^{99m}Tc-MIBI in the Evaluation of Breast Cancer Biology

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CONTENTS

7.1 Introduction

Scintigraphic in vivo evaluation of complex cellular processes such as proliferation, apoptosis, receptor/ligand interactions, transport of substrates and metabolism of nutrients in human cancers is a wide and continuing evolving area of investigation in nuclear medicine (Denoyer, et al. 2006; Been et al. 2004; Corsten et al. 2006; Weissleder 2006). A major purpose in this area is the non-invasive detection of well-known biochemical, molecular and histological markers of tumor aggressiveness, invasiveness and resistance to therapy, which may provide rational criteria for a fine tuning of therapeutic strategies in individual patients.

In the last decade, ^{99m}Tc-labeled lipophilic cations, originally developed as myocardial perfusion agents and subsequently used as tumor-seeking agents in a variety of human neoplasms, emerged as suitable tools to explore specific cellular processes and functions in malignant tumors. The class of ^{99m}Tc-labeled lipophilic cations includes several tracers such as ^{99m}Tc-MIBI, ^{99m}Tc-tetrofosmin and ^{99m}Tc-furifosmin, which share common biophysical, chemical and pharmacokinetic properties (Sharma 2004). In particular, ^{99m}Tc-MIBI and analogous ^{99m}Tc-labeled agents share similar mechanisms of uptake in both normal and malignant cells. A number of studies consistently show the passive influx of these lipophilic cations in response to large negative plasma membrane and mitochondrial membrane potentials as well as the reversible accumulation within mitochondria of both normal and malignant cells (Piwnica-Worms et al. 1990; Delmon-Moingeon et al. 1990; Carvalho et al. 1992).

Another common property of these tracers is the ability to interact with P-glycoprotein (Pgp), which is responsible for their active outward transport in the extracellular compartment (Piwnica-Worms et al. 1993). Human P-glycoprotein is a 170-kDa transmembrane protein that is encoded by the MDR1 gene and acts as an energy-dependent drug efflux pump of broad specificity (Szakacs et al. 2006). Overexpression of this protein confers resistance to a large number of chemotherapeutic agents including anthracyclines, Vinca alkaloids, epipodophyllotoxins, actinomycin D and taxol. A number of studies document that ^{99m}Tc-MIBI is a transport substrate of Pgp in a variety of tumor cells, and similar Pgp recognition properties have been reported for ^{99m}Tc-tetrofosmin and ^{99m}Tc-furifosmin (Sharma 2004) as well as positron-emitter labeled compounds (Bigott et al. 2005; Sharma et al. 2005; Elsinga et al. 2004). Several studies report that ^{99m}Tc-MIBI is a also a substrate for the multidrug resistance associated protein (MRP1), a multispecific organic anion transporter that differs from Pgp in that it has a substrate specificity for glutathione-, glucuronate- or sulfate-conjugates of drugs, and it is sensitive to glutathione depletion (Hendrikse 2000).

Because of the extensive literature on the use of ^{99m}Tc-MIBI for the biological characterization of breast lesions, the present chapter will primarily focus on the ability of this compound to trace specific cellular processes in breast cancer. However, the principles outlined for ^{99m}Tc-MIBI can be equally applicable to other agents of the same class of compounds. Clinical studies performed to correlate ^{99m}Tc-MIBI uptake or clearance with histological, molecular and biochemical markers of several cellular processes including apoptosis, proliferation, P-glycoprotein expression and neoangiogenesis will be reviewed and discussed. Since the existence of such correlations does not necessarily imply a direct dependence of the imaging findings from a specific cellular process or a cause-effect relationship, attempts will be made to define which cellular process directly affects ^{99m}Tc-MIBI uptake or clearance. Furthermore, efforts will be made to explain the apparent discrepancy in the results of different studies and to address specific issues such as the clinical relevance of correlations and the possibility to set criteria for daily clinical applications. Finally, the opportunity to translate the same principles to other human neoplasms will be taken into account by reporting parallel evidence of similar correlations in other types of cancer.

7.2 ^{99m}Tc-MIBI and Apoptosis: Biological Significance of False Negatives

Apoptosis is an energy-dependent, highly regulated process leading to selective cell death. Several stimuli including drugs, toxins, gamma irradiation, cytokines of the TNF family and growth factor withdrawal may trigger an apoptotic response. Three main apoptotic pathways originating from three different subcellular compartments have been identified as the death receptor-mediated pathway, the mitochondrial apoptotic pathway and the recently recognized endoplasmic reticulum pathway (Danial and Korsmeyer 2004). All pathways lead to the activation of the executioner caspases, which in turn cleave cellular substrates and cause the biochemical and morphological changes that are characteristic of apoptosis (Igney and Krammer 2002).

The mechanisms involved in the induction of apoptosis by most anticancer agents are believed to be largely mediated by the mitochondrial pathway (Johnstone et al. 2002). However, it has been increasingly recognized that the endoplasmic reticulum (ER) cooperates in drug-induced apoptosis and the interaction between mitochondria, and ER is an emerging topic of investigation (Bassik et al. 2004; Scorrano et al. 2000). When a death signal converges onto mitochondria, it causes an early increase in the permeability of the mitochondrial membrane and the release of cytochrome-c and other apoptogenic factors that trigger the downstream sequence of reactions (Kroemer and Reed 2000). Consistent evidence indicates that mitochondrial membrane permeabilization is regulated by the opposing actions of pro- and anti-apoptotic members of Bcl-2 family (Cory and Adams 2002). Although the complex interplay among these members remains controversial and several competing models have been proposed to explain how apoptogenic factors are released into the cytosol, it is generally accepted that this event results in mitochondrial dysfunction and dissipation of mitochondrial membrane potentials (Kroemer and Reed 2000; Cory and Adams 2002).

Due to the reversible accumulation of ^{99m}Tc-MIBI within mitochondria and the dependence of tracer uptake on mitochondrial membrane potentials, the relationship between ^{99m}Tc-MIBI uptake and apoptosis has been explored both in vivo and in vitro. In particular, we obtained consistent evidence that breast carcinomas that fail to accumulate ^{99m}Tc-MIBI, have high levels of the anti-apoptotic protein Bcl-2 (Del Vecchio et al. 2003). The expression of the anti-apoptotic protein was also inversely correlated with the early tumor-to-background ratio in malignant lesions capable of accumulating ^{99m}Tc-MIBI. A cause-effect relationship between Bcl-2 overexpression and reduction of ^{99m}Tc-MIBI uptake in breast carcinomas has also been confirmed by transfecting breast cancer cell lines with the human bcl-2 gene (Aloj et al. 2003). A dramatic reduction of ^{99m}Tc-MIBI uptake was observed in Bcl-2 overexpressing clones as compared to control cells. Interestingly, treatment with staurosporine, a potent inducer of apoptosis, caused an early, partial and transitory recover of tracer uptake in transfected cells.

