

Molecular and cellular biomarkers for angiogenesis in clinical oncology

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A number of “molecularly targeted” anti-angiogenic drugs such as bevacizumab (Avastin), sunitinib (Sutent), and sorafenib (Nexavar) are now available for clinical oncologists (1). Their clinical use, however, suffers from several relevant limitations. Although some large clinical trials have demonstrated a benefit of these drugs in terms of prolonged survival of cancer patients, there is a compelling need for determining the optimal biologic dose (OBD) of these drugs, monitoring their biologic activity, selecting and stratifying the patients who are most likely to benefit from treatment. In medical oncology, problems related to the definition of the OBD for such drugs include the low frequency of tumor responses (tumor shrinkage); the lack, in some cases, of dose limiting toxicities (DLT) normally used to define a maximum tolerated dose (MTD), observed frequently when using cytotoxics but not as frequently when using certain anti-angiogenic drugs; and significant (if not optimal) therapeutic activity at doses below the MTD. Most of these drugs are extremely expensive, and the escalating cost of clinical care underlines the urgent need for development and clinical validation of biomarkers of angiogenesis for patient selection and stratification and for OBD tailoring (2).

It was first reported in the mid 1970s that cells with endothelial characteristics circulate in the blood; it took two more decades to establish a procedure to quantify the CEC population (3-5). In healthy subjects, this numerically rare cell population is stable in quantity and represents 1/1,000-100,000 of circulating blood cells. In many pathological conditions, such as cancer, the number of CECs is increased (*Fig. 1*). The majority of CECs shows characteristics of mature, terminally differentiated and frequently apoptotic cells (the first morphological studies described them as “anucleated carcasses”), only a subpopulation of which expresses antigens that suggest a stem- or progenitor-like phenotype (CEPs, Circulating Endothelial Progenitors, see *Figs. 1 and 2*).

Differentiating CEPs from CECs based on different expression of surface molecules is very difficult due to the antigenic promiscuity amongst hematopoietic cells and

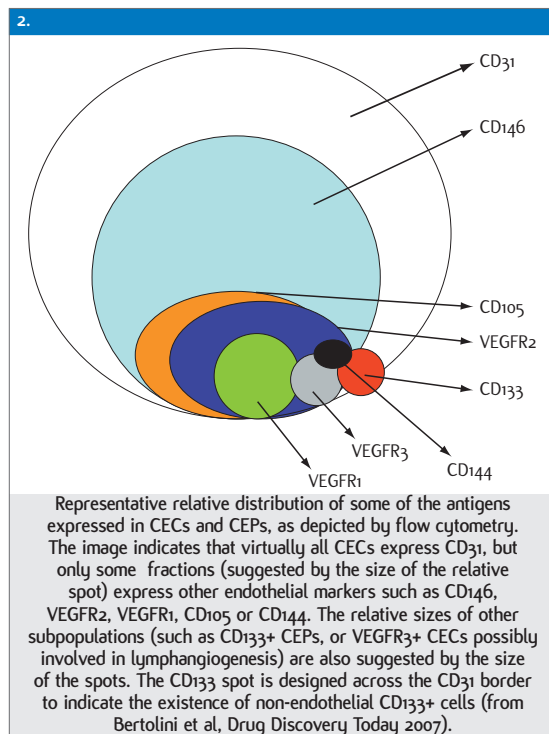
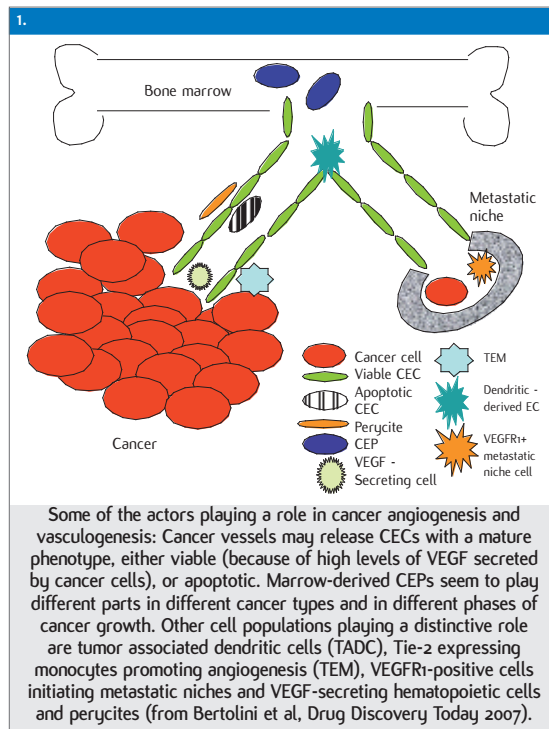
progenitors, platelets, CECs and CEPs as mentioned above. In fact, there is no single antigen to discriminate between CECs, platelets and hematopoietic cells (3).

