Biomarkers detecting minimal residual disease in solid tumors: what do they mean in the clinical management of patients?

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After curative resection of the primary tumor in nonmetastatic cancer, there are three questions to be answered. First, is adjuvant therapy necessary to decrease the risk of future relapse? Second, if so, what therapy? And third, how to monitor the response?

The decision to use adjuvant therapy is based on the clinical-pathological findings of the primary tumor stage according to the TNM classification, tumor differentiation and vascular, lymphatic and/or perineural infiltration. Patients are classified according to the risk of relapse and based on the results of large double-blinded randomized trials assigned to adjuvant therapy (chemotherapy, radiotherapy, immunotherapy or hormonal therapy as monotherapy or in combination). Adjuvant therapy may be nonspecific such as chemotherapy with/without radiotherapy or specific. Specific therapy is based on the characteristics of the primary tumor; examples are trastuzumab for HER-2-positive breast cancer, tamoxifen for estrogen receptor positive breast cancer [1]. In colon cancer, the determination of tumor gene status for *RAS* and *BRAF* is done individually, as part of next-generation sequencing, the use of microsatellite instability, or mismatch repair testing [2]. In patients with advanced non-small-cell lung cancer, *EGFR* mutation testing, ALK testing and PD-L1 testing (category 1 based evidence), *BRAF* and *ROS1* testing are utilized to determine specific therapies [3]. The third question is how to monitor treatment, as imaging studies are initially negative for disseminated cancer and follow-up is based on detecting treatment failure and the appearance of metastasis. The level of prostate-specific antigen after primary treatment is used as a serum biomarker to determine the need for treatment and to monitor treatment response in non-metastatic cancer only in prostate cancer.

The rationale for the use of adjuvant therapy is that the presence of occult cancer cells, which have dissemination prior to surgery, will cause future relapse and their elimination will improve disease-specific survival. These occult cancer cells are termed minimal residual disease (MRD). To detect MRD there are three forms, the detection of circulating tumor cells (CTC), detection of disseminated tumor cells or micro-metastasis in bone marrow (DTC) and circulating tumor DNA. Each method has its pitfalls. CTC detection is method dependent; those based on enrichment using EpCAM such as CellSearch[®] do not detect EpCAM-negative CTCs, such as those that have undergone the epithelial–mesenchyme transition and can be found in benign disease [4–7]. Those based on cell size or density, such as Oncoquick[®], Screenshot[®] or ISET[®], do not detect cell clusters or CTCs smaller than 8 μm . DTCs are detected in bone marrow aspirates or biopsies and identified using immunocytochemistry or RT-PCR, which is a more invasive procedure, but serious adverse events are reported in only 0.07% of cases [8]. Both CTCs and DTCs are detected with increasing frequency with higher stage nonmetastatic cancer and their presence is associated with a worse prognosis [8–12]. More recently, the circulating tumor-free DNA (ctDNA) has been used as a measure of MRD. At present, only the detection of tumor-specific mutations on cell-free DNA indicates the presence of ctDNA. In cancer patients, a major source of ctDNA is from necrotic or apoptotic tumor cells, a minor component is that secreted from viable cancer cells, however there is no form of distinguishing between ctDNA from live or dying/dead tumor cells [13].

Primary tumor phenotypic and genotypic characteristics may be different from those characteristics in cells, which have disseminated and implanted in distant sites. Thus the selection of specific treatments based on the characteristics of the primary tumor may not be the most appropriate. HER2 expression in bone marrow micrometastasis has been reported to be up to 60% in comparison with the 20% expressed in primary breast tumors [14]. Similarly, HER-2-positive prostate cells are associated with resistance to androgen blockade but not diethylstilbestrol [15]. In colon cancer, *KRAS* and *BRAF* mutations detected in primary and metastatic cancer are used as predictive markers for anti-EGFR therapy. However, there is discordance between *KRAS* mutations in CTCs and primary tumors; there are more mutations in CTCs in stage I and II patients and less in stage IV patients. Furthermore, the more aggressive *KRAS* codon 12 mutations were more frequently detected than the *KRAS* codon 13 mutations in CTCs [16].