Overexpression of the anti-apoptotic protein Bcl-2 has been reported in various types of cancer and correlates with relative resistance to chemotherapy and radiation therapy due to a defective apoptotic program (Reed 2006). In the breast, Bcl-2 is expressed in normal glandular epithelium and is upregulated by estrogen possibly by direct transcrip-

tional induction (Teixeira et al. 1995). High levels of Bcl-2 have been found in a considerable percentage of breast carcinomas ranging between 32% and 86% (Arun et al. 2003). Recently, the expression of 13 biomarkers including Bcl-2 was evaluated in 930 breast cancers by immunohistochemistry on a tissue microarray, and positivity for Bcl-2 was reported to be a favorable prognostic marker in breast cancer independently of lymph node status, tumor size and grade combined in the Nottingham Prognostic Index (Callagy et al. 2006). Another recent study reported an inverse correlation between bcl-2 levels and rate of proliferation as determined by protein profiling using tissue microarray technology including Ki67 staining (Ruiz et al. 2006). Despite the recent reports on the positive prognostic role of Bcl-2, it remains controversial whether Bcl-2 overexpression is a significant independent predictor of response to treatment in breast cancer. There is evidence indicating that the ratio between Bcl-2 and the pro-apoptotic protein Bax, which is reported to be modulated in a p53-dependent manner, is a more reliable predictor of tumor response (Ziyaie et al. 2000). Also, pro-apoptotic members of Bcl-2 family were reported to be included in the clusters of genes associated with a pathological complete response to chemotherapy (Gianni et al. 2005). Finally, several studies reported that the absence of Bcl-2 in locally advanced breast cancer was significantly associated with a better pathological response to chemotherapy (Ogston et al. 2004; Pusztai et al. 2004; Prisack et al. 2005).

The exact mechanism by which Bcl-2 overexpression prevents ^{99m}Tc-MIBI uptake in breast carcinoma is presently unknown. However, a clue can emerge considering that Bcl-2 is an integral protein of the outer mitochondrial membrane and endoplasmic reticulum. At the mitochondrial level, it exerts a strong inhibitory effect on the permeabilization of mitochondrial membrane. Bcl-2 is reported indeed to inhibit cytochrome c release from mitochondria (Yang et al. 1997), the opening of mitochondrial permeability transition pore (Hirsch et al. 1997; Marzo et al. 1998), and the disruption of mitochondrial membrane potentials (Shimizu et al. 1998). However there is growing evidence that the Bcl-2 family controls apoptosis from the ER by regulating Ca2+ dynamics and crosstalk with mitochondria (Bassik et al. 2004; Scorrano et al. 2000; Oakes et al. 2005). At the endoplasmic reticulum level, the anti-apoptotic protein Bcl-2 can bind and sequester proapoptotic proteins, thus preventing the oligomerization and insertion of "multidomain" pro-apoptotic proteins such as Bax and Bak into the outer mitochondrial membrane and therefore pore formation and cytochrome-c release (Bassik et al. 2004; Scorrano et al. 2000; Oakes et al. 2005). Furthermore phosphorylated Bcl-2 predominantly localizes to the ER where it is reported to physically interact with inositol trisphosphate receptors I and III and directly or indirectly control the phosphorylation status and Ca2+ leak through these receptors (Oakes et al. 2005; Chen et al. 2004; Orrenius et al. 2003). Thus high levels of Bcl-2 in breast carcinomas may similarly affect the trafficking of ^{99m}Tc-MIBI cations across endoplasmic reticulum and prevent tracer uptake within mitochondria despite the stabilization of mitochondrial membrane potentials (Fig. 7.1).

There are several reports in the literature addressing the issue of ^{99m}Tc-MIBI uptake in cells or tumors undergoing apoptosis after exposure to drugs or gamma irradiation (Zhu et al. 2002; Vergote et al. 2001; Moretti et al. 2005). In particular, ^{99m}Tc-MIBI uptake has been reported to decrease in breast cancer cell lines after treatment with anti-cancer drugs (Vergote et al. 2001). The decrease of tracer uptake has been reported to be dose-dependent and time-dependent usually reaching a maximum at 72 h. Although cells may still appear viable, the downstream progression through the apoptotic cascade may lead to the disruption of mitochondrial membrane potential and dissipation of the driving force behind ^{99m}Tc-MIBI uptake.

Recently, we have obtained evidence that treatment of Bcl-2 transfected breast cancer cells and control parental cells with different anti-cancer agents results in opposite changes of ^{99m}Tc-MIBI uptake (unpublished data). After 24 h of drug exposure, Bcl-2 overexpressing clones show an increase of ^{99m}Tc-MIBI uptake as compared to untreated controls. Conversely, parental cells with no detectable levels of Bcl-2 show a reduction of tracer uptake after treatment.

The picture emerging from these findings is that, in the absence of biological barriers limiting the free diffusion of the tracer from blood to tumor, the uptake of ^{99m}Tc-MIBI reflects the status of mitochondrial membranes and endoplasmic reticulum. In particular it reflects the permeability of mitochondrial membrane, the preservation of mitochondrial membrane potentials and likely the integrity of interorganelle cross-talk. Alterations of these factors normally occur in cancer during drug-induced apoptosis and finally result in mitochondrial dysfunction with consequent reduction of ^{99m}Tc-MIBI

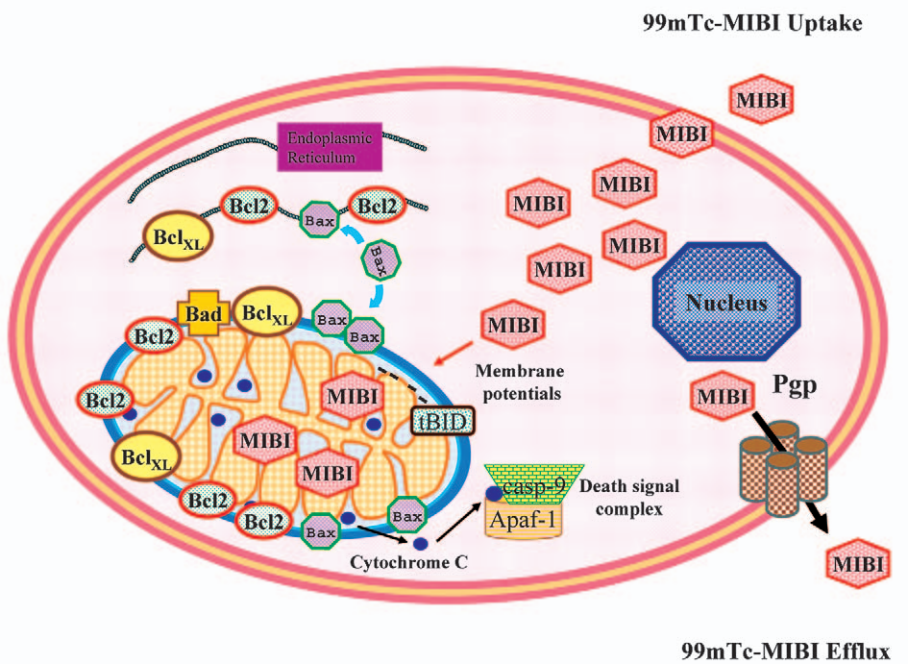

Fig. 7.1. Schematic representation of the uptake and efflux modalities of ^{99m}Tc-MIBI. Uptake: the passive movement of ^{99m}Tc-MIBI from the extracellular to the intracellular compartment and its reversible accumulation within mitochondria is driven by the electronegative plasma membrane and mitochondrial membrane potentials. Mitochondria are also main executioners of apoptosis, and mitochondrial membrane permeabilization is required for apoptosis. Permeability of mitochondrial membrane is regulated by the opposing actions of pro- and anti-apoptotic members of Bcl-2 family. The pro-apoptotic members such as Bax and Bad promote release of cytochrome c, whereas the anti-apoptotic members such as Bcl-2 and BclXL inhibit permeabilization of mitochondrial membrane and release of cytochrome c. Once in the cytosol, cytochrome c activates caspase-9 (casp-9) by binding to Apaf-1. High levels of Bcl-2 prevent ^{99m}Tc-MIBI to accumulate within mitochondria. Efflux: overexpression of P-glycoprotein in resistant tumor cells represents a powerful mechanism of ^{99m}Tc-MIBI extrusion from cells. The Pgp-dependent outward transport of the tracer can be visualized and estimated in ^{99m}Tc-MIBI positive malignant lesions

uptake. Overexpression of the antiapoptotic protein Bcl-2, with its dual inhibitory role on mitochondrial permeability and disruption of mitochondrial membrane potentials as well as its potential ability to control mitochondrial function from the endoplasmic reticulum, prevents ^{99m}Tc-MIBI accumulation in untreated breast carcinoma, and this effect may be counteracted during the early phases of drug-induced apoptosis allowing a transitory increase of tracer uptake.

