Pathology International

Pathology International 2016; **66**: 491–505 doi:10.1111/pin.12440

Review Article

Role of tumor-associated macrophages in human malignancies: friend or foe?

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Tumor-associated macrophages (TAMs) play a pivotal role in tumor growth in human malignancies. Published studies have analyzed the relationship between TAM infiltration and the prognosis of patients for many human tumors. Most studies reported a positive correlation between TAM density and a poor prognosis. Studies focusing on macrophage phenotypes emphasized the protumor role of M2 anti-inflammatory macrophages in many types of human tumors. However, TAMs influence tumor progression in various ways that depend on differences in tumor sites, histology, and microenvironments. In this review, we summarize the function of TAMs in various human malignancies by reviewing the data provided in studies of TAMs in human malignancies.

Key words: human malignant tumors, immunohistochemistry, M1/M2 macrophage subtypes, tumor-associated macrophages

Immunohistochemical analysis of various human tumors via CD68 and other macrophage markers revealed that tumor-associated macrophages (TAMs) undoubtedly influenced the prognosis of patients. Significant positive correlations between TAM density and poor survival of patients were noted for many tumors.[1,2] Indeed, more than 80% of reports demonstrated the association of TAM density with an unfavorable outcome of patients.[1] Recent studies with markers of M2 macrophages such as CD163 and CD204 also indicated that the density of M2-TAMs had a closer relationship to poor outcome than did the density of $CD68^+$ TAMs.[3,4]

RECRUITMENT OF MONOCYTES/MACROPHAGES IN TUMOR TISSUES

Recruitment of monocytes/macrophages at tumor sites is guided by tumor-derived cytokines such as chemokine (C-C motif) ligand 2 (CCL2), CCL5, CCL7, and chemokine (C-X-C motif) ligand 1(CXCL1).[5] Among these chemokines, CCL2 is the most frequently found in human tumors, and its expression level correlates with the number of infiltrated TAMs.[6] CCL2 production was also detected in TAMs themselves,[6] as part of an amplification loop for their recruitment, such as in atherosclerosis.[7] Besides chemokines, colony-stimulating factor (CSF) -1, granulocyte-macrophage CSF (GM-CSF), and vascular endothelial growth factor (VEGF) recruit monocytes/macrophages.[5]

Studies of macrophage ontogeny showed that tissue-resident macrophages in many organs derive directly from yolk sac progenitors or via fetal liver hematopoiesis and are maintained in those organs throughout life.[8] The traditional concept that all tissue-resident macrophages derive from blood monocytes, as do inflammatory exudate macrophages,[9] was recently abandoned. Most TAMs, however, are believed to derive from circulating monocytes recruited by various chemoattractants as in inflammatory condition, whereas resident macrophages from surrounding tissues accumulate inside and around tumors when they are small.[10]

MACROPHAGE POLARIZATION

Responding to environmental stimuli, macrophages show multidirectional polarization. Polarized macrophage subtypes are categorized as classically activated (M1) and alternatively activated (M2) macrophages, categories that reflect the T-helper cell classification, T helper 1 and T helper 2 (Th1/Th2).[11,12] Interferon (IFN)-γ, alone or together with lipopolysaccharide (LPS) or tumor necrosis factor (TNF), activates macrophages to be classically activated M1 macrophages, whereas interleukin (IL)-4 and IL-13 induce the alternatively activated phenotype (M2).[11,13] However, other cytokines and factors not fit in the context of Th1/Th2 responses such as IL-10, transforming growth factor (TGF)-β, and glucocorticoids also induce macrophages similar to M2 phenotype. In view of these data, Mantovani et al.[14] proposed to refer to the three well-defined forms of M2 as the following: M2a, induced by IL-4 or IL-13; M2b, induced by exposure to immune complexes and agonists of Toll-like receptors (TLRs) or IL-1R; and M2c, induced by IL-10 and glucocorticoids. Regardless of such

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Received 16 February 2016. Accepted for publication 24 June 2016.

careful categorization, the M1/M2 dichotomy has been widely accepted, and the concept of classic and alternative activation has become broad and overinterpreted.

M1 macrophages encourage inflammation by producing pro-inflammatory cytokines such as IL-12, IL-1β, TNF-α, IL-6, and IL-23 and other effecter molecules such as reactive oxygen species and nitrogen intermediates. In contrast, M2 macrophages suppress inflammation by producing anti-inflammatory cytokines such as IL-10, IL-13, and TGF-β as well as other anti-inflammatory molecules including interleukin-1 receptor antagonist and prostaglandin E2 (PGE_2). M2 macrophages also participate in tissue repair by inducing angiogenesis and activating stromal cells to produce matrix proteins.[6] Phenotypically, M1 macrophages show enhanced expression of inducible nitric oxide synthase (iNOS), human leukocyte antigen (HLA)-DR, CD80, CD86, CD169, and TLRs 2 and 4, whereas M2 macrophages upregulate CD163, CD204, CD206, and arginase 1[15–17] (Fig. 1).

Because diversity and plasticity are hallmarks of macrophages, that the M1/M2 concept is too bipolar, oversimplified, and sometimes overinterpreted may be reasonably argued.[12,18] Nevertheless, this paradigm is widely accepted because it is convenient for understanding the pathological processes of macrophage-related diseases.

ROLE OF TAMS IN TUMOR PROGRESSION

A considerable proportion of TAMs in human cancers are polarized to the anti-inflammatory M2 phenotype after stimulation by tumor-derived molecules such as CSF-1 and IL-10.[5,6] Tumor cells may thus educate macrophages to become beneficial for tumor growth and expansion.[5] TAMs, especially M2-TAMs, promote tumor progression in various ways (Fig. 2).

In tumor tissues, a dramatic enhancement of vascular density promotes oxygenation of and nutrient supply to tumor cells. In hypoxic conditions, macrophages produce angiogenic molecules such as VEGF, epidermal growth factor (EGF), TNF-α, basic fibroblast growth factor, and CXCL8.[19] Thymidine phosphorylase (TP) and various chemokines including CCL2 and CXCL8 produced by M2-TAMs also induce angiogenesis.[19,20] Many studies of human tumors documented significant associations between microvessel density and numbers of M2-TAMs.[3,19]

M2-TAMs also produce immunosuppressive molecules including PGE_2, TGF-β, and IL-10 to suppress T-cell mediated antitumor immunity.[1,6,20] Molecules produced by M2-TAMs such as CSF-1, IL-6, and IL-10 suppress dendritic cell maturation.[20] Although the migratory process of regulatory T cells into tumor tissues is not fully understood, M2-TAM-derived PGE_2, TGF-β, IL-10, and cytokines including CCL17, CCL18, and CCL22 recruit regulatory T cells.[1,6,21] In human cancers, the numbers of M2-TAMs and regulatory T cells correlate well.[22]

M2-TAMs are also involved in formation of a niche to maintain cancer stem cell survival.[23] This niche is enriched in growth factors, cytokines, prostaglandins, and extracellular matrix components. Essential cellular players include TAMs and other immune cells, cancer-associated fibroblasts, mesenchymal stem cells, and endothelial cells. M2-TAM-derived growth factors including basic fibroblast growth factor, hepatocyte growth factor, EGF, platelet-derived growth factor, and TGF-β play important roles in maintaining cancer stem cells and promoting tumor growth.

In addition, M2-TAMs play a critical role in the process of tumor cell invasion and metastasis. TAMs produce matrix metalloproteinases (MMPs) such as MMP2 and MMP9 to degrade the extracellular matrix. Signal transduction and activator of transcription 3 (STAT3) has received attention as a significant transcription factor mediating interactions between TAMs and tumor cells. STAT3 promotes tumor cell proliferation, survival, and invasion by regulating many angiogenic and immunosuppressive factors.[24] STAT3 activation also induces polarization of TAMs to the M2 phenotype.[25] Direct co-culture of tumor cells

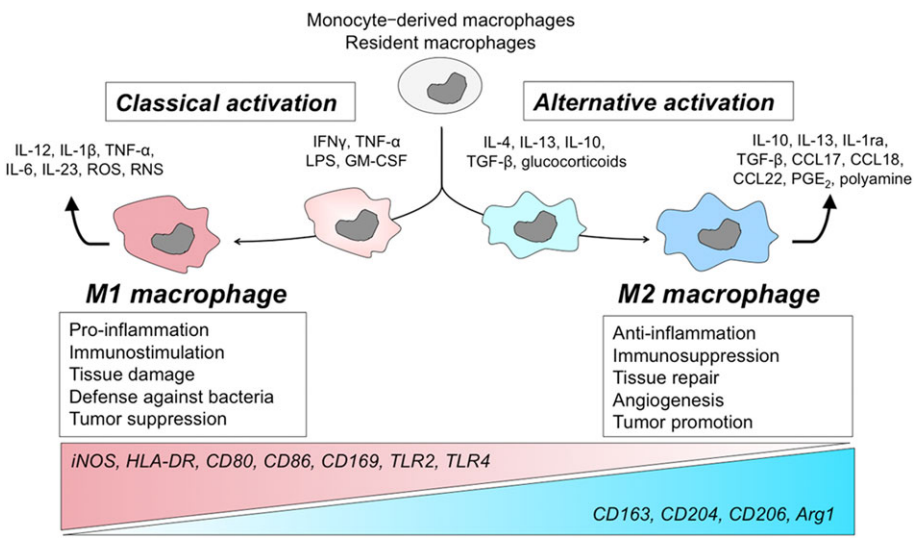

Figure 1 M1/M2 polarization of macrophages. Macrophages differentiate into M1 and M2 subtypes in response to environmental stimuli. Most TAMs manifest M2-like functions and phenotype and contribute to tumor progression. Arg1, arginase 1; CCL, chemokine (C-C motif) ligand; GM-CSF, granulocyte-macrophage colony-stimulating factor; HLA, human leukocyte antigen; IFN, interferon; IL, interleukin; IL-1ra, IL-1 receptor antagonist; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide; RNS, reactive nitrogen species; ROS, reactive oxygen species; PGE_2, prostaglandin E_2; TGF, transforming growth factor; TLR, Toll-like receptor; TNF, tumor necrosis factor.

