

# Locally sourced: site-specific immune barriers to metastasis

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## Abstract

Tumour cells migrate very early from primary sites to distant sites, and yet metastases often take years to manifest themselves clinically or never even surface within a patient's lifetime. This pause in cancer progression emphasizes the existence of barriers that constrain the growth of disseminated tumour cells (DTCs) at distant sites. Although the nature of these barriers to metastasis might include DTC-intrinsic traits, recent studies have established that the local microenvironment also controls the formation of metastases. In this Perspective, I discuss how site-specific differences of the immune system might be a major selective growth restraint on DTCs, and argue that harnessing tissue immunity will be essential for the next stage in immunotherapy development that reliably prevents the establishment of metastases.

## Sections

Introduction

Principles of site-specific immunity

Setting the immune tone one site at a time

Systemic challenges to tissue immunity

Therapeutic implications of site-specific immunity

Concluding remarks

## Introduction

Whether, and if so when, metastases will occur remains a distinct challenge in the effectiveness of treatment of patients with cancer. Metastases invariably originate from disseminated tumour cells (DTCs) that have spread beyond the primary tumour site through the circulation and have successfully colonized distant sites. But despite all spreading systemically, DTCs grow asynchronously in distant sites, and may initiate metastases within months, years or decades or not at all within a patient's lifetime. This differential emergence of metastases stems from persistent DTCs that entered a state of dormancy<sup>1,2</sup> upon arrival at the distant site, only to later awaken and form metastases. From a clinical perspective, this pause in cancer progression is both a threat to a long-lasting cure and a singular therapeutic window to prevent future metastases. Understanding what brings DTCs into and out of dormancy is obviously fundamental to inform the therapeutic intervention in patients at risk of currently incurable metastases.

When it comes to explaining metastatic success, a hypothesis articulated more than a century ago by Paget<sup>3</sup> rings true even today: the 'seed' (that is, the DTC) will grow only if it lands on fertile 'soil' (that is, the microenvironment within distant tissues). Although the genetic traits of the seed are certainly important, the current lack of a systematic analysis of DTCs from patients with cancer who are asymptomatic hinders the conclusion of whether the acquisition of specific mutations is required to exit dormancy. On the contrary, the findings that the earliest transformed cells are proficient disseminators<sup>4–8</sup> and yet are often unable to resume growth for a long time argue that the properties of the soil are rather the limiting factor of metastatic progression<sup>9</sup>. Analyses of paired primary tumours and metastases demonstrate that driver mutations precede diagnosis by many years or even decades<sup>8,10–14</sup>. Furthermore, autopsy studies have long hinted that dormant DTCs spread throughout most tissues and at much higher frequency than commonly recognized, but fail to colonize certain sites in a meaningful time frame<sup>3,15–19</sup>. This steady resistance to metastasis suggests that tissue-specific barriers to DTC outgrowth exist and contribute to the chronicity of cancer. Additional specificity of barriers to metastasis comes from even within the same tissue, as recently demonstrated in the liver, where spatially distinct sub-microenvironments allow the coexistence of breast dormant DTCs and metastases<sup>20</sup>. And, clearly, occasional disruptions of the tissue integrity can breach the barriers to metastasis and present dormant DTCs with an opportunity to awaken.

What, then, is the nature of these tissue barriers to metastasis? Being the front line defence against DTCs arriving at distant sites, the immune system is one major barrier that DTCs must overcome on the path to successful colonization. Perhaps the most striking evidence of this stems from observations that metastasis can be involuntarily transmitted by organ transplantation from apparently disease-free donors into immunosuppressed recipients<sup>21–24</sup>. Such cases support an important prediction of the classic cancer immunoediting postulate<sup>25</sup>; that is, the existence of an equilibrium phase, during which the host immune system limits the outgrowth of dormant DTCs into metastases. Clinical evidence of immune-mediated dormancy comes from the detection of an increased frequency of certain immune cells, namely natural killer (NK) cells and T cells, lining dormant breast DTCs in the bone marrow of patients with no evidence of metastases<sup>26,27</sup>. By contrast, the upsurge in metastases in animal models either depleted of those antimetastatic immune cell populations<sup>20,28–33</sup> or enriched in other immune cell populations conducive to DTC reactivation<sup>34–41</sup> cements a role for immunity in determining metastatic progression

(Fig. 1) across several types of cancer, and raises the possibility of targeting DTCs by altering their immune microenvironment.

Recent breakthroughs in cancer immunotherapy<sup>42–45</sup> have generated excitement in applying checkpoint blockade, vaccines and engineered T cells and NK cells for metastatic prevention, often searching for a one-size-fits-all approach to target DTCs. But the immune context experienced by DTCs is anything but the same in every tissue. First, each site contains a specific assortment of tissue-resident immune cells<sup>46</sup>, translated in anatomically specialized DTC–immune cell interactions. Second, tissue immunity directs the development of regional and systemic immune responses through cytokine-mediated communication<sup>47</sup>, further diversifying local antimetastatic immune responses. Third, DTC–immune cell interactions co-evolve over time<sup>48,49</sup>, and it is likely that the same immune functions that initially impede metastasis either change or provide the selective pressure that promotes outgrowth of immunoevasive DTCs. In sum, the guiding principle of antimetastatic immunity is the same one touted by real-estate experts: location, location, location.

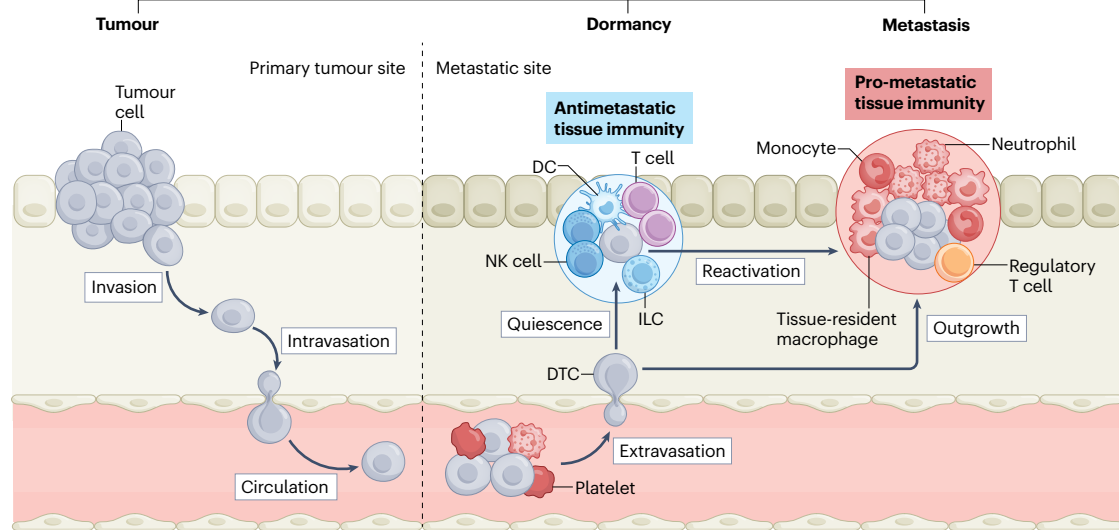
In this Perspective, I posit that site-specific differences in the immune system are a major selective growth restraint on DTCs. I outline how anatomical organization controls local tissue immunity and discuss emerging examples of site-specific immunoregulation of metastasis. I also discuss the array of challenges to tissue integrity that may breach immune barriers to DTC outgrowth, and how normalizing tissue immunity might be explored therapeutically to prevent metastases.

## Principles of site-specific immunity

### Site-specific immune cell diversity, development and function

The immune system has been shaped through evolution by the need to discriminate non-self pathogens (such as viruses or bacteria) from self tissues. The first defence strategy is anatomical; that is, the physical, chemical and biological barriers to the entry of invaders in the organism. The second defence is innate immunity, which provides an immediate, less specific response that prevents invaders from functioning or multiplying<sup>50</sup>. Finally, the third defence is adaptive immunity, which provides a long-term, more specific response to the particular invader that the organism has encountered<sup>51</sup>. Achieving immunity to DTCs arriving at distant sites does not fit neatly into this self–non-self paradigm because DTCs arise from once-normal cells from the host. However, the immune system does spot and react to DTCs, presumably through either activation of innate immune pattern recognition receptors<sup>52</sup> or adaptive strategies that involve recognition of altered-self antigens<sup>53</sup>. Equipped with a multitude of different cell types that mediate fractions of those innate and adaptive functions, at a high-level, the immune system might be viewed as a single structure that spreads throughout the body to provide host defence against insults that occur at diverse places episodically and without warning. But given the wide range of threats that beset an individual throughout life, populating all tissue sites permanently with large amounts of all the immune cell types required to mount a suitable defence response would be not only an unacceptable energetic burden<sup>54</sup> but also incompatible with the highly organized epithelial and mesenchymal cellular sheets that ensure an organ's function. Instead, the immune system has evolved to strategically position sentinel resident immune cells within tissues, which can locally initiate and amplify the immune response by recruiting circulating immune cells to the site of tissue damage, and readily restore tissue homeostasis.

