Interaction of tumor-associated macrophages and cancer chemotherapy

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ABSTRACT
It has been recently recognized that the tumor microenvironment (TME) is an essential factor that defines the efficiency of chemotherapy. The local TME, consisting of immune cells with diverse phenotypes and functions, can strongly modulate the response to chemotherapy. Tumor-associated macrophages (TAMs) that display pronounced heterogeneity and phenotypic plasticity are the major innate immune component in the microenvironment of solid tumors. In our review, we elucidate the complex role of TAMs in the progression of different types of solid tumors, summarize the current knowledge about the effects of different anticancer chemotherapeutic agents on monocytes/macrophages, and describe the mechanisms of chemotherapy resistance mediated by TAMs.

Introduction
Enhancing the efficiency of antitumor therapy is the most relevant challenge in clinical oncology. The main goal of the established cytostatic therapeutic schemas is to achieve the maximal cytoreduction of the primary tumor and metastatic foci by inducing apoptosis or necrosis or blocking uncontrolled cancer cell proliferation. The outcome of cytostatic treatment depends on the biological (including genetic) characteristics of tumor cells and their sensitivity or resistance to therapeutic agents. On average, only 40–60% of the cancer patients benefit from antitumor chemotherapy (CT). Drug resistance in surviving cancer cells, in fact, leads to an inability to achieve complete pathological regression. However, even when complete pathological regression is achieved, tumors can relapse in 10–40% of the cases.

The therapeutic sensitivity of tumors significantly depends on the complex interaction of cancer cells with different components of the tumor microenvironment (TME), particularly with immune cells. It was shown that effective cytostatic treatment is associated with a manifestation of the cytotoxic activity of immune effector cells that can be enhanced by radiation. Cytostatic agents can stimulate the antigenic properties of cancer cells, facilitating their recognition by the immune system and can also enhance the systemic effects of chemotherapy, resulting in immunosuppressive cell depletion.

The key cells of the immune system that define the intratumoral immune status and interaction of cancer cells with the immune component of the microenvironment are the tumor-associated macrophages (TAMs). There are two main directions of phenotypic and functional macrophage polarization: classically activated pro-inflammatory M1 macrophages with antitumor properties and alternatively activated anti-inflammatory M2 macrophages with tumor-supporting functions. In the majority of solid tumors, TAMs have a pronounced M2 phenotype that strongly supports primary tumor growth and metastatic spread. However, there are also experimental data suggesting that TAMs can combine the properties of M1 and M2 macrophages. The effect of TAMs on tumor progression can depend on the tumor type, the type of tumor microenvironment and the localization of TAMs in specific intratumoral compartments. Reprogramming the M2 macrophage phenotype toward the pro-inflammatory M1 phenotype is recognized as a promising therapeutic approach for cancer treatment.

In our review, we focused on recent advances in understanding the role of the microenvironment, particularly, the role of TAMs in defining the efficiency of chemotherapeutic treatment on solid tumors.

Macrophages and tumor progression
TAMs originate from two major sources: (a) tissue-resident macrophages that are long-living and embryonically derived (yolk sac), and (b) macrophages derived from the circulating monocytes that originate out of bone marrow and are recruited to tumor tissue by growth factors and chemokines, such as M-CSF, CCL2, and CCL5. The tumor microenvironment affects the programming of both resident and infiltrating macrophages into tumor-specific macrophages. It is...
believed that resident macrophages are the first to be reprogrammed by growing tumors into pro-tumoral phenotypes. TAMS originating from resident macrophages can mediate DNA damage, transformation and the survival of transformed cells and cancer-related inflammation. Monocytes/macrophages recruited to the tumor site further promote the proliferation and survival of tumor cells and angiogenesis.

A significant number of data indicate a supportive role for macrophages in cancer development, as shown in different experimental models and in clinical observations. In solid tumors, TAMs can promote primary tumor growth, induce angiogenesis, lymphangiogenesis, stromal remodeling, metastasis, and suppression of immunity. TAMs express molecules that directly affect cancer cell proliferation, including epidermal growth factor (EGF), members of the fibroblast growth factor (FGF) family, and transforming growth factor beta (TGFβ). The ability of TAMs to promote tumor progression and accelerate vessel growth is mediated through the upregulation and release of several pro-angiogenic factors, such as vascular endothelial growth factor A (VEGF-A), tumor necrosis factor α (TNFα), FGF, thymidine phosphorylase (TP), urokinase plasminogen activator (uPA), adenomedullin (ADM), and semaphorin 4D (Sem4D).

TAMs produce several factors that are responsible for the induction of lymphangiogenesis, including VEGF-C, VEGF-D, VEGF-A, MMP2, MMP9, CXCL8 and many others. TAMs support stromal remodeling, tumor cell invasion and metastasis by releasing various enzymes, including plasmin, uPA, matrix metalloproteinases (MMPs), cathepsin B, platelet-derived growth factor (PDGF) and TGF-β1. TAMs also secrete a number of cytokines and chemokines, such as CCL3, CCL4, CCL5, CCL22, TGFB, and IL10, which recruit natural regulatory T cells (nTreg) to the tumor microenvironment and suppress CD4+ and CD8 + T cell effector functions.

TAMS are able to support cancer stem cell functions. Cancer stem cells (CSCs) represent a tumor subset with an enhanced ability to initiate tumor progression, dissemination, and relapse. Thus, in non-small-cell lung carcinoma, TAMs accompany enhanced stemness by increasing the expression of CD133+ cells (CSCs) and inflammation-associated genes, including Sox2 and NF-κB. In glioma cells, M2-TAMs promote stemness and migration by secreting TGF-β1.

The tumor-supporting functions of TAMs have been demonstrated for many types of malignancies, such as breast cancer, lung adenocarcinoma, cervical cancer, ovarian cancer, prostate cancer, melanoma, renal cell carcinoma, and esophageal cancer. However, there is much experimental clinical evidence that indicates a dual role of TAMs in tumor progression and survival, and the antitumor activities of TAMs have been identified (Table 1). M. Bogels and coworkers showed that the pro- or antitumor activity of macrophages depends on the type of tumor. For example, breast carcinoma cells induce the secretion of tolerogenic cytokines in human monocyte-derived macrophages, while intestinal carcinoma cells stimulate the secretion of inflammatory cytokines.

