Circulating and disseminated tumour cells — mechanisms of immune surveillance and escape

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Abstract | Metastatic spread of tumour cells is the main cause of cancer-related deaths. Understanding the mechanisms of tumour-cell dissemination has, therefore, become an important focus for cancer research. In patients with cancer, disseminated cancer cells are often detectable in the peripheral blood as circulating tumour cells (CTCs) and in the bone marrow or lymph nodes as disseminated tumour cells (DTCs). The identification and characterization of CTCs and DTCs has yielded important insights into the mechanisms of metastasis, resulting in a better understanding of the molecular alterations and profiles underlying drug resistance. Given the expanding role of immunotherapies in the treatment of cancer, interactions between tumour cells and immune cells are the subject of intense research. Theoretically, cancer cells that exit the primary tumour site — leaving the protection of the typically immunosuppressive tumour microenvironment — will be more vulnerable to attack by immune effector cells; thus, the survival of tumour cells after dissemination might be the 'Achilles' heel' of metastatic progression. In this Review, we discuss findings relating to the interactions of CTCs and DTCs with the immune system, in the context of cancer immuno-editing, evasion from immune surveillance, and formation of metastases.

Dissemination of single tumour cells into the bloodstream is usually not detectable in patients with early stage cancer, even using state-of-the-art, high-resolution imaging approaches. Nevertheless, disseminated tumour cells (DTCs) that have passed through the vascular or lymphatic system and migrated to distant organs can survive chemotherapy and initiate tumour regrowth, leading to disease recurrence and metastasis[1,2]. During the past decades, efforts have been made to develop and refine ultrasensitive molecular and immunocytological devices to detect circulating tumour cells (CTCs) in the blood, or DTCs in the bone marrow and lymph nodes — among the billions of nonmalignant blood or tissue cells[3–7]. In addition to the bone marrow and lymph nodes, DTCs must also be present in other organs in which metastases eventually occur (such as the liver, lungs, or brain), but detecting individual or micrometastatic populations of DTCs at these locations is difficult. In mouse models of cancer, DTCs have been detected and analysed in virtually all organs[8,9]. Importantly, the presence of DTCs in the bone marrow is predictive of metastatic relapse for various carcinomas, including breast, prostate, colorectal, and oesophageal cancer, even when detected at the time of primary surgery, years before the occurrence of overt distant metastases[10–13]. Interestingly, DTCs could potentially recirculate from the bone marrow (or other organs) back to the primary tumour site, and might, therefore, also underlie local recurrence in some patients[14].

Factors that promote or suppress the entry and survival of CTCs in the bloodstream, as well as the activation of dormant DTCs, are the subject of intense investigation. Of note, the immune system might have an important 'gatekeeper' role in these processes. Tumour-infiltrating lymphocytes (TILs), which have the propensity to mount an adaptive antitumour response, are present in many malignant tumours[15]; however, the immunosuppressive tumour microenvironment inhibits the local activation and/or effector functions of these cells, leading to T-cell exhaustion and senescence[16]. The tumour microenvironment is, therefore, an immune-privileged site, and tumour cells leaving this sanctuary might become susceptible to attack by immune cells in the blood (as CTCs), or after extravasation at sites distant from the primary tumour (as DTCs)[17]. Interestingly, in patients with glioma, tumour cells can disseminate from the brain to other tissues via the bloodstream, but very rarely give rise to

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Key points

- Cancer cells leaving the immunosuppressive microenvironment of the primary tumour become vulnerable to immune surveillance and require mechanisms of escape from immune-mediated elimination if they are to form metastases
- Circulating tumour cells (CTCs) and disseminated tumour cells (DTCs) are often detectable in the peripheral blood and bone marrow, respectively, of patients with any of a range of different malignancies
- CTCs and DTCs exploit a large variety of immune-escape mechanisms, including alterations in the expression of MHC molecules, NK-cell ligands, FAS, FAS ligand (FASL), and immune-checkpoint molecules, such as CD47 and programmed cell death 1 ligand 1 (PD-L1)
- CTC homing to distant organs can be supported by direct interactions with immune cells during the process of extravasation, and by the effects of inflammatory cytokines in the target organ
- Future studies must address the important question of how the immune system shapes the molecular composition of CTCs and DTCs during cancer dormancy and metastatic progression

overt extracranial metastases, except when organs from such patients are transplanted into immunosuppressed recipients[18–21]. Thus, glioma cells could conceivably be efficiently eliminated by immune cells once they exit the brain, despite ineffective intracranial immune control of the primary tumour.

Herein, we discuss the current literature on the interactions of CTCs and DTCs with the immune system, focusing on data generated in analyses of blood and bone marrow samples from patients with cancer. To the best of our knowledge, this Review is the first bridging two exciting, but hitherto rather separated fields of research: tumour-cell dissemination and anticancer immunity. Combined consideration of these processes might stimulate new studies and result in novel discoveries in the future.

Principles of cancer immuno-editing

Immune escape or suppression has long been proposed to constitute a critical step in both tumour formation and progression[22]. This concept of 'cancer immuno-editing' encompasses many complex biological processes, involving a range of cell types and molecular mechanisms[23–26] that are outside the scope of this Review; therefore, we focus on the aspects that are relevant to CTCs and DTCs.

Three key stages of cancer immuno-editing have been described: elimination, equilibrium, and escape[23]. The first phase — elimination — describes the containment and eradication of microcolonies of neoplastic cells by innate and adaptive immune cells. The second phase, equilibrium, is reached after neoplastic cells have survived their initial encounter with the immune system, and when interactions between tumour cells and immune cells reach a dynamic equilibrium, with the immune system exerting a constant selective pressure on the tumour cells[27–29]. This selective pressure, placed on the genetically unstable and highly heterogeneous cells in the tumour mass, results in further refinement of the tumour-cell subpopulations[30]. Thus, although primarily intended to restrain tumour development, the immune processes that are active in the equilibrium phase can facilitate tumour progression through clonal selection, leading to the third stage — immune escape[23]. In the escape phase, the selected tumour-cell clones, which have evolved mechanisms of immune evasion and/or suppression, gain the ability to grow and proliferate in an immunocompetent environment.

During the process of immune evasion, tumour cells can develop resistance to immune effectors, for example, interferon γ (IFNγ)[31]. Insensitivity to IFNγ enhances the resistance of tumours to immune attack via adaptation to the hallmark cytokine of an antitumour immune response, as demonstrated in early studies using murine models[32,33]. In addition, tumour cells can shed or otherwise restrict the presentation of ligands involved in their recognition by natural killer (NK) cells or cytotoxic T lymphocytes (CTLs), or downregulate the expression of other factors (such as proinflammatory cytokines and chemokines) that promote activation of tumour-specific immune responses[23]. With increasing size and genetic instability, however, certain tumour cells in the tumour mass will express neoantigens, which in turn activate tumour-specific immune cells. In this scenario, in particular, the mechanisms of tumour immunosuppression come into play[34,35]. Indeed, active suppression of antitumour immune responses, either directly via upregulation of antagonistic factors on tumour cells, or indirectly by recruitment of anti-inflammatory immune-cell subtypes, further complements tumour immune evasion. Thus, by orchestrating a complex interplay of immunosuppressive factors, cancer cells can effectively silence the antitumour immune response in their immediate environment[36].

