

## Metastatic Breast Cancer – Opportunities from Novel Diagnostic Modalities

a report by

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Breast cancer remains the most frequent type of cancer in women, with approximately 212,600 new cases (1,300 male) diagnosed each year in the US. Breast cancer is among the leading causes of cancer death in women (15% of all cancer deaths in women) with an estimated 40,200 cancer-related deaths (400 male) in 2003.<sup>1</sup>

Despite years of clinical research, the odds of achieving complete resolution of metastatic disease with current treatment strategies remain low.<sup>2-4</sup> The majority of patients with advanced disease achieve various degrees of objective remission, usually transient, with conventional treatments, and develop evidence of progressive disease within 12 to 24 months of initiating treatment.<sup>4</sup> For these patients, systemic treatment has rarely translated into a significant improvement in survival<sup>5</sup> but has generally substantially improved the quality of life. The identification of these patients at the time of their initial diagnosis of metastatic disease remains a challenge. A few retrospective studies have demonstrated that these long-term survivors are usually young, have an excellent performance status and, more importantly, have limited metastatic disease.<sup>5-7</sup> Several clinical factors have been proposed that would help in predicting long-term outcome and efficacy of treatments. These include a greater number of involved nodes at diagnosis, visceral metastases, primary tumor measuring >2cm, and performance status of the patients.<sup>8-10</sup>

The use of an intense follow-up program in women with high risk of breast cancer has been advocated in the past, based on the criteria that the early detection and consequent treatment of asymptomatic disease could have translated into a survival benefit for the patients. However, two large randomized trials investigating the relationship between an intensive follow-up policy using standard imaging and routine tests (including bone scans, liver ultrasound, chest X-rays, and blood routine tests) and overall survival failed to detect any advantage for the more aggressive approach.<sup>11,12</sup> For the asymptomatic patient, current universally adopted guidelines recommend a regular history, an annual mammography, and physical examination every three to six months for two years, then every six to 12 months for three years, and then annually. In the asymptomatic patient, or when a recurrence is suspected, complete and accurate non-invasive imaging

data are needed to improve clinical management. The advent of more sensitive functional imaging modalities raises the issue of the possible value of early detection of metastatic disease and possibility of more effective systemic treatments in that setting. One example is represented by positron emission tomography (PET) using 2-fluoro-2-deoxy-D-glucose (FDG) that allows metabolic and functional imaging of tumor cells. FDG PET has been shown to improve detection of distant relapses over conventional imaging obtained with computed tomography (CT) and magnetic resonance imaging (MRI).<sup>13</sup> However, the limited accuracy of lesion localization using PET alone impairs its clinical value, particularly with those patients in whom a precise re-staging is necessary. A hybrid PET/CT scanner has been recently developed for the simultaneous acquisition of anatomic (CT) and functional (PET) data. The superimposition of CT with high spatial resolution improves the localization of areas of increased uptake in PET by directly combining functional and morphological information, allowing more reliable anatomical lesion definition. A definite gain in diagnostic accuracy for neoplastic lesions ranging from approximately 20% to 40% can be achieved with the use of integrated PET/CT compared with PET alone. The potential of PET/CT to become a widespread diagnostic tool and to improve clinical efficacy needs to be properly addressed and evaluated in well designed, prospective, randomized clinical trials.

The increasing availability of laboratory techniques able to detect the presence of cancer cells, or new predictive or surrogate markers in several tissue and body fluids, offer the opportunity to evaluate microscopic disease (MCD) in breast cancer. The detection of MCD in breast cancer has been evaluated in lymph nodes, bone marrow (primary breast cancer), and peripheral blood (metastatic disease). The majority of these studies have demonstrated that the detection of MCD in breast cancer patients contributes prognostic information and, in selected cases, it can predict the efficacy of treatments. In primary breast cancer the detection of microscopic disease in lymph nodes and bone marrow contributed to describe the role of minimal residual disease in this disease. In patients with metastatic breast cancer (MBC) these novel approaches have demonstrated unique features and could significantly



