Cancer progression and the invisible phase of metastatic colonization

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Abstract | Metastatic dissemination occurs very early in the malignant progression of a cancer but the clinical manifestation of metastases often takes years. In recent decades, 5-year survival of patients with many solid cancers has increased due to earlier detection, local disease control and adjuvant therapies. As a consequence, we are confronted with an increase in late relapses as more antiproliferative cancer therapies prolong disease courses, raising questions about how cancer cells survive, evolve or stop growing and finally expand during periods of clinical latency. I argue here that the understanding of early metastasis formation, particularly of the currently invisible phase of metastatic colonization, will be essential for the next stage in adjuvant therapy development that reliably prevents metachronous metastasis.

Patients with solid cancers mostly die of systemic metastatic disease. Clinical manifestation of metastatic disease as detected by clinical imaging methods is a sign of death for the vast majority of patients, and its prevention therefore is a clinical imperative. Over the past few years, it has become firmly established that dissemination of cancer cells from primary to distant sites often occurs very early[1–7], long before diagnosis of the primary tumour. Consistently, patients without manifest metastases receive not only local treatment (for example, surgery or radiotherapy) but in many cases also some type of systemic therapy to target minimal residual disease. Regimens to achieve this are called 'adjuvant' when given after local removal of the primary tumour and 'neoadjuvant' when administered before removal. Whether or not these therapies prevent metastasis (or are needed at all) is commonly assessed by studying patient outcome in clinical trials, which may take decades. So far, therapies targeting minimal residual disease are administered in a blind, solely empirical manner without consideration of the molecular characteristics of the disseminated cancer cells (DCCs) (in contrast to those of the primary tumour) and rarely of the habitat (for example, organ) to which the DCCs homed.

Decades of cancer research have generated an impressive understanding of molecular mechanisms of tumorigenesis, cell migration and metastasis among other cancer-related processes. However, observations of disease courses and tumour growth kinetics reveal that we lack relevant information. For example, what are the determinants of aggressive cancers as opposed to cancers that are deemed less malignant but nevertheless kill their host? What are the selective conditions of the target organs in which cancer cells establish metastases? Answering these questions may not only guide the development of future adjuvant therapies but may also possibly spare a large proportion of patients who may have disseminated disease and who are currently overtreated from significant, therapy-associated adverse effects as the DCCs may never progress. For this reason, in this Perspective I try to provide a framework for addressing these questions and suggest that key answers will be found by studying the so far occult stage of cancer progression — the currently invisible phase in the formation of metastases when colonies are smaller than a few millimetres and undetectable by clinical imaging. Interesting biological phenomena, such as cancer cell dormancy, explosive growth, immune control or the impact of any type of treatment on colonizing cancer cells, are most likely key aspects for the development of future adjuvant therapies. Thus, it is high time to carefully reassess concepts upon which our treatment routines are based and elucidate the unknown stage of systemic cancer progression.

Disease courses and cancer biology

About 17 million Americans (and four million Germans; that is, about 5% of the population in Western countries) have currently received a diagnosis of some type of cancer and are either undergoing treatment or live in fear of relapse and death[8]. Survival depends on the tumour type, disease stage and available therapy. The present discussion will consider breast and lung cancer (specifically non-small-cell lung cancer (NSCLC)) and melanoma, because they represent different biological types. As a measure of improvement in diagnosis and treatment, 5-year survival is typically used — with older data reflecting more closely what can be considered the 'natural' history of the disease, because patients may not have been receiving systemic therapies. The 5-year relative survival (relative survival is adjusted for normal life expectancy by comparing survival among patients with cancer with longevity of the general population, controlling for age, race and sex; relative survival is different from 'observed' survival used in most studies that compare different types of treatment between groups) is 92% for melanoma, 91% for breast cancer and 23% for NSCLC[8] for all stages combined. These cumulative numbers both reveal and conceal important differences between tumour types, as discussed in the following sections (TABLE 1).

Melanoma. Before the era of immunotherapies and targeted therapies, 5-year survival of patients with metastatic melanoma was dismal (generally less than 10% (REF[9])) and progression was so rapid that it was often measured by 1-year or 2-year survival[10]; however, 5-year survival recently increased to 17% (REF.[8]). At diagnosis, patients with melanoma are relatively young (median age 63 years[11]), and the lowest tumour stage, tumour stage 1 (T1; in melanoma), is defined as a tumour thickness of 1 mm or less (where T is part of the tumour-node-metastasis (TMN) staging system, in which T represents the size of the

Table 1 | **Growth measures, clinical parameters and survival for selected cancer types**

Cancer type	Median age at diagnosis (years)	Definition of T1 stage	5-year survival at M0 stage (%)	5-year survival at M1 stage[a] (%)	Late relapse after 5 and 10 years[b] (%)	Proliferation-index (Ki67 in primary tumour) (%)	TVDT (days) of primary tumour	Volume doublings until T1 diagnosis[c]	Duration of growth until T1 diagnosis (years)
Melanoma	63 (REF.[11])	≤1 mm	98 (REF.[11])	<10	7–25 (REFS[13,14])	Dermal: 9 Epidermal: 24 (REF.[165])	Not available	20	6[d]
Breast cancer:	–	≤20 mm	–	–	–	–	–	33	–
HR$^+$-BC	61 (REF.[11])	–	99 (REF.[11])	19	13–34[e] (REF.[20])	17	111–398	–	10–36
TN-BC	51 (REF.[21])	–	76 (REF.[166])	14 (REF.[167])	9 (REF.[21])	50 (REF.[168])	77–177 (REF.[169])	–	7–16
NSCLC:	70 (REF.[11])	≤30 mm	–	4 (REF.[11])	8–11 (REFS[24,25])	Median: 40	–	35	–
Adenocarcinoma	–	–	75–80[f] (REF.[22])	–	–	38	185–215	–	18–20
SCC	–	–	20–30[g] (REF.[22])	–	–	50 (REF.[30])	90–144 (REFS[27,170])	–	9–14

HR$^+$-BC, hormone receptor-positive breast cancer; NSCLC, non-small-cell lung cancer; SCC, squamous cell carcinoma; TN-BC, triple-negative-breast cancer; TVDT, tumour volume doubling time. M, presence of distant metastasis; N, number of reginal lymph nodes; T, size of the primary tumour. [a]Before immunotherapy era. [b]For patients without recurrence within first 5 years (lung cancer) or 10 years (breast cancer and melanoma), respectively. [c]Starting from a single cell[26]. [d]Calculated with the lowest TVDT of HR$^+$-BC. [e]For T1N0 and T1N4–9. [f]For adenocarcinoma and SCC with primary tumour resected at T1–2N0M0. [g]For adenocarcinoma and SCC in regionally advanced stages II and III.

primary tumour, N represents the number of regional lymph nodes (LNs) and M indicates the presence of distant metastasis). Tumour thickness is the most important risk factor in non-metastatic melanoma, and the risk of death from melanoma with age is approximately constant for each T category with minimal peaks over a period of 15 years[12] (although T4 is the exception; FIG. 1a). The rates of late recurrences, defined as occurring after a 10-year latency, are reported to be as high as 25% (REF.[13]), although more recent analysis identified the risk of late recurrence at 15 and 25 years to be 6.8% and 11.3%, respectively[14], for patients free of disease for 10 years. Growth rates of primary melanomas are not available, but clinically detectable metastases of malignant melanomas in patients have been determined to double their volume every 48 days (tumour volume doubling time (TVDT)), with enormous variation[15].

Breast cancer. Current 5-year relative survival of patients with metastatic breast cancer is about 25%, but 99% when the cancer is diagnosed at a localized stage[8,11]. At diagnosis, patients are rather young (median 61 years[11]), and T1 is defined as a primary tumour diameter of 20 mm or less. Breast cancer presents in two paradigmatic progression modes: one is steroid hormone (for example, oestrogen or progesterone) receptor-positive breast cancer (HR$^+$-BC) and the other is triple-negative (TN) breast cancer (TN-BC), which is characterized by lack of HRs and human epidermal growth factor receptor 2 (HER2; also known as ERBB2), rendering TN-BC difficult to treat as oestrogen receptor (ER) and HER2 are important therapeutic targets. The discussion below will focus on the distinction between these progression modes. For completeness, four major types of breast cancer are recognized in clinical studies, although more subtypes are distinguishable by genetics and histology[16]. They are defined by expression of ER and progesterone receptor (PR) and HER2, to give HR$^+$/HER2$^-$, HR$^-$/HER2$^+$, HR$^+$/HER2$^+$ and HR$^-$/HER2$^-$. At first sight, HR$^+$-BCs have a better prognosis than HR$^-$-BCs, which comprise TN-BC and HER2-enriched tumours[17], although this needs to be qualified through observation of clinical data. HR$^-$-BC-derived metastasis is mostly diagnosed within 5 years of surgery, and metastasis after 8 years is extremely rare[17,18], whereas HR$^+$-BCs continue to relapse beyond 8 years after surgery, resulting — at least in young patients — in a similar risk of death for patients with HR$^+$-BCs and patients with HR$^-$-BCs at 8 years[19]. This is illustrated by plotting the hazard rates, that is, the risk of relapse over time (FIG. 1b), which shows that the risk of death before 5 years is greater for patients with HR$^-$ tumours and after 5 years is greater for patients with HR$^+$ tumours[18,19].