Parked in tissues, long-lived tissue-resident immune cell populations self-maintain locally, and develop together with the site they populate<sup>55</sup>.



**Fig. 1 | Tissue immunity determines metastatic progression.** Before metastases manifest themselves clinically, tumour cells need to overcome multiple barriers throughout their journey from the primary site until they successfully colonize a distant site. First, they invade locally and intravasate the endothelium to enter the circulation, where they travel alone or in clusters with other cells, in search of a new site to extravasate and expand. The few disseminated tumour cells (DTCs) that survive the journey then face attrition from the specific immune environment within the distant site:

where antimetastatic tissue-resident immune cell populations are dominant, DTCs blend into the physiological context and persist in a quiescent state for several years or even decades (dormancy stage of cancer); conversely, microenvironments depleted of antimetastatic immune cells or enriched in other immune cells conducive to DTC reactivation support metastatic outgrowth into clinically detectable metastases. Treating the specific immune microenvironment at distant sites may be a way to effectively control DTCs. DC, dendritic cell; ILC, innate lymphoid cell; NK, natural killer.

These populations include tissue-resident macrophages<sup>56</sup>, mast cells<sup>57</sup>, NK cells<sup>58</sup>, innate lymphoid cells (ILCs)<sup>59</sup>, NKT cells<sup>60</sup>,  $\gamma\delta$  T cells<sup>61</sup>, mucosal-associated invariant T cells<sup>62</sup> and tissue-resident memory T cells<sup>63</sup>, all of which conserve a core lineage identity and yet retain sufficient plasticity to specialize and fulfil functions specific to their tissue of residence. Taking mouse tissue-resident macrophages as an example, despite all sharing macrophage lineage markers (such as colony-stimulating factor 1 receptor (CSF1R), Fc $\gamma$  receptor 1 (also known as CD64), the integrin CD11b, the adhesion G protein-coupled receptor F4/80 and the receptor tyrosine kinase MERTK), one finds that they diversify in phenotype to perform tissue-specific functions<sup>64</sup>; for example, Kupffer cells clear foreign debris and balance lipid metabolism in the liver<sup>65,66</sup>, red pulp macrophages drive senescent red blood cell removal and iron recycling in the spleen<sup>67</sup>, and alveolar macrophages clear excess lung surfactant<sup>66</sup>. Tissue-resident macrophage specification arises from selective chromatin states and expression of certain transcription factors<sup>68,69</sup>, such as Spi-C for red pulp macrophages<sup>70</sup>, ID3 for Kupffer cells<sup>71</sup> and PPAR $\gamma$  for alveolar macrophages<sup>72</sup>. The past two decades have seen a revolution in the tools available for studying the diversity of immune cells in tissues. Lineage tracing and highly multiplexed immunofluorescence imaging have enabled semiquantitative analyses of the composition and spatial organization of the immune system within tissues, whereas two-photon intravital microscopy has provided insights into immune cell population dynamics, cell-cell interactions and functional responses *in situ*<sup>73</sup>. These advanced imaging modalities, combined with conventional flow cytometry approaches and emerging systems-level immunomics<sup>74</sup>, continue to uncover new phenotypic diversity in immune cells across multiple mouse<sup>75–83</sup> and human<sup>82,84–93</sup> tissues. Thorough investigation of the ontogeny and function of all these new tissue-resident immune cell

subsets in different physiological and disease contexts, especially during the formation of metastases, will be crucial to determining their overall importance.

### Niche imprinting of site-specific immunity

If tissue compartmentalization of immunity is a given, how is it established and maintained? Resident immune cells are locally conditioned by specialized tissue microenvironments composed of several non-immune resident cell types, all embedded in extracellular matrix components, which not only provide them with a physical niche and trophic factors that confer self-maintenance but also imprint them with tissue-specific functions that are essential for the homeostasis of their tissue of residence. For example, in the skin, ILC subsets rely on hair follicle-derived factors for localization and maintenance, and tune the skin microbiota by limiting sebaceous gland growth<sup>94</sup>. In the intestine, tissue-resident macrophages respond to microbial cues to enhance regulatory T cell (T<sub>reg</sub> cell) function<sup>95</sup> and to control gastrointestinal tract transit through the regulation of enteric neuronal function<sup>96</sup>. Immune cells are also imprinted by the microenvironment within non-barrier tissues such as the brain, where microglia rely on neuron-derived factors to ensure neuronal development and synaptic pruning<sup>97–100</sup>, or the adipose tissue, where mesenchymal stromal cell populations provide a niche for ILC2s<sup>101–103</sup> and T<sub>reg</sub> cells<sup>104</sup> to regulate fat metabolism. Factors such as interleukin-15 (IL-15)<sup>105–108</sup>, IL-7 (refs. 105,109) and transforming growth factor- $\beta$  (TGF $\beta$ )<sup>107,110,111</sup> are commonly required for the maintenance of most of the tissue-resident immune cells. However, given that all organs contain multiple immune cell subsets occupying very unique niches, it is likely that many other tissue-specific trophic factors and their discrete cellular sources remain to be identified.

Just as non-immune resident cells imprint immune cells with specific functions that are essential for organ homeostasis, so do they shape antimetastatic immunity. The dominant permanent resident cellular components of the metastatic niche are cancer-associated fibroblasts, endothelial cells and nerve cells, all of which have been shown to strongly influence antimetastatic immunity (Box 1). The widespread use of single-cell technologies has led to a whole new appreciation of the heterogeneity of these cell populations. As new cell subsets are revealed, functional characterization of the interactions of non-immune and immune resident cell populations under distant site-specific conditions should provide a first glimpse into how tissue microanatomy contributes to local immunity and the induction of site-specific antimetastatic immune responses.

## Site-specific immune cell density

Because immunity is tailor-made for each organ site, the cross-tissue composition of the immune system is distinct. Comprehensive studies of multiple organs from mice have illustrated the presence of distinct

densities of immune cells across different sites. For example, the liver is a preferential tissue for NK cell residency<sup>58</sup>, whereas in the brain NK cells are scarce and resident myeloid-derived cells are by far the most dominant immune cell population<sup>112</sup>. The skin is largely populated by resident  $\gamma\delta$  T cells<sup>113</sup>, but these cells constitute only a small percentage of all the splenic T cells<sup>114</sup>. In the lung, tissue-resident macrophages represent more than 90% of all immune cells<sup>115</sup>, whereas in the bone, they account for only 15–20% of the immune cellular content<sup>116</sup>. In recent years, analysis of tissue samples from hundreds of healthy human organ donors has revealed that interorgan heterogeneity of immune cell density is also conserved in humans<sup>90,117,118</sup>. One important consideration is that organs consist of different regions with distinct macroanatomical and physiological traits, and these spatial topographies may account for further diversity in immune cell density even within the same organ. For instance, the way the intestine handles the challenge of tolerating food antigens and the commensal microbiota, while eliminating ingested pathogens, is by macroanatomical segregation of these reactions into functionally distinct gut-draining

## Box 1

# Non-immune tissue-resident cells: tailors of local antimetastatic immunity

## Fibroblasts

The accumulation of cancer-associated fibroblasts (CAFs)<sup>257</sup> has become increasingly recognized as supportive of outgrowth of disseminated tumour cells (DTCs) in the lung and liver through different immuno-inhibitory mechanisms, such as the recruitment of neutrophils<sup>132,157</sup> and inflammatory monocytes<sup>157</sup>, exclusion of cytotoxic T cells from tumour areas<sup>258</sup> and direct suppression of natural killer (NK) cell expansion<sup>20</sup>. Recent advances in single-cell technologies have resulted in the description of heterogeneity in the CAF network across organs at the baseline, by revealing tissue-specific CAF subsets with distinct matrix-producing and immunomodulatory phenotypes<sup>259,260</sup> and major differences in matrix composition across tissues<sup>261,262</sup>. As we start to uncover heterogeneity of CAF subsets and their immunomodulatory functions across tumour types<sup>263–268</sup>, it is anticipated that distinct CAF subsets also assist the formation of metastases.

