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DATABASES
miRBase: http://microrna.sanger.ac.uk/sequences/
miR-107 | *miR-10a* | *miR-10b* | *miR-126* | *miR-141* | *miR-146a* | *miR-148a* | *miR-155* | *miR-15a* | *miR-16-1* | *miR-200a* | *miR-200b* | *miR-200c* | *miR-205* | *miR-206* | *miR-20a* | *miR-20b* | *miR-21* | *miR-210* | *miR-221* | *miR-222* | *miR-27a* | *miR-27b* | *miR-29c* | *miR-335* | *miR-34a* | *miR-34b* | *miR-34c* | *miR-373* | *miR-429* | *miR-520c* | *miR-9-3* | *miR-92a*
UniProtKB: http://www.uniprot.org
AGO2 | BCL-2 | cathepsin B | cathepsin Z | CD44 | CDK6 | CRK | CTGF | cyclin D1 | dicer | E2F1 | E2F3 | ephrin A3 | EZH2 | FAK1 | FXR1 | HMGA2 | HOXD10 | HRAS | IRAK1 | KIT | MMP2 | MMP9 | MYC | neuropilin 1 | p53 | PDCD4 | PIK3R2 | PTEN | RECK | RHOA | RHOB | RHOC | ribonuclease 3 | ROCK1 | ROCK2 | SEMA3C | SERPINB5 | SMAD4 | SOX4 | SPRED1 | TGIF2 | TNC | TPM1 | TRAF6 | TSP1 | TWIST1 | uPAR | vimentin | ZEB1 | ZEB2

FURTHER INFORMATION
G. A. Calin's homepage: http://www.mdanderson.org/labs/calinlab/
miRGen: http://www.diana.pcbi.upenn.edu/miRGen.html
RNA22: http://cbcsrv.watson.ibm.com/rna22.html

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OPINION

Parallel progression of primary tumours and metastases

Christoph A. Klein

Abstract | Systemic cancer progression is accounted for in two basic models. The prevailing archetype places the engine of cancer progression within the primary tumour before metastatic dissemination of fully malignant cells. The second posits parallel, independent progression of metastases arising from early disseminated tumour cells. This Perspective draws together data from disease courses, tumour growth rates, autopsy studies, clinical trials and molecular genetic analyses of primary and disseminated tumour cells in support of the parallel progression model. Consideration of this model urges review of current diagnostic and therapeutic routines.

Although advances have been made in reducing mortality rates and improving survival, cancer is the leading cause of death among men and women under 85 years of age in the United States[1]. Most deaths from cancer are due to metastatic disease, and prevention of later arising metastasis has moved to the centre of clinical attention. Prevention of metastasis combines early surgery (or radiotherapy in some cases) with systemic therapy given before or after surgery (respectively neoadjuvant or adjuvant therapy). Systemic therapy mainly targets tumour cells that have detached from the primary lesion to lodge elsewhere, undetectable by clinical imaging and inaccessible to excision.

Once primary tumours are resected, metachronous metastases must arise from tumour cells that disseminated to ectopic sites before surgery, called disseminated tumour cells (DTCs). The term DTC is used for any tumour cell that has left the primary lesion and travelled to an ectopic environment. However, it is not predictable *a priori* which DTCs will grow into overt metastases (the majority will not) and which molecular traits are required for that process; that is, the metastasis founder cells are unknown. In this situation, models of cancer progression become important, as they provide predictions about the target cells of systemic therapies. They generate therapeutic hypotheses, motivate preclinical research and contribute to the design of clinical trials. An example is the current practice of using molecular traits of the primary tumour to predict responses of DTCs to adjuvant therapies that target specific molecular mechanisms[2–5].

Two fundamental models of metastasis

The use of primary tumours to predict therapy response is based on one of the two models discussed here, referred to as the linear progression model. In this model, tumour ontogeny proceeds to full malignancy within the primary tumour microenvironment, after which tumour cell dissemination founds a metastasis. Therefore, the primary tumour prescribes the molecular characteristics of DTCs spread throughout the body. In the second model, parallel progression, tumour cells depart the primary lesion before the acquisition of fully malignant phenotypes to undergo somatic progression and metastatic growth at a distant site. The proposition of early dissemination and divergent progression of primary tumours and DTCs towards metastasis questions the role of the primary tumour for therapy prediction. It is important to note that, unfortunately, neither model is supported by direct and incontrovertible evidence and they have been derived indirectly.

The linear progression model. The linear progression model is based on Leslie Fould's description of a stepwise progression of morphological abnormalities (reviewed in REF. 6) accompanying cancer. Accumulation of genetic and epigenetic alterations was subsequently associated with this process[7]. At its simplest, the model states that cancer cells pass through multiple successive rounds of mutation and selection for competitive fitness[8] in the context of the primary tumour. After a significant number of such rounds the cells may be able to proliferate relatively autonomously at a competitive rate. These tumour cell clones expand and individual cancer cells leave the primary site to seed secondary growths (FIG. 1). The final clonal expansion of fully malignant clones is linked to tumour size. For example, mutations of the tumour suppressor gene *TP53* are rare in T1 stage (<2 cm; sizes refer to diameter throughout) breast cancers, and significantly more frequent in T3 stage (>5 cm) tumours. Clonal expansion of *TP53* mutated cells therefore often occurs when tumours grow beyond 2 cm (REF. 9). Such observations and the well-known association of tumour size with higher frequency of metastasis, which is the basis of the routinely used TNM classification system (see below), have promoted the concept that only tumour cells that are shed late in primary tumour progression have the possibility of eventually spawning macroscopic

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metastases[10]. However, the possibility that dissemination of tumour cells occurs early (when tumours may be small) after acquisition of fully malignant traits cannot be excluded. Such cells, having departed early, are also thought to share most of their characteristics with the primary tumour.

A related, although somewhat distinct, concept is that of metastatic cascades[11]. Systemic disease progression may continue after the first metastasis until the tumours comprise a lethal mass of about 1 kg, equivalent to a burden of 10^{12}–10^{13} cells. It has therefore been suggested that, once DTCs have adapted to the distant site and have formed a macroscopic metastasis, this tumour can then propagate secondary metastases: a lethal shower of metastases[12].

Therefore, the linear progression model comprises three key empirically testable aspects: first, dissemination of fully malignant cells, which — second — arise mostly in advanced cancers and, third, enable metastasis from metastasis.