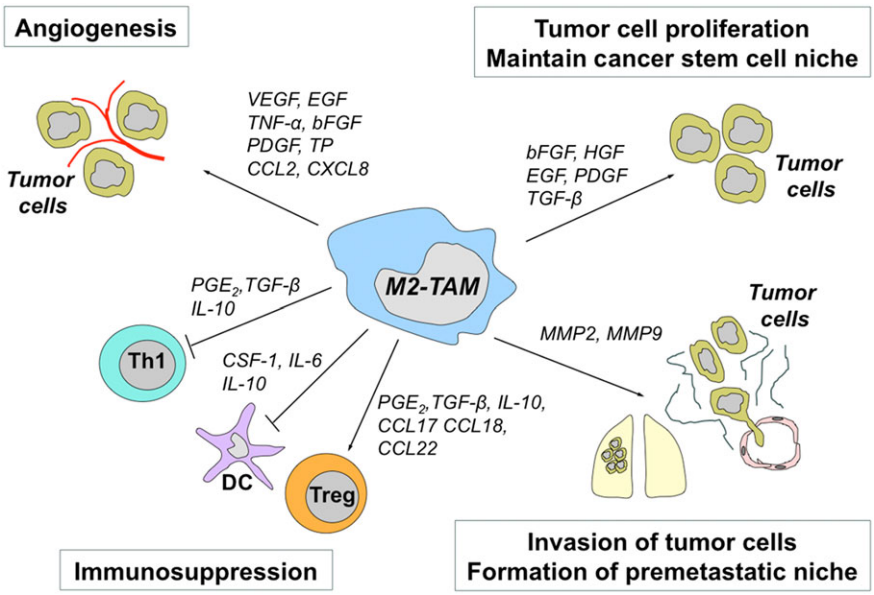

Figure 2 Role of M2-TAMs in tumor progression. M2-TAMs induce angiogenesis in the tumor microenvironment, suppress antitumor immunity, and directly stimulate tumor cell proliferation. They also participate in formation of a cancer stem cell niche and pre-metastatic niche to promote tumor progression. bFGF, basic fibroblast growth factor; CCL, chemokine (C-C motif) ligand; CXCL, chemokine (C-X-C motif) ligand; CSF-1, colony stimulating factor-1; DC, dendritic cell; EGF, epidermal growth factor; HGF, hepatocyte growth factor; IL, interleukin; MMP, matrix metalloproteinase; PDGF, platelet-derived growth factor; PGE_2, prostaglandin E_2; TGF-β, transforming growth factor-β; Th1, T-helper cell 1; TNF-α, tumor necrosis factor-dα; TP, thymidine phosphorylase; Treg, regulatory T cell; VEGF, vascular endothelial growth factor.

and macrophages demonstrated activation of STAT3 in macrophages and that macrophage-derived factors including EGF, IL- 6, and IL-10 activate STAT3 in tumor cells. CSF-1 receptor and sphingosine-1-phosphate receptor (S1PR1) on the cell surface were thought to be important during reciprocal activation of STAT3.[25,26]

Of greater interest, recent studies demonstrated that macrophages participate in formation of a pre-metastatic niche in which a suitable microenvironment is prepared before metastasis occurs. Suppressor cell types such as protumor macrophages and regulatory T cells accumulate in this niche to evade the antimetastatic immune response.[27] S1PR1-Janus kinase-STAT3 signaling was said to be crucial for myeloid cell colonization at future metastatic sites.[28]

ROLE OF TAMS IN EPITHELIAL TUMORS

Breast cancer

Many studies of breast cancer indicated a close relationship between TAM density and poor outcome of patients. A general application of this result, however, needs careful analysis because many histological subtypes exist in breast cancer and hormone dependence varies. Invasive ductal carcinoma, the most common histological subtype, is rich in stroma with well-developed vasculature, which promotes tumor progression. TAMs, especially M2-TAMs, induce vascularization via production of blood vessel growth factors as described earlier. Invasive ductal carcinoma showed a strong association among microvessel density, $CD68^+$ TAM infiltration, reduced relapse-free survival, and reduced overall survival (OS).[29] Expression of TP, one of the angiogenic factors, in $CD68^+$ TAMs, correlated with poor prognosis of patients with invasive ductal cancer.[30] The authors indicted that TP^+ status was an independent prognostic factor. Also, VEGF expression in $CD68^+$ TAMs correlated closely with microvessel density.[31] Furthermore, microvessel density and VEGF expression were related to tumor grade and lymph node metastasis in patients with invasive ductal carcinoma.[31,32] In contrast, microvessel density in invasive lobular carcinoma had no correlation with VEGF expression, TAM density, mitotic activity index, lymph node metastasis, or grade.[32] Triple-negative breast cancer with a high number of $CD68^+$ TAMs demonstrated a significantly higher risk of distant metastasis, and lower rates of disease-free survival (DFS) and OS, than did cancers with a smaller number of TAMs.[33]

Recent reports indicated the significance of $CD163^+$ M2-TAMs for the poor prognosis of invasive ductal carcinoma. High numbers of $CD163^+$ M2-TAMs had potent associations with rapid proliferation, poor differentiation, estrogen receptor negativity, and histological ductal type.[34] $CD163^+$ M2-TAM density in tumor stroma correlated positively with higher tumor grade, larger tumor size, Ki67 positivity, estrogen receptor and progesterone receptor negativity, and triple-negative basal-like breast cancer.[35]

As an intriguing result, the location of TAM infiltration influences tumor progression. The density of $CD68^+$ TAMs in the tumor stroma, but not in the tumor nest, was an independent prognostic factor for reduced survival of patients with breast cancer.[35] Another report[36] indicated that high $CD68^+$ TAM counts in the tumor stroma, but not in the tumor nest, were associated with higher tumor grades and negative estrogen receptor status, whereas those in the tumor nest were significantly correlated with microvessel density. The authors suggested that tumor stromal macrophages and tumor nest macrophages residing in different microenvironments have distinct roles.

Lung cancer

Many reports demonstrated a positive correlation between TAM density and increased vascularization, as well as poor prognosis, in non-small-cell lung cancer (NSCLC).[37] The site of TAM infiltration also influences prognosis. Multiple reports found increased stromal TAM density to be independent predictor of reduced survival, whereas TAM density in tumor islets correlated with a good prognosis.[38] Phenotypic analysis of islet macrophages indicated that the density of M1-TAMs (defined by $CD68^+$ and either $HLA\text{-}DR^+$ or $iNOS^+$) was significantly increased compared with that of M2-TAMs (defined by $CD68^+$ and either $CD163^+$ or $VEGF^+$); 70% of islet macrophages were positive for M1 markers versus 38% being positive for M2 markers in the extended survival group of patients with relatively early stages of NSCLC.[39] The authors suggested that TNF-α produced by M1-TAMs may have antitumor activity.[40] With regard to the site of TAM infiltration, a study of advanced NSCLC showed that most ($>$95%) $CD68^+$ TAMs were found in the tumor stroma and showed positive co-staining with CD163.[37] Patients with progressive disease had significantly higher TAM counts, and high TAM counts were significantly related to poor progression-free survival (PFS) and OS.[37] Studies using CD204 as an M2 marker indicated a significant association of $CD204^+$ TAM density and poor outcome of patients with adenocarcinoma[41] and squamous cell carcinoma of the lung.[42] As an interesting finding, the number of circulating $CD14^+CD204^+$ cells in the pulmonary vein of NSCLC patients correlated with the number of $CD204^+$ TAMs in the tumor stroma and was a significant independent risk factor for early recurrence.[43] That density of TAMs, especially M2-TAMs in tumor stroma, is associated with poor prognosis in NSCLC is generally believed. In the early stage of NSCLC, however, M1-TAM density in tumor islets correlates with long survival.

Only a few reports examined the association between small-cell lung cancer and TAMs. The analysis of $CD204^+$ TAMs showed no relationship of these cells with OS or relapse-free survival of patients with this cancer.[44]

Hepatocellular carcinoma (HCC)

HCC is an inflammation-related cancer. Chronic inflammation after hepatitis virus infection is the major risk factor for HCC development. Various components including immune cells, carcinoma-associated fibroblasts, hepatic stellate cells, endothelial cells, and extracellular matrix form microenvironments in HCC. TAMs are a main component in the microenvironments, but they do not necessarily possess the M2 phenotype, and, unlike the situation in many other tumors, the activated state of TAMs varies according to the complex stimulations associated with the inflammatory milieu.

Several reports showed positive correlations between $CD68^+$ TAM density and poor prognosis.[45–47] In contrast, the density of $CD163^+$ M2-TAMs had no prognostic value.[47] A favorable association between $CD68^+$ TAM density and DFS and OS was also reported.[48] With regard to the relationship between macrophages and metastasis, the density of $CD68^+HLA\text{-}DR^+$ M1-like TAMs in HCC cases with metastasis was reportedly significantly higher than in cases without metastasis.[49] The authors speculated that increased motility of HCC cells activated by M1-like macrophages enhanced metastasis.

Sites of TAM infiltration in HCC influence prognosis in different ways. One study reported that both intratumoral and marginal densities of $CD68^+$ TAMs were associated with poor OS and DFS, whereas peritumoral TAM density was unrelated to OS and DFS.[46] In contrast, another study[45] reported that the presence of peritumoral TAMs was related to an early recurrence and was an independent prognostic factor for OS and DFS, whereas that of intratumoral TAMs was unrelated to survival.

Therefore, not all but a considerable number of reports demonstrated a relationship between TAM density and poor outcome of patients with HCC, but the effect of macrophage phenotypes was not clear compared with tumors in other sites, probably because TAMs in HCC are exposed to complex activation in the tumor microenvironment modified by chronic inflammation.

Colorectal cancer

Although high numbers of TAMs correlate with poor prognosis in many cancers,[3,4] colorectal cancer is one of the few exceptions to this association.[50] Many studies reported that a high density of $CD68^+$ TAMs at the invasive front was related to a favorable prognosis of patients with colorectal cancer.[50,51] Macrophage phenotype analysis found that patients with a high infiltration of $iNOS^+$ M1-TAMs at tumor fronts had a significantly better prognosis than those with few $iNOS^+$ M1-TAMs.[51] Conversely, certain reports indicated that intratumoral $CD68^+$ TAM counts correlated with depth of invasion, lymph node metastasis, and staging of colorectal cancer.[52]

The intestine is an organ that is exposed to continuous immune stimuli. Edin et al.[51] observed increased infiltration of M1 macrophages at tumor fronts that was accompanied by a concomitant increase in M2 macrophage numbers. They found that the presence and functions of M1 macrophages dominated those of M2 macrophages in colorectal cancer, thereby leading to an improved prognosis.[50] Macrophages expressed costimulatory molecules such as CD80 and CD86 and stimulated antitumor immunity at the invasive front of colorectal cancer.[53] Though not related to TAMs, that neoplastic colorectal cancer cells themselves express CD163 should be noted. $CD163^+$ tumor cells were detected in 32 of 163 cases (23%) of rectal cancer, and such patients had a poorer prognosis than did patients with $CD163^-$ cells.[54]