Immune surveillance of CTCs and DTCs

Over the past decade, detailed characterization of antitumour immune responses in clinical studies has provided irrevocable evidence for the important influence of the immune system on the course of disease[15]. Herein, we have focused our discussion on the interaction of CTCs and/or DTCs with the leukocyte populations mediating immune surveillance (FIG. 1).

Innate surveillance by NK cells and macrophages. NK cells are the type of immune cell that has been investigated most prominently in the context of tumour–host interactions and immune surveillance. NK cells are lymphocytic cells that form an integral part of the innate immune system and contribute to immune defense against neoplastic cells and viruses[37]. The interaction of NK cells with tumour cells is controlled by a complex network of receptors and ligands, including major histocompatibility complex class I (MHC I)-related inhibitory molecules[38]; downregulation or loss of MHC I and/or MHC I-related protein expression on tumour cells, which has also been observed on DTCs in bone marrow[39], prevents inhibition of NK cells, thus enabling subsequent lysis of the tumour cell through release of cytolytic granules and induction of apoptosis. Activation of NK cells is antigen-independent; therefore, innate NK-cell responses complement adaptive, tumour-specific T-cell responses in tumour immune surveillance. The cytotoxic and cytolytic capacities of

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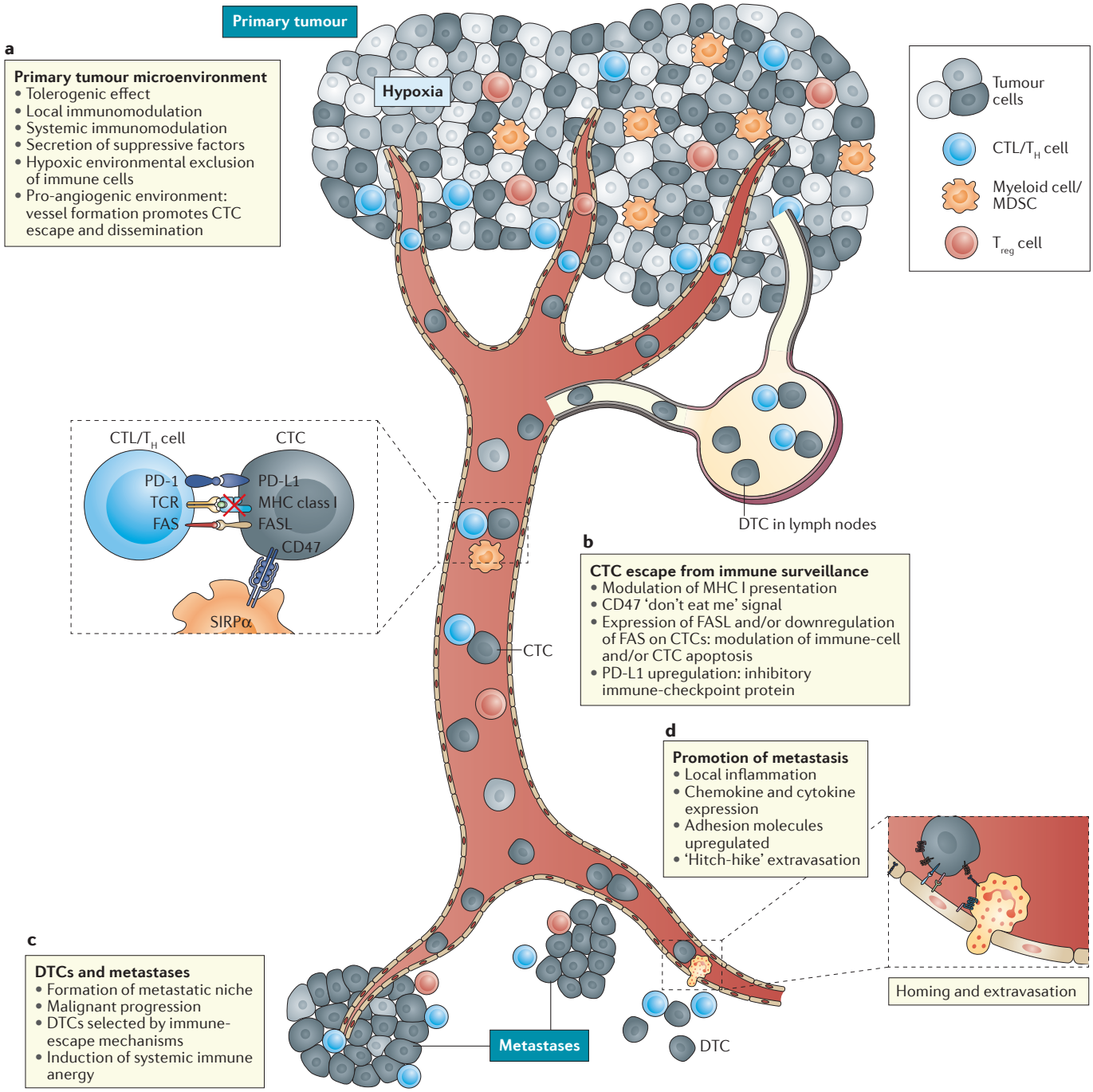

Figure 1 | **Systemic immunomodulation and immune escape in the metastatic cascade.** This figure depicts the key factors influencing cancer cell–immune cell interactions within the primary tumour microenvironment (including the local lymph nodes), within the circulation, and within the metastatic niche throughout the metastatic cascade. **a** | Mechanisms active in the primary tumour restrain antitumour immune responses by modulating the local microenvironment and creating an immunosuppressive milieu. Secreted cytokines and chemokines, which orchestrate the induction of T-cell anergy and exhaustion, inhibit tumour-cell killing. In addition to other known immune-escape mechanisms (including expression of immune-checkpoint proteins, such as CD47 and PD-L1), and loss of MHC expression, adaptation to hypoxia enables cancer cells to 'hide' in hypoxic areas in which immune-effector functions are impaired. **b** | Circulating tumour cells (CTCs) under immune surveillance in the peripheral blood encounter immune cells through direct cell–cell interactions and are subject to immune-mediated elimination. Escape mechanisms involving expression of CD47, PD-L1, FAS, and FASL, as well as alterations affecting classic and nonclassic HLA/MHC molecules promote the survival of CTCs in the blood; CTCs that are unable to counteract immune-mediated killing will not survive (very light grey cells). Thus, presumably, the immune system selects for clonal variants with an immune-escape phenotype (dark grey and black cells). **c** | In the process of homing to secondary locations, CTCs and DTCs can interact directly with immune cells. **d** | Indeed, direct contact between cancer cells and immune cells, on a background of local inflammation, can enhance cell recruitment, adhesion, extravasation and survival, thereby supporting the formation of metastasis. CTL, cytotoxic T lymphocyte; CTC, circulating tumour cell; DTC, disseminated tumour cell; FASL, FAS ligand; MDSC, myeloid-derived suppressor cell; MHC I, MHC class I; PD-1, programmed cell-death protein 1; PD-L1, programmed cell death 1 ligand 1; SIRPα, signal-regulatory protein α; TCR, T-cell receptor; T_H, T-helper; T_{reg}, T-regulatory.