The two progression modes of HR$^+$-BC and TN-BC have recently been investigated closely[20,21]. As HR$^+$-BCs were routinely treated for 5 years with endocrine therapy (such as aromatase inhibitors or tamoxifen), the question arose as to whether patients would benefit from extended therapy. From analysis of 62,923 women with HR$^+$-BC who were disease-free after 5 years of scheduled endocrine therapy, the risk of distant recurrence rose steadily throughout the study period from 5 to 20 years[20]. Like for melanoma, the risk of distant recurrence strongly correlated with the original tumour and LN (T and N) status, ranging from 10% to 41% (REF.[20]). For T1N0 disease, the risk of late (20 years) distant recurrence ranged from 10% to 17% and correlated with primary tumour grade. Unlike for melanoma and HR$^+$-BC, for TN-BC the risk of relapse peaks within the first 5 years[17]. Late relapses in patients who have been free of relapse during the first 10 years after primary diagnosis are rare and are observed in about 9% of patients without recurrence within the first 5 years at year 10 and in about 17% of patients without recurrence within the first 5 years at year 15 (REF.[21]). As TN-BCs are defined as cancers with less than 10% ER- and PR-positive cells, the study authors checked whether late-relapsing TN-BCs displayed low ER and/or PR positivity as opposed to true negativity. HR positivity in TN-BCs is significantly associated with late relapse[21], further emphasizing the biological difference between HR$^+$-BC and TN-BC. The growth rate in breast cancer closely reflects the proliferation indices of each subtype, with higher numbers of Ki67$^+$ proliferating cells in the primary tumour corresponding to lower TVDTs (TABLE 1). Clinically, the results for the continuous impact of HR expression support recommendations to extend the duration of endocrine therapy beyond 5 years if adverse effects do not preclude continuation[20].

Non-small-cell lung cancer. The current 5-year relative survival of patients with NSCLC is so low that this discussion is restricted to very early stages. For patients with T1–2N0M0 disease treated with surgery, 5-year survival is 75–80% (with women surviving longer) and for patients with regional disease (that is, with positive LNs) it is 20–30% (REF.[22]). Patients with distant metastasis at diagnosis survive for 5 years in only 4% of cases[11]. Until very recently, 5-year survival was so rare that few patients could be observed who lived longer than 5 years, and relapse-free survival exceeding 5 years was often considered proof of cure[23]; 8–11% of patients who were relapse-free at 5 years experienced a late recurrence[24,25].

In absolute terms, the numbers listed in TABLE 1 reflect the clinical experience of outcome for patients presenting with these different cancers at an early stage. This can be illustrated by a simple calculation. If 1,000 patients of each cancer type present at the clinic, and if we assume the 5-year relative survival approximates the 5-year relapse-free interval (which is not correct because some patients experience relapse before 5 years and are still alive at 5 years; however, the interval is rarely reported), then 990 patients with HR$^+$-BC, 980 patients with melanoma, 760 patients with TN-BC, 750 patients with T1–2N0M0 NSCLC and 200 patients with LN-positive NSCLC would still be alive and enter our 'late-relapse study'. Of these, about 150 patients with melanoma and HR$^+$-BCs, about 70 patients with TN-BC and T1–2N0M0 NSCLC and about 20 patients with regionally advanced NSCLC would have relapsed before year 10. Thereafter, patients with HR$^+$-BC and melanoma will relapse at about the same frequency, whereas very few additional patients with TN-BC or NSCLC will present to the hospital. In summary, the presentation of late-relapsing patients in the clinic depends on the ability of a cancer to kill its host within 5 years and to generate metastases thereafter. As will be shown later, these striking differences in aggressiveness between the cancer types must result from the invisible phase of progression and not after manifestation of overt metastases.

Lead time to distant relapse

What underlies these different disease courses? Tumour growth kinetics in patients has been assessed for decades[26] by various imaging techniques, and the implications have been discussed before[27,28]. The TVDT as the direct assessment of growth that integrates all impacting factors, including immunogenicity and response, apoptosis rate or angiogenesis, is also reflected in the immunohistochemical evaluation of the number of cycling cells as determined by Ki67 staining. However, this relationship is not absolute but tumour dependent; for instance, the attrition rate (for example, through fluctuating apoptosis, necrosis or localized phagocytosis) also determines growth. During clinically observable tumour growth, data support the assumption of constant (exponential) growth rates with a flattening for very large

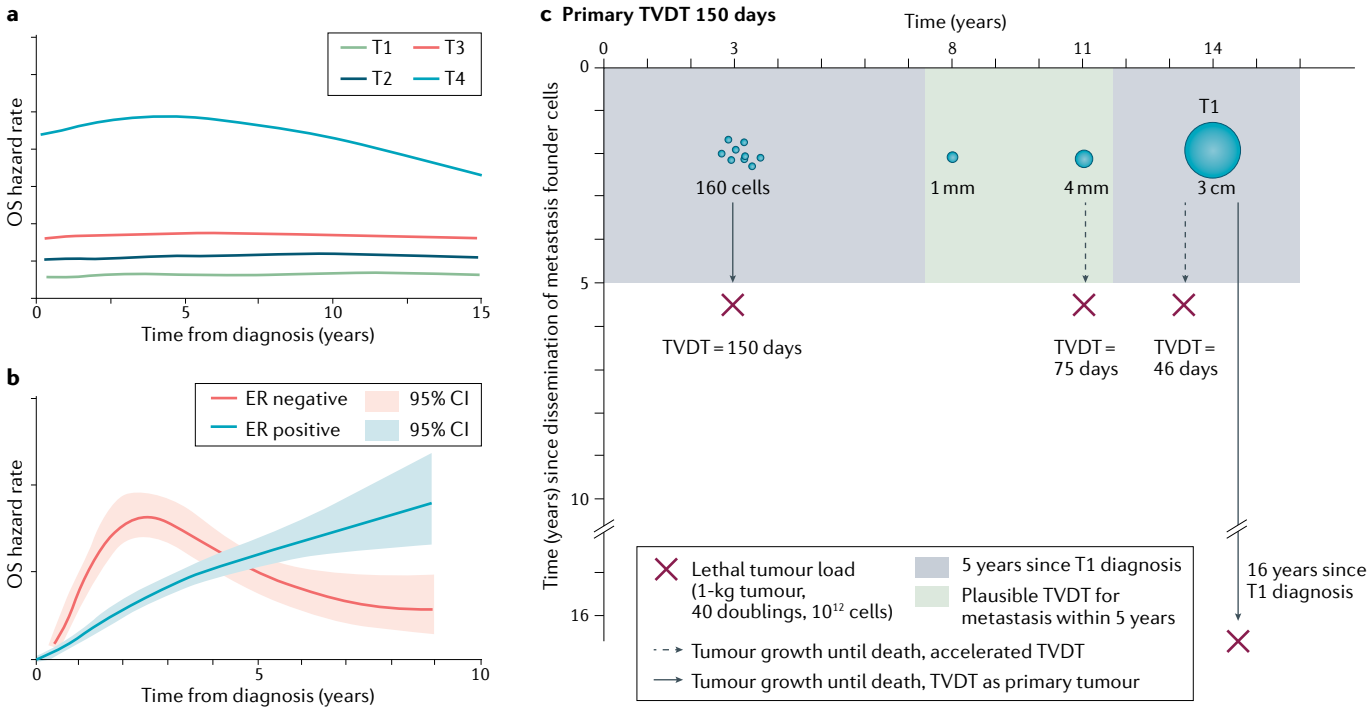

Fig. 1 | **Tumour growth kinetics over disease courses. a**,**b** | Hazard rates for overall survival (OS) in melanoma (part **a**) and breast cancer (part **b**). In melanoma, the risk of death appears constant for each tumour stage (that is, tumour thickness) at resection, however with differences between stages[12]. In breast cancer, oestrogen receptor (ER) status determines OS hazard rates[19]. The OS hazard rate before 5 years from diagnosis is greater for patients with ER-negative tumours and after 5 years is greater for patients with ER-positive tumours. **c** | Different scenarios for how metastatic relapse may occur within 5 years of diagnosis assuming different tumour volume doubling times (TVDTs) and constant growth. A lung cancer with a TVDT of 150 days needs about 14 years to reach the T1 stage (3-cm diameter). If the metastasis-founding disseminated cancer cell (DCC) leaves the day before surgery, it would need about 16 years to kill the patient (assuming growth from a single cell, the same TVDT between the primary tumour and metastases and constant growth). To kill within 5 years, the tumour volume would need to double constantly within 46 days. Empirical data argue against both scenarios. As TVDT may accelerate up to twofold for metastases, dissemination would more likely occur at a diameter between 1 and 4 mm. If the growth rate is indeed the same as estimated from data during the visible growth phase of the primary tumour (that is, 150 days), dissemination should often occur from lesions comprising fewer than 200 cells. CI, confidence interval; T, size of the primary tumour. Part **a** adapted with permission from REF.[12], Elsevier. Part **b** adapted from REF.[19], Oxford University Press, and available under the Crown copyright agreement.

