

BREAST

Why do some breast cancer cells remain dormant?*

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Abstract

Dormant breast cancer cells are a reality that cannot be overlooked. They may stay dormant either after a spread of cancer cells caused by surgery or after being generated by spontaneous or induced mutations in the course of breast gland growth. Some cases are presented in support of both possibilities, followed by a critical appraisal of the factors that may either keep them dormant or later stimulate their growth into a breast cancer.

Keywords: *Dormant cancer cells, immunity, angiogenesis, genetics, prevention, surgery*

Introduction

Clinical observations that lack scientific explanations are extremely helpful to stimulate and direct the interest of basic and clinical investigators into other areas that may eventually turn out to be of capital importance.

The following case reports justify the present critical review:

- (1) Case 1: A 72-year-old woman who came to me, many years ago, because of a bloody vaginal discharge that was later proved to be due a cervical carcinoma. She told that when she first noticed the spotting she also noticed the appearance of a 5 mm nodule in the scar of the total mastectomy done 20 years before, which had been followed by extensive external radiation. Biopsy: breast cancer!
- (2) Case 2: A patient followed since 1986 had a previous mastectomy in 1985. It was done because there were suspicious calcifications. The surgeon had difficulty in locating them but finally a piece of tissue was shown by the pathologist to contain a ductal carcinoma *in situ* (DCIS). This patient went to London for a second opinion. The sections were re-examined and there the

consultant felt that there had been no need for such an extensive ablation; she was cured, and neither chemotherapy nor radiation was needed. When the scar of the incision was examined a very small 3–4 mm red nodule was noticed, that was not there one year before. A puncture biopsy was done. The result: breast cancer!

Both cases are extremely intriguing. One of them suggests that radiation does not destroy every cancer cell and that these can remain dormant for many years until some defense mechanisms decrease and let other cancers develop, as happened in the case of the cervical cancer patient and in the following case.

- (3) Case 3: A 58-year-old nulliparous patient had a routine mammogram taken in August 2003 that showed some suspicious lesions on the left breast. This was repeated one month later with the diagnosis of non-suspicious lesions. In November 2004 the patient felt a lump in her right breast. Another mammogram was taken; the diagnosis was malignancy and she underwent surgery. The tumor was 2.5 cm in diameter, with a differentiation of (3 + 2 + 1) according to Elston and Elly criteria. Thirteen nodes removed from the axilla were all negative.

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*In view of the complexity of this review, the author (a gynecologist, not an immunologist) has decided to use the same words as the quoted authors as much as possible, in order to avoid any unintended misquotation.

Tumor 100% positive for estrogen receptor; she is being treated with tamoxifen. In October 2004 this same patient had hematuria. The conclusion was the presence of an invasive carcinoma of the transition epithelium of the bladder with invasion of the muscular layer (pT2a). She underwent more surgery and a total hysterectomy was done at the same time (March 2005). The fact that a second different cancer manifested soon after the first was diagnosed strongly suggests that dormant cancer cells might be present in the bladder and breast and that a fall in immunity may have caused the appearance of the bladder cancer and breast cancer, in a breast that one year before was considered to be normal by digital mammography!

Another example, in men, is the risk of developing a second primary cancer following radiotherapy for testicular seminoma. The second primary cancers reported were two bronchogenic carcinomas, one contralateral seminoma, one thymoma, one papillary carcinoma of the thyroid, one carcinoma of the stomach, one transitional cell carcinoma of the urinary bladder, one carcinoma of the colon and one malignant melanoma. Three of these tumors developed within the irradiated field [1].

There are similar reports in the literature. Eleven of 28 women developed invasive breast carcinoma (IBC), all in the same breast and quadrant from which their low-grade DCIS biopsy was taken. Seven IBC cases were diagnosed within 10 years of the DCIS biopsy, one was diagnosed within 12 years of the DCIS biopsy, and the remaining three IBC cases were diagnosed over 23–42 years. Five of these women, including one woman who developed IBC 29 years after her DCIS biopsy, developed distant metastasis, which resulted in death 1–7 years after the diagnosis of IBC. The natural history of low-grade DCIS can extend greater than four decades, with IBC developing at the same site as the previous DCIS in the majority of women. There is a 30% chance of developing IBC in the 15 years after low-grade DCIS is found [2].

