

OPINION

Does tumour dormancy offer a therapeutic target?

Paul E. Goss and Ann F. Chambers

Abstract | The increasing number of cancer survivors is cause for celebration, but this expanding population has highlighted the problem of tumour dormancy, which can lead to relapse. As we start to understand more about the biology of dormant cancer cells, we can begin to address how best to treat this form of disease. Preclinical models and initial clinical trials, as exemplified in patients with breast cancer, are paving the way to address how best to treat long-term cancer survivors to minimize the risk of cancer recurrence.

Tumour dormancy has been recognized as a clinical phenomenon in numerous types of cancer for many years. Clinicians and experimental biologists have used the term dormancy loosely, to describe the hypothetical state of cancer cells lying in wait over a period of time after treatment of the primary tumour, pending subsequent growth and clinical recurrence. This phenomenon is responsible for the intractable nature of many malignancies and is the means by which cancers can defeat the intent to cure with the initial treatment of a primary tumour. One way clinicians have sought to address this phenomenon is to apply increasingly lengthy courses of anticancer treatments. With the development of less toxic and more targeted therapies that can be used for long periods of time, the idea of extended therapy to prevent late cancer recurrences has begun to gain momentum. Simultaneously, laboratory researchers have striven to define and understand the biology of cancer cells that can allow them to exist in a state of dormancy and subsequently grow to become clinically detectable. It is timely to consider current concepts addressing both experimental and clinical data regarding cancer dormancy, with a view to identifying targets of dormancy and potential clinical strategies. In this Opinion article, we focus particularly on the clinical and therapeutic implications of evolving concepts about tumour dormancy, with an emphasis on relevant clinical trials in breast cancer.

Experimental studies of dormancy

Metastasis is an inefficient process¹. Metastases form from only a subset of cells that arrive in an organ, and many cells remain as either solitary dormant cells or micrometastases². Experiments in preclinical models suggest that dormant cells are common and that they represent a valid, but difficult, therapeutic target. Laboratory studies have shown that cancer cells in a metastatic organ can coexist in three distinct states: actively growing and angiogenic metastases, which are most readily detected by various imaging modalities or by their effects on vital organ function; micrometastases, which may be in a dormant state in which cell proliferation is balanced by apoptosis and no net size increase in tumour volume occurs; and solitary, dormant cells, which are in a truly quiescent state (FIG. 1). The micrometastases and dormant cells can also be detected after the fact by histology but can be detected *in vivo* by new and evolving preclinical imaging modalities, such as intravital videomicroscopy and magnetic resonance imaging^{3–8}. Thus, what can be readily detected might represent only the ‘tip of the iceberg’ in a metastatic organ, both in experimental models and in patients. Cancer cells in these three states represent very different therapeutic targets (FIG. 1). Key to determining whether dormant solitary cells or micrometastases represent valid targets is knowledge of the

underlying biology of dormancy and the probability of cells progressing to active metastatic growth. This progression is poorly understood in preclinical models and even less so clinically.

Preclinical models. Preclinical models that are specifically focused on tumour dormancy have been difficult to develop, as for practical reasons much of metastasis research is focused on using models that provide experimental results within short time frames. However, some models for studying dormancy have been characterized, and are now providing insights into how dormancy might be regulated (reviewed in REFS 9,10). Furthermore, increasing evidence suggests that dormant cells are often present in organs in which metastases are also actively growing. These experimental findings are consistent with clinical evidence for dormancy. However, details about where dormant cells reside in patients is limited, as clinical assessment of minimal disease is usually restricted to accessible tissues and organs, such as the blood or bone marrow^{9–11}. The true extent of dormancy in other tissues cannot currently be assessed.

There are few cell lines that have been characterized that exhibit a dormant phenotype in experimental mice in the absence of actively growing metastases, partly because cell lines that are in common use have been selected for rapid metastatic ability. Naumov *et al.*¹² screened a series of cell lines for tumour growth following subcutaneous injection. They found that many non-angiogenic cell lines initially showed a prolonged dormant phenotype, followed by the development of angiogenic capability and much more rapid growth on re-injection. Other studies have also implicated the failure of angiogenesis as a factor that contributes to the maintenance of the dormant state and the activation of angiogenesis as a trigger for the initiation of growth by dormant cells^{13–15}. Indraccolo *et al.*¹⁶ recently reported a role for Notch signalling in tumour cells, which is mediated by Notch ligands that are released by endothelial cells, emphasizing the potential role of the angiogenic switch in regulating release from dormancy¹². Therefore,

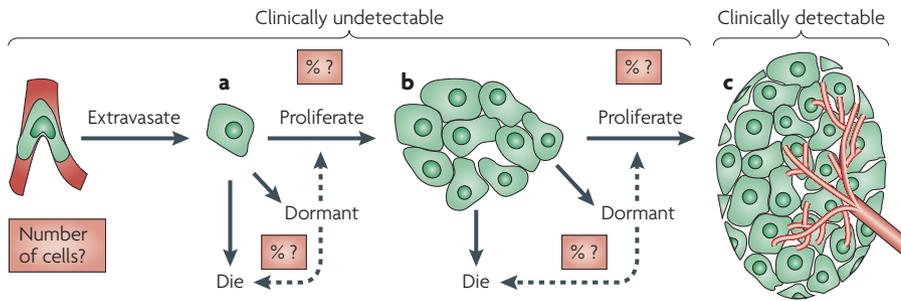


Figure 1 | Cancer cells can coexist in three distinct states in a metastatic organ. Metastatic cells can be present as dormant, quiescent solitary cells (part a); dormant, pre-angiogenic micrometastases, in which cell division may be balanced by apoptosis (part b); and as actively growing, vascularized metastases (part c). The cells that can be detected by current clinical or preclinical imaging modalities are thus only a subset of the metastatic burden that may exist in an organ. The net progression to clinically relevant, large metastases is governed by the numbers of cancer cells that are delivered to that organ and by the proportion of cells that follow the various fates shown. The dashed arrows indicate a link between proliferation, dormancy and cell death. Image is modified, with permission, from *Nature Reviews Cancer* REF. 2 © (2002) Macmillan Publishers Ltd. All rights reserved.

anti-angiogenic therapies could have the potential to hold pre-angiogenic dormant micrometastases in check.

Aguirre-Ghiso and colleagues⁹ have developed a model for studying tumour dormancy in the immunodeficient chick embryo, using human epidermoid carcinoma HEp3 cells, and have used this model to identify molecular factors that can contribute to the maintenance of, and release from, dormancy⁹. Factors identified using this model include the balance between two MAPK signalling pathways, the ERK and p38 signalling pathways, which in turn can be regulated by the urokinase receptor (uPAR) and interactions with fibronectin^{17–19}. Therapeutic strategies to regulate this balance could be contemplated, but would be complicated by limited specificity for tumour cells.

