Engineering the pre-metastatic niche

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The pre-metastatic niche — the accumulation of aberrant immune cells and extracellular-matrix proteins in target organs — primes the initially healthy organ microenvironment and renders it amenable for subsequent colonization by metastatic cancer cells. By attracting metastatic cells, mimics of the pre-metastatic niche offer both diagnostic and therapeutic potential. However, deconstructing the complexity of the niche by identifying the interactions between cell populations as well as the mediatory roles of the immune system, soluble factors, extracellular-matrix proteins and stromal cells has proved challenging. Experimental models are needed to recapitulate niche-population biology in situ and to mediate in vivo tumour-cell homing, colonization and proliferation. In this Review, we outline the biology of the pre-metastatic niche and discuss advances in the engineering of niche-mimicking biomaterials that regulate the behaviour of tumour cells at an implant site. Such 'oncomaterials' offer strategies for the early detection of metastatic events, the inhibition of the formation of the pre-metastatic niche and the attenuation of metastatic progression.

The hypothesis that tumour cells exhibit preferences when metastasizing to organs dates back to 1889, when Steven Paget posited in his 'seed and soil' hypothesis that the spread of tumour cells is not random but governed by regulated processes, and that it is pre-determined. For example, breast cancer metastases tend to form primarily in lung, liver, brain, bone and lymph-node tissues, which indeed indicates a tropism for specific microenvironments. This 'primed' microenvironment, also known as the pre-metastatic niche (Fig. 1), is involved in promoting tumour-cell homing and colonization of the target organ. Once metastases form at niche sites, the clinical conversation typically changes from curative treatments to the prolongation of survival. Complications from metastasis are ultimately responsible for 90% of cancer-associated deaths.

The pre-metastatic niche consists of a complex microenvironment that includes inflammatory immune cells, stromal cells, extracellular matrix (ECM) proteins, tumour-secreted exosomes and homing factors. Tumour-secreted factors and tumour-derived exosomes (Fig. 1a) mobilize and recruit bone-marrow-derived cells (BMDCs) to niches in secondary organs (Fig. 1b), where they interact with the local stroma to create permissive and attractive sites for metastatic cells (Fig. 1c,d). In fact, the arrival of the vascular endothelial growth factor receptor 1 (VEGFR1)-positive BMDCs to the pre-metastatic site precedes and predicts the arrival of tumour cells. Tumour-secreted factors also increase the proliferation of fibroblast-like stromal cells, which contribute to the local deposition of fibronectin. VEGFR1+ niche cells express integrin a4β1, which binds to fibronectin and allows the stromal cells to assemble at the site. Other BMDC populations that have also been implicated in the formation of the pre-metastatic niche include CD11b+ myeloid cells, myeloid-derived suppressor cells (MDCs), neutrophils, tumour-associated macrophages and regulatory T cells. Tumour-secreted factors and exosomes can also directly modify the host stroma to establish a supportive microenvironment. In addition, fibroblasts, endothelial cells and lung epithelial cells have been associated with the establishment of the pre-metastatic niche via secretion of inflammatory cytokines and chemokines. The compelling evidence that pre-metastatic niche formation is required for metastases has prompted biologists and biomedical scientists to elucidate the individual and combinatorial cues that affect cell-niche behaviour, with the ultimate aim of developing effective therapeutic interventions.

Because of the complex molecular pathways that promote metastasis, and their overlap with primary tumour progression, the study of the relative contributions of each pathway in vivo has been challenging. Strategies based on engineered biomaterials have enabled the deconstruction of these complex environments and the study of distinct processes, such as primary tumour formation, invasion and extravasation, as well as metastatic cell homing, colonization and proliferation. Studying these processes by using engineered ectopic sites in vivo can therefore provide key information that can ideally complement insights obtained by genetic modification of the tumour or the host (Table 1). Moreover, the design of artificial biomaterials that mimic the pre-metastatic niche opens up translational opportunities, such as the diversion of metastatic cells away from target organs and the development of early-detection strategies that had been unattainable with conventional approaches.

Here, we review strategies for the design and implementation of engineered biomaterials as pre-metastatic niche mimics. We discuss the choice of synthetic or natural materials, the fabrication method, the inclusion of bioactive cues, and material properties such as degradability and porosity, and examine how biomaterials have been used to probe tumour-cell recruitment to an engineered niche and tumour-cell behaviour on arrival to the niche. We also describe how engineered niches may be leveraged in new detection and therapeutic strategies.

### Recruitment to an engineered niche

Cancer cells migrate from a primary tumour to a secondary target organ via a progressive cascade of events, including micro-environmental remodelling processes at each stage of disease.
progression. Following the degradation of the tumour basement membrane, cells invade and gain access to the vasculature to become circulating tumour cells (CTCs). CTCs respond to chemokine gradients and home in to niche microenvironments at a target organ by escaping the vasculature via extravasation, at which point they are classified as disseminated tumour cells (DTCs). DTCs may be capable of adhering and colonizing a site, provided that they have access to a permissible niche.

Biomaterials that mimic properties of the pre-metastatic niche (Table 2) can be created by using tissue-engineering approaches. They are typically made of synthetic degradable materials (such as poly(lactic-co-glycolic acid); PLG), synthetic non-degradable materials (such as polyacrylamide) or natural materials (for example, silk). Any of these materials can be formed into a porous scaffold that supports the retention of loaded factors or cells, integrates within a host tissue on implantation, facilitates the formation of a defined microenvironment in vivo and provides an ectopic site for the recruitment of metastatic tumour cells. The choice of material depends on the desired application and feature of the pre-metastatic niche to mimic.

| Strategy                           | Advantages                                                                 | Disadvantages                                                                 |
|------------------------------------|---------------------------------------------------------------------------|------------------------------------------------------------------------------|
| Biomaterial pre-metastatic niche mimic | Limited off-target effects  
Biological materials can be manipulated for different applications  
Ease of evaluating multiple niche cues in one device  
Large number of cells can be retrieved from the device | Does not recapitulate all elements of the native pre-metastatic niche  
The foreign-body response may influence the biomaterial environment and differ from a natural pre-metastatic niche |
| High-risk tissue-bed biopsy        | Enables determination of cues leading to organ-specific metastasis  
Captures heterogeneity between metastatic foci | Variability between samples may confound discovery of critical signals  
The identification of pre-metastatic sites is limited |
| Tumour-cell modification           | Direct evidence for molecular drivers of metastasis  
User-defined alterations | Potential for off-target effects on tumour progression  
The generation of a reliable cell or mouse model is challenging |
| Genetically engineered mouse models | Direct evidence for the role of a factor or cell type in metastasis  
Ability to knock-out and knock-in specific genes | Costly and time intensive  
Potential for off-target effects on health of the animal or for tumour progression |

Figure 1: Formation of the pre-metastatic niche. a. The hypoxic tumour sheds exosomes to simultaneously prepare the niche at a target organ by fusing to organ-specific cells (such as fibroblasts) and to stimulate the mobilization of BMDCs. Other tumour-secreted factors (such as lysyl oxidase) crosslink ECM proteins. b. BMDCs accumulate at conditioned sites, adhering to the crosslinked ECM. c. BMDCs and other immune cells (such as MDSCs) secrete factors to induce metastatic-cell homing to niche sites. d. Metastatic cells colonize and proliferate at metastatic niche sites.
is to simulate the bone microenvironment, relatively stiff biomaterials with similar mechanical properties to bone may be advantageous\(^6\). Also, the materials can be combined with biological factors to model the properties of the target organ\(^6\) and to evaluate the contribution of each factor during homing and colonization\(^7,8\).

