# Tumor cells commence to disseminate early in oncogenesis and are accountable for secondary tumor formation

*Do not awaken her, induce and prolong her dormancy*

**Linda van Weele**
**May 2011**

# Tumor cells commence to disseminate early in oncogenesis and are accountable for secondary tumor formation

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Date: May 2011

Figure front page derived from Disney (1).

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# Summary

Whereas in cancer the solely primary tumor can often be conquered with therapy, the presence of metastases is frequently lethal. The multi-step process of metastasis starts with tumor cells disseminating from the primary lesion. The most commonly accepted theory states that dissemination starts late in tumorigenesis, when the primary tumor cells have acquired full malignancy. However evidence obtained from mainly breast cancer patients and mouse models, indicates dissemination already starts during early tumor stages, when tumors are still marked as benign. Although tumor dissemination might commence during an early tumor stage, dissemination is only the first step in metastasis. The last step, colonization of a cancer cell at a distant site leading to the outgrowth of secondary tumors, is only completed by a small number of cells. Other evidence indicates that early spreading tumor cells can also fulfill this ultimate step of metastasis. Nevertheless, the spread of cancer cells is often not directly followed by the formation of malignant metastases. One concept that explains this observation is the occurrence of dormancy. Here, tumor cells are kept innocuous by the influence of antiangiogenic factors, the tissue microenvironment they try to settle in, and/or the immune system. This might offer an interesting concept for therapy: if tumor cells cannot be completely eliminated from the body, inducing and prolonging an inactive state might be an option.

Abbreviations used: bone marrow (BM), carcinoma in situ (CIS), circulating tumor cell (CTC), disseminated tumor cells (DTC), peripheral blood (PB), single-nucleotide polymorphism (SNP)

# Introduction

Cancer is a widely spread disease and has a high mortality rate. From 2008, it was estimated that throughout Europe 3.2 million people were diagnosed with cancer and 1.7 million cancer patients died of cancer. Out of this number, breast cancer accounted for 7.5% of deaths, making it the number three cause of death among the different cancers (2). Lethality is often not a result of the damage caused by the primary tumor itself, but by complications caused by metastasis. Metastasis is the formation of distant secondary tumors that derive from cells that are released by the primary tumor. Metastasis starts with dissemination: the process of cells that leave the primary tumor and spread throughout the body. The basement membrane plays an important role in dissemination. It is a thin layer of specialized extracellular matrix found between the connective tissue and the epithelium, covering cavities and surfaces (e.g., the breast), or the endothelium, covering the blood vessels. In this position, it gives support to tissues and regulates molecular transport and the signaling between tissues (3). When the basement membrane is intact, cells cannot migrate out of their home tissue. Therefore it is thought that the basement membrane needs to be disrupted before cells can leave the primary lesion and disseminate to distant sites. Indeed, the occurrence of metastasis is correlated with late and end stage malignant tumors, which are marked by the disruption of the basement membrane (Table 1). In contrast, cells cannot leave the primary tumor in early benign tumors, since the basement membrane is still intact (Table 1) (4). This positions the fragmentation of the basement membrane as a hallmark of the transition from benign to malignant.

| Time point | Basement membrane intact? | Evidence of metastasis? M0: yes M1: no | Tumor stages | Hallmarks |
|---|---|---|---|---|
| Early | Yes | M0 | Neoplasm, adenoma | Uncoordinated proliferation of cells in a tissue, exceeds growth speed surrounding tissue, benign |
| Intermediate | Yes / no (both possible) | M0/M1 (both possible) | Carcinoma in situ (CIS) | Some invasion into surrounding tissue, transition from benign to malignant |
| Late and end | No | M1 | Early and late carcinoma | Invasive into surrounding tissue, metastasis, malignant |

**Table 1.** The different stages of primary tumor development.

Cancer cells need to undertake several steps to complete metastasis. Firstly, the cells need to escape the primary lesion by invading the host tissue barriers surrounding the primary lesion. Hereafter, they need to pass the basement membrane to reach blood or lymphatic vessels for transportation. In the vessels, the cells can encounter the hostility of the immune system and the damaging power of the vigorous blood flow. This is then followed by attempts to halt in a capillary bed of the target organ. Here the cell needs to exit the vessel and settle in the target tissue. Metastasis is completed when these disseminated cells establish a tumor at this distant site from the primary tumor (Figure 1) (5). This last step is also referred to as colonization. The failure of these cells somewhere down this path is rather rule than an exception; thus metastasis is known as an inefficient process. However, dissemination may be a very efficient process.

The predominant theory places metastasis late in tumor development. Since disruption of the basement membrane and obvious invasion of the surrounding tissue only present in late stages, dissemination is thought not to occur during the early stages. Furthermore, late dissemination fits in the concept of clonal expansion of the tumor. This theory states that Darwinian evolution applies to tumorigenic genetic changes in a cell. This means that the cell that acquires mutations that enable it to grow faster than its neighboring cells, will outgrowth the other tumor cells as so to become the predominant cell type in the lump.
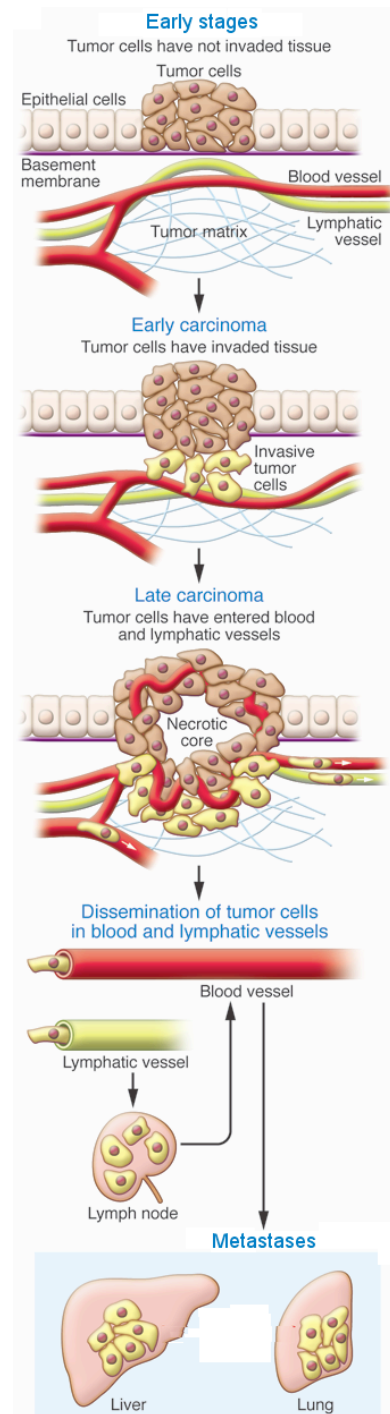


**Figure 1. Classical model of metastasis.** Cells start disseminating from the primary tumor at a late time point when the basement membrane is disrupted and the surrounding tissue is invaded. Dissemination takes place to lymphatic and blood vessels to reach ectopic tissues to establish metastases. Figure derived from Röcken (6).