Gastric cancer

Gastric cancer, which has close links to *Helicobacter pylori* infection, is associated with infiltration of various immune cells. Infiltrations of $CD8^+$ cytotoxic T cells, dendritic cells, and $CD45RO^+$ T cells were related to good prognosis, whereas infiltrations of TAMs, myeloid-derived suppressor cells, and forkhead box P 3 ($FOXP3^+$) regulatory T cells were related to poor prognosis.[55] Many studies indicated that the number of $CD68^+$ TAMs correlated with the depth of invasion, lymph node metastases, microvessel density, and poor outcome of patients.[56]

With regard to phenotypic analysis of TAMs, Pantano et al.[57] examined M1-TAMs ($CD68^+NOS2^+$) and M2-TAMs ($CD68^+CD163^+$) by double immunostaining in 52 cases of stomach cancer. A correlation with prognosis was not found for M2-TAMs alone; the group with a high M1/M2 ratio had a longer survival. The M1/M2 ratio was thus a positive independent predictor of survival. Another study indicated that a high number of $CD204^+$ M2-TAMs was related to a poor 5-year OS.[58]

Pancreatic cancer

Pancreatic cancer possesses abundant stroma, which may account for up to 80% of the tumor mass and which contains many kinds of non-tumor cells including immune cells such as macrophages, cancer-associated fibroblasts, and endothelial cells.[59] Among these cells, TAMs are critical for tumor growth and influence the outcome of patients. Many researchers noted the unfavorable role of M2-TAMs in this disease.[60–62] Cases with high numbers of M2-TAMs (either $CD163^+$ or $CD204^+$), but not $CD68^+$ TAMs, manifested significantly increased lymphatic vessel density, a high incidence of lymph node metastasis, and poor prognosis.[60] High numbers of $CD204^+$ TAMs were associated with large tumors, early recurrence, and shortened survival in patients with invasive ductal carcinoma of pancreas head.[61] Close localization of $CD44^+CD133^+$ cancer stem cells and $CD204^+$ TAMs was related to shorter OS and DFS in pancreatic ductal adenocarcinoma.[62] Taken together, these reports show M2-TAMs contributed to poor prognosis in pancreatic ductal carcinoma.

Prostate cancer

As in many other cancers, dense infiltration of TAMs correlates with unfavorable clinical characteristics in patients with prostate cancer.[63,64] A significant number of studies, however, reported the opposite relationship.[65]

In one report analyzing biopsy specimens, the number of $CD68^+$ TAMs correlated with blood levels of prostate-specific antigen, Gleason's score, and advanced clinical stage.[64] Another report found a significantly higher number of $CD68^+$ TAMs in prostate cancer compared with prostatic intraepithelial neoplasia and normal prostate tissue.[66] Patients treated with androgen deprivation therapy showed a relationship between high $CD68^+$ TAM density and an increased risk of biochemical recurrence.[63] With regard to macrophage phenotype analysis, denser infiltration of $CD204^+$ TAMs was observed in malignant glands than in normal glands, in contrast to $CD68^+$ TAMs, which tended to infiltrate normal glands.[67]

However, opposite correlations were also reported. Several groups found that reduced numbers of $CD68^+$ TAMs and/or $CD204^+$ cells including macrophages and dendritic cells were associated with cancer progression and poor prognosis.[65]

Thyroid cancer

In the early 1990s, a positive correlation between the density of $CD68^+$ TAMs and increased vascularization of thyroid tumors was published.[68] Ryder et al., in a comparative analysis of well-differentiated, poorly differentiated, and anaplastic thyroid carcinomas, reported a denser infiltration of $CD68^+$ TAMs in anaplastic carcinomas (95%) and poorly differentiated carcinomas (54%) compared with well-differentiated carcinomas (27%). In that report, increased TAM numbers in poorly differentiated carcinomas were associated with capsular invasion, extrathyroidal extension, and reduced cancer-related survival.[69] Another group reported a positive correlation between the density of $CD68^+$ TAMs and lymph node metastases in patients in advanced stages of the disease.[70]

In contrast, the infiltration of immune cells, including $CD68^+$ TAMs and $CD8^+$ lymphocytes, was associated with an increased DFS in differentiated thyroid carcinoma.[71] Those authors believed that such contradictory results may have been related to the different microenvironments in differentiated and undifferentiated carcinomas and that the prognosis of patients with differentiated thyroid carcinomas may be influenced by the complex interactions among infiltrated mixed immune cells.

Cholangiocarcinoma

An association between TAM infiltration and poor survival was also found for cholangiocarcinoma. We reported that the densities of $CD68^+$ TAMs and $CD163^+$ M2-TAMs in intrahepatic cholangiocarcinoma were associated with microvessel density and the number of $FOXP3^+$ regulatory T cells.[22] High numbers of $CD163^+$ M2-TAMs correlated with poor DFS, although no such relationship was seen with OS. Another study reported that patients with a high density of $MAC387^+$ macrophages (recently migrated monocyte-derived macrophages) had the worst OS. Double immunofluorescent staining revealed that $MAC387^+$ macrophages co-expressed matrix metalloproteinase-9, which indicated that such macrophages are critical for degrading the extracellular matrix and facilitating tumor metastasis.[72] The same group found an increased $CD14^+CD16^+$ monocyte

subset in peripheral blood in patients with a high density of MAC387$^+$ macrophages and suggested the CD14$^+$CD16$^+$ monocyte level as a possible predictor of tissue invasion in cholangiocarcinoma.[73] One recent study reported a significant association of a high density of CD163$^+$ M2-TAMs in cholangiocarcinoma with the presence of extrahepatic metastases.[74] As an interesting result, they found that some tumor cells expressed CD163, which correlated with metastasis. They suggested that this observation was evidence of involvement of epithelial-to-mesenchymal transition.[75]

Renal cell carcinoma (RCC)

TAM infiltration in RCC contributes to tumor progression and metastasis via stimulating angiogenesis, tumor growth, and cell migration and invasion. Moreover, TAMs were involved in the epithelial-to-mesenchymal transition of RCC cells and in development of tumor resistance to targeted drugs.[76] The density of CD68$^+$ TAMs increased with tumor size and was implicated in RCC progression.[77] Patients with recurrence had significantly higher VEGF levels and TAM density than did those without recurrence. Because the TAM count correlated well with microvessel density, angiogenesis was thought to be the main mechanism of tumor progression.[77] Gene expression analysis of RCC revealed negative correlations of CD68 and FOXP3 (a regulatory T cell marker) with survival.[78]

In our analysis of macrophage phenotypes, CD163$^+$ M2-TAM density correlated better than CD68$^+$ TAM density with poor prognosis.[79] Similar results were reported for CD206, another M2 macrophage marker.[80] The expression of M2-TAM-related genes such as those encoding CD163, IFN regulatory factor 4, and fibronectin 1 correlated with large tumor size and poor outcome, whereas expression of the iNOS gene, an M1-TAM-related gene, showed an inverse correlation.[78] We demonstrated expression of T-cell immunoglobulin and mucin domain-containing molecule-3 (TIM-3) on tumor cells and a positive correlation of CD204$^+$ TAMs with shorter PFS in patients with RCC,[81] and we suggested that TIM-3 is implicated in resistance to antitumor therapy.

Urothelial cell carcinoma of the bladder

TAM infiltration in this carcinoma is related to poor prognosis, as in many other cancers. CD68$^+$ TAM density was significantly higher in invasive bladder cancer than in superficial bladder cancer, and TAM density was related to microvessel density.[82] These authors noted that cases with high TAM density manifested frequent distant metastasis and that their 5-year survival rate was significantly low. Another study also reported a significant correlation between TAM density and microvessel density as well as poor prognosis.[83] With regard to bacille de Calmette et Guérin (BCG) therapy, which is the gold standard of bladder cancer treatment, a high infiltration of CD68$^+$ TAMs was related to an increased risk of recurrence in patients with non-muscle-invasive urothelial cancer before BCG immunotherapy.[84] Analysis of patients with bladder carcinoma in situ who received BCG therapy indicated frequent recurrences in patients with a high density of CD68$^+$ TAMs.[85]

Subtype analysis of macrophages showed that the density of CD204$^+$ stromal macrophages predicted poor prognosis and had a positive association with tumor size and stage, nodal metastasis, and histological grade.[86]

Endometrial cancer

Many studies reported that patients with endometrial cancer showed a correlation of dense infiltration of TAMs with poor outcome. Intratumoral density of CD68$^+$ TAMs was significantly associated with FIGO stage, histological grade, Ki-67 expression, and intratumoral expression of Ki-67 and p53.[87] High CD68$^+$ TAM counts were also related to reduced survival.[87] Another study also found positive links between CD68$^+$ TAMs at the invasive margin and FIGO stage, histological grade, microvessel density, myometrial invasion, and lymph node metastasis.[88] Patients with high numbers of CD68$^+$ TAMs at the invasive margin had significantly worse PFS and OS than did those with low marginal CD68$^+$ TAM numbers.[88] In contrast, certain other studies failed to demonstrate significant correlations between CD68$^+$ TAM density and prognosis,[89] although they did find associations with myometrial invasion and microvessel density.[89]

In a macrophage phenotype analysis, CD163$^+$ M2-TAM density correlated significantly with myometrial invasion, microvessel density, and regional lymph node metastasis,[90] but a correlation with prognosis was not described.

Epithelial ovarian cancer

Although ovarian cancer manifests many kinds of histological subtypes, we discuss only epithelial ovarian cancer here because most studies of TAMs focused on epithelial-type cancers. Our analysis confirmed that macrophages represent the most abundant infiltrating immune cells in human epithelial ovarian cancer, although various immune cells occur in the stroma, as is the case for other solid tumors.[91] However, most reports analyzing CD68$^+$ TAMs failed to demonstrate significant associations with prognosis.[92] In contrast, CD163$^+$ M2-TAM density correlated significantly with poor prognosis. PFS and OS were significantly higher in the low-CD163 expression group than in the high-CD163 expression group.[92] The expression of CSF-1, a cytokine that induces M2 differentiation of macrophages, was high in malignant ovarian tumors compared with borderline and benign tumors is noteworthy.[93] In contrast, a high M1/M2 ratio of TAMs was associated with extended survival in ovarian cancer patients.[94] These reports thus indicate

that M2-TAMs were related to poor prognosis in epithelial ovarian cancer but that the total number of $CD68^+$ TAMs were not.

Uterine cervical cancer

Infection with human papillomavirus (HPV) has a strong association with the development of uterine cervical cancer. HPV is responsible for almost all cervical cancer cases, and HPV-associated inflammation and consequent immune reaction influence disease progression.[95] HPV-specific T cells reportedly infiltrated cervical cancer tissues, but HPV-specific T-cell responses were detected in only half of patients with cervical cancer or high-grade squamous intraepithelial lesions.[95] $CD68^+$ macrophage infiltration was also found in cervical cancer tissues, and high numbers of stromal $CD68^+$ TAMs were related to tumor size.[96] In a comparative study of the normal cervix, low-grade squamous intraepithelial lesions, high-grade squamous intraepithelial lesions, and cervical cancer, $CD68^+$ macrophage counts increased linearly with disease progression.[97] The number of $CD68^+$ TAMs in the tumor stroma correlated significantly with lymphatic vessel density and lymphatic metastasis.[98] However, these studies did not address a link between $CD68^+$ TAMs and prognosis.