**Immune-cell trafficking.** Immune cells — such as BMDCs\(^9,10\), MDSCs\(^9,46\), macrophages\(^17\), T cells\(^46,47\) and monocytes\(^20\) — all contribute to niche formation and tumour-cell homing. For instance, hypoxic tumour cells secrete lysyl oxidase, which crosslinks collagen IV in the lung and facilitates the accumulation of CD11b\(^+\) monocytes for niche formation\(^13,20\). Also, purified populations of haematopoietic stem and progenitor cells have been tracked in vivo in an orthotopic E0771 adenocarcinoma breast-tumour model and shown to differentiate readily into immunosuppressive myeloid cells\(^48\). Once immune cells have accumulated at distal organs, they secrete a multitude of factors that facilitate the subsequent recruitment and colonization of DTCs\(^5,12,15\). Real-time interactions between immune cells and DTCs undergoing colonization have been studied by intravital imaging, further elucidating the role of myeloid cell populations in providing a primed response for tumour cells at target organs\(^49\). Despite the importance of the roles of immune cells in the pre-metastatic niche, few studies have investigated the interplay between tumour and immune cells within the niche itself, in part owing to a lack of suitable research tools.

The host response to an implanted biomaterial includes several blood–material interactions, which can lead to the formation of a fibrous capsule consisting of inflammatory immune cells and fibroblasts around the border of the implant\(^44\). Beyond the overall inflammatory response to implanted biomaterials\(^44,54\), recent studies have shown a connection between the immune cells recruited to a biomaterial in the context of cancer and those required to establish a pre-metastatic site (Fig. 2a). For example, in an immunocompetent BALB/c mouse, a variety of inflammatory immune cell populations were recruited to a subcutaneously implanted poly(lactic-co-glycolic acid) (PCL) microporous scaffold (Fig. 2a). During a four-week implantation period, before 4T1 breast-tumour-cell inoculation, Ly6C\(^+\)F4/80\(^+\) inflammatory monocytes and CD11c\(^+\)F4/80\(^+\) dendritic cells accumulated at the implant site. Following tumour inoculation, more inflammatory monocytes as well as Gr1\(^+\)CD11b\(^+\)Ly6C\(^-\) MDSCs accumulated at the scaffold (Fig. 2b), whereas the accumulation of dendritic-cell and F4/80\(^+\)CD11b\(^+\) macrophage populations decreased, thus recapitulating elements of the pre-metastatic niche and enabling tumour-cell recruitment\(^35\) (Fig. 2c).

**Soluble factors.** Chemokines and cytokines that actively influence both immune and metastatic cell behaviour play an important role in niche formation (Supplementary Table 1). For example, secreted factors from stromal cells have been implicated in the recruitment of immune cells associated with the pre-metastatic niche, including the stromal cell-derived factor 1 (SDF-1), the transforming growth factor β (TGF-β), the calcium-binding proteins S100A8 and S100A9, interleukin 1 (IL-1) and caveolin-1. Also, VEGF was found to recruit VEGFR1\(^+\) BMDCs, G-CSF to mobilize MDSCs, V8 to promote angiogenesis and the mobilization of myeloid cells\(^17\). IL-6 is responsible for tumour-promoting inflammation\(^15\), CCL2 to recruit monocytes and BMDCs and to facilitate the extravasation of cancer cells\(^20\) and TNFα to induce S100A8 and S100A9 expression, which in turn attracts the metal-binding activator 1 (Mac1)\(^-\) positive myeloid cells and tumour cells\(^65,66\). In addition, inflammatory Mac1\(^-\) monocytes and lung endothelial cells secrete calcium-binding S100A8 and S100A9 factors in the presence of a primary tumour, which initiates the recruitment of additional

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**Table 2 | Materials and biological modifications for the engineering of the pre-metastatic niche.**

| Source       | Material                              | Structure                | Fabrication method                        | Bioactive modifications             | Refs |
|--------------|---------------------------------------|--------------------------|------------------------------------------|------------------------------------|------|
| Synthetic    | Poly(lactic-co-glycolic acid)         | Scaffold                 | Gas foaming                              | CCL2, MDSCs                         | 36   |
|              | Poly(lactic-co-glycolic acid)         | Layered scaffold         | Microspheres pressed in a gas-foamed scaffold | Haptoglobin                         | 44   |
|              | Poly(ε-caprolactone)                  | Scaffold                 | Gas foaming                              | BMP-2                              | 35   |
|              | Poly(ε-caprolactone)                  | Scaffold                 | Microfabrication                         | Exosomes                            | 34   |
|              | Poly(ε-caprolactone)                  | Scaffold                 | Electrospinning                          | Osteoblasts                         | 42   |
|              | Poly-l-lactic acid                    | Microparticles           | Precipitation                            | EPO, SDF-1                          | 31   |
|              | Hydroxyapatite                       | Nanoparticles in a PLG scaffold | Precipitation, gas foaming             | Serum protein                       | 65   |
|              | Polycrylamide                         | Porous gel               | Microfabrication                         | Collagen I, BMSCs                    | 32,96|
|              | Polylurethane                         | Scaffold                 | Commercially available                   | MSCs                                | 97   |
|              | Polyallylamine/polystyrene            | Microparticles           | Layer-by-layer coating                   | CAFs                                | 104  |
| Natural      | Bone fragments/polystyrene            | Human/mouse sources      | Direct harvest                           | None                                | 108,139|
|              | Silica                                | Scaffold                 | Salt leaching                            | BMP-2                               | 41,98,140|
|              | Lung/interstitial matrix              | Coatings                | Decellularization                        | None                                | 88   |
|              | Osteoblast matrix                     | Mineralized sheets       | Decellularization                        | None                                | 95   |
|              | Collagen                              | Bulk gel                 | Embedded in microfluidic chamber         | Osteo-differentiated MSCs, endothelial cells | 179 |

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monocytes to pre-metastatic sites\(^4\)\(^6\)\(^9\). S100A8 and S100A9 increase the formation and activation of invadopodia via p38 signalling, which may promote tumour-cell adhesion\(^4\). Moreover, immature protein-gamma response (GR1)\(^+\), CD11b\(^+\) MDSCs are responsible for suppressing interferon-\(\gamma\) and increasing inflammatory cytokine expression, and induce the expression of the matrix metallopeptidase MMP9 in cells to allow for matrix remodelling at the niche\(^3\)\(^9\).

Three-dimensional (3D) scaffolds have been used to recruit metastatic melanoma tumour cells in vivo\(^3\)\(^5\) and to characterize the role of soluble factors in mediating metastasis to bone tissue in vitro, where tumour cells actively prepare the site for colonization through the release of cytokines such as IL-8 (refs\(^3\)\(^6\)\(^8\)). Colonizing breast-tumour cells produce osteoclast-activating factors, including IL-6, IL-11 and TNF\(\alpha\), to initiate bone resorption and create space for a metastatic lesion\(^6\). Subcutaneously implanted chemokine-releasing scaffolds have instead been used to compare two factors implicated in melanoma metastasis, SDF-1 and erythropoietin (EPO), with EPO scaffolds having increased tumour-cell recruitment\(^3\)(Fig. 3a).