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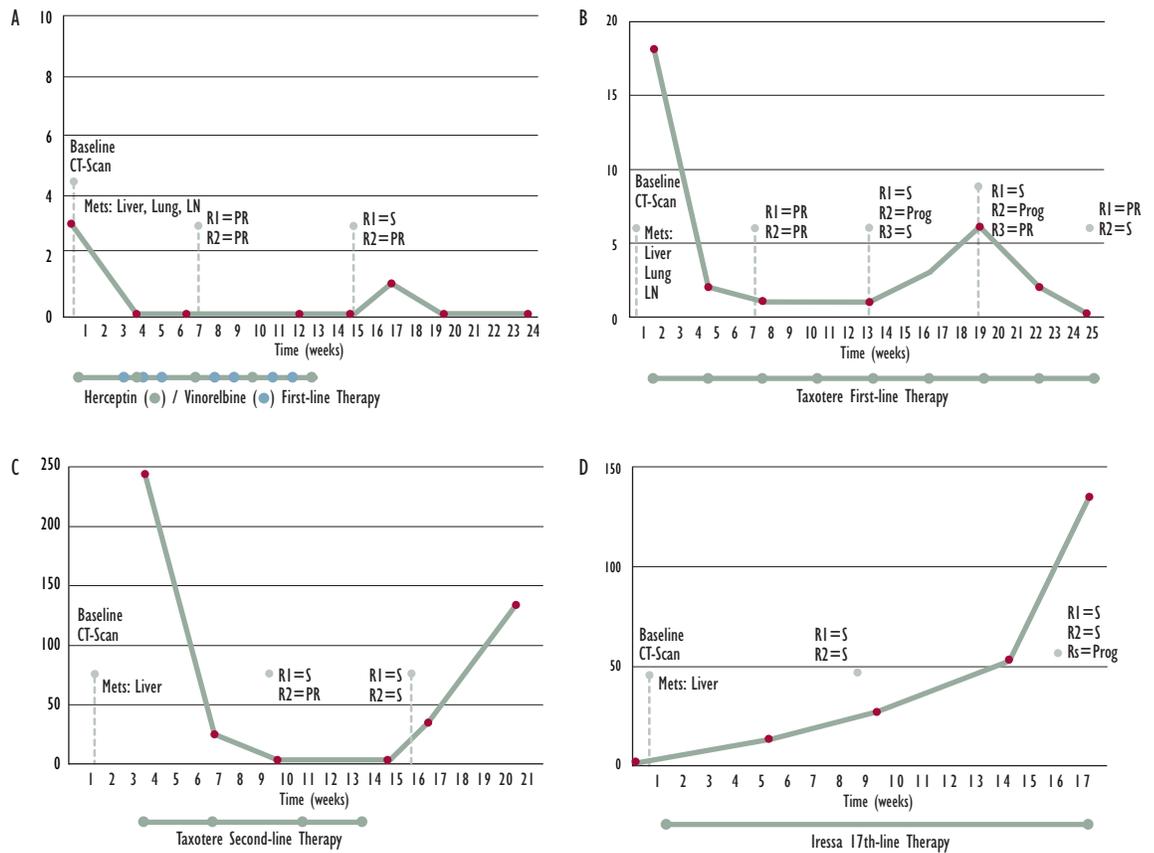


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**Figure 1: Longitudinal Trends of CTC Observed in Metastatic Breast Cancer Patients Starting a New Line of Therapy**