In analysis of macrophage phenotypes, $CD14^+CD33^-$ $CD163^-$ M1-TAMs showed a correlation with a large influx of intraepithelial T lymphocytes, improved disease-specific survival, and served as an independent prognostic factor for survival in patients with cervical cancer. In locally advanced cervical cancer, polarization of TAMs to M2 macrophages correlated with poor responses to chemoradiation therapy and reduced survival.[99] One study introduced a new double immunohistochemical approach for identifying M1 and M2 macrophages, and $CD163^+$pSTAT1$^+$ TAMs and $CD68^+$pSTAT1$^+$ TAMs were defined as M1-TAMs, whereas $CD163^+CMAF^+$ TAMs and $CD68^+CMAF^+$ TAMs were defined as M2-TAMs.[100]

Esophageal cancer

Many studies of esophageal squamous cell cancer reported an association between TAM density and poor prognosis.[101–103] Significantly higher $CD68^+$ TAM counts were detected in patients with lymph node metastasis than in those without metastasis, and such counts were an independent prognostic factor for survival.[101]

Concerning macrophage phenotypes, $CD204^+$ M2-TAMs were significantly related to poor prognosis for patients.[102] A comparative analysis of $CD163^+$ M2-TAMs and $CD204^+$ M2-TAMs found that a high density of $CD204^+$ M2-TAMs was significantly associated with features indicating greater malignancy, including depth of tumor invasion, lymph and blood vessel invasion, and lymph node metastasis, as well as clinical stage, whereas $CD163^+$ M2-TAMs had no correlation with these clinicopathological features except for depth of tumor invasion and blood vessel invasion.[102] In this report, patients with high $CD204^+$ TAM counts had poor DFS.

In esophageal adenocarcinoma without neoadjuvant treatment, an increased number of $CD163^+$ M2-TAMs and poor survival were correlated. The M2($CD163^+$)/M1($CD40^+$) ratio was significantly higher in node-positive esophageal adenocarcinomas than in node-negative esophageal adenocarcinomas and was inversely correlated with OS.[103]

ROLE OF TAMS IN NON-EPITHELIAL TUMORS

Glioma

TAM density in gliomas increases in association with a higher grade.[104] CCL2 and other chemoattractants including GM-CSF and VEGF are believed to be responsible for macrophage infiltration into gliomas.[105,106] In fact, glioblastoma cell line-derived protein was used for initial purification of CCL2.[107]

In the brain, microglial cells, which are macrophages in the central nervous system, constitute 5–20% of the glial cell population.[108] It is indicated that microglial cells originate from fetal macrophages, but not monocytes.[8] Although differentiating microglial cells from monocyte-derived macrophages is difficult, there is a good possibility that microglial cells, as well as monocyte-derived macrophages, influence tumor progression.

A number of authors reported a positive association between TAM density and microvessel density, and angiogenic factors such as VEGF and TP[109,110] were reportedly responsible for this result. Microglial cells promoted invasion of glioblastoma cells through signaling pathways involving EGF receptor and CSF-1 receptor.[111]

Although densities of both $CD68^+$ TAMs and $CD163^+$ M2-TAMs as well as $CD204^+$ TAMs increased in association with grade, $CD163^+$ M2-TAMs and $CD204^+$ TAMs increased more than did $CD68^+$ TAMs.[104] A positive correlation between densities of $CD163^+$ M2-TAMs and $CD204^+$ TAMs and poor prognosis was observed, but no such correlation was found for CD68+ TAMs.[104]

Unlike the situation in other tumors such as breast cancer, NSCLC, and HCC, the degree of TAM infiltration did not differ between central and marginal areas of gliomas.[112] Close cell–cell communications between macrophages and tumor cells exist in gliomas. Co-culture of macrophages and glioma cells resulted in activation of STAT3 in both cell types.[25] These reports together show that TAMs in gliomas increased microvessel density and promoted tumor growth and tumor cell invasion. TAMs have emerged as exciting targets for therapeutic intervention, and further investigation may yield new glioma treatment strategies.

Melanoma

Melanoma is a high-grade tumor showing high invasive and metastatic capacity. Different kinds of immune cells infiltrated

the melanoma stroma, and the increased number of macrophages was associated with local progression of primary melanomas.[113] TAM density was significantly higher in thick melanomas than in thin melanomas and was positively correlated with melanoma invasiveness and metastasis.[114] Dense CD163$^+$ M2-TAM infiltration in tumor stroma and CD68$^+$ TAM infiltration at the invasive front were related to poor OS.[115] These authors indicated that both serum levels of soluble CD163 and the presence of CD68$^+$ TAM infiltration at the invasive tumor front were independent predictors of survival in stage I/II melanoma.

For melanoma at special sites, intratumoral CD68$^+$ TAM density was associated with tumor thickness and with OS in sinonasal melanoma of stages I and II.[116] In uveal melanoma, high CD163$^+$ M2-TAM density correlated with increased microvessel density and worse prognosis.[117]

Hodgkin lymphoma

Although the presence of macrophages in tumor tissues of Hodgkin lymphoma has long been known,[118] their role in disease progression received little attention until Steidl et al.[119] demonstrated the association of TAMs with poor prognosis in this lymphoma. They studied gene expression profiles via fresh-frozen tissues of Hodgkin lymphoma obtained during diagnostic lymph node biopsy and found a significant association of the gene signature of TAMs with primary treatment failure. In a parallel examination of paraffin-embedded materials via CD68 immunohistochemistry, they found strong correlations between CD68$^+$ TAM density and a shortened PFS and disease-specific mortality rate and concluded that TAM density predicts treatment outcome. Most subsequent studies by other groups demonstrated the positive relationship between densities of CD68$^+$ TAMs and/or CD163$^+$ M2-TAMs and poor outcome,[120–123] although several studies failed to demonstrate the correlation between either CD68$^+$ TAMs or CD163$^+$ M2-TAMs with prognosis.[124]

In a comparative analysis of CD68 and CD163, several studies indicated the superiority of CD163 compared with CD68 for predicting poor prognosis.[120,122,123]

Barros et al.[125] introduced a new immunohistochemical approach for identifying M1 and M2 macrophages in an analysis of pediatric Hodgkin lymphoma. By using double immunohistochemical staining combining CD68 or CD163 with pSTAT1 (M1-like) or CMAF (M2-like),[100] they found better OS in cases with higher numbers of CD163$^+$pSTAT1$^+$ M1-like macrophages but worse PFS in cases with higher numbers of CD163$^+$CMAF$^+$ M2-like macrophages.[125]

B-cell non-Hodgkin lymphoma

In follicular lymphoma, gene expression profiling of tumor biopsy specimens from untreated patients indicated an important role of non-malignant tumor-infiltrating cells for predicting the outcome of patients.[126] In this study, most genes associated with poor prognosis were those preferentially expressed in macrophages, dendritic cells, or both. Ensuing immunohistochemical studies using CD68 and/or CD163 reported significant relationships between TAM density and poor prognosis.[127–130] Studies evaluating the influence of rituximab (anti-CD20 antibody) therapy found that high TAM content correlated with longer survival[129,130] or that the correlation between TAMs and poor prognosis was abrogated.[128]

With respect to diffuse large B-cell lymphoma, associations between CD68$^+$ TAMs and prognosis varied. Some studies reported a significant link between CD68$^+$ TAM density and poor outcome,[131,132] whereas others reported no association with prognosis.[133,134] In contrast, the number of CD163$^+$ TAMs and the CD163/CD68 ratio showed significant correlations with a poor clinical course in most studies.[132,134,135] An interesting finding was that CD68$^+$ TAM numbers were associated with a favorable prognosis when patients received rituximab together with multi-agent chemotherapy,[135] as in follicular lymphoma.[128–130] Such an inverse effect of M2-TAMs associated with rituximab therapy may be related to the fact that M2 macrophages phagocytose rituximab-opsonized lymphoma cells more efficiently than do M1 macrophages.[136] In B-cell lymphoma, different therapeutic regimens are thought to influence the role of TAMs. Reverse correlations between M2-TAM density and prognosis were observed in two groups of patients receiving different therapeutic regimens.[137]

T-cell non-Hodgkin lymphoma

Our studies of adult T-cell leukemia/lymphoma showed that an increased percentage of CD163$^+$ macrophages among total TAMs was significantly associated with a poor clinical prognosis, although the numbers of TAMs positive for CD68, CD204, or CD206 had no correlation with outcome.[138,139] A study of angioimmunoblastic T-cell lymphoma found that a higher ratio of CD163$^+$ M2-TAMs to CD68$^+$ TAMs had a significant correlation with worse OS.[140]

In a study of cutaneous T-cell lymphoma, a high number of CD163$^+$ M2-TAMs was linked to a poor clinical prognosis.[141] The serum soluble CD163 level correlated with soluble IL-2 receptor and CCL17 levels and was associated with disease progression.[141] Two studies of natural killer/T-cell lymphoma demonstrated an association of CD68$^+$ TAM number with poor prognosis.[142,143]

DISCUSSION AND FUTURE PERSPECTIVES

Tables 1 and 2 summarize the roles of TAMs in epithelial tumors and non-epithelial tumors, respectively. In many epithelial tumors including breast cancer, lung cancer, gastric cancer, pancreatic cancer, cholangiocarcinoma, renal cell carcinoma,