A related strategy used biomaterials to deliver a virus encoding for a cytokine to modulate immune-cell trafficking\(^9\). PLG scaffolds with an immobilized lentivirus encoding for CCL22 modulated the immune cell composition within the scaffold\(^9\), resulting in an increase in MDSCs at the niche, which in turn enhanced tumour-cell recruitment to the scaffold in a manner similar to what had been described for the natural niche\(^6\)\(^8\). These factors are thought to modulate the chemokines at the local environment; however, altering the local trafficking of immune cells may potentially have an impact systemically. Collectively, these studies indicate that individual secreted factors have distinct cell-recruitment abilities and that direct release from the biomaterial enables the study of immune and metastatic cell trafficking (Fig. 3b).

Silk scaffolds have been developed to study the impact of the bone morphogenetic protein BMP-2 on bone metastasis\(^9\), as BMP-induced transcriptional pathways are activated during breast and prostate cancer invasion as well as during bone metastasis\(^9\)\(^8\)\(^7\). Using a layered scaffold system, BMP-2 release stimulated the adhesion
of PC3 prostate cancer cells to the scaffold and enhanced the expression of osteogenic markers. More recently, the immune cell secretome from a tumour-bearing mouse, thought to contain factors that mediate the attraction of MDA-MB-231 breast cancer cells to engineered niches, was characterized with a combined approach of systems biology and biomaterial techniques. By using mass spectrometry proteomics, 144 proteins were identified as uniquely secreted by the immune cells from diseased mice and were considered candidate mediators of metastatic-cell homing. Through a complementary systems biology approach via the measurement of large-scale transcription-factor activity and subsequent computational network analysis, the list of candidate factors was narrowed to five. Haptoglobin, a secreted glycoprotein highly abundant in patients with inflammatory diseases and in many cancer patients, was identified as a critical mediator of homing. This key discovery then allowed PLG scaffolds to be engineered to specifically release haptoglobin at the site of implantation in orthotopic breast-cancer mouse models. These protein-releasing scaffolds recruited significantly more metastatic tumour cells to the implant, compared with blank scaffolds, indicating a role for haptoglobin in breast-cancer cell homing. Taken together, the elucidation of the ability of secreted factors to recruit tumour cells to engineered niches indicates that the mimetic niches can serve to validate components of the pre-metastatic niche and facilitate the discovery of novel contributors to pre-metastatic niche formation and function.

**Exosomes.** Soluble factors that elicit dramatic changes in immune-cell trafficking and in the target organ ECM have been characterized in exosomes—cell-shed membrane vesicles, typically 30–150 nm in diameter, that carry signalling molecules secreted and internalized by different cell types and that participate in intracellular communication. Exosomes have been delivered locally as a means to promote tumour-cell recruitment. For example, exosomes were shown to prepare organs for tumour-cell colonization and mobilize BMDCs to pre-metastatic niche sites. For pre-metastatic niche formation in the lung, RNA from tumour-shed exosomes activated the toll-like receptor TLR3 in alveolar type II cells, which stimulated neutrophil recruitment to a target site. Recently, it was also shown that exosomes from pancreatic ductal adenocarcinomas promote liver pre-metastatic niche formation and increase metastatic burden, demonstrating a role for exosomes in establishing the niche. In addition, macrophage-like Kupffer cells present at the liver uptake exosomes and subsequently increase TGF-β and fibronectin expression to recruit BMDCs. The ability for exosomes to interact with resident cells to determine the organotropism at target organs has been further demonstrated, with specific integrins shown to enable tissue targeting.

As such, tumour-derived exosomes incorporated in engineered pre-metastatic niches may further elucidate their role during metastatic progression. In fact, exosomes in a 3D biomaterial scaffold can serve as a metastatic trap (M-Trap). The M-Trap device preferentially captured metastatic cells in both peritoneal and orthotopic models of ovarian cancer (Fig. 3c). As a result, mice implanted with M-Trap scaffolds survived significantly longer than those without the implants, with improved overall survival demonstrated on removal of the implant carrying the metastatic disease. As the collective understanding of how exosomes participate in the preparation of the niche expands, biomaterials will serve as a tool for the evaluation of metastatic-cell recruitment to a niche as a function of exosome presence (Fig. 3d).

**Extracellular matrix.** Organ-specific colonization, or organotropism, has been modelled by *in vitro* mimics of the organ ECM. Aberrantly accumulated proteins produced by tumour-subverted stroma (including organ fibroblasts and endothelial cells), such as fibronectin, collagen IV, tenasin and peristin, promote tumour-cell adhesion at organ sites. Tumour-cell lines show a preference for the ECM according to integrin expression, which has led to the hypothesis that integrin binding dictates organotropism, with β₃, α₂ and α₅ integrin-subunit expression determining cellular adhesion to lung-, liver- and brain-ECM mimics. By taking advantage of cell-surface receptors expressed on tumour cells, tissue-inspired biomaterials (mimicking bone, brain, or lung ECM) can recapitulate integrin-mediated phenotypes and provide an *in vitro* fingerprint for cells with

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**Figure 3 | Biomaterials loaded with soluble factors and exosomes mediate tumour-cell homing.** a, Control, EPO-loaded and SDF-1α-loaded scaffolds recruit labelled B16F10 melanoma cells, as quantified by *in vivo* fluorescence imaging (in relative fluorescence units, RFU). *P* < 0.05. b, Proposed mechanism for the recruitment of metastatic cells by biomaterials pre-loaded with soluble factors of interest. c, Exosome-laden scaffolds (M-Trap) capture SKOV3 ovarian cancer cells delivered into the peritoneal cavity. Bioluminescence imaging shows control mice with metastasis to the pancreas and gonadal fat pads one week after inoculation. Blank scaffolds redirected tumour cells to the implant site, although abdominal metastases were still detected. M-Trap scaffolds were able to recruit tumour cells with no visible metastases at one week after inoculation. d, Proposed mechanism for exosomes in mediating the preparation of the pre-metastatic niche at the scaffold. Figure reproduced from: a, ref. **35**, Elsevier; c, ref. **34**, Oxford University Press.
predictable metastatic targets. It has also been shown that tumour-derived exosomes display distinct integrin patterns that preferentially bind to organ-specific cells, which demonstrates that organotropism can be mediated through ‘packets’ of extracellular signals.

Tumour-cell adhesion has also been tested through biomaterial scaffolds coated with decellularized matrices. Organ decellularization is a commonplace tissue-engineering method used to retain the active components of the matrix and has been recently used to assess tumour-cell activity on primary tumours, lung and bone-derived matrices. By using this approach in vivo, decellularized lung and liver matrices obtained from tumour-bearing mice and used to coat microporous PCL scaffolds enhanced tumour-cell colonization at the scaffold on subcutaneous implantation (Fig. 4a,b). Interestingly, proteomics was used as a technique to evaluate the matrix composition and identify the unique components of organ-specific peritoneal niches. In this example, myeloperoxidase, an enzyme that generates reactive oxygen species, was determined and validated as a factor that mediates tumour-cell colonization through an engineered myeloperoxidase-coated PCL scaffold.

Another study investigated metastatic breast-cancer-cell colonization in scaffolds seeded with primary human osteoblasts to mimic a mineralized bone ECM (the myofibrillar network produced by the seeded osteoblasts was found to be comparable to the assembly of trabecular bone tissue). The detachment force of various breast cancer cells as a measure of tumour-cell adhesion to the engineered sites can be measured by atomic force microscopy. Tumour cells seeded on the bone mimic revealed gene-expression changes in osteopontin consistent with what has been observed in tumour cells colonizing bone tissue in vivo. Taken together, these findings demonstrate that biomaterials and tissue-engineering approaches can lead to the recapitulation of elements of the in vivo pre-metastatic niche.