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NK cells from CTC-positive patients with metastatic breast, colorectal, or prostate cancer are decreased compared with those of cells from CTC-negative patients, with a close correlation between peripheral CTC load and NK cell activity[40,41]. Paradoxically, overall NK-cell counts are increased in many patients with cancer[40,41], but whether this finding reflects a direct response to the presence of CTCs, or a compensatory mechanism to counteract the inhibition of NK-cell effector functions remains unclear. Proposed mechanisms underlying the suppression of NK-mediated antitumour activity include direct inhibition of NK cells through cell–cell contact, mediated by killer-cell immunoglobulin-like receptors (KIR) or the pathway involving the E3 ubiquitin-protein ligase CBL-B, and indirect inhibition via the production of inhibitory cytokines, such as interleukin (IL)-1β, IL-8, IL-10, and prostaglandin E_2 (PGE_2)[42,43].

The potential immunosuppressive effects of CTCs are, however, not limited to diminished NK-cell cytolytic capacity. In addition, CTCs have been associated with altered expression of Toll-like receptors (TLR) on other immune-cell populations. Studies have revealed decreases in TLR2 and TLR4 expression levels[41], and general dysregulation of TLR expression on a variety of immune cells in patients with detectable CTCs versus that observed in those without CTCs, indicating a more widespread response to the presence of malignant cells in the peripheral blood[44]. These findings suggest that the diminished NK-cell activity in patients with cancer might be manifested at multiple levels by the presence of CTCs because stimulation of TLRs expressed on monocytes and monocyte-derived dendritic cells (DCs) results in cytokine-mediated initiation of NK-cell activation[45,46]. In addition, NK cells can also express TLRs, at variable levels, and thus downregulation of TLRs might impede the functionality of NK cells directly[47]. Whether the observed changes in TLR expression are a consequence of a systemic immune response to a 'danger signal' associated with cancer, or a result of specific immune modulation by the tumour or CTCs remains unclear.

NK cells might be capable of intercepting CTCs and thus blocking the establishment of metastases[48]. Simulation of this process in a colon cancer model showed that perforin-dependent, NK-cell-mediated cytotoxicity is more efficient than indirect killing by the secretion of cytotoxic factors in limiting CTC numbers and restraining the expansion of DTCs — indicating the advantage, or even necessity, of direct cell–cell contact for elimination of CTCs[49]. Nevertheless, NK cells cause tumour-cell lysis via secretion of tumour necrosis factor (TNF)-related apoptosis inducing ligand (TRAIL; also known as TNF ligand superfamily member 10)[50]. Interestingly, Mitchell *et al.*[51] have developed a means of modifying peripheral blood leukocytes by coupling them to TRAIL-coated liposomes via E-selectin, in order to create 'unnatural killer cells' that can efficiently lyse CTCs and reduce metastatic burden in mouse models.

Together with NK cells, macrophages have a critical role in controlling metastatic progression. In the context of immunotherapeutic approaches using tumour-specific antibodies, the macrophage response is crucial for antibody-dependent phagocytosis of CTCs[52]. This process is mediated mostly by macrophages resident in the liver[52,53], consolidating early observations regarding the central role of Kupffer cells in intercepting CTCs in an antibody-independent manner[54]. In a study by Denève *et al.*[55], comparisons of CTC counts between peripheral and mesenteric blood samples from patients with colorectal cancer confirmed that a considerable proportion of the viable CTC population seems to be filtered and trapped in the liver. These findings highlight the prominence of the liver microenvironment in mediating the outcome of interactions between tumour cells and immune cells, which often promotes tumour-cell death, but sometimes facilitates DTC survival and growth[56]. Indeed, cytotoxic sinusoidal lymphocytes interact closely with resident Kupffer cells and enhance their capacity to eliminate a large fraction of CTCs and/or DTCs[54]. These functions of the liver — and potentially other organs — probably derive from its anatomical portal capillary bed structure, which enhances the possibility of direct cell–cell interactions between immune and cancer cells.

T-helper cells and cytotoxic T cells. Several investigations have demonstrated positive correlations between TIL counts and overall survival in patients with melanoma, breast, oesophageal, ovarian, or colorectal cancers[57–59]. Additional work has revealed that patients with cancer can spontaneously develop a mostly T-cell-driven antitumour immune response that is associated with a favourable outcome[60]. In contrast to NK-cell surveillance of CTCs, which has been reported in many studies, the role of the adaptive immune system in controlling peripheral tumour-cell dissemination is less clear.

Reports have provided circumstantial evidence that CTCs can affect antitumour responses and the efficiency of adaptive immune surveillance[61–63]. De Giorgi *et al.*[61] demonstrated that lymphocytopenia and increased numbers of CTCs (≥5 cells per ml of blood) are independent risk factors associated with decreased progression-free survival and overall survival in patients with metastatic breast cancer. These data indicate that lymphocytopenia contributes to tumour progression, potentially by facilitating CTC persistence. Interestingly, investigators of two studies in patients with breast cancer have described alterations in the $CD4^+$ T-helper-cell compartment that are associated with the presence of CTCs in peripheral blood[62,63]. For instance, flow cytometric assessments performed by Gruber *et al.*[62] revealed a statistically significant increase in the number of $CD4^+$ T cells expressing apoptosis-mediating surface antigen FAS (also known as TNF receptor superfamily member 6) in patients who were CTC-positive versus those who were CTC-negative ($P = 0.042$). This finding could potentially indicate a shift towards a pro-apoptotic phenotype and vulnerability to tumour-induced immunosuppression, via activation of T-cell death via binding of FAS to its cognate ligand FASL presented by tumour cells (or other cell types)[62]. Whether this immunosuppressive mechanism is mediated directly by CTCs is an important question for upcoming functional studies. Another possibility is that

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the peripheral immunosuppression is mediated by the primary tumour itself, thus rendering CTCs a peripheral biomarker of tumour-mediated immunosuppression in the primary tumour microenvironment. Addressing both of these possibilities will result in major advances in understanding the role of immunosurveillance in the initial steps of tumour-cell dissemination.

Immune-evasion mechanisms of CTCs/DTCs

CTCs leaving the immunosuppressive primary tumour microenvironment become exposed to the active immunosurveillance mechanisms that are operative in noncancer tissues. In addition, the possibility that CTCs will be lysed by tumour-specific immune cells increases markedly outside the immunosuppressive sanctuary of the tumour because peripheral immune cells far outnumber CTCs. The circulatory system can, therefore, be considered a hostile environment for cancer cells. The fact that primary tumours are predicted to shed thousands of cells into the bloodstream every day[64], but evidently only a very small percentage develop the ability to grow into distant metastases supports this conclusion. Nevertheless, studies have identified new pathways by which CTCs might evade or survive encounters with immune cells (FIG. 2). We could not describe all potential mechanisms of immune evasion in detail herein; therefore, we have focused on the most-established mechanisms in the context of CTC/DTC evasion.