Thus the use of MRD to determine specific treatments may improve treatment results. The repeated use of MRD classification would allow changes in treatment before the appearance of metastatic disease. Changes in tumor cell sensitivity by clonal evolution or clonal selection detected during follow-up would identify patients at the risk of relapse. Serial sampling of CTCs has shown changes in the *KRAS* mutational status during treatment and thus predict patient response to targeted therapy [17].

This leads to another important question: when should samples be taken? CTCs are rapidly cleared from the circulation, mostly within 24 h [18]. There is no information on the time scale of elimination of micrometastasis. As for tfDNA, the question of how long tfDNA continues to be released from dead and dying tumour cells after treatment is not known.

More recently, differing types of MRD have been identified; those patients with CTCs are at early risk of relapse whereas those with only bone marrow micrometastasis of late relapse [12,19]. It appears that patients with CTCs have a more aggressive disease than those with only DTCs; this has been reported in esophageal cancer [20], colon cancer [12], prostate cancer [19], pancreatic cancer [11] and breast cancer [21].

This raises the interesting question of do all MRDs need to be treated? With increasingly sensitive molecular techniques to detect tumor cells, it may be that below a certain tumor load, the body is able to control tumor cell proliferation. This is the concept of dormancy. However, with time, due to changes in the tumor cell or stromal microenvironment, the cells begin to proliferate and disseminate, giving rise to metastatic diseases. This has been reported in patients with chronic myeloid leukemia post bone marrow transplant, whereby PCT-detected tumor cells are found in patients in 'complete' remission years after transplant [22]. Thus a form of active surveillance may be more effective, starting treatment when CTCs appear. This leads to the next question: do all MRDs need to be eradicated? In myeloma, treatment is continued until the paraprotein is stable, the 'plateau' phase of the disease. In patients achieving plateau, active treatment is stopped and maintenance treatment such as thalidomide, lenolidamide and/or bisphosphonates is used until the paraprotein levels start to rise. If CTCs are eradicated, leaving only bone marrow DTCs, is this equivalent to plateau phase in myeloma? Bisphosphonates have been reported to eliminate CTCs and DTCs [23–26] and as such could have a role in extending the 'plateau phase' of solid tumors.

Combined methods of detection and characterization may yield more clinically meaningful data, for example CTCs with DNA/RNA analysis permits the morphological confirmation of a tumour cell with a specific marker, for example *BRAF* to define specific treatments. Due to the heterogeneity of tumor cells, not all tumour cells will be positive for a given marker; for example, HER-2, *BRAF*; thus tfDNA negative for *BRAF* does not imply the absence of CTCs but the absence of CTCs positive for *BRAF*. This would also permit the identification of clonal selection post-treatment, such as ARv7 variants in prostate cancer patients and the selection of androgen blockade [27]. Single CTC sequencing shows the genetic heterogeneity of these cancer cells and has been reported to identify therapeutic resistance, multiple mutations in the ALK-independent pathways and the *RTK-KRAS* genes seen with crizotinib and lorlatinib resistance in patients with non-small-cell lung cancer [28].

At present, there is not a single 'gold-standard' method for the detection of CTCs and/or DTCs however, the detection of CTCs and DTCs overcomes many of the limits of surgical biopsies. Both blood and bone marrow can be repeatedly sampled for the detection of cancer cells thus detecting changes in the phenotypic and genotypic characteristics before the appearance of clinical disease.

In summary, it is important to define and understand what biomarkers mean, both positive and negative results, and the purpose of their use. Their possible role in the detection of MRD after primary curative therapy identifies high-risk patients in whom additional treatment is most likely to be beneficial. A possible role during follow-up is the early detection of treatment resistance enabling changes in treatment before the appearance of clinical metastasis.

When detecting MRD, it is important to recognize that there are subtypes and it may not be necessary to eradicate all subtypes of MRD in order to produce clinical benefits. In the real world, health budgets do not allow high technology, high cost tests, bone marrow sampling with immunocytochemistry or detection of circulating tumor cells using density gradient differential centrifugation and immunocytochemistry or FISH may be more readily available in district hospitals. Equally important are results based on a positive/negative or yes/no decision-making choice and thus making the oncologist's job easier.

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