In Table 1, we summarized the latest results concerning the tumor-promoting and antitumor activities of TAMs in different types of cancer and the association of the main macrophage markers with tumor progression. Thus, CD68 was indicative of poor prognosis and reduced survival in many types of cancer, including non-small-cell lung cancer (NSCLC), ovarian cancer, stomach cancer, melanoma, and breast cancer. In NSCLC, the tumor-infiltrating CD68-expressing macrophage density negatively correlated with patient survival and positively correlated with tumor IL-8 expression, which may contribute to the increased tumor angiogenesis. High levels of CD68-positive infiltrating TAMs in gastric cancer (GC) were associated with metastasis and poor prognosis and strongly correlated with EMT features (loss of E-cadherin and positivity of vimentin). In melanoma tissue, a high number of CD68+ macrophages was associated with a worse prognosis and high melanoma-specific mortality.

Controversial reports were found for CD68 expression in patients with breast cancer (BC). Triple-negative breast cancer (TNBC) with a large number of infiltrating CD68+ TAMs had a high risk of distant metastasis and low rates of disease-free survival (DFS) and overall survival (OS). In another study of breast tumors, high numbers of CD68 macrophages were significantly associated with worse breast cancer-specific survival and shorter DFS. A study of 100 breast cancer samples demonstrated a significant association of CD68+ TAM infiltration with TNM stages and tumor size, and the high-infiltration of TAMs in tumors correlated with poor outcome and decreased OS. On the other hand, the average score of CD68 expression was found to be lower in cases with lymph node (LN) metastases compared to negative LN, both without NAC and after NAC in BC patients.

The opposite results, indicating an antitumor effect of macrophages, were demonstrated for colorectal cancer where TAMs had pro-inflammatory properties and expressed a number of cytokines such as IFN-γ, IL-1, and IL-6, which activated cytotoxic Th1 cells, mediating the antitumor immune response. In a study of 446 patients with colorectal cancer treated in the Department of Surgery, Umea University Hospital (Sweden), Forsell J and colleagues showed that high levels of CD68+ TAMs localized in the tumor/stroma line were correlated with better survival in colon cancer. In another study of 208 patients with colorectal cancer who were treated in the Humanitas Research Hospital (Italy), a high amount of TAMs was associated with better DFS and OS, independent of nodal status and vascular invasion, in patients with colorectal cancer.

Several independent clinical studies demonstrated that the M2 phenotype of TAMs is associated with poor survival in patients with breast cancer, ovarian cancer, gastric cancer, renal cell carcinoma, hepatocellular carcinoma, multiple myeloma, and osteosarcoma. CD206 and CD163 are the most frequently used biomarkers to histologically identify the M2 phenotypes of TAMs in tumor tissues. Zhang et al. showed that a decrease in the number of CD206+ TAMs in gastric cancer was associated with a longer DFS, which can be considered a significant prognostic factor. A positive correlation between CD206+ TAM macrophage infiltration and poor survival rates was found for ovarian cancer, renal cell carcinoma, and hepatocellular carcinoma. Similarly, an increased density of CD163+ was found in the advanced stages of ovarian cancer, multiple myeloma, gastric cancer, breast cancer, osteosarcoma and positively correlated with worse progression-free survival (PFS) and OS.
Stabilin-1 is a multifunctional scavenger receptor that plays important roles in the clearance of “unwanted” self-substances. The expression of stabilin-1 was found on alternatively activated macrophages. A high number of stabilin-1 + TAMs was found in metastasizing primary human breast tumors and was shown to support tumor growth in a mammary adenocarcinoma mouse model. Chitinase-like proteins (CLPs) are evolutionarily conserved lectins, and their elevated levels of gene expression or secretion are indicative of tumor progression, metastasis or response to therapy. Thus, circulating levels of YKL-40 are increased in glioblastoma, breast, colorectal, lung, prostate, bladder, stomach, and endometrial cancers among others and predict a poor outcome or short DFS. For example, YKL-40 may contribute to the proliferation of malignant cells, stimulate angiogenesis, and regulate extracellular tissue remodeling. YKL-39 is considered a biological marker for the progression of osteoarthritis. Recently, we identified that an elevated gene expression of YKL-39, a new pro-angiogenic and monocyte-recruiting factor in breast cancer tumors, after neoadjuvant chemotherapy (NAC) is predictive for the increased risk of distant metastasis and for a poor response to NAC. In patients who received anthracycline-containing NAC, the absence of a clinical response was associated with the presence of M2-type TAMs identified by the expression of YKL-39 or CCL18.

These numerous studies demonstrate the important roles of both the amount and phenotypes of TAMs in tumor progression and metastasis and indicate the necessity to understand the mechanism of the interaction between TAMs and chemotherapeutic agents to predict the efficiency of chemotherapy and to design therapeutic schemas enhancing the antitumor activities of TAMs.

### Cancer chemotherapy, chemoresistance, and immunomodulation

In patients with the operable forms of cancer, systemic chemotherapy is divided into preoperative (neoadjuvant) and postoperative (adjuvant) chemotherapy. The goal of adjuvant therapy is the long-term suppression of distant metastasis and the eradication of micrometastases after surgical treatment, which results in an increase in patient survival and a prolongation of disease-free (metastases-free) period.