The D2A1 and D2.0R mouse mammary carcinoma cells²⁰ represent a genetically related pair of cells with aggressive, rapidly metastatic (D2A1) and more indolent, dormant (D2.0R) phenotypes, and have been used with success in many studies of metastasis and dormancy^{21–23}. Barkan *et al.*²³ used this pair of cell lines, and also identified a series of additional cell lines, that show dormant versus rapidly growing properties both *in vivo* and in an *in vitro* assay that correlated with *in vivo* behaviour. It is anticipated that this *in vitro* assay will help to clarify the molecular nature of dormancy and the therapeutic strategies that are targeted to dormant cells. Molecular factors identified with this model include adhesive interactions with components of the extracellular matrix^{23–26}. Again, strategies to use this mechanistic information therapeutically remain to be developed and issues of tumour specificity need to be addressed.

Goodison *et al.*²⁷ and Suzuki *et al.*²⁸ have used a pair of clonal, green fluorescent protein (GFP)-transfected derivatives of the MDA-MB-435 human cell line, one of which (NM2C5) shows a non-metastatic phenotype *in vivo* and the other of which (M4A4) is highly metastatic in immunodeficient mice. They found that NM2C5 tumours disseminated cells to distant organs, where they remained in a viable but dormant state, again supporting the idea that tumour dissemination is not synonymous with clinically overt metastasis. Even with the metastatic M4A4 line, many cells disseminated and remained dormant, including in organs that had actively growing metastases^{27,28}, which is consistent with the information illustrated in FIG. 1. Similarly, Huseman *et al.*²⁹ reported early dissemination of mammary carcinoma cells to multiple organs in a syngeneic model, and subsequent release from dormancy in the bone marrow, in response to irradiation. In addition, Eyles *et al.*³⁰ have implicated cytostatic CD8⁺ T cells in regulating the outgrowth of early disseminated melanoma cells. Indeed, when researchers take the time to look, dormant cancer cells can be found in many metastatic animal models both in organs that are free of overt metastases and in involved organs where they coexist with actively growing metastases^{3–5,7,22,27–29}.

Preclinical models of therapeutic effects on dormant tumour cells. Few studies have assessed the effects of therapy on dormant cells. Cytotoxic chemotherapy was shown to effectively inhibit the growth of metastases while having no effect on the numbers of dormant cancer cells residing in the same organs in models of breast cancer metastases in the liver²² (FIG. 2) and melanoma metastases in the

lungs⁴. Furthermore, chemotherapy treatment delivered early in the course of metastasis had no effect on late-developing metastases in the D2.0R poorly metastatic model, presumably because the cancer cells were in a dormant state at the time of treatment, and thus mimicking a failure of early administration of adjuvant therapy to prevent late recurrences in some patients (FIG. 2). Dormant cancer cells are therefore common in preclinical models of metastasis and are a difficult target for therapy. Although quiescent, dormant cells might be difficult to kill, but they would presumably be affected by treatment delivered after they begin to reinitiate growth. As discussed below, recent and ongoing clinical trials in hormone-dependent breast cancer suggest that anti-oestrogen therapies that are introduced as a treatment late in follow-up can reduce subsequent clinical recurrences, suggesting that either true dormant cells or tumour micrometastases remain vulnerable to these therapies. Such an approach may be contemplated in other malignancies, particularly with the development of therapies with few toxicities.

Clinical tumour dormancy

Circulating breast cancer cells have been detected in patients up to 22 years after diagnosis who are clinically disease free, suggesting that tumour cells are in a state of dynamic dormancy³¹. Clinical dormancy is reflected by relapses at distant sites, following the original primary cancer diagnosis. In some tumours — for example, breast cancer, melanoma and renal cancer — these recurrences are common and can occur many years after diagnosis. Such late recurrences are less common in other tumour types (TABLE 1). For example, in colon cancer, more than 85% of recurrences occur within the first 3 years of follow-up³², whereas patients with breast cancer are at risk for a much longer time³³. Mathematical modelling using clinical recurrence data has suggested that dormancy can be better explained by true cancer quiescence, followed by periods of active growth, rather than steady but slow growth throughout a period of clinical dormancy^{34,35}. Many examples of evidence for minimal residual disease in blood, bone marrow, lymph nodes, the peritoneal cavity and other sites for different types of cancer have been shown^{9,36}. Investigators in Italy measured the size of clinically detected breast cancer lesions in patients who were followed every 3 months and they were able to deduce that the tumour could not have been growing by exponential or Gompertzian growth before the diagnosis of metastases but rather that rapid growth after coming out of dormancy

was likely to have occurred³⁷. In a meta-analysis of early stage breast cancer, the presence of dormant occult bone marrow micrometastases was an independent prognostic indicator³⁸. Publications by Ragaz *et al.*³⁹ and Overgaard *et al.*⁴⁰ raised the possibility that dormant metastases in regional nodes of the breast could seed tumour cells to distant sites over time following initial surgery. Ultimately, a reduction in cancer mortality and morbidity depends substantially on our ability to prevent, delay or treat distant metastatic disease that can occur at times ranging from immediately after diagnosis to years or decades later (TABLE 1).

Recent and evolving technological advances have made it possible to detect circulating tumour cells (CTCs) in blood samples from cancer patients, as well as disseminated tumour cells (DTCs), which are primarily isolated from bone marrow samples. Such cells have been shown to provide some valuable prognostic information (reviewed in REFS 10,41,42). These studies provide evidence for cancer cells at sites that are remote from the primary tumour. Molecular characterization of these cells is the subject of much ongoing work, as methodological hurdles of characterizing isolated individual cells are being overcome. Whether these cells represent a reservoir of dormant cells that can re-initiate growth in response to microenvironmental stimuli remains to be clarified. Although sampling of DTCs from bone marrow is an invasive procedure, blood sampling of CTCs is minimally invasive. Quantification and/or molecular characterization of CTCs could therefore be feasible in the context of clinical trials and might provide further insights into the clinical utility of CTC (or DTC) measurements in patients¹⁰. For example, a recent publication describes the ability to harvest, isolate and

study ERBB2 levels in CTCs⁴³, and another publication has reported the detection of mutations in epidermal growth factor receptor (EGFR) in CTCs from patients with lung cancer⁴⁴. Using these techniques it is hoped that targeting dormant cells to kill them, or keep them dormant indefinitely, will be possible. This represents an important challenge in the future treatment of early stage cancer that could provide a major step forwards in improving the outcome of patients with solid tumour malignancy.