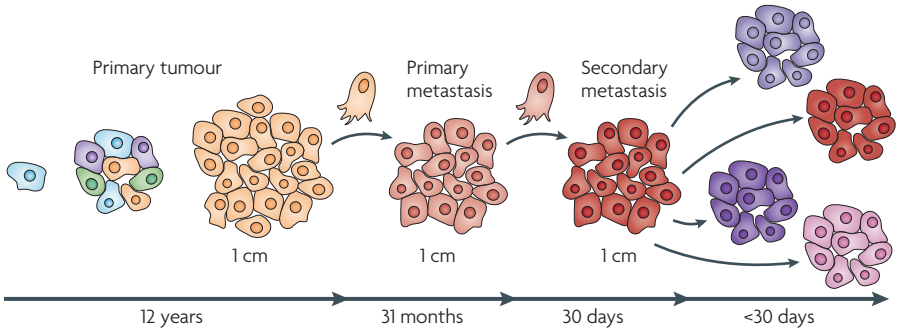

Figure 1 | **Late dissemination and metastatic cascade model (breast cancer).** During local progression, aggressive cells are selected and dissemination initiates (here at the stage of early diagnosis: 1 cm^3). In primary tumours and primary and secondary metastases, cells become increasingly malignant until a final shower of metastasis from metastasis kills the patient. Time considerations in the example use tumour volume doubling times of 157 days for primary breast cancer[21], 30 days for first metastasis (median diagnosis of metastasis 30 months after surgery[61]), 1 day for secondary metastasis[59] and less than 1 day for cells killing the patient.

The parallel progression model. The parallel progression model dates back at least to the 1950s (REF. 13), when considerable effort was made to quantify human cancer growth rates. These studies concluded that metastasis must be initiated long before the first symptoms appeared or the primary tumour was diagnosed[13,14]; given their growth rates, metastases were simply too large to be accounted for by initiation at a late stage of primary tumour development.

Parallel progression does not question general mechanisms of cancer growth such as clonal selection for competitive fitness in primary tumours, or accumulation of genetic alterations. However, parallel progression does not necessarily place metastatic founder cell dissemination near the end of primary tumour development (FIG. 2). Moreover, dissemination of tumour cells that are still evolving may lead to allopatric selection and expansion of variant cells adapted to specific microenvironments. Therefore, owing to selection pressure and the inherent genetic instability of tumour cells, parallel progression predicts greater disparity between metastatic founder and primary tumour cells than does linear progression and emphasizes the mechanistic importance of site-specific selection of genetic and epigenetic alterations. Finally, the parallel progression model makes three testable predictions. The first is parallel and independent accumulation of genetic and epigenetic alterations in the primary tumour compared with the metastasis. The second is seeding of tumour cells to different distant sites in parallel, independent, niche-related adaptation during outgrowth towards metastasis. The third is parallel ontogenesis of metastatic cells that disseminated from the primary site at different times. Because, in parallel progression, metastatic founder cells in most cases disseminate long before clinically detectable disease, there is ample time for multiple waves of dissemination or continuous spread.

In this article, data from disease courses, genetic analyses of DTCs and manifest metastases, and therapy trials are discussed in the context of each model. With few exceptions, the arguments are based on data from patients rather than animal models. This is because, first, some animal models, such as xenotransplantation of cell lines that are usually derived from advanced tumours or metastases, partially rely on the correctness of the linear progression model (and therefore would provide circular arguments); second, any model of cancer progression gains its relevance by its explanatory power of the human disease; and, third, the fidelity of such models to human disease is questionable.

Disease courses and growth rates

The course of human cancer is determined by anatomical disease stage and the rate of growth. The TNM classification[15] accounts for the extent of the disease at diagnosis by measuring tumour size (T stage), and cancer spread to local lymph nodes (N stage) or distant metastatic sites (M stage). Survival time is determined by the speed needed to reach a total tumour mass of about 1 kg. At this stage organ failure or several systemic processes lead to death[16]. The association of tumour size and metastasis or death seems to be valid for all types of cancer (for example see Tumour-specific analysis in Further information (in German)). For example, the 15 year survival prognosis for patients with T1N0M0 breast cancer (tumour <2 cm, no lymph node and no overt distant metastasis) is 90%, whereas for patients with T2N0M0 breast cancer (tumour diameter 2–5 cm, no lymph node and no overt distant metastasis) it is 70%.

Radiographic studies determine human cancer growth rates by determining the tumour volume increase between measurements to obtain the tumour volume doubling time (TVDT). Thus, determination of the TVDT is a non-invasive assay accommodating all factors that influence steady-state tumour kinetics, such as rates of proliferation, neo-angiogenesis, apoptosis and necrosis, and immune system effects. Collectively, TVDTs vary considerably between patients (FIG. 3c) but less so between primary tumours and matched metastases or between metastases at different sites[14]. TVDTs for metastases and primary tumours are comparable (typically ~60–200 days[14,17,18]) (TABLE 1) but up to two times faster for metastases. Unfortunately, paired samples have rarely been analysed but, when they have been, metastatic growth rates correlated with the corresponding rates for the primary tumour[19]. Thus, cancer growth rates at the detectable stage are an inherent property reflecting the provenance of the cancers, no matter where they grow[19]. This general conclusion is supported by independent histological studies in which the proliferation index — measured by determining the mitotic index or counting cycling cells using the Ki-67 antigen — was used to compare growth rates (TABLE 1). The proliferation indices of paired metastasis and