### Table 1. Correlations between common macrophage markers and the progression of several cancers.

| Markers | Types of cancer | Correlations | Method of analysis |
|---------|----------------|--------------|--------------------|
| CD68 | Non-small cell lung cancer (NSCLC) (N = 35) | Increasing CD68+ macrophage density correlated with reduced survival. | IHC |
| | Gastric cancer (N = 178) | High CD68+ TAM infiltration is associated with metastasis and poor prognosis. | IHC |
| | Melanoma (N = 167) | A high number of CD68+ macrophages is associated with worse prognosis and high melanoma-specific mortality. | IHC |
| | Breast cancer (N = 287) | A large number of infiltrating CD68+ TAMs correlated with high risk of distant metastasis and low rates of DFS and OS. | IHC |
| | Breast cancer (N = 1322) | High numbers of infiltrating CD68+ macrophages were significantly associated with worse breast cancer-specific survival and shorter DFS. | IHC and tissue microarrays |
| | Breast cancer (N = 100) | High CD68+ TAMs are associated with poor outcome and decreased OS. | IHC |
| | Breast cancer (N = 36) | CD68+ TAMs are negatively correlated with LN metastasis. | IHC and IF |
| | Breast cancer (N = 50) | CD68+ TAMs were negatively correlated with lymphatic metastasis after NAC. | IHC and IF |
| | Colon cancer (N = 446) | Significant positive association between survival and CD68+ macrophages was found. | IHC |
| | Colorectal cancer (N = 208) | High CD68+ TAMs were associated with better DFS. | IHC |
| CD206 | Renal cell carcinoma (N = 185) | High CD206+ TAM density were correlated with reduced survival. | IHC |
| | Gastric cancer (N = 180) | Low CD206+ TAM infiltration was associated with a longer DFS. | IHC |
| | Hepatocellular carcinoma (N = 80) | High CD11c+ TAM density and low CD206+ TAM density were associated with better OS. | IHC |
| CD163 | Ovarian cancer (N = 199) | High CD163+ macrophage infiltration was associated with low DFS and OS. | IHC |
| | Ovarian cancer (N = 110) | High CD163+ macrophage infiltration was associated with low DFS and OS. | IHC |
| | Multiple myeloma, (N = 240) | The PFS and OS were significantly higher in patients with low CD163+ TAM density. | IHC |
| | Breast cancer (N = 139) | More CD163 positive macrophages are associated with tumor invasion and poor prognosis. | IHC |
| | (N = 144) | CD163+ macrophages in tumor stroma positively correlated with higher grade, larger tumor size. | IHC |
| | Triple-negative BC (N = 278) | CD163+ TAM infiltration and low level of E-cadherin had a significantly higher risk of recurrence and LN metastasis. | IHC |
| Stabilin-1 | Breast cancer (N = 31) | Stabilin-1 expression was higher in metastasizing primary tumors. | IHC |
| YKL-40 | Gastric cancer (N = 172) | High YKL-40 protein level is an independent biomarker of short survival and is associated with tumor invasion, LN metastasis. | IHC |
| | Osteosarcoma tumor (N = 48) | Patients with tumors of high YKL-40 score had a better survival than patients with low score. | IHC |
| | Small cell lung cancer (N = 131) | High plasma YKL-40 levels before chemotherapy independently predicted short survival. | RIA |
| | Colorectal cancer (N = 197) | High plasma YKL-40 in patients before treatment was associated with short PFS and OS. | Immulite CEA assay |
| YKL-39 | Breast cancer (N = 40) | Elevated levels of YKL-39 expression in tumors after NAC is predictive for increased risk of distant metastasis and for poor response to treatment. | Real-time PCR |
| | Breast cancer (N = 68) | The absence of clinical response was associated with the presence of M2+ macrophage phenotype (YKL-39-CCL18+ or YKL-39 + CCL18). | Real-time PCR |

Notes: 5-FU – 5-fluorouracile, DFS – disease-free survival, IF – immunofluorescent analysis, IHC – immunohistochemistry, LN – lymph node, NAC – neoadjuvant chemotherapy, OS – overall survival, PFS – progression-free survival, TAMs – tumor-associated macrophages.
Neoadjuvant chemotherapy (NAC) is used to reduce the volume of the primary tumor and the level of regional lymphadenopathy, enabling radical surgery.

The mechanisms of action of the main chemotherapeutic agents are illustrated in Figure 1 and summarized in Table 2. Anthracyclines (for example, doxorubicin) intercalate between base pairs of nucleic acids and disrupt topoisomerase-II-mediated DNA repair, preventing the DNA double helix from being resealed and thereby inhibiting DNA replication. Anthracyclines (for example, doxorubicin) intercalate between base pairs of nucleic acids and disrupt topoisomerase-II-mediated DNA repair, preventing the DNA double helix from being resealed and thereby inhibiting DNA replication.79 Taxanes (paclitaxel and docetaxel) target tubulin and stabilize the microtubule polymer, protecting it from disassembly, which blocks mitosis and reverses to the G0 phase of the cell cycle without cell division.80 Alkylating agents (cisplatin, oxaliplatin, carboplatin, which are platinum drugs, and cyclophosphamide) form DNA crosslinks both between and within DNA strands (known as interstrand and intrastrand cross-linkages, respectively). This process is irreversible and leads to cell apoptosis.81 5-Fluorouracil (5-FU) acts as an inhibitor of thymidylate synthase (TS), which methylates deoxyuridine monophosphate (dUMP) to form thymidine monophosphate (dTMP), which in turn blocks the synthesis of the pyrimidine thymidine that is required for DNA replication.82 Gemcitabine, after the attachment of the three phosphates, becomes pharmacologically active as gemcitabine triphosphate (dFdCTP) and is incorporated into new DNA strands.83 Trabectedin binds to the minor groove of the DNA, causing the DNA helix to bend toward the major groove, which causes the inhibition of transcription and reparation.84

Chemoresistance and chemotherapy-induced immunosuppression can result in the relapse of tumors and is critical for survival in cancer patients.7,85 In the past few decades, several studies have examined the molecular mechanisms that promote the chemoresistance of cancer cells, such as the induction of anti-apoptotic regulators, ABC transporters, aberrant transcription factor nuclear factor-κB (NF-κB) activity, and the mechanisms of damaged DNA repair.7,86,87 CT can also lead to the selective expansion of resistant cancer clones.88 It was found that myeloid cells, particularly TAMs, generally accumulate in tumors after chemotherapy and contribute to tumor recurrence by initiating the physiological regenerative program that is a beneficial action of macrophages in

Figure 1. The mechanisms of action of the main chemotherapeutic agents.

There are several mechanisms by which chemotherapeutic agents act in the cancer cell (a) intercalate into DNA and disrupt topoisomerase-II-mediated DNA repair (doxorubicin); (b) promote microtubule polymerization and stabilization (paclitaxel and docetaxel); (c) form DNA crosslinks (cisplatin, carboplatin); (d) inhibit thymidylate synthase (TS) (5-fluorouracil); (e) act as pyrimidine nucleoside antimetabolite (gemcitabine); (f) bind to the minor groove of the DNA (trabectedin). Commonly, all of them cause inhibition the DNA replication and transcription and cancer cell death. The detailed explanation is given in the paragraph “Cancer chemotherapy, chemoresistance and immunomodulation”.
Table 2. The effects of different anticancer chemotherapy agents on monocytes/macrophages.