Table 1 Roles of TAMs in human epithelial tumors

Tumor type	Link of TAMs to tumor progression and prognosis	Comments
Breast cancer	Poor prognosis (invasive ductal carcinoma)	High TAM density correlated with tumor progression with or without link to poor prognosis in invasive ductal cancer[29–32] and triple-negative cancer,[33] but not in invasive lobular carcinoma.[32] M2-TAMs were related to poor prognosis more closely than were total TAMs.[34] Stromal TAMs linked to poor prognosis more closely than were intratumoral TAMs.[35,36]
Lung cancer	Poor prognosis (non-small cell lung cancer) No relationship (small cell lung cancer)	TAM density correlated with increased vascularization as well as poor prognosis.[37] Stromal TAM density was an independent predictor of reduced survival, whereas TAM density in the tumor islets correlated with good prognosis in non-small cell lung cancer.[38] No relationship between TAMs and prognosis of patients was found in small cell lung cancer.[44]
Hepatocellular carcinoma	Relatively poor prognosis	Several reports indicated a link between TAMs and poor prognosis,[45–47] but a study reported an opposite link.[48] Sites of TAM infiltration in HCC influenced prognosis differently.[45,46] Effect of TAM phenotypes was not clear.
Colorectal cancer	Better prognosis	High TAM density at the tumor invasive front correlated with favorable prognosis.[51]
Gastric cancer	Poor prognosis	High $CD68^+$ TAM density correlated with tumor progression with or without link to poor prognosis.[56]
Pancreatic cancer	Poor prognosis (ductal carcinoma)	M2-TAMs were related to poor prognosis more closely than were total TAMs.[60–62]
Prostate cancer	Relatively poor prognosis	Many reports indicated a link between TAMs and poor prognosis,[63,64] but not a few studies reported an opposite link.[65]
Thyroid cancer	Not clear	Dense TAM infiltration was observed in poorly differentiated carcinoma[69] as well as in advanced thyroid cancer.[70] Relationship between TAM density and prognosis of patients was not clear.
Cholangiocarcinoma	Poor prognosis	High TAM density correlated with poor prognosis.[22,72] TAM density was associated with microvessel density and the number of $FOXP3^+$ regulatory T cells.[22]
Renal cell carcinoma	Poor prognosis	High TAM density correlated with tumor progression with or without link to poor prognosis.[76–81]M2-TAMs were related to poor prognosis more closely than were total TAMs.[79,80]
Urothelial cell carcinoma of the bladder	Poor prognosis	High TAM density correlated with tumor progression with or without link to poor prognosis.[82–86] Recurrence after BCG therapy occurred frequently in patients with a high density of TAMs.[84,85]
Endometrial caner	Poor prognosis	High TAM density correlated with tumor progression with or without link to poor prognosis.[87–90] M2-TAMs but not total TAMs correlated with poor prognosis.[90]
Epithelial ovarian cancer	Poor prognosis	High density of M2-TAMs but not total TAMs correlated with tumor progression with or without link to poor prognosis.[92]
Uterine cervical cancer	Not clear	No association between $CD68^+$ TAMs and prognosis.[96,98]
Esophageal cancer	Poor prognosis	High TAM density linked to poor prognosis.[101–103] M2-TAMs were related to poor prognosis more closely than were total TAMs.[103]

BCG, bacille de Calmette et Guérin; FOXP3, forkhead box P 3; HCC, hepatocellular carcinoma; RCC, renal cell carcinoma;. TAM, tumor-associated macrophage.

urothelial cell carcinoma of the bladder, endometrial cancer, epithelial ovarian cancer, and esophageal cancer, TAMs are thought to contribute tumor progression and correlate with poor prognosis of patients (Table 1). M2-TAMs are linked to poor

Table 2 Roles of TAMs in human non-epithelial tumors

Tumor type	Link of TAMs to tumor progression and prognosis	Comments
Glioma	Poor prognosis	High TAM density correlated with tumor progression with or without link to poor prognosis.[104,112] M2-TAMs were related to poor prognosis more closely than were total TAMs[104]
Melanoma	Poor prognosis	High TAM density correlated with tumor progression with or without link to poor prognosis.[113–117]
Hodgkin lymphoma	Poor prognosis	High TAM density linked to poor prognosis.[119–123] M2-TAMs were related to poor prognosis more closely than were total TAMs.[120,122,123]
B-cell non-Hodgkin lymphoma	Poor prognosis	High TAM density linked to poor prognosis.[127–132,134,135] M2-TAMs were related to poor prognosis more closely than were total TAMs.[132,134,135] High TAM density correlated with longer survival in rituximab-treated patients.[129,130,135]
T-cell non-Hodgkin lymphoma	Poor prognosis	M2-TAMs were related to poor prognosis more closely than were total TAMs.[138–142]

TAM: tumor-associated macrophage.

prognosis more than are total TAMs in breast cancer, pancreatic cancer, renal cell carcinoma, endometrial cancer, epithelial ovarian cancer, and esophageal cancer. In inflammation-related cancers such as HCC and uterine cervical cancer, the influence of TAMs on tumor progression is limited partly because of the different balance of tumor M1- and M2-TAMs. As already indicated, TAM density in colorectal cancer correlated with favorable prognosis.[50] Because the large intestine is an organ exposed to continuous inflammatory stimuli from bacterial flora and digested materials, M1-TAM functions may dominate over M2-TAM functions.[50] The histological features of tumors and infiltration sites of macrophages also influences the role of TAMs. In breast and lung cancers, stromal TAM density correlated more closely with poor prognosis than did intratumoral TAM density.[35,38] For non-epithelial tumors such as glioma, melanoma, and lymphoma, most reports indicated a significant relationship between TAM density and poor prognosis (Table 2). Close associations including direct contact between tumor cells and TAMs exist in non-epithelial tumors. Such cell-to-cell interaction is believed to promote M2 polarization of macrophages and proliferation of tumor cells via activation of STAT3.[25]

Most human studies today have used CD68 as a pan-macrophage marker, and CD163, CD204, and/or CD206 as M2 phenotype markers to compare total TAMs and M2-TAMs. In most papers referred to in this review, CD163 was mainly used to detect M2-TAMs. Certain studies compared $CD163^+$ TAMs and $CD204^+$ TAMs. In esophageal squamous cell carcinoma, a high density of $CD204^+$ TAMs correlated with more malignant phenotypes, whereas a high density of $CD163^+$ TAMs did not.[102] In glioma[104] and pancreatic ductal carcinoma,[60] both $CD163^+$ TAMs and $CD204^+$ TAMs correlated with poor prognosis of patients. In clear cell renal cell carcinoma[79] and adult T-cell leukemia/lymphoma,[138,139] in contrast, $CD163^+$ TAMs correlated with poor prognosis but $CD204^+$ TAMs did not. These differences between $CD163^+$ and $CD204^+$ TAMs indicate that CD163 and CD204 are differently induced in M2 macrophages in a manner that depends on tumor type and tumor-specific microenvironments. For example, the matricellular protein cysteine-rich angiogenic inducer 61, which is enriched in tissues of esophageal squamous cell carcinoma, is believed to induce CD204 expression in infiltrated macrophages.[144] The molecular functions of CD163 and CD204 themselves are also suggested directly influence the function of M2-TAMs. CD163 is a membrane protein belonging to the scavenger receptor cysteine-rich domain family and acts as an endocytic receptor for a hemoglobin-haptoglobin (Hb-Hp) complex.[145] Binding of this Hb-Hp complex to CD163 elicits a direct anti-inflammatory effect via secretion of IL-10.[145] CD204 is a class A scavenger receptor that recognizes various negatively charged macromolecules, including modified low-density lipoproteins and apoptotic cells.[146] CD204 suppresses the TLR4-mediated inflammatory response by inhibiting the binding of LPS to TRL4 in a competitive manner.[146] Because both molecules possess some kind of anti-inflammatory function, CD163 and CD204 may be directly involved in the functions of M2-TAMs.

For determining macrophage phenotypes in tumor tissue specimens, currently used immunohistochemical methods have limitations to discriminate M1 and M2 macrophages. Because M1 and M2 stimuli do not exist alone in tumor tissues, TAMs are activated variously by multiple stimuli. Each of CD163, CD204, or CD206 recognizes different spectrums of macrophages stimulated toward M2 phenotype. Another problem is that no suitable immunohistochemical markers exist to detect M1 macrophages. Several recent studies introduced double immunohistochemical methods using a macrophage-specific molecule and molecules associated with functions of

M1 or M2 macrophages that are not necessarily specific for macrophages. For example, Ohri et al.[39] used CD68/HLA-DR as an M1 marker and CD68/CD163 as an M2 marker. Barros et al.[100] used CD68/STAT1 and CD163/STAT1 as M1 markers and CD68/CMAF and CD163/CMAF as M2 markers. Although these trials produced new findings, more specific immunohistochemical markers to differentiate M1 and M2 macrophages are needed.

Accumulated data indicate the close association of TAMs and poor prognosis in many human cancers, and TAMs, especially M2-TAMs, are thought to be a new therapeutic target.[21,147] Resetting macrophages phenotypes by exposing M2 macrophages to M1 stimuli, or vice versa, can re-polarized already differentiated macrophages.[148] For example, treatments have attempted to re-polarized M2 TAMs in loco by suppressing the molecules implicated in M2 differentiation including nuclear factor-κB, STAT3, STAT6, and IFN regulatory factor 4.[148–150] Further clarification of TAM phenotypes and their role in individual human malignancy will provide valuable data for developing new therapeutic strategies.

ACKNOWLEDGMENTS

The authors' work was supported in part by Grants-in Aid for Scientific Research from the Ministry of Education, Culture, Science, and Technology of Japan (B25293089, C25460497).