## Vascular endothelial cells

In the context of metastasis, vascular endothelium association is crucial across all organs; a normalized perivascular niche is critical for maintaining DTC dormancy, whereas endothelial cell sprouting triggers DTC outgrowth<sup>269</sup>. Endothelial cells also control DTC progression through regulation of homing and extravasation of specific immune cell subsets<sup>270,271</sup>, and direct immunosuppression through expression of programmed cell death ligand 1 (PDL1)<sup>272</sup> or FAS ligand (FASL)<sup>273</sup>. Single-cell studies have reported remarkable differences in endothelial cell expression patterns of co-stimulatory molecules and cytokines across tissues<sup>274</sup>, but whether tissue-specific endothelial cell subsets contribute to the formation of metastases remains to be seen.

Lymphatic vasculature completes the other half of the specialized vascular system in mammals, and directly contributes to immune

homeostasis and disease in a context-dependent manner<sup>275</sup>.

Lymphatics contribute to metastasis by serving as intratumoural gateways to distant organs<sup>276</sup>, providing a dormancy niche for DTCs in the lung and lymph nodes<sup>277,278</sup>, and inducing peripheral tolerance<sup>279,280</sup>.

## Nerve cells

Although little is known about neuronal regulation of DTCs, it has been described that sympathetic innervation reactivates breast<sup>247</sup> and prostate<sup>247,248</sup> dormant DTCs in the bone, and excitatory synapses with glutamatergic nerves fuel metastases in the brain<sup>281</sup>. Neuron-immune cell interactions are well-described mediators of host defence and inflammation. A notable example is the neuron-macrophage crosstalk that maintains either protective or tolerogenic environments that are vital to intestinal homeostasis: noradrenaline signalling in  $\beta_2$ -adrenergic receptor-expressing submucosal muscularis macrophages enforces an anti-inflammatory programme, which balances the pro-inflammatory phenotype of mucosal macrophages in the lamina propria<sup>282</sup>. Akin to specialized intestinal macrophages, several other immune cells engage in a constant conversation with neurons at discrete anatomical sites to sustain tissue health<sup>283</sup>; it is, thus, tempting to speculate that disrupting neuron-immune cell interactions might result in tissue-specific disease, including the emergence of metastases. In support of this, neuroendocrine stress induces immune dysfunction and disease<sup>246</sup>, and perioperative denervation (for example, using  $\beta$ -adrenergic blockers) has been hypothesized to stimulate antimetastatic immunity<sup>251</sup>. Further understanding of neuron-immune cell interactions in the context of tissue-specific metastasis is among the most exciting challenges in cancer immunology for the coming years.

**Table 1 | Types of immune cells and their contribution to disseminated tumour cell progression in four frequent sites of metastasis**

Cell type	Description	Function in DTC progression
Dendritic cell	Presents antigens on its surface	Suppression of bone <sup>165</sup> and brain <sup>192</sup> metastases
Group 1 innate lymphoid cell or tissue-resident natural killer cell	Generally weakly or non-cytotoxic; secretes cytokines in response to pathogens or cancer cells	IFN $\gamma$ -mediated DTC dormancy in the liver <sup>20</sup> ; suppression of liver metastases <sup>125</sup>
Group 2 innate lymphoid cell	Responds to large extracellular parasites and allergens	Suppression of brain metastases by enhancing dendritic cell antigen presentation and T cell responses <sup>192</sup> ; pro-metastatic through natural killer cell suppression in the lung <sup>152</sup>
Inflammatory monocyte	Produces inflammatory molecules in response to infection and cancer	Establishment of a pre-metastatic niche in the liver <sup>132,133</sup> ; support of DTC seeding and outgrowth in the lung through suppression of T cell function <sup>35,40,157</sup>
Neutrophil	Releases chemicals that act against pathogens and cancer cells	Establishment of a pre-metastatic niche in the lung <sup>36</sup> ; awakening of dormant DTCs in the lung <sup>34,153</sup> ; colonization of the lung <sup>38,39,154</sup> , bone <sup>165</sup> and brain <sup>189</sup>
Natural killer cell	Kills pathogens and cancer cells through cytotoxic granules	DTC elimination in the lung <sup>149–151</sup> , liver <sup>150</sup> and brain <sup>150,191</sup> ; suppression of bone metastases <sup>65,170–172</sup>
Natural killer T cell	Shares properties of both T cells and natural killer cells; kills pathogens and cancer cells	IFN $\gamma$ -mediated antimetastatic immunity in the liver <sup>226</sup>
Tissue-resident macrophage	Phagocytic cell that engulfs and destroys foreign pathogens and cancer cells	Clearance of DTCs upon liver entry <sup>130,131</sup> ; establishment of a lung pre-metastatic niche through T cell suppression <sup>37</sup> and neutrophil recruitment <sup>146</sup> ; pro-metastatic in the lung <sup>147</sup> and liver <sup>141</sup> through acquisition of a lipid metabolism phenotype; promotion <sup>41,186,187</sup> or suppression <sup>184,185</sup> of brain metastases
Cytotoxic T cell	Processes specific immune responses to antigens; kills pathogens and cancer cells	Suppression of DTC outgrowth in the lung <sup>28,155,156,158</sup> , brain <sup>190</sup> and bone <sup>165,171</sup>
$\gamma\delta$ T cell	Promotes inflammatory responses through cytokine production	Pro-metastatic in the lung through suppression of cytotoxic T cells <sup>39</sup>
Regulatory T cell	Suppresses immune responses, to maintain peripheral tolerance, prevent autoimmunity and limit chronic inflammatory diseases	Promotion of bone <sup>165</sup> , lung <sup>148</sup> and brain <sup>190</sup> metastases

DTC, disseminated tumour cell; IFN $\gamma$ , interferon- $\gamma$ .

lymph nodes<sup>119</sup>; this anatomical parsing results in important regional differences in the distribution of immune cell populations along the length of the intestine.

Emerging studies show that site-specific immune cell density also shapes the differential emergence of metastases. Clinical data from patients with metastatic ovary<sup>120</sup> and colorectal<sup>49</sup> cancer show that distinct composition and distinct spatial organization of the immune cell landscape across different organs (that is, inter-organ immune heterogeneity) give rise to metachronous metastases within the same individual. Additionally, spatially distinct immune microenvironments exist even within the same organ (that is, intra-organ immune heterogeneity), and disproportionally impact DTC progression. Within the liver, for instance, breast DTCs lodged in NK cell-abundant microenvironments remain dormant, whereas those experiencing a local shortage of NK cells resume growth into metastases<sup>20</sup>. An understanding of interorgan and intra-organ immune heterogeneity is, thus, necessary to determine whether there are universal immune responses that can be exploited to effectively control dormant DTCs or whether a more customized approach that considers site-specific immunity is required. In the following section, I describe the unique immune microenvironment (Table 1) within and across four frequent sites of metastasis (that is, liver, lung, bone and brain; Fig. 2) and two where metastases are rather rare (that is, spleen and thyroid; Fig. 3), in search of targets for long-lasting therapeutic prevention of metastases.

## Setting the immune tone one site at a time Liver

Excluding lymph nodes, the liver is the most common site of metastasis across solid tumours<sup>121</sup>. Despite having facilitated access to the liver through discontinuous sinusoids, DTCs often find resistance to immediate outgrowth and persist for a long time as dormant cells at this site<sup>122–124</sup>. This liver capacity to support dormancy is, at least in part, rooted in its exceptionally tolerogenic milieu. The particular abundance of circulating NK cells and liver-resident NK cells (also termed ‘liver ILC1s’)<sup>58</sup> sets these innate immune cells as major effectors of hepatic immune function. In the steady state, they maintain an immature phenotype with low levels of pro-inflammatory cytokines and cytotoxic mediators, but they promptly respond to virally infected cells or DTCs by expressing tumour necrosis factor-related apoptosis-inducing ligand (TRAIL)<sup>125</sup> and producing interferon- $\gamma$  (IFN $\gamma$ )<sup>126</sup>. Recently, colleagues and I showed that IFN $\gamma$  secreted by liver NK cells is essential to maintain breast DTCs dormant<sup>20</sup>. We found that the size of the NK cell pool in the liver can itself determine metastatic outgrowth, and sustaining NK cell abundance with adjuvant IL-15-based immunotherapy succeeded in preventing hepatic metastases and prolonging survival in preclinical models<sup>20</sup>. It is relevant to note that, physiologically, NK cells counter liver fibrosis by eliminating activated hepatic stellate cells (which activate from quiescent hepatic stellate cells to proliferative myofibroblasts)<sup>127,128</sup> and by restraining the proliferation of activated hepatocytes in an IFN $\gamma$ -dependent manner<sup>129</sup>; this antifibrotic role of



liver NK cells already in place for normal physiology also helps to sustain DTCs in a dormant state. Similarly, the function of Kupffer cells in tissue repair and clearance of dead and senescent cells that is central to maintaining liver homeostasis also constrains the growth of DTCs upon their entry to the liver sinusoids<sup>130,131</sup>.