Box 1 | **The framework model of dormancy and the nature of disseminated cancer cells**

The nomenclature of dormancy concepts is confusing, and therefore a framework model of dormancy has been suggested[35]. It focuses on cellular dormancy of disseminated cancer cells (DCCs), as originally described by Willis[34], and thus on distant relapses. It rejects a dormancy definition based on extended distant disease-free intervals[33], because slow but constant proliferation is not differentiated from transient periods of cellular arrest (dormancy) followed by accelerated growth[35]. It further differentiates cellular dormancy from the inability of proliferating cells to induce a tumour vasculature (angiogenesis suppression) or to generate a stably progressing colony, which could also result in late relapses once the inability has been overcome. Long-term survival of DCCs has been documented in patients with non-progressing cancer[47,179], and its potential clinical relevance was proposed after DCCs were found to survive adjuvant therapy[180]. Furthermore, non-progressing DCCs were found to proliferate and manifest themselves after inadvertent transmission during organ transplantation[35]. The frequency of DCCs (as detected by epithelial cytokeratin markers in mesenchymal bone marrow) expressing proliferation markers (for example, Ki67, NOL1 (also known as NOP2 or NSUN1) and proliferating cell nuclear antigen (PCNA)) is about 10% (REFS[181,182]) — similar to that of many primary tumours, but this finding indicates that some DCCs could be in a dormancy state.

tumour masses[27]. The growth rate for a given individual primary tumour is similar to that for its metastases[27], which is supported by similar Ki67 proliferation indices of each[28–30]. However, an acceleration of metastatic growth of about twofold compared with primary tumours has been observed[15,31,32].

I previously argued that a constant growth rate (leading to a 1-cm tumour in 6–20 years) and the aggressiveness to kill via metastasis within 5 years is not compatible with a late dissemination model with cancer cells spreading immediately before surgery[28]. Instead, dissemination must be early, and primary tumours and metastases must grow in parallel, although primary tumours lead this growth such that most are diagnosed before metastases[28]. This lead time, defined as the interval between diagnosis of the primary tumour and diagnosis of the metastasis, is rather short in TN-BC and NSCLC and longer in melanoma and HR^+-BC, and should be determined by two factors: the time point of dissemination and the growth rate of metastases (FIG. 1c).

The assumption of constant exponential growth is frequently contrasted by the dormancy hypothesis, holding that DCCs experience growth arrest and are awakened months or years later to progress to metastases (BOX 1). Historically, relapses 5 years after curative treatment have been considered to involve a mechanism of dormancy[33], a cellular state of arrest or quiescence[34] of unknown and varied duration, involving eventual reactivation. An analysis of tumour growth kinetics revealed that cellular dormancy cannot be deduced from a long disease-free interval after surgery, regardless of its duration[35]. Patients with non-metastatic (M0) cancer have been shown to harbour thousands of DCCs[35,36] in various organs after successful primary therapy (that is, surgery and/or radiotherapy). These are targeted by some type of systemic treatment either before (neoadjuvant) or after (adjuvant) local treatment, and the vast majority do not grow into macrometastases, although a tiny minority may. However, it is unclear whether the relapse-initiating cells are recruited from the pool of dormant, cell cycle-arrested cancer cells and, if they are, it is unclear which mechanisms induce and maintain the state of dormancy and how control is lost (see later).

Assuming constant exponential growth (as deduced from the growth rates measured during the visible period by clinical imaging), one could argue that TN-BC and NSCLC must spread extremely early (FIGS 1c,2a). That is, it would have to spread many years before diagnosis to kill a patient within 5 years, whereas melanoma and HR^+-BC could spread shortly before diagnosis to generate late (more than 15 years) metastasis. However, T1 melanomas are diagnosed 15 volume doublings before T1 NSCLCs (which translates into several years of lead time in diagnosis; TABLE 1) and yet melanoma DCCs are frequently found in such an early stage in the draining LN[37]. From analysis of more than 1,000 patients, we determined that one third of melanomas spread before a tumour thickness of 500 µm and one third spread thereafter, whereas the remaining third do not seed via the lymphatic system. In less than 10% of cases dissemination occurred exclusively by the way of blood[6,37]. Similarly, the number of breast cancer DCCs in bone marrow is detected at the earliest stages (even in ductal carcinoma in situ (DCIS)) and hardly increases as tumours become larger[4]. These data re-emphasize the notion that cancers in general seed very early, often before they reach a size of 1–4 mm, and that early seeding does not imply metastatic success within 5 years.

In summary, we need to discover what determines the growth rate after dissemination to understand why some patients relapse early and others relapse late. Furthermore, metastases that arise from the growth of previously arrested cells must develop explosively to align with observed clinical disease courses. Conversely, the concept of constant exponential growth has been derived exclusively from observable periods of tumour mass growth and it is not known whether the growth rates observed then also apply to the invisible phase of metastatic colony formation; we must, therefore, investigate the invisible phase of metastasis.

Mechanisms of early colonization

Since Stephen Paget's study titled "The distribution of secondary growths in cancer of the breast"[38] successful metastatic colonization has been linked to the putative characteristics of the seed (that is, the DCC) and the soil (that is, the specific metastatic site). Cancer type-specific disease courses already demonstrate the importance of tissue-of-origin specific differences (see earlier for melanoma, breast cancer and NSCLC), and decades of cellular, molecular and biomarker research of primary tumours have further identified specific molecular traits of cancer cells. These include expressed proteins, gene signatures, mutations or copy number alterations (CNAs) statistically associated with poor outcome and metastatic growth. Yet substantially less research has been performed on the seeds directly (that is, patient DCCs), which hinders the assignment of a causal role to the findings for specific steps in the formation of metastases.

If dissemination occurs early, time considerations may help to explain why surgery in early disease stages can be curative and prevent metastasis (FIG. 2a). Increasing evidence indicates that primary tumours have systemic effects beyond seeding of cells, for example via mechanisms of immunosuppression, extracellular matrix (ECM) remodelling or preparing metastatic niches through delivery of soluble factors, including exosomes (for an excellent review see[39]). Therefore, early DCCs could benefit in their niches from this systemic support the longer the primary tumour is growing before diagnosis as more systemic effects could be exerted and possibly higher doses of these primary tumour-derived factors could be delivered. Growth stimulation of early DCCs by such primary tumour-induced effects in parallel with primary tumour growth may be essential

for early colonization. Abrogation of this support by early surgery might be as relevant as surgical prevention of late seeding.