To establish distant metastases, tumour cells need to overcome several obstacles: leaving the primary tumour site; homing to a distant organ site; resisting apoptosis and immune destruction at the ectopic site; and establishing initial colonization at the metastatic site^{2,30,45,46}. The metastatic dissemination of cancer cells can occur early in the course of disease progression, before or at the time of diagnosis of the primary tumour in patients; this has also been seen in experimental models^{29,30,36}. The time taken to subsequently establish a blood supply and grow at the metastatic site to clinically detectable disease is considered the true dormancy period. Clinically, tumours have been considered dormant when recurrence occurs after 5 years from diagnosis, but the variation in time to recurrence probably reflects a lead time bias from original dissemination rather than a measure of variations in tumour biology. Regulation of the switch from quiescent dormancy to active regrowth is poorly understood, but could include factors such as surgery and other causes of tissue injury, diet, immunological factors and other as yet unidentified chance elements^{34,47,48}.

The initiation of metastatic growth is not equivalent to dissemination of tumour cells^{2,46}, as illustrated by the experimental

studies discussed above. The phenomenon of tumour dormancy can introduce years of uncertainty for patients and their physicians, as it is not known whether a patient is cured or still harbours residual disease that carries the risk of recurrence. The various possible states of dormancy, as well as their temporal presence or absence over time, suggests that single or combinations of therapies, introduced at varying time intervals, could be necessary to interrupt the process of clinical recurrence. This poses a tremendous challenge to the researchers and clinicians involved in clinical trials. Nevertheless, recent clinical trials in breast cancer support the idea that there might be a long temporal window for some patients, during which continued therapy or therapy that is applied late in follow-up may be of benefit in preventing cancer recurrence. Such trials can increasingly be carried out owing to the development of treatments that have less toxicity than earlier cytotoxic chemotherapies. However, much remains to be learned about the efficacy of chronic therapies that are targeted towards dormant cancer cells and their benefits and risks to patients need to be carefully evaluated.

Recurrence patterns in breast cancer.

Clinically, most malignancies have a chronic recurrence pattern (TABLE 1). It is immediately obvious that there is no distinct point when recurrences begin or end. Breast cancer is clinically divided into various subtypes. Distinct differences in recurrence patterns are seen between oestrogen receptor-positive (ER⁺) and ER⁻ tumours^{33,49} (FIG. 3). ER⁺ disease has a lower risk of recurrence in the initial 5 years after diagnosis than ER⁻ disease but thereafter has a greater chronic annual risk of recurrence than ER⁻ tumours. After 15 years the recurrence curves come together again (FIG. 3). It is unclear whether these differences could be accounted for by different growth rate patterns or by differences in dormancy patterns.

Improving the systemic therapies that are given immediately after treating the primary tumour continues to be the commonest way to attempt to reduce subsequent recurrences. Emphasis has generally been placed on long-term morbidities of cancer treatments rather than on addressing residual risk of recurrence. Improving therapeutic efficacy in late-relapsing cancers has been less of a focus. However, examples of diseases in which this has begun to be addressed include ER⁺ breast cancer and malignant melanoma.

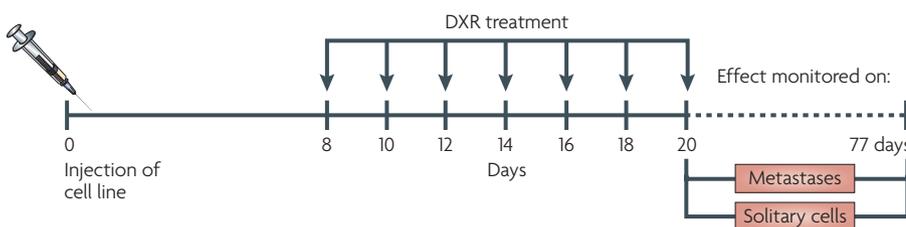


Figure 2 | The differential sensitivity of dormant versus actively growing cancer cells to doxorubicin. In this experiment, cells were injected into the mesenteric vein of mice to target cells to the liver. Two mouse mammary carcinoma cell lines were compared: D2A1, which forms metastases within 3 weeks after injection, and D2.0R, which remains in a dormant state for up to 11 weeks before forming metastases. Treatment with doxorubicin (DXR) reduced the D2A1 metastatic burden from large metastases, but did not reduce the numbers of solitary cells. Late-developing D2.0R metastases were not inhibited, owing to DXR treatment being given to the mice when the cancer cells were in a dormant, non-responsive state²². See REF. 4 for a similar study, which also showed differential responses to chemotherapy of dormant versus actively growing melanoma cells.

Clinical trials and long-term dormancy

ER⁺ breast cancer is a model for chronic relapsing solid tumour malignancy. Adjuvant systemic therapy with tamoxifen reduces the rate of relapse and improves survival both during and after 5 years of therapy in women with ER⁺ early stage breast cancer⁴⁹. Data from clinical trials that have given tamoxifen for longer than 5 years were mixed. The most definitive prospective trial, NSABP B14, showed no additional benefit or worsening of outcomes by continuing tamoxifen beyond 5 years and led to a US National Cancer Institute alert limiting tamoxifen use to 5 years^{50,51}. A Scottish trial of longer than 5 years of tamoxifen treatment showed a similar result⁵². By contrast, a trial conducted by the Eastern Cooperative Oncology Group, USA, showed a slight advantage for longer tamoxifen use by comparing 3 years of treatment and longer treatment⁵³. Similarly, results from the ongoing Adjuvant Tamoxifen Longer Against Shorter trial (ATLAS; see Further information) of around 5 years of tamoxifen treatment versus longer treatment are awaited to determine the efficacy of treatment with adjuvant tamoxifen of longer than 5 years. A trial of delayed adjuvant tamoxifen started >2 years after diagnosis showed marked benefit for systemic

intervention late in follow-up, which was remote from initial diagnosis and treatment of the primary tumour⁵⁴.

