



Cancer-associated circulating atypical cells with both epithelial and macrophage-specific markers

Lucile Broncy¹, Patrizia Paterlini-Bréchet^{1,2}

¹INSERM Unit 1151, Faculté de Médecine, Université Paris Descartes, Paris, France; ²Laboratoire de Biochimie A, Hôpital Necker-Enfants Malades, Paris, France

Correspondence to: Patrizia Paterlini-Bréchet. INSERM Unit 1151, Faculté de Médecine, Université Paris Descartes, Paris, France.

Email: patrizia.paterlini@inserm.fr.

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In 2014, Adams *et al.* (1) reported on atypical circulating macrophages, that they named circulating cancer-associated macrophage-like cells (CAMLs), detected in the blood of breast and pancreatic cancer patients following enrichment by blood filtration. The Authors identified CAMLs as “giant cells of myeloid lineage (CD14+/CD11c+)* presenting with enlarged nuclei, CD45+ and exhibiting cytoplasmic staining by cytokeratins 8, 18 and 19 and epithelial cell adhesion

molecule (EpCAM)”. In the same year and month, Lustberg *et al.* reported about a population of circulating “atypical cells” expressing cytokeratins 8, 18 and 19, CD45 and CD68 markers without concomitant expression of EpCAM in the blood of metastatic breast cancer patients (2). Recent reports about circulating atypical macrophages have now shed more light on these cells, on the possible mechanism of their formation and on their relevance in tumor invasion.

*Note: all cellular markers mentioned in the text, figure or table are further defined here according to the information found in RefSeq Gene ID from PubMed 2018. B7-H4 is a cell surface antigen (encoded by the VTCN1 gene, meaning V-set domain containing T cell activation inhibitor 1) which interacts with ligands bound to receptors on the surface of T cells and has been correlated with tumor progression. The CD163 protein is a member of the scavenger receptor cysteine-rich superfamily and is exclusively expressed at the cell surface by monocytes and macrophages. CD146 refers to the Melanoma Cell Adhesion Molecule (MCAM) which is expressed in the cytoplasm of adipose and stromal progenitor cells. The CD68 protein is a transmembrane glycoprotein which is highly expressed by human monocytes and tissue macrophages. CD45 refers to the protein tyrosine phosphatase receptor type C (PTPRC) which is a transmembrane receptor expressed by mature leukocytes. The CD14 protein is a cell surface antigen expressed on monocytes and macrophages, but also present on other subtypes of myeloid cells such as dendritic cells. CD11b refers to the integrin subunit alpha M (ITGAM) and CD11c to the integrin subunit alpha X (ITGAX) which are both parts of leukocyte-specific integrins. CD133 refers to prominin 1, a transmembrane glycoprotein which localizes to membrane protrusions and is often expressed on adult stem cells, where it is thought to function in maintaining stem cell properties by suppressing differentiation. CD204 refers to the macrophage scavenger receptor 1 (MSR1) which is a macrophage-specific trimeric integral membrane glycoprotein. CD206 refers to the mannose receptor C-type 1 (MRC1) which is a type I membrane receptor that mediates the endocytosis of glycoproteins by macrophages. Cytokeratins (CK) are intermediate filaments expressed in epithelial tissues and are often used as a specific marker of epithelial cells. The epithelial cell adhesion molecule (EpCAM) is a membrane protein expressed on most normal epithelial cells that functions as a homotypic calcium-independent cell adhesion molecule. Vimentin is a type III intermediate filament protein which is responsible for maintaining cell shape and integrity of the cytoplasm in mesenchymal cells but has also recently been associated with tumor cells when expressed at the cell surface [i.e., cell surface vimentin, (CSV)].

Earlier studies had pointed out the heterogeneous nature of circulating “atypical cells”, in particular regarding circulating tumor cells (CTCs), endothelial and epithelial cells, fibroblasts, macrophages and megakaryocytes (3,4). However, only cytomorphological studies were possible at that time, as no immunolabelling-mediated characterization was available.

In 2012, Chen *et al.* used flow cytometry to detect circulating macrophages expressing CD68 and B7-H4, an antigen known to be expressed in tumor cells, in the blood of 56 lung cancer patients (5). Although the authors did not further characterize those cells, they showed that CD68+ and B7-H4+ circulating macrophages significantly correlated with tumor size and lymph node metastasis in their patient cohort.