**Manipulation of cell populations.** A variety of cell types have been implicated in pre-metastatic niche formation. These cells can be manipulated with transgenic strategies, antibody depletion, adoptive transfer and other such techniques, but these can affect a cell population systemically. Alternatively, biomaterial scaffolds can be used as in vivo implants to recreate defined conditions and facilitate tumour-cell recruitment. For example, bone-marrow niches have been recreated by the transplantation of human bone marrow stromal cells (BMSCs) on silk, BMSCs on polyacrylamide (Fig. 4c,d) and mesenchymal stem cells (MSCs) on polyurethane. These cells were initially cultured on the engineered scaffold (Table 2) in vitro; on implantation, the niches were able to recruit human breast cancer cells as well as erythroleukaemia cells (Fig. 4e,f), acute myeloid leukemia cells and prostate cancer cells. Cell-laden materials can thus be used to capture tumour cells at an ectopic site in animal models of both haematological and metastatic carcinoma origin. Interestingly, it has been suggested that the frequency of capturing tumour cells through scaffolds seeded with BMDCs may correlate with the frequency of CTCs in the blood.

Aside from bone-marrow mimics, tissue-engineered constructs have been used to deliver stromal cells (such as neutrophils, fibroblasts, lymphatic endothelial cells or osteoblasts) at target organs, where they provide a permissive microenvironment for human breast-cancer-cell colonization. Local fibroblasts that participate in the formation of pre-metastatic niches become cancer-supportive through the secretion of growth factors and ECM-remodelling proteins. In a model of ovarian and colorectal peritoneal metastasis, cancer-associated fibroblasts (CAFs) were encapsulated within alginate–gelatin microparticles (MPs; 500–700 μm in diameter), coated with a membrane composed of polyelectrolytes to retain the CAFs and prevent degradation. Once implanted in the intraperitoneal space of nude mice, CAFs and CAF-secreted ECM were found to be key in the formation of peritoneal niches for metastasis. Injection of MP-CAFs into the peritoneal cavity redirected cancer cells to the microparticles and resulted in a biomimetic trap that prolonged animal survival. Similarly, MDSCs harvested from spleens of mice and seeded onto PLG scaffolds before implantation in an orthotopic model of breast cancer that used highly metastatic, brain-tropic MDA-MB-231BR cells were retained on the scaffold after implantation and recruited significantly more tumour cells to the implant site relative to control scaffolds. More recently, protocols to engineer humanized bone tissue have been developed by using electrospun PCL–tricalcium phosphate scaffolds seeded with human osteoblastic cells to mimic clinical bone metastases. By using the bone mimic in humanized mouse models, the study modelled several stages of the human bone metastatic cascade, including spontaneous metastasis from orthotopic prostate tumours, systemic metastasis and local bone colonization.

**Behaviour at engineered niches**

Once a DTC adheres to and grows within a niche in the target organ, the cell is said to have colonized the organ. Colonization has been associated with specific genetic changes, including a mesenchymal-to-epithelial (MET) transition. In contrast to the EMT transition during invasion, MET is the process by which tumour cells return to their epithelial-like state to form a distant tumour mass. MET is typically characterized by gene-expression studies, generally showing a return to E-cadherin expression and downregulation of vimentin. Metastatic colonization is also mediated by the activity of specific transcription factors, including decreased transforming growth factor β/mothers against decapentaplegic homolog 3 (TGFβ/SMAD3) canonical signalling activity, and the loss of the paired related homeobox factor (PRRX1) activity, both potent EMT inducers. As DTCs successfully colonize the target organ, proliferation at the metastatic site may occur on the basis of cues received from the pre-metastatic niche. The cues involved are still largely unknown; however, there is evidence that the perivascular niche, as well as sprouting and stable endothelial networks, regulate dormancy through control of thrombospondin 1 (TSP-1), TGF-β, periostin, tenasin, versican and fibronectin, all factors previously implicated in the pre-metastatic niche. Without proper activation, tumour cells may undergo apoptosis at the target organ, remain dormant at the metastatic site for up to several years, or continue circulating through the body.

The inability of DTCs to grow at a metastatic site, a part of metastatic inefficiency, has been modelled using in vitro coloni-\n\zation experiments where the presence of specific ECM proteins can activate dormant CTCs back into a proliferative state. Using a 3D basement-membrane culture system, solitary tumour cells can remain dormant due to cell-cycle arrest through elevated abundance of cyclin-dependent kinase inhibitor proteins p16 and p27. In addition, the proliferation rates of a variety of breast-cancer cell lines, measured using 2D and 3D basement membrane gels, pointed to signs of dormancy in the 3D culture in vitro. However, the introduction of fibronectin to the 3D culture environment enhanced proliferation rates of dormant cells and increased cytoskeletal rearrangements, consistent with a static-to-dynamic switch in phenotype.

The effect of tissue paracrine signalling on metastatic cells, as determined using 3D co-culture systems, can also be used to refine pre-metastatic niche models. For instance, to recreate MDA-MB-231 tumour-cell extravasation, the bone pre-metastatic niche was recently reproduced in 3D using a microfluidic model consisting of osteo-differentiated MSCs embedded in a collagen gel lined with endothelial cells. Likewise, 3D collagen gels containing human lung adenocarcinoma cells, lung fibroblasts and macrophages were used to track matrix metalloproteinase 1 (MMP-1) and VEGF production in different culture conditions (such as hypoxia). Co-culture systems on a silk scaffold of human breast...
adenoacarcinoma cells with osteoblast-like cells and MSCs have also resulted in enhanced migration, adhesion and drug resistance. When compared with the same cells co-cultured in 2D on standard tissue-culture plastic, the study further reported phenotypic changes in the niche osteoblasts, including decreased proliferation and mineralization, concomitantly to enhanced tumour-cell activity.
Figure 5 | Proposed use of engineered mimics as oncomaterials. a. Engineered pre-metastatic niche mimics may be designed using a variety of parameters. Material properties such as chemistry, porosity, degradability and stiffness may all be modulated to engineer a pre-metastatic niche mimic. Bioactive components such as immune cells, soluble factors, exosomes, ECM and other niche cells may be incorporated into the niche design. Parameters for designing the most effective oncomaterial may be tuned depending on the cancer type or the needs for a specific patient. b. After removal of the primary tumour, a biomaterial scaffold may be implanted subcutaneously, ideally before metastasis occurs. c. Imaging of the oncomaterial may be performed during the patient’s course of treatment to detect colonizing tumour cells. For example, when using ISOCT (right), the shape factor ($D$) may be used to quantify microstructural alterations at the scaffold due to the arrival of metastatic tumour cells; scale bar, 200 μm. The scaffold may also be removed for pathological scoring (left) and/or analysis of disseminating tumour cells arriving to the scaffold; scale bar, 100 μm. Panel c (ISOCT image and histology image) reproduced from ref. 106, Macmillan Publishers Ltd.