DISCLOSURE STATEMENT

None declared

REFERENCES

1 Bingle L, Brown NJ, Lewis CE. The role of tumour-associated macrophages in tumour progression: Implications for new anticancer therapies. *J Pathol* 2002; **196**: 254–5.
2 Lewis CE, Pollard JW. Distinct role of macrophages in different tumor microenvironments. *Cancer Res* 2006; **66**: 605–12.
3 Komohara Y, Jinushi M, Takeya M. Clinical significance of macrophage heterogeneity in human malignant tumors. *Cancer Sci* 2014; **105**: 1–8.
4 Lahmar Q, Keirsse J, Laoui D, Movahedi K, Van Overmeire E, Van Ginderachter JA. Tissue-resident versus monocyte-derived macrophages in the tumor microenvironment. *Biochim Biophys Acta* 1865; **2016**: 23–34.
5 Pollard JW. Tumour-educated macrophages promote tumour progression and metastasis. *Nat Rev Cancer* 2004; **4**: 71–8.
6 Mantovani A, Sozzani S, Locati M, Allavena P, Sica A. Macrophage polarization: Tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. *Trends Immunol* 2002; **23**: 549–55.
7 Takeya M, Yoshimura T, Leonard EJ, Takahashi K. Detection of monocyte chemoattractant protein-1 in human atherosclerotic lesions by an anti-monocyte chemoattractant protein-1 monoclonal antibody. *Hum Pathol* 1993; **24**: 534–9.
8 Ginhoux F, Greter M, Leboeuf M *et al.* Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science* 2010; **330**: 841–5.
9 van Furth R, Cohn ZA. The origin and kinetics of mononuclear phagocytes. *J Exp Med* 1968; **128**: 415–35.
10 Gabrusiewicz K, Ellert-Miklaszewska A, Lipko M, Sielska M, Frankowska M, Kaminska B. Characteristics of the alternative phenotype of microglia/macrophages and its modulation in experimental gliomas. *PLoS One* 2011; **6** :e23902
11 Mills CD, Kincaid K, Alt JM, Heilman MJ, Hill AM. M-1/M-2 macrophages and the Th1/Th2 paradigm. *J Immunol* 2000; **164**: 6166–73.
12 Murray PJ, Allen JE, Biswas SK *et al.* Macophage activation and polarization: Nomenclature and experimental guidelines. *Immunity* 2014; **41**: 14–20.
13 Doyle AG, Herbein G, Montaner LJ *et al.* Interleukin-13 alters the activation state of murine macrophages in vitro: comparison with interleukin-4 and interferon-gamma. *Eur J Immunol* 1994; **24**: 1441–5.
14 Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M. The chemokine system in diverse forms of macrophage activation and polarization. *Trends Immunol* 2004; **25**: 677–86.
15 Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. *Nat Rev Immunol* 2008; **8**: 958–69.
16 Biswas SK, Sica A, Lewis CE. Plasticity of macrophage function during tumor progression: Regulation by distinct molecular mechanisms. *J Immunol* 2008; **180**: 2011–17.
17 Martinez FO, Helming L, Gordon S. Alternative activation of macrophages: An immunologic functional perspective. *Annu Rev Immunol* 2009; **27**: 451–83.
18 Martinez FO, Gordon S. The M1 and M2 paradigm of macrophage activation: time for reassessment. *F1000Prime Rep* 2014; **6**: 13.
19 Quail DF, Joyce JA. Microenvironmental regulation of tumor progression and metastasis. *Nat Med* 2013; **19**: 1423–37.
20 Allavena P, Sica A, Garlanda C, Mantovani A. The Yin-Yang of tumor-associated macrophages in neoplastic progression and immune surveillance. *Immunol Rev* 2008; **222**: 155–61.
21 Noy R, Pollard JW. Tumor-associated macrophages: From mechanisms to therapy. *Immunity* 2014; **41**: 49–61.
22 Hasita H, Komohara Y, Okabe H *et al.* Significance of alternatively activated macrophages in patients with intrahepatic cholangiocarcinoma. *Cancer Sci* 2010; **101**: 1913–19.
23 Raggi C, Mousa HS, Correnti M, Sica A, Invernizzi P. Cancer stem cells and tumor-associated macrophages: A roadmap for multitargeting strategies. *Oncogene* 2016; **35**: 671–82.
24 Yu H, Lee H, Herrmann A, Buettner R, Jove R. Revisiting STAT3 signalling in cancer: New and unexpected biological functions. *Nat Rev Cancer* 2014; **14**: 736–46.
25 Komohara Y, Horlad H, Ohnishi K *et al.* Importance of direct macrophage-tumor cell interaction on progression of human glioma. *Cancer Sci* 2012; **103**: 2165–72.
26 Lee H, Deng J, Kujawski M *et al.* STAT3-induced S1PR1 expression is crucial for persistent STAT3 activation in tumors. *Nat Med* 2010; **16**: 1421–8.
27 Kitamura T, Qian BZ, Soong D *et al.* CCL2-induced chemokine cascade promotes breast cancer metastasis by enhancing retention of metastasis-associated macrophages. *J Exp Med* 2015; **212**: 1043–59.
28 Deng J, Liu Y, Lee H *et al.* S1PR1-STAT3 signaling is crucial for myeloid cell colonization at future metastatic sites. *Cancer Cell* 2012; **21**: 642–54.
29 Leek RD, Lewis CE, Whitehouse R, Greenall M, Clarke J, Harris AL. Association of macrophage infiltration with angiogenesis and prognosis in invasive breast carcinoma. *Cancer Res* 1996; **56**: 4625–9.

30 Toi M, Ueno T, Matsumoto H *et al.* Clinical significance of thymidine phosphorylase as a marker of protumor monocytes in breast cancer. *Clin Cancer Res* 1999; **5**: 1131–7.
31 Valković T, Dobrila F, Melato M, Sasso F, Rizzardi C, Jonjić N. Correlation between vascular endothelial growth factor, angiogenesis, and tumor-associated macrophages in invasive ductal breast carcinoma. *Virchows Arch* 2002; **440**: 583–8.
32 Bolat F, Kayaselcuk F, Nursal TZ, Yagmurdur MC, Bal N, Demirhan B. Microvessel density, VEGF expression, and tumor-associated macrophages in breast tumors: Correlations with prognostic parameters. *J Exp Clin Cancer Res* 2006; **25**: 365–72.
33 Yuan ZY, Luo RZ, Peng RJ, Wang SS, Xue C. High infiltration of tumor-associated macrophages in triple-negative breast cancer is associated with a higher risk of distant metastasis. *Onco Targets Ther* 2014; **7**: 1475–80.
34 Sousa S, Brion R, Lintunen M *et al.* Human breast cancer cells educate macrophages toward the M2 activation status. *Breast Cancer Res* 2015; **17**: 101.
35 Medrek C, Pontén F, Jirström K, Leandersson K. The presence of tumor associated macrophages in tumor stroma as a prognostic marker for breast cancer patients. *BMC Cancer* 2012; **12**: 306.
36 Ch'ng ES, Tuan Sharif SE, Jaafar H. In human invasive breast ductal carcinoma, tumor stromal macrophages and tumor nest macrophages have distinct relationships with clinicopathological parameters and tumor angiogenesis. *Virchows Arch* 2013; **462**: 257–67.
37 Chung FT, Lee KY, Wang CW *et al.* Tumor-associated macrophages correlate with response to epidermal growth factor receptor-tyrosine kinase inhibitors in advanced non-small cell lung cancer. *Int J Cancer* 2012; **131**: E227–35.
38 Dai F, Liu L, Che G *et al.* The number and microlocalization of tumor-associated immune cells are associated with patient's survival time in non-small cell lung cancer. *BMC Cancer* 2010; **10**: 220.
39 Ohri CM, Shikotra A, Green RH, Waller DA, Bradding P. Macrophages within NSCLC tumour islets are predominantly of a cytotoxic M1 phenotype associated with extended survival. *Eur Respir J* 2009; **33**: 118–26.
40 Ohri CM, Shikotra A, Green RH, Waller DA, Bradding P. Tumour necrosis factor-alpha expression in tumour islets confers a survival advantage in non-small cell lung cancer. *BMC Cancer* 2010; **10**: 323.
41 Ohtaki Y, Ishii G, Nagai K *et al.* Stromal macrophage expressing CD204 is associated with tumor aggressiveness in lung adenocarcinoma. *J Thorac Oncol* 2010; **5**: 1507–15.
42 Hirayama S, Ishii G, Nagai K *et al.* Prognostic impact of CD204-positive macrophages in lung squamous cell carcinoma: Possible contribution of CD204-positive macrophages to the tumor-promoting microenvironment. *J Thorac Oncol* 2012; **7**: 1790–7.
43 Maeda R, Ishii G, Neri S *et al.* Circulating $CD14^{+}CD204^{+}$ cells predict postoperative recurrence in non-small-cell lung cancer patients. *J Thorac Oncol* 2014; **9**: 179–88.
44 Takahashi A, Ishii G, Kinoshita T *et al.* Identification of prognostic immunophenotypic features in cancer stromal cells of high-grade neuroendocrine carcinomas of the lung. *J Cancer Res Clin Oncol* 2013; **139**: 1869–78.
45 Zhu XD, Zhang JB, Zhuang PY *et al.* High expression of macrophage colony-stimulating factor in peritumoral liver tissue is associated with poor survival after curative resection of hepatocellular carcinoma. *J Clin Oncol* 2008; **26**: 2707–16.
46 Ding T, Xu J, Wang F *et al.* High tumor-infiltrating macrophage density predicts poor prognosis in patients with primary hepatocellular carcinoma after resection. *Hum Pathol* 2009; **40**: 381–9.
47 Kong LQ, Zhu XD, Xu HX *et al.* The clinical significance of the CD163+ and CD68+ macrophages in patients with hepatocellular carcinoma. *PLoS One* 2013; **8** :e59771
48 Li YW, Qiu SJ, Fan J *et al.* Tumor-infiltrating macrophages can predict favorable prognosis in hepatocellular carcinoma after resection. *J Cancer Res Clin Oncol* 2009; **135**: 439–49.
49 Wang H, Wang X, Li X *et al.* $CD68^{+}HLA\text{-}DR^{+}$ M1-like macrophages promote motility of HCC cells via NF-κB/FAK pathway. *Cancer Lett* 2014; **345**: 91–9.
50 Edin S, Wikberg ML, Oldenborg PA, Palmqvist R. Macrophages: Good guys in colorectal cancer. *Oncoimmunology* 2013; **2** : e23038
51 Edin S, Wikberg ML, Dahlin AM *et al.* The distribution of macrophages with a M1 or M2 phenotype in relation to prognosis and the molecular characteristics of colorectal cancer. *PLoS One* 2012; **7** :e47045
52 Kang JC, Chen JS, Lee CH, Chang JJ, Shieh YS. Intratumoral macrophage counts correlate with tumor progression in colorectal cancer. *J Surg Oncol* 2010; **102**: 242–8.
53 Ohtani H, Naito Y, Saito K, Nagura H. Expression of costimulatory molecules B7-1 and B7-2 by macrophages along invasive margin of colon cancer: A possible antitumor immunity? *Lab Invest* 1997; **77**: 231–41.
54 Shabo I, Olsson H, Sun XF, Svanvik J. Expression of the macrophage antigen CD163 in rectal cancer cells is associated with early local recurrence and reduced survival time. *Int J Cancer* 2009; **125**: 1826–31.
55 Chang WJ, Du Y, Zhao X, Ma LY, Cao GW. Inflammation-related factors predicting prognosis of gastric cancer. *World J Gastroenterol* 2014; **20**: 4586–96.
56 Ishigami S, Natsugoe S, Tokuda K *et al.* Tumor-associated macrophage (TAM) infiltration in gastric cancer. *Anticancer Res* 2003; **23**: 4079–83.
57 Pantano F, Berti P, Guida FM *et al.* The role of macrophages polarization in predicting prognosis of radically resected gastric cancer patients. *J Cell Mol Med* 2013; **17**: 1415–21.
58 Lin CN, Wang CJ, Chao YJ, Lai MD, Shan YS. The significance of the co-existence of osteopontin and tumor-associated macrophages in gastric cancer progression. *BMC Cancer* 2015; **15**: 128.
59 Erkan M, Hausmann S, Michalski CW *et al.* The role of stroma in pancreatic cancer: Diagnostic and therapeutic implications. *Nat Rev Gastroenterol Hepatol* 2012; **9**: 454–67.
60 Kurahara H, Shinchi H, Mataki Y *et al.* Significance of M2-polarized tumor-associated macrophage in pancreatic cancer. *J Surg Res* 2011; **167**: e211–19.
61 Yoshikawa K, Mitsunaga S, Kinoshita T *et al.* Impact of tumor-associated macrophages on invasive ductal carcinoma of the pancreas head. *Cancer Sci* 2012; **103**: 2012–20.
62 Hou YC, Chao YJ, Tung HL, Wang HC, Shan YS. Coexpression of CD44-positive/CD133-positive cancer stem cells and CD204-positive tumor-associated macrophages is a predictor of survival in pancreatic ductal adenocarcinoma. *Cancer* 2014; **120**: 2766–77.
63 Gannon PO, Poisson AO, Delvoye N, Lapointe R, Mes-Masson AM, Saad F. Characterization of the intra-prostatic immune cell infiltration in androgen-deprived prostate cancer patients. *J Immunol Methods* 2009; **348**: 9–17.
64 Nonomura N, Takayama H, Nakayama M *et al.* Infiltration of tumour-associated macrophages in prostate biopsy specimens is predictive of disease progression after hormonal therapy for prostate cancer. *BJU Int* 2011; **107**: 1918–22.
65 Takayama H, Nonomura N, Nishimura K *et al.* Decreased immunostaining for macrophage scavenger receptor is associated with poor prognosis of prostate cancer. *BJU Int* 2009; **103**: 470–4.
66 Gollapudi K, Galet C, Grogan T *et al.* Association between tumor-associated macrophage infiltration, high grade prostate cancer, and biochemical recurrence after radical prostatectomy. *Am J Cancer Res* 2013; **3**: 523–9.