the predominant cell type in the lump. This is also applicable to the change from benign to malignant. For this transition a tumor needs to obtain mutations that activate oncogenesis and/or inactivate tumor suppressor genes. The more mutations a tumor cell has acquired, the likelier these mutations will enable tumor cells to metastasize. Such a correlation, between the risk on metastasis and the size of the primary tumor, has been observed (7,8). Therefore it is generally thought that those cells that disseminate derive from the most advanced tumor cell that is present in late tumors (Figure 2A). However, evidence challenges this concept and supports a model where dissemination occur early (Figure 2B). Firstly, the common occurrence of cancer of unknown primary origin shows that a tumor does not have to be large to metastasize. Cancer of unknown primary origin presents in approximately 5% of patients with distant metastasis (9). Secondly, M0 patients can relapse after complete resection of their primary tumor (10). This contradicts the idea that cells can only disseminate late from the most advanced clone. Thirdly, angiogenesis can already commence when tumors are still marked as benign (11). Angiogenesis is the growth of new blood vessels, sprouting from existing ones. In mammals angiogenesis only takes place during embryogenesis, the female reproductive cycle, and/or wound healing. Furthermore, it plays a vital role in malignancy. Like every tissue, a tumor needs the supply of nutrition and it needs to remove waste. Nutrition is acquired from blood and waste is deposited in blood.
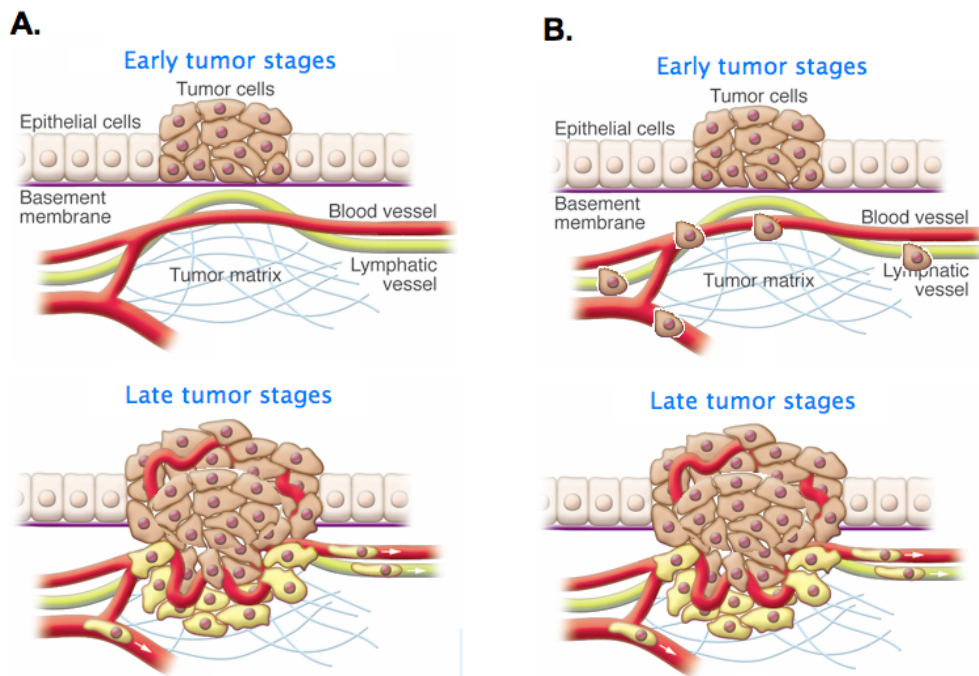


**Figure 2. Late and early dissemination.** A. Late dissemination. In early tumor stages the basement membrane is intact and tumor cells have not left from the primary tumor site yet. In late tumor stages, when the basement membrane is disrupted, cells excess blood and lymph vessel to spread to distant locations. B. Early dissemination. Although the basement membrane is not disturbed in the early tumor, tumor cells have disseminated from the primary tumor. Dissemination commences during late tumor stages. Figure adjusted from Röcken (6).

The growth of malignant tissue requires enhanced proportions of both nutrition delivery and waste deposit. This increased demand can only be met by angiogenesis (12,13). Moreover, these newly formed vessels are used for dissemination. Therefore, it is not surprising that elevated vascularization has shown to be highly associated with an increased risk on malignancy (14). Thus the early activation of angiogenesis suggests cells willing to disseminate are being assisted. Fourthly, dormancy seems to occur. Tumor dormancy signifies a cancer cell is shielded for clinical detection due to a prolonged quiescent or proliferation-apoptosis balanced state (15). Either way, dormant tumor cells are innocuous. Dormancy can be induced after extravasation, when tumor cells arrive in a target tissue or stay transitory in the bone marrow (BM) or in the lymph nodes. From here tumor cells die, escape or prolong the dormant state (Figure 3). Tumor cell dormancy is a frequent occasion. This is shown by autopsies performed on people aged over 70, revealing the presence of multiple micrometastases that never made it to detection (16-18). The occurrence of dormancy may give a cell the possibility to disseminate early without leading directly to the development of a secondary tumor when arriving at a distant location. All these different types of evidence provide reasonable ground to question the predominate theory that states that dissemination and colonization only take place during late CIS and thereafter. This motivates to look further into the possibility of tumor cells commencing to disseminate during early tumor stages potentially leading to a prior time point of colonization.

If early spreading tumor cells are responsible for colonization, the gauging of disseminated tumor cells might be used to give a reliable prognosis for the occurrence of metastasis. Currently, several indicators and methods are in use to give such a prediction. The most commonly used method for classifying tumors is the TNM Classification, using an increasing scale to indicate gravity. The TNM Classification describes the size and the histological severity of the primary tumor (T0-4), the degree of metastases in adjacent lymph nodes (N0-3), and the presence of metastases (M0/1) (19). Although the occurrence of metastases in adjacent lymph nodes is highly predictive for the development of metastases at other sites in most cancers (20), in breast cancer this is not always applicable. In breast cancer metastases are not always found in adjacent lymph nodes while being detected at other distant locations (7,21,22). In addition, it has been shown that lymph node status does not need to be included to come to a reliable prognosis (7, 105). Therefore it seems that the dissemination does not exclusively occur via the adjacent lymph nodes (7). This triggers the search for better prognostic factors. Other well-investigated markers are circulating tumor
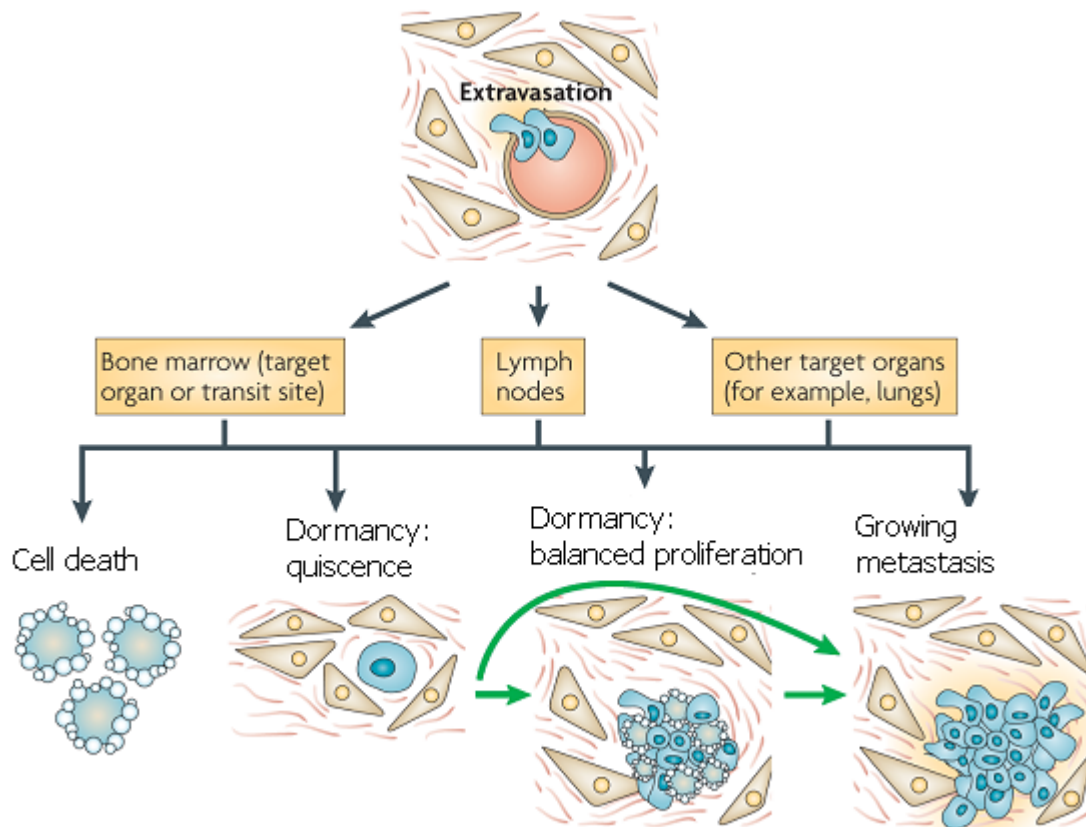
**Figure 3. The paths a disseminated tumor cell can encounter upon extravasation.** Upon extravasation, tumor cells can arrive in transit or in a target tissue. Transit sites can be BM or lymph nodes. Target tissue can either be one of these two or other tissues. Disseminated tumor cells can encounter at any of these places death, dormancy, or outgrowth into a micrometastasis. In case of dormancy, tumor cells may turn quiescent or find a balance between proliferation and apoptosis keeping its tumor size equal. Arrows in green show tumor cells escaping the dormant state, resulting in the outgrowth of metastases. Figure adjusted from Aguirre-Gisho (15).

cells (CTCs) in peripheral blood (PB) and disseminated tumor cells (DTCs) in BM. The large benefit of these markers is that they can be monitored over time. In contrast to lymph nodes that are often removed when a patient is at risk of metastasis and so can only provide information once. Especially the monitoring of CTCs would be desirable, since blood withdrawal is a low invasive tool and can be easily performed on a regular basis. The detection of CTCs in PB has been shown to function as a reliable prognostic factor (23-26). It has even been noted that elevated CTC levels during therapy predict augmented progression and mortality in breast cancer patients (27). Nonetheless, studies show that DTCs in BM have a stronger predictive value than the presence of PB CTCs (28-31). Thus although the monitoring of CTCs would be favorable, the detection in BM seems to be more reliable. Therefore the focus here will be on BM DTCs for evidence of dissemination and subsequent metastasis.