Genomic analysis shows that DCCs isolated before manifestation of metastasis often lack characteristic genomic alterations typical of the investigated primary tumour type and suggests that acquisition of such changes constitutes an essential condition for full proliferative and metastatic potential (reviewed in[40]). Indeed, acquisition of typical alterations, although absent at initial homing to the target organ, is associated with metastatic colony formation[4,6]. Comparative analysis of spatially close (in relation to the primary tumour) LN DCCs and more distant bone marrow DCCs indicates that spatially close LN DCCs are genomically more advanced, consistent either with proliferation (and hence indirectly genomic progression) of DCCs being promoted by primary tumour-derived factors[41,42] (FIG. 2b) or with preferential seeding of more advanced cancer cells to LNs as opposed to bone marrow. However, stepwise acquisition of genomic alterations during formation of metastases in LNs was further demonstrated in melanoma. On arrival at the first draining LN, melanoma cells are genomically aberrant, yet they often lack characteristic CNAs and activating mutations, such as those in *BRAF*[6]. These are acquired as melanoma cells expand in the LN and form an early metastatic colony. On transplantation in immunocompromised mice, only cells from colonies, but not early invading cancer cells, establish xenografts[6]. To progress towards metastases, early, genomically immature DCCs must be able to receive and process stimulatory microenvironmental signals and proliferate. In line with this, we recently found that bone marrow-derived breast DCCs are stimulated via microenvironmentally derived interleukin-6 (IL-6) *trans* signalling. The inflammatory cytokine IL-6 is abundant in bone marrow as is the soluble IL-6 receptor subunit-α, which together can activate mammary-derived cells via the signal transducer gp130 (also known as IL-6 receptor subunit-β). Notably, the data further indicate the importance of the specific cellular niche as the cancer cell expression of gp130 is downregulated by osteoblasts and mesenchymal stem cells but not by endothelial cells. IL-6 *trans* signalling-induced proliferation could be one mechanism enabling the cells to acquire additional genetic alterations that render them increasingly autonomous[43].

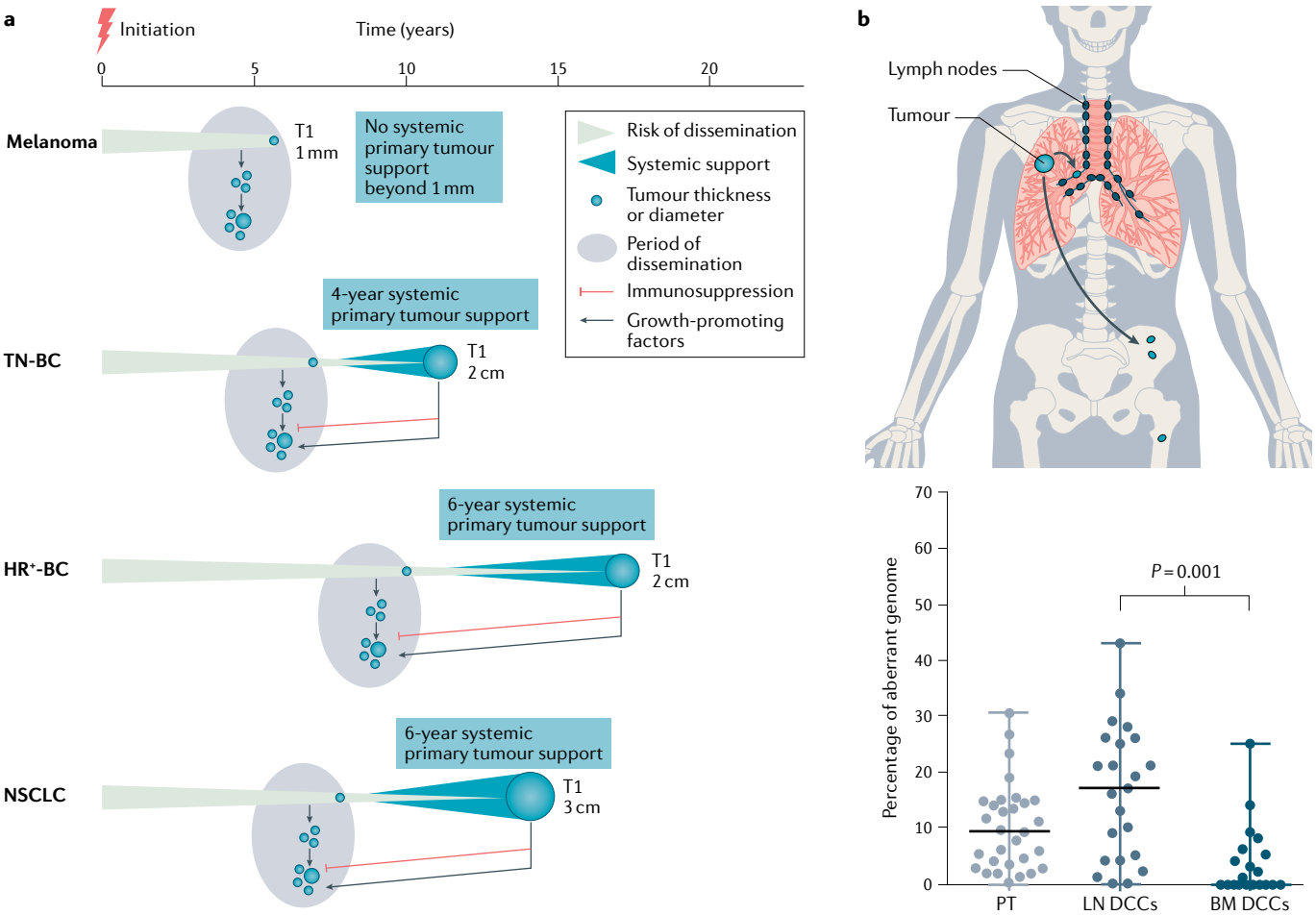

Fig. 2 | **Metastatic dissemination and systemic support of colonization. a** | Time to initial cancer diagnosis and surgical removal and potential systemic support of colonization at metastatic sites by undetected primary tumours. For melanoma, triple-negative-breast cancer (TN-BC), hormone receptor-positive breast cancer (HR+-BC) and non-small-cell lung cancer (NSCLC) time of growth until diagnosis of a T1 tumour (where T indicates the size of the primary tumour) is depicted. Estimates of overall survival hazard rates and in vivo models indicate that dissemination occurs more often from early lesions[4,6]. Primary tumours may accelerate the growth of disseminated cancer cells (DCCs) at distant sites via various mechanisms, including immunosuppression or secretion of growth-promoting factors[39]. This effect may be time or tumour size dependent. **b** | Spatial proximity to primary tumour (PT) and genomic progression of DCCs. Close proximity to the primary tumour accelerates genomic progression of early metastatic lesions, as regional lymph node DCCs (LN DCCs) display higher numbers of copy number alterations than bone marrow DCCs (BM DCCs)[41,42]. Part **b** adapted from REF.[41], Springer Nature Limited.