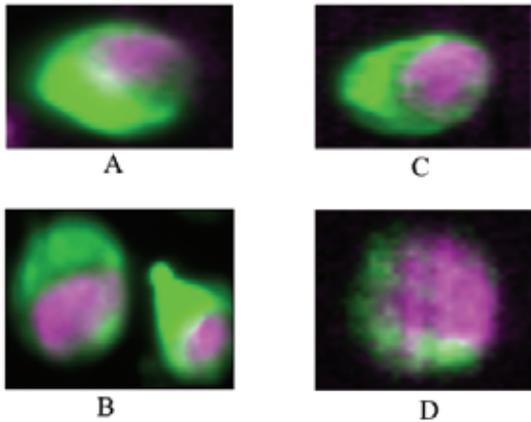
Panel A, CTC do not exceed 5/7.5ml of blood. Panel B, decrease of CTC. Panel C, CTC decrease followed by an increase. Panel D, CTC increase. Site of metastasis is indicated in the panels. Response to therapy (PR = partial response, S = stable disease, Prog = disease progression) was assessed using World Health Organization (WHO) criteria for bidimensional imaging by two independent radiologists R1 and R2. In case they did not agree a third radiologist R3 read the image. Rs = Prog indicates that the radiologist at the site saw progression after reading the same CT-scans.

modify current approaches to the diagnosis and treatment by providing an invaluable tool for patients' stratification (prognostic feature) and assessment of treatment efficacy (predictive feature).

The hematogenous route offers a potential source of circulating tumor cells (CTCs). Various different molecular and cellular approaches have been proposed for the detection of CTCs.<sup>14</sup> Immunohistochemistry (IHC), flow cytometry (FC) and reverse transcription polymerase chain reaction (RT-PCR) have been used to detect CTC but with several methodologic issues associated with each of these techniques. Recently, the CellSearch™ system (Veridex LLC, a Johnson & Johnson company, Warren, NJ) has been used for the detection and enumeration of CTCs in a prospective, multicenter trial in 177 patients with MBC.<sup>15</sup> The procedure enriches the sample for cells expressing the epithelial-cell adhesion molecule with antibody-coated magnetic beads, and it labels the cells with the fluorescent nucleic acid dye 4,2-diamidino-2-phenylindole dihydrochloride. Fluorescently labeled monoclonal antibodies specific for

leukocytes (CD45-allophycocyan) and epithelial cells (cytokeratin 8,18,19-phycoerythrin) are used to distinguish epithelial cells from leukocytes. The identification and enumeration of CTCs were performed with the use of the CellSpotter™ analyzer, a semi-automated fluorescence-based microscopy system that permits computer-generated reconstruction of cellular images. The results of this trial indicate that in metastatic breast cancer the level of CTCs before a new therapy is initiated and, even more importantly, the level measured at the first follow-up visit are useful predictors of progression-free survival and overall survival. Circulating tumor-cell levels of five cells per 7.5ml of blood – a cut-off point that was prospectively identified in patients in a training set and confirmed in patients in a validation set – gave a reliable estimate of disease progression and survival earlier than estimates made with the use of traditional imaging methods (three to four weeks versus eight to 12 weeks after the initiation of therapy, respectively). Patients with levels of CTCs equal to or higher than five per 7.5ml of whole blood, compared with the group with fewer than five CTCs/7.5ml, had a shorter median

**Figure 2: Selected CTCs are Represented in these Four Images**



These cells are characterized by expression of the cytoskeleton protein by cytokeratin phycoerythrin (CKPE), the nucleus visualized by DNA staining (DAPI), the overlay of cytokeratin (green) and DAPI staining (purple and white), and the absence of the leukocyte-specific antigen by CD45-Allophycocyan (APC).

progression-free survival (2.7 months versus 7.0 months,  $p < 0.001$ ) and shorter overall survival (10.1 months versus  $> 18$  months,  $p < 0.001$ ). At the first follow-up visit after the initiation of therapy, this difference between the groups persisted. In a multivariate analysis, the predictive value of the level of CTCs, either at baseline or at the first follow-up visit, was independent of the time to metastasis, the site of metastasis (visceral compared with non-visceral), and hormone-receptor status (see Figure 1). These data would suggest that the detection of CTCs could be used for patients' stratification in the design of clinical trials, enabling the detection of magnitude and type of treatment benefit for each cohort of patients. This will practically allow for the design of more clinically tailored treatments in metastatic disease.