A similar study was performed where androgen-sensitive prostate cancer LNCaP cells were embedded in poly(ethylene glycol) (PEG) hydrogels and cultured with PCL scaffolds pre-seeded with human osteoblasts. Following microarray analysis of cells obtained from two engineered scaffolds, the study revealed that paracrine signalling between cancer cells and osteoblasts altered the expression patterns of genes associated with homing and colonization (such as S100A6), compared with monoculture controls.

Opportunities for niche mimics

Implantable niches may serve as ‘oncomaterials’, a term that we propose and define as biomaterials that enable the detection and treatment of cancer metastasis (Fig. 5). Pre-metastatic niche oncomaterials may be designed by manipulating a variety of material parameters, including material chemistry, porosity, degradability and stiffness, as well as biological parameters such as the natural immune response to the implant, soluble-factor delivery, ECM composition and niche-cell delivery (Fig. 5a). Parameters for designing the most effective oncomaterial may be tuned depending on the cancer or the needs for a specific patient. After removal of the primary tumour, a biomaterial scaffold may be implanted subcutaneously, ideally before metastasis occurs (Fig. 5b). In a clinical setting, the probability of a tumour spreading to target organs has been shown to correlate with tumour size; for example, breast-cancer tumours less than 1 cm in diameter have a lower risk of metastasis. Detection strategies to map metastatic spread mostly rely on whole-body imaging modalities such as positron emission tomography, X-ray computed tomography and magnetic resonance imaging; however, the initial cell clusters are beyond the resolution of these imaging systems. These limitations are particularly problematic for highly aggressive cancers that follow a parallel progression model, where tumour-cell dissemination and colonization occurs during the undetectable stages of the primary disease.

Materials for metastatic-cell detection. The early detection of rare CTCs in the blood may enable earlier treatments for metastatic cancer. This has motivated the continued development of nanomaterials for the isolation and characterization of CTCs. To date, genetic screening of tumour biopsies has been the most common approach to identifying biomarkers for the development of personalized therapies. However, the progression of a cancer from a neoplastic or dysplastic lesion to metastasis is increasingly understood as the result of continued evolutionary pressure that dictates mutations of its genetic and molecular landscape. This dynamic behaviour
is the primary factor responsible for the emergence of therapeutic-resistant clones, and challenges the development of personalized therapies. For these reasons, techniques to capture, characterize and culture CTCs are intended to complement the analysis of primary tumour biopsies and to provide a comprehensive disease description for individual patients. For example, CellSearch — a Food and Drug Administration-approved, commercially available CTC-enrichment system — enables the reliable detection of CTCs in blood samples from metastatic cancer patients. Most notably, ex vivo culture of CTCs in conjunction with in vitro biomaterial mimics of the pre-metastatic niche have facilitated the capture, culture and study of CTCs.

CTCs are isolated from blood, whereas cells found within the pre-metastatic niche mimics have left the vasculature and may represent a distinct cell population with distinct prognostic value. Despite advances in ex vivo detection, CTCs may remain in the circulation for years, and those captured in blood samples may therefore not be representative of tumour-cell populations capable of homing and colonization, because the detection of CTCs does not indicate the existence of permissive niches. Recently, biomaterial scaffolds for the early detection of cancer metastasis were reported in an orthotopic mouse model of breast cancer. Microporous PLG scaffolds were implanted, either subcutaneously or in the intraperitoneal fat, and tumour cells populated these scaffolds before their colonization at common organ sites (such as the lung, liver and brain). Interestingly, using inverse spectroscopic optical coherence tomography (IS OCT) and unique microstructural alterations were detected at the scaffold due to tumour-cell arrival, allowing for a non-invasive and label-free detection method of metastatic colonization. This type of scaffold technology, coupled with IS OCT or other imaging techniques, may enable a viable method for early detection during low metastatic tumour burden (Fig. 5c). Regular imaging at check-ups may be performed during the patient’s course of treatment. In a translational setting, these scaffolds alone, or modified with ECM proteins or for cytokine delivery, could provide direct access to actively colonizing tumour cells for patient-specific phenotypic and genomic analyses.

Early intervention for metastatic-cell capture can enhance survival. Implantable scaffolds have been shown to significantly increase survival in mouse models of metastasis. For instance, microporous PCL scaffolds have increased the survival of immune-competent mice inoculated with 4T1 metastatic breast cancer cells. This scaffold provided a site for early detection and acted as a ‘sink’ for metastatic tumour cells (Fig. 2c) and MDSCs (Fig. 2d). As a result, the scaffold reduced the average tumour burden in the liver and brain (Fig. 2e,f). A post-surgical model of breast cancer metastasis was then used to investigate the impact of the scaffold on survival, where the primary tumour was removed following the localization of tumour cells in the scaffolds. Forty percent of scaffold-implanted mice survived the tumour resection procedure past 200 days relative to sham controls, for which survival did not exceed 30 days (Fig. 2g). The study suggested that increased survival of scaffold-implanted mice may result from a decreased burden of MDSCs present in the primary tumour and in the spleen, as well as from decreased brain and liver tumour-cell burden (Fig. 2h). Therefore, the study implicates that biomaterials designed to reduce the overall generation of MDSCs during metastatic disease progression or to divert them to an ectopic location may impact survival. Similarly, exosome-impregnated scaffolds drastically changed the pattern of peritoneal ovarian cancer metastasis by redirecting the vast majority of tumour cells to the implant (Fig. 3c), which resulted in a significant survival benefit for mice that received an implant (mean survival time of ~200 days compared with ~120 days for the control). In addition, removal of the implant after focalization of the disease to the biomaterial further enhanced survival (mean survival time, ~310 days).

Opportunities for metastasis-detection technologies. Although recent evidence suggests that pre-metastatic niche models can enable the early detection and treatment of metastatic disease, open questions remain regarding the efficacy of these models when compared with other emerging metastasis-detection technologies. Additional technologies for the detection of metastases include exosome detection and CTC enumeration (Table 3). Both technologies are part of a larger initiative to utilize liquid biopsies to gain more information about a patient’s disease state, evolving molecular features and response to therapy. The advantages of liquid biopsies for the detection of metastases include the ease of sample collection and the ability to collect multiple samples over the course of a patient’s treatment. Although liquid biopsies have shown promise in these areas, they also have distinct disadvantages that could potentially be circumvented by pre-metastatic niche mimics. For example, exosome detection is likely to be less sensitive than CTC detection due to exosome heterogeneity and their presence in large numbers also in healthy patients. Similarly, the presence of CTCs indicates the risk for metastasis but does not indicate the presence of permissive microenvironments in organs for these cells to home to and colonize. These considerations show specific advantages of the use of pre-metastatic niche mimics (Table 3). However, potential issues associated with the clinical use of these devices, such as the overall safety of creating a site for metastatic cells to home to, will need to be evaluated thoroughly in clinical trials. Future methods may provide complementary implementations of these techniques and provide a more comprehensive evaluation on the metastatic state of a patient.