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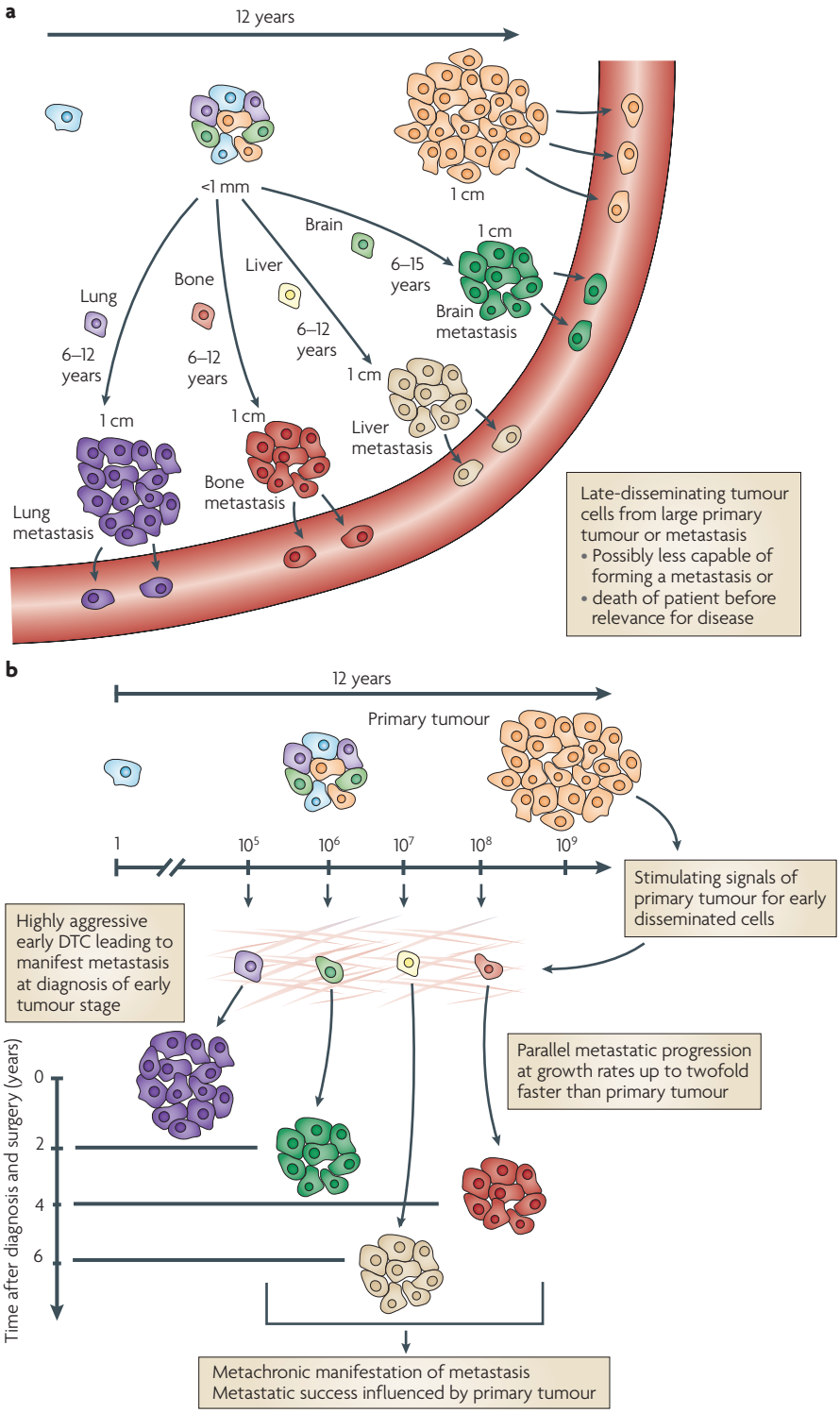

Figure 2 | **Parallel progression model (breast cancer). a** | In the early dissemination and parallel progression model, dissemination starts when tumour diameter is 1–4 mm. Metastases in different organs are seeded in parallel and develop within 6 years (tumour volume doubling time (TVDT) twice as fast as primary tumour) to 12 years (TVDT in primary tumour equal to TVDT in metastasis). In brain, microenvironmental factors might delay the outgrowth of metastases. Tumour cells seeded from the primary tumour at diagnosis or the various metastases will either not contribute to death (as they are lagging behind) or might not be able to grow out. **b** | In the parallel progression model, several waves of disseminated tumour cells (DTCs) may disseminate before diagnosis and may progress in parallel at different rates in different organs. Factors secreted by the primary tumour may stimulate colonization and account for the relationship of tumour size and probability of metastatic outgrowth.

primary tumours were again similar (FIG. 3d; TABLE 1). Although these techniques do not consider attrition rates, they are an adjuvant to TVDT.

TVDT is based on direct measurements and estimates of the duration of growth (that is, age of the tumour), typically assuming a constant exponential rate (FIG. 3a). However, growth rates can plateau as tumours become large[20] (FIG. 3b), exhibiting an 'S-shaped' growth curve where proliferation initially accelerates then slows when tumour size results in nutrient restrictions. At the detection threshold of mammography screening that is used to determine the TVDT of breast cancers[21], tumours are very small (<10–14 mm) and could plausibly be growing rapidly[21]. Therefore, TVDT was not determined during the plateau phase of growth but during exponential growth. Moreover, the assumption of constant exponential growth is supported by an independent approach based on the time from diagnosis of metastasis to death (recorded at large cancer registries[22,23]). In breast cancer, time after diagnosis of metastasis to death varied only by a factor of two and was independent of the size of the primary tumour when diagnosed, which indicates that growth rates before primary tumour diagnosis are similar. The period between initiation of metastasis and diagnosis was estimated to be 6 years[22]. Although these approaches may be simplistic, it is important to note that the errors they introduce are uniform. Both linear and parallel progression models must account for disease courses, including the observation that metastases rarely display growth rates more than twofold higher than those of the primary tumour.

The linear progression model. Correlation between tumour size and metastasis provides a strong argument in favour of the linear progression model. In the example above, the 20% higher rate of death of patients with T2 tumours compared with those with T1 tumours can be explained by the generation of cells with metastatic proficiency during clonal expansion from 2 cm to 5 cm. Surgery that removes the tumour before 2 cm will arrest clonal progression and prevent the dissemination of fully malignant cells.

Well-established breast cancer growth rates have been determined through extensive mammography studies[14,21]. Primary tumours grow more rapidly in young women than in old women and, at 60 years, the average TVDT is 157 days[21] (TABLE 1). Accordingly, the tumour takes ~12 years[21]

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to attain a size of 1 cm and ~3 years to grow from 1.4 cm (stage T1; small tumour) to 7 cm (~7 volume doublings). In linear progression, dissemination of a metastasis founder cell shortly before surgery would therefore lead to manifestation of a 1 cm metastasis within 6–12 years. This is difficult to reconcile with data from registries that report a median time from resection to distant metastasis of 35 and 20 months, respectively, for patients with T1 (<2 cm) and T3 (>5 cm) tumours[22]. Therefore, a DTC seeding a new growth on the day before surgery would require a TVDT at least five times higher than that of the primary breast tumour (30 doublings within 30 months versus 30 doublings within 12 years).

Two further prevalent conditions are not readily compatible with linear progression: first, diagnosis of metastasis at clinical presentation in early-stage cancer (T1M1 or T2M1), and, second, cancer of unknown primary site (that is, with no detectable primary tumour). Of T1 and T2 breast cancer patients, 5% present with metastasis at diagnosis[22], and cancer of unknown primary site accounts for 5–10% of diagnoses in Europe and the United States[24,25]. In these situations, according to linear progression, metastatic growth rates would have to far exceed that of the primary tumour; there is no evidence that this can be the case.