| Chemotherapeutic agent | Applications | Type and mechanism of action of chemotherapeutic agent | Effect on macrophages in vitro | Effect on macrophages in mouse models | Clinical data |
|------------------------|--------------|----------------------------------------------------------|------------------------------|--------------------------------------|--------------|
| Paclitaxel (Taxol)     | Ovarian cancer, breast cancer, lung cancer, Kaposi sarcoma, cervical cancer, prostate cancer | Taxanes Stabilize GDP-bound tubulin in the microtubule and inhibit tubulin polymerization/depolymerization. Cell-cycle arrest and cell death | Stimulates Mφ to induce pro-inflammatory genes (TNF-α, IL-12, IFN-γ, COX-2), CSFs, TFs, activates DCs, CTLS, NK. | Recruitment of CSF1R-expressing Mφ. | Depletion of immunosuppressive (M2-like) TAMs and activation of antitumoral (M1-like) monocytes/MDCs which are able to enhance cytotoxic T cell responses in 4T1-Neu mammary tumor implants. |
| Docetaxel (Taxotere)  | Testicular cancer, ovarian cancer, breast cancer, bladder cancer, non-small cell lung cancer | Alkylating agents Induce inter- and intra-strand DNA crosslinks. Inhibition of DNA replication and transcription | Enhances production of NO, TNF-α, IL-1b, IL-12 and FN-γ, activation of MAP kinases and NF-κB pathways. In a combination with carboplatin skew the differentiation of monocytes toward the M2-like phenotype. | Increases antigen-presenting ability of peritoneal Mφ due to releasing IL-1, IL-6, and IL-8, IL-1 and TNF-α. | In esophageal cancer patients infiltration of CD68+ and CD163+ Mφ were significantly associated with tumor depth, lymphatic and venous invasion, and with poor prognosis during the treatment with combination of cisplatin, S-FU and adriamycin. |
| Cyclophosphamide       | Multiple myeloma, leukemia, breast cancer, neuroblastoma, lymphoma, ovarian cancer, retinoblastoma. | Enhances the production of the pro-inflammatory cytokines IL-6 and IL-12 and decreases the production of the anti-inflammatory cytokines IL-10 and TGF-b potentiating innate responses. Enhances the recruitment and phagocytic activity of monocytes/macrophages. | In a combination with immunotherapy results in up-regulation of the M1-associated molecules (CD40, CD80, CD86, MHC class II, IFN-γ, TNF-α, IL-12) and down-regulation of the M2-associated molecules (IL-4Rα, B7-H1, IL-4, IL-10). Stimulates the recruitment of DCs, macrophages and NK cells to the tumor site. | Activates NK-dependent antitumor immunity in cancer patients. |
| Doxorubicin (Adriamycin) | Breast cancer, bladder cancer, stomach cancer, lung adenocarcinoma, ovarian cancer, soft tissue sarcoma, multiple myeloma, Kaposi’s sarcoma, lymphoma, acute lymphocytic leukemia | Anthracyclines Intercalate between base pairs of nucleic acids Inhibition of RNA and DNA synthesis | Causes the ICD of tumor cells, the recruitment and differentiation of myeloid cell into antigen-presenting cells, resulting in the activation of effective adaptive responses. In a combination with immunotherapy results in up-regulation of the M1-associated molecules (CD40, CD80, CD86, MHC class II, IFN-γ, TNF-α, IL-12) and down-regulation of the M2-associated molecules (IL-4Rα, B7-H1, IL-4, IL-10). The accumulation of CD11b+F4/80+ Gr-1(Ly6C/Ly6G)+ cells involved in the antigen presentation and the induction of anti-tumor T-cell immunity that was correlated with a reduced tumor growth. | In esophageal cancer patients treated with S-FU, cisplatin, and DOX the infiltration of CD68+ and CD163+ Mφ were significantly associated with tumor depth, lymphatic and venous invasion, and with poor prognosis. |

(Continued)
Table 2. (Continued).

| Drug          | Tumor Type                      | Mechanism                                                                 |
|---------------|--------------------------------|---------------------------------------------------------------------------|
| S-fluorouracil| Colon, esophageal cancer, stomach cancer, pancreatic cancer, breast cancer, and cervical cancer, head and neck cancer | Antimetabolites Analóg of pyrimidine nucleoside Disrupting DNA and/or RNA synthesis |
| Gemcitabine   | Pancreas cancer, non-small cell lung cancer, bladder cancer, soft-tissue sarcoma, metastatic breast cancer, ovarian cancer | Re-education of Mφ to the antitumour phenotype by an upregulation of the expression of the M1 markers HLA-DR, CD40, CCR7, a downregulation of the expression of M2 markers CD163 and CD206, and an activation of the pro-inflammatory program in macrophages. |
| Trabectedin   | Advanced soft tissue sarcoma, ovarian cancer | Natural compound (alkaloid) Binds to the minor groove of the DNA Inhibition of transcription and repair, cell cycle arrest Significant reduction in the number of monocytes (CD45+CD11b+CD115+) in the blood stream, mature monocytes (CD11b+CD115+) in the bone marrow and splenic F4/80+Mφ. The decrease of the CCL2 production. |

Notes: CCL2 – C-C motif chemokine ligand 2, CCRI – chemokine receptor type 2, CSF – colony-stimulating factor, CT – chemotherapy, CTLs – cytotoxic T-lymphocytes, DCs – dendritic cells, ICD – immunogenic cell death, Mφ – macrophages, MDSC – myeloid-derived suppressor cells, NK – natural killers, TAMS – tumor-associated macrophages.

wound healing but detrimental in the case of tumor relapse. Concomitant mechanisms include the macrophage-induced suppression of T cell immunity, the maintenance of tumor cell survival and the activation of tumor revascularization (Figure 2).

Alternatively, activated M2 macrophages can mediate chemoresistance by secreting growth factors and inhibiting cell death signaling pathways in tumor cells, protecting them from the cytotoxic effects of chemotherapy. Thus, in patients with esophageal cancer who received neoadjuvant chemotherapy (two courses of 5-fluorouracil (5-FU), cisplatin, and adriamycin), the infiltration of CD68+ and CD163+ macrophages in tumor mass significantly correlates with tumor depth, lymphatic and venous invasion, and poor prognosis. Treatment with cyclophosphamide (CTX), paclitaxel (PTX) and doxorubicin (DOX) for a mouse Lewis lung carcinoma model (LLC1s) and mouse models of breast cancer metastasis (MMTV-PyMT) resulted in a significant increase in the number of CD206+ TAMs, accumulating mostly in the vascularized chemokine CXCL12-rich regions of tumors after chemotherapy that caused tumor revascularization and relapse. In breast cancer patients undergoing neoadjuvant chemotherapy and in the PyMT mouse model after paclitaxel (PTX) treatment, a dramatic accumulation of macrophages protecting tumors was found. The application of chemotherapeutic agents can also be cytotoxic for monocytes and macrophages, as in the case of the DNA-damaging agent trabectedin, which has strong antitumor activity. In transplanted tumor models of fibrosarcoma, ovarian carcinoma, and Lewis lung carcinoma, treatment with trabectedin significantly delayed tumor growth and decreased the production of the major monocyte chemoattractant CCL2 by TAMs. Decreased levels of CCL2 resulting in macrophage depletion in tumor tissues, which was suggested as an essential mechanism of the antitumor activity of trabectedin.

