

## IN THE SPOTLIGHT

## Escaping Out of the Brain

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**Summary:** Technological development in the field of circulating biomarkers has allowed the identification of circulating tumor cells in the peripheral blood of patients with glioblastoma. This opens a new avenue of research with implications for the understanding and clinical managing of this fatal disease. *Cancer Discov*; 4(11); 1259-61. ©2014 AACR.

See related article by Sullivan et al., p. 1299 (3).

In general, metastasis to distant organs is a common feature of most aggressive tumors. In advanced cancer, tumor cells usually acquire the ability to spread and disseminate throughout the body via the bloodstream, reinitiate a tumor in a different location, and establish metastasis. Despite being one of the most aggressive tumors, glioblastoma (GBM) does not follow the general rule. GBMs are highly malignant, usually recalcitrant to radiotherapy and chemotherapy, and are characterized by an extremely invasive nature, resulting in the inability of surgery to completely eradicate tumors (1). However, the infiltrative feature of GBM seldom leads to extracranial metastasis (0.4% of cases; ref. 2).

To address this paradox, Sullivan and colleagues (3) looked for circulating tumor cells (CTC) in the peripheral blood of patients with GBM. They aimed to assess if the lack of GBM extracranial metastasis was due to the inability of tumor cells to escape from the brain. CTCs have been studied in other malignancies besides GBM; they are tumor cells that are shed into the bloodstream from the primary tumor or from metastatic lesions and are believed to be the mediators of metastasis (4, 5). The analysis of CTCs as minimally invasive biomarkers has the potential to provide crucial molecular information about the metastatic process, detect changes in tumor burden, and monitor therapeutic responses in patients with solid cancers (4, 5).

In a first approach, the authors used a state-of-the-art pre-clinical animal model for GBM. Patient-derived xenografts (PDX) of GBM were obtained through the inoculation of tumor cells from fresh GBM surgical specimens into the brains of immunocompromised mice (3). Patient-derived cells have been shown to generate tumors in mice that recapitulate the genomic and histopathologic characteristics of the patient tumor (6, 7). The authors identified CTCs in the blood of GBM PDX and subsequently carried out the identification of CTCs in patients with GBM (Fig. 1).

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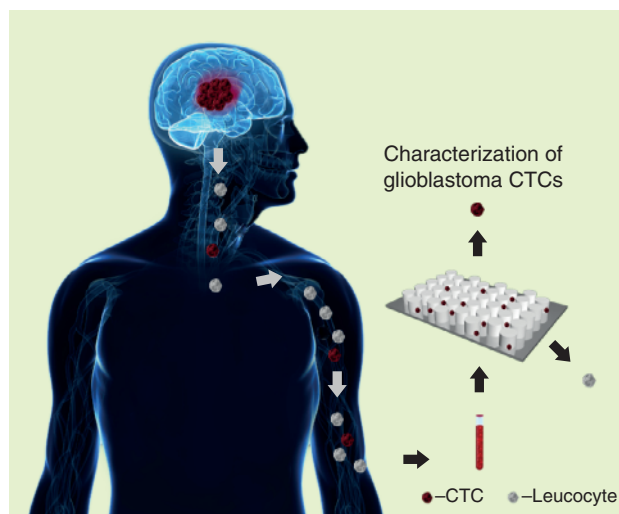
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Based on a negative selection that discarded nontumor cells through size and leukocyte marker expression, Sullivan and colleagues used the CTC-iChip, a microfluidic device developed by the authors' group, to identify CTCs (8). Importantly, the authors avoided the use of previously described markers of CTCs such as epithelial cell adhesion molecule, because they knew that nonepithelial cancers such as GBM do not express this type of marker. A panel of five immunofluorescence-based markers (STEAM, which stands for SOX2, Tubulin  $\beta$ -3, EGFR, A2B5, and c-MET) was developed to identify GBM CTCs. The markers were selected based on their common expression in GBM tumors. Importantly, the authors observed that CTCs shared the same genomic alterations as the primary tumor because *EGFR* copy gain was detected in CTCs from patients with tumors with *EGFR*-amplified cells. The analysis of the blood of 33 patients reported 13 cases presenting CTCs (39%), an extremely high incidence of CTCs as compared with the incidence of GBM metastasis. Interestingly, the authors observed that the GBM CTCs expressed predominantly genes associated with mesenchymal differentiation. These mesenchymal CTCs may be related to a more aggressive phenotype (or even a stem cell-like phenotype), and may be the ones capable of intravasating the brain vasculature and disseminating in the bloodstream. Further work to address this point is required.

Sullivan and colleagues (3) also analyzed the CTCs present in a rare GBM case with extracranial dissemination. In that case, they also observed CTCs presenting a mesenchymal phenotype and, although they performed a genomic analysis of the lesions of the patient, they unfortunately could not assess how CTCs were representative of the architecture of genomic clones and subclones of the tumors of the patient.