67 Fujii T, Shimada K, Asai O *et al.* Immunohistochemical analysis of inflammatory cells in benign and precancerous lesions and carcinoma of the prostate. *Pathobiology* 2013; **80**: 119–26.
68 Herrmann G, Schumm-Draeger PM, Müller C *et al.* T lymphocytes, CD68-positive cells and vascularisation in thyroid carcinomas. *J Cancer Res Clin Oncol* 1994; **120**: 651–6.
69 Ryder M, Ghossein RA, Ricarte-Filho JC, Knauf JA, Fagin JA. Increased density of tumor-associated macrophages is associated with decreased survival in advanced thyroid cancer. *Endocr Relat Cancer* 2008; **15**: 1069–74.
70 Qing W, Fang WY, Ye L *et al.* Density of tumor-associated macrophages correlates with lymph node metastasis in papillary thyroid carcinoma. *Thyroid* 2012; **22**: 905–10.
71 Cunha LL, Morari EC, Guihen AC *et al.* Infiltration of a mixture of immune cells may be related to good prognosis in patients with differentiated thyroid carcinoma. *Clin Endocrinol (Oxf)* 2012; **77**: 918–25.
72 Subimerb C, Pinlaor S, Khuntikeo N *et al.* Tissue invasive macrophage density is correlated with prognosis in cholangiocarcinoma. *Mol Med Rep* 2010; **3**: 597–605.
73 Subimerb C, Pinlaor S, Lulitanond V *et al.* Circulating $CD14^+CD16^+$ monocyte levels predict tissue invasive character of cholangiocarcinoma. *Clin Exp Immunol* 2010; **161**: 471–79.
74 Thanee M, Loilome W, Techasen A *et al.* Quantitative changes in tumor-associated M2 macrophages characterize cholangiocarcinoma and their association with metastasis. *Asian Pac J Cancer Prev* 2015; **16**: 3043–50.
75 Techasen A, Loilome W, Namwat N, Dokduang H, Jongthawin J, Yongvanit P. Cytokines released from activated human macrophages induce epithelial mesenchymal transition markers of cholangiocarcinoma cells. *Asian Pac J Cancer Prev* Suppl 2012; **13**: 115–8.
76 Santoni M, Massari F, Amantini C *et al.* Emerging role of tumor-associated macrophages as therapeutic targets in patients with metastatic renal cell carcinoma. *Cancer Immunol Immunother* 2013; **62**: 1757–68.
77 Toge H, Inagaki T, Kojimoto Y, Shinka T, Hara I. Angiogenesis in renal cell carcinoma: The role of tumor-associated macrophages. *Int J Urol* 2009; **16**: 801–7.
78 Dannenmann SR, Thielicke J, Stöckli M *et al.* Tumor-associated macrophages subvert T-cell function and correlate with reduced survival in clear cell renal cell carcinoma. *Oncoimmunology* 2013; **2** :e23562
79 Komohara Y, Hasita H, Ohnishi K *et al.* Macrophage infiltration and its prognostic relevance in clear cell renal cell carcinoma. *Cancer Sci* 2011; **102**: 1424–31.
80 Xu L, Zhu Y, Chen L *et al.* Prognostic value of diametrically polarized tumor-associated macrophages in renal cell carcinoma. *Ann Surg Oncol* 2014; **21**: 3142–50.
81 Komohara Y, Morita T, Annan DA *et al.* The coordinated actions of TIM-3 on cancer and myeloid cells in the regulation of tumorigenicity and clinical prognosis in clear cell renal cell carcinomas. *Cancer Immunol Res* 2015; **3**: 999–1007.
82 Hanada T, Nakagawa M, Emoto A, Nomura T, Nasu N, Nomura Y. Prognostic value of tumor-associated macrophage count in human bladder cancer. *Int J Urol* 2000; **7**: 263–9.
83 Chen WT, Hung WC, Kang WY *et al.* Overexpression of cyclooxygenase-2 in urothelial carcinoma in conjunction with tumor-associated-macrophage infiltration, hypoxia-inducible factor-1α expression, and tumor angiogenesis. *APMIS* 2009; **117**: 176–84.
84 Ayari C, LaRue H, Hovington H *et al.* Bladder tumor infiltrating mature dendritic cells and macrophages as predictors of response to bacillus Calmette-Guérin immunotherapy. *Eur Urol* 2009; **55**: 1386–95.
85 Takayama H, Nishimura K, Tsujimura A *et al.* Increased infiltration of tumor associated macrophages is associated with poor prognosis of bladder carcinoma in situ after intravesical bacillus Calmette-Guerin instillation. *J Urol* 2009; **181**: 1894–900.
86 Wang B, Liu H, Dong X *et al.* High $CD204^+$ tumor-infiltrating macrophage density predicts a poor prognosis in patients with urothelial cell carcinoma of the bladder. *Oncotarget* 2015; **6**: 20204–14.
87 Salvesen HB, Akslen LA. Significance of tumour-associated macrophages, vascular endothelial growth factor and thrombospondin-1 expression for tumour angiogenesis and prognosis in endometrial carcinomas. *Int J Cancer* 1999; **84**: 538–43.
88 Soeda S, Nakamura N, Ozeki T *et al.* Tumor-associated macrophages correlate with vascular space invasion and myometrial invasion in endometrial carcinoma. *Gynecol Oncol* 2008; **109**: 122–8.
89 Hashimoto I, Kodama J, Seki N *et al.* Macrophage infiltration and angiogenesis in endometrial cancer. *Anticancer Res* 2000; **20**: 4853–6.
90 Espinosa I, José Carnicer M, Catasus L *et al.* Myometrial invasion and lymph node metastasis in endometrioid carcinomas: Tumor-associated macrophages, microvessel density, and HIF1A have a crucial role. *Am J Surg Pathol* 2010; **34**: 1708–14.
91 Takaishi K, Komohara Y, Tashiro H *et al.* Involvement of M2-polarized macrophages in the ascites from advanced epithelial ovarian carcinoma in tumor progression via Stat3 activation. *Cancer Sci* 2010; **101**: 2128–36.
92 Lan C, Huang X, Lin S *et al.* Expression of M2-polarized macrophages is associated with poor prognosis for advanced epithelial ovarian cancer. *Technol Cancer Res Treat* 2013; **12**: 259–67.
93 Kawamura K, Komohara Y, Takaishi K, Katabuchi H, Takeya M. Detection of M2 macrophages and colony-stimulating factor 1 expression in serous and mucinous ovarian epithelial tumors. *Pathol Int* 2009; **59**: 300–5.
94 Zhang M, He Y, Sun X *et al.* A high M1/M2 ratio of tumor-associated macrophages is associated with extended survival in ovarian cancer patients. *J Ovarian Res* 2014; **7**: 19.
95 Piersma SJ. Immunosuppressive tumor microenvironment in cervical cancer patients. *Cancer Microenviron* 2011; **4**: 361–75.
96 Zijlmans HJ, Fleuren GJ, Baelde HJ, Eilers PH, Kenter GG, Gorter A. Role of tumor-derived proinflammatory cytokines GM-CSF, TNF-α, and IL-12 in the migration and differentiation of antigen-presenting cells in cervical carcinoma. *Cancer* 2007; **109**: 556–65.
97 Hammes LS, Tekmal RR, Naud P *et al.* Macrophages, inflammation and risk of cervical intraepithelial neoplasia (CIN) progression—Clinicopathological correlation. *Gynecol Oncol* 2007; **105**: 157–65.
98 Ding H, Cai J, Mao M *et al.* Tumor-associated macrophages induce lymphangiogenesis in cervical cancer via interaction with tumor cells. *APMIS* 2014; **122**: 1059–69.
99 Petrillo M, Zannoni GF, Martinelli E *et al.* Polarisation of tumor-associated macrophages toward M2 phenotype correlates with poor response to chemoradiation and reduced survival in patients with locally advanced cervical cancer. *PLoS One* 2015; **10** : e0136654
100 Barros MH, Hauck F, Dreyer JH, Kempkes B, Niedobitek G. Macrophage polarisation: An immunohistochemical approach for identifying M1 and M2 macrophages. *PLoS One* 2013; **8** : e80908
101 Guo SJ, Lin DM, Li J *et al.* Tumor-associated macrophages and CD3-ζ expression of tumor-infiltrating lymphocytes in human esophageal squamous-cell carcinoma. *Dis Esophagus* 2007; **20**: 107–16.
102 Shigeoka M, Urakawa N, Nakamura T *et al.* Tumor associated macrophage expressing CD204 is associated with tumor