Macrophages, derived from blood monocytes through differentiation, are innate immune cells involved in tissue homeostasis, inflammatory responses and wound healing (6). Usually categorized in a binary classification [M1, mainly pro-inflammatory, and M2, mainly anti-inflammatory and pro-tumorigenic, phenotypes (7)], macrophages are nonetheless recognized as a highly heterogeneous population displaying extensive plasticity in terms of molecular markers (8). Macrophages infiltrating the tumor tissue, termed tumor-associated-macrophages (TAM), have been long recognized as a functional feature of cancer (9), often related to poor prognosis of cancer patients (7). The possible involvement of macrophages in tumor cell intravasation, migration and extravasation at distant organ sites has been proposed to explain the role of TAM as prognostic indicators (10,11). In human cancer tissue sections, the presence of TAM is usually evidenced through immunostaining of the CD68 transmembrane glycoprotein (12). Yet, antibodies targeting CD14, CD16, CD163, CD204 or CD206 are also used to identify macrophages *in situ* (12)

Adams *et al.* and Lustberg *et al.* described for the first time circulating atypical cells with concomitant expression of macrophage-specific and epithelial cell-specific markers. Adams *et al.* speculated that CAMLs may represent different stages of myeloid differentiation and/or derive from non-specific engulfment of epithelial cellular debris. They also described that some CAMLs bind to and migrate in blood attached to CTC (1). Lustberg *et al.* noted that circulating CD45 positive, CK positive, CD68 positive cells were absent in healthy subject and in higher number in metastatic patients (2). Shortly thereafter, in 2015, Clawson *et al.* reported on cells that were thought to derive from fusion

of macrophages with tumor cells (MTFs) by culturing blood from melanoma patients. Cultured cells were large with pseudopod extensions and lamellipodia, with highly heterogeneous aneuploidy/polyploidy, expressing macrophage M2 markers (CD204, CD206 and CD163), melanocyte markers (ALCAM, MLANA) and epithelial markers (cytokeratins and EpCAM). MTFs generated metastases upon injection in nude mice. Since cells with these characteristics were also found in the melanoma tissues, the Authors concluded that MTFs are present in the blood of patients with melanoma and can potentially generate metastasis (13).

In a report published in 2016 by Adams *et al.*, CAMLs were further defined as cytokeratin-positive enlarged multinuclear cells expressing either CD14 or CD45 or both, to account for the heterogeneous marker expression profiles exhibited by CAMLs (14). The Authors also showed that CAMLs were present in 93% of blood samples from advanced stage breast cancer patients while none of the 16 healthy subjects presented CAMLs in their blood (14). However, 5 of 19 patients (26%) with benign breast conditions such as ductal hyperplasia, also scored positive for CAMLs, showing that CAMLs cannot be used to distinguish between breast cancer and benign breast diseases. The following year, Mu *et al.* determined the prognostic value of baseline CAMLs enumeration in metastatic breast cancer patients, showing a significant correlation of CAMLs numbers with patients' survival (15) and Zhang *et al.* demonstrated that, actually, macrophages can acquire expression of epithelial markers (cytokeratins, EpCAM) as well as stem cell markers (Oct4) upon phagocytosis of apoptotic cancer cells (16). Using density gradient centrifugation to isolate monocytes from patients with breast, cervical, ovarian, endometrial and pancreatic cancers, the Authors evidenced the presence of CD163 and EpCAM double-positive cells, which they called “tumacrophage”, in the blood of cancer patients (16). Importantly, like CAMLs, tumacrophages were consistently absent from the blood of healthy donors, suggesting their possible clinical value as a biomarker of cancer (1,14,16). Later on, in 2018, Li *et al.* reported on a class of circulating cells, which they called macrophage-like CTCs (ML-CTC) on the basis that those cells express macrophage markers (CD14 and CD68) and tumor markers (C-kit, DOG-1 and cell-surface vimentin) without concomitant CD45 expression, that they found in the blood of patients with metastatic gastrointestinal stromal tumors (GISTs) (17). The authors found that these patients had significantly