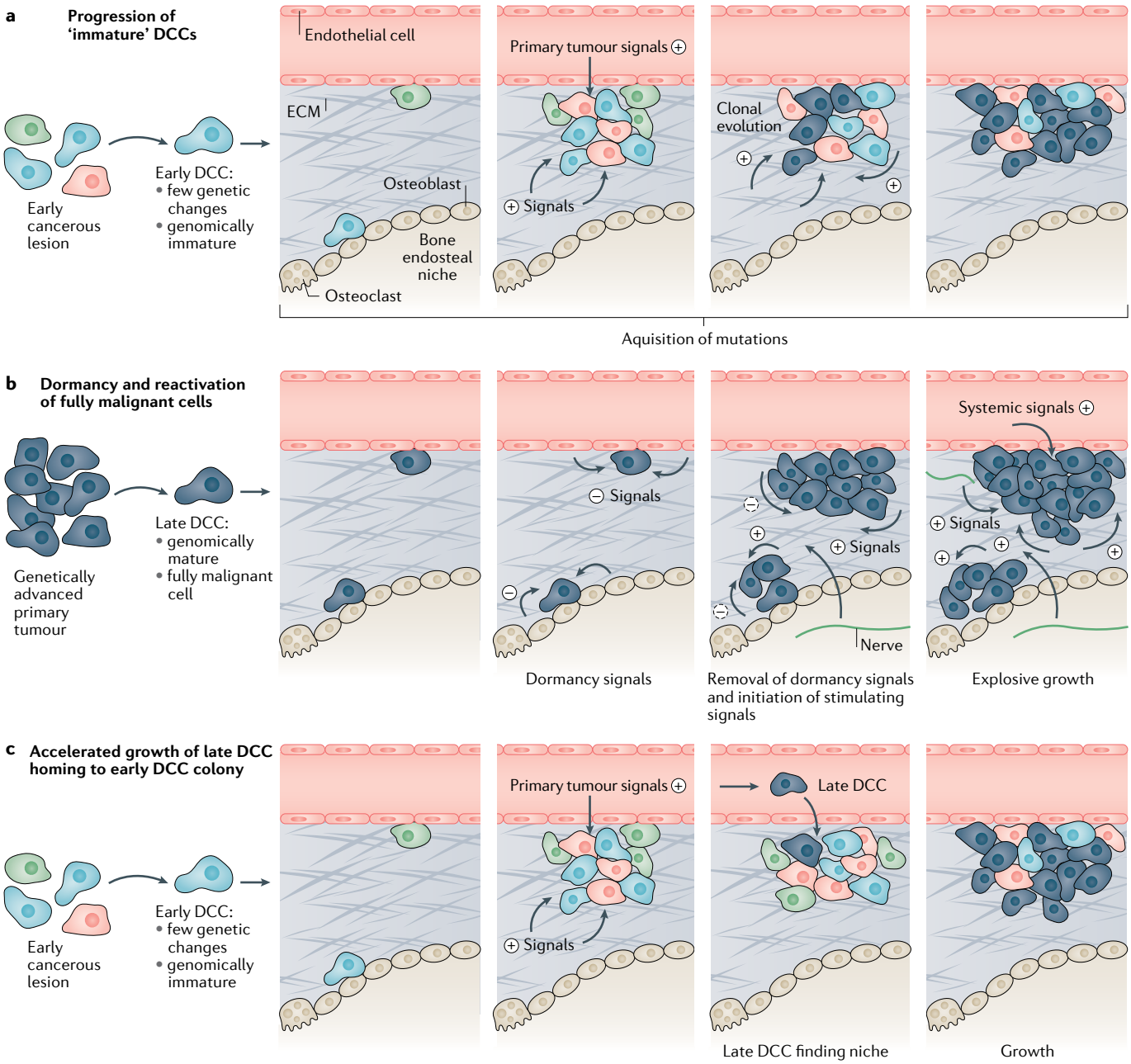

Fig. 3 | **Metastatic colony formation during the invisible phase of systemic cancer. a** | Early disseminated cancer cells (DCCs); that is, cancer cells that spread at a high frequency before diagnosis of the primary tumour, often need to acquire driver alterations at the site of metastasis. The cell division rate is expected to be higher than estimated from measurable tumour volume doubling times. At least to begin with, clonal evolution and possibly co-evolution of the microenvironment may further accelerate initial growth. **b** | Fully malignant, late DCCs as founder cells of late relapses are thought to undergo dormancy (that is, a long-lasting growth arrest) until reawakening. Dormancy-inducing signals from the microenvironment keep the growth of cells arrested until unknown signals stimulate the cell cycle. Growth rates must be accelerated in many cases for alignment with observed disease courses. **c** | Potential scenario of cellular cooperation between early and late DCCs. Early DCCs prepare the niche for late-arriving DCCs, which take over the formation of metastases. Mathematical models indicate that 10–150 cells seed a metastasis and that the number of clones shared between the primary tumour and the metastases increases over time[59]. ECM, extracellular matrix.

This two-step process (step 1, signal processing, and step 2, accumulation of (epi) genetic hits and genomic progression) could account for the extended clinical latency periods until distant relapse (FIG. 3a).

However, if one considers early dissemination to be a common feature of DCCs from NSCLC and TN-BC as well as those from HR$^+$-BC and some melanomas, the former must acquire typical changes faster than the latter during the invisible phase of metastasis. This again may have one of at least two causes: first, cancer type-intrinsic differences which may be related to the biology of the cell of origin, or second, aggressive cancers may inactivate systemic or organ-specific defence mechanisms more efficiently than less aggressive cancer, enabling fast progression of their DCCs. By contrast, if late relapses are generated by DCCs equipped with typical driver changes (here called 'fully malignant' DCCs as opposed to early, genomically immature DCCs) spreading at late tumour

stages, the long latency might be generated after a period of cellular arrest in dormancy. Furthermore, cancer types and subtypes that kill patients early (NSCLC and TN-BC) could differ from those that kill patients later (HR^+-BC and some melanomas) as a result of a differing sensitivity to dormancy-inducing signals (FIG. 3b).

There is a puzzling discrepancy between the absence of fully malignant DCCs at the time of surgery to remove primary tumours — when they are even less frequently detected than the extremely rare 'immature' DCCs — and the apparent genomic similarity between some primary tumours and manifest metastases. This discrepancy has produced an ongoing debate as to whether metastases are founded by early DCCs or late DCCs (TABLE 2). Early DCCs would need to evolve and acquire important driver changes at the distant site, whereas late DCCs would be able to found metastases after adaptation to the novel environment followed by clonal expansion. While most available data point towards metastatic dissemination occurring early, with some studies in mouse models suggesting that 80% of metastases are derived from early DCCs[4], genomic analysis of DCCs and primary tumour and metastases pairs has generated contradicting conclusions. When DCCs are isolated from patients without overt metastasis during surgery to remove primary tumours, their genomic profiles indicate early genomic separation from the predominant clone of the primary tumour, indicating dissemination early in 'genomic' time[4,6,41,42,44–47]. By contrast, comparative sequencing of primary tumours and their matched metastases led to the conclusion of late genomic dissemination of metastasis founder cells in about 60% of

Table 2 | **Metastasis founder cells: early or late disseminated cancer cells?**

Pros	Cons
Late DCCs are founders	
The associations between outcome and tumour size and between early surgery and outcome favour late DCCs as metastasis founders	Disease courses based on epidemiological data cannot be aligned with late dissemination. Growth of metastatic cancer is largely constant, and hence metastasis from late tumours or metastases from metastasis are in conflict with observed clinical data[171,172]. Other factors (for example, systemic effects) associated with large tumour size may account for these statistical associations[36,44]
Many genetic changes are shared among primary tumours and manifest metastases (see the main text for associated references)	Studies often focus on similarity, but disparity can be substantial. Emphasis on autopsy studies and selection of easily accessible samples may bias results and conclusions; that is, may reflect a biology different from the first metastatic manifestation
Cancer cells become naturally more and more aggressive as concluded from the famous generation of the B16-F1 to F10 melanoma models[173]. Therefore, late cells must be more metastatic than early DCCs and may have a higher likelihood to found metastases	In contrast to the Fidler study[173], the landmark experiments of Vaage using serial, orthotopic transplantation of primary tumours and metastases showed that from the original autochthonous tumour, and during strictly orthotopic growth, metastases displayed no average greater tendency to generate metastases than the unselected tumour of their origin[174]. Therefore, the observed increase of aggressiveness in patients or models may be related to selection forces (for example cell culture conditions or iatrogenic intervention) different from metastatic manifestation
Late DCCs are equipped with changes needed for formation of metastases when spreading. Early DCCs must evolve and acquire critical alterations. For this, they must proliferate. However, DCCs are often in a state of quiescence, known as dormancy[75]	Not all early DCCs are quiescent. By contrast, microenvironmental signals, such as IL-6 *trans* signalling activates many breast cancer DCCs in the bone marrow[43]. Additional activating signals were identified in mouse models (see the main text for details and associated references)
Early DCCs are founders	
During primary tumour surgery, genotypes highly similar to the advanced clone of the primary tumour (resembling late DCCs) are rarely detected at distant sites with current markers. However, the source of additional DCCs is then removed and only early-DCC-like cells are left behind[40]	Metastasis founder cells from advanced cancers may express a different phenotype undetectable with current markers
DCCs detected with current markers and often lacking high genomic similarity with primary tumours have prognostic impact[175]	Clinical associations are important but do not formally prove that such DCCs are metastasis founder cells
Typical driver changes are often not present in DCCs at arrival at the distant site but are detected when colonies are formed. Furthermore, acquisition of many driver changes by DCCs is associated with cell division numbers at the distant site but not the primary site[6]	There is no formal proof that specific alterations are acquired outside the primary tumour. In patients with large tumours and early colonies, massive waves of dissemination from the primary tumour may have rapidly overwhelmed early DCCs to generate the appearance of a colony. Such late DCCs would then look more similar to the advanced clones of the primary tumour
Dissemination from large tumours is suppressed, making late massive waves of dissemination unlikely. Strong oncogenic activation or other mechanisms favour proliferation as opposed to migration[4,176,177]. Hence, acquisition of such drivers suppresses dissemination from advanced tumours. Consistently, there is no association between the numbers of DCCs or CTCs and primary tumour size[4,178], despite the up to several hundredfold higher cell numbers in large primary tumours as opposed to small primary tumours	Very rare metastasis founder cells from advanced tumours may simply escape detection during primary tumour surgery
Growth takes time. Early DCCs have time to acquire alterations, whereas it is unclear how long latency periods result from the control of cancer cells with full malignant potential	Although growth control of cell lines in mouse models is short, currently unknown organ-defence mechanisms may control fully malignant cells over years in patients

CTCs, circulating tumour cells; DCCs, disseminated cancer cells; IL-6, interleukin-6.

cases[48–55]. Several aspects could account for this discrepancy, including technical and clinical differences. First, bulk sequencing and bioinformatics may be less suited to uncover the true phylogeny than previously thought[56,57], and secondly, currently analysed primary tumour–metastases pairs are biased towards synchronous metastasis, whereas metachronous metastasis (that is, the most common cases) are under-represented. In addition, the discrepancy could also reflect our currently limited understanding. It has been suggested that metastases are multiclonal and may be generated by several waves of seeding[58–60]. Possibly, early DCCs prepare the niche for late DCCs, with clonal cooperation being important for clinical manifestation of metastases (FIG. 3c). Advantageous clonal cooperation may also be involved in late DCCs from metastatic breast cancers that have been shown to spread in clusters comprising cancer and non-cancer cells[61,62]. It would appear that cooperation includes not only homotypic support but also the help of immune cells, specifically neutrophils, that activate proliferation of cancer cells via IL-6 and gp130 (REF.[62]). The significance of circulating cancer cell clusters in early disease for the generation of metastasis in patients has not been resolved so far.