It is tempting to accept that out of millions of mitoses going on in the body some may not be normal and thus cause (many) mutants. But cancer develops and progresses to be lethal in only a very small percentage of most of us. Microscopic tumors are mostly dormant and need additional signals to grow and become lethal. So what are these additional signals and why are most of us protected from them [3]?

It has been estimated that more than a third of women aged 40–50 years who did not have cancer-related disease in their lifetime were found at medicolegal autopsies with ‘*in situ*’ ductal carcinomas in their breasts. Microscopic breast cancers were

found in 30% of women autopsied; 45% of the women with cancer had two or more lesions, and over 40% had bilateral lesions. Half of the lesions were detected usually as microcalcification on postmortem radiography of the resected breasts [4]. But breast cancer is diagnosed in only 1% of women in this age range.

In breast cancer, a small percentage of women treated surgically are at risk for recurrence even 15–20 years after being ‘cured’ of cancer. Thus, individuals treated for cancer may harbor a large reservoir of disseminated dormant cancers. It seems that neovascularization plays an important role in the transition from dormant metastasis to rapidly growing tumor. That is the role of angiogenesis. Vasculature is a paracrine regulator of apoptosis. Inadequate vascularization can elicit tumor cell apoptosis and cell death [5]. In the absence of angiogenesis an *in situ* tumor can remain dormant indefinitely [3].

Angiogenesis

Some human tumor lines do not form visible tumors when inoculated into immunosuppressed mice. Although the tumor lines fail to grow progressively, they form small dormant microscopic foci maintained at constant mass by balanced proliferation and apoptosis. Dormancy is lost when there is a switch in the angiogenic balance toward increased vascularity with reduced tumor cell apoptosis.

The progression depends crucially on the balance between the *in situ* tumor’s total angiogenic output and an individual’s total angiogenic defense (the inhibitors) that are either associated with specific tissues or circulating in the blood. They include thrombospondin, tumstatin, canstatin, endostatin, angiostatin and interferon α/β [6]. Nature has provided some interesting models like Down’s syndrome: these patients have a very low incidence of solid tumors probably due to high circulating levels of endostatin [3].

Another puzzling observation is that when breast cancer survivors are submitted to hormone replacement therapy (HRT) for the alleviation of severe vasomotor symptoms they have fewer recurrences and longer survival compared with matched controls. Why? In the Women’s Health Initiative estrogen-only arm, an observation of almost statistical significance was that conjugated estrogens alone do not increase the number of diagnoses of breast cancer but may even decrease them [7].

What is the role of immune defense mechanisms?

Immunity

It is certain that estrogens, and to a lesser degree progesterone, modify immunity. Estrogens and progestogens are known to be immune modulators in opposite directions. What is the mechanism of the

effect of estrogens and progesterone on immunity and what is the role of decreased immunity in the late appearance of breast cancers? Is this the explanation for their role in the risk of breast cancer under HRT?

The cells of the immune system

There are two arms to the immune system: the non-specific (innate or natural) immune system and the specific (acquired or adaptive) immune system. The effector cells of the non-specific immune response are monocytes, macrophages, granulocytes (neutrophils, eosinophils and basophiles), dendrite cells and natural killer (NK) cells, producing cytokines to enhance non-specific immune and specific immune responses (all cells). The cellular components of the specific immune response are T lymphocytes and immunoglobulin-producing B lymphocytes. However, postmenopausal women showed a reduction in the number of total lymphocytes in comparison to fertile women (as a result of decreased B and T helper (Th) lymphocytes). HRT in postmenopausal women affected lymphocyte subtypes: total lymphocytes count, the percentage of T cells and the percentage of Th lymphocytes. One of the main functions of Th lymphocytes is the production of cytokines. While HRT reduced the production of interleukin (IL)-2 by lymphocytes, IL-2 production was increased by lymphocytes of postmenopausal women compared with fertile women. B lymphocytes are antibody-producing cells and constitute 5–15% of circulating lymphocytes. During menopause, monocyte counts declined following estrogen replacement therapy. This decreasing effect of estrogen and progesterone on monocyte numbers may be due to sex hormones inducing meiotic arrest and apoptosis in monocytes. A very important function of monocytes is to direct immune responses by the production of cytokines. Important cytokines in this respect are IL-1 β , tumor necrosis factor- α , IL-12, IL-18 and IL-6 [8].