Classic MHC molecules and NK-cell ligands. MHC I molecules expressed on the surface of essentially all nucleated cells present peptide epitopes that are processed from intracellular proteins for interrogation by immune cells. Indeed, presentation of tumour-associated antigens (TAAs) to T-cell receptors (TCR) in the context of MHC I molecules is crucial for initiation of an adaptive $CD8^+$ CTL response[65]. Thus, downregulation or even complete loss of MHC I expression at the cell surface is a mechanism used by tumour cells to 'hide' from CTLs and thereby evade death[66] (FIG. 2). As a biological 'backup' to counteract this mechanism, NK cells become

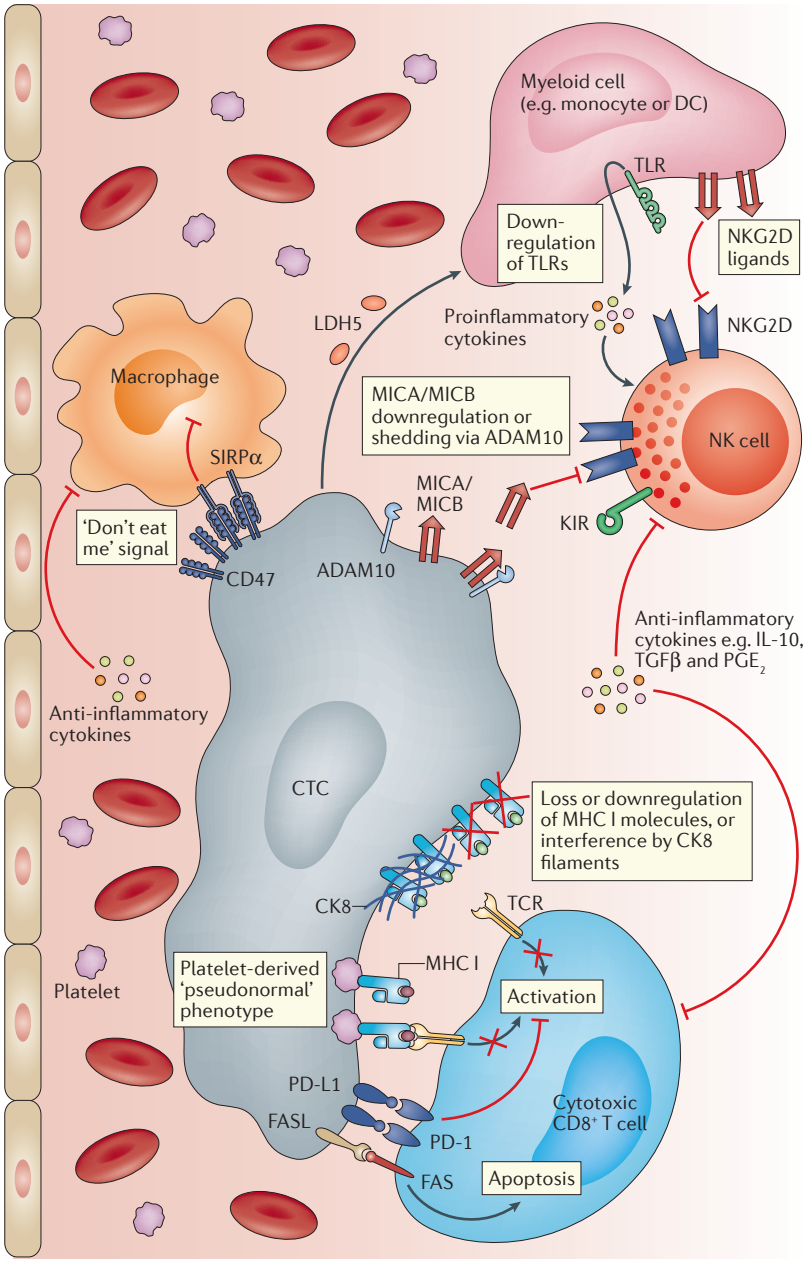

Figure 2 | **Immune-escape mechanisms of CTCs in the peripheral blood.** The schematic illustrates the potential mechanisms of immune escape of CTCs, and the interactions between CTCs and immune cells in the peripheral blood. The interplay between CTCs and NK cells is highlighted, with secretion of LDH5 and ADAM10-mediated shedding of the NKG2D ligand MICA/MICB from CTCs preventing recognition and elimination of the cells via NK-cell-mediated lysis. LDH5 exerts this effect indirectly by upregulating the expression of NKG2D ligands on circulating monocytes, which results in downregulation of NKG2D expression on NK cells. Three different strategies of CTC escape from MHC I-mediated recognition by NK cells and T cells are depicted: interference with TCR recognition of MHC I molecules by cell-surface-bound cytokeratins (CK8, CD18, and CK19); acquisition of a 'pseudonormal' phenotype resulting from membrane transfer from platelets to CTCs; and the downregulation or loss of MHC I expression. Additional mechanisms of immune escape include expression of the inhibitory immune-checkpoint protein PD-L1, presentation of the 'don't eat me' signalling receptor CD47, and an altered expression of the apoptotic proteins FAS and/or FASL. ADAM10, disintegrin and metalloproteinase domain-containing protein 10; CK8, cytokeratin 8; CK18, cytokeratin 18; CK19, cytokeratin 19; CTC, circulating tumour cell; DC, dendritic cell; FASL, FAS ligand; IL-10, interleukin 10; KIR, killer-cell immunoglobulin-like receptor; LDH5, lactate dehydrogenase 5; MHC I, MHC class I; MICA, MHC I polypeptide-related sequence A; MICB, MHC I polypeptide-related sequence B; NK, natural killer; NKG2D, NK-cell receptor D (also known as NKG2-D type II integral membrane protein); PD-1, programmed cell-death protein 1; PD-L1, programmed cell death 1 ligand 1; PGE_2, prostaglandin E_2; SIRPα, signal-regulatory protein α; TCR, T-cell receptor; TGFβ, transforming growth factor β; TLR, Toll-like receptor.

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activated when MHC I molecules are underexpressed or absent[38]. Thus, to escape both NK-mediated and CTL-mediated cytotoxicity, CTCs must find a way to present MHC I molecules without presenting TAAs. In an early study, investigators analysed DTCs from bone-marrow aspirates and demonstrated a differential MHC I expression in DTCs from patients with breast cancer versus those with colon or stomach cancer: absence of MHC I expression was more frequently observed in patients with breast cancer, which is consistent with the high rate of overt bone metastasis associated with this disease[39]. Furthermore, the correlation between lower levels of MHC I expression and higher tumour grade, as well as with patterns of metastasis, indicates that MHC molecules are relevant to the metastatic behaviour of CTCs and/or DTCs[39]. Of note, downregulation of MHC I expression seemed to be associated with an increased propensity for regional lymph-node metastasis, independent of the primary carcinoma of origin[39].

In addition, findings by Placke *et al.*[67] indicate that tumours can acquire a 'pseudonormal' phenotype, conferred by the transfer of platelet-derived MHC I molecules to the tumour cells (FIG. 2): the tumour cells integrate MHC I-containing membrane vesicles from platelets into their own cell membrane, thereby using the 'normal' platelet phenotype to escape NK-cell-mediated cytotoxicity. This finding built on established roles of platelets in supporting cancer metastasis[68,69]. Other researchers have extended this work by studying the effects of platelet activation in the metastatic process in mice deficient for fibrinogen, $G_{\alpha q}$, protease-activated receptors, or the transcription factor NF-E2 — which are all involved in platelet production or activation of coagulation[70,71]. Therein, abrogation of platelet or fibrinogen function resulted in decreased metastatic spread, and this effect was dependent on the presence of NK cells[70,71]. These studies demonstrate, therefore, that tumour cells can interact with haemostatic factors to escape immune surveillance. More recently, Leblanc and Peyruchaud[72] reviewed the mechanisms by which platelets initiate and maintain epithelial-to-mesenchymal transition (EMT) of CTCs (by secretion of TGFβ), and support the homing to and outgrowth of DTCs in bone marrow.

