METASTATIC BREAST CANCER AND CIRCULATING TUMOUR CELLS

Linda M McInnes and Christobel M Saunders
School of Surgery, The University of Western Australia, Perth, Western Australia, Australia.
Email: christobel.saunders@uwa.edu.au

Abstract
Circulating tumour cells – often referred to as a ‘liquid biopsy’ for cancer – can be found in a large proportion of patients with breast cancer that has spread to other organs. Changes in circulating tumour cell levels in metastatic breast cancer patients receiving chemotherapy have been shown to correlate with survival. Consequently, they have been shown to be an independently important way of predicting both the course of the metastatic disease, which will ultimately prove to be fatal, and the magnitude of response to systemic therapy. Circulating tumour cell research will not only elucidate the metastatic process but will also provide a platform for development of new cancer treatment regimens and drug targets. In this article, we will show how circulating tumour cell analysis is currently used in clinical practice, in clinical trials and the challenges that remain both in detecting these rare cells in the blood and in unravelling their molecular signatures, which may differ considerably from the primary cancer. As the isolation and characterisation of circulating tumour cells steadily improves, new metastatic breast cancer treatments will be developed, old regimes refined and patients will ultimately benefit.

Breast cancer, the most common cancer affecting women in Australia, claims the lives of approximately seven women every day in Australia.1 Deaths due to breast cancer are caused by metastatic spread of the tumour from the primary site to other parts of the body. Although some women who will ultimately have metastatic breast cancer present with it at time of diagnosis, the majority have localised disease at diagnosis. An Australian study found approximately one in 20 women with localised node-negative disease and one in six women with regional disease at diagnosis went on to develop metastatic breast cancer within five years of diagnosis.2

Metastatic breast cancer is a heterogeneous disease that can be confined to one site, to one organ (most commonly bone) or display diffuse and multiple organ involvement. Current identification of metastatic breast cancer relies on clinical manifestations of the metastases, biopsy results, imaging tests and serum tumour markers. Although metastatic breast cancer is considered incurable, the introduction of novel therapies and drug combination regimes (see Madigan et al, this issue) has led to considerable prognostic improvement for most patients. Metastatic breast cancer is treated with systemic therapy (chemotherapy, biological therapies and/or endocrine therapy), often local palliative radiation and less commonly palliative surgery.3 The choice of treatment depends on: the type of primary cancer; receptor status (hormones; oestrogen (ER), progesterone and human epidermal growth factor receptor 2 (Her2)); size and location of metastases (visceral versus nonvisceral); patient age, comorbidities and preferences; and previous treatments and response.3

Role of circulating tumour cells and epithelial to mesenchymal transition in cancer metastasis
The mechanisms of metastasis have been rigorously debated since 1874, when British surgeon Campbell Greig De Morgan postulated that breast cancer arose locally, spread to lymph nodes and thereafter to other organs.4 We now understand cancer spreads via growth in situ, and via blood and lymphatics.5 Furthermore, breast cancer is known to not only spread from breast to lymph nodes and other organs, but also from metastatic lesions, re-seeding breast tissue and distant sites.6 The tumour cells are called circulating tumour cells (CTC) when found in blood, and disseminated tumour cells when located in the bone marrow. Depending on the method of isolation and the tumour burden, these cells can be found in up to 50-70% of women with breast cancer which has spread.7

Increasingly, experimental data show that CTCs have undergone epithelial-mesenchymal transition (EMT), a transient process by which fixed, rigid epithelial cancer cells lose their apical-basal polarity and transition to an intermediary elongated (fibroblast-like), motile, mesenchymal cell, allowing escape from the primary tumour site, resisting apoptosis (programmed cell death) and allowing transportation via the blood circulatory system to establish metastases (figure 1).8-12 The EMT mechanism was originally recognised in embryogenesis and wound healing.13

Isolation, characterisation and analysis of CTCs and DTCs from metastatic breast cancer patients are important for developing deeper understanding of metastatic spread and may lead to improved treatments. Analysis of CTCs is
favoured over that of DTCs due to the invasiveness of bone marrow sampling required for DTC collection. This article will therefore focus on the isolation and analysis of CTCs in metastatic breast cancer.