Although the tolerogenic milieu is intentionally kept to avoid inappropriate inflammatory responses against frequently encountered antigens, when the liver surpasses its ability to successfully clear an aggravating invader, it breaks the tolerance and triggers inflammation. It is essential that this inflammation is controlled in a timely and effective manner, otherwise the liver can initiate a cycle of chronic inflammation, fibrosis and scarring, which overall results in loss of tissue integrity and increased risk of metastasis. Activation of hepatic stellate cells, by either Kupffer cell-derived TGF $\beta$ <sup>132</sup> or recruited inflammatory monocyte-secreted granulins<sup>133</sup>, results in a fibrotic environment that assists the pre-metastatic niche<sup>134</sup> formation and metastasis of pancreatic cancer in the liver. Additionally, the accumulation of activated hepatic stellate cells prevents NK cell expansion and precipitates metastases<sup>20</sup>, suggesting that failure to resolve fibrosis causes incidental DTC awakening.

A particular feature of the liver is the spatial division of labour according to blood flow-generated gradients of oxygen, nutrients and hormones, together with morphogenetic fields, which run radially along the porto-central axis of the liver lobules, a phenomenon termed 'zonation'<sup>135</sup>. Not surprisingly, resident immune cells also comply with liver zonation. Resident NKT cells and Kupffer cells concentrate around periportal regions to protect liver cell populations around the central vein from incoming blood-borne threats<sup>136</sup>. Liver ILCs also reside primarily in the perivascular spaces surrounding the portal triad<sup>137,138</sup>, where they engage in bidirectional interplay with Kupffer cells, dendritic cells (DCs) and neutrophils, in fine coordination of an optimized immune response. Recent studies expanded on myeloid cell zonation by unravelling a population of lipid-associated macrophages that locates at the bile ducts in the healthy liver but repositions itself pericentrally to regions with steatosis (fatty liver disease)<sup>139,140</sup> and accumulates in liver metastasis<sup>141</sup>. As more insights into the regional heterogeneity of immune cells in the context of normal liver physiology<sup>80,135,142,143</sup> become available, how immune zonation impacts the emergence of metastases will warrant further investigation.

## Lung

Pulmonary metastases are detected in 20–54% of patients with cancer and tend to occur in the basal and peripheral regions of the lung lobes, where increased vascular density raises the chances of DTC extravasation<sup>144</sup>. Because lung metastases rarely appear without other co-occurring secondary sites<sup>121</sup>, they are likely the result of default accessibility and permissiveness of this site. The lung is continuously exposed to the external environment, and it must protect the organism from inhaled pathogens and pollutants, while not mounting a disproportionate response against innocuous airborne antigens. Lung-resident immune cells are responsible for striking this delicate balance between immunity and tolerance. Among them, tissue-resident macrophages, including alveolar and interstitial macrophages<sup>145</sup>, are by far the most abundant, and there is growing appreciation of their importance also in the context of metastasis. Alveolar macrophages contribute to the pre-metastatic niche by suppressing antimetastatic cytotoxic T cell responses<sup>37</sup> or recruiting neutrophils to the lung<sup>146</sup>. A recent study found extensive heterogeneity within macrophages isolated from

metastasis-bearing lungs<sup>147</sup>, including a subset of lipid-associated macrophages enriched in transcripts associated with extracellular matrix remodelling and immunosuppression, whose functional significance awaits to be validated. Besides tissue-resident macrophages, T<sub>reg</sub> cell accumulation favours local tolerance in the lung, a feature that is fully exploited by DTCs to colonize this site<sup>148</sup>.

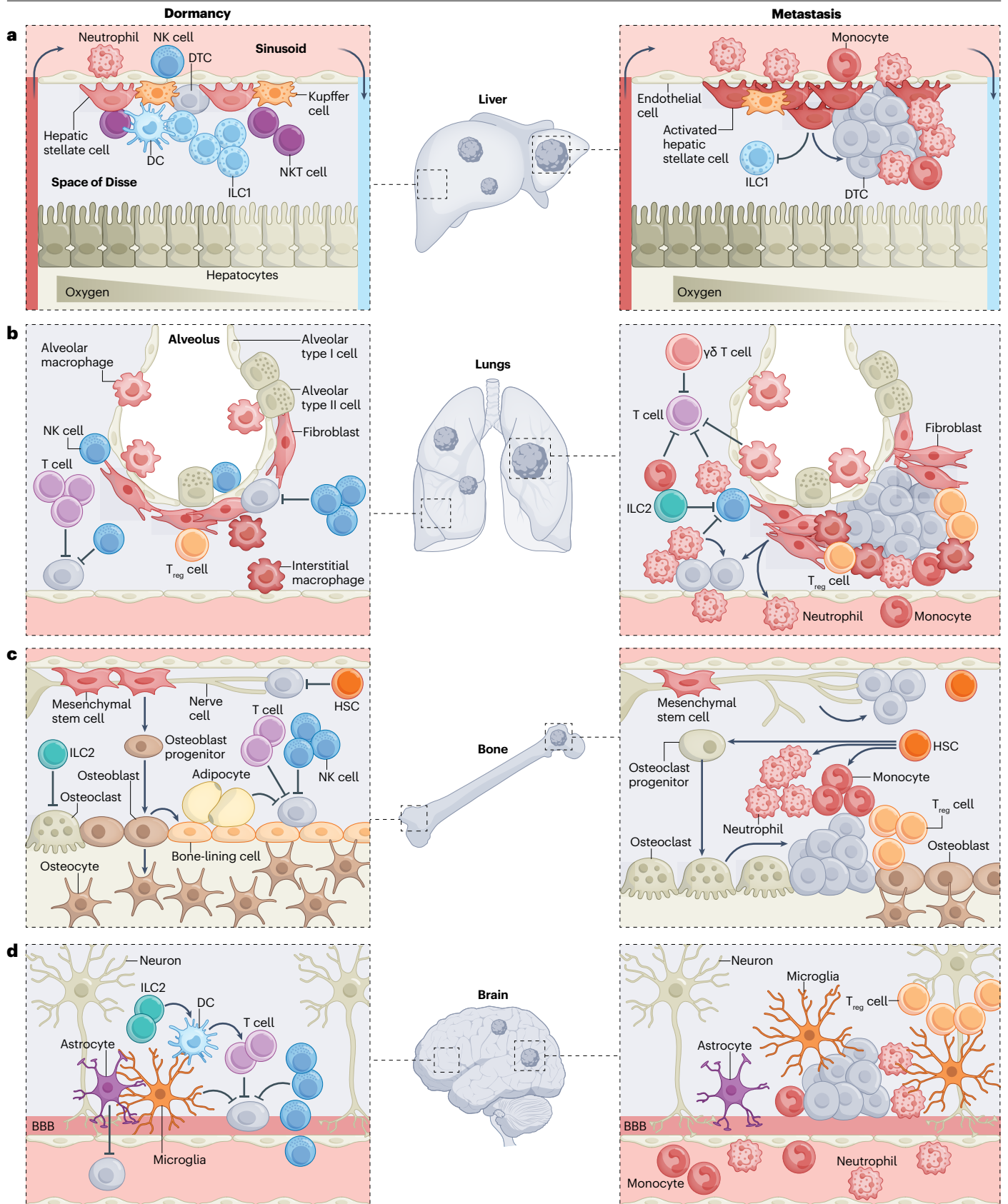
Lung NK cells constitute approximately 10–20% of all lung-resident lymphocytes, and are critical for antigen-independent recognition and elimination of DTCs, as demonstrated by overwhelming metastatic burden in their absence or impairment<sup>149–151</sup>. However, NK cell antimetastatic function in the lung is countered by ILC2s<sup>152</sup> and recruited neutrophils<sup>36</sup>. Neutrophils in the lung have received particular attention, as they have been implicated in the awakening of dormant DTCs through extracellular matrix proteolysis<sup>34</sup> and lipid metabolism<sup>153</sup>, and subsequent lung colonization<sup>38,154</sup>. Neutrophils also synergize with  $\gamma\delta$  T cells to dampen cytotoxic T cell-mediated antimetastatic immunity<sup>39</sup>, which has thus far been regarded as a main growth constraint of DTCs in the lung<sup>28,155,156</sup>. Similarly, inflammatory monocytes also promote breast DTC seeding and outgrowth by suppressing cytotoxic T cell function<sup>35,40</sup>. The recruitment of immunosuppressive neutrophils and inflammatory monocytes to the lung is orchestrated by fibroblasts, which establish a hospitable inflammatory milieu permissive of metastasis<sup>157</sup>. Even short-term inflammation, such as that induced after surgery, awakens breast dormant DTCs, the growth of which would otherwise be constrained by antimetastatic cytotoxic T cell responses<sup>158</sup>. Importantly, perioperative anti-inflammatory treatment reduces DTC outgrowth in the lung, offering an inexpensive intervention to counter inflammation and prevent the emergence of metastases particularly at this site.