Chemotherapy was also considered to create the conditions for the activation of cytotoxic immune responses against tumor cells. Thus, the application of antitumor drugs that damage DNA in tumor cells results in immunogenic cell death (ICD) due to the expression of neoantigens on tumor cells following cellular DNA disruption. Carboplatin is a platinum compound with DNA-damaging agents. An increased tumor pathological complete response (pCR) was shown in 53.2% of the patients with stage II–III triple-negative breast cancer (TNBC) treated with carboplatin against 36.9% without carboplatin. In the BrighTNess trial, the pCR rate in TNBC increased from 31% without carboplatin to 58% with carboplatin. In gastric cancer, a high amount of TAMs before treatment correlated with prolonged survival in patients who received 5-fluorouracil (FU)-based postoperative chemotherapy. In patients with stage III colorectal cancer treated with 5-FU adjuvant therapy, the high macrophage density before the treatment significantly correlated with a better improved prognosis. In pancreatic adenocarcinoma, high levels of CD68+ TAMs were associated with a better prognosis. The GEM-modified polarization of macrophages was characterized by an upregulation of the expression of the M1 markers...
HLA-DR, CD40 and the chemokine receptor CCR7, a downregulation of the expression of M2 markers CD163 and CD206, and an activation of the pro-inflammatory program in macrophages.77

Thus, TAMs have a controversial role not only in tumor progression but also in cancer chemoresistance. Macrophages modify the effect of chemotherapeutic drugs, but the direction of these changes (enhancement or decrease) depends on both the type of CT agent and the type of cancer. However, the ways in which different types of TAMs respond to CT agents must be understood at the mechanistic level.

**How does chemotherapy edit macrophages?**

Chemotherapeutic agents can edit macrophages in tumor-protective or antitumor directions, where three major mechanisms must be considered: 1) changes in the macrophage phenotype; 2) induced recruitment of monocytes or macrophages to the tumor site; and 3) systemic depletion of monocytes/macrophages. The main effects of chemotherapeutic agents on monocytes/macrophages are summarized in Table 2.

1) Changes in macrophage phenotype during chemotherapy treatment

In vitro, paclitaxel was found to re-educate macrophages to induce genes encoding the mediators of inflammation such as TNF-α, IL-12, inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), transcription factors, colony-stimulating factors, adrenomedullin, and cytokines that can activate other immune cells, including dendritic cells (DCs), natural killer cells (NKs) and tumor-specific cytotoxic T-lymphocytes (CTLs). Such activity of PTX-treated TAMs can result in the suppression of tumor growth and the enhancement of immune cell function against tumors.61

In vitro, the short exposure of cisplatin (a platinum agent) on peritoneal macrophages isolated from BALB/c mice and coincubated with L929 cells (a mouse fibroblast cell line) resulted in the enhanced production of NO, TNF-α, IL-1β, IL-12 and IFNγ, linked to the enhanced expression of Toll-like receptor (TLR)-2 and TLR-4 genes, and the activation of the mitogen-activated protein (MAP) kinases and NF-κB pathways.64 The increased antigen-presenting ability of peritoneal macrophages isolated from BALB/c mice treated with cisplatin was regulated not only by the continuous presence of cisplatin in the culture medium but also by the macrophage release of soluble factors, such as IL-1, IL-6, and IL-8, and TNF-α.93 Cisplatin-activated murine peritoneal macrophages in coculture with L929 cells were able to release the tumor cell-specific cytotoxic factors FasL and TNF and to facilitate the apoptosis of tumor cells.94 The induction of tumor cell death by apoptosis was also confirmed by an enhanced activation of apoptosis-mediating molecules such as caspase-8, caspase-3, CAD, PARP, Bid, Bax, cytochrome c, as well as DNA fragmentation and the downregulation of Bcl-2.94

![Figure 2. The mechanisms of macrophage-mediated resistance to chemotherapy.](image-url)
At the same time, Dyikgraaf and colleagues showed that in an in vitro model, M2 macrophages were more sensitive to cisplatin and carboplatin compared to M1-macrophages and DCs. The impact of platinum chemotherapy on human cervical and ovarian cancer cell lines resulted in the increased production of prostaglandin E2 (PGE2) and IL-6 by tumor cells in a STAT-dependent manner, which skewed the differentiation of monocytes toward the M2-like macrophage phenotype and led to chemoresistance. The blockage of these molecular pathways increased the efficacy of cisplatin and carboplatin.65

Cyclophosphamide (CTX) was found to enhance the production of the pro-inflammatory cytokines IL-6 and IL-12 and to decrease the production of the anti-inflammatory cytokines IL-10 and TGF-b in mouse peritoneal macrophages in vivo, potentiating immune responses by activating Th1 cells and antigen-specific macrophages.67 DOX and CTX can activate macrophages to maintain the antitumor response in breast and leukemia models.68 DOX causes the ICD of tumor cells and the recruitment and differentiation of myeloid cells into antigen-presenting cells, resulting in the activation of effective adaptive responses.72,73 Leukemic cells treated with CTX released cytokines CCL4, CXCL8, VEGF and TNF that enhanced the recruitment and phagocytic activity of monocytes/macrophages.68 In the B16F10 mouse model of melanoma, combined chemotherapy (vin-cristine, cyclophosphamide and doxorubicin) and immunotherapy (monoclonal anti-CD40+ cytotoxic–phosphate-guanosine-containing oligodeoxynucleotide 1826 (CpG-ODN)) treatment resulted in the enrichment of an M1-polarized TAM subpopulation.69 This TAM subpopulation was characterized by the upregulation of molecules associated with the M1 phenotype (CD40, CD80, CD86, major histocompatibility complex (MHC) class II, IFN-y, tumor necrosis factor-a (TNF-a) and IL-12) and the downregulation of molecules associated with the M2 phenotype (IL-4Ra, B7-H1, IL-4 and IL-10).59

Thus, chemotherapeutic agents can have several potentially beneficial effects via M1-programming TAMs. However, whether the pro-inflammatory activity of TAMs is helpful for antitumor responses or whether it can create conditions of low-grade inflammation remains an open question. Moreover, the same chemotherapeutic agent, depending on the type of tumor and therapy schemas, can also enhance the tumor-supporting M2 phenotype of TAMs. The interactions of the chemotherapeutic agents and TAMs must be carefully investigated for each type of tumor, and individual differences between patients must be considered. Deep insight into the mechanisms of TAM response to CT must be obtained in ex vivo experimental models.