Many well-known factors including tumor size, cellularity, blood supply and cell viability can cause false-negative results at scintimammography. How and when false-negative results at ^{99m}Tc-MIBI scan may provide a piece of information on breast cancer biology? A possible way to address this issue is to perform a stress test. In other words, a ^{99m}Tc-MIBI scan performed before and immediately after treatment with anti-cancer agents may reveal changes in tracer accumulation, which depends on Bcl-2 levels in mitochondrial and ER membranes. For instance, ^{99m}Tc-MIBI negative lesions, which become positive early after treatment with anti-cancer agents, would presumably express high levels of Bcl-2. A decrease of tumor-to-background ratio in ^{99m}Tc-MIBI positive lesions after treatment would indicate the absence of significant levels of Bcl-2. Further studies are needed to validate this hypothesis and to translate this approach into clinical applications. However, many clinical studies have highlighted the prognostic value of absent or reduced early ^{99m}Tc-MIBI uptake in different types of tumors. In particular, the absent or reduced early ^{99m}Tc-MIBI uptake in lung cancer predicts poor response to chemotherapy and

radiation therapy independently of tracer clearance (Bom et al. 1998; Yamamoto et al. et al. 1998; Yuksel et al. 2002; Nishiyama et al. 2000). Also, lymphoma lesions not visualized at ^{99m}Tc-MIBI scan are refractory to subsequent chemotherapy (Kapucu et al. 1997; Kao et al. 2001).

7.3 ^{99m}Tc-MIBI and Proliferation: Biological Significance of True Positives

Several studies have been undertaken to elucidate the relationship between ^{99m}Tc MIBI uptake and proliferation in breast cancer. Cutrone et al. (1998) evaluated several histological variables, including tumor cell proliferation, in 42 surgical excised breast lesions. They found a moderate, but significant correlation between degree of ^{99m}Tc-MIBI uptake and cellular proliferation in such lesions. In malignant lesions with a diameter lower than 1.5 cm, Bonazzi et al. (2001) found that breast carcinomas with detectable ^{99m}Tc-MIBI uptake showed an increased proliferative activity as compared to ^{99m}Tc-MIBI negative malignant lesions. In 42 breast carcinomas with a diameter higher than 1.8 cm, we could not find any significant difference in mitotic index between MIBI-positive and MIBI-negative lesions, but the apoptotic index was dramatically reduced in MIBI-negative malignant lesions (Del Vecchio et al. 2003). Furthermore, a strong, significant and direct correlation between the rate of proliferation and the apoptotic index was found in MIBI-positive lesions, and the apoptotic index was significantly and directly correlated with the early tumor-to-background ratio, whereas proliferation showed a borderline correlation. Evidence of a strong and direct correlation between the proliferation rate and ^{99m}Tc-MIBI uptake has been reported also for brain tumors (Nagamachi et al. 2001; Ak et al. 2003).

These observations, taken together, raised the question whether proliferation is more important than apoptosis in determining the degree of ^{99m}Tc-MIBI uptake in malignant breast tumors. Although cell proliferation and cell death appear to be opposing and mutually contradictory processes, substantial evidence indicates that their pathways are generally coupled in human malignancies (Lowe et al. 2004). Many dominant oncogenes, which are well known promoters of cell proliferation, actually also possess proapoptotic activity, and their mitogenic and pro-apoptotic properties are often genetically inseparable (Pelengaris et al. 2002; Nahle et al. 2002). Similar findings have been reported for certain functionally inactive tumor suppressors (Lowe et al. 2004; Hickman et al. 2002). Therefore in cells with deregulated oncogenes or inactive suppressors, the activation of the proliferative machinery by appropriate growth signals primes also the cellular apoptotic program or alternatively sensitizes growing cells to apoptotic signals. In the absence of further alterations impairing the apoptotic response such as, for instance, Bcl-2 overexpression, the enhanced proliferative activity in response to growth signals is accompanied by an enhanced apoptotic response to death signals in malignant tumors. In this respect, a direct and significant correlation between the proliferative and apoptotic index has been reported in breast cancer (de Jong et al. 2000; Archer et al. 2003). Therefore it is not surprising that both the proliferative and apoptotic indexes have been found to correlate directly with ^{99m}Tc-MIBI uptake. Furthermore, detection of apoptosis in tumor tissues is usually performed by staining of DNA fragments or by counting cells with peculiar morphological changes and identifies the fraction of cells that had already completed the apoptotic program. Although directly correlated to the rate of proliferation, this fraction is usually limited in breast cancer, accounting for less than 5–10% of total tumor cells. At present, we do not have any marker to recognize cells that have been primed for apoptosis or sensitized to death signals by deregulated oncogenes or inactive suppressors. It remains to be elucidated whether early ^{99m}Tc-MIBI uptake can be a surrogate marker to identify such cells.

7.4 ^{99m}Tc MIBI and P-Glycoprotein Expression: Biological Significance of Tracer Clearance

Despite the large number of anticancer drugs that have been developed and tested, multidrug resistance remains the primary cause of treatment failure in cancer patients. Multiple cellular mechanisms may contribute to the development of the multidrug-resistant phenotype using different modes of action. Reduced uptake of water-soluble drugs, altered metabolism of drugs, increased repair of DNA damage, reduced apoptosis and enhanced efflux of

hydrofobic drugs through energy-dependent transporters may potentially cause resistance of cancer cells (Szakacs et al. 2006). One of the most extensively studied mechanisms of multidrug resistance in human tumors involves the overexpression of P-glycoprotein (Pgp), a member of the ATP-binding cassette (ABC) family of transporters. A number of physiological, biochemical and genetic studies indicates that high levels of Pgp enable cancer cells to extrude many chemotherapeutic agents, circumventing their lethal effects (Gottesman et al. 2002). The human genoma contains 48 genes that encode ABC transporters, which have been divided into seven subfamilies named from ABCA through ABCG, and at least 12 ABC transporters have a role in drug resistance in cultured tumor cells. Whether each of these transporters has a role in clinical anticancer drug resistance in patients remains to be established (Leonard et al. 2003). The association of high levels of Pgp (ABCB1) with poor clinical outcome appears to be consolidated in many human cancers including breast carcinoma, sarcoma and certain types of leukemia. In particular, a meta-analysis of 31 breast cancer trials showed a three-fold reduction in response to chemotherapy among tumors expressing Pgp after treatment (Trock et al. 1997).