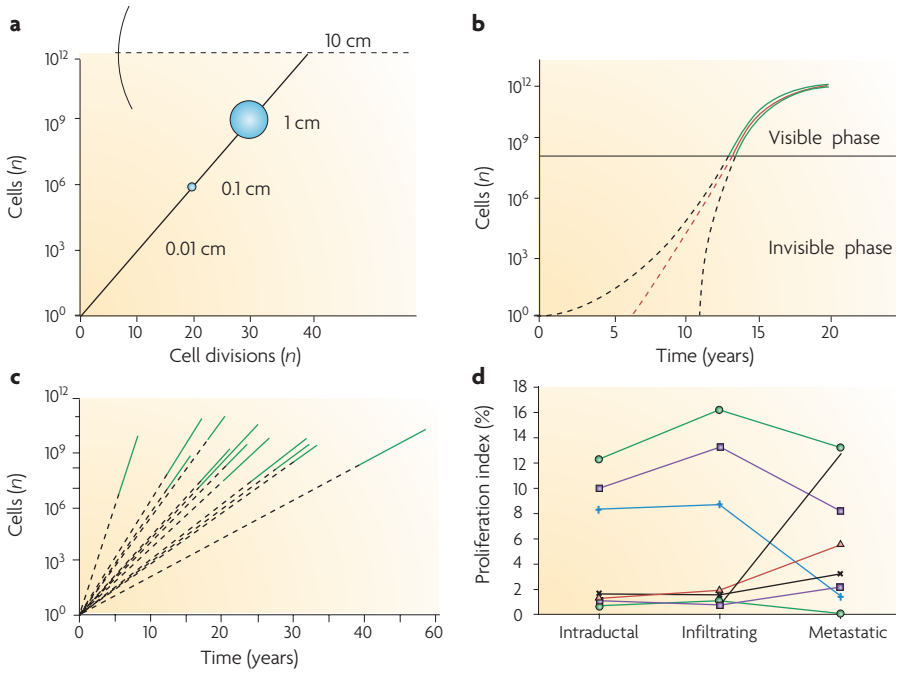

Figure 3 | **Growth rates in human cancer. a** | The graph shows the relationship between tumour size and cell doublings assuming exponential growth. **b** | During the visible phase, tumour growth may be initially exponential but slow down at larger sizes (Gompertzian growth[86]), whereas growth kinetics during the invisible phase may be faster or slower. Most researchers assume constant exponential growth (red line). **c** | The graph shows growth rates for 12 cases with primary cancer of the breast. Each curve represents one case. The solid line indicates the period of observation. Dotted lines mark the invisible phase. **d** | The graph shows the proliferation index (measured by Ki-67 labelling) of matched triplets including intraductal areas, invasive areas and metastases, from eight patients. Note that proliferation indices are higher in metastases in four patients and lower in the other four. Data taken from REF. 87. Parts **a**–**c** reproduced, with permission, from REF. 14 © (2009) Wiley–Liss, Inc.

The parallel progression model. The growth kinetics of tumours and metastases are accommodated by the parallel progression model. If a primary breast cancer grows in 12 years to 1 cm, a metastasis also needs ~6–12 years to reach this size. Parallel progression accounts with higher fidelity for observed disease dynamics by moving the starting point of metastatic dissemination to the clinically undetectable phase of cancer growth, years before diagnosis of a primary tumour (FIG. 2).

The parallel progression model must, however, account for correlations between primary tumour size and early onset and frequency of metastatic disease. Early diagnosis of metastatic disease after presentation of a large (as opposed to small) primary tumour is explained by a lead time effect: the extra time that the large tumour takes to grow compared with the small one. In the example above (in which a tumour takes 3 years longer to reach 7 cm than 1.4 cm), metastasis may be diagnosed 3 years earlier in the patient with the large tumour because, in the parallel progression model, the metastasis of the T3 tumour had another 3 years to grow[22,23].

However, if dissemination starts during the undetectable phase of tumour growth, why does surgery of a T1 stage tumour improve survival by 20% relative to T2 stage tumour removal? One as yet untested explanation is that signals from the primary tumour promote secondary tumour growth. To explain the relationship between tumour size and metastasis, stimulation by these signals need to be related to tumour size. The signals could include secretion of growth factors and cytokines that remodel stromal architecture and modulate stromal and bone marrow-derived cell function and activities, to promote metastatic growth indirectly within a metastatic niche[26–28].

Genetic evidence for models of metastasis

Selection and late-stage clonal expansion of an advanced cancer cell population preceding dissemination are central to linear progression, whereas parallel progression predicts that genetically less-advanced cells disseminate from early stages of tumour development. In patients, metastasis founder cells are recruited from the pool of DTCs; that is, one or few of the DTCs will eventually grow out. Genetic analyses of DTCs and manifest metastasis might therefore shed light on the credibility of the two models.

According to the seminal study of Schlimok and Riethmüller[29], DTCs can be detected in mesenchymal organs including bone marrow and lymph node in patients with (M1) or patients without (M0) evidence of clinical metastasis[30,31]. Epithelial markers, such as epithelial cytokeratins or the epithelial cell adhesion molecule (EPCAM, also known as TACSTD1) used to detect DTCs, suggest that DTCs are a very rare cell population. Only 1–10 such cells were detected per 2 million bone marrow cells in ~30% of M0 patients with marginal differences between cancer types when the same protocol[32] for DTC detection was used. Moreover, the presence of cytokeratin-positive DTCs predicted poor outcome for a range of epithelial cancer types, and they therefore seem to be a cell population that is relevant to disease progression[30,33].

Not until the advent of single cell techniques for whole genome analysis has it become possible to carry out molecular

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Table 1 | **Growth rates and proliferation indices of human tumours and metastases**

	TVDT analysis						Ki 67 labelling (%)*	
	Primary tumour			Metastasis			Primary tumour	Metastasis
Tumour type	TVDT (days)	Patients (*n*)	Method	TVDT (days)	Patients (*n*)	Method		
Breast cancer <50 years 50–70 years >70 years	150 80 157 188	236	Minimum two mammographies[21]	82	29	Thoracic radiography[18]	Mean 4.9; median 4.0 (REF. 87)	Mean 5.7; median 5.5 (REF. 87)
Colon cancer	130	27	Two radiographies[88]	109 155 (overt) 86 (occult)	10 11 18	Thoracic radiography[17] Liver CT scan[20]	ND	ND
Prostate cancer	>730	43	Serum PSA doubling time[89]	ND	NA	NA	Median 8.4 (range 0–67) (REF. 90)	Median 5.3 (range 0-30; lymph node metastasis)[90] median 9.0 (range 0–97; bone metastasis)[91]

*Data from REFS 87,90 are from matched pairs of primary tumours and metastases. CT, computed tomography; NA, not applicable; ND, no data available; PSA, prostate-specific antigen; TVDT, tumour volume doubling time.

analyses of DTCs years before manifest metastasis[34,35]. The bone marrow samples in these studies comparing primary tumours and DTCs were taken just before surgery, so the genotypic analysis compared matched samples from the same disease stage. Surprisingly, in breast, prostate and oesophageal cancer, bone marrow DTCs display significantly fewer genetic aberrations than primary tumour cells[36–39]. In breast cancer, ~50% of cytokeratin-positive cells had normal karyograms (as judged by metaphase comparative genomic hybridization (CGH)) whereas all matched primary tumour karyograms were abnormal[36,37]. Although they seemed karyotypically normal, these DTCs were shown to contain small, subchromosomal deletions that are typical for breast cancer, proving their malignant origin and indicating that they disseminated before genome-wide instability was acquired[36]. Likewise, the most prevalent chromosomal aberrations of primary breast tumours (including 8q gain, 13q loss, 16q loss and 17p loss)[37] and the most frequent alterations in prostate cancers (8p loss, 8q gain, 13q and 16q loss)[39] are rarely found in DTCs with abnormal karyograms isolated at the time of curative surgery, whereas other alterations are detectable. Multiple control experiments show that the observed divergence is not a technical artefact[34,36–38,40].