Along the same line, it is hoped that the improvement in the knowledge of the biological steps involved in the process of tumor progression and metastasis will contribute to the discovery and development of more appropriate agents to intervene at various steps of the metastatic process. Perou and colleagues have proposed a novel molecular classification of breast cancer based on DNA microarray hierarchical cluster analysis.<sup>16</sup> They identified two large subgroups of breast cancers with separate gene expression profiles. The first group was characterized by the relatively high expression of genes normally expressed in breast luminal epithelial cells and included mostly estrogen receptor (ER)-positive tumors. Genes present in basal-like epithelial or myoepithelial cells, including primarily ER-negative tumors, characterized the second group. Based on these findings, a new molecular classification was proposed dividing breast cancers into 'luminal-like' and 'basal-like' types. The classification scheme was further tuned by adding other molecular subgroups including 'luminal

types A, B, and C', 'erbB2-positive', and 'normal-breast-like'.<sup>17</sup> From a biological point of view, this scheme suggests that most breast cancers may originate from one of two different cell types, luminal or basal-like epithelial cells of the terminal ducts. From a clinical point of view, the value of a novel tumor classification scheme lies in its ability to predict disease outcome or response to therapy. Published evidence suggests that 'basal-like' (ER-negative) and 'erbB2 positive' subgroups have the shortest relapse-free and overall survival, whereas the 'luminal type' (ER-positive) shows more favorable clinical outcome.<sup>18,19</sup> Moreover, recent evidence has demonstrated that cancers can be regarded as an abnormal organ in which a small population of cancer stem cells drives tumor growth, giving rise to phenotypically diverse cancer cells of different proliferative potential or behaviors – tumorigenic and non-tumorigenic populations.<sup>20</sup> However, if therapies fail to eradicate the tumorigenic cells, then these cells would be able to regenerate the tumor. Experimental evidence is in favor of an adult mammary epithelial stem cell in the normal breast and its identification is in progress.<sup>21</sup> Applying the principles of stem cell biology to breast cancer should allow elimination of this crucial population of malignant cells, leading to more effective cancer therapies.<sup>22</sup> It must be asked how these two areas of research can be integrated and how they can both provide the clue to the establishment of more 'biologically tailored' treatments with definitive improvement in outcomes. Presently, no data are available on the different molecular subgroups' sensitivity to various cytotoxic agents or other biological agents.

Increasing research interest relates to the possibility that the tumor microenvironment might play a role in the process of carcinogenesis and metastatic development, essentially representing a potential therapeutic target.<sup>23,24</sup> This area of research is still very controversial – this article summarizes the opposite view on this topic. Recently, it has been postulated that the capacity to metastasize might be acquired relatively early during multistep tumorigenesis and is intrinsic to the tumor.<sup>25</sup> This hypothesis is based on findings that in human breast cancer, gene expression profiles of the whole primary tumor can predict disease outcome. A 'poor-prognosis signature' has been validated and is strongly predictive for the development of distant metastases.<sup>26,27</sup> In addition, gene expression profiles of primary breast tumors are maintained in their distant metastasis.<sup>28</sup> However, the use of DNA microarray cannot define the possible role of different variant subpopulations of cells of high metastatic potential and of different tissue-specific expression profiles in predicting the site of metastasis. Moreover, complex interplays between stromal and tumor cells have been identified suggesting a paracrine mechanism of action.<sup>23</sup> It has recently been demonstrated that a particular

component of this process, involving chemokines and their receptors, could play a critical role in the metastatic process. In fact, altered chemokine receptor/ligand expression levels are being studied as possible indicators of progression to tumorigenicity, different metastatic capacity, and preferential homing.<sup>29,30</sup>

In summary, improvements in laboratory technologies have demonstrated that the clinical heterogeneity of MBC can be predicted at the time of diagnosis by determination of CTCs. This represents the initial step

in the further classification of metastatic disease that will translate into a change in the pattern of practice in the management of this disease (clinically tailored treatments). Furthermore, the demonstration that breast cancers derive from cells with differences in biological features will contribute to better understanding of the process of carcinogenesis and define the parameters for more biologically tailored treatments. The future clinical impact of such discoveries in an almost incurable disease needs to be prospectively evaluated in randomized clinical trials. ■

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