This thesis will look into the time point of dissemination and the consequences for metastasis. Evidence shows the bulk of the primary tumor possesses the potential to metastasize, indicating this treat is acquired early in tumorigenesis. This is confirmed by the detection of DTCs in BM during early tumor stages. Furthermore, the presence of BM DTCs predict poor outcome. This contradicts the predominant theory that states that dissemination only occurs from highly mutated cells late in tumor development. This thesis will describe the evidence supporting the alternative theory that dissemination occurs early in tumor development and discusses the consequences for the successive steps in metastasis. Furthermore the concept of dormancy will be addressed by discussing both endogenous and exogenous factors inducing and prolonging the dormant cell state. Ultimately the consequences of early dissemination and the potential of dormancy for future therapy will be discussed.

## Argumentation

### 1. Tumor cells hold the capacity to disseminate early

Hypothetically, tumors cells should have the capacity to disseminate in early phases in tumorigenesis as first proposed by Bernards & Weinberg (32). They suggest that in case of clonal expansion due to Darwinian evolution, there would be no benefit for a cell in obtaining the capacity to metastasize, since metastatic capacity would not make a cell more competitive in outgrowing its neighboring cells. Hence, they argue that the mutations that result in proliferative advantage need to be the same mutations that give a cell the know-how to metastasize. If they argued correctly, the metastatic proclivity should be present in the bulk of the primary tumor. This contradicts the traditional view proposing metastasis is executed by rare cells appearing late in tumorigenesis (Figure 1). Indeed, research confirmed the existence of such an inclination by comparing the gene expression profiles of primary tumors and secondary tumors. It was shown that a primary tumor that will metastasize can be distinguished from a benign tumor, even before the basement membrane is disrupted (33-36). For these gene expression profiles a section of a tumor was used, representing the average expression profile of the entire tumor. This results in the conclusion that the proclivity to metastasize is determined early in tumorigenesis.

Another argument supporting such a proclivity is the early initiation of angiogenesis. Angiogenesis is thought to be a strict process that can be switched on or off. This depends on the number of countervailing factors, either shifting the balance to an angiogenesis activating or inhibiting state, a process known as the angiogenic switch (Figure 4) (37). This switch is only turned on transiently in normal angiogenesis. In contrast to malignancy, when the switch is continuously balanced to the active angiogenic state aiding the tumor to expand and offering the possibility to disseminate (37). Angiogenesis has been demonstrated to commence during benign tumor stages (11). This was first shown by an experiment wherein pre-malignant human urothelial cells, deriving from early tumor stages, showed to stimulate capillary proliferation on rabbit iris while normal tissue did not show such an effect (38). Hence, angiogenesis is a widely studied event. The protein probably mostly studied in angiogenesis research, is he angiogenesis activator and angiogenic marker VEGF (13,18). Over-expression of VEGF and enhanced vessel density have been noted in premalignant breast tumor stages in both mouse models (37,39) as in patient samples (37,40,41).

Both the observation that the process of angiogenesis is already activated during premalignant tumor stages as well by gene expression profiles showing a malignant future

can be predicted early, show the proclivity to metastasize is already present in early stages. However, this does not inevitably mean dissemination occurs early.
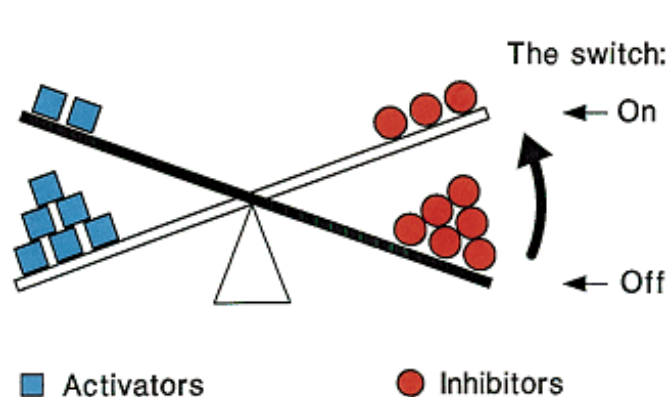


**Figure 4. The postulated balance mechanism for the angiogenic switch.** When angiogenic activators shift the balance to the left, angiogenesis induces. Normally this shift is restored after angiogenesis has been completed, done by a rebalancing of inhibitors. On contrary to cancer, where this balance is not restored and angiogenesis is a continuous process. Figure derived from Hanahan and Folkman (37).

## 2. Proof for early dissemination

The time point of dissemination can roughly be estimated by studying the number and types of chromosomal aberrations in BM DTCs, since the number of chromosomal aberrations is approximately proportional to the stage of tumorigenesis in the majority of tumor types (42). Thus the later a cell disseminates, the more aberrations it will share with the primary tumor. Additionally, this means fewer and different deviations in spread tumor cells are a hallmark of early dissemination. Research comparing BM DTCs from breast cancer patients to cells from the primary lesion, found that DTCs had acquired significantly fewer aberrations than cells from the breast tumor (10,43). This was even seen in samples deriving from M0 patients, who did not show any evidence of metastasis besides the presence of BM DTCs (10).

Other evidence for early dissemination comes from a study on p53 status in primary tumors versus DTCs in BM. The p53 gene is one of the most commonly mutated genes in human cancers (44). In breast cancer, mutations in p53 arise late in breast oncogenesis (8,45). Remarkably, this gene seems to be rarely mutated in DTCs in BM (46). In lung cancer mutations in p53 in BM DTCs and their matched primary tumor were compared, mutations in p53 appeared to be rare which was not the case for primary tumors (47). This gives further evidence dissemination occurs before p53 mutations start accumulating in the primary lung tumor.

More proof has been gathered in studies with cancer mouse models. Using the RET.AAD mouse model, a model of spontaneous melanoma, Eyles et al. showed that disseminating cells could already be detected three weeks after birth. At this time point, the primary tumor still appeared to be in the neoplastic stage. The time of separation was traced by comparing

the single-nucleotide polymorphism (SNP) pattern of the primary tumor with its metastases, these patterns exhibited strong differences. This indicates both tumors acquired most of their mutations independently, suggesting the cells resulting in metastases must have migrated from the primary lesion early in tumorigenesis (48). For breast cancer confirmative results have been published. In mouse models BM DTCs were likewise detected in early stages of breast cancer development. Moreover, when 607 breast cancer patient samples were screened, no association between the stage of the tumor and the presence of BM DTCs was found (49). This implies the spread of tumor cells is not bound to late tumor stages.

In summary, differences in the genetic aberrations between BM DTCs and primary tumors and the early detection of DTCs in BM, argue dissemination is not exclusively bound to late tumor stages in breast malignancy. However, the value of DTCs in metastasis can be discussed.

### 3. The value of BM DTC detection for disease survival prognosis

Although the evidence supporting early dissemination is rather striking, this does not inevitably mean these are the cells responsible for establishing metastases. E.g., Naume et al have questioned the value of DTC detection in BM for metastasis prediction and thus the potential of DTCs to establish metastases. They showed that the prognostic value of DTC detection fluctuates between earlier specified breast cancer subtypes (50,51). Only in one out of five subtypes, an association between the presence of DTCs in BM and disease-free survival was found (51). However, as Naume et al point out, several factors could have influenced these results that question the malignant capacity of DTCs. Firstly, therapy after surgery might have been more beneficial in the four other subgroups. After all, BM was only aspirated just before surgery while the therapy applied after surgery might not have been as successful in every subtype. This suggestion was confirmed in studies taking BM aspirates three weeks after complete treatment and up to 42 weeks after the primary diagnosis, showed to provide prognostic relevance – the presence of DTCs correlated with poor prognosis (52,53). Secondly, disseminating breast cancer cells of different subtypes might be predestined to tissues other than BM, resulting in the lack of detection in BM. Indeed, for some subtypes they found metastases preferably developed in visceral organs (51). Furthermore, several groups have confirmed that the presence of DTCs in BM is a strong independent factor for poor disease-free survival (22,28,54-56). This indicates DTC detection in BM can still be a valuable tool in predicting the progression of breast cancer.