In summary, rapid progression at secondary sites within 5 years depends not only on the time point of dissemination but also on either cell-intrinsic differences or the ability to respond to growth-promoting or growth-suppressing systemic (immune) or organ-specific microenvironmental conditions. We currently lack a systematic analysis of DCCs from patients without overt metastasis across all cancer types to decide whether genomic maturation or alterations in specific pathways processing intrinsic or microenvironmental information are related to survival data. However, important insights into microenvironmental factors controlling the formation of metastases have recently been obtained.

Host factors acting on DCCs

Like the seed, Paget's soil factors are highly variable, with each target organ providing different conditions. Soil factors, more broadly recognized as 'host' factors, comprise (1) effects of the immune system and organ-defence mechanisms which may suppress or stimulate the growth of invading cancer cells and (2) organ-specific mechanisms that happen to either stimulate or suppress the growth of DCCs for often not fully understood reasons.

Immunoediting and organ defence. The concept of immunoediting[63] evolved from the immunosurveillance theory originally proposed more than 60 years ago by Burnet and Thomas[64] predicting that the immune system acts as a sentinel in recognizing and eliminating nascent transformed cells. It has been focused on the interaction of the immune system with emerging cancers at the primary site and comprises three phases: elimination, equilibrium and escape[65]. As the determinants of the invisible phase in the formation of metastases, particularly the questions around the identity and molecular characteristics of metastasis founder cells have not been resolved, the impact of immunoediting during metastatic colonization is unknown. No studies in humans have addressed the three 'E's at distant sites during the formation of metastases. For example, early and late DCCs should differ significantly regarding their neoantigenic load that may elicit immune responses via major histocompatibility complex class I (MHC I)[63,65] and MHC II presentation:[66] While late DCCs would have already passed the elimination and equilibrium phases and escaped immune control during malignant evolution at the primary site, early DCCs would need to overcome these hurdles, paralleling the primary lesion, although possibly under distant site-specific conditions. Detailed characterization of neoantigen presentation of DCCs and pinpointing escape from immunosurveillance of ploidy changes[67] occurring during genomic progression of DCCs[45] should provide a first glimpse into this question.

The first hints of DCC–innate immune cell interactions have been documented. Autocrine expression of the WNT inhibitor Dickkopf-related protein 1 (DKK1) by DCCs resulted in a downregulation of UL16-binding proteins that otherwise activated natural killer (NK) cells[68], supporting previous evidence for NK cell immunosurveillance at metastatic sites[69]. Site-specific differences of the innate immune system may constitute a major component of organ-defence mechanisms against invading cancer cells[70]. Here, a new field is emerging which will merit a more detailed review in the future.

Dormancy and cell activation in response to host-dependent factors. The aforementioned influences synergize with other challenges for the arriving cancer cells, comprising characteristics of the tissue microenvironment of the target organ such as blood supply, oxygenation, stiffness, ECM composition, growth factor availability and specifically the various cell types forming a niche[71–73]. As we currently lack experimental models for the evolution of early DCCs (that is, for the two-step progression), all models use fully malignant cancer cells to investigate mechanisms of growth arrest and reactivation. Thus, current dormancy models exclude an assumed critical phase of cancer evolution by using primary tumour-derived or metastasis-derived cells. So far, the studies have identified important signalling pathways and have revealed similarities to physiological processes that regulate tissue homeostasis, regeneration and stem cell behaviour. These findings have been summarized in excellent reviews[74–76], permitting focus here on some recent work that has specifically addressed mechanisms regulating the colonization of DCCs in a positive or negative way. The duration of the observed dormancy period in mouse models is rather short (3–18 weeks)[77–86], and we therefore lack information as to whether fully malignant DCCs can be arrested for longer in patients.

Cell cycle arrest can be imposed on otherwise proliferative cells by extrinsic signals. Some data suggest that dormancy is the default cellular reaction of cancer cells in a hostile, unfamiliar environment[87,88], which includes hypoxia[89,90]. Although the exact location or niche of human DCCs for any given organ has not been described so far, two sites are typically investigated in models: the vascular niche and the osteoblastic niche. Real-time imaging of human lung and melanoma cancer cells injected into the circulation of immunodeficient mice showed that extravasating cancer cells lodge at perivascular positions in the brain[91]. Endothelial cells were found to put breast cancer cells into a 6-week-long dormancy in vitro[92], which was induced by endothelial cell-secreted thrombospondin 1 (TSP1). Interactions with the perivascular niche via integrins protected dormant and proliferating cancer cells from cytotoxic agents[93]. In bone, where cancer cells may compete with resident haematopoietic stem cells (HSCs) for their niche[94], dormancy-inducing signals expressed by osteoblasts include growth arrest-specific protein 6 (GAS6)[95], bone morphogenetic protein 7 (BMP7)[96], transforming growth factor-β2 (TGFβ2)[79,97–99] and WNT5A[100], constituting the pathways responsible. Microenvironmental BMPs were shown also to inhibit the growth of breast cancer cells in the lungs[80]. Recently, signalling through leukaemia inhibitory factor receptor (LIFR) has been associated with induction of dormancy in HR^+-BC cells[101].

Pathological processes such as wound healing or inflammation may either prevent dormancy or induce cellular exit from it[102–104]. The fostering of metastatic outgrowth by inflammation has been reported for cells entering a pre-inflamed tissue microenvironment[105,106] and for residual cells that escape dormancy[107], and both are caused by direct effects of inflammatory cells and via changes in the ECM. Sprouting vasculature, which occurs independently of TSP1, enables cancer cells to escape from dormancy[92]. Other growth-promoting signals include tenascin C[108] and periostin[109] in the lungs and vascular cell adhesion protein 1 (VCAM1)[81] in bone. Cancer cells can also exploit collagen I to activate signal transducer and activator of transcription 3 (STAT3) via a recently defined signalling cascade, which sustains stem cell traits and drives reactivation in several organs[110]. The sequential activation of immune and non-immune cells was recently identified via a novel approach to the labelling of niche cells. This revealed that parenchymal epithelial cells are also activated to support lung colonization and to orchestrate metastatic-niche formation via a protumorigenic inflammatory response[111]. A complex interaction between cancer cells, parenchymal lung cells and fibroblasts was noted to support persistence of DCCs and early metastatic colony formation[112]. As a developing field, the link between chronic stress and metastatic progression is increasingly being explored in several cancer types[113,114]. It was found that β_2-adrenergic signalling can reactivate dormant prostate cancer cells and drive their progression via osteoblast-derived GAS6 downregulation[114]. In addition, effects on migration[113] and direct cell cycle activation and apoptosis resistance via β_2-adrenergic receptor (ADRB2) signalling have also been observed[115], and the regulation of β-adrenergic signalling via monoamine oxidase A (MAOA) has potential ramifications for metastasis, epithelial–to–mesenchymal transition (EMT) and stemness[116,117], which may all contribute to the formation of metastases.

Acceleration of metastatic growth

The incomplete synopsis of mechanisms leading to awakening from cell cycle arrest presented so far reveals a plethora of regulatory mechanisms acting during the invisible phase in the formation of metastases. Yet we are still confronted with accounting for the kinetics of metastasis growth observed in patients. If we assume similar growth rates between DCCs observed during the visible phase of metastasis and DCCs of the invisible phase, the clinical relevance of cancer cell dormancy for late relapses is hard to defend because the observed relapse kinetics do not require such an additional and unnecessary explanation: the concept of dormancy would be removed by applying Ockham's razor. However, what if microenvironmental changes cause non-linear kinetics?