Cytokeratin 8 (CK8), together with its heterodimeric partners CK18 and CK19, has been demonstrated to inhibit MHC I interactions with TCRs on $CD8^+$ CTLs[73]. In particular, CK8/18 and CK8/19 expression on the surface of a lymph-node derived metastatic carcinoma cell line masked MHC I contact motifs for TCRs[73]. Overexpression of these cytokeratins has long been observed in malignant tissues[74]. This mechanism illustrates how cancer cells evolve new methods of immune evasion, and that interference with MHC-mediated antigen presentation seems to be a crucial approach to immune escape.

In the context of neotransformation, stress, or complete loss of MHC I molecules, NK cells can mediate cancer-cell cytotoxicity through NK-cell receptor D (NKG2D) engagement of stress-induced ligands, such as MHC I polypeptide-related sequence A (MICA)/MICB, which are usually expressed solely on virus-infected and/or neoplastic cells[75]. Correspondingly, reduced expression of MICA/MICB in stem-like breast cancer cells is associated with increased resistance to NK-cell-mediated cytotoxicity[76] (FIG. 2). Downregulation of MICA/MICB in these cancer cells is mediated by aberrant expression of an oncogenic microRNA, miR20a[76]. Interestingly, hypoxic cells, which are known to be more resistant to chemotherapy and radiotherapy, adopt similar immune-escape mechanisms: expression of the hypoxia-inducible factor 1α (HIF-1α) increases the cell-surface expression of disintegrin and metalloproteinase containing-domain protein 10 (ADAM10), which can cleave MICA/MICB from the surface of prostate cancer and breast cancer cell lines[77]. As a consequence, hypoxia contributes to cancer-cell resistance to NK-cell-mediated elimination and, therefore, immune escape[77].

A third pathway for cancer-cell evasion of NK-cell-mediated cytotoxicity has been discovered, wherein glioblastoma cells can induce NKG2D-ligand expression on tumour-infiltrating myeloid cells and circulating monocytes via secretion of lactate dehydrogenase 5 (LDH5)[78]. The LDH5-mediated induction of NKG2D ligands on myeloid cells results in activation and subsequent downregulation of NKG2D on NK cells, thereby subverting antitumour immune responses[78]. Similarly, the aberrant presence of NKG2D ligands on circulating monocytes can also be detected in patients with breast or prostate cancers, or hepatitis C virus-induced hepatocellular carcinoma[78].

In 2015, another contrasting mechanism of NKG2D interference was described in mice, wherein a soluble MHC I-related NKG2D ligand (Mult1) stimulates, rather than antagonizes, NK-mediated antitumour responses, reversing the global desensitization of this cell type and resulting in tumour rejection[79]. This study thus identifies a potential approach to restoring antitumour responses using soluble ligands. Wang *et al.*[76] and Barsoum *et al.*[77], the authors who described the escape mechanisms relating to MICA/MICB downregulation mediated by miR20a and ADAM10-dependent shedding, respectively, went one step further by demonstrating the potential to reverse these effects therapeutically using all-*trans* retinoic acid and a nitric oxide mimetic (nitroglycerin), respectively, in breast cancer and prostate cell lines. When or if these approaches will be explored in clinical trials remains uncertain; nonetheless, immunotherapeutic strategies to counteract the mechanisms of tumour immune escape are receiving increasing attention.

Nonclassic MHC molecules. In addition to the aforementioned roles of classic MHC I molecules, encoded by the human leukocyte antigen (HLA) genes *HLA-A*, *HLA-B*, and *HLA-C*, important mechanisms of tumour immune escape have been attributed to nonclassic MHC I molecules[80]. Among the nonclassic MHC I genes, *HLA-G* is most prominently implicated in cancer invasiveness, immune escape, and metastatic progression[81]. *HLA-G* is highly expressed in numerous malignancies, including melanoma, glioma, and colorectal, hepatocellular, breast, lung, and ovarian cancers[82–86]; expression of HLA-G is associated with poor patient survival and has been confirmed as independent prognostic factor[82–85]. HLA-G

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exerts its functions through binding to a multitude of receptors expressed on immune cells, including KIRs, CD8, and leukocyte immunoglobulin-like receptor subfamily B member 1 (LIR-1); binding to these co-receptors results in protection of the HLA-G-expressing cell from immunocytotoxicity mediated by NK cells and T cells, and induces an anti-inflammatory immune phenotype[80,81,87,88]. Furthermore, soluble HLA-G (sHLA-G), which is produced by alternative splicing and lacks the transmembrane domain, can be secreted by cells of the primary tumour and functions as a systemic modulator of antitumour responses[89]. König *et al.*[90] demonstrated a direct link between HLA-G levels, the presence of CTCs, and clinical outcomes of patients with breast cancer after neoadjuvant chemotherapy (NACT): high pre-NACT plasma levels of sHLA-G in extracellular vesicles, but not free sHLA-G, were associated with the presence of stem-cell-like CTCs and disease progression. In addition, elevated levels of both free (>24 ng/ml) and vesicle-bound (>15 ng/ml) sHLA-G were associated with unfavourable clinical outcomes after NACT[90]. These findings indicate that CTCs exploit sHLA-G derived from extracellular vesicles in the blood to escape NK-cell-mediated elimination. Future studies are needed to unravel the functional role of HLA-G as a critical mediator of CTC survival in the peripheral blood, and as a key player in the evasion of cytotoxicity mediated by NK cells and T cells.

FAS/FASL-induced apoptosis. The FAS/FASL apoptotic pathway is highly relevant to immune evasion. The transmembrane receptor FAS can initiate apoptosis, and activation of this receptor on T cells via binding to FASL is a proposed mechanism of tumour-mediated immunosuppression in various malignancies[91]. Histopathological analyses have revealed that FASL is upregulated in metastases compared with the primary tumour in patients with melanoma or colorectal cancer[92,93]. Furthermore, in patients with breast cancer, upregulation of FASL has been correlated with increased apoptosis of T cells[94]. FASL expression on tumour cells might, therefore, actively induce apoptosis in immune cells. Vice versa, tumour cells that express FAS will probably be vulnerable to apoptosis evoked by tumour-specific immune cells, which can also express FASL. Thus, simultaneous loss or downregulation of FAS and upregulation of FASL on tumour cells might contribute to tumour evasion of immune-mediated cytolysis and increase the potential for metastatic progression[95–97].

In the first investigation of a putative role of FASL on CTCs in immune evasion, the expression of FAS on peripheral lymphocyte subsets from patients with primary breast cancer was analysed, and was found to be upregulated on $CD4^+$ T-helper cells in the presence of CTCs[62]. These findings support those of earlier studies suggesting that $FASL^+$ tumour cells can deplete activated FAS-expressing immune cells[93]. Furthermore, other investigations have revealed that $FAS^+/CD8^+$ CTLs are significantly more prevalent in patients with breast cancer than in women without this disease ($P<0.05$)[98]. Interestingly, evidence indicates that FAS-mediated apoptosis of $CD8^+$ CTLs, but not $CD4^+$ T cells, can also be induced by $FASL^+$ regulatory T (T_{reg}) cells[99]. An additional mechanism potentially underlying tumour immune escape involves soluble FAS and/or FASL molecules: these soluble forms can protect tumour cells against FAS-mediated apoptosis, and increased levels of soluble FAS are associated with a poor prognosis in patients with melanoma[100–102]. The extent to which surface expression of FAS and/or FASL, or soluble forms of these proteins can protect CTCs from elimination by immune cells remains incompletely understood; however, the accumulating evidence of metastasis-promoting effects suggests a pivotal role of the FAS/FASL pathway in CTC immune escape[103].

