using combination oral UFT – cyclophosphamide metronomic chemotherapy." *Cancer Res.*, 66: 3386-3391, 2006; Man et al. "On the development of models in mice of advanced visceral metastatic disease for

anti-cancer drug testing." *Cancer & Metastasis Rev*. 26: 737-747, 2007. The last paper contains relevant references to the use of 'GEMMs' for therapy testing.