The Letrozole after Tamoxifen in Treating Women with Breast Cancer (NCIC CTG MA17) trial demonstrated a significant reduction in recurrence risk and improvement in disease-free survival by extending adjuvant endocrine therapy with an aromatase (oestrogen synthetase) inhibitor, letrozole, for an additional 5 years after the initial 5 years of tamoxifen treatment⁵⁵ (FIG. 4a). Importantly, women in this trial with primary tumours that were ER⁺ and progesterone receptor (PRGR)-positive (PRGR⁺) accrued most of the benefit⁵⁶. A similar association with ER⁺ and PRGR⁺ tumours was also shown in the ATLAS trial discussed above⁵⁴. Importantly, subsequent analyses of NCIC CTG MA17 also showed that 1–7 years after 5 years of tamoxifen treatment and anywhere between 6 years and 11 years after diagnosis, women still have a significant recurrence risk; this ongoing risk was markedly reduced by introducing delayed letrozole treatment up to 11 years and 16 years from diagnosis⁵⁷. This further supports the idea of persistent or dormant metastases occurring many years after primary presentation of breast cancer. Further follow-up of the NCIC CTG MA17 trial has

resulted in women who received 10 years of endocrine therapy (5 years of tamoxifen treatment and 5 years of letrozole treatment) being re-randomized in the NCIC CTG MA17R trial to a further 5 years of letrozole treatment or no treatment, further addressing the concept of prolonged tumour dormancy and the need for chronic endocrine therapy for up to 15 years after diagnosis (FIG. 4a). From the results of the NCIC CTG MA17 trial it can be concluded that, despite 5 years of adjuvant tamoxifen treatment, women can continue to harbour clinically dormant micrometastases, which are generally sensitive to anti-oestrogen therapy regardless of when this therapy is applied. By contrast, although some benefit is accrued from anti-oestrogen therapy in patients with ER⁺ and PRGR⁻ tumours when it is given early in treatment, later, after being exposed to 5 years of tamoxifen, ER⁺ and PRGR⁻ disease may no longer be responsive to further anti-oestrogen therapy. Primary tumour evaluation for prognostic and predictive gene signatures or other biomarkers in the NCIC CTG MA17 trial is underway.

In a further twist on tumour dormancy in ER⁺ breast cancer, experimental data from Sabnis *et al.*⁵⁸ have suggested that constant anti-oestrogen therapy might induce an acquired ER⁻ phenotype and anti-oestrogen insensitivity over time. This has resulted in the Study of Letrozole Extension (SOLE) trial, a Phase III trial evaluating the role of continuous letrozole versus intermittent (9 months off and 1 month on) letrozole treatment following 4 to 6 years of adjuvant endocrine therapy for postmenopausal women with hormone receptor⁺, node⁺, early stage breast cancer. This trial will address both the phenomenon of dormancy and the potential effects of therapy on its biology.

The Tykerb Evaluation After Chemotherapy (TEACH) trial is evaluating the biology and potential responsiveness to anti-ERBB2 therapy of dormant micrometastases in a specific ERBB2⁺ subtype of breast cancer (FIG. 4b). Women with ERBB2⁺ primary tumours (as determined by fluorescence *in situ* hybridization (FISH) or immunohistochemistry (IHC)) who are clinically free of recurrent disease following initial primary therapy and adjuvant chemotherapy are randomized to receive lapatinib (Tykerb; GlaxoSmithKline) or a placebo any time after prior diagnosis of their primary tumour⁵⁹. Stratification factors include: less than 4 years from diagnosis versus greater than or equal to 4 years from diagnosis; node⁺ versus node⁻ tumours; and ER⁺ versus ER⁻ tumours. In this trial, the event rate over time in patients not treated with anti-ERBB2 therapy will be

Table 1 | Relative survival rates by tumour site at 5, 10 and 15 years*

Tumour site	5 years [†]	10 years [‡]	15 years
Breast (female)	90%	82%	75%
Melanoma of the skin	93%	90%	89%
Kidney and renal pelvis	69%	61%	54%
Colon and rectum	68%	58%	54%
Lung and bronchus	16%	11%	9%
Oesophagus	19%	12%	9%
Liver and intrahepatic bile duct	14%	9%	8%
Pancreas	6%	4%	3%
Stomach	27%	21%	18%
Brain and other nervous system	36%	32%	30%
Oral cavity and pharynx	63%	51%	43%
Larynx	63%	49%	41%
Urinary bladder	82%	75%	71%
Thyroid	97%	97%	96%
Ovary	46%	38%	38%
Prostate	100%	95%	82%
Testis	96%	95%	94%
Corpus and uterus, NOS	84%	80%	77%
Cervix uteri	72%	67%	65%

NOS, not otherwise specified. *Table is based on Surveillance, Epidemiology and End Results (SEER) data (see Further information), which was provided by the American Cancer Society. [†]Cases diagnosed from 1999 to 2005 and followed during 2006. [‡]Cases diagnosed from 1994 to 2006 and followed during 2007. ^{||}Cases diagnosed from 1989 to 2006 and followed during 2007.

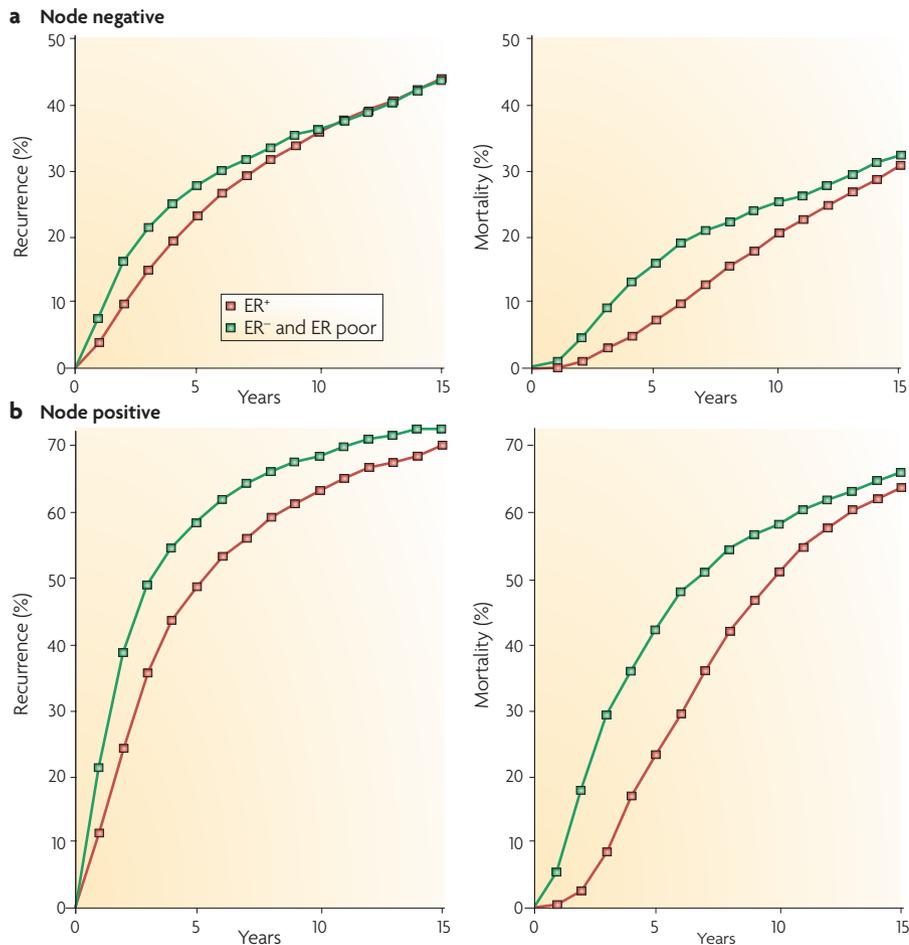


Figure 3 | Long-term risk of breast cancer recurrence by ER status. These curves are from a meta-analysis by the Early Breast Cancer Trialists' Collaborative Group (EBCTCG) and show that for both node⁻ (part **a**) and node⁺ (part **b**) disease, oestrogen receptor-negative (ER⁻) disease has a higher early relapse and mortality risk and a lower late risk than ER⁺ disease. After 15 years the two curves approximate one another. Figure is reproduced, with permission, from REF. 49 © (2005) Elsevier.