The clinical relevance of Pgp in determining breast cancer response to treatment and the availability of ^{99m}Tc-labeled compounds such as ^{99m}Tc-MIBI, ^{99m}Tc-tetrofosmin and 9mTc- furifosmin prompted investigating whether these tracers could detect and monitor Pgp expression and function in vivo (Fig. 7.1).

The first issue addressed by clinical studies in breast cancer was whether ^{99m}Tc-MIBI uptake is reduced in Pgp-overexpressing tumors (Moretti et al. 1996; Kostakoglu et al. 1997; Sun et al. 2000; Kao et al. 2001). Although the results of these studies indicated an inverse relationship between net tracer uptake and Pgp levels, the time-dependence of such relationships was not proven since delayed images were not obtained in these studies.

A tracer kinetic analysis was performed over a 4-h period in a series of 30 untreated patients with ^{99m}Tc-MIBI-positive breast carcinomas and revealed a direct statistically significant correlation between tracer efflux and Pgp levels (Del Vecchio et al. 1997). A threshold value could also be established to discriminate Pgp-overexpressing tumors from breast carcinomas with basal levels of Pgp, and this threshold corresponded to a time to half clearance of 204 min. Tracer clearance was also tested for its ability to predict response to subsequent treatment in patients with locally advanced breast cancer candidates for neoadjuvant chemotherapy (Ciarmiello et al. 1998). A rapid clearance of ^{99m}Tc-MIBI from tumors was significantly associated with a highly cellular macroscopic residual tumor at pathological examination of surgical specimens indicating a lack of tumor response to neoadjuvant chemotherapy. On the contrary, the prolonged retention of the tracer was associated with an effective pathological tumor response to treatment in two-thirds of the patients. Similar findings have been obtained by Sciuto et al. (2002) in 30 patients with locally advanced breast cancer undergoing neoadjuvant chemotherapy. They used early (10 min) and delayed (4 h) ^{99m}Tc-MIBI uptake ratio to derive the wash-out rate and found that a cut-off of 45% provides a satisfactory discrimination between responders and non-responders.

Takamura et al. (2001) evaluated 46 patients with locally advanced or recurrent breast carcinoma and determined both early and delayed tumor-to-background ratios on SPECT images. After chemotherapy, tumor response was determined by clinical examination. Both early and delayed tumor-to-background ratios were significantly higher in responders than in non-responders. Conversely, Pgp levels determined by immunoperoxidase on biopsy specimens prior to treatment were significantly lower in responders than in non-responders.

Mubashar et al. (2002) evaluated early and delayed uptake ratios in 20 patients with breast carcinoma before and after treatment with toremifene, an antiestrogen with Pgp-modulating property. An inverse correlation between the delayed uptake ratio and Pgp expression in tumors was confirmed before treatment. Although a clear cut-off value of tumor-to-background ratio between Pgp-overexpressing and Pgp-negative tumors could not be established, analysis of the change between early and delayed scan appeared to be a better predictor of Pgp status. After toremifene treatment, the authors found that the delayed uptake ratio significantly increased only in Pgp-overexpressing tumors, whereas it decreased in tumors with low Pgp levels. Interestingly, three of the four patients whose tumors were not visualized before toremifene failed to accumulate ^{99m}Tc-MIBI also after treatment with the Pgp-modulator.

There are several reports in the literature correlating a single early uptake ratio of ^{99m}Tc-MIBI with response to treatment (Cayre et al. 2002; Cayre et al. 2002). Although the absent or reduced uptake of ^{99m}Tc-MIBI in breast carcinoma is indeed correlated

with a poor response to therapy, it is not clear, in the absence of a direct evidence of Pgp expression in individual tumors, whether resistance is due to the overexpression of Pgp or to other Pgp-independent mechanism of resistance such as Bcl-2 overexpression altering MIBI uptake. In order to distinguish in vivo Pgp-dependent from Pgp-independent mechanisms of multidrug resistance, the faster decline of tracer uptake over time should be demonstrated and estimated in individual patients. This should be taken into account when setting the criteria for a standardized procedure allowing the functional imaging of Pgp in clinical practice and the selection of patients that may benefit from treatment with Pgp inhibitors.

Once patients with high efflux of Pgp substrates have been identified, two alternative approaches can be adopted, namely inhibition of Pgp function or use of drugs that are able to evade efflux. Despite the considerable efforts to develop drugs that inhibit the function of efflux transporters that lead to the identification of first, second and third generation of Pgp inhibitors, clinical trials with these drugs did not show significant clinical benefit in term of overall survival and response rate (Szakacs et al. 2006). However, phase III trials with the last generation of inhibitors showing greater substrate specificity, lower toxicity and improved pharmacokinetic profiles are currently ongoing. ^{99m}Tc-MIBI has been used to test the effect of Pgp inhibitors in patients. An enhanced liver uptake of ^{99m}Tc-MIBI was reported following administration of several Pgp inhibitors (Chen et al. 1997; Peck et al. 2001; Agrawal et al. 2003). This finding has been ascribed to Pgp inhibition at physiological sites of protein expression and considered as a surrogate marker of effective Pgp inhibition (Wong et al. 2005; Hendrikse et al. 2004). An increased accumulation of ^{99m}Tc-MIBI in drug resistant tumors has also been reported after the administration of third generation inhibitor XR9576 (Agrawal et al. 2003; Pusztai et al. 2005.

On the other end, the number of drugs that are able to evade efflux is currently limited, and most anticancer agents of the MDR spectrum are practically irreplaceable in chemotherapy regimens (Szakacs et al. 2006). Therefore the clinical impact of functional imaging of multidrug resistance still remains unexploited and appears to mainly rely upon the development of novel anticancer agents designed to escape efflux mechanisms.

7.5 ^{99m}Tc-MIBI and Tumor Blood Supply

Since a prerequisite of ^{99m}Tc-MIBI uptake is an effective delivery of the tracer to the tumor mass, several studies have been undertaken to evaluate the relationship between ^{99m}Tc-MIBI uptake and tumor blood supply. Mankoff et al. (2002) evaluated ^{99m}Tc-MIBI kinetics and blood flow in locally advanced breast carcinoma. They studied 37 patients with ^{99m}Tc-MIBI and 15O-water PET imaging and found a direct correlation between early ^{99m}Tc-MIBI uptake and blood flow in agreement with the findings reported for myocardial perfusion studies. Conflicting results have been reported on neoangiogenesis as a factor affecting ^{99m}Tc-MIBI uptake. The formation of new blood vessels is invariably required for growth of breast cancer, and it has a recognized role as an indicator of node metastases and survival (Weidner et al. 1991; Neri and Bicknell 2005; Jain et al. 2006). Cutrone et al. (1998) found no significant difference in microvessel density in ^{99m}Tc-MIBI-positive and ^{99m}Tc-MIBI-negative malignant lesions. When neoangiogenesis was compared with tumor-to-background ratio, a direct correlation has been found in 31 untreated breast cancer patients (Yoon et al. 1999). Scopinaro et al. evaluated microvessel density in 19 untreated breast carcinomas and correlated this histological variable with nodal metastases and ^{99m}Tc-MIBI findings (Scopinaro et al. 1994). The enhanced density of newly formed blood vessels was associated with both nodal metastases and detectable ^{99m}Tc-MIBI uptake in the primary tumors. No correlation between intratumoral microvessel density and ^{99m}Tc-MIBI uptake ratio could be found in patients with breast carcinomas in two independent studies (Kim et al. 2002; Bekis et al. 2005). These findings, taken together, indicate that, although a preserved blood supply is required for tracer delivery to breast carcinoma, it is still controversial whether ^{99m}Tc-MIBI uptake can be used as an indicator of tumor angiogenesis.