In a clinical setting, the choice of material (Fig. 5a, Table 2) is critical for the design of a functional implantable device that recruits and detects metastatic cells. For example, materials such as PLG are susceptible to hydrolytic degradation, thus limiting the amount of time that the material can remain in a patient. The scaffold should ideally maintain its structure for several months during a patient’s treatment, given that metastasis may occur on a time-scale from months to years. The degradation of polymer scaffolds is usually desirable for tissue-engineering applications, where host cells eventually replace the material; but degradation is likely to be undesirable for long-term implantable metastatic detectors. Non-degradable or semi-degradable materials could be used to fabricate implantable scaffolds that are less susceptible to hydrolytic degradation. The material should also elicit an appropriate inflammatory response at the implant site to initiate the recruitment of metastatic cells, and should be amenable to harvesting intact populations of

**Table 3 | Risks and opportunities of detection technologies for metastasis.**

| Detection platform | Stage at detection | Safety | Sensitivity | Specificity | Therapeutic benefit | Refs |
|--------------------|-------------------|--------|-------------|-------------|-------------------|------|
| Exosome detection  | Primary           | +      | +           | ++          | -                 | 124  |
| CTC detection      | Circulation       | +      | +           | +           | -                 | 128,141|
| Biomaterial pre-metastatic niche mimic | Dissemination | ?      | +           | ++          | +                 | 32,34-36|

*+, high; ++, very high; −, low; ?, unclear.
tumour cells for downstream analysis. In addition, scaffold porosity increases the interior surface area for blood-vessel and immune-cell infiltration and should also facilitate the access of tumour cells to the scaffold. Material selection is hence paramount for the successful translation of pre-metastatic niche mimics as oncomaterials.

Thus far, no clinical trials have been initiated for the application of biomaterials as pre-metastatic niche mimics. Although several biomaterials utilized as pre-metastatic niche mimics are already approved by the US Food and Drug Administration for use in human patients (Table 2), limitations in imaging tumour-cell arrival at the implant remain. ISOCT is a practical approach for detecting nanostructural alterations occurring as a result of tumour-cell arrival; however, the penetration depth of this optical technique will need to be enhanced for clinical translation. Ultrasound and other imaging technologies already available in the clinic may be implemented for tumour-cell detection at a scaffold. Although safety remains to be assessed, the future for pre-metastatic niche mimics as oncomaterials remains promising.

Outlook
Pre-metastatic niche mimics offer the ability to identify and validate critical factors leading to metastatic-cell colonization at an ectopic site. Roles of inflammatory immune cells, secreted factors, exosomes, ECM proteins and delivered cells have been evaluated using niche mimics to determine contributions to metastatic-cell homing and colonization. Furthermore, the capture of early metastatic cells at a pre-defined site may enable the early detection of metastatic-cell dissemination. The development of novel imaging modalities, or the engineering of probes to label colonizing tumour cells may enable real-time tracking of tumour cells or of vascular leakiness at the niche during the evolution of the disease. Capturing tumour cells at an ectopic site could potentially reduce the burden of disease in solid organs and provide an extended window of time over which a therapeutic intervention may succeed. The use of oncomaterials supplemented with current therapeutic methods such as surgery and chemotherapy may serve as a disruptive strategy for combating metastasis. Extending beyond the concept of capturing tumour cells, scaffolds may be bioengineered to manipulate other types of circulating niche components, including exosomes and immune cells that reflect disease (such as MDSCs). Furthermore, future work in the genetic profiling of captured metastatic cells at implanted niches may lead to the identification of the types of cell arriving at the scaffold (for example, tumour stem cells and cells positive for the epithelial cell adhesion molecule), which may in turn guide the discovery of targets to treat metastases based on the biology of the disease. We believe that the successful integration of pre-metastatic niche components and biomaterials will enable the discovery of biomarkers and other molecular cues of metastasis, and could be developed further as diagnostic and therapeutic technologies.

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References
1. Paget, S. The distribution of secondary growths in cancer of the breast. 1889. Cancer Metastasis Rev. 8, 98–101 (1989).
2. Maru, Y. The lung metastatic niche. J. Mol. Med. 93, 1185–1192 (2015).
3. Azizidoost, S. et al. Hsp70 promotes lung metastasis. J. Mol. Med. 93, 1213–1220 (2015).
4. Kaplan, R. N., Rafii, S. & Lyden, D. Preparing the "soil": the premetastatic niche. Cancer Res. 66, 11089–11093 (2006).
5. Sleeman, J. P. The lymph node pre-metastatic niche. J. Mol. Med. 93, 1173–1184 (2015).
6. Nguyen, D. X., Bos, P. D. & Massague, J. Metastasis: from dissemination to organ-specific colonization. Nat. Rev. Cancer 9, 274–284 (2009).
7. Minn, A. J. et al. Genes that mediate breast cancer metastasis to lung. Nature 436, 518–524 (2005).
8. Peinado, H. et al. Pre-metastatic niches: organ-specific homes for metastases. Nat. Rev. Cancer 17, 302–317 (2017).
9. Bos, P. D. et al. Genes that mediate breast cancer metastasis to the brain. Nature 459, 1005–1009 (2009).
10. Ranklin, E. B. & Giaccia, A. J. Hypoxic control of metastasis. Science 352, 175–180 (2016).
11. 8. Minn, A. J. et al. The lung metastatic niche.
12. Davis, L. J. et al. Pre-metastatic niche mimics offer the ability to identify and validate critical factors leading to metastatic-cell colonization at an ectopic site. Roles of inflammatory immune cells, secreted factors, exosomes, ECM proteins and delivered cells have been evaluated using niche mimics to determine contributions to metastatic-cell homing and colonization. Furthermore, the capture of early metastatic cells at a pre-defined site may enable the early detection of metastatic-cell dissemination. The development of novel imaging modalities, or the engineering of probes to label colonizing tumour cells may enable real-time tracking of tumour cells or of vascular leakiness at the niche during the evolution of the disease. Capturing tumour cells at an ectopic site could potentially reduce the burden of disease in solid organs and provide an extended window of time over which a therapeutic intervention may succeed. The use of oncomaterials supplemented with current therapeutic methods such as surgery and chemotherapy may serve as a disruptive strategy for combating metastasis. Extending beyond the concept of capturing tumour cells, scaffolds may be bioengineered to manipulate other types of circulating niche components, including exosomes and immune cells that reflect disease (such as MDSCs). Furthermore, future work in the genetic profiling of captured metastatic cells at implanted niches may lead to the identification of the types of cell arriving at the scaffold (for example, tumour stem cells and cells positive for the epithelial cell adhesion molecule), which may in turn guide the discovery of targets to treat metastases based on the biology of the disease. We believe that the successful integration of pre-metastatic niche components and biomaterials will enable the discovery of biomarkers and other molecular cues of metastasis, and could be developed further as diagnostic and therapeutic technologies.