compared with the rate in patients receiving 1 year of anti-ERBB2 therapy at any time after diagnosis. The effect of 1 year of lapatinib treatment on the ongoing subsequent relapse risk from dormant metastases will be assessed. The analysis will determine the effect depending on whether lapatinib treatment was started within the first year from diagnosis, between 1 and 4 years from diagnosis, or more than 4 years from diagnosis. Primary tumour analyses, including prognostic and predictive gene signatures, as well as host pharmacogenomic signatures, are being planned. Additionally, proteomic analyses from plasma samples taken from the diagnosis of the trial participants and during the 10 years of follow-up of all participants are being planned.

Ultra-late recurrences of cutaneous melanoma are also known to occur⁶⁰. To address clinical dormancy in this disease, a trial of interferon- α (IFN α) therapy or no further therapy following completely excised stage II, stage III or recurrent melanoma was

initiated in 1995. The trial aimed to determine the overall effects of adjuvant low-dose extended-duration IFN α therapy on disease-free and overall survival and to determine whether there is any correlation with patient age or sex. Unfortunately, although launched in 1995, the trial has not reached its original accrual goal and is no longer actively recruiting patients, underscoring the difficulties in studying late tumour recurrences in clinical trials.

Strategies that have been considered in prostate and ovarian cancer include randomizing patients who are clinically free of recurrent disease but who have rising prostate-specific antigen (PSA) or CA 125 serum tumour markers (and possibly markers of dormant tumours becoming clinically active) to different treatments. Studies have demonstrated that T cell-mediated immunity is an important component in the regulation of tumour dormancy and that there is a role for CD8⁺ T cells through

the endogenous production of IFN γ , in collaboration with humoral immunity, to both induce and maintain a state of tumour dormancy⁶¹; mechanisms of regulation of tumour dormancy are also being further clarified⁶². Therefore, vaccines are considered a potential mechanism for inhibiting cells from emerging from dormancy, although the possible roles for immune components in regulating dormancy are not thoroughly understood⁶³. Other potential immune modulatory approaches that could be applied during periods of putative clinical dormancy are being considered. For example, lenalidomide, an analogue of thalidomide, induces apoptosis in cancer cells through the activation of natural killer cells, and its effects on markers of the immune response, which could be predictive of response to therapy, are being studied in cancer patients⁶⁴.

Risks and benefits of long-term therapy

The decision to use prolonged chronic adjuvant anticancer therapies to prevent recurrences has to be viewed in the context of benefit versus risk to the patient and cost to the health care system^{65,66}. The number needed to treat (NNT) to benefit one patient is a way of expressing cost versus benefit and has been used in several disease settings. The NCIC CTG MA17 trial showed that the NNT was 37 for node⁻ patients and 12 for node⁺ patients. These numbers are highly competitive compared with anti-hypertensive treatment or lipid-lowering agents, which are commonly prescribed. Interestingly, in the NCIC CTG MA17 trial, menopausal-like symptoms were generally mild and quality of life was well maintained⁶⁷. This reflects the general likelihood that extended therapies will be well tolerated in patients who have survived longer disease free and have frequently tolerated prior therapy well. However, more serious organ toxicities, such as increasing numbers of fragility fractures in women treated with prolonged oestrogen synthesis inhibition, have to be weighed into decision making when considering extended therapy administration. Ways to improve the NNT for both extended aromatase inhibitors and for late extended anti-ERBB2 therapy in the TEACH trial are underway. These include primary tumour signatures and pharmacogenomic host signatures to assist in the selection of patients at risk for recurrence of cancer or for specific toxicities. The potential of this personalized medicine approach is likely to improve the cost effectiveness of treatments that are aimed at clinical dormancy in solid tumour malignancy.

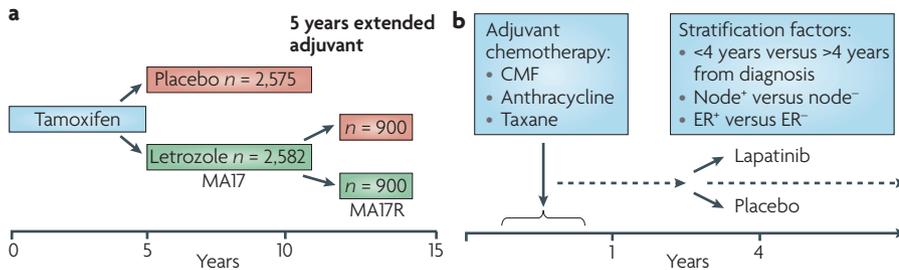


Figure 4 | MA17 and TEACH trials. a | Schema of the NCIC CTG MA17 and MA17R (re-randomization) trials. The NCIC CTG MA17 trial showed benefit for extended late adjuvant therapy given for 5 years after 5 years of prior tamoxifen treatment, which was given to prevent recurrences in early stage breast cancer. The ongoing MA17R trial further explores the effects of later systemic therapy on the risk of late (dormant) recurrences. **b** | Schema of the Tykerb Evaluation After Chemotherapy (TEACH) trial of chronic anti-ERBB2 therapy. The TEACH trial will determine the pattern of chronic late relapses after initial diagnosis in early stage ERBB2-positive (ERBB2⁺) breast cancer. In this trial, the event rate over time in patients not treated with anti-ERBB2 therapy will be compared with the rate in patients receiving 1 year of anti-ERBB2 therapy at any time after diagnosis, treatment of the primary tumour and completion of standard adjuvant chemotherapy. This trial will also determine the benefit/cost ratio of using lapatinib treatment, given across a range of time periods from primary diagnosis, as indicated by the dashed arrow. CMF, cyclophosphamide, methotrexate and 5-fluorouracil; ER, oestrogen receptor.