7.6 Clinical Implications

All the cellular processes that have been examined in relation to scintigraphic findings with ^{99m}Tc-MIBI

are indeed relevant for tumor response to treatment. Most anticancer drugs exert their lethal effect by inducing apoptosis mainly through the mitochondrial pathway that is governed by members of the Bcl-2 family. The lack of ^{99m}Tc-MIBI uptake in palpable malignant lesions of the breast may indicate the presence of high levels of the anti-apoptotic protein Bcl-2 in the mitochondrial membrane or endoplasmic reticulum. Whether this observation is relevant to predict poor tumor response in breast carcinomas remains to be established in perspective clinical studies.

Conversely, a high early uptake in untreated breast carcinoma is correlated with the rate of proliferation. A high growth fraction usually indicates a more aggressive tumor behavior, but also a better and more rapid tumor response to treatment. An orthodox concept in oncology suggests indeed that cytotoxic agents are more effective against rapidly proliferating cells. Therefore a high early ^{99m}Tc-MIBI uptake ratio in breast cancer, when associated with high tracer retention, would indicate an effective tumor response to subsequent chemotherapy.

In addition, an enhanced tracer clearance in ^{99m}Tc-MIBI-positive malignant lesions of the breast is indicative of a Pgp-mediated outward transport of the tracer. Therefore tumors with a fast tracer clearance are likely to become refractory to subsequent chemotherapy due to the drug transport activity of Pgp. Since the clearance of ^{99m}Tc-MIBI cannot be obviously evaluated in negative ^{99m}Tc-MIBI malignant lesions, the expression of Pgp in those tumors will remain indeterminate. An important step toward the clinical application of functional imaging of Pgp in breast cancer is the standardization of the procedure. On the basis of the reported observations, the most reliable and direct index of Pgp function appears to be the tracer washout or clearance. Furthermore, some cut-off values of clearance and washout have been reported to correctly discriminate between responders and non-responders in patients with locally advanced breast cancer, and these values may be used in larger clinical studies with the aim to confirm this evidence.

Although the influence of neoangiogenesis on ^{99m}Tc-MIBI uptake is still controversial, it is worth to noting that a preserved tumor blood supply is relevant not only for tracer delivery, but also to limit tumor hypoxia, which can contribute to treatment failure.

7.7 Conclusions

Both the uptake and efflux mechanisms of ^{99m}Tc-MIBI and analogous ^{99m}Tc-labeled agents in breast carcinomas involve cellular processes that are important for tumor response to treatment. The tracer uptake reflects the status of mitochondria and endoplasmic reticulum, in terms of permeability of mitochondrial membrane, preservation of mitochondrial membrane potentials and integrity of cross-talk between the two cellular organelles. Mutations or altered expression of key molecules participating in the apoptotic process may profoundly affect the function of mitochondria and endoplasmic reticulum and hence tracer uptake both in basal conditions and during drug-induced apoptosis. In the absence of such alterations, apoptosis and proliferation are coordinately modulated in breast cancer, and this may explain the additional relationship between the tracer uptake and rate of proliferation. Although apparently contradictory, both processes are considered important to sensitize cells to the lethal effects of drugs. Accordingly, malignant breast lesions, which are able to accumulate and retain the tracer, will likely respond to therapy. The role of ^{99m}Tc-MIBI and analogous agents in predicting tumor response is reinforced by a number of studies evaluating its ability to trace the activity of Pgp. In this case the tracer efflux reflects the Pgp-mediated outward transport of the tracer, and this clearance mimics the kinetic behavior of anti-cancer drugs of the MDR spectrum.

In conclusion, on the basis of the imaging parameter chosen for the analysis of the ^{99m}Tc-MIBI scan in breast cancer patients, the biological information provided may be related to different cellular processes, but all of them appear ultimately related to the susceptibility of breast carcinoma to treatment. Nevertheless it remains important to discriminate on the images alterations of the uptake mechanism from enhancement of tracer clearance because these observations may orient clinicians towards a very different adjustment of therapy. A number of efforts have been focused on the development of Pgp-inhibitors and Bcl-2 antagonists. The appropriate selection of patients based on the mechanism primarily involved in the development of a multi-drug resistant phenotype would hopefully improve the efficacy of individually tailored therapeutic strategies.

