Evaluation by Multicolor Flow Cytometry of Circulating Endothelial Cells as a Prognostic and Predictive Biomarker in Cancer Patients

By Elena Di Gennaro

Background
Several reports demonstrated that circulating endothelial cell (CEC) count in the peripheral blood of cancer patients correlates with prognosis and represents a promising tool for selecting patients who might benefit from anti-angiogenic therapies. In parallel, chemotherapy mobilization of bone-marrow–derived progenitor circulating endothelial cells appeared as a consistent finding and a key mechanism mediating tumor resistance. Recent clinical trials suggested that the anti-VEGF antibody, bevacizumab, is able to decrease CECs in patients’ peripheral blood. Therefore, combination strategies of chemotherapy with anti-angiogenic therapy, by interfering with bone marrow mobilization of endothelial cells, could optimize cancer therapy.

However, there is no standardized methodology of CEC analysis or consensus on their phenotype. Moreover, published data on this subject is often conflicting and obtained from heterogeneous studies that evaluate patients with different tumors and/or stages.

Hypothesis and significance
Based on our previous experience and our preliminary data on the evaluation of CECs in cancer patients, we hypothesize that CEC counts could represent an important prognostic and predictive biomarker in colorectal (CRC) and ovarian cancer (OC) patients.

Moreover, the evaluation of the levels (baseline and during treatment) of CEC counts could help to select the patients most likely to benefit from anti-angiogenic therapies, and/or to identify possible mechanisms of resistance.

These hypotheses could be validated only through a standardized method of analysis and through the definition of a reference range of counts in healthy individuals.

Specific aims
a) Standardize the identification and count of CECs in the peripheral blood of healthy donors in collaboration with other Italian research groups and cancer patients.

b) Define a reference range for the number of CECs in healthy individuals and compare it with the basal levels measured in naïve (untreated) CRC and OC patients.

c) Determine whether the viability of CECs varies within 24 hours after collection and storage at 4°C or after separation of peripheral blood mononuclear cells (PBMCs) using Ficoll® and storage of cells at -80°C.

d) Evaluate any correlation between CEC counts with stage of disease, phenotypic characteristics of the tumor, and patient outcomes.
e) Define the prognostic and predictive value of CEC counts on the clinical efficacy of anticancer treatment, including anti-angiogenic agents.

Methodologies and statistical analyses
Inclusion criteria of donors and patients: 50 healthy donors and 150 naive patients (50 stages I, II, and III and 50 metastatic stage IV CRC patients; 30 operable stages I, II, and III and 20 metastatic stage IV OC patients) will be enrolled. CEC level will be evaluated at baseline. The stage IV CRC and OC patients will be enrolled among those candidates for first-line treatment with bevacizumab-based therapy, and CECs will be evaluated at baseline and after 15 days from the beginning of treatment. Both healthy donors and cancer patients enrolled should sign an informed consent form.

Flow cytometry instrument setting: an instrument setup has been generated to maximize fluorescence resolution sensitivity on the flow cytometer. Compensation will be calculated using BD™ CompBead particles (BD Biosciences). Instrument performance, stability, and data reproducibility will be sustained and checked in real time by using the BD™ CS&T module and further validated by the acquisition of Spherotech™ Rainbow Beads.

Sample staining: 20 x 10^6 leucocytes per sample from peripheral blood (drawn in EDTA tubes, (BD Diagnostic Systems) will be processed using the lyse/wash method within 4 hours (24 hours or after storage at -80°C) after blood collection. Staining will be performed using a lyophilized cocktail of reagents from BD Biosciences, defined on the basis of a panel previously optimized (SYTO®-16, CD146 PE, CD34 PE-Cy™7, CD309 APC, CD45 APC-H7, and 7-AAD). CD31 V450 and/or CD133 biotinilated + streptavidin V500 will be added in liquid form. Samples will be washed and analyzed by flow cytometry (BD FACSCanto™ II system, BD Biosciences). CEC numbers will be obtained by a double platform counting method.

Statistics will be performed by Fisher, Mann-Whitney, Pearson, and Sperman tests, depending on the analysis, and p ≤0.05 will be considered significant.

Expected outcomes, translational relevance, and impact
CRC and OC are among the most common cancers, and despite the improvement in diagnosis and treatment, they remain two leading causes of cancer deaths. Thus, there is an unmet need for new diagnostic, prognostic, and/or predictive biomarkers that could offer opportunities for early diagnosis or for select patients who could most likely benefit from a specific treatment or, on the contrary, avoid unnecessary ineffective but costly therapies. In both CRC and OC, the treatment with bevacizumab added to chemotherapy has recently significantly improved clinical outcome. However, at the moment there is no validated predictive biomarker for bevacizumab and any of the anti-angiogenic drugs approved for cancer treatment.

Our study will represent the basis for the validation of CEC count as a new potential diagnostic, prognostic, and/or predictive cancer biomarker. If our hypothesis is confirmed, the determination of these cellular populations with a standardized methodology that uses instrumentation and products (BD Custom Lyotubes) from BD could represent the most important tool for their detection and an important opportunity for BD. The capability to measure CECs after storage for 24 hours or after freezing could
facilitate the collection of samples throughout large clinical trials.

The BD Biosciences Research Grant Program aims to reward and enable important research by providing vital funding for scientists pursuing innovative experiments to advance the scientific understanding of disease.

Visit bdbiosciences.com/grant to learn more and apply online.