Although the potency of the value of DTC detection in BM might vary between breast cancer subtypes, most groups do confirm its value in progression prediction. Although evidence on whether also early disseminating cell can be responsible for colonization, cannot be provided with these data.

### 4. Metastases are probably established at a parallel time point to the primary tumor

To know if early disseminating cells can actually establish metastases, it is important to know at what time point these secondary tumors are established. Although it is generally thought that metastasis is a late event in tumorigenesis, the detection of BM DTCs in early tumor stages and the association between DTCs in BM and poor clinical prognosis indicate this might be different. This leads to the next question: can those tumor cells that leave the primary lesion complete metastasis? The linear progression model and the parallel progression model discuss this topic, two terms proposed by Klein (Figure 5) (8). The linear progression model states the primary tumor needs to reach to full malignancy before metastasis starts. The parallel progression model states dissemination and the foundation of tumors at distant sites start early. In the latter model, further somatic mutations are acquired after cells disseminated to ectopic sites. These mutations are needed to bring the metastasis to advanced malignancy, necessary for full malignant growth. As a result of this order, the possibility to form secondary tumors is present at a much earlier time point. Hence in this model, metastases do not start growing much later than the primary tumor they originate from supporting the concept of early dissemination.
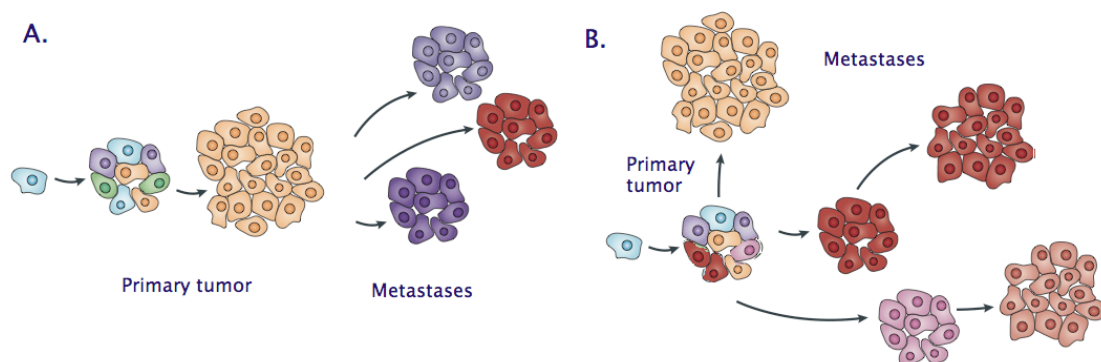


**Figure 5. Linear progression model and parallel progression model.** A. Linear progression model. The primary tumor reaches full malignancy before it starts to metastasize. B. Parallel progression model. The primary tumor starts already to metastasize in an early state where after metastases grow parallel to the primary tumor. Figure adjusted from Klein (8).

Research studying growth rates supports the parallel progression model. Both primary breast tumors and their secondary descendants grow by a linear exponential growth that levels off as time passes, a pattern referred to as Gompertzian growth (57). This pattern is

followed in a similar speed between primary and its matching secondary tumors (58-60). If the linear progression model is true, metastases should be small compared to the primary tumor they derive from. However, metastases are found to be too big to meet both equal Gompertzian growth and linear progression. Especially in case of cancer of unknown primary origin, where the primary tumor cannot be located while metastases can (59,60).

Other support for the parallel progression model is provided by epidemiological studies. It was calculated that metastases already start growing on average 5.8 years before the diagnosis of the primary tumor has been made (60). In earlier studies specified for breast cancer, metastases were estimated to start growing before the detection of the primary tumor in 50 up to 90% of the cases (61,62). Thus the primary tumor does by no means need to be large to metastasize. Moreover, clinical trials attacking the invasiveness of the primary tumor were disappointing after promising preclinical studies. The drug in trial blocks matrix metalloproteinases, proteins responsible for the degradation of the extracellular matrix that are highly elevated in malignant tissues. Preclinical in mouse studies, this drug was highly successful in preventing metastasis. However, in clinical studies this effectiveness was not found (63). These drugs would have prevented late dissemination, if in line with the linear progression model. Thus the failure of these drugs in humans further supports the parallel progression model.

While in humans only indirect proof can support the parallel progression model, in mice direct experimental evidence has been obtained. In a breast cancer mouse model, micrometastases could already be detected in the lungs while the primary lesion was often still at CIS stage (49). Since disseminated tumor cells need some time to grow into detectable micrometastases, this suggest the metastasis founder cells already left the primary lesion early in or even before the CIS stage. Moreover it shows that these early disseminating cells were capable of establishing metastases. Thus current research seems to support the parallel progression model, implying early disseminated cells can found metastases.

However, the parallel progression model needs complementation, since not every disseminated cell colonizes a metastasis parallel to the primary tumor. Not every patient with BM DTCs develops metastases and metastatic cancer can arise years after the treatment of the primary tumor (55,64). Thus dissemination is not always directly followed by the outgrowth of metastases, a mechanism for which exist several explanations.

### 5. The failure of disseminating cells to colonize may be caused by dormancy

Although the potential to metastasize is present in most malignant primary tumor cells and cancer cells seem to start disseminating during early tumor stages, the step to successful

colonization is sparsely made. While millions of cells can be shed into the blood stream every day, e.g., rat mammary carcinomas shed on average 3.7 million tumor cells per 24 hours per gram tissue in blood (65), the vast amount of these cells will never make it far in the process of metastasis. Hence, metastasis seems to be a very inefficient process. Perhaps the primary tumor only sheds cells incompetent of forming metastases, like apoptotic cells. Although in breast cancer patients the majority of CTCs in PB seems not to be viable, a subset is (66). The majority of DTCs in PB shows even no signs of apoptosis in breast and prostate cancer patients (67).

Another explanation for inefficiency of metastasis can be found in the heterogeneity of the cell population. Heterogeneity here indicates that not every cell has the adequate qualities enabling indefinite replication. This is in line with the so-called cancer stem cell theory: only the cancer stem cells in a tumor population have to capacity to self-renewal. Subsequently, only the dissemination of these cells will lead to the colonization of metastases (68).

A third explanation is the existence of dormancy, possibly inducible at different time points in metastasis (Figure 3). Mouse models support this concept. The spontaneous melanoma mouse model, mentioned earlier to provide in vivo evidence for early dissemination, showed a delayed development of metastases in visceral organs. In these organs the median age of onset was 233 days (48). Also patient data suggest the occurrence of tumor dormancy. Cases are known wherein tumor cells continued to occupy the human body for a long time after therapy, while often not leading to recurrence. Normally breast cancer patients relapse in the first decade after mastectomy (64). However, CTCs have been found in former early stage breast cancer patients as late as 22 years after removal of the primary tumor (69). These patients are at low risk of relapse as shown by the lack of signs of recurrence during the length of the study, suggesting tumor cells pursue dormant. Interestingly, these CTCs still seemed to be replaced regularly. By withdrawing blood from breast cancer patients at different intervals before and after mastectomy, the half-life of CTCs was determined between 1 and 2.4 hours. In the patient group used for the dormancy study, this could not be determined (69). Nevertheless the half life of CTCs in these patients is most likely situated in the same range, showing CTCs must be replaced by tumor cells located somewhere unknown. Since the source of these cells was not detected and a short half-live is suggested, their preservation is probably maintained by apoptosis (69)(69,70). This suggests the existence of dormant cells that continue to replicate at such a low pace,

they do not expand nor cause disturbance. Thus dormancy can possibly be caused by both cellular quiescence and a balanced apoptosis-proliferation cycle.

Another, argument supporting the occurrence of dormancy can be found in several studies modeling breast tumor growth. Gompertzian growth kinetics is currently the most widely used method for therapy modeling; however, clinical data do not entirely seem to overlap with this theory. Data suggest that tumors know periods of no obvious progression interchanging with periods of swift expansion. This led to the suggestion by several groups that the Gompertzian model should be expanded with periods of dormancy (71-74).

Metastasis shows to be an inefficient process. Explanations are a possible heterogeneity of cells in the primary tumor, not providing every cell the capacity to self-renewal, and the occurrence of dormancy.