The Fas system

The Fas/Fas ligand (FasL) system, a major regulator of apoptosis, is involved in cancer cell death induced by the immune system and anticancer drugs. Fas is a cell-surface receptor. In both sexes, soluble Fas (sFas) levels increased with age. High-sFas patients showed a worse prognosis than low-sFas patients. Compared with healthy female controls, breast cancer patients, especially those with liver metastases, had higher circulating sFas levels [9].

The ratio FasL:Fas is an apoptosis-related marker. Fas-mediated apoptosis is triggered by FasL, the natural ligand of the receptor. The Fas receptor is a widely expressed cell-surface molecule. The FasL is a

tumor necrosis factor. It is detected predominantly in activated T cells and activated NK cells.

Estrogens have an immune-inhibiting effect. Thus, the induction of FasL and reduction of CD40 expression by estrogen could paralyze the immune system and set free breast cancer cells that are being held at bay by the immune system (Naftolin F, personal communication, 2005). Clinical significance of the Fas/FasL system in hormone-sensitive carcinomas such as those of the breast and ovary has been reported. The link between estrogen, monocytes/macrophages and the Fas/FasL system has been investigated. Estrogen treatment increased FasL expression in monocytes through estrogen-induced apoptosis in monocytes expressing estrogen receptor (ER) β , but not in monocyte-differentiated macrophages expressing ER α . It has been shown that estrogen replacement therapy in postmenopausal women decreased the number of circulating monocytes. Estrogen may regulate immune cell survival through the Fas/FasL system [10].

However, during neoplastic growth, high doses of estrogen can promote tumor regression in postmenopausal women with hormone-dependent breast cancer, but the mechanism is unknown [11].

During neoplastic growth and metastasis, the immune system responds to the tumor by developing both cellular and humoral immune responses. Despite this active response, tumor cells escape immune surveillance. It was shown (Baulieu EE, personal communication, 2005) that FasL expression by breast tumor plays a central role in the induction of apoptosis of infiltrating Fas-immune cells, providing the mechanism for tumor immune privilege. FasL in breast tissue is functionally active, and estrogen and tamoxifen regulate its expression. Moreover, an increase in FasL in breast cancer cells induces apoptosis in Fas-bearing T cells and tamoxifen blocks the induction of apoptosis. These studies provide evidence that tamoxifen inhibits FasL expression, allowing the killing of cancer cells by activated lymphocytes. This partially explains the protective effect of tamoxifen against breast cancer [12].

Breast tumors are frequently associated with a predominantly lymphocytic infiltrate, which constitutes an immune response against the tumor. Despite this massive infiltrate, the immune response appears to be inefficient and the tumor is able to evade it. In breast cancer, tumor escape from immunological surveillance results from the induction of apoptosis of Fas-bearing activated lymphocytes by FasL-bearing breast cancer cells. Breast tumors may elude immunological surveillance by inducing, via the Fas/FasL system, the apoptosis of activated lymphocytes. Recent data have demonstrated FasL RNA in other tumor types. Upregulation of FasL expression in hyperplastic and normal breast ducts close to the

tumor also suggests a possible role in early neoplastic transformation and proliferation [13].

Natural killer cells

NK cells arise from the bone marrow precursor cells that also give rise to T cells. NK cells are a crucial component of the immune surveillance of cancerous cells and their tumoricidal activity has been shown to be modulated by estrogens. NK cells and specialized T cells also recognize and lyse stressed host cells [15].

Immature dendritic cells stand ready to mature upon activation by exposure to danger signals [16]. This activates the co-stimulatory pathways critical for effective T cell activation, enabling the development of a finely tuned antigen-specific immune response. It is the ability of mature dendritic cells to activate both CD4⁺ and CD8⁺ T cells in the context of proper T cell co-stimulation that allows them to orchestrate a more potent immune response than any other antigen-presenting cell [17,18].

Activated CD4⁺ T cells provide help to maximize the humoral (antibody) response mediated by B lymphocytes, and the magnitude and durability of the CD8⁺ cytotoxic T cell response. The immune response is further integrated by the engagement of antigen-specific Ig with specific receptors on neutrophils, macrophages and NK cells, resulting in an alternative path to cell-mediated cytotoxicity (antibody- or complement-dependent). The integration of innate and adaptive immune responses by dendritic cells and immunoglobulin molecules argues that synergistically engaging innate and antigen-specific immune effectors is likely to result in the most effective tumor rejection response [18].