In oesophageal cancers, heterogeneous chromosomal rearrangements were shown to occur not only in primary tumours and DTCs isolated from lymph nodes and bone marrow of a given patient but also in DTCs from different sites[38], suggesting adaptive selection of different features to achieve disseminated survival and growth. This does not exclude the possibility that some general mechanisms provide advantages that are recurrent. Although *ERBB2* amplification was the most frequent chromosomal alteration in DTCs from lymph nodes and bone marrow, neither *ERBB2* gains nor their absence in primary tumours were predictive for the *ERBB2* status in DTCs from the same patient[38]. When primary tumour samples were analysed, *ERBB2* amplification did not correlate with reduced survival, whereas all patients displaying a gain of *ERBB2* in a single DTC from bone marrow or lymph node died within 23 months. Only *ERBB2* gain in DTCs and large (T3 or T4) primary tumours were independent risk factors for death in this cohort of patients[38]. This observation has several implications. First, *ERBB2* amplification can occur in DTCs after dissemination from the primary site. Second, *ERBB2* can be amplified in the primary tumour after dissemination of DTCs, although these late-developing ERBB2-positive primary tumour cells do not necessarily disseminate detectably. Third, independent *ERBB2* amplification in some matched primary tumour–DTCs, together with the different prognostic value of ERBB2-positive cells in bone marrow and lymph nodes versus primary tumours, suggests that ERBB2 has an important role in DTCs.

In addition to the data comparing DTCs with primary tumours, evidence for or against both models may be found in comparative data from primary tumours and manifest metastases. Linear progression of metastasis founder cells during primary tumour growth predicts overlapping traits for primary tumours and manifest metastasis, whereas parallel metastatic progression outside the primary tumour emphasizes their divergence.

Only matched pairs of primary tumours and metastases from the same patient are informative for the questions posed in this context, and few data are available. However, a high degree of divergence is observed at all levels of molecular resolution. For example, *KRAS* mutation states for primary cancers and their metastases were found to diverge in up to 50% of colorectal cancers[41–43] and 75–80% of lung cancers[44,45], and lung carcinomas show divergence in *EGFR* mutations (exons 18–21) in 75% of cases[46]. A detailed study of 146 samples from 26 patients assessed allelic imbalances for 52 microsatellite markers and found that allelic imbalances were significantly higher in primary tumours than in lymph node metastases[47]. A high degree of discordance in patterns of allelic imbalances existed not only between primary tumours and metastases, but also between different metastases from the same patient[47]. Many studies using genome-wide assays, such as CGH, provide additional examples of multiple distinct genetic aberrations[48,49]. Finally, it has been observed that multiple, coexisting metastases apparently derive from genetically unrelated tumour clones, implying that some primary tumours arise from more than one tumour cell[50]. Although most studies concur on mutational heterogeneity between paired samples, few report the superimposable or overlapping genotypical aberrations that are predicted by linear progression[51].

Global gene expression profiles are closer for matched primary tumour–metastasis pairs than when comparing primary tumours (or metastases) with those from different patients[52]. However, such comparisons neglect the influence of disparate genetic backgrounds on the expression of thousands

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of genes[53]; clearly, paired samples are likely to be similar compared with samples from unrelated individuals. Furthermore, if primary tumours and metastases influence or create environments (niches) independently of anatomical location, concordant gene expression profiles are broadly expected. Therefore, gene expression profiling may not be optimal for characterizing clonal relationships between primary tumours and metastases.

The linear progression model. The genetics of tumour cells detected in lymph nodes or bone marrow at the time of surgery contradict the linear progression model. Unexpectedly, putatively fully malignant (genetically advanced) tumour cells displaying the genetic alterations of primary tumours rarely disseminate to bone marrow or lymph nodes[36,37,39]. However, this could be an artefact of the detection assays, which relied on the expression of cytokeratin 8, cytokeratin 18, cytokeratin 19 or EPCAM, which are strongly expressed by primary tumour cells. The findings do not exclude the possibility that DTC populations exist in which the epithelial markers cytokeratin 8, cytokeratin 18, cytokeratin 19 and EPCAM are not expressed and therefore are not detected. Such marker-negative cells are postulated to display genomic aberrations that are distinct from those of marker-positive DTCs, but harbour the same genomic alterations of primary tumour cells. The absence of such marker-negative DTCs would be incompatible with linear progression.

The high frequency of distinct genetic alterations in primary tumours and manifest metastasis is also difficult to reconcile with linear progression. At best, disparities between matched pairs may indicate undetected clones within the primary tumour that metastasize, but even this would mean that a major correction of the model was required.

The parallel progression model. The data outlined above indicate that dissemination is not commonly a late event in the progression of breast, prostate and oesophageal cancers. This begs the question: when does dissemination commence? In mouse mammary tumour virus (MMTV)–*Erbb2* and MMTV–PyMT (polyoma middle T antigen) transgenic mouse models with spontaneously arising mammary cancers, DTCs were demonstrated in bone marrow and lung tissue before the primary tumour became morphologically invasive[54]. Transmission electron microscopy revealed that individual cells break through the basement membrane during pre-invasive atypical hyperplasia. Subsequent detection of cytokeratin-positive cells in the bone marrow of patients with ductal carcinoma *in situ*, a non-invasive lesion, established the relevance of the mouse model for human cancer[54] and indicates that tumour cells disseminate early in the morphological development of breast cancer (although ductal carcinoma *in situ* patients do, rarely, develop metastasis after surgery).