Conclusions and unanswered questions

Identifying the mechanisms of tumour dormancy, those at risk of recurrence and therapeutic interventions to prevent recurrences, are imperatives in cancer treatment research. The design of clinical trials to address tumour dormancy is challenging. Findings from the NCIC CTG MA17 clinical trial in ER⁺ breast cancer are a paradigm for investigating the role of chronic systemic therapy for chronic relapsing cancer. This trial has also demonstrated that the late introduction of such therapy in patients with what seems to be ongoing tumour dormancy can also be beneficial. The TEACH trial is another important proof-of-concept trial that is looking at the natural history of dormancy in a more aggressive subtype of breast cancer and exploring the role of anti-ERBB2 therapy late in follow-up. In both trials tumour and host pharmacogenomic signatures are underway to identify those at risk and thus those who should be targeted for treatment. Similarly, those most at risk of serious toxicities can also avoid therapy, and in this way the cost/benefit ratio can be improved considerably. As discussed above, the stimuli for triggering the regrowth of dormant micrometastases are unclear. The initiation of angiogenesis, the availability of growth-promoting factors from increases in bone turnover or variations in immune modulation over time suggest that the timing of inhibitors of tumour regrowth may not necessarily require constant therapy from the time of diagnosis onwards, as is currently practised. Indeed such trials may render spuriously negative results. Delivering systemic therapy over a range of time periods, as was the case in the NCIC CTG MA17 and

TEACH trials, may help to discern whether changing variables over time are important. An obstacle that we have encountered with this approach, however, is the difficulty of obtaining regulatory approval for therapies not given as up-front adjuvant treatment.

We need a better method for cataloguing the occurrence and nature of subclinical micrometastatic disease in patients, and better understanding about the organs in which these cells reside. Do all patients, most patients or many patients harbour dormant cells? Preclinical data suggest that there might be dormant cells in organs that are unlikely to support the growth of metastases, which is consistent with, for example, the detection of cancer cells in the bone marrow of patients with colorectal cancer, as metastases rarely develop at this site^{68,69}, as well as those in which metastases more commonly arise. Furthermore, is there information about the tumour or patient at initial diagnosis that can predict which patients will and which patients will not develop late metastases after a period of dormancy? Or is the re-awakening of dormant cells more dependent on life events that occur subsequent to primary cancer treatment (such as diet, surgery and immune suppression)? In other words, what proportion of late recurrences is destined to occur compared with the proportion that may or may not occur depending on factors that happen after treatment of the primary tumour? Much more remains to be learned about the biology of dormancy and re-awakening, in order to better decide who will benefit from treatment that is targeted to dormant cancer. In the meantime, long-term therapy, particularly for hormone-responsive breast

cancer, is becoming standard of care, and the benefit for a small proportion of women who are at risk for late recurrences must be balanced against the risks of side effects from long-term treatment. Biomarkers, including those mentioned above that have been identified as being related to dormancy or escape from dormancy, such as Notch signalling and angiogenic switching, need to be more clearly studied in patients before rational clinical trials can be designed.

Recent data with the anti-angiogenesis inhibitor bevacizumab established benefit in the advanced disease setting⁷⁰. However, no benefit was shown when bevacizumab was given as 1 year of adjuvant therapy in early stage disease^{71,72}. These results in early stage disease seem to contradict the results in preclinical models, but the selection of specific patients or the precise timing of anti-angiogenic therapy might be needed to demonstrate benefit.

In summary, there remains a disconnection between identifying dormant metastases and their biology and the design of appropriate clinical trials. Novel imaging techniques that can detect dormant tumours or the harvesting of circulating tumour cells, bone marrow cells and other metastatic biopsy sites of disease should allow the evaluation of putative biological markers or pathways such as the two MAPK signalling pathways — the ERK and p38 pathways — and the uPAR and fibronectin interactions mentioned above, and thereby identify novel targets and agents for addressing dormant cells.

Paul E. Goss is at the Harvard Medical School, Massachusetts General Hospital Cancer Center, 55 Fruit Street, Lawrence House, RH-302, Boston, Massachusetts 02114, USA.

Ann F. Chambers is at the London Regional Cancer Program, and Department of Oncology, University of Western Ontario, 790 Commissioners Road East, London, Ontario N6A 4L6, Canada.

*Correspondence to A.F.C.
e-mail: ann.chambers@lhsc.on.ca
doi:10.1038/nrc2933
Published online 4 November 2010*

- Weiss, L. Metastatic inefficiency. *Adv. Cancer Res.* **54**, 159–211 (1990).
- Chambers, A. F., Groom, A. C. & MacDonald, I. C. Dissemination and growth of cancer cells in metastatic sites. *Nature Rev. Cancer* **2**, 563–572 (2002).
- Heyn, C. *et al.* In vivo MRI of cancer cell fate at the single-cell level in a mouse model of breast cancer metastasis to the brain. *Magn. Reson. Med.* **56**, 1001–1010 (2006).
- Townson, J. L. *et al.* Three-dimensional imaging and quantification of both solitary cells and metastases in whole mouse liver by magnetic resonance imaging. *Cancer Res.* **69**, 8326–8331 (2009).
- Luzzi, K. J. *et al.* Multistep nature of metastatic inefficiency: dormancy of solitary cells after successful extravasation and limited survival of early micrometastases. *Am. J. Pathol.* **153**, 865–873 (1998).