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1. Paget, S. The distribution of secondary growths in cancer of the breast. 1889. Cancer Metastasis Rev. 8, 98–101 (1989).
2. Maru, Y. The lung metastatic niche. J. Mol. Med. 93, 1185–1192 (2015).
3. Azizidoost, S. et al. Hsp70 promotes lung metastasis. J. Mol. Med. 93, 1213–1220 (2015).
4. Kaplan, R. N., Rafii, S. & Lyden, D. Preparing the "soil": the premetastatic niche. Cancer Res. 66, 11089–11093 (2006).
5. Sleeman, J. P. The lymph node pre-metastatic niche. J. Mol. Med. 93, 1173–1184 (2015).
6. Nguyen, D. X., Bos, P. D. & Massague, J. Metastasis: from dissemination to organ-specific colonization. Nat. Rev. Cancer 9, 274–284 (2009).
7. Minn, A. J. et al. Genes that mediate breast cancer metastasis to lung. Nature 436, 518–524 (2005).
8. Peinado, H. et al. Pre-metastatic niches: organ-specific homes for metastases. Nat. Rev. Cancer 17, 302–317 (2017).
9. Bos, P. D. et al. Genes that mediate breast cancer metastasis to the brain. Nature 459, 1005–1009 (2009).
10. Ranklin, E. B. & Giaccia, A. J. Hypoxic control of metastasis. Science 352, 175–180 (2016).
11. 8. Minn, A. J. et al. The lung metastatic niche.
12. Davis, L. J. et al. Pre-metastatic niche mimics offer the ability to identify and validate critical factors leading to metastatic-cell colonization at an ectopic site. Roles of inflammatory immune cells, secreted factors, exosomes, ECM proteins and delivered cells have been evaluated using niche mimics to determine contributions to metastatic-cell homing and colonization. Furthermore, the capture of early metastatic cells at a pre-defined site may enable the early detection of metastatic-cell dissemination. The development of novel imaging modalities, or the engineering of probes to label colonizing tumour cells may enable real-time tracking of tumour cells or of vascular leakiness at the niche during the evolution of the disease. Capturing tumour cells at an ectopic site could potentially reduce the burden of disease in solid organs and provide an extended window of time over which a therapeutic intervention may succeed. The use of oncomaterials supplemented with current therapeutic methods such as surgery and chemotherapy may serve as a disruptive strategy for combating metastasis. Extending beyond the concept of capturing tumour cells, scaffolds may be bioengineered to manipulate other types of circulating niche components, including exosomes and immune cells that reflect disease (such as MDSCs). Furthermore, future work in the genetic profiling of captured metastatic cells at implanted niches may lead to the identification of the types of cell arriving at the scaffold (for example, tumour stem cells and cells positive for the epithelial cell adhesion molecule), which may in turn guide the discovery of targets to treat metastases based on the biology of the disease. We believe that the successful integration of pre-metastatic niche components and biomaterials will enable the discovery of biomarkers and other molecular cues of metastasis, and could be developed further as diagnostic and therapeutic technologies.
72. Andersen, C. B. et al. Structure of the haptoglobin–haemoglobin complex. Nature 489, 456–459 (2012).
73. Gupta, T. et al. Serum fucosylated haptoglobin as a novel prognostic biomarker predicting high-Gleason prostate cancer. Prostate 74, 1052–1058 (2014).
74. Pompach, P. et al. Site-specific glycoforms of haptoglobin in liver cirrhosis and hepatocellular carcinoma. Mol. Cell. Proteomics 12, 1281–1293 (2013).
75. Sun, L. et al. Combination of haptoglobin and osteopontin could predict colorectal cancer hepatic metastasis. Ann. Surg. Oncol. 19, 2411–2419 (2012).
76. Vakeda, Y. et al. Fucosylated haptoglobin is a novel type of cancer biomarker linked to the prognosis after an operation in colorectal cancer. Cancer 118, 3036–3043 (2012).
77. Azmi, A. S., Bao, B. & Sarkar, F. H. Exosomes in cancer development, metastasis, and drug resistance: a comprehensive review. Cancer Metastasis Rev. 32, 623–643 (2013).
78. Thakur, K. et al. Double-stranded DNA in exosomes: a novel biomarker in cancer detection. Cell Res. 24, 766–769 (2014).
79. Abels, E. R. & Breakefield, X. O. Introduction to extracellular vesicles: biogenesis, RNA cargo selection, content, release, and uptake. Cell. Mol. Neurobiol. 36, 301–312 (2016).
80. Wendler, F. et al. Extracellular vesicles swarm the cancer microenvironment: from tumor-stroma communication to drug intervention. Oncogene 36, 877–884 (2017).
81. Valadi, H. et al. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat. Cell Biol. 9, 654–659 (2007).
82. Becker, A. et al. Extracellular vesicles in cancer: cell-to-cell mediators of metastasis. Cancer Cell 30, 836–848 (2016).
83. Brinton, L. T., Slosane, H. S., Kester, M. & Kelly, K. A. Formation and role of exosomes in cancer. Cancer Sci. 102, 659–671 (2011).
84. Barkan, D., Green, J. E. & Chambers, A. F. Extracellular matrix: a gatekeeper in the transition from dormancy to metastatic growth. Eur. J. Cancer 46, 1181–1188 (2010).
85. Oskarsson, T. Extracellular matrix components in breast cancer progression and metastasis. Breast 22 (Suppl. 2), S66–S72 (2013).
86. Desgrosellier, J. S. & Cheresh, D. A. Integrins in cancer: biological implications and therapeutic opportunities. Nat. Rev. Cancer 10, 9–22 (2010).
87. Barney, L. E. et al. A cell-ECM screening method to predict breast cancer metastasis. Integr. Biol. 7, 198–212 (2015).
88. Aguado, B. A. et al. Extracellular matrix mediators of metastatic cell colonization characterized using scaffold mimics of the pre-metastatic niche. Acta Biomater. 33, 13–24 (2016).
89. Crapo, P. M., Gilbert, T. W. & Badyak, S. E. An overview of tissue and whole organ decellularization processes. Biomaterials 32, 3233–3243 (2011).
90. Lu, W. D. et al. Development of an allogeneic tumor extracellular matrix organ as a three-dimensional scaffold for tumor engineering. PLoS ONE 9, e103672 (2014).
91. Mishra, D. K. et al. Human lung cancer cells grown on acellular rat lung matrix create perfusable tumor nodules. Ann. Thorac. Surg. 93, 1075–1081 (2012).
92. Villasante, A., Marturano-Kruik, A. & Vunjak-Novakovic, G. Bioengineered human tumor within a bone niche. Biomaterials 35, 5785–5794 (2014).
93. Kubala, L. et al. The potentiation of myeloperoxidase activity by the glycosaminoglycan-dependent binding of myeloperoxidase to proteins of the extracellular matrix. Biochim. Biophys. Acta 1830, 4524–4536 (2013).
94. van der Veen, B. S., de Winther, M. P. & Heeringa, P. Myeloperoxidase: molecular mechanisms of action and their relevance to human health and disease. Antioxid. Redox Signal. 11, 2899–2937 (2009).
95. Taubenberger, A. V., Quent, V. M., Thibaudeau, L., Clements, J. A. & Hutmacher, D. W. Delineating breast cancer cell interactions with engineered bone microenvironments. J. Bone Miner. Res. 28, 1399–1411 (2013).
96. Lee, J. et al. Implantable microenvironments to attract hematopoietic stem/cancer cells. Proc. Natl. Acad. Sci. USA 109, 19636–19642 (2012).
97. Vaiselrhuh, S. B., Edelman, M., Lipton, J. M. & Liu, J. M. Ectopic human mesenchymal stem cell-coated scaffolds in NOD/SCID mice: an in vivo model of the leukemia niche. Tissue Eng. Part C Methods 16, 1523–1531 (2010).
98. Seib, P. F., Berry, J. E., Shiozawa, Y., Taichman, R. S. & Kaplan, D. L. Tissue engineering a surrogate niche for metastatic cancer cells. Biomaterials 31, 313–319 (2010).
99. Holzapfel, B. M. et al. Species-specific homing mechanisms of human prostate cancer metastasis in tissue engineered bone. Biomaterials 35, 4108–4115 (2014).
100. Lee, E. et al. Breast cancer cells condition lymphatic endothelial cells within pre-metastatic niches to promote metastasis. Nat. Commun. 5, 4715 (2014).
101. Wulek, S. K. & Malanchi, I. Neutrophils support lung colonization of metastasis-initiating breast cancer cells. Nature 538, 413–417 (2015).
102. Kalluri, R. & Zeisberg, M. Fibroblasts in cancer. Nat. Rev. Cancer 6, 392–401 (2006).
103. De Boeck, A. et al. Differential secretome analysis of cancer-associated fibroblasts and bone marrow-derived precursors to identify microenvironmental regulators of colon cancer progression. *Proteomics* **13**, 379–388 (2013).