Certain microenvironmental conditions can overcome the potentially 'hardwired' intrinsic differences between the growth rates of HR^+ and HR^- breast tumours. This is the case in inflammatory breast cancer (IBC), a clinical diagnosis typically made when the sudden onset of a swollen, red breast leads to a biopsy confirming breast cancer. It accounts for about 2–4% of all breast cancers, but 10% of the annual breast cancer mortality[118]. The reason for its great aggressiveness is still not fully understood, but data indicate that changes in the breast parenchyma generate a tumour-promoting environment[119] potentiating rapid progress in all molecular subtypes of IBC[120] and overriding the prognostic differences associated with HR^+ and HR^- subtypes. This example indicates that the microenvironment can enable otherwise slow-growing cancers to grow aggressively, challenging the assumption that growth is axiomatically constant. Therefore, microenvironmental conditions may enable invisible DCCs to grow faster than is suggested by calculations from manifest metastasis. However, the example also demonstrates how little we understand about DCC growth in patients: if the breast parenchyma is changing the typical growth rates of breast cancer subtypes, why do prognostic differences between breast cancer subtypes disappear given that systemic disease kills the patient? How does the mammary microenvironment exert systemic effects on the growth rates of distant DCCs?

Additional clinical observations provide striking examples of explosive growth of DCCs after dissemination resulting from marked changes in the microenvironment. Inadvertent transfer of cancer during organ transplantation has been taken as proof of the existence of dormant DCCs[35,121]. However, these cases not only demonstrate that DCCs lodge in dormancy in unexpected sites (such as the heart) for years and become reactivated in the organ recipient but also reveal strikingly anomalous kinetics: while TVDTs of human cancers normally range from 50 to 200 days[27], the time to presentation of the donor malignancy transmission in these cases averages 60 days[122], resulting in a TVDT of less than 1 day to a few days — comparable to the growth of the human breast cancer MDA-MB-231 cell line in mice.

One example is the report of a donor patient in whom brain death was diagnosed after a stroke[123], who was found on autopsy to harbour a lung adenocarcinoma 1 day after his liver had been transplanted; the liver was replaced in the recipient with another one after 7 days (and subsequently tested negative for DCCs) but the recipient died 11 months later from metastatic lung adenocarcinoma, which was genetically proven to stem from the first donor. Thus, a 7-day exposure to undetectable cancer cells co-transplanted with the liver sufficed to kill the recipient[123]. For comparison, the reported average TVDT for lung adenocarcinoma is 185 days[27]; that is, to reach a size of 1 cm (about 30 doublings) normally takes about 15 years. It is unknown what changes may be responsible for the enormous growth acceleration of transplanted DCCs: a lack of immunosuppression, cold and warm ischaemia during transplantation, and surgery-associated stress and/or anaesthetics may all contribute to activation of dormant DCCs and may be summarized as iatrogenic activation.

Other, less exotic examples of treatment-induced explosive growth activation are reports of sudden metastasis to sites of healing dental implants[124,125] or tooth-extraction sites[126]. The kinetics is strikingly similar to that in the transplantation cases mentioned above: in an analysis of 55 patients, the mean time between diagnosis of the primary tumour to the appearance of the oral metastasis was 29 months, whereas the mean time between extraction and discovery of a metastasis was 2 months[126]. In 9 of 13 cases where radiographic documentation of the pre-extraction site was available, the bone appeared normal without evidence of a malignant lesion.

An inflammatory response to therapy may also be responsible for accelerated growth rates after chemotherapy or radiotherapy. For example, one study investigating whether delays in the application of radiotherapy after induction chemotherapy affected outcome found a more than threefold acceleration of the TVDT of NSCLC by chemotherapy[127]. Of note, the acceleration of growth was greater for tumours that were smaller after chemotherapy completion[127]. A similar acceleration of regrowth after chemotherapy was reported for brain metastases of NSCLC[128]. Apparently,

chemotherapy-induced inflammation can accelerate growth particularly during the invisible phase and early visible phase.

Systemic effects of surgical trauma, surgery-associated stress and anaesthesia have also been associated with activation of dormant DCCs and tumour promotion[129–131]. While we still lack quantitative data for perioperative stimulation as well as a mechanistic understanding, initial experimental models indicate that the systemic inflammatory response induced after surgery promotes the emergence of tumours whose growth is otherwise restricted by a tumour-specific T cell response[132]. Although this study did not investigate the stimulation of DCCs (but focused on the response of tumour cells at the injection site), its findings are consistent with data from patients with prostate cancer, where the detection of cytokeratin-positive DCCs in bone marrow before radical prostatectomy is a risk factor for metastasis. By contrast, detection of cytokeratin-positive DCCs 6 months to 11 years after surgery is not associated with disease outcome in prostate cancer. Possibly, outgrowth of DCCs present at the detection threshold can be triggered by surgery-associated mechanisms[47].

In addition to iatrogenic changes of the microenvironment, age and lifestyle may impact the growth rate of DCCs. The clinical association of age and body mass index (BMI) with outcome has been repeatedly described[133–135] and is being increasingly dissected mechanistically[136,137]. Notably, menopause-associated changes in inflammatory cytokines in the bone microenvironment could be causally associated with the formation of bone metastases[138,139]. Chemotherapy, which is gerontogenic, may aggravate such changes by inducing cellular senescence in vivo. It accelerates molecular ageing of haematopoietic tissues equivalent to chronological ageing over 15 years[140]. Therefore, ageing could activate dormant DCCs via senescence-associated cytokine changes, reduced immune control or alterations in the stroma and parenchyma[141]. The combined result of adjuvant therapies comprising elimination of growing micrometastases and dormant DCC activation via selection of aggressive cancer cells or a progression-promoting microenvironment may be one explanation for why some trials report initial increases of disease-free survival that are not translated into greater overall survival[142,143].

Finally, an emergent research field focuses on cancer cells surviving systemic therapy. The interesting finding is that such cells, while they may regain a normal-like state or morphology or acquire a state of dormancy, adopt a phenotype that is apparently poised for rapid regrowth after appropriate stimulation. While this work is still in its infancy, the specific state into which these therapy-arrested cells are forced may provide hints for achieving complete eradication or lasting cell cycle arrest[144–146].

Therapeutic relevance

Clearly, it would be beneficial to learn which intrinsic and extrinsic factors drive progression during the invisible phase. This may provide new options to prevent metastasis. Conversely, current systemic therapies may have a severe shortcoming by promoting progression in some cases.

Two studies may serve as examples of the effects of adjuvant therapy on DCCs. In one, it was found that 10-year treatment with tamoxifen for patients with HR^+-BC reduces breast cancer recurrence and overall mortality better than 5-year treatment. The effect was predominant after year 10, and 10 years of tamoxifen treatment halved breast cancer mortality during the second decade after diagnosis[147], although the effect may apply only to subgroups of patients[148]. Secondly, it has been suggested that adjuvant administration of bisphosphonates prevents the reactivation of DCCs by interfering with osteoclast and T cell biology[149], thereby reducing bone metastasis and increasing survival. However, the benefit is relatively small; bisphosphonates reduce bone recurrences with statistical significance from 9.0% to 7.8% at year 10, and survival benefit is definite only for postmenopausal women[149]. Both findings are consistent with — but do not prove — a therapeutic relevance for dormant DCCs. The effect of extended tamoxifen administration is also consistent with the assumption of a further growth deceleration of HR^+-BCs, which are, at any rate, slow growing (FIG. 1b), whereas the potential generation of a hostile environment by bisphosphonates may equally hit proliferating and dormant DCCs. With respect to the latter, almost all of the effect is already seen at year 5 (4.7% versus 5.9% bone recurrence rate for treatment versus control[149]), indicating a predominant effect on non-dormant, possibly still-evolving DCCs.

The therapeutic relevance of dormant DCCs might alternatively be established from data demonstrating their iatrogenic activation. The 15-year survival results and follow-up studies from the Early Breast Cancer Trialists' Collaborative Group demonstrated that adjuvant polychemotherapy reduces the 10-year risk of death from breast cancer on average by about a third, regardless of tamoxifen use, ER status, nodal status and other tumour characteristics in clinical trials comparing different chemotherapies[150,151]. On closer examination, the initial benefit of adjuvant therapy diminishes with statistical significance at specific time points following surgery before the fourth year and has little or no effect on late relapses[152]. Moreover, time-varying effects were observed in 50% of studies, and at least one regimen was found that generated an initial advantage that subsequently switched to a disadvantage[152], consistent with therapy-induced activation of dormant DCCs. As for overall survival, plotting the hazard rates illustrates the different effects therapies may have on early and late relapses of patients (FIG. 4a).