CD47-mediated 'don't eat me' signalling. Overcoming the physiological process of programmed cell removal — in addition to programmed cell death — marks a key step in cancer development. Detailed studies by Irving Weissman and colleagues[104] have highlighted the role of the leukocyte surface antigen CD47 in cancer, particularly, in cancer-cell evasion of phagocytic clearance. CD47 binds to its ligand signal-regulatory protein α (SIRPα, also known as macrophage fusion receptor), which is expressed on macrophages and dendritic cells, consequently inhibiting phagocytosis by these cell types[105]. Thus, upregulation of CD47, an antiphagocytic 'don't eat me' signal, might confer CTCs with a non-immunogenic profile by enabling them to escape the consequences of cell-damage-induced upregulation of pro-phagocytic signals and, therefore, the immune sequelae evoked after CTC recognition in the context of adaptive immunity[104]. Steinert *et al.*[106] have compared the gene-expression profiles of primary tumours and CTCs from patients with colorectal cancer. Notably, CD47 was the only gene upregulated in CTCs versus the matched primary tumours, indicating a survival advantage conferred by CD47 expression for peripheral blood CTCs[106]. The role of CD47 might be even more pronounced in patients with non-Hodgkin lymphoma: in mouse models of this disease, tumour-cell expression of CD47 is required for extranodal dissemination, and this process can be inhibited by anti-CD47 antibodies[107]. Moreover, expression of CD47, together with CD44, epithelial cell-adhesion molecule (EpCAM), and MET, was detected in CTC populations from patients with metastatic breast cancer, and the number of CTCs co-expressing CD47 and MET was correlated with decreased survival and increased numbers of metastatic sites[108]. Furthermore, an increased frequency of $CD47^+/MET^+$ CTCs among all CTCs detected in patients with luminal breast cancer was not only associated with more metastatic sites and disease progression, but also negatively correlated with prognosis[109]. Together, these findings suggest that CD47 is part of a potential metastasis-initiator cell signature, but intense functional analysis is required to delineate the exact role of CD47 expression on CTCs.

Hypoxia-induced immune escape. A number of genes that are expressed during EMT, in cancer-stem cells, or in response to hypoxia have been shown to be upregulated in CTCs, and many of the encoded proteins are able

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to modulate the immune response[110]. Specific metabolic and molecular alterations enable DTCs to adapt to and survive in a microenvironment with a lower oxygen concentration, such as the bone marrow. Findings of studies investigating HIF-1α expression in CTCs and functional studies on DTCs from the bone marrow of patients with breast, lung, or prostate cancer indicate that many CTCs and DTCs display a hypoxia-associated phenotype, and can efficiently adapt to hypoxic conditions[111,112]. For instance, upon hypoxic stress, glucose-regulated protein 78 (Grp78) is upregulated in cell lines established from the bone marrow of patients with cancer, and expression of Grp78 is associated with mesenchymal attributes and poorly differentiated primary breast and lung tumours[113]. Whether adaptation to hypoxia also promotes CTC and/or DTC evasion of immune cells is currently under investigation; nevertheless, the hypoxia-resistant phenotype of DTCs certainly has implications for immunotherapeutic strategies. For example, elimination of such DTCs might be challenging, owing to the ineffective execution of immune effector functions in hypoxic niches. In light of the increasing efforts to generate chimeric antigen receptor (CAR) T cells and NK cells for use in cell-based immunotherapeutic strategies[114], consideration of additional engineering of hypoxia-resistance into forthcoming CAR T-cell or NK-cell designs would be desirable, in order to support execution of their immune effector functions against DTC in hypoxic microenvironments.

Immune checkpoints. Releasing the brakes on anti-tumour immune responses by targeting immune-checkpoint molecules has been the primary focus of immunotherapeutic strategies[115]. Such strategies aim to overcome the anergy of tumour-specific effector T cells induced via immunosuppressive immune-checkpoint pathways. Programmed cell-death protein 1 (PD-1) and its ligand (PD-L1) are the most prominent examples of immune-checkpoint molecules that underlie this immune-escape mechanism. PD-L1 can be expressed by tumour cells or other cells in the tumour microenvironment, and transmits inhibitory signals via PD-1 expressed on T cells, thereby limiting immune effector functions[116]. Within the immunological synapse, a multitude of alternative inhibitory receptors have been identified, including cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and related B7 family members, and galectin-9 (REF. 117).

The associations between the biology of EMT and CTCs remains under investigation, but the presence of CTCs undergoing EMT seems to be associated with poor clinical outcomes[118,119]. Interestingly, evidence indicates that crosstalk occurs between regulators of EMT and PD-L1 (REFS 120–122). Currently, however, no information is available on PD-L1 expression on CTCs undergoing EMT, as compared with those with an epithelial phenotype. Nevertheless, patients with primary head-and-neck squamous cell carcinomas (HNSCCs) that co-express PD-L1 and EMT markers have a worse clinical outcome than patients with tumours that express either PD-L1 or EMT markers alone[123]. This observation suggests that immune-checkpoint inhibitors might mitigate the additive effect of PD-L1 and EMT on the clinical outcomes of patients with cancer. Moreover, CD44 expression in HNSCCs is indicative of not only a stem-cell-like phenotype, but also suppression of T-cell-mediated immunity and induction of PD-L1 expression[124]. How secretion of EMT-inducing cytokines by immune cells in the tumour microenvironment affects PD-L1 expression on tumour cells released into the circulation is a focus for ongoing research[125,126].

The idea of disabling immune-escape mechanisms on tumour cells using immune-checkpoint inhibitors is now being explored in many clinical trials[116]. Efforts to restore latent antitumour immunity are focused on antibody-based interventions targeting CTLA-4 and/or PD-1 on T cells, or PD-L1 on tumour cells (or other cells in the tumour microenvironment)[115,127]. The anti-CTLA-4 antibody ipilimumab was approved by the FDA for the treatment of advanced-stage melanoma in 2011. More recently, two antibodies targeting PD-1 (pembrolizumab and nivolumab) have also been approved for the treatment of advanced-stage melanoma, as well as advanced-stage non-small-cell lung cancer. In May 2016, nivolumab was also approved for the treatment of patients with Hodgkin lymphoma, and the anti-PD-L1 antibody atezolizumab was approved for treatment of urothelial carcinoma. In addition, multiple other targets for immune-checkpoint blockade therapy, such as lymphocyte-activation gene 3 (LAG3) and CD276 (B7-H3), are currently being tested in phase I and phase I/II clinical trials involving patients with a variety of malignancies[115]. Additional immunomodulatory antibodies directed at proteins such as KIR, which can augment NK-cell-mediated tumour-cell lysis, could further expand the spectrum of immune-checkpoint blockade[128,129].

In view of the high costs and the toxicity profiles of these therapies, predictive biomarkers that can be used to discriminate responders from nonresponders are urgently needed. In this context, CTC PD-L1 assays should be tested as a potential 'liquid biopsy' approach for stratification and monitoring of patients with cancer undergoing immune-checkpoint blockade. Mazel *et al.*[130] have shown that PD-L1 expression on CTCs in patients with metastatic breast cancer is common, but heterogeneous. This finding highlights the potential for assessment of PD-L1 expression on CTCs as a novel biomarker to be tested in future trials of immune-checkpoint inhibitors[131]. Heterogeneous PD-L1 expression on CTCs has also been observed in oral squamous cell carcinoma[132]. The extent to which PD-L1 expression protects CTCs from antitumour immunity, and whether other mechanisms of immune escape are exploited by CTCs/DTCs are areas of ongoing investigation.