6. Naumov, G. N. *et al.* Persistence of solitary mammary carcinoma cells in a secondary site: a possible contributor to dormancy. *Cancer Res.* **62**, 2162–2168 (2002).
7. Cameron, M. D. *et al.* Temporal progression of metastasis in lung: cell survival, dormancy, and location dependence of metastatic inefficiency. *Cancer Res.* **60**, 2541–2546 (2000).
8. Holmgren, L., O'Reilly, M. S. & Folkman, J. Dormancy of micrometastases: balanced proliferation and apoptosis in the presence of angiogenesis suppression. *Nature Med.* **1**, 149–153 (1995).
9. Aguirre-Ghiso, J. A. Models, mechanisms and clinical evidence for cancer dormancy. *Nature Rev. Cancer* **7**, 834–846 (2007).
10. Muller, V., Alix-Panabieres, C. & Pantel, K. Insights into minimal residual disease in cancer patients: implications for anti-cancer therapies. *Eur. J. Cancer* **46**, 1189–1197 (2010).
11. Pantel, K., Alix-Panabieres, C. & Riethdorf, S. Cancer micrometastases. *Nature Rev. Clin. Oncol.* **6**, 339–351 (2009).
12. Naumov, G. N. *et al.* A model of human tumor dormancy: an angiogenic switch from the nonangiogenic phenotype. *J. Natl Cancer Inst.* **98**, 316–325 (2006).
13. Naumov, G. N., Akslen, L. A. & Folkman, J. Role of angiogenesis in human tumor dormancy: animal models of the angiogenic switch. *Cell Cycle* **5**, 1779–1787 (2006).
14. Indraccolo, S., Favaro, E. & Amadori, A. Dormant tumors awoken by a short-term angiogenic burst: the spike hypothesis. *Cell Cycle* **5**, 1751–1755 (2006).
15. Favaro, E., Amadori, A. & Indraccolo, S. Cellular interactions in the vascular niche: implications in the regulation of tumor dormancy. *APMIS* **116**, 648–659 (2008).
16. Indraccolo, S. *et al.* Cross-talk between tumor and endothelial cells involving the Notch3-Dll4 interaction marks escape from tumor dormancy. *Cancer Res.* **69**, 1314–1323 (2009).
17. Ranganathan, A. C., Adam, A. P., Zhang, L. & Aguirre-Ghiso, J. A. Tumor cell dormancy induced by p38SAPK and ER-stress signaling: an adaptive advantage for metastatic cells? *Cancer Biol. Ther.* **5**, 729–735 (2006).
18. Allgayer, H. & Aguirre-Ghiso, J. A. The urokinase receptor (u-PA)-a link between tumor cell dormancy and minimal residual disease in bone marrow? *APMIS* **116**, 602–614 (2008).
19. Adam, A. P. *et al.* Computational identification of a p38SAPK-regulated transcription factor network required for tumor cell quiescence. *Cancer Res.* **69**, 5664–5672 (2009).
20. Rak, J. W., McEachern, D. & Miller, F. R. Sequential alteration of peanut agglutinin binding glycoprotein expression during progression of murine mammary neoplasia. *Br. J. Cancer* **65**, 641–648 (1992).
21. Morris, V. L., Tuck, A. B., Wilson, S. M., Percy, D. & Chambers, A. F. Tumor progression and metastasis in murine D2 hyperplastic alveolar nodules mammary tumor cell lines. *Clin. Exp. Metastasis* **11**, 103–112 (1993).
22. Naumov, G. N. *et al.* Ineffectiveness of doxorubicin treatment on solitary dormant mammary carcinoma cells or late-developing metastases. *Breast Cancer Res. Treat.* **82**, 199–206 (2003).
23. Barkan, D. *et al.* Inhibition of metastatic outgrowth from single dormant tumor cells by targeting the cytoskeleton. *Cancer Res.* **68**, 6241–6250 (2008).
24. Barkan, D. *et al.* Metastatic growth from dormant cells induced by a Col-I-enriched fibrotic environment. *Cancer Res.* **70**, 5706–5716 (2010).
25. Barkan, D., Green, J. E. & Chambers, A. F. Extracellular matrix: a gatekeeper in the transition from dormancy to metastatic growth. *Eur. J. Cancer* **46**, 1181–1188 (2010).
26. Shibue, T. & Weinberg, R. A. Integrin $\beta 1$ -focal adhesion kinase signaling directs the proliferation of metastatic cancer cells disseminated in the lungs. *Proc. Natl Acad. Sci. USA* **106**, 10290–10295 (2009).
27. Goodison, S. *et al.* Prolonged dormancy and site-specific growth potential of cancer cells spontaneously disseminated from nonmetastatic breast tumors as revealed by labeling with green fluorescent protein. *Clin. Cancer Res.* **9**, 3808–3814 (2003).
28. Suzuki, M., Mose, E. S., Montel, V. & Tarin, D. Dormant cancer cells retrieved from metastasis-free organs regain tumorigenic and metastatic potency. *Am. J. Pathol.* **169**, 673–681 (2006).
29. Husemann, Y. *et al.* Systemic spread is an early step in breast cancer. *Cancer Cell* **13**, 58–68 (2008).
30. Eyles, J. *et al.* Tumor cells disseminate early, but immunosurveillance limits metastatic outgrowth, in a mouse model of melanoma. *J. Clin. Invest.* **120**, 2030–2039 (2010).
31. Meng, S. *et al.* Circulating tumor cells in patients with breast cancer dormancy. *Clin. Cancer Res.* **10**, 8152–8162 (2004).
32. Sargent, D. J. *et al.* End points for colon cancer adjuvant trials: observations and recommendations based on individual patient data from 20,898 patients enrolled onto 18 randomized trials from the ACCENT Group. *J. Clin. Oncol.* **25**, 4569–4574 (2007).
33. Saphner, T., Tormey, D. C. & Gray, R. Annual hazard rates of recurrence for breast cancer after primary therapy. *J. Clin. Oncol.* **14**, 2738–2746 (1996).
34. Retsky, M. W., Demicheli, R., Hrushesky, W. J., Baum, M. & Gukas, I. D. Dormancy and surgery-driven escape from dormancy help explain some clinical features of breast cancer. *APMIS* **116**, 730–741 (2008).
35. Hanin, L. & Korosteleva, O. Does extirpation of the primary breast tumor give boost to growth of metastases? Evidence revealed by mathematical modeling. *Math. Biosci.* **223**, 133–141 (2010).
36. Riethmuller, G. & Klein, C. A. Early cancer cell dissemination and late metastatic relapse: clinical reflections and biological approaches to the dormancy problem in patients. *Semin. Cancer Biol.* **11**, 307–311 (2001).
37. Demicheli, R., Terenzi, M. & Bonadonna, G. Estimate of tumor growth time for breast cancer local recurrences: rapid growth after wake-up? *Breast Cancer Res. Treat.* **51**, 133–137 (1998).
38. Braun, S. *et al.* A pooled analysis of bone marrow micrometastasis in breast cancer. *N. Engl. J. Med.* **353**, 793–802 (2005).
39. Ragaz, J. *et al.* Adjuvant radiotherapy and chemotherapy in node-positive premenopausal women with breast cancer. *N. Engl. J. Med.* **337**, 956–962 (1997).
40. Overgaard, M. *et al.* Postoperative radiotherapy in high-risk premenopausal women with breast cancer who receive adjuvant chemotherapy. Danish Breast Cancer Cooperative Group 82b Trial. *N. Engl. J. Med.* **337**, 949–955 (1997).