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References

1. Denoyer, D, Perek, N, Le Jeune, N, and Dubois, F. Spectrum of radiopharmaceuticals in nuclear oncology. Curr Cancer Drug Targets 2006;6: 181–96.
2. Been, LB, Suurmeijer, AJ, Cobben, DC, Jager, PL, Hoekstra, HJ, and Elsinga, PH. [^{18}F]FLT-PET in oncology: current status and opportunities. Eur J Nucl Med Mol Imaging 2004;31: 1659–72.
3. Corsten, MF, Hofstra, L, Narula, J, and Reutelingsperger, CP. Counting heads in the war against cancer: defining the role of annexin A5 imaging in cancer treatment and surveillance. Cancer Res 2006;66: 1255–60.
4. Weissleder, R. Molecular imaging in cancer. Science 2006;312: 1168–71.
5. Sharma, V. Radiopharmaceuticals for assessment of multidrug resistance P-glycoprotein-mediated drug transport activity. Bioconjug Chem 2004;15: 1464–74.
6. Piwnica-Worms, D, Kronauge, JF, and Chiu, ML. Uptake and retention of hexakis (2-methoxyisobutyl isonitrile) technetium(I) in cultured chick myocardial cells. Mitochondrial and plasma membrane potential dependence. Circulation 1990;82: 1826–38.
7. Delmon-Moingeon, LI, Piwnica-Worms, D, Van den Abbeele, AD, Holman, BL, Davison, A, and Jones, AG. Uptake of the cation hexakis(2-methoxyisobutylisonitrile)-technetium- 99m by human carcinoma cell lines in vitro. Cancer Res 1990;50: 2198–202.
8. Carvalho, PA, Chiu, ML, Kronauge, JF, Kawamura, M, Jones, AG, Holman, BL, and Piwnica-Worms, D. Subcellular distribution and analysis of technetium-99m-MIBI in isolated perfused rat hearts. J Nucl Med 1992;33: 1516–22.
9. Piwnica-Worms, D, Chiu, ML, Budding, M, Kronauge, JF, Kramer, RA, and Croop, JM. Functional imaging of multidrug-resistant P-glycoprotein with an organotechnetium complex. Cancer Res 1993;53: 977–84.
10. Szakacs, G, Paterson, JK, Ludwig, JA, Booth-Genthe, C, and Gottesman, MM. Targeting multidrug resistance in cancer. Nat Rev Drug Discov 2006;5: 219–34.
11. Bigott, HM, Prior, JL, Piwnica-Worms, DR, and Welch, MJ. Imaging multidrug resistance P-glycoprotein transport function using microPET with technetium-94m-sestamibi. Mol Imaging 2005;4: 30–9.
12. Sharma, V, Prior, JL, Belinsky, MG, Kruh, GD, and Piwnica-Worms, D. Characterization of a 67Ga/68Ga radiopharmaceutical for SPECT and PET of MDR1 P-glycoprotein transport activity in vivo: validation in multidrug-resistant tumors and at the blood-brain barrier. J Nucl Med 2005;46: 354–64.
13. Elsinga, PH, Hendrikse, NH, Bart, J, Vaalburg, W, and van Waarde, A. PET studies on P-glycoprotein function in the blood-brain barrier: how it affects uptake and binding of drugs within the CNS. Curr Pharm Des 2004;10: 1493–503.
14. Hendrikse, NH. Monitoring interactions at ATP-dependent drug efflux pumps. Curr Pharm Des 2000;6: 1653–68.
15. Danial, NN, and Korsmeyer, SJ. Cell death: critical control points. Cell 2004;116: 205–19.
16. Igney, FH, and Krammer, PH. Death and anti-death: tumour resistance to apoptosis. Nat Rev Cancer 2002;2: 277–88.
17. Johnstone, RW, Ruefli, AA, and Lowe, SW. Apoptosis: a link between cancer genetics and chemotherapy. Cell 2002;108: 153–64.
18. Bassik, MC, Scorrano, L, Oakes, SA, Pozzan, T, and Korsmeyer, SJ. Phosphorylation of BCL-2 regulates ER Ca2+ homeostasis and apoptosis. Embo J 2004;23: 1207–16.
19. Scorrano, L, Oakes, SA, Opferman, JT, Cheng, EH, Sorcinelli, MD, Pozzan, T, and Korsmeyer, SJ. BAX and BAK regulation of endoplasmic reticulum Ca2+: a control point for apoptosis. Science 2003;300: 135–9.
20. Kroemer, G, and Reed, JC. Mitochondrial control of cell death. Nat Med 2000;6: 513–9.
21. Cory, S, and Adams, JM. The bcl2 family: regulators of the cellular life-or-death switch. Nat Rev Cancer 2002;2: 647–56.
22. Del Vecchio, S, Zannetti, A, Aloj, L, Caraco, C, Ciarmiello, A, and Salvatore, M. Inhibition of early ^{99m}Tc-MIBI uptake by Bcl-2 anti-apoptotic protein overexpression in untreated breast carcinoma. Eur J Nucl Med Mol Imaging 2003;30: 879–87.
23. Aloj, L, Zannetti, A, Caraco, C, Del Vecchio, S, and Salvatore, M. Bcl-2 overexpression prevents $^{(99m)}$Tc-MIBI uptake in breast cancer cell lines. Eur J Nucl Med Mol Imaging 2003.
24. Reed, JC. Drug Insight: cancer therapy strategies based on restoration of endogenous cell death mechanisms. Nat Clin Pract Oncol 2006;3: 388–98.
25. Teixeira, C, Reed, JC, and Pratt, MA. Estrogen promotes chemotherapeutic drug resistance by a mechanism involving Bcl-2 proto-oncogene expression in human breast cancer cells. Cancer Res 1995;55: 3902–7.
26. Arun, B, Kilic, G, Yen, C, Foster, B, Yardley, D, Gaynor, R, and Ashfaq, R. Correlation of Bcl-2 and p53 expression in primary breast tumors and corresponding metastatic lymph nodes. Cancer 2003;98: 2554–9.
27. Callagy, GM, Pharoah, PD, Pinder, SE, Hsu, FD, Nielsen, TO, Ragaz, J, Ellis, IO, Huntsman, D, and Caldas, C. Bcl-2 is a prognostic marker in breast cancer independently of the Nottingham Prognostic Index. Clin Cancer Res 2006;12: 2468–75.
28. Ruiz, C, Seibt, S, Al Kuraya, K, Siraj, A K, Mirlacher, M, Schraml, P, Maurer, R, Spichtin, H, Torhorst, J, Popovska, S, Simon, R, and Sauter, G. Tissue microarrays for comparing molecular features with proliferation activity in breast cancer. Int J Cancer 2006;118: 2190–4.
29. Ziyaie, D, Hupp, TR, and Thompson, AM. P53 and breast cancer. Breast 2000;9: 239–46.
30. Gianni, L, Zambetti, M, Clark, K, Baker, J, Cronin, M, Wu, J, Mariani, G, Rodriguez, J, Carcangiu, M, Watson, D, Va-