## 6. Endogenous factors influencing dormancy

The failure of disseminated malignant cells to colonize at ectopic sites could be caused by dormancy. What keeps these cells dormant? Weinberg (17) brings up several concepts about why dormancy can develop. Firstly, he discusses the event of dormancy in primary tumors. Here, he suggests a tumorigenic cell acquires several mutations that may seem promising initially, yet ultimately causes its fall resulting in abrogated growth leading to dormancy (Figure 6). Secondly, he addresses dormancy of disseminated cells. Once these cells acquire a dormant state due to adaptation problems in the new surroundings, they need to pass through years of slow proliferation before the chance to acquire the right treats needed to defeat the new environment may occur. If dormancy results in complete senescence, it seems improbable the cell will receive the right mutations to become hazardous. Thus he questions if cells converted to dormancy possess the capacity to 'awaken' and turn destructive, arguing this might be an irreversible process bringing cells to senescence. As a result, Weinberg suggest that early disseminating cells are premalignant, cells that have not yet acquired all the treats needed to accomplish hostile metastasis successfully (Figure 6). Thus although the proclivity to metastasize might be present, only late clones leaving the primary lesion are sufficient malignant to cause ectopic detriment when disseminated (17). This may be an interesting concept for malignant tumors and some metastatic tumors, earlier discussed evidence shows the SNP pattern of secondary tumors in a mouse spontaneous melanoma model supports to originate from early disseminated tumor cells (48). Furthermore, experiments using cell lines and xenograft mouse models showed tumor cells that adopted a reversible dormant state. Here tumor cells underwent autophagy, a

process whereby cells use their contents for hydrolysis as to produce energy if normal supply is insufficient, upon the expression of tumor suppressor gene *ARHI*. This dormant process was reversed upon withdrawal of *ARHI* induction and tumor cells started to proliferate resulting in a fast growth of the xenograft (75,76). Also the earlier discussed occurrence of relapse after even twenty years contradicts this idea (64).

Since it seems unlikely tumor cells acquire the accurate treats to metastasize successfully once slowly proliferating, exogenous factors might play a significant role in inducing and maintaining dormancy.
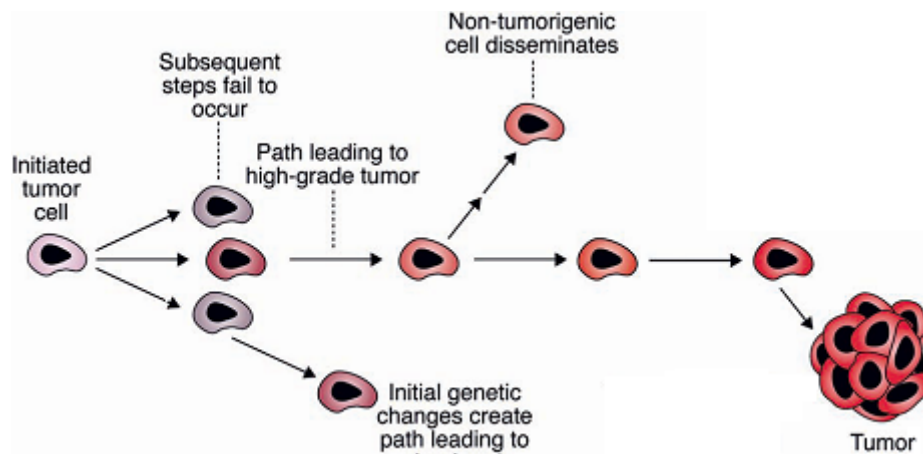


**Figure 6. Endogenous factors leading to failure of malignancy and metastasis.** An initiated tumor cell may not acquire the right mutations to become malignant either at the primary or an ectopic location, or only non-malignant disseminate. Figure derived from Weinberg (17).

## 7. Exogenous factors influencing dormancy

Several exogenous aspects outside of the tumor cells act to instigate dormancy. These are the prevention of angiogenesis, the influence of the surrounding microenvironment, and the recognition by the immune system.

Vasculature is essential for tumor outgrowth. Without blood supply, metastases do not surpass the micrometastatic size (11,18,77). Thus the stalling of disseminated cells can be induced by a shift in the balance to the site of angiogenic inhibitors (Figure 7A). As a consequence, cells can lose the dormant state by a shift to the site of angiogenic activators (Figure 3). One provoking hypothesis is that this shift can be initiated by mastectomy (74,78,79). Striking data revealing a pattern in time point of relapse after surgery triggered this idea. A two-peaked pattern in time seems to become evident after mastectomy without further treatment. This counts for both the time point of detected relapse at the same location as where the primary tumor was removed (80) as for death as a result of metastasis (79). This suggests signaling of the primary breast tumor to its disseminated cells inhibits

them to grow out into metastases. Nevertheless, these results must not be interpreted to label surgery undesirable therapy (81). Patients that do not undergo any form of treatment show to have a considerable higher death risk than those who do get treated (79). These data are only meant to shed light on a possible relation between a primary tumor and its progeny.

The microenvironment of a tumor consists out of normal cells and molecules neighboring it. This microenvironment differs between tissues. Disseminating cells often attempt to lodge in ectopic tissues, where they encounter a distinct microenvironment. As a result, a tumor cell might not be as effective at this new site, simply due to this dissimilar ambient that requires different treats to survive (Figure 7B). E.g., if tumor cells cannot adhere appropriately to the extracellular matrix dormancy or cell death may be initiated (15,82). Thus only those tumor cells that know how to adapt to locations where the microenvironment differs will be able to conquer its new environment, allowing cancer cells to survive and grow. This is also a possible explanation to why metastases seem to be prone to develop in certain tissues than in others when originating from the same primary tumor. Adaptation is probably easier and thus likelier to happen in these tissues (83,84).

Although the hypothesis that the immune system has an important influence on cancer has often been doubted in the past, recent research seems to show the immune system often fights tumor cells successfully off; these cells need to surpass immunosurveillance to blast their malignancy (Figure 7C) (85). The influence of the immune system on cancer has been proposed to know three phases: elimination, equilibrium, and escape (86). Elimination covers the successful recognition and removal of cancer cells by the immune system (87). The equilibrium is the period of dormancy; the immune system can arrest tumor growth though complete elimination is not reached. This period is thought to end by either the eventual successful removal of the dormant cell by the immune system or by the third phase: escape. In the latter tumor cells find a way to surpass the immune system leading to the outgrowth into a malignant metastasis. This process of mastering the immune system is thought to happen either up on alteration in the tumor cells or by a weakened immune system (86). Research using mice deficient for several components of the immune system showed that the loss of certain components of the immune system resulted in more frequent and/or more rapid appearing tumors than in wild type mice. Especially defects in the development or function of Interferon γ (IFN-γ) (88-92), CD8+ memory T-cell (48,93-95), CD4+ memory T-cell (92,94), and natural killer (NK) cells (96,97) resulted in mice more susceptible to cancer. IFN-γ promotes both innate and adaptive mechanisms of the immune

system, while CD8+ and CD4+ memory T-cells are part of the adaptive and NK cells of the innate immune system.

Several factors can induce dormancy. Today three processes are thought to induce dormancy. These are the lack of tumor cells to activate angiogenesis, the microenvironment surrounding a tumor cell, and the protecting influence of the immune system. If tumors cannot always be successfully eliminated, keeping them dormant might be an interesting alternative. Consequently, the stimulation of these three proposed naturally occurring processes might be an interesting target for therapy.
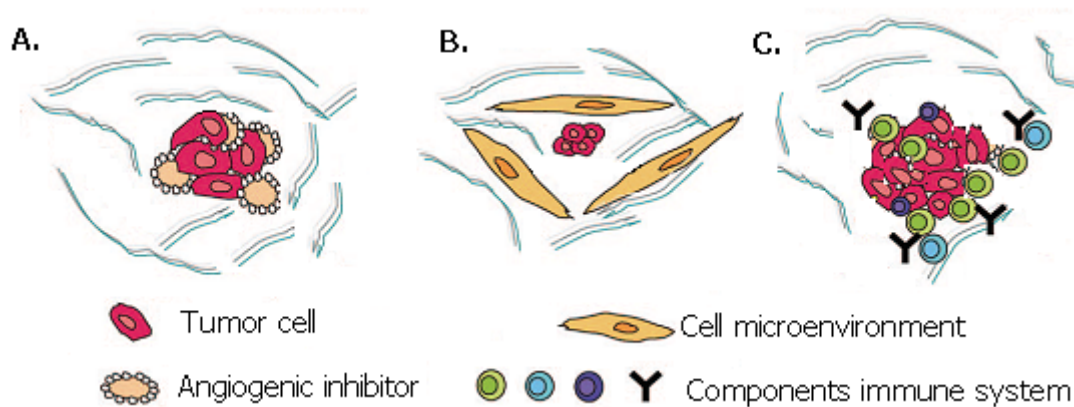


**Figure 7. Angiogenesis inhibition, microenvironment, and immune system can induce and prolong tumor cell dormancy.** A. Angiogenesis inhibition. The angiogenic switch is balanced to inhibition due to the presence of angiogenesis inhibitors. B. Microenvironment. The tumor cell cannot accustom to its new microenvironment, preventing the tumor cells from colonizing. C. Immune system. Components of the immune system cannot eliminate tumor cells, though they can maintain these cells dormant. Figure adjusted from Teng et al (98).