## Bone

After the liver and lung, the bone is the most frequent site of metastasis of all solid tumours<sup>15</sup>. Conventional bone marrow biopsies indicate that one-third of patients with early-stage breast cancer already have DTCs in their bone marrow at the time of primary tumour resection, and approximately one quarter of these patients will progress to have metastases<sup>159</sup>. Although the presence of DTCs in the bone is a common event that invariably correlates with poor outcome<sup>159–161</sup>, the inconsistently low incidence of clinically detectable bone metastases suggests that growth restraints to DTCs exist at this site. In addition, not all bones are equally susceptible to metastasis; whereas the axial skeleton (such as the spine, ribs and bony pelvis) is a preferential target for breast cancer spread, metastases rarely develop within the appendicular skeleton (such as hands and feet)<sup>162,163</sup>. Conceivably, these discrepancies might reflect differences in bone marrow composition (that is, the content of blood cell-producing red marrow and adipocyte-rich yellow marrow) within and across different bones, and also during life, which likely influence DTC outgrowth through ways still underexplored.

Despite orchestrating the vast immune cell network arising from haematopoiesis, the bone is a site of particularly dampened immunity. The small pool of cytotoxic T cells and mainly immature NK cells contrasts with a large proportion of myeloid progenitors (of both monocytic origin and granulocytic origin) and T<sub>reg</sub> cells<sup>164</sup>, creating an immunosuppressive potential ready to assist the formation of metastases. For example, heightened myeloid progenitor differentiation into immunosuppressive neutrophil and monocyte populations precedes breast DTC outgrowth in the bone<sup>165</sup>. Similarly, myeloid progenitors can differentiate into bone-resorbing osteoclasts<sup>166</sup>, feeding the vicious cycle of bone destruction that triggers breast DTCs to exit dormancy and initiate metastases<sup>167</sup>. Likewise, bone T<sub>reg</sub> cells

# Perspective



## Fig. 2 | Site-specific differences of the immune system contribute to differential emergence of metastases within and across common metastatic sites.

The immune system is customized by anatomical site, featuring distinct cell types that are distributed at defined ratios and spatial locations, where they engage in dynamic interactions with diverse non-immune resident cells to constantly safeguard tissue homeostasis. It is the product of these interactions that sets the immune tone for recognition of disseminated tumour cells (DTCs), and makes an ideal locale for DTCs to either be kept dormant or establish metastases within and across different sites. **a**, In the liver, DTCs migrate from the portal triad vessels to the sinusoid and into the subendothelial space of Disse, where they encounter immune and non-immune resident cells, all positioned in compliance with liver zonation. Liver-resident natural killer (NK) cells (or liver group 1 innate lymphoid cells (ILC1s)) are the main immune gatekeepers of DTC dormancy, whereas activation of hepatic stellate cells precipitates metastasis through suppression of liver-resident NK cell expansion or recruitment of immunosuppressive populations. **b**, In the lungs, NK cells and T cells are the major immune barriers to metastasis, and their function is countered by diverse immunosuppressive populations, including ILC2s, neutrophils, monocytes,

regulatory T cells ( $T_{reg}$  cells) and  $\gamma\delta$  T cells. Tissue-resident macrophages and activated fibroblasts feed the recruitment of these immunosuppressive populations by maintaining a hospitable inflammatory milieu permissive of metastasis. **c**, The bone is the primary niche for haematopoietic stem cells (HSCs), and is co-opted by DTCs to remain dormant. NK cells and T cells actively survey this niche, in concert with ILC2s that preserve the bone integrity through suppression of bone-resorbing osteoclasts. Conversely, an imbalance towards osteoclast differentiation supports the cycle of bone destruction that triggers dormant DTC reactivation and metastasis initiation. Other awakening factors in the bone include the accumulation of neutrophils, monocytes and  $T_{reg}$  cells, as well as enhanced innervation. **d**, Access to the brain parenchyma is restricted by the blood–brain barrier (BBB), and thus DTC extravasation to the brain takes longer than in other tissues. Once in the brain, DTCs park tightly at the perivascular space, and are actively surveyed and often eliminated by NK cells and T cells, with the latter helped by ILC2-mediated enhanced antigen presentation by dendritic cells (DCs). Microglia, the main immune cell residents of the brain, act in a context-dependent manner, being either suppressive of or permissive of metastatic outgrowth. NKT cell, natural killer T cell.

have non-immunological activity by endowing haematopoietic stem cells with a licence to evade immunity through prevention of oxidative stress<sup>168,169</sup>, a privilege that might also be afforded to DTCs lodged in the bone.

In contrast to the default immunosuppressive microenvironment of the bone, a few antimetastatic immune responses have been described at this site. NK cell activity underpins the efficacy of adjuvant IL-2-based immunotherapy in eradicating neuroblastoma metastases to the bone<sup>170</sup>. Similarly, NK cells and cytotoxic T cells are required for interferon regulatory factor 7-mediated suppression of breast cancer bone metastases in preclinical models<sup>171</sup>. Loss of host type I interferon signalling impairs NK cell immunity and drives breast cancer metastasis in the bone<sup>172</sup>. DTC-endogenous interferon signalling also directly sustains prostate DTC dormancy in the bone, and the loss of type I interferon genes triggers DTC outgrowth into metastases<sup>173</sup>. Although the local source of interferons in the dormant DTC niche is still unclear, the fact remains that interferon abundance mediates dormancy in the bone marrow. The reports that osteal macrophages ensure ossification and maintenance of haematopoietic stem cell niches<sup>116,174</sup> and that ILC2s suppress the generation of bone-resorbing osteoclasts<sup>175</sup> highlight the critical function of tissue immunity in preserving bone integrity; this tissue protective mechanism may be relevant to minimize the growth of bone marrow DTCs as well.

### Brain

Brain metastases are clinically observed in about 20% of all patients with cancer, even though the true incidence is estimated to be much higher and rising owing to more accurate imaging technologies and systemic therapies that extend patient survival<sup>176</sup>. For decades, the brain was considered an immune-privileged site, sealed off from peripheral immunity through the blood–brain barrier, and self-sufficient for maintenance and repair based on its own resident immune cells. This simplistic view was revisited with the discovery that bone marrow-derived immune cells enter the brain to support its function<sup>177</sup>, and immune molecules are ferried into and out of the brain through a lymphatic drainage system<sup>178</sup>. Recent studies have shown that immune cells are housed within specialized niches across spatially distinct brain regions (that is, the choroid plexus, the meninges and the cerebrospinal fluid)<sup>179</sup>. Although cellular and spatial maps of these regions during development and ageing are starting to emerge<sup>82,180</sup>, how immune phenotype