Although disparities between primary tumours and manifest metastasis are an expected consequence of the early dissemination of still evolving tumour cells and site-specific selection pressures, how can the similarities between primary tumours and metastases be explained? Divergence depends not only on factors outlined above, but also on which mutations DTCs carry from the primary site and the degree to which these changes restrict the mutational ranges of metastatic founder cells. However, the range of mutations in human cancers that lead to 'fitter' cells is apparently limited, and selection therefore results in the independent acquisition of identical solutions. For example, *BRAF* mutations occur at codon 600, *KRAS* at codons 12, 13 or 61 (see Catalogue of somatic mutations in cancer in Further information) and 80% of PI3K mutations are also found in three hotspots[55]. Likewise, chromosomal abnormalities do not seem to be random. Analysis of about 6,000 CGH profiles from 22 cancer types revealed a

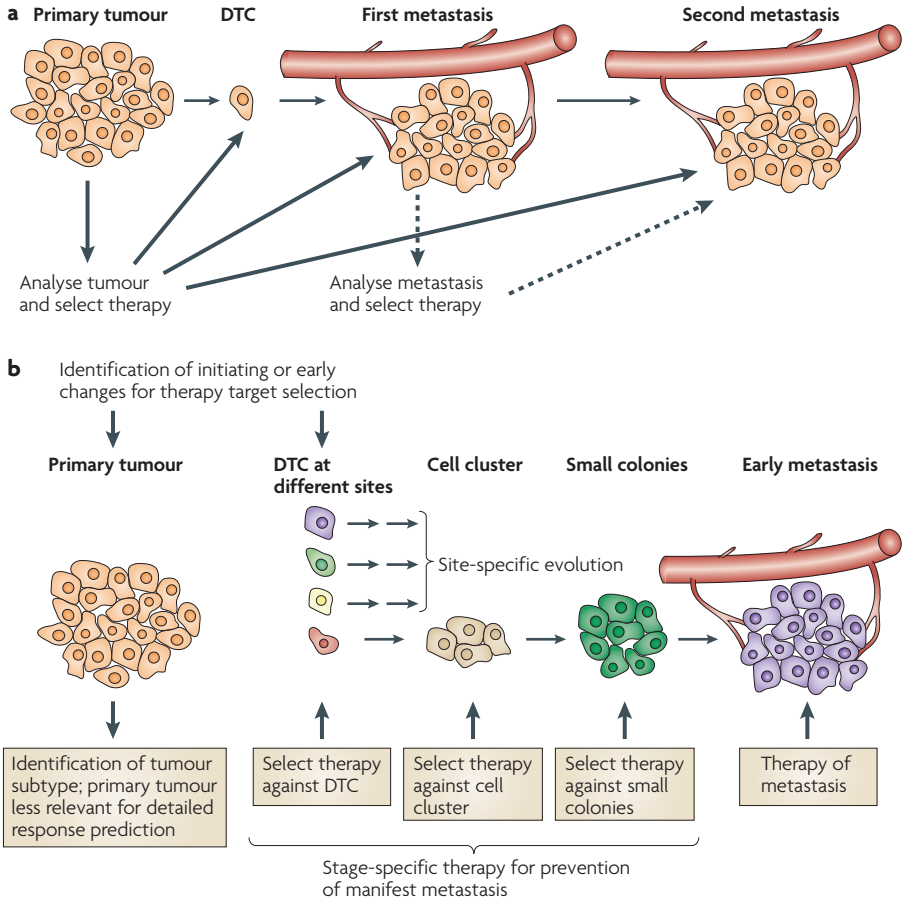

Figure 4 | **Consequences of the two models for therapy research and clinical decisions. a** | The late dissemination and metastatic cascade model predicts that disseminating tumour cells (DTCs) resemble the most aggressive clone and therefore the tumour at time of diagnosis. Primary tumours are therefore good surrogate markers to select therapies. As the cells adapt by epigenetic modes, no further progression is needed and genetic targets (mutations and gene amplification leading to oncogene addiction) are identical to those in the primary tumour. Catch-all therapies can be selected on the basis of analysis of the primary tumour. **b** | Parallel progression complicates therapy selection. Although tumour cells at primary and distant sites might converge on similar genomes (chromosomal aberrations and point mutations) this cannot be confirmed without direct analysis of systemic disease. Moreover, genomes may vary between different sites and undergo independent progression. Several questions remain to be assessed: whether specific genetic changes are selected at different sites; whether different targeting strategies need to be applied for DTCs, small clusters and small colonies; and whether early genetic changes shared among all cells are key to eradicating systemic cancer.

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Table 2 | **Clinical findings and research questions in the context of the linear and parallel progression models (part 1)**

	Linear progression model		Parallel progression model	
	Prediction	**Observation**	**Prediction**	**Observation**
Clinical finding				
Association of tumour size and metastasis	• The larger the PT the more tumour cells disseminate	• No increase of dissemination with tumour size[54]; no increase of CTC with metastatic load[74]	Dissemination is early (diameter 1–4 cm); lead time effect accounts for different latency periods of T1 and T3 tumours	Consistent with modelling of large patient numbers[22,23]; consistent with tumour growth rates[14]; DTC detection in patients with small tumours and DCIS[54]
	• Genetically advanced PT will seed genetically advanced DTCs; DTCs similar to most advanced clone	• DTCs display fewer aberrations than PT; divergence from PT[37–39]		
	• Growth rate much higher in metastases to account for shorter metastatic latency than primary tumour growth	• Growth rates in metastases only slightly higher in matched pair analysis[14]		
Death of patient	Final shower of metastases by highly malignant cells resulting from more and more aggressive cells	Not frequent	Patient dies when critical tumour mass is reached (10^{12}–10^{13} cells) as sum from independent metastases	At autopsy, on average three organs involved with six metastases[11,59,60]
Metastatic inefficiency	CTCs in blood of patients with or without metastases; patients are fully malignant and form metastases once 'educated' by the environment[75]	Millions of cells from advanced tumours shed into the blood stream do not grow into metastasis[92,93]	CTC and DTC at diagnosis reflect ongoing selection; metastatic success of individual cell currently unpredictable	Prognostic impact of DTC or CTC[30]; however, this is no proof for metastatic outgrowth
Association of early surgery and cure	Early surgery prevents dissemination of metastasis founder cells	Not proven	Early surgery prevents outgrowth of early DTCs	Not proven
Dormancy clinically defined as latency period longer than 6 years[94]	• Time needed for fully malignant cells to adapt to the new environment	• No patient data available	• Dormancy is a significant extension of latency over the lead-time effect; strict clinical definition therefore not useful	• No data available on growth rates of DTCs; proliferation indices (15.9% of DTC positive for Ki-67 or p120) are similar to those of PTs[95]
	• Failure to acquire much higher growth rates than PT	• As growth rates of metastasis are not higher than that of PT, clinical dormancy should be more frequent	• Defined as above, dormancy is rare; latency period is explained by somatic progression of early DTCs	• Somatic progression from nearly normal genomes to characteristic CGH aberrations observed in M0 versus M1 stage DTCs[39]

CGH, comparative genomic hybridization; CTC, circulating tumour cell in blood; DCIS, ductal carcinoma *in situ*; DTC, disseminated tumour cell in bone marrow or lymph node; EMT, epithelial–mesenchymal transition; PT, primary tumour; TERT, telomerase reverse transcriptase.

limited set of changes (+1q, +3q, +7, +8q, −13q, −17p, −18q and +20q) of which different subsets occurred in most entities[56]. It is remarkable that this holds for diverse tumours and patients (which, of course, are not clonally related). Non-random mutations and non-random patterns of alterations increase the probability of a given genetic lesion independently arising in different cells within the same tumour and at distant sites.