lagussa, P, Rouzier, R, Symmans, WF, Ross, JS, Hortobagyi, GN, Pusztai, L, and Shak, S. Gene expression profiles in paraffin-embedded core biopsy tissue predict response to chemotherapy in women with locally advanced breast cancer. J Clin Oncol 2005;23: 7265–77.
31. Ogston, KN, Miller, ID, Schofield, AC, Spyrantis, A, Pavlidou, E, Sarkar, TK, Hutcheon, AW, Payne, S, and Heys, SD. Can patients' likelihood of benefiting from primary chemotherapy for breast cancer be predicted before commencement of treatment? Breast Cancer Res Treat 2004;86: 181–9.
32. Pusztai, L, Krishnamurti, S, Perez Cardona, J, Sneige, N, Esteva, F J, Volchenok, M, Breitenfelder, P, Kau, S W, Takayama, S, Krajewski, S, Reed, JC, Bast, RC, Jr, and Hortobagyi, GN. Expression of BAG-1 and BcL-2 proteins before and after neoadjuvant chemotherapy of locally advanced breast cancer. Cancer Invest 2004;22: 248–56.
33. Prisack, HB, Karreman, C, Modlich, O, Audretsch, W, Danae, M, Rezai, M, and Bojar, H. Predictive biological markers for response of invasive breast cancer to anthracycline/cyclophosphamide-based primary (radio-)chemotherapy. Anticancer Res 2005;25: 4615–21.
34. Yang, J, Liu, X, Bhalla, K, Kim, CN, Ibrado, AM, Cai, J, Peng, TI, Jones, DP, and Wang, X. Prevention of apoptosis by Bcl-2: release of cytochrome c from mitochondria blocked. Science 1997;275: 1129–32.
35. Hirsch, T, Marchetti, P, Susin, SA, Dallaporta, B, Zamzami, N, Marzo, I, Geuskens, M, and Kroemer, G. The apoptosis-necrosis paradox. Apoptogenic proteases activated after mitochondrial permeability transition determine the mode of cell death. Oncogene 1997;15: 1573–81.
36. Marzo, I, Brenner, C, Zamzami, N, Jurgensmeier, JM, Susin, SA, Vieira, HL, Prevost, MC, Xie, Z, Matsuyama, S, Reed, JC, and Kroemer, G. Bax and adenine nucleotide translocator cooperate in the mitochondrial control of apoptosis. Science 1998;281: 2027-31.
37. Shimizu, S, Eguchi, Y, Kamiike, W, Funahashi, Y, Mignon, A, Lacronique, V, Matsuda, H, and Tsujimoto, Y. Bcl–2 prevents apoptotic mitochondrial dysfunction by regulating proton flux. Proc Natl Acad Sci USA 1998;95: 1455-9.
38. Oakes, SA, Scorrano, L, Opferman, JT, Bassik, MC, Nishino, M, Pozzan, T, and Korsmeyer, SJ. Proapoptotic BAX and BAK regulate the type 1 inositol trisphosphate receptor and calcium leak from the endoplasmic reticulum. Proc Natl Acad Sci USA 2005;102: 105–10.
39. Chen, R, Valencia, I, Zhong, F, McColl, KS, Roderick, HL, Bootman, MD, Berridge, MJ, Conway, SJ, Holmes, AB, Mignery, GA, Velez, P, and Distelhorst, CW. Bcl–2 functionally interacts with inositol 1,4,5–trisphosphate receptors to regulate calcium release from the ER in response to inositol 1,4,5–trisphosphate. J Cell Biol 2004;166: 193–203.
40. Orrenius, S, Zhivotovsky, B, and Nicotera, P. Regulation of cell death: the calcium-apoptosis link. Nat Rev Mol Cell Biol 2003;4: 552–65.
41. Zhu, X, Wu, H, Xia, J, Zhao, M, and Xianyu, Z. The relationship between $^{(99m)}$Tc-MIBI uptakes and tumor cell death/proliferation state under irradiation. Cancer Lett 2002;182: 217–22.
42. Vergote, J, Di Benedetto, M, Moretti, JL, Azaloux, H, Kouyoumdjian, JC, Kraemer, M, and Crepin, M. Could ^{99m}Tc-MIBI be used to visualize the apoptotic MCF7 human breast cancer cells? Cell Mol Biol (Noisy-le-grand) 2001;47: 467–71.
43. Moretti, J L, Hauet, N, Caglar, M, Rebillard, O, and Burak, Z. To use MIBI or not to use MIBI? That is the question when assessing tumour cells. Eur J Nucl Med Mol Imaging 2005;32: 836–42.
44. Bom, HS, Kim, YC, Song, HC, Min, JJ, Kim, JY, and Park, KO. Technetium-99m-MIBI uptake in small cell lung cancer. J Nucl Med 1998;39: 91–4.
45. Yamamoto, Y, Nishiyama, Y, Satoh, K, Takashima, H, Ohkawa, M, Fujita, J, Kishi, T, Matsuno, S, and Tanabe, M. Comparative study of technetium-99m-sestamibi and thallium-201 SPECT in predicting chemotherapeutic response in small cell lung cancer. J Nucl Med 1998;39: 1626–9.
46. Yuksel, M, Cermik, F, Doganay, L, Karlikaya, C, Cakir, E, Salan, A, and Berkarda, S. ^{99m}Tc-MIBI SPET in non-small cell lung cancer in relationship with Pgp and prognosis. Eur J Nucl Med Mol Imaging 2002;29: 876–81.
47. Nishiyama, Y, Yamamoto, Y, Satoh, K, Ohkawa, M, Kameyama, K, Hayashi, E, Fujita, J, and Tanabe, M. Comparative study of Tc-99m MIBI and TI-201 SPECT in predicting chemotherapeutic response in non-small-cell lung cancer. Clin Nucl Med 2000;25: 364–9.
48. Kapucu, LO, Akyuz, C, Vural, G, Oguz, A, Atasever, T, Buyukpamukcu, M, and Unlu, M. Evaluation of therapy response in children with untreated malignant lymphomas using technetium-99m-sestamibi. J Nucl Med 1997;38: 243–7.
49. Kao, CH, Tsai, SC, Wang, JJ, Ho, YJ, Ho, ST, and Changlai, SP. Evaluation of chemotherapy response using technetium-99M-sestamibi scintigraphy in untreated adult malignant lymphomas and comparison with other prognosis factors: a preliminary report. Int J Cancer 2001;95: 228–31.
50. Cutrone, JA, Yospur, LS, Khalkhali, I, Tolmos, J, Devito, A, Diggles, L, Vargas, MP, Shitabata, P, and French, S. Immunohistologic assessment of technetium-99m-MIBI uptake in benign and malignant breast lesions. J Nucl Med 1998;39: 449–53.
51. Bonazzi, G, Cistaro, A, Bello, M, Bessone, M, Tetti, M, Villata, E, Coluccia, C, Ardine, M, Moz, G, Massaioli, N, and Bisi, G. Breast cancer cellular proliferation indexes and ^{99m}Tc-sesta Mibi capture: what correlation? J Exp Clin Cancer Res 2001;20: 91–4.
52. Nagamachi, S, Jinnouchi, S, Nabeshima, K, Nishii, R, Flores, L, 2nd, Kodama, T, Kawai, K, Tamura, S, Yokogami, K, Samejima, T, and Wakisaka, S. The correlation between ^{99m}Tc-MIBI uptake and MIB-1 as a nuclear proliferation marker in glioma–a comparative study with 201Tl. Neuroradiology 2001;43: 1023–30.
53. Ak, I, Gulbas, Z, Altinel, F, and Vardareli, E. Tc-99m MIBI uptake and its relation to the proliferative potential of brain tumors. Clin Nucl Med 2003;28: 29–33.
54. Lowe, SW, Cepero, E, and Evan, G. Intrinsic tumour suppression. Nature 2004;432: 307–15.
55. Pelengaris, S, Khan, M, and Evan, G. c-MYC: more than just a matter of life and death. Nat Rev Cancer 2002;2: 764–76.
56. Nahle, Z, Polakoff, J, Davuluri, RV, McCurrach, ME, Jacobson, MD, Narita, M, Zhang, MQ, Lazebnik, Y, Bar-Sagi, D, and Lowe, SW. Direct coupling of the cell cycle and cell death machinery by E2F. Nat Cell Biol 2002;4: 859–64.