The time-limited effect of chemotherapy (and bisphosphonate therapy) during the first 4 years after surgery is consistent with two assumptions: first, late-relapse-initiating cancer cells are derived from DCCs that escape eradication because they were in dormancy at drug administration, whereas actively proliferating cells are hit (FIG. 4b); or second, cycling and non-cycling DCCs were somehow protected[93]. Delaying adjuvant chemotherapy after surgery beyond 61–90 days generates adverse outcomes, consistent with a lack of chemotherapeutic eradication of DCCs that were activated during surgery[153,154] (FIG. 4b). If surgery activates DCCs and stable colonies are formed, then therapies may fail to eradicate those colonies as a protective environment is formed[155]. However, growth acceleration by intrinsic or extrinsic mechanisms would be needed to explain many relapses starting from individual persisting cells and occurring within 10 years after surgery.

Finding evidence of iatrogenic acceleration in the background of partially successful therapies is difficult. Comparison of 6 months versus 12 months versus 24 months of treatment with trastuzumab, an antibody to the oncogene product HER2 and usually administered together with chemotherapy, indicated that there is no reawakening of dormant DCCs between year 1 and year 2 and that late relapses arise from $HER2^-$ founder cells[156–158]. This hypothesis is supported by observations that chemotherapy-induced switching from $HER2^+$ to $HER2^-$ tumours, possibly resulting from the higher sensitivity of HER2-amplified cancers to anthracyclines[159], may accelerate disease progression[160,161]. Iatrogenic acceleration may therefore result from selection of aggressive variant

cells generated during metastatic colony formation. Selection of aggressive clones or microenvironmental damage might explain why 'new' metastases after chemotherapy are more aggressive than pre-existing lesions[162].

Concluding remarks

The treatment of manifest metastasis often fails, whereas adjuvant therapies have demonstrated efficacy and in some cases may have cured patients (for example, treated patients with HER2-positive breast cancer)[163]. However, for many patients with non-metastatic cancer treated locally and systemically, relapse remains a Damocles sword. Improved eradication of proliferating cancer cells by adjuvant therapies shifts the emphasis towards prevention of late relapses. Understanding the mechanisms that drive colony formation during the invisible phase of cancer metastasis is obviously fundamental. Mechanistic studies await the generation of models suited to investigate the formation of metastases from early, genomically immature DCCs. Furthermore, much could be learned by linking DCC research to adjuvant therapy studies, as exemplified by the work of Naume and colleagues[164], who identified DCCs as a surrogate marker for adjuvant treatment effects. If such studies are linked to omics analysis of DCCs and to assessment of the pre-therapeutic and post-therapeutic microenvironment, we might gain substantial insights for the development of drugs that prevent activation of dormant DCCs or drugs that also target slow-growing, evolving or stimulated, explosively growing DCCs. As pointed out, a diversity of mechanisms seem to affect metastatic colonization, providing additional therapeutic opportunities. In line with this, the fundamental role of the microenvironment in metastatic colony formation and growth during the invisible phase should encourage the search for and clinical testing of drugs that either kill DCCs without generating growth-promoting damage (for example, senescence or inflammation) or withdraw essential stimuli provided by the microenvironment. However, a major challenge will emerge when we come to test these novel metastasis prevention modes in the clinic. Methods to detect DCCs are currently not sensitive enough to use eradication of DCCs as a readout, and follow-up studies in the adjuvant setting take a very long time. Therefore, reliable detection of early systemic cancer, molecular risk assessment and identification of predictive markers during the invisible phase of metastatic progression should become an intensified research field soon. If we had good surrogate end points, adjuvant therapy studies, liberated from trial designs used for patients with manifest metastasis, may become feasible and pave the way for rational improvements of adjuvant therapies.

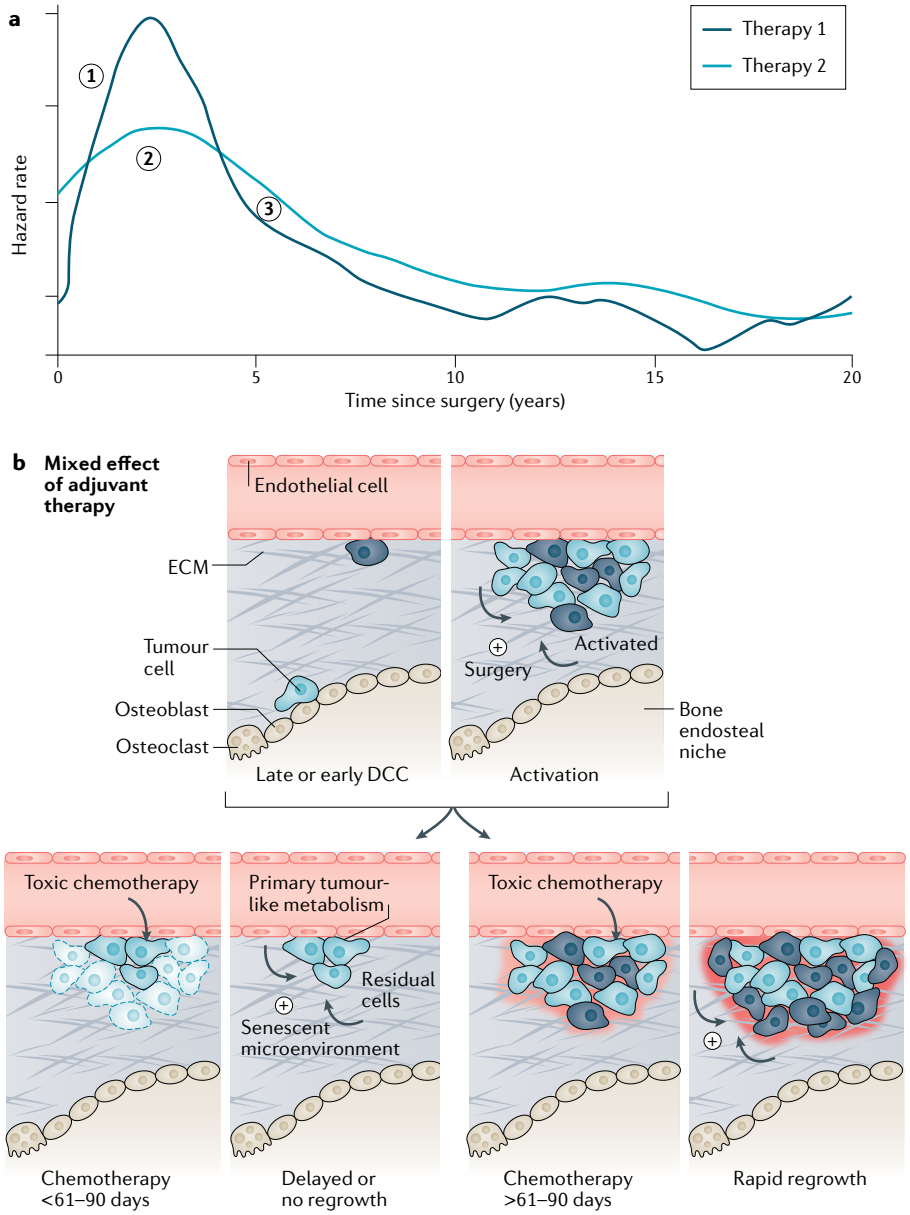

Fig. 4 | **Effects of adjuvant therapies on metachronous relapses. a** | Hazard rate of disease-free survival for two different adjuvant therapies in the NSAB-09 study[152]. Initially, therapy 1 is superior (period 1), and therapy 2 shows a delayed positive effect (period 2). However, the risk of late relapse beyond 5 years is increased for therapy 2 (period 3); these time-varying effects indicate that therapy-associated mechanisms may prevent or favour late outgrowth of disseminated cancer cells (DCCs). **b** | Acceleration of DCC growth by surgery and optimal window of chemotherapeutic DCC eradication. Systemic effects of surgery and anaesthesia may awaken DCCs from dormancy or accelerate DCC growth (upper panel). Immediate administration of chemotherapy may effectively eradicate activated cells (lower-left panels). Perisurgical activation is not counteracted by adjuvant therapy. Colonies may form and generate a protective environment, and delayed systemic therapy becomes ineffective (lower-right panels). Relapse may occur in both scenarios, although with different probabilities and by different mechanisms. Effectively, arrested residual clones may awaken from dormancy as they 'remember' previous activation states[145] and in both scenarios an altered microenvironment may become supportive of growth. ECM, extracellular matrix. Part **a** adapted with permission from REF.[152], Oxford University Press.

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https://doi.org/10.1038/s41568-020-00300-6

Published online 6 October 2020

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Acknowledgements
The author thanks S. Pausch for help with the figures, T. Perry for critical reading of the manuscript and M. Guzvic for hints about relevant references.

Competing interests
The author is a member of the scientific advisory board of HiberCell.

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