2) Recruitment of monocytes or macrophages to the tumor site

A variety of studies demonstrate that anticancer therapies induce the recruitment of monocytes to the tumor sites where chemotherapy-damaged cells are localized, which monocytes consider as persistent nonhealing wounds to be repaired.75 In this case, tumor-infiltrating macrophages initiate the regenerative program that supports the proliferation of cancer cells.96 Thus, it was shown that chemotherapy, ionizing radiation, and the vascular disrupting agent combretastatin A4-P cause the increased production of the mononuclear phagocyte growth factor CSF-1 and the chemokines CCL2 and CXCL12 that can trigger monocyte recruitment and TAM accumulation in tumor sites.85 In patients with advanced pancreatic cancer receiving gemcitabine, an overall increase in CD14+ monocytes, CD11c+ myeloid DCs and CD123+ plasmacytoid DCs was observed more frequently in comparison with untreated patients.10

In mouse mammary tumors, chemotherapy increased the expression of CSF-1 by tumor cells, which then recruited large numbers of CSF1R-expressing macrophages.62 In vivo, murine MDA-MB-435 breast tumors treated with Abraxane (paclitaxel-albumin) showed a significantly higher infiltration of CD45+ CD169+ macrophages in comparison with the tumors from the untreated group. Flow cytometry analysis confirmed a significantly increased population of F4/80+ macrophages in MDA-MB-435 tumors but not in MDA-MB-435R (paclitaxel-resistant) tumors. The authors supposed that in this case, TAMs mediated the clearance of chemotherapy-induced apoptotic tumor cells.97 A metronomic CTX regimen was shown to stimulate the recruitment of DCs, macrophages, and NK cells to the tumor site in mouse models and activate NK-dependent antitumor immunity in cancer patients.79,81 HER2/Neu-driven mammary carcinomas under the protein kinase inhibitor lapatinib and/or doxorubicin treatment were also highly infiltrated with immature macrophages in a STAT1-dependent manner. These cells had a phenotype of CD11b+F4/80+ Gr-1(Ly6C/Ly6G)+ and were possibly involved in antigen presentation and the induction of anti-tumor T cell immunity. The accumulation of immature macrophages and reduction of mature TAMs at the tumor site after lapatinib/doxorubicin treatment correlated with reduced tumor growth.74

Nakasone and colleagues showed that in MMTV-PyMT (mouse model of breast cancer metastasis) tumors treated with DOX, the recruitment of monocytes was increased, and MMP9 produced by the recruited myeloid cells limited drug delivery to the tumors due to the decreased blood vessel permeability, suggesting that an increased vascular permeability is associated with a better response to DOX.75 Moreover, the different populations of myeloid cells were differentially correlated with drug response. Thus, the CD206+ macrophages promoted the increased vascular leakage that resulted in a better DOX response, but the CCR2-dependent recruitment of monocytes was associated with tumor relapse. In contrast, CCR2 null mice responded better to treatment with doxorubicin or cisplatin. Therefore, the impact of the chemotherapy-induced infiltration of monocytes/macrophages in the tumor site is still controversial and depends on the nature of chemotherapeutic agents and the context of the local tumor microenvironment, and it still has to be profoundly studied in human cancer.

3) Depletion of monocyte-macrophage lineage cells due to chemotherapy treatment

The antitumor activity of the chemotherapeutic agent docetaxel was shown in 4T1-Neu mammary tumor implants and involves the depletion of immunosuppressive (M2-like) TAMs and the activation of antitumoral (M1-like) monocytes/
MDSCs, which can enhance the cytotoxic T cell responses in tumors.63

In mouse tumor models, treatment with trabectedin led to a significant reduction in the number of monocytes (CD45+ CD11b+ CD115+) in the bloodstream, mature monocytes (CD11b+ CD115+) in the bone marrow and splenic F4/80+ macrophages via the TRAIL-R2-dependent pathway activating caspase-8-dependent apoptosis. The effect of trabectedin was selectively cytotoxic for cells of the monocyte/macrophage lineage but not for neutrophils and lymphocytes. Moreover, the production of CCL2, a major chemotactic factor that induces monocyte recruitment in tumors, was also decreased in treated mice. In patients with soft tissue sarcoma receiving trabectedin in a neoadjuvant regimen, a strong decrease in the density of TAMs and blood vessels was observed after treatment.78

Macrophages contribute to tumor drug resistance and relapse after chemotherapy treatment

The tumor-protective function of macrophages was found in many in vivo and in vitro studies for some antitumor agents, including doxorubicin (adriamycin), platinum compounds, 5-fluorouracil (5-FU), gemcitabine, and paclitaxel.65,69 The major mechanisms of chemotherapy resistance are illustrated in Figure 2. The pathways responsible for the tumor-promoting function of TAMs after chemotherapy include the increased recruitment of immunosuppressive TAMs, protumor polarization, the activation of a tumor-promoting Th17 response, and the activation of anti-apoptotic programs in malignant cells and others.89 M2 macrophages are known to have strong tumor-supporting functions as they contribute to the establishment of a local immunosuppressive microenvironment.98 Macrophage-mediated resistance to paclitaxel, doxorubicin and etoposide was found in coculture studies combining mammary carcinoma cell lines and bone marrow-derived macrophages.90

Thus, Baghdadi M and colleagues investigated the influence of the tumor supernatants of doxorubicin-resistant (DR) and doxorubicin-sensitive (DS) cell lines on monocyte polarization. They found that in monocytes stimulated with tumor supernatants from the doxorubicin-resistant cell line of lung adenocarcinoma A549-DR, the expression levels of CD68 and the M2 marker CD163 were significantly higher in comparison with the DOX-sensitive group A549-DS. Moreover, monocytes stimulated with chemoresistant tumor supernatants from A549-DR cells differentiated into M2 macrophages and acquired an immunosuppressive phenotype via an elevated expression of PD-L1 and a low expression of the MHC class I and II costimulatory molecules CD80 and CD86. Moreover, CD68+ CD163+ TAMs released various factors that contributed to tumor progression and chemoresistance, such as immunosuppressive cytokines (IL10 and TGFβ), proangiogenic factor VEGF, and factors that promote invasion (MMP9, MMP13).7 (Figure 2(a))