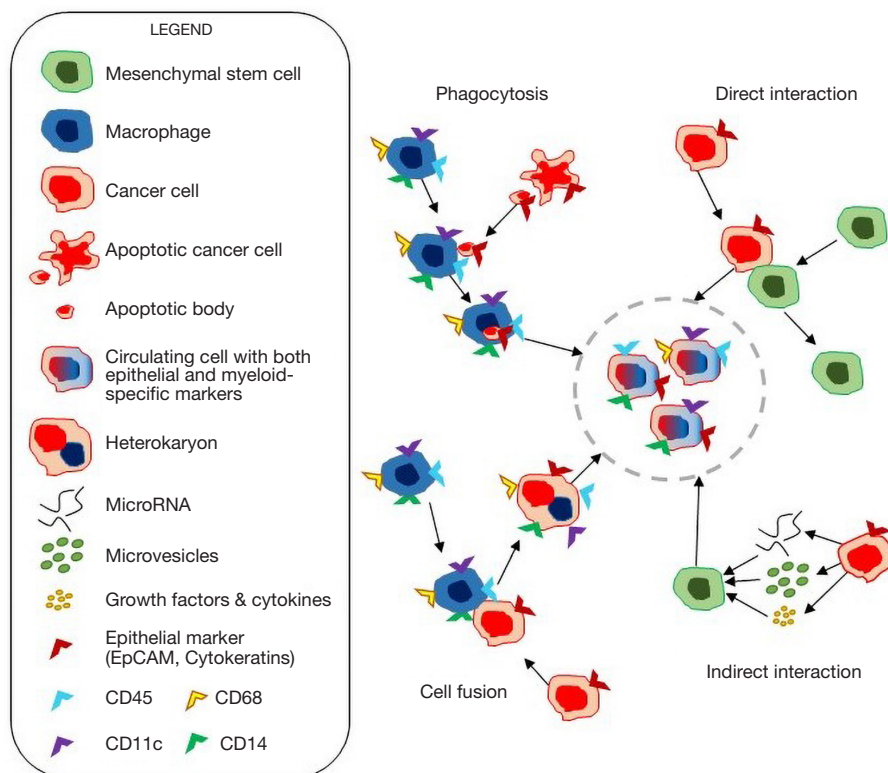


Figure 1 Possible mechanisms involved in the origin of circulating atypical cells with both epithelial and macrophage-specific markers.

greater numbers of ML-CTC than patients with localized GIST or cancer-free blood donors and hypothesized that CAMLs or ML-CTC may interact with CTC or other cells in the blood to promote metastases.

Recently, further light has been shed on the puzzling heterogeneity of circulating atypical macrophages. Gast *et al.* have demonstrated that the fusion of neoplastic cells with macrophages occurs *in vivo* and can generate hybrid cells with various combinations of phenotypes exhibiting enhanced metastatic behavior (18). Using a GFP⁺ mouse model with isogenic RFP⁺ tumors, the authors determined that fusion hybrid cells outnumber the canonical CTC in the blood of tumor-bearing mice, representing 90% of the tumor cells in circulation. Importantly, by sampling blood from pancreatic cancer patients at various stages, although in limited number, Gast *et al.* also demonstrated that circulating hybrid cells expressing cytokeratins and the CD45 leukocyte marker correlated with advanced disease while canonical CTC (expressing cytokeratins but not CD45) did not correlate with disease stage or patient survival (18).

The concept that hybrid malignant cells could be the

result of a fusion between myeloid cells and cancer cells dates back to 1911 (19). However, convincing evidence of such mechanism taking place *in vivo* and in tissues has only recently emerged (20). Furthermore, macrophage fusion with cancer cells in tissues had been proposed to explain the simultaneous presentation of macrophage epitopes and epithelial markers on hybrid cancer cells (13,21). Still, the report from Gast *et al.* provides extensive evidence about this phenomenon, the proof that these hybrid cells circulate in blood and that they display increased metastatic behavior.