Therefore, the presence of restricted genetic changes does not provide conclusive insight into the clonality or descent of primary tumours and metastasis, whereas differences indicate the selection of different founder cells. Many factors that affect tumour propagation, genetic and epigenetic background (cellular origin), and microenvironment are shared by primary and secondary sites, so the genetic similarities between primary tumours and metastases may reflect convergent evolution.

Metastasis from metastasis

Both models of tumour progression consider the emergence of multiple metastases. In parallel progression, metastatic founder cells are selected in parallel at various sites and grow independently. Linear progression predicts metastasis from metastasis, in part predicated on the assumption that a metastasis is at least as able to spawn metastases as is the primary tumour, as it has been selected to do so. This view emerged from autopsy studies and selection of highly metastatic cell lines *in vivo*[57].

During the 1970s and 1980s, studies on thousands of cadavers addressed the extent of metastatic spread in a broad range of human cancer types[11,58–60] and addressed whether metastases affecting multiple organs originate directly from the primary tumour (direct seeding) or from the first metastasis (the cascade model). These studies show, first, that cancer patients with metastatic disease have metastases in, on average, 2–3 organs[59,60]. Second, the total number of metastases was 1–13 (5.6 on average) per patient[11]. Third, each cancer had a characteristic predilection for sites of distant metastasis, with a few — including lung, liver and bone — being most frequently targeted. Some sites, such as central nervous and endocrine systems, were more frequently affected in cancers with lung or liver involvement than those without. Direct sourcing from the primary tumour could not, it was concluded, explain these patterns[11], and thus the results favoured the cascade model. The authors predicted that targeting the first metastasis might prevent additional metastasis[60] (FIG. 4a).

The linear progression model. The autopsy studies were taken to support linear progression from primary tumours to metastatic cascades. However, growth rates

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Table 2 | **Clinical findings and research questions in the context of the linear and parallel progression models (part 2)**

	Linear progression model		Parallel progression model	
	Prediction	**Observation**	**Prediction**	**Observation**
Research question				
Whole genome sequencing of PT for personalized therapy	• Most if not all mutations and genetic changes will also be present in metastasis	• Divergence for many mutations and genetic aberrations already established[41,47,49]	• Site-specific accumulation of alterations; convergence on same mutations possible but currently unpredictable	• Divergence for many mutations and genetic aberrations already established[41,47,49]
	• Reliable prediction of response to therapy	• To be tested	• Diagnostic pathology of systemic cancer needed for adequate prediction of response	• To be proved
Usefulness of cell lines from metastases and advanced tumours	The most metastatic cell lines are the most adequate models	Discrepancy of disease course between patients and models usually not addressed; signatures for site-specific metastasis identified that are predictive if present in PT[96]; epigenetic mechanisms active[76]	Of limited usefulness; selection of nearly normal genomes to manifest metastasis fundamental for understanding	Genomic rearrangements characteristic for early lymph node and bone marrow dissemination identified[38]; selection of characteristic genomic rearrangements of metastatic cells outside PT demonstrated from patient samples[39]
Designer cell lines (genetically modified normal cells)	Optimal set of changes is identified when fully malignant cells are generated	Certain mammary cells transformed by TERT, SV40 early region and HRAS metastasize[97]	Useful if initiating changes were tested and somatic progression were allowed	Not done so far; initiating changes in sporadic cancers mostly unknown
Search for metastasis initiating mechanism	Differential analysis of low and high metastatic cell lines or early and late primary tumours useful	No initiating mutations found so far; EMT programmes activated in invading cells[77]	During early tumorigenesis a transient stage of high dissemination activity might exist	No proportional increase of DTC with total tumour population; in transgenic mice expressing *Erbb2* highest activation of TWIST1 early after transformation[54]

CGH, comparative genomic hybridization; CTC, circulating tumour cell in blood; DCIS, ductal carcinoma *in situ*; DTC, disseminated tumour cell in bone marrow or lymph node; EMT, epithelial–mesenchymal transition; PT, primary tumour; TERT, telomerase reverse transcriptase.

and therapy trials argue against a linear progression from first to secondary metastasis. For example, the median time taken for 50% of breast cancer patients to develop a single metastasis after diagnosis of M0 disease and surgery is shortest for the liver, taking 29 months. However, the median time taken for M0 stage patients to be diagnosed with multiple metastases, including liver (such as liver and lung or liver and brain), after surgery is 28 months[61]. Metastasis-from-metastasis seeding of multiple tumours should take longer to manifest, but this does not seem to be the case[61]. Autopsy findings indicated that the period of growth of lung metastases derived from liver metastasis in colorectal cancer is 31 days[59], which would require a growth rate 130-fold greater than that of the primary tumour (FIG. 1; TABLE 1); nothing approaching this has ever been reported.

Additionally, at least two clinical examples, described below, argue against the notion that targeting putative first metastases prescriptively affects disease outcome. These are dissection for lymph node metastasis, and the risk of developing brain metastasis after treatment of visceral metastasis by trastuzumab — a humanized monoclonal antibody directed against ERBB2, which is frequently expressed in breast cancers.

Regional lymph node dissection in breast cancer is a remnant of Halsted's theory of continuous cancer spread by lymphatic dissemination[62]. Because lymph node status is an important prognostic indicator and lymph node metastases might seed tertiary cancer growth, surgeons still remove 10–12 lymph nodes in patients with clinically tumour-free lymph nodes and a positive sentinel node. At least three randomized trials question lymph nodes as a source of distant metastases[63–65]. In none of these studies did removal of clinically negative nodes affect distant metastasis or survival, although local control was significantly improved in the group of women receiving total mastectomy plus regional radiotherapy compared with total mastectomy alone[63] (indicating that metastases grew in these nodes). Likewise, a recent randomized trial comprising 1,408 women with endometrial cancer also found no evidence of a benefit for systematic lymphadenectomy in terms of overall, disease-specific and recurrence-free survival[66].

Lymphatic metastases do rarely seed distant metastases that compromise survival. However, do distant metastases seed additional distant metastases? Recent results from clinical trials with trastuzumab argue that this is unlikely. Trastuzumab poorly traverses the blood–brain barrier, and its level in serum compared with cerebrospinal fluid of patients differs more than 400-fold[67]. These properties suggest that it cannot directly target brain micrometastases and metastases, making it useful for testing whether brain metastasis is seeded from the primary site or from metastasis outside the central nervous system. If trastuzumab reduced lung and liver metastases, any supplemental reduction of brain metastases would support the metastatic cascade model. However, although trastuzumab attenuates disease progression in patients with M1 disease[68] and seems to cure a substantial proportion of M0 stage patients with ERBB2-positive breast cancer[3,4,69], its effects are restricted to visceral metastases. Indeed, patients treated with trastuzumab have increased, not decreased, numbers of brain metastases[70]. However, as these metastases occur with substantial delay,

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it is argued that this reflects increased longevity providing sufficient time for the metastases to grow[70].