Circulating tumour cell characterisation and isolation

CTCs were first observed and described in 1869 by the Australian physician Thomas Ashworth. CTCs are, in most patients, extremely rare (~1-10 CTCs to 10^9-10^10 normal blood cells), and are difficult to detect with immunohistochemistry, however the development of immunomagnetic capture techniques, allowing enrichment of tumour cells, has made this easier. CellSearch (Veridex, US), a semi-automated instrument with an immunomagnetic bead capture system based on epithelial cell adhesion molecule (EpCAM), followed by immunofluorescence analysis, has become the most commonly-used CTC assessment method for epithelial cancers such as breast cancer, gaining Food and Drug Administration approval in the US in 2004 for metastatic breast cancer. Its use in Australia outside a research setting is limited by the very small number of machines available, the lack of Therapeutic Goods Administration approval, emergence of evidence of EMT in CTCs which may cloud its efficiency, and still poorly understood clinical utility. Other CTC immunomagnetic isolation methodologies include the Isoflux system, AdnaTest, CTC-chip, Herringbone-Chip, and Magsweeper, and methodologies which utilise CTC physical properties (isolation by size of epithelial cells), Dean Flow Fractionation, microtube device, and filter based technique or techniques, using a combination of immunological and physical properties to isolate cells. Although comparisons of CellSearch with the other CTC isolations methods have reported discordance in detection rates with some methods reported to be more sensitive or specific, CellSearch remains the predominant CTC isolation method in clinical trials due to the FDA approval and depth of experience.

In 2004, Cristofanilli et al. using the CellSearch system, conducted a landmark study enumerating baseline CTCs of patients with metastatic breast cancer prior to the commencement of a new line treatment and at the first follow-up visit, and determined that a CTC baseline of 5 per 7.5 mL whole blood distinguished patients with a slow disease progression from those with rapid disease progression. Subsequent studies reported ≥5 CTCs correlate better with overall survival than radiological changes. This has been confirmed by the recently presented SWOG S0500 randomised trial, which confirmed patients whose CTC count was low at start of treatment for metastatic breast cancer had a better survival, and for those whose count was high and remained elevated three weeks into chemotherapy, survival was worse.

Circulating tumour cells as a guide to treatment selection

It is hoped that monitoring CTC levels and characteristics can be useful in deciding when to change treatment due to progressive disease and potentially to help decide initial treatment for metastatic breast cancer.

Changes in CTC levels in metastatic breast cancer patients receiving chemotherapy correlates with survival. Yet changing chemotherapy regimen in response to CTC count does not appear to improve overall survival or time to progression, as shown in a number of studies including the SWOG S0500 study. In this study, CTC count was prognostic, but in those whose CTC levels remained high after a cycle of chemotherapy, changing treatment did not improve survival. Although chemotherapy can be useful in metastatic breast cancer patients, this study suggests other more effective treatment options are needed. This is still being studied in patients on third line chemotherapy in the CirCé01 trial (France, NCT01349842). The randomised CirCé01 trial will enumerate CTCs before and after the first dose of a third line of chemotherapy. If the CTC values do not respond to treatment, then a subsequent fourth and fifth line of chemotherapy will be tested in the same manner. In this instance, CTCs are being used to tailor individual treatment and reduce the usage of costly, toxic, ineffective chemotherapeutic agents during palliative care.
The potential of using changes in CTC levels to guide non-chemotherapeutic treatments is also being explored. Minimal survival gains with first line endocrine therapy alone are seen in patients with ≥5 CTCs/7.5 mL, regardless of tumour hormone receptor status. Patient treatment selection on the basis of CTC baseline is currently being examined in the STIC CTC Metabreast clinical trial (France, NCT01710605), which will stratify treatment of hormone sensitive, Her2- metastatic breast cancer patients based on CTC count to chemo- or endocrine therapy.