aggressiveness of esophageal squamous cell carcinoma. *Cancer Sci* 2013; **104**: 1112–9.
103 Cao W, Peters JH, Nieman D, Sharma M, Watson T, Yu J. Macrophage subtype predicts lymph node metastasis in oesophageal adenocarcinoma and promotes cancer cell invasion in vitro. *Br J Cancer* 2015; **113**: 738–46.
104 Komohara Y, Ohnishi K, Kuratsu J, Takeya M. Possible involvement of the M2 anti-inflammatory macrophage phenotype in growth of human gliomas. *J Pathol* 2008; **216**: 15–24.
105 Leung SY, Wong MP, Chung LP, Chan AS, Yuen ST. Monocyte chemoattractant protein-1 expression and macrophage infiltration in gliomas. *Acta Neuropathol* 1997; **93**: 518–27.
106 Sielska M, Przanowski P, Wylot B *et al.* Distinct roles of CSF family cytokines in macrophage infiltration and activation in glioma progression and injury response. *J Pathol* 2013; **230**: 310–21.
107 Yoshimura T, Robinson EA, Tanaka S, Appella E, Kuratsu J, Leonard EJ. Purification and amino acid analysis of two human glioma-derived monocyte chemoattractants. *J Exp Med* 1989; **169**: 1449–59.
108 Lawson LJ, Perry VH, Gordon S. Turnover of resident microglia in the normal adult mouse brain. *Neuroscience* 1992; **48**: 405–15.
109 Hirano H, Tanioka K, Yokoyama S, Akiyama S, Kuratsu J. Angiogenic effect of thymidine phosphorylase on macrophages in glioblastoma multiforme. *J Neurosurg* 2001; **95**: 89–95.
110 Jenny B, Harrison JA, Baetens D *et al.* Expression and localization of VEGF-C and VEGFR-3 in glioblastomas and haemangioblastomas. *J Pathol* 2006; **209**: 34–43.
111 Coniglio SJ, Eugenin E, Dobrenis K *et al.* Microglial stimulation of glioblastoma invasion involves epidermal growth factor receptor (EGFR) and colony stimulating factor 1 receptor (CSF-1R) signaling. *Mol Med* 2012; **18**: 519–27.
112 Roggendorf W, Strupp S, Paulus W. Distribution and characterization of microglia/macrophages in human brain tumors. *Acta Neuropathol* 1996; **92**: 288–93.
113 Bröcker EB, Zwadlo G, Holzmann B *et al.* Inflammatory cell infiltrates in human melanoma at different stages of tumor progression. *Int J Cancer* 1988; **41**: 562–7.
114 Varney ML, Johansson SL, Singh RK. Tumour-associated macrophage infiltration, neovascularization and aggressiveness in malignant melanoma: Role of monocyte chemotactic protein-1 and vascular endothelial growth factor-A. *Melanoma Res* 2005; **15**: 417–25.
115 Jensen TO, Schmidt H, Møller HJ *et al.* Macrophage markers in serum and tumor have prognostic impact in American Joint Committee on Cancer stage I/II melanoma. *J Clin Oncol* 2009; **27**: 3330–7.
116 Shi L, Lei D, Ma C *et al.* Clinicopathological implications of tumour-associated macrophages and vascularization in sinonasal melanoma. *J Int Med Res* 2010; **38**: 1276–86.
117 Bronkhorst IH, Ly LV, Jordanova ES *et al.* Detection of M2-macrophages in uveal melanoma and relation with survival. *Invest Ophthalmol Vis Sci* 2011; **52**: 643–50.
118 Coppleson LW, Rappaport H, Strum SB, Rose J. Analysis of the Rye classification of Hodgkin's disease. The prognostic significance of cellular composition. *J Natl Cancer Inst* 1973; **51**: 379–90.
119 Steidl C, Lee T, Shah SP *et al.* Tumor-associated macrophages and survival in classic Hodgkin's lymphoma. *N Engl J Med* 2010; **362**: 875–85.
120 Zaki MA, Wada N, Ikeda J *et al.* Prognostic implication of types of tumor-associated macrophages in Hodgkin lymphoma. *Virchows Arch* 2011; **459**: 361–6.
121 Tan KL, Scott DW, Hong F *et al.* Tumor-associated macrophages predict inferior outcomes in classic Hodgkin lymphoma: A correlative study from the E2496 Intergroup trial. *Blood* 2012; **120**: 3280–7.
122 Klein JL, Nguyen TT, Bien-Willner GA *et al.* CD163 immunohistochemistry is superior to CD68 in predicting outcome in classical Hodgkin lymphoma. *Am J Clin Pathol* 2014; **141**: 381–7.
123 Koh YW, Park CS, Yoon DH, Suh C, Huh J. CD163 expression was associated with angiogenesis and shortened survival in patients with uniformly treated classical Hodgkin lymphoma. *PLoS One* 2014; **9** :e87066
124 Azambuja D, Natkunam Y, Biasoli I *et al.* Lack of association of tumor-associated macrophages with clinical outcome in patients with classical Hodgkin's lymphoma. *Ann Oncol* 2012; **23**: 736–42.
125 Barros MH, Segges P, Vera-Lozada G, Hassan R, Niedobitek G. Macrophage polarization reflects T cell composition of tumor microenvironment in pediatric classical Hodgkin lymphoma and has impact on survival. *PLoS One* 2015; **10** :e0124531
126 Dave SS, Wright G, Tan B *et al.* Prediction of survival in follicular lymphoma based on molecular features of tumor-infiltrating immune cells. *N Engl J Med* 2004; **351**: 2159–69.
127 Farinha P, Masoudi H, Skinnider BF *et al.* Analysis of multiple biomarkers shows that lymphoma-associated macrophage (LAM) content is an independent predictor of survival in follicular lymphoma (FL). *Blood* 2005; **106**: 2169–74.
128 Canioni D, Salles G, Mounier N *et al.* High numbers of tumor-associated macrophages have an adverse prognostic value that can be circumvented by rituximab in patients with follicular lymphoma enrolled onto the GELA-GOELAMS FL-2000 trial. *J Clin Oncol* 2008; **26**: 440–6.
129 Taskinen M, Karjalainen-Lindsberg ML, Nyman H, Eerola LM, Leppä S. A high tumor-associated macrophage content predicts favorable outcome in follicular lymphoma patients treated with rituximab and cyclophosphamide-doxorubicin-vincristine-prednisone. *Clin Cancer Res* 2007; **13**: 5784–9.
130 Kelley T, Beck R, Absi A, Jin T, Pohlman B, Hsi E. Biologic predictors in follicular lymphoma: Importance of markers of immune response. *Leuk Lymphoma* 2007; **48**: 2403–11.
131 Cai QC, Liao H, Lin SX *et al.* High expression of tumor-infiltrating macrophages correlates with poor prognosis in patients with diffuse large B-cell lymphoma. *Med Oncol* 2012; **29**: 2317–22.
132 Wada N, Zaki MA, Hori Y *et al.* Tumour-associated macrophages in diffuse large B-cell lymphoma: A study of the Osaka Lymphoma Study Group. *Histopathology* 2012; **60**: 313–9.
133 Hasselblom S, Hansson U, Sigurdardottir M, Nilsson-Ehle H, Ridell B, Andersson PO. Expression of CD68+ tumor-associated macrophages in patients with diffuse large B-cell lymphoma and its relation to prognosis. *Pathol Int* 2008; **58**: 529–32.
134 Marchesi F, Cirillo M, Bianchi A *et al.* High density of CD68+/CD163+ tumour-associated macrophages (M2-TAM) at diagnosis is significantly correlated to unfavorable prognostic factors and to poor clinical outcomes in patients with diffuse large B-cell lymphoma. *Hematol Oncol* 2015; **33**: 110–2.
135 Nam SJ, Go H, Paik JH *et al.* An increase of M2 macrophages predicts poor prognosis in patients with diffuse large B-cell lymphoma treated with rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone. *Leuk Lymphoma* 2014; **55**: 2466–76.
136 Leidi M, Gotti E, Bologna L *et al.* M2 macrophages phagocytose rituximab-opsonized leukemic targets more efficiently than m1 cells in vitro. *J Immunol* 2009; **182**: 4415–22.
137 Kridel R, Xerri L, Gelas-Dore B *et al.* The prognostic impact of CD163-positive macrophages in follicular lymphoma: A study from the BC Cancer Agency and the Lymphoma Study Association. *Clin Cancer Res* 2015; **21**: 3428–35.
138 Komohara Y, Niino D, Saito Y *et al.* Clinical significance of $CD163^+$ tumor-associated macrophages in patients with adult T-cell leukemia/lymphoma. *Cancer Sci* 2013; **104**: 945–51.

139 Saito Y, Komohara Y, Niino D *et al.* Role of CD204-positive tumor-associated macrophages in adult T-cell leukemia/lymphoma. *J Clin Exp Hematop* 2014; **54**: 59–65.
140 Niino D, Komohara Y, Murayama T *et al.* Ratio of M2 macrophage expression is closely associated with poor prognosis for angioimmunoblastic T-cell lymphoma (AITL). *Pathol Int* 2010; **60**: 278–83.
141 Sugaya M, Miyagaki T, Ohmatsu H *et al.* Association of the numbers of $CD163^+$ cells in lesional skin and serum levels of soluble CD163 with disease progression of cutaneous T cell lymphoma. *J Dermatol Sci* 2012; **68**: 45–51.
142 Lin ZX, Bai B, Cai QC *et al.* High numbers of tumor-associated macrophages correlate with poor prognosis in patients with mature T- and natural killer cell lymphomas. *Med Oncol* 2012; **29**: 3522–8.
143 Wang H, Li P, Wang L *et al.* High numbers of CD68+ tumor-associated macrophages correlate with poor prognosis in extranodal NK/T-cell lymphoma, nasal type. *Ann Hematol* 2015; **94**: 1535–44.
144 Shigeoka M, Urakawa N, Nishio M *et al.* Cyr61 promotes CD204 expression and the migration of macrophages via MEK/ERK pathway in esophageal squamous cell carcinoma. *Cancer Med* 2014; **4**: 437–46.
145 Philippidis P, Mason JC, Evans BJ *et al.* Hemoglobin scavenger receptor CD163 mediates interleukin-10 release and heme oxygenase-1 synthesis: antiinflammatory monocyte–macrophage responses in vitro, in resolving skin blisters in vivo, and after cardiopulmonary bypass surgery. *Circ Res* 2004; **94**: 119–26.
146 Ohnishi K, Komohara Y, Fujiwara Y *et al.* Suppression of TLR4-mediated inflammatory response by macrophage class A scavenger receptor (CD204). *Biochem Biophys Res Commun* 2011; **411**: 516–22.
147 Komohara Y, Fujiwara Y, Ohnishi K, Takeya M. Tumor-associated macrophages: Potential therapeutic targets for anti-cancer therapy. *Adv Drug Deliv Rev* 2016; **99**: 180–5.
148 Mantovani A, Allevena P. The interaction of anticancer therapies with tumor-associated macrophages. *J Exp Med* 2015; **212**: 435–55.
149 Lawrence T, Natoli G. Transcriptional regulation of macrophage polarization: Enabling diversity with identity. *Nat Rev Immunol* 2011; **11**: 750–61.
150 Fujiwara Y, Komohara Y, Ikeda T, Takeya M. Corosolic acid inhibits glioblastoma cell proliferation by suppressing the activation of signal transducer and activator of transcription-3 and nuclear factor-kappa B in tumor cells and tumor-associated macrophages. *Cancer Sci* 2011; **102**: 206–11.