41. Slade, M. J. *et al.* Comparison of bone marrow, disseminated tumour cells and blood-circulating tumour cells in breast cancer patients after primary treatment. *Br. J. Cancer* **100**, 160–166 (2009).
42. Wikman, H., Vessella, R. & Pantel, K. Cancer micrometastasis and tumour dormancy. *APMIS* **116**, 754–770 (2008).
43. Flores, L. M. *et al.* Improving the yield of circulating tumour cells facilitates molecular characterisation and recognition of discordant HER2 amplification in breast cancer. *Br. J. Cancer* **102**, 1495–1502 (2010).
44. Maheswaran, S. *et al.* Detection of mutations in EGFR in circulating lung-cancer cells. *N. Engl. J. Med.* **359**, 366–377 (2008).
45. Hanahan, D. & Weinberg, R. A. The hallmarks of cancer. *Cell* **100**, 57–70 (2000).
46. Pantel, K. & Brakenhoff, R. H. Dissecting the metastatic cascade. *Nature Rev. Cancer* **4**, 448–456 (2004).
47. Kendal, W. S. Chance mechanisms affecting the burden of metastases. *BMC Cancer* **5**, 138 (2005).
48. Chambers, A. F. Influence of diet on metastasis and tumor dormancy. *Clin. Exp. Metastasis* **26**, 61–66 (2009).
49. Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. *Lancet* **365**, 1687–1717 (2005).
50. Fisher, B. *et al.* Five versus more than five years of tamoxifen therapy for breast cancer patients with negative lymph nodes and estrogen receptor-positive tumors. *J. Natl Cancer Inst.* **88**, 1529–1542 (1996).
51. Fisher, B., Dignam, J., Bryant, J. & Wolmark, N. Five versus more than five years of tamoxifen for lymph node-negative breast cancer: updated findings from the National Surgical Adjuvant Breast and Bowel Project B-14 randomized trial. *J. Natl Cancer Inst.* **93**, 684–690 (2001).
52. Stewart, H. J. *et al.* Randomised comparison of 5 years of adjuvant tamoxifen with continuous therapy for operable breast cancer. The Scottish Cancer Trials Breast Group. *Br. J. Cancer* **74**, 297–299 (1996).
53. Tormey, D. C., Gray, R. & Falkson, H. C. Postchemotherapy adjuvant tamoxifen therapy beyond five years in patients with lymph node-positive breast cancer. Eastern Cooperative Oncology Group. *J. Natl Cancer Inst.* **88**, 1828–1833 (1996).
54. Delozier, T. *et al.* Delayed adjuvant tamoxifen: ten-year results of a collaborative randomized controlled trial in early breast cancer (TAM-02 trial). *Ann. Oncol.* **11**, 515–519 (2000).
55. Goss, P. E. *et al.* A randomized trial of letrozole in postmenopausal women after five years of tamoxifen therapy for early-stage breast cancer. *N. Engl. J. Med.* **349**, 1793–1802 (2003).
56. Goss, P. E. *et al.* Randomized trial of letrozole following tamoxifen as extended adjuvant therapy in receptor-positive breast cancer: updated findings from NCIC CTG MA.17. *J. Natl Cancer Inst.* **97**, 1262–1271 (2005).
57. Goss, P. E. *et al.* Late extended adjuvant treatment with letrozole improves outcome in women with early-stage breast cancer who complete 5 years of tamoxifen. *J. Clin. Oncol.* **26**, 1948–1955 (2008).
58. Sabisz, G., Goloubeva, O., Gilani, R., Macedo, L. & Brodie, A. Sensitivity to the aromatase inhibitor letrozole is prolonged after a "break" in treatment. *Mol. Cancer Ther.* **9**, 46–56 (2010).
59. Moy, B. & Goss, P. E. TEACH: Tykerb evaluation after chemotherapy. *Clin. Breast Cancer* **7**, 489–492 (2007).
60. Tsoo, H., Cosimi, A. B. & Sober, A. J. Ultra-late recurrence (15 years or longer) of cutaneous melanoma. *Cancer* **79**, 2361–2370 (1997).
61. Farrar, J. D. *et al.* Cancer dormancy. VII. A regulatory role for CD8⁺ T cells and IFN- γ in establishing and maintaining the tumor-dormant state. *J. Immunol.* **162**, 2842–2849 (1999).
62. Muller-Hermelink, N. *et al.* TNFR1 signaling and IFN- γ signaling determine whether T cells induce tumor dormancy or promote multistage carcinogenesis. *Cancer Cell* **13**, 507–518 (2008).
63. Quesnel, B. Tumor dormancy and immunoescape. *APMIS* **116**, 685–694 (2008).
64. Zhu, D., Corral, L. G., Fleming, Y. W. & Stein, B. Immunomodulatory drugs Revlimid (lenalidomide) and CC-4047 induce apoptosis of both hematological and solid tumor cells through NK cell activation. *Cancer Immunol. Immunother.* **57**, 1849–1859 (2008).
65. Delea, T. E. *et al.* Cost-effectiveness of extended adjuvant letrozole therapy after 5 years of adjuvant tamoxifen therapy in postmenopausal women with early-stage breast cancer. *Am. J. Manag. Care* **12**, 374–386 (2006).
66. Chapman, J. A. *et al.* Competing causes of death from a randomized trial of extended adjuvant endocrine therapy for breast cancer. *J. Natl Cancer Inst.* **100**, 252–260 (2008).
67. Whelan, T. J. *et al.* Assessment of quality of life in MA.17: a randomized, placebo-controlled trial of letrozole after 5 years of tamoxifen in postmenopausal women. *J. Clin. Oncol.* **23**, 6931–6940 (2005).
68. Pantel, K., Braun, S., Schlimok, G. & Riethmuller, G. Micrometastatic tumour cells in bone marrow in colorectal cancer. *Lancet* **341**, 501 (1993).
69. Pantel, K. *et al.* Differential expression of proliferation-associated molecules in individual micrometastatic carcinoma cells. *J. Natl Cancer Inst.* **85**, 1419–1424 (1993).
70. Murphy, J. E. & Ryan, D. P. American Society of Clinical Oncology 2010 colorectal update. *Expert Rev. Anticancer Ther.* **10**, 1371–1373 (2010).
71. Allegra, C. J. *et al.* Initial safety report of NSABP C-08: a randomized phase III study of modified FOLFOLX6 with or without bevacizumab for the adjuvant treatment of patients with stage II or III colon cancer. *J. Clin. Oncol.* **27**, 3385–3390 (2009).
72. Wolmark, N. *et al.* A phase III trial comparing mFOLFOLX6 plus bevacizumab with bevacizumab in stage II or III carcinoma of the colon: results of NSABP Protocol C-08. *J. Clin. Oncol. Abstr.* **27**, LBA4 (2009).

Acknowledgements

P.E.G. is supported by the Avon Foundation for Women in New York, USA. A.F.C. is Canada Research Chair in Oncology.

Competing interests statement

The authors declare no competing financial interests.

FURTHER INFORMATION

Ann F. Chambers's homepage:
http://www.lhsc.on.ca/Research_Training/LRCP/Research_Scientists/AFChambers.htm
 ATLAS: <http://www.ctsu.ox.ac.uk/~atlas/>
 Surveillance, Epidemiology and End Results (SEER):
<http://seer.cancer.gov/>

ALL LINKS ARE ACTIVE IN THE ONLINE PDF