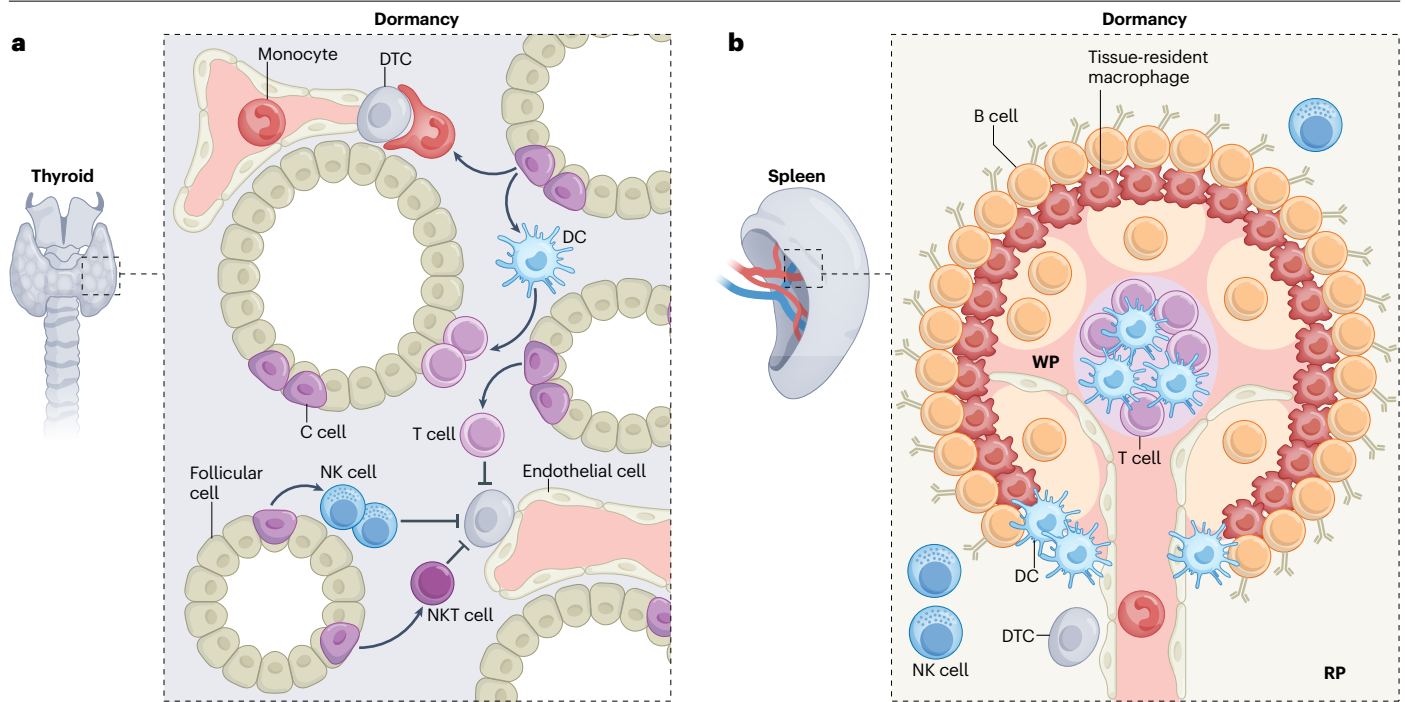
diversity and spatial topographies within the brain impact the formation of metastases remains unknown and ripe for study.

Accounting for approximately 75–80% of all immune cells in the brain<sup>181</sup>, resident microglia have garnered the most acclaim in the context of brain metastasis. They build up in and around metastases<sup>182,183</sup>, and exhibit polarized phenotypes that can be either growth suppressive<sup>184,185</sup> or permissive of metastatic colonization<sup>41,186,187</sup>. A recent study combining single-cell analyses with transgenic mouse models points to a much wider heterogeneity within the microglial compartment, by revealing seven transcriptionally different subsets of microglia that mediate brain metastatic outgrowth<sup>188</sup>. In humans, activated resident microglia seem more relevant for brain tumour development, whereas the recruitment of macrophages and neutrophils<sup>189</sup> and the accumulation of  $T_{reg}$  cells and exhausted cytotoxic T cells<sup>190</sup> are dominant in brain metastases. It has been suggested that other populations of immune cells contribute as a barrier to DTC outgrowth in the brain. NK cells actively survey and eliminate DTCs that periodically re-enter proliferation and re-express NK group 2 member D (NKG2D) receptors<sup>191</sup>, whereas ILC2s limit brain metastases by enhancing DC antigen presentation and the generation of cytotoxic T cell responses<sup>192</sup>.

### Thyroid

Although only 1.4–3% of malignant solid tumours clinically present as metastases in the thyroid<sup>193</sup>, autopsy studies reveal a much higher prevalence of subclinical disease (that is, small metastatic nodules and/or interspersed DTCs) in patients who die of non-thyroidal cancer<sup>194–197</sup>. This apparent discrepancy suggests that DTCs successfully reach and lodge in the thyroid, and yet they are prevented from colonizing this site in a meaningful time frame. The fast arterial blood flow, rich oxygenation and high iodine content of the thyroid gland have been long suggested to underlie its resistance to metastasis<sup>197</sup>. Another hypothesis is the naturally hyperactive immune microenvironment established by the three thyroid hormones (that is, tri-iodothyronine, thyroxine and calcitonin<sup>198</sup>), which impacts many inflammation-related processes, such as chemotaxis, phagocytosis, cytokine production and the generation of reactive oxygen species<sup>199</sup>. Both tri-iodothyronine and thyroxine stimulate IFN $\gamma$  responses and NK cell activity<sup>200</sup>, and facilitate maintenance of T cells and NKT cells<sup>201</sup>. In addition, tri-iodothyronine uniquely stimulates the maturation and antigen presentation capacity of DCs<sup>202</sup>, and triggers monocyte phagocytic activity<sup>201</sup>. Thus, it is plausible that





**Fig. 3 | Diversity and spatial distribution of tissue immunity may underlie the resistance to disseminated tumour cell outgrowth at infrequent sites of metastases. a**, In the thyroid, the continuous production and high concentration of thyroid hormones (such as tri-iodothyronine, thyroxine and calcitonin) maintains natural killer (NK) cell, T cell and NKT cell activity, stimulates the maturation and antigen presentation capacity of dendritic cells (DCs), and sparks monocyte phagocytic activity. It is plausible that this naturally hyperactive local immunosurveillance maintains dormancy of disseminated tumour cells (DTCs) and antagonizes metastatic outgrowth at this site. **b**, Beyond their high numbers, splenic immune cells display an intricate positioning within the three main structures of the spleen: the inner white pulp (WP), where cytokine-mediated

compartmentalization separates T cell and B cell areas into distinct zones; the red pulp (RP), which harbours many immune cells with innate immune functions that selectively express distinct pattern recognition receptors, helping to tailor the nature of both the early innate immune response and the subsequent adaptive immune response; and the in-between marginal zone, harbouring concentric macrophage subsets and DCs with defined positioning to ensure efficient pathogen encounter and antigen presentation. It is possible that this sharp anatomical parsing of immunosurveillance readies the splenic antimetastatic niche by facilitating low-probability DTC–immune cell interactions. Although these hypotheses are enticing, specific mechanisms driving immune-mediated low frequency of metastases in the thyroid and spleen remain to be seen.

the continuous production and high concentration of thyroid hormones sustains an increased local immunosurveillance that maintains DTC dormancy and antagonizes metastatic outgrowth at this site. Although the explanation is enticing, its confirmation remains to be seen.

## Spleen

Similarly to the thyroid, the spleen is also an uncommon site for metastases from solid tumours to manifest themselves clinically<sup>3,121,163,203</sup>. Many hypotheses have been offered to explain the antimetastatic nature of the spleen<sup>163,203,204</sup>, yet none seems to have been proved and all have issues. Perhaps the most obvious explanation would be the large number of immune cells within the spleen. However, an equally abundant immune cell population is present in lymph nodes, and these are not similarly protected from metastases. Beyond numbers, immune cells display an intricate positioning within the three main structures of the spleen: the red pulp, the white pulp and the in-between marginal zone<sup>205</sup>. Inside the white pulp, cytokine-mediated compartmentalization separates T cell and B cell areas into distinct zones, which synergize to perform adaptive immune functions. In contrast, the red pulp harbours many immune cells with innate immune functions, which selectively express distinct pattern recognition receptors, helping to tailor the nature of

both the early innate immune response and the subsequent adaptive immune response. Just as distinct subsets of macrophages<sup>206</sup> and correct positioning of DCs<sup>207</sup> are critical for efficient pathogen encounter and antigen presentation, it is possible that sharp anatomical parsing of immunosurveillance readies the splenic antimetastatic niche by facilitating low-probability DTC–immune cell interactions. Assessment of the spatiotemporal dynamics of splenic immune cells during the formation of metastases will probe the validity of this hypothesis.

Hence, whether in tissues long recognized as infrequent sites of metastases (Fig. 3) or even within common metastatic sites where dormant DTCs coexist with growing metastases (Fig. 2), the diversity and spatial distribution of tissue immunity are major determinants of the differential emergence of metastases.