In parallel to the work from Sullivan and colleagues (3), Muller and colleagues (9) have also recently identified CTCs in the bloodstream of patients with GBMs. The authors used the glial fibrillary acidic protein (GFAP), which is mainly expressed by astrocytes and glial tumors, as a marker to isolate GBM cells in peripheral blood. CTCs expressing GFAP were subjected to next-generation sequencing, comparative genomic hybridization, and fluorescent *in situ* hybridization and were shown to share the same somatic genomic alterations as the ones found in the corresponding primary brain tumor (i.e., mutations in *MECOM* and *MYH11*; ref. 9). This clearly supported the hypothesis that the CTCs stemmed from the primary brain tumor. Of note, the authors observed an increased tendency toward *EGFR* amplification in CTCs,



**Figure 1.** CTCs of patients with glioblastoma are identified in the peripheral blood.

indicating that perhaps in the context of a heterogeneous tumor mass, the cells with *EGFR* copy gain were more prone to disseminate to the bloodstream than other cell clones. In this work, the authors analyzed 141 patients with GBM and observed CTCs in 29 cases (20.6%).

Both groups have pioneered the detection of circulating brain tumor cells and their dissemination into the bloodstream (3, 9). Remarkably, both reports indicate that CTCs are not the result of blood–brain barrier disruption due to surgical procedures, as, in both cases, CTCs were observed in preoperative patients with GBM. Moreover, due to the low number of patients, the authors did not show any correlation between the presence of CTCs and the clinical characteristics of the patients.

The incidence of detected CTCs differed in both studies. Although Sullivan and colleagues (3) reported 39% of GBM cases with CTCs, Muller and colleagues (9) identified CTCs in 20.6% of patients with GBM. The methodologies used to isolate and characterize CTCs were different and based on different markers, and a question that arises is which is the best and more thorough technology to identify GBM CTCs. Taking into account the molecular diversity of GBM tumors, one could argue that some GBM cells might not express the STEAM or GFAP markers, and, hence, in both studies, the number of CTCs could be underrepresented. Moreover, considering the intratumor heterogeneity of GBMs, it may be that CTCs with different expression markers might coexist and fluctuate in time. It is expected that in the coming years, novel and more accurate technologies to identify GBM CTCs will be developed.

Both reports (3, 9) have shed light onto a long-observed paradox. However, some questions were left unanswered. If tumor cells escape the brain, why are there not more metastases? Several options have been discussed: (i) the tumor cells are not able to home in on extracranial niches and generate metastases; (ii) the cells might require brain-specific growth factors to thrive; and (iii) the short survival periods of patients with GBM do not allow sufficient time to develop metastasis. Interestingly, an observation described in Muller

and colleagues (9) might provide some hints for the decreased number of metastases in patients with GBM. It has been reported that transplantation of organs from patients with GBM can lead to overt metastases in the recipient patient (10). This suggests that the immunosuppression after organ transplantation may potentiate GBM cells to circulate and generate metastasis. Hence, GBM CTCs might be kept in check by the immune system. This means that CTCs need time to “learn” how to escape immune surveillance, and the short survival time of patients with GBM would not allow this process to occur. The implication of this possibility is that advances in the treatment of the primary tumor might provide time for CTCs to become metastatic.

The findings reported by both groups (3, 9) have enhanced our understanding of the biology of GBM. In addition, these “liquid biopsies” are potentially powerful tools to characterize the tumors of patients with GBM, because the restricted and invasive access to tumor material in this type of cancer makes the molecular characterization of brain tumors through regular biopsies extremely challenging. CTCs or even circulating cell-free tumor DNA (5) may represent sources of tumor-derived material and may be seen as minimally invasive barcodes of the brain tumor tissue. These circulating biomarkers can be molecularly characterized and may reveal the repertoire of somatic genomic aberrations in a given moment and assist in the longitudinal analysis of the molecular characteristics of the tumor.

The accomplishment of identifying CTCs in the blood of patients with GBM still needs further validation in expanded studies, but it represents a step forward to understand the clinical behavior of brain tumors and their mechanisms of extracranial dissemination. Liquid biopsies in GBM will allow us to uncover the patterns of hematogenous dissemination in a tumor otherwise thought to be restricted to the brain; this may provide grounds to develop minimally invasive biomarkers of diagnosis and prognosis and, for example, help us recognize pseudoprogression, in which radiographic imaging frequently fails to distinguish between treatment-related response and tumor progression in brain tumors. The analysis of GBM CTCs could be also relevant for the selection of specific therapies and for monitoring the mechanisms of resistance to cytotoxic and targeted therapeutics. In these exciting times of precision medicine, the future application of GBM liquid biopsies should guide cancer care according to the GBM-associated molecular and genomic alterations “written” in the blood.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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