NK cells play a crucial role in host defense against pathogens and immune surveillance against cancer. Given that estrogens have been reported to suppress NK cell activity, a study was done to elucidate the mechanisms by which estrogen mediates this effect. Both ER α and ER β were expressed in murine NK cells; 17 β -estradiol elicited a significant decrease in NK cell activity in both wild-type and ER α -knockout mice ($p < 0.001$).

The murine NK cells potentially can respond to estrogen signaling through ER β or a novel estrogen receptor, since ER α -deficient NK cells display a cytolytic capacity similar to that of wild-type NK cells. NK cells have the potential to respond directly to estrogens. Estrogens may act directly on NK cells via the ability of estrogen to alter the mitogen-activated protein kinase [19]. The existence of diverse signaling pathways through which estrogen could modulate NK cell activity creates potential avenues for the design of selective estrogen receptor modulators (SERMs) [20,21] to modify NK cell activity in a potentially beneficial manner; i.e. for

cancer patients an increase in NK cell activity should be desirable.

The data suggest a new role for ER β in the regulation of NK cell lytic activity or the existence of a novel estrogen receptor(s) in the immune system [22].

Approximately 5% of leukocytes are NK cells. Peripheral blood NK cells can be recognized by the fact that they express CD16⁺/CD56⁻ or CD16⁺/CD56⁺. NK cells are capable of killing virus-infected cells or tumor cells in the absence of prior immunization and without major histocompatibility complex restriction. In conclusion, it seems likely that estrogen decreases NK cell numbers and NK activity, but that sex hormones do not affect NK cell cytokine production [8].

Higher numbers of dormant NK cells are markers of activation of the immune system. They may be affected by several factors such as diurnal variability and the intake of concomitant medication. There is a pattern of association of prognostically favorable factors such as ER-positive tumor and older age with higher NK cell counts or with β_2 -microglobulin or soluble IL-2 receptor [22].

A recent systematic review has found preliminary evidence that exercise can improve blood immune function in cancer survivors. The improvements that have been shown include increased NK cell cytotoxic activity, which was increased *in vitro* by exercise training. Recent data suggest a positive correlation between good NK cell function and disease-free and overall survival, as well as poor NK cell function and disease relapse, and that NK cell cytotoxic activity was higher in tumor-free survivors compared with those who had tumor-related deaths at 50 years of age. Exercise training increased NK cell cytotoxic activity and unstimulated [³H]thymidine uptake by peripheral blood lymphocytes in postmenopausal breast cancer survivors [23].

There is humoral immunity in some patients with breast cancers. Unexpectedly, the antitumor humoral immunity, which leads to decreased malignant cell survival, was also found in a minority of healthy people [24].

Polymorphic epithelial mucin immunoglobulin G antibodies

Free, natural polymorphic epithelial mucin (MUC1) antibodies are present in the circulation of healthy subjects as well as in cancer patients. Nulliparous women had significantly lower absolute levels of MUC1 immunoglobulin G (IgG) antibodies than all other groups studied [25]. It is attractive to link these observations with those obtained from epidemiologic studies indicating that nulliparity as well as older age at first delivery increase the lifetime risk of breast cancer, whereas high parity [26] and prolonged

breastfeeding seem to reduce the risk of breast cancer [27]. Although it can be explained otherwise [29], the observed association could suggest a role of MUC1 immune responses in the immune surveillance of breast cancer. Isolated disseminated tumor cells can be found at the time of primary surgery in the bone marrow of 30% of lymph node-negative breast cancer patients [14]. Antibodies may be effective in eradicating these circulating tumor cells while exerting a limited effect against the primary tumor. Antibodies to MUC1 could be involved in restoring cell adhesion, uncovering cell-surface receptors involved in immune recognition and neutralizing the immunosuppressive effect of soluble MUC1. The benefit in survival observed in patients with stage I and II disease and positive levels of MUC1 IgG antibodies could be signaling the presence of a MUC1-specific Th lymphocyte response. This possibility is strengthened by recent results [25] that indicate a predominance of IgG2 subclass in these responses. On the other hand, the effectors do not necessarily have to be specific T cells, at an early tumor stage. Immune surveillance and checking disease spread could be achieved by the broadly reactive properties of NK cells. In conclusion, these preliminary results seem to indicate that naturally occurring MUC1 antibodies may check disease spread in patients with breast cancer, possibly by destroying circulating or seeded isolated disseminated tumor cells (micrometastases) that eventually could lead to metastatic disease and death [25].