## Conclusion & therapeutic perspectives: inducing and prolonging dormancy

Although breast cancer mortality has decreased in the last decades due to increased research and improved treatment, mortality persists high (2). An often-used therapy is chemotherapy. Chemotherapy drugs target rapidly dividing cells, while leaving quiescent or slowly diving cells untouched. In case of early dissemination, cells will have migrated out of the primary lesion and most will be in a non-dividing state when chemotherapy commences. Thus chemotherapy cannot eliminate these cells, explaining why chemotherapy is often not curative (52,99). The same accounts for surgery in early-stage breast cancer. If cells have already migrated out of the primary lesion, this treatment will fail to prevent metastasis. Subsequently, patients that have completed their therapy and are at that time declared disease-free might still have some dormant cells that after being awoken can develop into devastating metastases. Hence, dormant tumor cells are an important target for the cure of cancer. However, studying dormant cells is complicated for several reasons. Firstly, although PB CTCs and BM DTCs can already be recognized and isolated, cells that have nested elsewhere are difficult to locate and transfer to culture. Secondly, even if these cells can be isolated and transferred to culture, expansion would change their footprint since a hallmark of a dormant cell is its lack of active proliferation. Thirdly, isolation would change their habitat and so their behavior while the host seems to have an important role in the maintenance of dormancy. Consequently dormant cells can currently only be studied in their natural environment, thus unfortunately animal models cannot be replaced yet. Nonetheless, molecular studies assessing the question how dormancy can be induced should find advantages in culture. After all, these cells are tumor cells and the number of easily culturable tumor cell lines is extensive.

Not only the question how and why dormant cells depart from the dormant condition is important. It is also vital to know if all disseminated tumor cells hold a potential to colonize or if only certain tumorigenic cells possess this capacity. Possibly most cells are predestined to fail and do not hold the capacity to surpass dormancy if encountered. The cancer stem cell theory investigates if this distinction is present in tumor cells. If such heterogeneity is true, therapy only needs to fight these cells that can colonize upon dissemination.

In addition, naturally occurring processes that influence dormancy should be further investigated. Since the angiogenic switch, immunosurveillance, and the microenvironment seem to be inducing the dormant state exogenously, acquiring more knowledge about how these processes operate and how they can be stimulated might be the key to future cancer therapy. Although it might be interesting to stimulate all three simultaneously, it is unclear if

these forms of dormancy are mutually exclusive. Drug research has mostly been done with antiangiogenic drugs, giving various results in the clinic. Problems encountered here include enhanced growing tumors in mouse models making medication more likely to succeed (100), the large diversity between tumors in different patients (101), adaptive resistance whereby an initially effective drug shows to lose its effect over time (102,103), and the use of patients with end-stages cancers in clinical trials that have already founds ways to override all the natural barriers and so are less like to be responsive to drugs (104). Despite the fact that certain antiangiogenic drugs might not be beneficial to end-stage patients, they may provide striking effectiveness in inducing and preserving dormancy in patients in a less advanced phase of the disease.

Studying the possibility to induce and prolong tumor cells dormant might provide the cure for cancer. If not all cancer cells can be eliminated; research should look for methods to keep these cells irrelevant. In this way, one day cancer might be treated as a chronic manageable disease.

# References

(1) Disney. Available at: http://www.disney-princesses.com/modules.php?name=coppermine&file=displayimagepopup&pid=599&fullsize=1. Accessed 05/2011, 2011.

(2) Ferlay J, Parkin DM, Steliarova-Foucher E. Estimates of cancer incidence and mortality in Europe 2008. Eur J Cancer 2010;46(4):765-781.

(3) LeBleu VS, Macdonald B, Kalluri R. Structure and function of basement membranes. Exp Biol Med (Maywood) 2007 Oct;232(9):1121-1129.

(4) Barsky SH, Siegal GP, Jannotta F, Liotta LA. Loss of basement membrane components by invasive tumors but not by their benign counterparts. Lab Invest 1983 Aug;49(2):140-147.

(5) Chambers AF, MacDonald IC, Schmidt EE, Koop S, Morris VL, Khokha R, et al. Steps in tumor metastasis: new concepts from intravital videomicroscopy. Cancer Metastasis Rev 1995 Dec;14(4):279-301.

(6) Rocken M. Early tumor dissemination, but late metastasis: insights into tumor dormancy. J Clin Invest 2010 Jun 1;120(6):1800-1803.

(7) Carter CL, Allen C, Henson DE. Relation of tumor size, lymph node status, and survival in 24,740 breast cancer cases. Cancer 1989 Jan 1;63(1):181-187.

(8) Klein CA. Parallel progression of primary tumours and metastases. Nat Rev Cancer 2009 Apr;9(4):302-312.

(9) Riethmuller G, Klein CA. Early cancer cell dissemination and late metastatic relapse: clinical reflections and biological approaches to the dormancy problem in patients. Semin Cancer Biol 2001 Aug;11(4):307-311.

(10) Schmidt-Kittler O, Ragg T, Daskalakis A, Granzow M, Ahr A, Blankenstein TJ, et al. From latent disseminated cells to overt metastasis: genetic analysis of systemic breast cancer progression. Proc Natl Acad Sci U S A 2003 Jun 24;100(13):7737-7742.

(11) Naumov GN, Bender E, Zurakowski D, Kang SY, Sampson D, Flynn E, et al. A model of human tumor dormancy: an angiogenic switch from the nonangiogenic phenotype. J Natl Cancer Inst 2006 Mar 1;98(5):316-325.

(12) Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000 Jan 7;100(1):57-70.

(13) Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011 Mar 4;144(5):646-674.

(14) Weidner N, Semple JP, Welch WR, Folkman J. Tumor angiogenesis and metastasis--correlation in invasive breast carcinoma. N Engl J Med 1991 Jan 3;324(1):1-8.

(15) Aguirre-Ghiso JA. Models, mechanisms and clinical evidence for cancer dormancy. Nat Rev Cancer 2007 Nov;7(11):834-846.

(16) Folkman J, Kalluri R. Cancer without disease. Nature 2004 Feb 26;427(6977):787.

(17) Weinberg RA. The many faces of tumor dormancy. APMIS 2008 Jul-Aug;116(7-8):548-551.

(18) Raica M, Cimpean AM, Ribatti D. Angiogenesis in pre-malignant conditions. Eur J Cancer 2009 Jul;45(11):1924-1934.

(19) Wittekind C, Oberschmid B. TNM classification of malignant tumors 2010: General aspects and amendments in the general section]. Pathologe 2010 Sep;31(5):333-4, 336-8.

(20) Pascual MR, Rodriguez M, Zayas A, Moreno L, Lage A. Factors associated with prognosis in human breast cancer. II. Multivariate stratification analysis. Neoplasma 1983;30(4):485-492.

(21) De Vita VT,Jr. Breast cancer therapy: exercising all our options. N Engl J Med 1989 Feb 23;320(8):527-529.

(22) Braun S, Pantel K, Muller P, Janni W, Hepp F, Kentenich CR, et al. Cytokeratin-positive cells in the bone marrow and survival of patients with stage I, II, or III breast cancer. N Engl J Med 2000 Feb 24;342(8):525-533.

(23) Weigelt B, Bosma AJ, Hart AA, Rodenhuis S, van 't Veer LJ. Marker genes for circulating tumour cells predict survival in metastasized breast cancer patients. Br J Cancer 2003 Apr 7;88(7):1091-1094.

(24) Cristofanilli M, Budd GT, Ellis MJ, Stopeck A, Matera J, Miller MC, et al. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. N Engl J Med 2004 Aug 19;351(8):781-791.

(25) Dawood S, Broglio K, Valero V, Reuben J, Handy B, Islam R, et al. Circulating tumor cells in metastatic breast cancer: from prognostic stratification to modification of the staging system? Cancer 2008 Nov 1;113(9):2422-2430.

(26) Zhao S, Liu Y, Zhang Q, Li H, Zhang M, Ma W, et al. The prognostic role of circulating tumor cells (CTCs) detected by RT-PCR in breast cancer: a meta-analysis of published literature. Breast Cancer Res Treat 2011 Feb 11.