The parallel progression model. Parallel progression diminishes the emphasis on metastatic cascades in favour of direct (and early) seeding from the primary lesion. Favoured metastatic sites may thus reflect the probability and rate of specific tumour cells growing in a given niche. In counterpoint to this, the prognostic value of circulating tumour cells (CTCs) in the blood of patients with metastasis may be considered as support for metastatic cascades. Studies on melanoma[71], breast[72,73] and lung[74] cancers seem to demonstrate that high numbers of CTCs correlate with augmented progression and reduced survival. Yet it is not clear whether this is because the cells directly form metastases. As progression-free survival was only 2.7 months for positive patients[72], the reasons outlined above concerning tumour growth rates indicate that metastases from single CTCs are unlikely. An alternative, non-causal hypothesis is that the presence of CTCs is associated with actively proliferating metastases elsewhere that eventually form the lethal tumour mass.

Perspectives

The sum of available data, mostly from disease courses and the genetics of DTCs, favours the parallel progression model in the opinion of this author (for an overview, see TABLE 2). If correct, the model implies an obligation to reconsider current research models and clinical approaches that assume primary tumours are surrogate markers.

One facet of this reconsideration concerns mechanisms of adaptation to the new environment. Linear progression emphasizes education or induction by the microenvironment, with the adaptation of fully malignant cells as the prime driver of metastatic outgrowth[12,75] leading to the final shower of metastasis. This is exemplified by *in vivo* selection of a human metastatic breast cancer cell line, forming bone metastases that correlated not with genotype (as evidenced by CGH analysis) but, rather, with stably heritable differences in gene expression[76] between parental and bone-seeding cell lines. By contrast, one interpretation holds that selection at the genetic level is prominent in parallel progression, where latency periods may reflect the time needed for mutation, selection and inheritance. Years before metastasis, DTCs from the same individual often display bewildering genetic heterogeneity[40]. Subsequent clonal expansion, evidenced by apparently overlapping genotypes of single DTCs isolated from M0 stage patients, is consistent with the acquisition of advantageous genetic changes and more aggressive growth[38]. The next step, apparently, is accumulation of copy number changes characteristic for the type of tumour[56], which is associated with the diagnosis of clinical metastasis and acquisition of a fully malignant phenotype[37,39]. In prostate cancer, such characteristic aberrations have been detected in primary tumours and M1 stage DTCs but not in M0 stage DTCs. However, of importance here, matched primary tumours and M1 stage DTCs shared only 25% of all changes[39], indicating that the characteristic changes were not linearly transmitted from the primary tumour to DTCs or to metastases. Rather, this suggests that the genetic convergence of metastases and primary tumours occurs independently. So, although both mechanisms of adaptation (educative and genetically predisposed) plausibly play a part in cancer progression, the use of cell lines derived from advanced tumours may pervert the adaptive selection of genotypes and blur crucial mechanisms that are active in patients during the early colonization of a distant site.

Parallel progression also argues for a shift of research focus to the identification of metastasis founder cells. Cancer stem cells (CSCs) or tumour-propagating cells have usually been isolated from primary tumours and these populations are thought to consist of metastasis founder cells[77,78]. The greater tumorigenic potential of CSCs over non-CSCs was established by the ability of CSCs to form tumours at low cell numbers within weeks in immunodeficient NOD–SCID (non-obese diabetic–severe combined immunodeficient) mice[76]. However, this was recently questioned when it was shown that every melanoma cell could form tumours under different experimental conditions in some immunocompromised (NOD–SCID *Il2rg*$^{-/-}$) mice[78,79]. This highlights one artefactual drawback of xenotransplant models, as it is clear that neither every tumour cell[79] nor cells expressing given stem cell markers[78] proliferate in patients at rates similar to those observed in mouse models.

These experiments in immunodeficient mice call for greater appreciation of the homeostatic contribution of the human immune system[79] and other key homeostatic factors that might be circumvented in different species. In as much as functional *in vivo* assays are questionable, there is currently no animal model in which to directly identify human metastasis founder cells and it is not possible at present to forecast which DTC will form a metastasis. No evident common genetic theme identifies a metastasis founder cell, with the possible exception of *ERBB2* gain in some oesophageal cancers[38]. This paucity of indicators may improve as higher-resolution analytical techniques are applied to DTCs[80,81]. Likewise, CSC markers[78], which are possibly expressed by DTCs are currently not linked to metastatic progression. Indeed, little is known in general about the phenotype of DTCs[82,83] and few functional assays have been developed[84,85]. Currently, population studies linking patient outcome with the genotype, epigenotype or phenotype of DTCs are the most likely to reveal the identity and molecular characteristics of metastatic founder cells.

Linear progression is a relatively straightforward model for therapy design: stop dissemination before tumours grow large and kill the most aggressive cells. This reasoning is poorly supported by therapeutic progress in recent decades. By contrast, the parallel progression model predicts DTC clonal diversity, which cannot be addressed by catch-all therapies (FIG. 4). Serious consideration of parallel progression should affect clinical therapeutic strategies in several ways. First, the identification of systemic homeostatic factors is likely to reveal novel therapeutic targets. Second, it calls for a direct diagnostic pathology of early systemic cancer. Although prognosis might be reliably determined by primary tumour profiling, predicting responses to therapies will require the molecular characterization of DTCs, which may be significantly different from the primary tumour. Third, although linear progression focuses on alterations that generate a fully malignant cell, parallel progression predicts that it might be more prudent to search for initiating and early predisposing alterations, because these changes have a higher likelihood of being shared among the tumour cells and might influence the mutational and epigenetic spectra of subsequent changes. If so, tumour cells at multiple sites would be affected by these early changes. Last, but not least, the parallel progression model opens the door for a concept unthinkable in linear progression: that cancer diagnosis is possible in bone

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marrow (or elsewhere) by detection and characterization of DTCs. Detection of a lesion comprising 10^6–10^8 cells (<1–4 mm) is intractable by imaging but, with future molecular tools, DTCs promise to become detectable at a correspondingly early stage of disease.

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DATABASES

Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
BRAF | *ERBB2* | *KRAS* | *TP53*
National Cancer Institute Drug Dictionary: http://www.cancer.gov/drugdictionary/trastuzumab
UniProtKB: http://www.uniprot.org
cytokeratin 8 | cytokeratin 18 | cytokeratin 19 | EPCAM

FURTHER INFORMATION

Christoph A. Klein's homepage: http://www.oncogenomics.de/
Catalogue of somatic mutations in cancer: http://www.sanger.ac.uk/genetics/CGP/cosmic/
Tumour-specific analysis: http://www.tumorregister-muenchen.de/facts/specific_analysis.php
Tumour-specific analysis (breast cancer): http://www.tumorregister-muenchen.de/facts/surv/surv_C50f_G.pdf

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