Promotion of metastases by immune cells

Findings indicate that metastasis can be supported by immune cells. Immune cells can, therefore, be regarded as both heroes and villains in the metastatic process[26,133–135]. Herein, we will focus on reports of the metastasis-promoting roles of the immune system that have included measurements of CTCs.

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Regulatory T cells. Evidence supports a role for T_{reg} cells not only in the establishment of immunosuppression in the primary tumour, but also tumour-cell dissemination and the formation of metastases. For example, in addition to earlier studies describing an increased prevalence of T_{regs} cells in patients with various malignancies[136], investigations in patients with advanced-stage melanoma have revealed a correlation between disease progression and the frequencies of both myeloid-derived suppressor cells (MDSCs) and T_{reg} cells in the peripheral blood, which were associated with increased circulating levels of IL-1β, IFNγ, and CXCL10[137]. More specifically, the expression of IL-1β and a concurrent increase in numbers of T_{reg} cells in the peripheral blood might be strongly predictive of a poor clinical outcome[138]. Future investigations of tumour-specific T cells are required to demonstrate whether this systemic state of immunosuppression is restricted to the tumour-specific immune response, or alternatively reflects a general state of immune alteration. Nevertheless, the parallel observation of disease progression and the occurrence of CTCs in the context of recruitment of T_{reg} cells and MDSCs has been reported in several studies[134,137,139]. It seems logical, therefore, that suppression of the peripheral antitumour immune response can support the survival of DTCs, and thus promote the formation of metastasis. This hypothesis might be especially relevant to highly immunogenic cancers, such as melanoma, wherein defined TAAs with well-described potency to elicit an adaptive T-cell response have been delineated, whereas the immunogenicity of epithelial cancers or primary brain tumours is not so clear. Whether T_{reg}-cell activation is a consequence of metastatic disease, or whether T_{reg} cells pave the way for occurrence of CTCs (and subsequently metastasis) remains unclear — akin to the 'chicken or egg' causality dilemma. Further studies are needed to unravel this relationship and determine causality. Stanzer *et al.*[140] have evaluated the effect of CTCs on immune dysfunction and on the numbers of T_{reg} cells in the peripheral blood of patients with metastatic epithelial cancer compared with healthy individuals. They detected increased numbers of T_{reg} cells in the peripheral blood and an elevated proportion of apoptotic non-T_{reg} $CD4^+$ and $CD8^+$ T-cells in the patients with cancer, but, surprisingly, no correlation between the prevalence of T_{reg} cells or other cell populations and CTC counts. However, the authors highlighted the restricted sensitivity of their CTC-detection method, which limits the statistical evaluations of these two rare cell populations (CTCs and T_{reg} cells) in the peripheral blood.

Interestingly, first direct associations between T_{reg} cells, MDSCs and CTCs were described in patients with metastatic breast cancer in 2014, with a significantly higher frequency of $CD4^+$ T_{reg} cells in the peripheral blood of CTC-positive patients compared with that of CTC-negative patients ($P = 0.022$)[141]. Moreover, patients with CTCs of a stem-like phenotype had higher numbers of T_{reg} cells than those with CTCs lacking expression of stem-cell markers ($P = 0.023$), whereas the latter group had higher levels of MDSCs ($P = 0.02$). More recently, Hensler *et al.*[142] compared gene-expression profiles of enriched CTCs from patients with breast cancer to those of PBMCs from healthy individuals and identified downregulation of the genes encoding mTOR, PARP, and MYC proteins, and upregulation of FOXO3, among others in the CTC-enriched samples. Whether these genes are upregulated or downregulated on PBMCs or the CTCs themselves is unclear. Nevertheless, all of these genes could potentially be involved in the induction of immune tolerance[142]; for example, downregulation of PARP in T_{reg} cells is associated with increased activity of this cell type[143]. Additional functional assays, together with enhanced CTC-detection assays using improved marker panels, CTC culturing, or novel enrichment techniques, will certainly help to unravel the influence on the systemic T_{reg}-cell activation on tumour-cell dissemination[119].

Promotion of CTC seeding. Initial studies of CTCs have demonstrated a positive correlation between metastasis formation and an acute inflammatory state in the target organ of metastatic spread. For example, using an allergic pulmonary inflammation model, Taranova *et al.*[144] showed that CTCs exploit the increased vascular permeability and expression of adhesion molecules at the metastatic site to extravasate and form tumour filiae. In this study, the recruitment of CTCs to the lung required the presence of $CD4^+$ T cells at the site of metastasis, and application of the immunosuppressive corticosteroid budesonide fully abrogated increased pulmonary metastasis following allergen-induced inflammation[144]. In line with these observations, data from a murine colorectal cancer model showed a positive correlation between serum levels of IL-17A, an exemplary proinflammatory cytokine, and the frequency of CTCs[145]. In this model, depletion of IL-17A resulted in reduced intratumoural microvasculature density and decreased metastases formation[145]. In addition, the presence of IL-17A increased tumour-cell motility by inducing MMP-9 expression in CTCs, thereby potentially supporting CTC mobilization and extravasation[145].

Primary-tumour-derived factors, such as VEGF, TGFβ, TNF, and others, can induce modifications of the premetastatic niche that lead to infiltration of myeloid cells and haematopoietic bone-marrow-derived progenitor cells, which can subsequently support metastatic seeding. For example, induction of fibronectin expression can promote the recruitment of myeloid cells expressing very late antigen 4 (VLA4), thus leading to infiltration of myeloid cells to the premetastatic niche, where they can form clusters and promote CTC seeding[135,146,147]. The quality (and/or extent) of immune activation determines the exact outcome, however, as demonstrated by the fact that increased serum level of granulocyte macrophage colony-stimulating factor (GM-CSF) stimulates, rather than prevents the elimination of CTCs. By contrast, using a transgenic murine model in which expression of NF-κB in macrophages could be induced or suppressed, Connelly *et al.*[148] demonstrated that activation of NF-κB instills macrophages with an antitumour phenotype and leads to a marked reduction of lung metastasis in a mammary tumour model.

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Box 1 | **Immune system and tumour-cell dissemination: unresolved questions**

- Does the presence of circulating tumour cells (CTCs) reflect the inability of the immune system to mount an efficient antitumour immune response?
- Can CTCs and/or disseminated tumour cells (DTCs) arise only if the tumour has acquired mechanisms of immune escape?
- Does the immune system shape the ability of CTCs and DTCs to survive and form metastases in an immunocompetent host?
- Does tumour-cell dissemination and successful homing to secondary organs require systemic immunosuppression?
- Can CTCs be useful as biomarkers for the expression of immune-checkpoint molecules (such as programmed cell death 1 ligand 1 (PD-L1)) on primary tumour cells?
- Do CTCs represent a specialized subpopulation of tumour cells with low immunogenicity and other unique characteristics, for example, metabolic adaptation to hypoxia?
- To what extent does the immune system control cancer dormancy? If immune responses do regulate this phenomenon, which changes in the immune system lead to escape from dormancy and outgrowth of metastases?
- Do immune cells that infiltrate the primary tumour affect the rate of CTC release?
- Is the DTC–immune-cell interaction in the process of metastatic spread a continuum of the 'three E' paradigm of cancer immuno-editing — elimination, equilibrium, and escape: that is, should 'expansion' of DTC populations be regarded as a fourth step (elimination, equilibrium, escape, and expansion)?