(27) Hayes DF, Cristofanilli M, Budd GT, Ellis MJ, Stopeck A, Miller MC, et al. Circulating tumor cells at each follow-up time point during therapy of metastatic breast cancer patients predict progression-free and overall survival. Clin Cancer Res 2006 Jul 15;12(14 Pt 1):4218-4224.

(28) Wiedswang G, Borgen E, Karesen R, Kvalheim G, Nesland JM, Qvist H, et al. Detection of isolated tumor cells in bone marrow is an independent prognostic factor in breast cancer. J Clin Oncol 2003 Sep 15;21(18):3469-3478.

(29) Pierga JY, Bonneton C, Vincent-Salomon A, de Cremoux P, Nos C, Blin N, et al. Clinical significance of immunocytochemical detection of tumor cells using digital microscopy in peripheral blood and bone marrow of breast cancer patients. Clin Cancer Res 2004 Feb 15;10(4):1392-1400.

(30) Benoy IH, Elst H, Philips M, Wuyts H, Van Dam P, Scharpe S, et al. Real-time RT-PCR detection of disseminated tumour cells in bone marrow has superior prognostic significance in comparison with circulating tumour cells in patients with breast cancer. Br J Cancer 2006 Mar 13;94(5):672-680.

(31) Wiedswang G, Borgen E, Schirmer C, Karesen R, Kvalheim G, Nesland JM, et al. Comparison of the clinical significance of occult tumor cells in blood and bone marrow in breast cancer. Int J Cancer 2006 Apr 15;118(8):2013-2019.

(32) Bernards R, Weinberg RA. A progression puzzle. Nature 2002 Aug 22;418(6900):823.

(33) van 't Veer LJ, Dai H, van de Vijver MJ, He YD, Hart AA, Mao M, et al. Gene expression profiling predicts clinical outcome of breast cancer. Nature 2002 Jan 31;415(6871):530-536.

(34) van de Vijver MJ, He YD, van't Veer LJ, Dai H, Hart AA, Voskuil DW, et al. A gene-expression signature as a predictor of survival in breast cancer. N Engl J Med 2002 Dec 19;347(25):1999-2009.

(35) Ma XJ, Salunga R, Tuggle JT, Gaudet J, Enright E, McQuary P, et al. Gene expression profiles of human breast cancer progression. Proc Natl Acad Sci U S A 2003 May 13;100(10):5974-5979.

(36) Ramaswamy S, Ross KN, Lander ES, Golub TR. A molecular signature of metastasis in primary solid tumors. Nat Genet 2003 Jan;33(1):49-54.

(37) Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell 1996 Aug 9;86(3):353-364.

(38) Chodak GW, Haudenschild C, Gittes RF, Folkman J. Angiogenic activity as a marker of neoplastic and preneoplastic lesions of the human bladder. Ann Surg 1980 Dec;192(6):762-771.

(39) Miller FR. Xenograft models of premalignant breast disease. J Mammary Gland Biol Neoplasia 2000 Oct;5(4):379-391.

(40) Vleugel MM, Bos R, van der Groep P, Greijer AE, Shvarts A, Stel HV, et al. Lack of lymphangiogenesis during breast carcinogenesis. J Clin Pathol 2004 Jul;57(7):746-751.

(41) Pavlakis K, Messini I, Vrekoussis T, Yiannou P, Keramopoullos D, Louvrou N, et al. The assessment of angiogenesis and fibroblastic stromagenesis in hyperplastic and pre-invasive breast lesions. BMC Cancer 2008 Apr 2;8:88.

(42) Heim S, Mitelman F. Cancer Cytogenetics. 2nd ed. Hoboken, NJ, USA: John Wiley & Sons, Inc.; 1995.

(43) Schardt JA, Meyer M, Hartmann CH, Schubert F, Schmidt-Kittler O, Fuhrmann C, et al. Genomic analysis of single cytokeratin-positive cells from bone marrow reveals early mutational events in breast cancer. Cancer Cell 2005 Sep;8(3):227-239.

(44) Greenblatt MS, Bennett WP, Hollstein M, Harris CC. Mutations in the p53 tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. Cancer Res 1994 Sep 15;54(18):4855-4878.

(45) Olivier M, Langerod A, Carrieri P, Bergh J, Klaar S, Eyfjord J, et al. The clinical value of somatic TP53 gene mutations in 1,794 patients with breast cancer. Clin Cancer Res 2006 Feb 15;12(4):1157-1167.

(46) Klein CA, Blankenstein TJ, Schmidt-Kittler O, Petronio M, Polzer B, Stoecklein NH, et al. Genetic heterogeneity of single disseminated tumour cells in minimal residual cancer. Lancet 2002 Aug 31;360(9334):683-689.

(47) Offner S, Schmaus W, Witter K, Baretton GB, Schlimok G, Passlick B, et al. P53 Gene Mutations are Not Required for Early Dissemination of Cancer Cells. Proc Natl Acad Sci U S A 1999 Jun 8;96(12):6942-6946.

(48) Eyles J, Puaux AL, Wang X, Toh B, Prakash C, Hong M, et al. Tumor cells disseminate early, but immunosurveillance limits metastatic outgrowth, in a mouse model of melanoma. J Clin Invest 2010 Jun 1;120(6):2030-2039.

(49) Husemann Y, Geigl JB, Schubert F, Musiani P, Meyer M, Burghart E, et al. Systemic spread is an early step in breast cancer. Cancer Cell 2008 Jan;13(1):58-68.

(50) Sorlie T, Tibshirani R, Parker J, Hastie T, Marron JS, Nobel A, et al. Repeated observation of breast tumor subtypes in independent gene expression data sets. Proc Natl Acad Sci U S A 2003 Jul 8;100(14):8418-8423.

(51) Naume B, Zhao X, Synnestvedt M, Borgen E, Russnes HG, Lingjaerde OC, et al. Presence of bone marrow micrometastasis is associated with different recurrence risk within molecular subtypes of breast cancer. Mol Oncol 2007 Sep;1(2):160-171.

(52) Braun S, Kentenich C, Janni W, Hepp F, de Waal J, Willgeroth F, et al. Lack of effect of adjuvant chemotherapy on the elimination of single dormant tumor cells in bone marrow of high-risk breast cancer patients. J Clin Oncol 2000 Jan;18(1):80-86.

(53) Janni W, Rack B, Schindlbeck C, Strobl B, Rjosk D, Braun S, et al. The persistence of isolated tumor cells in bone marrow from patients with breast carcinoma predicts an increased risk for recurrence. Cancer 2005 Mar 1;103(5):884-891.

(54) Diel IJ, Kaufmann M, Goerner R, Costa SD, Kaul S, Bastert G. Detection of tumor cells in bone marrow of patients with primary breast cancer: a prognostic factor for distant metastasis. J Clin Oncol 1992 Oct;10(10):1534-1539.

(55) Braun S, Vogl FD, Naume B, Janni W, Osborne MP, Coombes RC, et al. A pooled analysis of bone marrow micrometastasis in breast cancer. N Engl J Med 2005 Aug 25;353(8):793-802.

(56) Bidard FC, Vincent-Salomon A, Gomme S, Nos C, de Rycke Y, Thiery JP, et al. Disseminated tumor cells of breast cancer patients: a strong prognostic factor for distant and local relapse. Clin Cancer Res 2008 Jun 1;14(11):3306-3311.

(57) Norton L. A Gompertzian model of human breast cancer growth. Cancer Res 1988 Dec 15;48(24 Pt 1):7067-7071.

(58) Collins VP, Loeffler RK, Tivey H. Observations on growth rates of human tumors. Am J Roentgenol Radium Ther Nucl Med 1956 Nov;76(5):988-1000.

(59) Friberg S, Mattson S. On the growth rates of human malignant tumors: implications for medical decision making. J Surg Oncol 1997 Aug;65(4):284-297.

(60) Engel J, Eckel R, Kerr J, Schmidt M, Furstenberger G, Richter R, et al. The process of metastasisation for breast cancer. Eur J Cancer 2003 Aug;39(12):1794-1806.

(61) Tubiana M, Chauvel P, Renaud A, Malaise EP. Growth rate and natural history of breast cancer]. Bull Cancer 1975 Oct-Dec;62(4):341-358.

(62) Bauer W, Igot JP, Le Gal Y. Chronology of breast cancer using Gompertz' growth model]. Ann Anat Pathol (Paris) 1980;25(1):39-56.

(63) Coussens LM, Fingleton B, Matrisian LM. Matrix metalloproteinase inhibitors and cancer: trials and tribulations. Science 2002 Mar 29;295(5564):2387-2392.