57. Hickman, ES, Moroni, MC, and Helin, K. The role of p53 and pRB in apoptosis and cancer. Curr Opin Genet Dev 2002;12: 60–6.
58. de Jong, JS, van Diest, PJ, and Baak, JP. Number of apoptotic cells as a prognostic marker in invasive breast cancer. Br J Cancer 2000;82: 368–73.
59. Archer, CD, Parton, M, Smith, IE, Ellis, PA, Salter, J, Ashley, S, Gui, G, Sacks, N, Ebbs, SR, Allum, W, Nasiri, N, and Dowsett, M. Early changes in apoptosis and proliferation following primary chemotherapy for breast cancer. Br J Cancer 2003;89: 1035–41.
60. Gottesman, MM, Fojo, T, and Bates, SE. Multidrug resistance in cancer: role of ATP-dependent transporters. Nat Rev Cancer 2002;2: 48–58.
61. Leonard, GD, Fojo, T, and Bates, SE. The role of ABC transporters in clinical practice. Oncologist 2003;8: 411–24.
62. Trock, BJ, Leonessa, F, and Clarke, R. Multidrug resistance in breast cancer: a meta-analysis of MDR1/gp170 expression and its possible functional significance. J Natl Cancer Inst 1997;89: 917–31.
63. Moretti, JL, Azaloux, H, Boisseron, D, Kouyoumdjian, JC, and Vilcoq, J. Primary breast cancer imaging with technetium-99m sestamibi and its relation with P-glycoprotein overexpression. Eur J Nucl Med 1996;23: 980–6.
64. Kostakoglu, L, Elahi, N, Kiratli, P, Ruacan, S, Sayek, I, Baltali, E, Sungur, A, Hayran, M, and Bekdik, CF. Clinical validation of the influence of P-glycoprotein on technetium-99m-sestamibi uptake in malignant tumors. J Nucl Med 1997;38: 1003–8.
65. Sun, SS, Hsieh, JF, Tsai, SC, Ho, YJ, Lee, JK, and Kao, CH. Expression of mediated P-glycoprotein multidrug resistance related to Tc-99m MIBI scintimammography results. Cancer Lett 2000;153: 95–100.
66. Kao, CH, Tsai, SC, Liu, TJ, Ho, YJ, Wang, JJ, Ho, ST, and ChangLai, SP. P-Glycoprotein and multidrug resistance-related protein expressions in relation to technetium-99m methoxyisobutylisonitrile scintimammography findings. Cancer Res 2001;61: 1412–4.
67. Del Vecchio, S, Ciarmiello, A, Potena, MI, Carriero, MV, Mainolfi, C, Botti, G, Thomas, R, Cerra, M, D'Aiuto, G, Tsuruo, T, and Salvatore, M. In vivo detection of multidrug-resistant (MDR1) phenotype by technetium- 99m sestamibi scan in untreated breast cancer patients. Eur J Nucl Med 1997;24: 150–9.
68. Ciarmiello, A, Del Vecchio, S, Silvestro, P, Potena, MI, Carriero, M V, Thomas, R, Botti, G, D'Aiuto, G, and Salvatore, M. Tumor clearance of technetium 99m-sestamibi as a predictor of response to neoadjuvant chemotherapy for locally advanced breast cancer. J Clin Oncol 1998;16: 1677–83.
69. Sciuto, R, Pasqualoni, R, Bergomi, S, Petrilli, G, Vici, P, Belli, F, Botti, C, Mottolese, M, and Maini, CL. Prognostic value of $^{(99m)}$Tc-sestamibi washout in predicting response of locally advanced breast cancer to neoadjuvant chemotherapy. J Nucl Med 2002;43: 745–51.
70. Takamura, Y, Miyoshi, Y, Taguchi, T, and Noguchi, S. Prediction of chemotherapeutic response by Technetium 99m–MIBI scintigraphy in breast carcinoma patients. Cancer 2001;92: 232–9.
71. Mubashar, M, Harrington, KJ, Chaudhary, K S, Lalani el, N, Stamp, GW, Sinnett, D, Glass, DM, and Peters, AM. ^{99m}Tc-sestamibi imaging in the assessment of toremifene as a modulator of multidrug resistance in patients with breast cancer. J Nucl Med 2002;43: 519–25.
72. Cayre, A, Cachin, F, Maublant, J, Mestas, D, Feillel, V, Ferriere, J P, Kwiaktowski, F, Chevillard, S, Finat-Duclos, F, Verrelle, P, and Penault-Llorca, F. Single static view ^{99m}Tc-sestamibi scintimammography predicts response to neoadjuvant chemotherapy and is related to MDR expression. Int J Oncol 2002;20: 1049–55.
73. Fuster, D, Munoz, M, Pavia, J, Palacin, A, Bellet, N, Mateos, JJ, Martin, F, Ortega, M, Setoain, FJ, and Pons, F. Quantified ^{99m}Tc-MIBI scintigraphy for predicting chemotherapy response in breast cancer patients: factors that influence the level of ^{99m}Tc-MIBI uptake. Nucl Med Commun 2002;23: 31–8.
74. Chen, CC, Meadows, B, Regis, J, Kalafsky, G, Fojo, T, Carrasquillo, JA, and Bates, SE. Detection of in vivo P-glycoprotein inhibition by PSC 833 using Tc-99m sestamibi. Clin Cancer Res 1997;3: 545–52.
75. Peck, RA, Hewett, J, Harding, MW, Wang, YM, Chaturvedi, PR, Bhatnagar, A, Ziessman, H, Atkins, F, and Hawkins, MJ. Phase I and pharmacokinetic study of the novel MDR1 and MRP1 inhibitor biricodar administered alone and in combination with doxorubicin. J Clin Oncol 2001;19: 3130–41.
76. Agrawal, M, Abraham, J, Balis, FM, Edgerly, M, Stein, WD, Bates, S, Fojo, T, and Chen, CC. Increased ^{99m}Tc-sestamibi accumulation in normal liver and drug-resistant tumors after the administration of the glycoprotein inhibitor, XR9576. Clin Cancer Res 2003;9: 650–6.
77. Wong, M, Evans, S, Rivory, LP, Hoskins, JM, Mann, GJ, Farlow, D, Clarke, CL, Balleine, RL, and Gurney, H. Hepatic technetium Tc 99m-labeled sestamibi elimination rate and ABCB1 (MDR1) genotype as indicators of ABCB1 (P-glycoprotein) activity in patients with cancer. Clin Pharmacol Ther 2005;77: 33–42.
78. Hendrikse, NH, Kuipers, F, Meijer, C, Havinga, R, Bijleveld, CM, van der Graaf, WT, Vaalburg, W, and de Vries, EG. In vivo imaging of hepatobiliary transport function mediated by multidrug resistance associated protein and P-glycoprotein. Cancer Chemother Pharmacol 2004;54: 131–8.
79. Pusztai, L, Wagner, P, Ibrahim, N, Rivera, E, Theriault, R, Booser, D, Symmans, FW, Wong, F, Blumenschein, G, Fleming, DR, Rouzier, R, Boniface, G, and Hortobagyi, GN. Phase II study of tariquidar, a selective P-glycoprotein inhibitor, in patients with chemotherapy-resistant, advanced breast carcinoma. Cancer 2005;104: 682–91.
80. Mankoff, DA, Dunnwald, LK, Gralow, JR, Ellis, GK, Schubert, EK, Charlop, AW, Tseng, J, Rinn, KJ, and Livingston, RB. [Tc-99m]-sestamibi uptake and washout in locally advanced breast cancer are correlated with tumor blood flow. Nucl Med Biol 2002;29: 719–27.
81. Weidner, N, Semple, JP, Welch, WR, and Folkman, J. Tumor angiogenesis and metastasis–correlation in invasive breast carcinoma. N Engl J Med 1991;324: 1–8.
82. Neri, D, and Bicknell, R. Tumour vascular targeting. Nat Rev Cancer 2005;5: 436–46.
83. Jain, RK, Duda, DG, Clark, JW, and Loeffler, JS. Lessons from phase III clinical trials on anti-VEGF therapy for cancer. Nat Clin Pract Oncol 2006;3: 24–40.
84. Yoon, JH, Bom, HS, Song, HC, Lee, JH, and Jaegal, YJ. Double-phase Tc-99m sestamibi scintimammography to assess angiogenesis and P-glycoprotein expression in patients with untreated breast cancer. Clin Nucl Med 1999;24: 314–8.

85. Scopinaro, F, Schillaci, O, Scarpini, M, Mingazzini, PL, Di Macio, L, Banci, M, Danieli, R, Zerilli, M, Limiti, MR, and Centi Colella, A. Technetium-99m sestamibi: an indicator of breast cancer invasiveness. Eur J Nucl Med 1994;21: 984–7.
86. Kim, SW, Park, SS, Ahn, SJ, Chung, KW, Moon, WK, Im, JG, Yeo, JS, Chung, JK, and Noh, DY. Identification of angiogenesis in primary breast carcinoma according to the image analysis. Breast Cancer Res Treat 2002;74: 121–9.
87. Bekis, R, Degirmenci, B, Aydin, A, Ozdogan, O, Canda, T, and Durak, H. Correlation between ^{99m}Tc-MIBI uptake and angiogenesis in MIBI-positive breast lesions. Nucl Med Biol 2005;32: 465–72.