Highlighting the close relationship between leukocytes and CTC recruitment to inflamed tissues, evidence suggests that CD44 can be expressed on the surface of both haematopoietic stem cells and cancer cells[108]. CD44 serves as a ligand for the endothelial adhesion molecule E-selectin and/or its counterpart expressed on leukocytes, L-selectin, which mediates the tethering and rolling stages of the leukocyte adhesion cascade. The fucosylated isoform of CD44 found on these cell types is known as haematopoietic cell E-/L-selectin ligand (HCELL)[149]. The finding of tumour-cell expression of CD44, together with the observation that CTCs can interact with E-selectin under physiological flow conditions[150], strengthens the hypothesis that CTCs can mimic haematopoetic cell trafficking in the process of metastatic seeding[151].

CTCs utilize immune cells to hitchhike. Data from a study using a murine lung carcinoma model suggest the direct involvement of neutrophils in the process of metastatic homing of tumour cells to the liver[152]. Moreover, *in vitro* analyses indicate a role for reactive oxygen species (ROS) in the neutrophil-mediated enhancement of metastasis in multiple cancer cell types[153]. Interestingly, intravital microscopy has been used to further visualize direct adherence of CTCs on top of neutrophils, indicating that CTCs could potentially use immune cells to 'hitchhike' during extravasation[154]. Multiple mechanisms have been proposed to be involved in this interaction. For example, intercellular adhesion molecule 1 (ICAM-1) expressed on CTCs, which can bind to Mac-1 or β_2-integrins on neutrophils[154,155]. In addition, cytokines, such as IL-8, are presumed to be mediators of this cellular crosstalk[156,157]. *In vivo* studies in murine models have revealed that IL-8 produced by melanoma cells is involved in neutrophil activation and chemotaxis, which results in increased metastatic homing to the lung[156]. The neutrophils promoted metastases formation by supporting CTC adhesion to the pulmonary endothelium[156]. In addition, adhesion of CTCs to neutrophils can be augmented by the interaction of a specialized CD44 isoform (CD44v), which has been detected on colon carcinoma cells and can bind to L-selectin on neutrophils[158,159]. Neutrophils can also actively entrap CTCs through the production of 'neutrophil extracellular traps' (NETs), which describe a network of DNA and proteins extruded from neutrophils upon activation to ensnare and neutralize pathogens. Interestingly, systemically increased NET formation has been demonstrated in tumour-bearing mice, as compared with healthy mice[160,161]. The process of NET release, termed NETosis, can be induced by G-CSF, IL-8, and other unknown factors[160,162]. *In vivo* investigations in a lung carcinoma xenograft model demonstrated an increase in tumour-cell entrapment in the liver when NET expression was induced chemically[163]. Furthermore, increased metastatic seeding in the context of systemic inflammation was shown to be mediated by NETs, which increased CTC adherence to blood vessels and supported extravasation into target organs during metastatic spread[156,163]. Additional data from *in vitro* studies indicate that entrapment and migration of CTCs across endothelial cell monolayers is primarily supported by intact NETs on neutrophils; thus, NETs presumably increase the proximity of CTCs to neutrophils and thereby support the direct interaction of these cells[163].

Taken together, these findings exemplify some of the potential metastasis-promoting tumour cell–immune cell interactions that could promote the recruitment and survival of CTCs in target organs, as well as the outgrowth of DTCs in the metastatic niche. Future studies will reveal whether this complex interplay can be targeted pharmaceutically to prevent the outgrowth of metastatic tumour cells in patients with cancer.

Conclusions

CTCs within the bloodstream have left the immune privileged site of the primary tumour and, therefore, might be more vulnerable to immune-mediated elimination. Studies have provided initial insights into the mechanisms by which CTCs might counteract immune-mediated cytoxicity. Investigation of the immune-escape mechanisms that apply directly to CTCs is, however, technically challenging and, to date, most information has been derived only from descriptive studies. The extent to which CTCs reflect overt metastases remains the subject of investigation; although, fortunately, tremendous advances in this field of research over the past years have now paved the way to study the functional interaction of CTCs and/or DTCs with cells of the immune system. In particular, developments in the *in vitro* culturing and expansion of CTCs not only enable functional testing of drug responses, but might also allow for evaluation of the immunogenicity of CTCs and their mechanisms of immune escape[164,165]. We believe that these advances will profoundly extend the focus of

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Box 2 | **Hypotheses on immune cell and CTC/DTC interactions**

- Acquiring mechanisms of immune escape is obligatory for circulating tumour cells (CTCs) and disseminated tumour cells (DTCs) to survive and contribute to metastasis
- Immune escape of CTCs and DTCs is affected by factors released from the primary tumour
- CTCs and DTCs are more vulnerable than primary tumours or solid metastases to immune effector cells because they lack a protective immunosuppressive tumour microenvironment
- CTCs and DTCs confer tolerance of the peripheral antitumour response to tumour-associated antigens, resulting in anergy of the adaptive immune response
- Continued exposure to CTCs results in peripheral effector T-cell exhaustion and senescence
- Measuring CTC numbers enables real-time monitoring of the effectiveness of the peripheral antitumour response
- The formation of metastases derived from CTCs and DTCs is indicative of successful evasion of cancer cells from local and peripheral antitumour immune responses
- Activation of tumour-promoting immune-cell populations is a critical step towards the initiation of metastasis

CTC research. Indeed, many questions concerning the immune interactions of CTCs need to be addressed in future studies (BOX 1).

Analyses of CTCs are not only useful for therapy stratification, but might also provide important information on the development of resistance when performed in real-time during the course of therapy. Pilot studies of the uses of CTCs as a marker of responsiveness to vaccines have already been conducted[166,167]. Monitoring of immune responses during immune-checkpoint or vaccination therapies will expand the importance of CTCs beyond the 'liquid biopsy', and will certainly strengthen the translational significance of CTC research in the upcoming era of cancer immunotherapy. We have postulated hypotheses that might provoke expansion of the focus of upcoming studies to encompass CTC and immune-cell interactions (BOX 2).

In the context of antigen presentation, the question regarding the ability of CTCs to express immunogenic TAAs will be relevant for future immunotherapeutic approaches. Genomic and gene-expression profiling of tissue from patients with breast, colorectal, or lung cancer has revealed the remarkable molecular heterogeneity of both primary tumours and CTCs[168–170]. Thus, CTCs might often, but not always, reflect the molecular characteristics of the primary tumour. This interpatient and intrapatient heterogeneity will pose major challenges for a targeted anticancer vaccination approach using TAAs. In addition, the extent to which the genetic programme of metastasis might adapt to immune encounter during the metastatic process —for example, if increased expression of immune escape markers by CTCs in reaction to IFNγ secreted from activated tumour-specific T cells[171] — remains elusive, and future studies need to address how these differences relate to cancer immunogenicity and a response to immunotherapies. Whether exosome composition, which can influence the organ-specific homing of CTCs[172], supports CTC immune escape must also be evaluated in the future.

Taken together, escape from and modulation of peripheral immune responses beyond the local tumour milieu, are critical steps in the development of metastases. CTCs and DTCs might represent the 'Achilles' heel' of interactions between tumour cells and immune cells owing to their ubiquitous distribution and easy accessibility as single cells or small cell clusters. In order to target these vital intermediaries of tumour dissemination, additional studies are needed to explain how CTCs/DTCs escape from immune surveillance.

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