A number of studies have reported discordance between tumour and CTC hormone receptor and Her2 receptor status, with hormone positive primary tumours and negative CTCs and vice versa, and this may signal altered treatment response in metastatic breast cancer. Trials currently underway evaluating such discordance include those using Lapatinib in the DETECT III trial (Germany, NCT01619111), Herceptin in the TREAT-CTC trial (European Union, NCT01548677) or Trastuzumab-emtansine (T-DM1) in the T-DM1 trial (France, NCT01975142) and a Dana-Farber trial assessing Trastuzumab and Vinorelbine (US, NCT01185509).

There is to date no methodology to identify the endocrine resistant metastatic breast cancer patients who would benefit from a switch to chemotherapy treatment as a first line treatment. The COMETI PD clinical trial (US, NCT01701050) seeks to use an algorithm, the CTC-Endocrine Therapy Index (CTC-ETI), which incorporates CTC counts and measures of CTC endocrine sensitivity (ER and B-cell lymphoma 2 (Bcl2)) and resistance to chemotherapeutics (Her2 and Ki67) to identify ER+, Her2+ metastatic breast cancer patients who would benefit from being treated with chemotherapy as a first line therapy. Circulating tumour cells challenges

While the analysis of CTCs is demonstrating great promise for personalised medicine, there are many challenges. The dynamic processes by which cancer cells undergo continuous evolution means structuring cancer treatment on the status of the primary tumour, or even a single metastatic biopsy, is inherently flawed. The feasibility of performing several biopsies on a patient in order to keep abreast of an ever-changing cancer cell population is difficult. CTC analysis offers a less invasive ‘liquid biopsy’, however heterogeneity is also evident in the CTC population. The challenge is how to establish an efficient method to isolate this rare and heterogeneous population and characterise it effectively. Reliance on the EpCAM marker and cytokeratin expression, the basis of CellSearch for the isolation of CTCs from blood is problematic – many CTCs show low EpCAM expression and may have undergone EMT, yet other isolation methodologies are not fully validated. There is therefore an evident need for an isolation methodology that can recognise CTCs that have undergone EMT and exhibit few epithelial characteristics and more pronounced mesenchymal traits. In addition, the clinical relevance of circulating tumour masses (CTM), clusters of CTCs which may also incorporate accessory host cells, needs to be examined and incorporated into isolation methodology. Several studies hypothesise that the CTM environment enhances CTC survival in the bloodstream and is therefore an important part of the metastatic process. Importantly, interaction of tumour cells with platelets, which are integral to CTM, has been shown to induce EMT in the tumour cells.

A novel CTC capture using mesenchymal-marker based ferrofluids (N-cadherin or O-cadherin) has been developed at Duke University, in acknowledgement of the EMT process. This system will be evaluated in a clinical trial on metastatic castration-resistant prostate cancer and metastatic breast cancer patients (US, NCT02025413).

In addition to CTCs which have undergone EMT, subpopulations of CTC with stem cell characteristics may not be adequately detected by current methods, and may indeed be very important in initiating and sustaining the metastatic process. We are on the brink of understanding how these cells form, circulate and contribute to metastasis and studies elucidating the importance of CTMs and stem cells, culturing CTCs and analysing single cells for genomic and other alterations, will further advance our understanding of how these cells contribute to disease.

Conclusion

CTC analysis – the ‘liquid biopsy’ for cancer – has been shown to be an important prognostic and potentially predictive marker in metastatic breast cancer. However, their promise is yet to be fulfilled, with research needed to elucidate CTC heterogeneity and with ongoing clinical trials trying to establish how best to use CTCs to guide treatment. Evidently, we need to develop better ways to enumerate and characterise these rare but fascinating insights into the metastatic process.

We currently do not recommend the use of CTCs in Australia outside a clinical trial, but encourage further work in the area of technology development, enhancing understanding of the underlying biology of CTCs, and the metastatic process. The ultimate goal is to translate CTC knowledge and advances into better care of our patients with metastatic breast cancer.

References

15. Ashworth T. A case of cancer in which cells similar to those in the tumors were seen in the blood after death. Australian Medical Journal. 1869;14:146-9.