In order to validate the potential of CECs and CEPs as preclinical surrogate markers of angiogenesis, we took advantage of the genetically-induced difference in angiogenic responsiveness among different mouse strains (it is currently unclear whether such a wide variability exists also in humans). We studied 8 different strains and angiogenesis was assessed using the corneal micropocket assay in the mouse eye (6). The mouse strains differed up to 10-fold in their response to VEGF. A highly significant positive correlation was found between the response after VEGF stimulation and the absolute number of CECs and CEPs in normal (unstimulated) mice. These results were confirmed using the Matrigel (subcutaneous) plug perfusion assay for angiogenesis indicating that the number of CECs and CEPs is indeed representative of the angiogenic response. To investigate whether quantification of CECs and CEPs could be used to determine the OBD of targeted anti-angiogenic drugs in mice, we treated mice with DC101, a rat monoclonal antibody specifically blocking mouse VEGFR2, which has an optimal anti-tumor therapeutic dose in the range of 800–1200 µg/mouse. As expected, this dose led to the greatest decrease in tumor volume and also to the lowest level of viable CECs. Increasing the dose did not cause further reductions in CEC levels. In addition, we found that quantification of CECs and CEPs can be used to determine the OBD in cancer-bearing mice treated with low-dose “metronomic” chemotherapy, a cancer treatment strategy thought to have an antiangiogenic basis. Taken together, these findings underline the potential of CEC and CEP measurement for helping to establish OBD (6).

CEC levels are increased in the peripheral blood of patients affected by some types of cancer, and return to normal values in patients undergoing complete remission (3). In metastatic breast cancer patients treated with low dose metronomic chemotherapy using CTX and methotrexate, the CEC count after two months of continuous (daily) therapy was a particularly good predictor of disease-free and overall

survival after a follow-up of more than two years (7). Patients showing a CEC count above physiological levels after two months of therapy had a significantly improved progression-free and overall survival. The increased number of CECs in patients with a clinical benefit was mostly due to an increased number of apoptotic CECs. This is because antiangiogenic agents are thought to reverse cancer vessel abnormalities and in this scenario, the remodelling process likely involves a shedding of apoptotic endothelial cells from cancer-associated vessels. In preclinical studies, we did not observe a rise in the number of CECs in cancer-free animals treated with metronomic chemotherapy – only in tumor-bearing mice was this observed. Taken together, these findings suggest that the cancer-associated vasculature is most likely the predominant source of the rise in apoptotic CECs seen in breast cancer patients treated with metronomic therapy, thus confirming the potential of this surrogate biomarker (3, 7).

The measurement of CECs, of their viability, and of CEC subpopulations (eg VEGFR2+ CECs, activated CD105+ CECs, or VEGFR3+ CECs, possibly involved in lymphangiogenesis) might be useful for OBD finding in phase I-II studies on antiangiogenic drugs, alone or in combination with chemotherapeutics. When considering that in undifferentiated patient pools, the number of non-responders could jeopardize a trial's endpoint, CEC-related measurements might also be of help to identify responders and non-responders to a given therapeutic regimen including anti-angiogenic drugs or strategies such as metronomic chemotherapy. This patient stratification may significantly reduce the (otherwise rampant) costs of novel antiangiogenic cancer therapies by targeting treatments to those patients mostly likely to benefit.



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