Jinushi et al. demonstrated that the in vivo resistance of pancreatic ductal adenocarcinoma cells (PDAC) to carboplatin was associated with STAT3 signaling and macrophage-produced IL6 promoting tumor cell growth.99 (Figure 2(b))

There is evidence that the exosomes of M2-macrophages are involved in the mechanisms of cisplatin resistance due to microRNA-21. Exosomes are considered to act as extracellular communicators between tumor cells and the tumor microenvironment.100 As shown by Zheng et al. in vitro models and an athymic nude mouse model, miRNA-21 from the macrophage culture suppresses apoptosis and enhances the activation of the PI3K/AKT signaling pathway in tumor cells, resulting in tumor progression.100 (Figure 2(b)) TAMs also increase the chemoresistance of PDAC (in vitro cell line and in vivo mouse model) to gemcitabine by reducing the level of apoptosis, particularly by decreasing the activation of caspase-3.101 (Figure 2(c))

Numerous studies have demonstrated that cathepsins play a pivotal role in tumor chemoresistance by diverse mechanisms. Cysteine proteases, or cathepsins, comprise a family of proteins that are localized in the endosomal/lysosomal compartment and are responsible for the proteolytic degradation of a wide variety of intracellular and extracellular substances.102 Cathepsins can be secreted by malignant cells and cells of the tumor microenvironment, such as fibroblasts and macrophages.103 In cancer, cathepsins, particularly cathepsin B and S, are involved in apoptosis, angiogenesis, cell proliferation, and invasion.103 Cathepsin B is capable of degrading various components of the extracellular matrix, including type IV collagen, laminin, and fibronectin, facilitating the growth and invasion of tumor cells into surrounding tissue and vasculature.104,105 Cathepsins were found to be overexpressed in various human tumors (including breast, colorectal, gastric, urinary bladder carcinomas, glioblastomas, lung and prostate tumors, melanoma, chordrosarcoma, and many others), and their proteolytic activity correlated with poor prognosis and the invasive phenotype of colon and bladder cancers, esophageal adenocarcinomas, and breast cancer.104,106

Shree et al. demonstrated that cathepsin protease activity (cathepsin B and S) may influence the production of chemoprotective factors by macrophages in PTX-treated mice and that macrophages contribute to breast cancer resistance to therapy via the secretion of cathepsins.90 Cathepsin B is important in the trafficking of TNF-α-containing vesicles to the surface of macrophages, which mediates chemoprotection through NF-κβ activation, or indirectly through IL-6 expression and STAT3 activation.107 Macrophage-derived cathepsins B and S were able to protect the breast cancer cell line against PTX-induced cell death in vitro (Figure 2(d)).90

DeNardo and colleagues supposed that the ability of TAMs to limit tumor response to chemotherapy can be mediated by the suppression of the antitumor functions of cytotoxic T cells.108 It was found that breast tumors with high amounts of TAMs and low amounts of cytotoxic T cells respond weakly to neoadjuvant chemotherapy.102 It was reported that in chemotherapy-treated mouse tumors, the M2 subpopulation of TAMs (CD206+ TIE2HiCXCR4Hi) accumulated around blood vessels, where they promoted tumor revascularization and relapse, in part, via VEGF-A release. A similar subpopulation of TAMs was clustered after chemotherapy in human breast carcinomas and bone metastases. Moreover, CXCL12, a ligand of CXCR4, was upregulated in the perivascular sites after chemotherapy and was responsible for the chemotaxis of CD206+ TAMs.95
These findings demonstrated that macrophages contribute to drug resistance and relapse after chemotherapy treatment via different pathways based on the interaction of TAMs and cancer cells. However, the mechanisms of the direct action of chemotherapeutic drugs on TAMs as well as the mechanisms of TAM-mediated chemoresistance in tumors still require deep investigation.

**Approaches to TAM targeting that can improve the antitumor effect of chemotherapy**

Chemotherapy is primarily applied to selectively kill tumor cells or arrest their growth. However, chemotherapy-induced resistance frequently limits the cytotoxic effect of drugs in tumor sites. The immune system, especially TAMs, considerably contributes to chemoresistance; therefore, the depletion of M2-like TAMs or their reprogramming into the M1-like phenotype can enhance the efficacy of treatment and can be an efficient way to suppress tumor regression. Understanding the complex interaction between cancer cells and the immune system provides new therapeutic approaches to improve the antitumor effect in chemotherapy-treated patients.

Currently, an increasing number of studies have focused on complex therapeutic approaches in cancer treatment, including not only chemotherapy regimens but also immunotherapy, designed to activate immune responses to increase the efficacy of CTLs against cancers, and immune checkpoint blockade therapy, inhibiting immune suppressor molecules. For example, recently, the application of anti-PD-1, anti-PD-L1 and anti-CTLA-4 agents demonstrated significant benefits in the survival of patients with metastasis, and these agents have become advanced drugs in cancer treatment.

Macrophages express the ligands of the inhibitory receptors programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte antigen 4 (CTLA-4), which are normally upregulated in activated immune effector cells such as T cells, B cells, and NKT cells. The activation of PD-1 and CTLA-4 by their ligands (PD-L1, PD-L2 and B7-1 [D80], B7-1 [CD86], respectively) directly inhibits T cell and B cell responses by suppressing proliferation and cytokine production.12

Experimental studies and clinical trials demonstrate the beneficial effects of the combination of chemotherapy and immunotherapy. Breedveld showed that in breast cancer, the overall response rate was significantly greater in the group of patients treated with trastuzumab (anti-HER-2 monoclonal antibodies) and chemotherapy.109 Weir et al. demonstrated that the combined treatment with a class I restricted peptide-based cancer vaccine, metronomic cyclophosphamide (mCPA) and anti-PD-1 antibody, increases the clonality and activity of tumor-infiltrating antigen-specific T cells in a murine tumor model expressing HPV16 E7 (C3).110 Soares et al. also proposed that the immunosuppressive pathways, including those of regulatory T cells (Tregs) and the immune check-point molecule CTLA-4 expression on T cells, can be inhibited by the addition of the vaccine or a low dose of cyclophosphamide to the PD-1 blockade.111