## Systemic challenges to tissue immunity

Beyond local tissue specificities, a set of an individual's systemic factors might influence the tissue immune microenvironment and further diversify metastasis presentation. These systemic factors include ageing, circadian rhythm, the gut microbiota, therapy and certain pathological states, all of which challenge tissue integrity, with major consequences for local tissue immunity.

## Ageing

The time-dependent accumulation of cell and tissue damage during ageing poses a first major challenge to immunity. Ageing is associated with systemic, low-grade chronic inflammation leading to a gradual decline in immune function over time (or immunosenescence), which likely underlies the higher incidence of infection, autoimmunity and cancer in elderly people. Age-induced immunosenescence occurs largely in effector T cells, as illustrated by the loss of T cell function and the pronounced anti-inflammatory response that accompany the development of skin squamous cell carcinoma<sup>208</sup>. However, populations of innate immune cells, such as NK cells, macrophages and neutrophils, appear to also undergo density and phenotypic changes with ageing<sup>209</sup>. In the bone marrow, haematopoietic stem cells are skewed towards myeloid lineage specification in elderly people<sup>210,211</sup>, and increased neutrophil recruitment in aged mice accelerates breast tumour growth through suppression of cytotoxic T cell responses<sup>212</sup>. Besides a direct contribution to immunosenescence, ageing also changes the stromal compartment and indirectly favours an immunosuppressive microenvironment conducive to cancer progression. In a mouse model that mimics the aged skin microenvironment, stromal senescence establishes a tumour-permissive, chronic inflammatory milieu that allows skin squamous cell carcinoma to progress unchecked by the immune system<sup>213</sup>. Just recently, stromal changes in the aged lung were shown to induce dormant melanoma cells to exit dormancy<sup>214</sup>, strongly suggesting that age must be considered as a parameter in the design and delivery of therapies for the formation of metastases.

## Circadian rhythm

The cyclic variation in behaviour and biological activity that follows every 24-h light–dark cycle is termed ‘circadian rhythm’. Virtually all organisms have developed endogenous cellular clocks that allow time sensing, so every physiological process is optimally timed to its local environment. Circadian immune circuits appear ubiquitously<sup>215</sup>, and therefore may underlie tissue integrity or the lack thereof that precedes cancer initiation and metastasis. Elegant circadian experiments have shown a clear diurnal pattern in melanoma-induced metastasis, with many lung metastases forming when the cells were injected in the morning and nearly no metastatic foci when they were injected in the evening<sup>216</sup>. Remarkably, this time-dependent difference is mitigated upon neutrophil depletion, providing a new rationale for time-controlled administration of neutrophil-targeted therapies. In another study, key circadian rhythm hormones, such as melatonin, testosterone and glucocorticoids, were shown to dictate the generation of circulating breast tumour cells in a time-dependent manner<sup>217</sup>. Although a role for tissue immunity in this context remains to be seen, evidence is growing that treatment of metastasis-prone cancers should be timed to be maximally effective.

## Gut microbiota

Recent years have seen a surge of interest in the gut microbiota as a critical regulator of systemic antitumour immunity, controlling the efficacy of chemotherapies and immunotherapies<sup>218–220</sup>. However, the association with commensal bacteria extends well beyond the gut, and analyses of the microbiota from different anatomical locations in the human body have revealed site-specific commensal communities that locally shape tissue immunity<sup>221</sup>. For example, early-life microbial exposure establishes lung NKT cell tolerance to future allergens<sup>222</sup>, and commensal–DC interactions calibrate immunity in the skin<sup>223</sup>.

Conversely, local dysbiosis promotes the development of lung cancer, by stimulating IL-1 $\beta$  and IL-23 production from myeloid cells, and downstream activation of lung-resident  $\gamma\delta$  T cells<sup>224</sup>. Altering the gut microbiota with faecal transplantation has shown promise to reverse dysbiosis and correct neutrophil-induced organ damage in models of infection<sup>225</sup>, and to restore NKT cell immunity against liver primary tumours and metastases<sup>226</sup>. In addition to studies of the gut microbiota, further analysis of the cross-tissue local microbiota and its dysbiosis may uncover novel immunoregulatory nodes in the formation of tissue-specific metastases.

## Therapy

Beyond doubt, the introduction of chemotherapy and radiotherapy as the standard of care has substantially increased the survival of patients with cancer. However, growing awareness exists that they may, paradoxically, have negative consequences in some patients, largely by inducing tissue damage and twisting the immune microenvironment<sup>227</sup>. Chemotherapy has been shown to restrain antitumoural immunity<sup>228,229</sup>, elicit the release of pro-metastatic extracellular vesicles<sup>230</sup> and nurture a microenvironment conducive to metastases through neutrophil infiltration<sup>231</sup>, T cell reprogramming<sup>232</sup> and complement signalling<sup>233,234</sup>. Besides chemotherapy, radiotherapy has also been recently implicated in acute injury to the lung and neutrophil reprogramming, easing DTC outgrowth<sup>235</sup>. Thereby, a more thorough understanding of the effects of any therapy on tissue immunity and metastatic dynamics in patients should inform treatment strategies in the future.

## Pathological states

Certain individual conditions alter the physiology of specific sites and challenge tissue immunity, thereby bringing increased risk to the sprouting of site-specific metastases. One condition with high relevance

## Glossary

### Dormancy

A state of pause in cancer progression in which individual disseminated tumour cells are quiescent and reversibly arrested in G0 phase of the cell cycle.

### Disseminated tumour cells

(DTCs). Cancer cells that have left the primary tumour and survived in the circulation to land in a distant site.

### Metastases

Outgrowths of disseminated tumour cells that are histologically or radiologically detectable.

### Niche

A term borrowed from ecology that refers to a unique and optimal tissue microenvironment with defined nurturing and positional cues in a given anatomical location that allows a cell or a group of cells to survive and function.

### Pre-metastatic niche

A microenvironment within a distant site that is permissive for the survival and outgrowth of incoming disseminated tumour cells before their arrival at that site.

### Tissue-resident immune cells

Immune cells that occupy tissues for prolonged periods and undergo little or no recirculation.

### Tolerogenic milieu

An environment readily suppressive of adaptive immune responses, promoting tolerance to frequently encountered antigens, while maintaining local and systemic homeostasis.

is infection, as evidenced by the high vulnerability of patients with cancer to COVID-19 (ref. <sup>236</sup>), and the worsened clinical outcomes of patients with cancer after postoperative infection<sup>237</sup>. Even in the absence of infection, tissue injury as a consequence of surgery can itself accelerate the progression of colorectal cancer metastases in the liver through enhanced formation of neutrophil extracellular traps<sup>238</sup>. Additional studies have shown that external inflammation-inducing agents, such as bacterial lipopolysaccharides or tobacco smoke, initiate neutrophil-mediated pulmonary metastases, either by the formation of neutrophil extracellular traps<sup>34</sup> or by enzymatic degradation of the dormancy-promoting factor thrombospondin 1 (ref. <sup>239</sup>). Besides neutrophils, other innate immune cell populations are reshaped by airway inflammation to facilitate metastases, as shown by allergen-induced activation of an ILC2–eosinophil axis that suppresses NK cell antimetastatic immunity against breast and melanoma DTCs in the lung<sup>152</sup>.

Conditions of chronic inflammation, such as that caused by obesity, are also responsible for reprogramming tissue immunity and increasing

the risk of metastasis. As adipose tissue pathologically expands, resident macrophages switch to an inflammatory phenotype and instruct the bone marrow to expand the population of myeloid progenitors and increase neutrophil density across multiple organs<sup>240</sup>. In both genetically induced obesity and diet-induced obesity animal models, increased density of peripheral neutrophils and pulmonary neutrophils facilitates breast DTC extravasation into the lung, with metastases following suit<sup>241,242</sup>. Adaptation of tumour cells to the lipotoxic obese environment also induces a metabolic tug of war between tumour cells and T cells for lipids, which results in T cell dysfunction and tumour outgrowth<sup>243</sup>. Similarly, lipid uptake by NK cells in the obesity context limits antitumour immune responses, suggesting that metabolic reprogramming may restore NK cell immunity and improve the clinical outcome in patients with obesity with cancer<sup>244</sup>. Obesity may also indirectly affect tissue immunity by altering site-specific non-immune resident cells, as for example recently illustrated by differential obesity-induced vascular endothelium dysfunction across different organs<sup>245</sup>.