Patients with metastatic disease, stable or responsive to first-line antiestrogens, have been treated with cyclic administration of β -interferon and IL-2 combined with continuous conventional antiestrogen therapy. In patients with clinical benefit, eosinophils, total lymphocytes and CD4⁺, CD8⁺ and CD16⁺/CD56⁺ cells increased significantly after IL-2 administration. The estimated median benefit and survivals were more than three times longer than previously shown in similar populations. The differing response to IL-2 can be explained by the hypothesis that resting cancer cells do not inhibit the immune system during clinical benefit, while at the onset of resistance they recover the constitutive ability to inhibit it [29]. Therefore, it can be hypothesized that tumor cells, being in G0-G1 state during clinical benefit from antiestrogen salvage therapy, do not inhibit the immune system. On the other hand, at progression, tumor cells resistant to antiestrogens recover their constitutive function and inhibit the immune system [29].

There are data suggesting that estradiol promotes tolerance by expanding the regulatory T cell compartment [30]. Tolerance toward tumor antigens is an important explanation for the failure of the immune system to reject breast tumors with T cells that are capable of recognizing them. Breast cancer arises

in immunocompetent hosts. There is increasing evidence pointing to tolerance towards tumor antigens as an important explanation for the failure of the immune system to reject breast tumors. A better understanding of the cellular and molecular mechanisms involved in breast tumor-induced antigen-specific T cell tolerance may lead to approaches to effectively harness the immune system against this malignancy [31].

It has been shown experimentally that psychosocial processes influence the susceptibility to some infections, to some neoplastic processes and to some aspects of humoral and cell-mediated immune responses [32].

Genetics

The role of methylation in gene expression is pivotal. How does it happen? A given gene that is methylated and therefore silenced can stop the neoplastic process and the demethylation that can occur by changing the cellular milieu, even by diet, can demethylate that gene and the whole process starts again [28].

Conclusions

From the above review it seems that:

- (1) Malignant cells may remain or spread all over the body at the time of surgery;
- (2) These cells may remain dormant and may not develop into tumor metastases;
- (3) Immune defense mechanisms may play an important role in keeping malignant breast cells dormant;
- (4) Naturally occurring antiangiogenic factors prevent those nests of malignant cells from receiving proper blood supply to stimulate their multiplication;
- (5) Methylation of some genes may inactivate them;
- (6) Exercise may stimulate NK cell activation;
- (7) The interplay of all these factors is of paramount importance to keep the body free from the development of a malignant breast tumor.

The disruption of these barriers is the trigger for malignant cell growth. There are several possible causes, from diet to drugs, from inactivity to old age, etc. Demethylation of genes can activate them; NK cells can decrease; T cells may not be able to recognize tumor antigens. Hormone influences are no doubt extremely important. Estrogens may also decrease NK activity and thus may indirectly become tumor growth promoters.

This review poses many important problems with regard to both disease-free women and those who

were 'cured' by surgery, radiation, chemotherapy, etc., since it is far from certain that such therapeutic interventions are capable of eradicating existing tumor cells for good. Its focus on disease-free women, who did have or never had a breast cancer, may stimulate interest in the favorable role of exercise, vitamin E, proper nutrition, SERMs, etc.

The biology of breast cancer cells has entered into a new, very stimulating and complex era with the advent of antiangiogenic factors, vaccines that may fight immune tolerance, aromatase inhibitors, SERMs and, even more surprisingly, the possibility that estrogens may have to induce cancer cell apoptosis in estrogen-deprived milieu! It may also be that in the future high doses of estrogens may prove helpful in tamoxifen-resistant tumors, as suggested by some ongoing clinical trials; these new approaches are already stimulating a wave of optimism, but this is outside the scope of the present review.