B7-H4 is a member of the B7 superfamily that is expressed on TAMs and implicated in the suppression of T cells. Blocking B7-H4 or depleting B7-H4+ TAMs may represent a novel strategy to enhance T cell immunity in cancer.12,112 In human ovarian cancer, the inhibition of B7-H4 was found to contribute to tumor regression.112

Several studies demonstrated the positive effect of the suppression of macrophage recruitment to the tumor site. Thus, the blockade of colony-stimulating factor-1 (CSF-1) was shown to limit macrophage infiltration and improve the response of breast cancer xenografts in mice to chemotherapy.113,114 Paclitaxel was found to improve the survival of mammary tumor-bearing mice in combination with the blockade of macrophage recruitment with CSF1R-signaling antagonists by limiting tumor development and reducing pulmonary metastasis.52,108 TAM depletion was found to enhance the efficacy of paclitaxel in MMTV-PyMT mouse mammary tumors and combination chemotherapy (cyclophosphamide, methotrexate, and 5-fluorouracil) in breast cancer xenografts in immunodeficient mice.109 Genetic knockout of CSF-1, the use of neutralizing antibodies, low molecular weight inhibitors or antisense RNA for inhibiting CSF-1R signaling reduce the aggressiveness of tumor xenografts, which is associated with the lack of TAMs.21,115,116 In mouse models of pancreatic tumors, targeting tumor-infiltrating macrophages by inhibiting either CSF1R or chemokine (C-C motif) receptor 2 (CCR2) improves the efficacy of gemcitabine therapy and reduces the number of metastases by activating antitumor T cell responses.117

The combination of TAM depletion and chemotherapy was found to reduce tumor-vessel density by 50%. TAM depletion in tumor mass may normalize the vessels by skewing perivascular TAMs from pro-angiogenic cells to antiangiogenic cells that leads to increased blood flow and the delivery of chemotherapeutic agents to tumors, promoting tumor destruction.62 Vascular endothelial growth factor A (VEGFA) attracts macrophage progenitor cells, which then differentiate into TAMs (M2 macrophages) under the influence of IL-4.118 The removal of these macrophages inhibits growth, angiogenesis, and tumor invasion. The reduction of lymphangiogenesis can be achieved by inhibiting the activity of macrophages via blocking the VEGF-C/VEGFR3 axis in chemotherapy-treated tumors.119

Duhamel M and colleagues demonstrated a new therapeutic strategy combining paclitaxel and proprotein convertase 1/3 (PC1/3) inhibition to switch macrophages toward an antitumoral phenotype. PC1/3 knock-down (KD) macrophages activated by paclitaxel showed the inhibition of the anti-inflammatory pathway STAT3 and secreted more pro-inflammatory cytokines that can inhibit glioma growth in a cocultured experiment.120 The approach of the combined treatment in MMTV-PyMT mice with paclitaxel plus an inhibitor of the c-kit receptor tyrosine kinases (PLX3397) showed a significant reduction in primary tumor burden, a reduction in the CD31+ vessel density within the mammary tumors, and an activation of the cytotoxic effector T lymphocytes compared to the treatment with a single agent.120

**Concluding remarks**

It is evident that to overcome the chemoresistance of solid tumors, the effects of chemotherapeutic agents on TAMs must be considered. Many studies have demonstrated the involvement of TAMs in tumor progression in different cancers,
including breast, prostate, colorectal, gastric, ovarian, melanoma, glioblastoma and others, and indicate a controversial role of TAMs in tumors. The fact that chemotherapeutic agents do not kill TAMs and can support the recruitment of the precursors of TAMs to the tumor site indicates that it is essential to identify not only how macrophages affect the sensitivity of cancer cells to the chemotherapeutic agent but also to focus on the long-term program induced in TAMs by chemotherapeutic agents.

It must be clearly understood for each type of cancer and each therapeutic agent or their combinations, whether the treatment leads to the activation of the antitumor activities of TAMs or programs TAMs to create beneficial conditions for tumor replacement and metastasis. Recent studies revealed that macrophages contribute to drug resistance and relapse after chemotherapy treatment via different pathways: promoting tumor revascularization, suppressing cytotoxic T cell immunity, and activating anti-apoptotic programs in cancer cells; however, the mechanisms of the direct action of chemotherapeutic drugs on TAMs remain unknown.

Understanding the mechanisms of the interaction of TAMs with chemotherapeutic agents in a tumor-specific context can lead to the prospective use of macrophages and other inflammatory and stromal components as targets for therapeutic effects to modify the tumor microenvironment in the direction of inhibiting tumor growth and reducing the risk of metastasis.121

Such a strategy can lead to the possibility of using macrophages and other inflammatory and stromal components as targets for chemotherapeutic agents, where decisions about the specific treatment will be aimed at initiating the tumor-killing activity of macrophages and eliminating the macrophage types that can support tumor replacement. The functional “reorientation” of macrophages to the antitumor phenotype triggers a cascade of events leading to a disruption of the ecosystem by promoting tumor growth that results in the blocking of tumor cell proliferation, their metastatic potential and creating conditions for achieving a kind of parity between the tumor and the host organism, resulting in the inhibition of disease progression.

In summary, understanding the fundamentally important role of TAMs in determining the effectiveness of antitumor cytostatic therapy opens the prospect of developing new therapeutic approaches for the treatment of malignant neoplasms based on the balanced synergistic action of cytostatic agents and innovative immunomodulatory approaches.6,12

**Abbreviations**

5-FU 5-fluorouracil  
BC breast cancer  
COX cyclooxygenase-2  
CSF1 colony stimulating factor  
CT chemotherapy  
CTL cytotoxic T-lymphocyte  
CTX cyclophosphamide  
DC dendritic cell  
DFS disease-free survival  
DOX doxorubicin  
ECM extracellular matrix  
ICD immunogenic cell death  
INFy interferon gamma  
iNOS inducible nitric oxide synthase  
NAC neoadjuvant chemotherapy  
NK natural killer  
OS overall survival  
PTX paclitaxel  
TAM tumor-associated macrophage  
TME tumor microenvironment  
TGF beta transforming growth factor beta  
TLR toll-like receptor  
TNBC triple-negative breast cancer  
TNFa tumor necrosis factor alpha  
VEGF vascular endothelial growth factor

**Disclosure of Potential Conflicts of Interest**

The authors declare no conflicts of interest.

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