Whether all circulating atypical cells with epithelial and macrophage-like features arise preferentially from cell fusion, or some of them derive from phagocytosis or other interactions between neoplastic cells and macrophages or mesenchymal stem cells (22,23), remains to be further elucidated (see Figure 1). Considering that those heterogeneous circulating atypical cells are increasingly recognized as valuable prognostic indicators in patients with breast, pancreatic and gastrointestinal cancers (Table 1), more studies are needed to generate a consensus sub-classification and identify possible correlations of specific cell subtypes with clinical data. However, the published

Table 1 Clinical aspects related to the detection of circulating atypical cells with epithelial and macrophage markers (CAC-EM)

Detection markers for CAC-EM (Ref)	Cancer patients (stages) & healthy controls	CAC-EM+ patients (%) or number of CAC-EM	Clinical impact of CAC-EM detection	Definition used for CAC-EM	CAC-EM cell characteristics
B7-H4, CD68+ (5)	56 lung cancer (20 stage I-II NSCLC + 36 stage III-IV NSCL patients); 21 tuberculosis + 30 healthy subjects	With CAC-EM: 6.3%; healthy donors: 8.9%; tuberculosis: 20.6% lung cancer	Cell number significantly correlated with disease stage in non-small-cell lung cancer	Circulating B7-H4+ CD68+ macrophages	B7-H4+ & CD68+
CK+, CD45+, EpCAM+/- (2,18)	32 metastatic breast cancer patients + 5 healthy subjects 20 pancreatic cancer patients for prognostic assessment	CAC-EM: mean 89.5 in cancer patients, mean 5 in healthy Not described (in mice experiments, 90% of CTC were found to be hybrids)	Prognostic value for overall survival CD45+, CK+ cells correlate with disease stage & predict overall survival	Circulating atypical cells Circulating hybrid cells (CHC) resulting from fusion	CK+, CD45+, CD68+, EpCAM- CK+ & CD45+ or EpCAM+ & CD45+
CK+, EpCAM+, CD14+, CD11c+, CD45+/- (1,14,15)	79 cancer patients (29 breast, 32 pancreatic & 17 prostate cancer) 41 breast cancer patients; 19 benign breast conditions; 16 healthy subjects	CAMLs detected in 97% of breast cancer patients CAML found in: 93% of invasive carcinoma patients (38/41), 26% patients with benign breast conditions (5/19), 0% healthy controls	In 29 breast cancer patients, CAMLs number was affected by treatment The presence of CAMLs (using a cutoff threshold of 1 CAML) distinguished cancer patients from patients with benign breast conditions with 88% sensitivity and 74% specificity	Circulating cancer-associated macrophage-like cells (CAMLs)	Giant cells of myeloid lineage (CD14+/CD11c+) with multiple enlarged nuclei, CD146+, CD45+ or CD45- with diffuse CK+ (CK8, 18, 19) & EpCAM+ cytoplasmic vacuoles. Shaped as oblong, amorphous or tadpole
CD14+, CD68+, CK+, EpCAM+, CD163+, CD204/6+ (13)	127 metastatic breast cancer patients (stage IV) 11 melanoma patients	CAMLs detected in 21 patients at baseline (16.5%) 8 samples enabled successful culture of hybrid cells (72%)	Patients with CAMLs had a significantly increased risk of disease progression Not determined	Macrophage-tumor cell fusions (MTFs)	Large cells with pseudopods & lamellipodia, rich mitochondria & lysosome. CD14+, CD68+, CK+, EpCAM+, CD163+, CD204/6+, MLANA+
CSV+, CD14+, CD68+, CD45- (17)	104 sarcoma patients (including 77 gastrointestinal stromal tumors: GIST)	73% of 44 GIST patients and 26% of 27 other sarcoma patients with ML-CTC	CSV+, CD14+, CD68+, CD45- cells predict GIST metastasis	Macrophage-like circulating tumor cells (ML-CTC)	Cell surface vimentin (CSV)+, CD14+, CD68+, CD133-, CD45-
CD14+, CD68+, CD163+ (16)	Colon, breast, ovarian & colorectal cancer patients	12 breast & 8 colorectal cancer patients	Not determined	Tumacrophage	CD14+, CD68+, CD163+, EpCAM+

scientific studies on atypical circulating cells with epithelial- and macrophage-specific markers strongly suggest their potential relevant role in tumor invasion, opening a new field of investigation for diagnostic and therapeutic improvements in cancer patients.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