Other questions to be answered

- (1) If antiangiogenic factors are important in these cases, why do normal cells around tumor cells still survive?
- (2) Could aspirin be used to prevent cancer? Three recently published studies indicate that aspirin may soon become a first line of defense against cancer [33]. Breast cancer and non-steroidal cyclooxygenase-2 (COX-2) induction may promote breast cancer development by enhancing local estrogen biosynthesis, and COX-2 inhibition may reverse the process [34].
- (3) Should statins be used to prevent the proliferation of human breast cancer cells? Atorvastatin and fluvastatin can inhibit the proliferation of MCF-7 cells in the absence of estradiol. This effect seems to depend on an apoptotic statin effect. Statins may possess anticarcinogenic properties concerning the development of breast cancer in postmenopausal women [35]. Statins inhibit the activity of the rate-limiting enzyme in the cholesterol biosynthetic pathway, HMG-CoA reductase, and are widely prescribed for lowering plasma lipid levels. Several statins have antitumor effects in experimental models, and observational studies suggest that this anticancer activity in the laboratory may translate into effective treatments and/or preventive strategies for certain human cancers. There is laboratory and clinical evidence that statins have anticancer activity [36].
- (4) Is vitamin E of value? The dietary form of vitamin E (δ -tocotrienol) and a novel non-hydrolyzable vitamin E analog (α -TEA) induced estrogen non-responsive MDA-MB-435 and estrogen-responsive MCF-7 human breast cancer cells undergo high levels of apoptosis and caused either no or lower levels of apoptosis in normal human mammary epithelial cells and immortalized but non-tumorigenic human MCF-10A cells. These data provide a better understanding of the anticancer actions of vitamin E [37]. Experimental evidence strongly suggests that dietary supplementation with tocotrienols may provide significant health benefits in lowering the risk of breast cancer in women [38]. Moreover, α -tocopheryl succinate (α -TOS) is a redox-silent vitamin E analog with high pro-apoptotic and antineoplastic activity. α -TOS causes efficient apoptosis in breast cancer cells independent of their erbB2 status. Since erbB2 is frequently overexpressed in breast cancers and renders the neoplastic disease resistant to established treatment, the findings are of clinical interest [39].
- (5) What about other micronutrients? High intake of folate or adequate circulating levels of folate may reduce the risk of breast cancer [40]. Epidemiological studies show that a high intake of antioxidant-rich foods is inversely related to cancer risk, and carotenoids have been shown to help reduce breast cancer risk. Experimental studies show that antioxidant vitamins and some phytochemicals selectively induce apoptosis in cancer cells but not in normal cells and prevent angiogenesis and metastatic spread, suggesting a potential role for antioxidants as adjuvants in cancer therapy [41].
- (6) Concerning food intake patterns, lack of adherence to a Mediterranean diet and lifestyle factors is associated with a population-attributable risk of 60% of death from cancer [42]. The first set of human evidence shows that breast cancer risk is significantly inversely associated with tea intake, largely confined to intake of green tea. Green tea may have downregulatory effects on circulating sex steroid hormones, whereas black tea may have upregulatory effects [43]. Some findings that seem to suggest the possibility that a fat-rich diet may produce the dual steroidal disorders of ovarian dysfunction and hypercorticism, which in turn will open the way to breast carcinogenesis by activating two proto-oncogenes at the initiation and promotion steps [44].
- (7) Is exercise important to improve survival by increasing NK cell cytotoxic activity?
- (8) What is the role of psychological stress? In a case-control study of 257 women with breast cancer who had surgery and 565 controls free of any cancer, it was found that women with

major life events, stress of daily activity and depression had 3.7 times higher risk for breast cancer than women who did not experience such stress (odds ratio = 3.70, 95% confidence interval 2.61–5.26) [45]. In addition to the known risk factors, psychological determinants such as depressed mood may play an important role in the etiology of breast cancer [46].

- (9) If the tumor cells are dormant because they are recognized by the immune system, could some markers (antibodies) be identified to signal their presence? Both disease-free and overall survival rates were greater in patients with ER- or progesterone receptor-positive tumor cells who had detectable levels of soluble lymphocyte activation gene-3 (sLAG-3) at diagnosis versus patients with undetectable sLAG-3 levels. These results indicate that sLAG-3 may be a valuable marker for prognosis in some subsets of breast cancers and, more importantly, that cell-mediated mechanisms such as Th1 responses do have an impact on survival [47]. If dormant cells they are recognized by the immune defenses why they are not destroyed?
- (10) During surgery of the primary tumor should one always avoid cold knife excision below the skin in order to prevent the spread of tumor cells?
- (11) Should surgery always be performed in the first half of the luteal phase in premenopausal women, as suggested by some studies that find better survival rates? Premenopausal women with early breast cancer have a significantly better prognosis if their tumors are excised during the luteal phase of the cycle. Changing the hormonal milieu at the time of surgery for breast cancer may have the ability to improve the prognosis for some patients with early breast cancer [48–50].
- (12) Are there any means to boost the immune system before or after surgery?
- (13) Should SERMs be used as prophylactics in order to stimulate NK cells?

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