104. De Vlieghere, E. et al. Tumor-environment biomimetics delay peritoneal metastasis formation by deceiving and redirecting disseminated cancer cells. *Biomaterials* **54**, 148–157 (2015).

105. Yoneda, T., Williams, P. J., Hiraga, T., Niewolna, M. & Nishimura, R. A bone-microchips for end-to-end in vitro tumor cell attachment and xenograft formation. *Technology* **3**, 179–188 (2015).

106. Weiss, L. Metastatic inefficiency: intravascular and intraperitoneal transitions in carcinoma progression. *J. Cell. Physiol.* **213**, 374–383 (2007).

107. Hugo, H. Engineered 3D silk-based metastasis models: a microfluidic 3D culture of circulating breast tumor cells for individualized testing of drug susceptibility. *Science* **345**, 216–220 (2014).

108. Martine, L. C. et al. Engineering a humanized bone organ model in mice to study bone metastases. *Nat. Protoc.* **12**, 659–663 (2017).

109. Ocana, O. H. et al. Metastatic colonization requires the repression of the epithelial-mesenchymal transition inducer Prx1. *Cancer Cell* **22**, 709–724 (2012).

110. Ghajar, C. M. et al. The perivascular niche regulates breast tumour dormancy. *Nat. Cell Biol.* **15**, 807–813 (2013).

111. Holmgren, L., O'Reilly, M. S. & Folkman, J. Dormancy of micrometastases: balanced proliferation and apoptosis in the presence of angiogenesis suppression. *Nat. Med.* **1**, 149–153 (1995).

112. Meng, S. Metastatic inefficiency. *Cancer Cell* **14**, 159–211 (2008).

113. Weiss, L. Metastatic inefficiency: intravascular and intraperitoneal implantation of cancer cells. *Cancer Treat. Rev.* **32**, 1–11 (1996).

114. Giancotti, F. G. Mechanisms governing metastatic dormancy and reactivation. *Cell** **155**, 750–764 (2013).

115. Luzzi, K. J. et al. Multistep nature of metastatic inefficiency. *Am. J. Pathol.* **153**, 865–873 (1998).

116. Weiss, L. Metastatic inefficiency. *Adv. Cancer Res.* **54**, 159–211 (1990).

117. Weiss, L. Metastatic inefficiency: intracellular and interstitial cell interactions in the bone-metastasis. *Clin. Cancer Res.* **14**, 603–611 (2008).

118. Arrigoni, C., Bersini, S., Gilardi, M. & Moretti, M. Multistep nature of metastatic inefficiency. *Nat. Protoc.* **12**, 149–153 (2017).

119. Tauro, B. J. et al. Two distinct populations of exosomes are released from LIM1863 colon carcinoma cell-derived organoids. *Mol. Cell. Proteomics* **12**, 587–598 (2013).

120. Nemeth, J. A. et al. Severe combined immunodeficient-hu model of human prostate cancer metastasis to human bone. *Cancer Res.* **59**, 1987–1993 (1999).

121. Tauro, B. J. Bone metastasis in a novel breast cancer mouse model containing human breast and human bone. *Cancer Res.* **32**, 471–486 (2012).

122. Kalluri, R. Imaging a full set of optical scattering properties of biological tissue by inverse spectroscopic optical coherence tomography. *Opt. Lett.* **37**, 4443–4445 (2012).

123. Aguirre-Ghiso, J. A. Imaging a full set of optical scattering properties of biological tissue by inverse spectroscopic optical coherence tomography. *Opt. Lett.* **37**, 4443–4445 (2012).

124. Kalluri, R. The biology and function of exosomes in cancer. *J. Clin. Invest.* **126**, 1208–1215 (2016).

125. Pantel, K., Brakenhoff, R. H. & Brandt, B. Detection, clinical relevance and specific biological properties of disseminating tumour cells. *Nat. Rev. Cancer* **8**, 329–340 (2008).

126. Bednarz-Knoll, N., Alix-Panabieres, C. & Pantel, K. Clinical relevance and biology of circulating tumor cells. *Breast Cancer Res.* **13**, 228 (2011).

127. Bednarz-Knoll, N., Alix-Panabieres, C. & Pantel, K. Clinical relevance and biology of circulating tumor cells. *Breast Cancer Res.* **13**, 228 (2011).

128. Kim, J. et al. Bone metastasis. *J. Bone Miner. Res.* **26**, 1063–1084 (2013).

129. Bichsel, C. A. et al. Multistep nature of metastatic inefficiency. *Exp. Cell Res.* **351**, 781–791 (2004).

130. Yi, J. & Backman, V. Imaging a full set of optical scattering properties of biological tissue by inverse spectroscopic optical coherence tomography. *Opt. Lett.* **37**, 4443–4445 (2012).

131. Aguirre-Ghiso, J. A. Imaging a full set of optical scattering properties of biological tissue by inverse spectroscopic optical coherence tomography. *Opt. Lett.* **37**, 4443–4445 (2012).

132. Bednarz-Knoll, N., Alix-Panabieres, C. & Pantel, K. Clinical relevance and biology of circulating tumor cells. *Breast Cancer Res.* **13**, 228 (2011).

133. Yoneda, T., Williams, P. J., Hiraga, T., Niewolna, M. & Nishimura, R. A bone-microchips for end-to-end in vitro tumor cell attachment and xenograft formation. *Technology* **3**, 179–188 (2015).

134. Yu, M. et al. Ex vivo culture of circulating breast tumor cells for individualized testing of drug susceptibility. *Science* **345**, 216–220 (2014).

135. Tauro, B. J. et al. Two distinct populations of exosomes are released from LIM1863 colon carcinoma cell-derived organoids. *Mol. Cell. Proteomics* **12**, 587–598 (2013).

136. Sosa, M. S., Bragado, P. & Aguirre-Ghiso, J. A. Mechanisms of disseminated cancer cell dormancy: an awakening field. *Nat. Rev. Cancer* **14**, 611–622 (2014).

137. Frangioni, J. V. New technologies for human cancer imaging. *J. Clin. Oncol.* **26**, 4012–4021 (2008).

138. Nemeth, J. A. et al. Severe combined immunodeficient-hu model of human prostate cancer metastasis to human bone. *Cancer Res.* **59**, 1987–1993 (1999).

139. Tauro, B. J. Bone metastasis in a novel breast cancer mouse model containing human breast and human bone. *Breast Cancer Res. Treat.* **132**, 471–486 (2012).

140. Kwon, H. et al. Development of an in vitro model to study the impact of BMP-2 on metastasis to bone. *J. Tissue Eng. Regen. Med.* **4**, 590–599 (2010).

141. Cristofanilli, M. et al. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. *N. Engl. J. Med.* **351**, 781–791 (2004).

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Author contributions
B.A.A., G.G.B. and L.D.S. wrote and edited the manuscript. B.A.A. prepared the figures. Additional information
Supplementary information is available at www.nature.com/reprints. Reprints and permissions information is available at www.nature.com/reprints. Correspondence should be addressed to L.D.S. or J.S.J.

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