- Adams DL, Martin SS, Alpaugh RK, et al. Circulating giant macrophages as a potential biomarker of solid tumors. *Proc Natl Acad Sci USA* 2014;111:3514-9.
- Lustberg MB, Balasubramanian P, Miller B, et al. Heterogeneous atypical cell populations are present in blood of metastatic breast cancer patients. *Breast Cancer Res* 2014;16:R23.
- Sandberg AA, Moore GE, Schubarg JR. Atypical cells in the blood of cancer patients; differentiation from tumor cells. *J Natl Cancer Inst* 1959;22:555-65.
- Hume R, West JT, Malmgren RA, et al. Quantitative observations of circulating megakaryocytes in the blood of patients with cancer. *N Engl J Med* 1964;270:111-7.
- Chen C, Zhu YB, Shen Y, et al. Increase of circulating B7-H4-expressing CD68+ macrophage correlated with clinical stage of lung carcinomas. *J Immunother* 2012;35:354-8.
- Oishi Y, Manabe I. Macrophages in inflammation, repair and regeneration. *Int Immunol* 2018. [Epub ahead of print].
- Singh S, Mehta N, Lilan J, et al. Initiative action of tumor-associated macrophage during tumor metastasis. *Biochim Open* 2017;4:8-18.
- Biswas SK, Mantovani A. Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm. *Nat Immunol* 2010;11:889-96.
- Balm FA, Drexhage HA, von Blomberg M, et al. Mononuclear phagocyte function in head and neck cancer. Chemotactic responsiveness of blood monocytes in correlation between histologic grade of the tumor and infiltration of these cells into the tumor area. *Cancer* 1984;54:1010-5.
- Condeelis J, Pollard JW. Macrophages: obligate partners for tumor cell migration, invasion, and metastasis. *Cell* 2006;124:263-6.
- Wyckoff JB, Wang Y, Lin EY, et al. Direct visualization of macrophage-assisted tumor cell intravasation in mammary tumors. *Cancer Res* 2007;67:2649-56.
- Heusinkveld M, van der Burg SH. Identification and manipulation of tumor associated macrophages in human cancers. *J Transl Med* 2011;9:216.
- Clawson GA, Matters GL, Xin P, et al. Macrophage-tumor cell fusions from peripheral blood of melanoma patients. *PLoS One* 2015;10:e0134320.
- Adams DL, Adams DK, Alpaugh RK, et al. Circulating Cancer-Associated Macrophage-Like Cells Differentiate Malignant Breast Cancer and Benign Breast Conditions. *Cancer Epidemiol Biomarkers Prev* 2016;25:1037-42.
- Mu Z, Wang C, Ye Z, et al. Prognostic values of cancer associated macrophage-like cells (CAML) enumeration in metastatic breast cancer. *Breast Cancer Res Treat* 2017;165:733-41.
- Zhang Y, Zhou N, Yu X, et al. Tumacrophage: macrophages transformed into tumor stem-like cells by virulent genetic material from tumor cells. *Oncotarget* 2017;8:82326-43.
- Li H, Meng QH, Noh H, et al. Cell-surface vimentin-positive macrophage-like circulating tumor cells as a novel biomarker of metastatic gastrointestinal stromal tumors. *Oncoimmunology* 2018;7:e1420450.
- Gast CE, Silk AD, Zarour L, et al. Cell fusion potentiates tumor heterogeneity and reveals circulating hybrid cells that correlate with stage and survival. *Sci Adv* 2018;4:eaat7828.
- Pawelek JM. Tumour-cell fusion as a source of myeloid traits in cancer. *Lancet Oncol* 2005;6:988-93.
- Yilmaz Y, Lazova R, Qumsiyeh M, et al. Donor Y chromosome in renal carcinoma cells of a female BMT recipient: visualization of putative BMT-tumor hybrids by FISH. *Bone Marrow Transplant* 2005;35:1021-4.
- Dittmar T, Zänker KS. Tissue Regeneration in the Chronically Inflamed Tumor Environment: Implications for Cell Fusion Driven Tumor Progression and Therapy Resistant Tumor Hybrid Cells. *Int J Mol Sci* 2015;16:30362-81.
- Fischer S, Cornils K, Speiseder T, et al. Indication of Horizontal DNA Gene Transfer by Extracellular Vesicles.

- PLos One 2016;11:e0163665.
23. Yang Y, Otte A, Hass R. Human Mesenchymal Stroma/
Stem Cells Exchange Membrane Proteins and Alter

Functionality During Interaction with Different Tumor
Cell Lines. Stem Cells Dev 2015;24:1205-22.

doi: 10.21037/jlpm.2018.10.05

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