## Box 2

### Current challenges and opportunities in disseminated tumour cell–immune cell biology

#### Appraising single disseminated tumour cell–immune cell interactions in patients

Given that single disseminated tumour cells (DTCs) are difficult to detect in tissues from patients who are asymptomatic and that tissue resections are not part of the treatment options in many patients with metastatic cancer, viable human tissue for researching DTC–immune cell biology is scarce. Rapid autopsies of deceased patients offer the opportunity to obtain large amounts of tissue from multiregion sampling with sufficient quality for both multi-omics analysis of DTCs within the metastatic ecosystem and the establishment of patient-derived model systems for mechanistic studies<sup>284</sup>. Increased access to and use of this admittedly tedious approach entails the chance to unravel the number of dormant DTCs in the body, and the underpinnings of DTC growth restraints in certain sites.

#### Expanding the understanding of site-specific antimetastatic immunity

Emerging evidence points to immune cell heterogeneity and spatial compartmentalization as having a profound influence on the propensity and timing of DTC outgrowth. The advent of high-resolution, single-cell and spatial technologies (such as co-detection by indexing (CODEX)<sup>285</sup>, cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq)<sup>286</sup>, imaging mass cytometry<sup>287</sup>, multiplexed ion beam imaging<sup>288</sup>, spatial transcriptomics<sup>289</sup> and spatial proteomics<sup>290</sup>) has started to revolutionize the field and transcend the conventional study of individual cell types or factors in isolation to provide a global view of the rich and ever-changing ecosystem hosting DTCs. For example, multiplex imaging coupled with next-generation sequencing has revealed that immune spatial topography determines the emergence of metachronous metastases within the same individual<sup>49</sup>. Standardization of these

technologies will illuminate how site-specific immunity influences DTC progression, and will help to predict which patients are likely to develop metastases and benefit from site-specific immunotherapy.

#### Development of preclinical models that translate to human immunity

Striking differences in immune biology between humans and animal models make the generation of an ideal preclinical model a challenging feat. Classical transplantable syngeneic mouse and human cancer lines, and genetically engineered mouse models, have provided translational utility over many years, yet with practical limitations in reflecting human disease kinetics, and often showing only modest responses to immunotherapy<sup>291</sup>. Three-dimensional heterotypic patient-derived organoid cultures better recapitulate growth and clinical treatment response<sup>292,293</sup>, but the reconstitution of single or a few selected immune cell populations may not be sufficient to capture the complexity of the metastatic microenvironment and its response to immunotherapy. In contrast, patient-derived xenografts retain the architecture of the original specimen and are being implemented as patient avatars in precision medicine programmes to identify the most effective treatment for individual patients<sup>294,295</sup>; however, because they use immunodeficient hosts, their application to studying DTC–immune cell biology awaits further development in humanized settings. Perhaps patient-derived explants<sup>296</sup> are the closest to representing a human-specific microenvironment, at the expense of limited tissue integrity over time and suboptimal drug diffusion and uptake. Although neither is perfect on its own, combining the strengths of individual models will allow us to effectively translate immuno-oncology research to site-specific DTC targeting in human patients.

Finally, chronic stress has been pointed out as an accelerator of cancer progression. Neuroendocrine factors affect the number, activity and trafficking of immune cells in a time- and tissue-specific manner<sup>246</sup>, and their influence on tissue-specific metastases is starting to emerge. For example, sympathetic activation triggers DTC outgrowth in the bone<sup>247,248</sup>, and exacerbated  $\beta$ -adrenergic signalling catalyses pulmonary metastases through suppression of NK cell activity<sup>249</sup> or macrophage-mediated formation of a pre-metastatic lung niche<sup>250</sup>. Because cancer diagnosis, surgery and associated treatment is a highly stressful experience, they potentially limit antimetastatic responses and trigger awakening of dormant DTCs in distant sites; this encourages perioperative denervation as an adjuvant therapy to halt the emergence of metastases in an enduring manner<sup>251</sup>.

In summary, each individual is exposed to a unique combination of challenges throughout life that tweak tissue immunity and catalyse metastatic outgrowth. How nuanced these factors are within and across tissues is a growing area of research, and will provide additional therapeutic opportunities for preventing tissue-specific metastases.

## Therapeutic implications of site-specific immunity

Clearly, immunotherapy that harnesses immunological surveillance to control DTCs has significant potential to prevent the emergence of metastases. With the growing list of approvals for immune checkpoint inhibitors, it would be tempting to consider its application to metastasis prevention in a broad manner. However, the differences in the immune microenvironment within and across distant sites discussed earlier herein argue that therapeutic interventions and the development of new immunotherapy should rather be seen in the context of site-specific immunity. From surveying different organ sites, I have identified a set of mechanisms controlling site-specific immune composition and that hold promise to either eradicate DTCs or maintain metastatic disease in a long-term dormant state.

One strategy to harness site-specific immunity directly would be to sustain pre-existing antimetastatic resident immune populations. For example, nurturing the inherent abundant pool of liver-resident NK cells with adjuvant IL-15-based immunotherapy prevents breast cancer hepatic metastases and prolongs survival in preclinical models<sup>20</sup>. Similarly, adjuvant administration of bisphosphonates halts the reactivation of breast DTCs, presumably by interfering with osteoclast biology<sup>167</sup> and activating  $\gamma\delta$  T cells<sup>252</sup> in the bone, thereby reducing bone metastases and extending patient survival<sup>253</sup>. The converse strategy would be to suppress pro-metastatic immune cell types in specific sites. For instance, blocking high neutrophil density and activity, either by dissolving neutrophil extracellular traps<sup>34</sup> or by inhibiting S100A8/S100A9-mediated accumulation of oxidized lipids<sup>153</sup>, might succeed in preventing DTC reactivation in the lung.

Because antimetastatic immunity is profoundly influenced by non-immune resident cells, reversing subtle, but critical, imbalances in DTC-immune cell-non-immune cell interactions might be another way to effectively control dormant DTCs. One example would be to attempt to cause activated hepatic stellate cells to revert into quiescence as a means to reset the proliferation ability of liver-resident NK cells, and thus ensure that there are sufficient numbers to maintain DTC dormancy in the liver. Importantly, restoration of hepatic stellate cell quiescence has been achieved through use of all-*trans* retinoic acid<sup>254</sup>, use of vitamin D<sup>255</sup> and exposure to nitric oxide<sup>256</sup>, encouraging the possibility that correcting one specific cellular target may indeed restore the overall tissue physiology and, thus, tissue immunity against DTCs. For these reasons, harnessing site-specific immunotherapy is likely to

become an essential component of immunotherapy development that reliably prevents the establishment of metastases.

## Concluding remarks

The evidence presented herein provides a renewed endorsement of Paget's seed and soil hypothesis, yet newly contextualized to tissue immunity. Driven by anatomical organization, immune cells are indexed to tissues at defined ratios and spatial locations, where they engage in dynamic interactions with non-immune resident cells to constantly safeguard tissue homeostasis. It is the product of these interactions that sets the immune tone for DTC recognition, and makes an ideal locale for DTCs to either establish metastases or be kept dormant. Mounting an efficient antimetastatic immune response requires tissue-resident immune cells to coordinate their individual activation into global tissue-level responses, a feat that can be achieved only by long-range intercellular communication, as mediated by soluble factors (such as cytokines and growth factors). Thus, a systematic quantitative framework to monitor the different cellular and soluble architects of antimetastatic immune responses, and how they intersect with DTC intrinsic traits and individual conditions, in each tissue, in space and time, will be needed to optimize the choice of interventional approach in patients at risk of developing metastases.

As we begin to appreciate site-specific antimetastatic immunity and its therapeutic potential, a few challenges and exciting opportunities lie ahead before we leverage DTC-immune cell biology into targeted immunotherapies (Box 2). The first challenge is to improve detection of DTCs and accurately assess metastatic dynamics in patients throughout treatment, which will be greatly facilitated by increased establishment of and access to rapid autopsy programmes. The second is to continue increasing our understanding of the spatiotemporal regulation of dormant and metastatic niches, so as to predict which patients are likely to develop metastases, and how to effectively manage site-specific metastatic disease across cancer types; here, orthogonal spatial technologies have been revolutionary, and it is anticipated that their widespread implementation, combined with improved computational approaches to analyse multiple large datasets, will bring resolution of the niche full circle. The third challenge is to develop preclinical models that can translate to human immunity, and thus guide rational combination therapy strategies in future clinical trials. Finally, these trials should be large and long enough to support the complexity of precision immuno-oncology protocols, and extend clinical end points beyond the traditional progression-free survival readout that appraises only metastases to also include DTC burden. Continued progress in overcoming these challenges demands a collective interdisciplinary effort to inform the next stage in immunotherapy development that will bring long-lasting cures for all patients across all cancer types.

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