(64) Karrison TG, Ferguson DJ, Meier P. Dormancy of mammary carcinoma after mastectomy. J Natl Cancer Inst 1999 Jan 6;91(1):80-85.

(65) Butler TP, Gullino PM. Quantitation of cell shedding into efferent blood of mammary adenocarcinoma. Cancer Res 1975 Mar;35(3):512-516.

(66) Mehes G, Witt A, Kubista E, Ambros PF. Circulating breast cancer cells are frequently apoptotic. Am J Pathol 2001 Jul;159(1):17-20.

(67) Alix-Panabieres C, Vendrell JP, Pelle O, Rebillard X, Riethdorf S, Muller V, et al. Detection and characterization of putative metastatic precursor cells in cancer patients. Clin Chem 2007 Mar;53(3):537-539.

(68) Clarke MF, Fuller M. Stem cells and cancer: two faces of eve. Cell 2006 Mar 24;124(6):1111-1115.

(69) Meng S, Tripathy D, Frenkel EP, Shete S, Naftalis EZ, Huth JF, et al. Circulating tumor cells in patients with breast cancer dormancy. Clin Cancer Res 2004 Dec 15;10(24):8152-8162.

(70) Wijsman JH, Cornelisse CJ, Keijzer R, van de Velde CJ, van Dierendonck JH. A prolactin-dependent, metastasising rat mammary carcinoma as a model for endocrine-related tumour dormancy. Br J Cancer 1991 Sep;64(3):463-468.

(71) Speer JF, Petrosky VE, Retsky MW, Wardwell RH. A stochastic numerical model of breast cancer growth that simulates clinical data. Cancer Res 1984 Sep;44(9):4124-4130.

(72) Demicheli R, Terenziani M, Valagussa P, Moliterni A, Zambetti M, Bonadonna G. Local recurrences following mastectomy: support for the concept of tumor dormancy. J Natl Cancer Inst 1994 Jan 5;86(1):45-48.

(73) Goldhirsch A, Gelber RD, Castiglione M. Testing tumor dormancy in breast cancer. J Natl Cancer Inst 1994 Apr 6;86(7):558.

(74) Demicheli R, Retsky MW, Swartzendruber DE, Bonadonna G. Proposal for a new model of breast cancer metastatic development. Ann Oncol 1997 Nov;8(11):1075-1080.

(75) Lu Z, Luo RZ, Lu Y, Zhang X, Yu Q, Khare S, et al. The tumor suppressor gene ARHI regulates autophagy and tumor dormancy in human ovarian cancer cells. J Clin Invest 2008 Dec;118(12):3917-3929.

(76) Kenific CM, Thorburn A, Debnath J. Autophagy and metastasis: another double-edged sword. Curr Opin Cell Biol 2010 Apr;22(2):241-245.

(77) Folkman J. Role of angiogenesis in tumor growth and metastasis. Semin Oncol 2002 Dec;29(6 Suppl 16):15-18.

(78) Fisher B, Fisher ER. Experimental evidence in support of the dormant tumor cell. Science 1959 Oct 9;130:918-919.

(79) Demicheli R, Retsky MW, Hrushesky WJ, Baum M. Tumor dormancy and surgery-driven interruption of dormancy in breast cancer: learning from failures. Nat Clin Pract Oncol 2007 Dec;4(12):699-710.

(80) Demicheli R, Abbattista A, Miceli R, Valagussa P, Bonadonna G. Time distribution of the recurrence risk for breast cancer patients undergoing mastectomy: further support about the concept of tumor dormancy. Breast Cancer Res Treat 1996;41(2):177-185.

(81) Norton L. Tumor dormancy: separating observations from experimental science. Nat Clin Pract Oncol 2007 Dec;4(12):671.

(82) Barkan D, Green JE, Chambers AF. Extracellular matrix: a gatekeeper in the transition from dormancy to metastatic growth. Eur J Cancer 2010 May;46(7):1181-1188.

(83) Hart IR, Fidler IJ. Role of organ selectivity in the determination of metastatic patterns of B16 melanoma. Cancer Res 1980 Jul;40(7):2281-2287.

(84) Fidler IJ. The pathogenesis of cancer metastasis: the 'seed and soil' hypothesis revisited. Nat Rev Cancer 2003 Jun;3(6):453-458.

(85) Old LJ. Cancer vaccines: an overview. Cancer Immun 2008 Mar 12;8 Suppl 1:1.

(86) Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol 2002 Nov;3(11):991-998.

(87) Dunn GP, Old LJ, Schreiber RD. The immunobiology of cancer immunosurveillance and immunoediting. Immunity 2004 Aug;21(2):137-148.

(88) Dighe AS, Richards E, Old LJ, Schreiber RD. Enhanced in vivo growth and resistance to rejection of tumor cells expressing dominant negative IFN gamma receptors. Immunity 1994 Sep;1(6):447-456.

(89) Kaplan DH, Shankaran V, Dighe AS, Stockert E, Aguet M, Old LJ, et al. Demonstration of an interferon gamma-dependent tumor surveillance system in immunocompetent mice. Proc Natl Acad Sci U S A 1998 Jun 23;95(13):7556-7561.

(90) Shankaran V, Ikeda H, Bruce AT, White JM, Swanson PE, Old LJ, et al. IFNgamma and lymphocytes prevent primary tumour development and shape tumour immunogenicity. Nature 2001 Apr 26;410(6832):1107-1111.

(91) Dunn GP, Koebel CM, Schreiber RD. Interferons, immunity and cancer immunoediting. Nat Rev Immunol 2006 Nov;6(11):836-848.

(92) Muller-Hermelink N, Braumuller H, Pichler B, Wieder T, Mailhammer R, Schaak K, et al. TNFR1 signaling and IFN-gamma signaling determine whether T cells induce tumor dormancy or promote multistage carcinogenesis. Cancer Cell 2008 Jun;13(6):507-518.

(93) Mahnke YD, Schwendemann J, Beckhove P, Schirrmacher V. Maintenance of long-term tumour-specific T-cell memory by residual dormant tumour cells. Immunology 2005 Jul;115(3):325-336.

(94) Feuerer M, Rocha M, Bai L, Umansky V, Solomayer EF, Bastert G, et al. Enrichment of memory T cells and other profound immunological changes in the bone marrow from untreated breast cancer patients. Int J Cancer 2001 Apr 1;92(1):96-105.

(95) Muller M, Gounari F, Prifti S, Hacker HJ, Schirrmacher V, Khazaie K. EblacZ tumor dormancy in bone marrow and lymph nodes: active control of proliferating tumor cells by CD8+ immune T cells. Cancer Res 1998 Dec 1;58(23):5439-5446.

(96) Smyth MJ, Thia KY, Street SE, Cretney E, Trapani JA, Taniguchi M, et al. Differential tumor surveillance by natural killer (NK) and NKT cells. J Exp Med 2000 Feb 21;191(4):661-668.

(97) Smyth MJ, Crowe NY, Godfrey DI. NK cells and NKT cells collaborate in host protection from methylcholanthrene-induced fibrosarcoma. Int Immunol 2001 Apr;13(4):459-463.

(98) Teng MW, Swann JB, Koebel CM, Schreiber RD, Smyth MJ. Immune-mediated dormancy: an equilibrium with cancer. J Leukoc Biol 2008 Oct;84(4):988-993.

(99) Norton L, Massague J. Is cancer a disease of self-seeding? Nat Med 2006 Aug;12(8):875-878.

(100) Shojaei F, Ferrara N. Antiangiogenic therapy for cancer: an update. Cancer J 2007 Nov-Dec;13(6):345-348.

(101) Kerbel R, Folkman J. Clinical translation of angiogenesis inhibitors. Nat Rev Cancer 2002 Oct;2(10):727-739.

(102) Casanovas O, Hicklin DJ, Bergers G, Hanahan D. Drug resistance by evasion of antiangiogenic targeting of VEGF signaling in late-stage pancreatic islet tumors. Cancer Cell 2005 Oct;8(4):299-309.

(103) Baeriswyl V, Christofori G. The angiogenic switch in carcinogenesis. Semin Cancer Biol 2009 Oct;19(5):329-337.

(104) Bergers G, Benjamin LE. Tumorigenesis and the angiogenic switch. Nat Rev Cancer 2003 Jun;3(6):401-410.

(105) Menard S, Bufalino R, Rilke F, Cascinelli N, Veronesi U, Colnaghi MI. Prognosis based on primary breast carcinoma instead of pathological nodal status. Br J Cancer 1994 Oct;70(4):709-712.