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Tumor Associated Macrophages: Evolutionary Role in Cancer Therapeutics

Fouzia Fazal, Muhammad Arsalan Khan, Sumayya Shawana, Muhammad Mubarak

ABSTRACT

Cancer therapeutics have evolved more significantly during the last two decades with increasing focus on precision medicine. In principle this involves targeted therapies tailored to patients' cancer-specific molecular attributes. It includes a repertoire of immunomodulating, and apoptotic agents added to cytotoxic chemotherapy, to increase effectiveness. Tumor Associated Macrophages (TAMs) are an interesting potential targets for expanding these therapies. These represent a spectrum of subtypes with anti-inflammatory M1 and pro-tumor M2 being the predominant among all. A large number of studies have established their central role in modulating the tumor microenvironment (TME) and contributing to tumor initiation, and progression. Potential therapeutic strategies that modulate TAMs reduce or block monocyte recruitment, induce apoptosis of TAMs, re-educate TAMs from pro-tumor M2 to anti-tumor M1, among others. This review takes a detailed look at this evolving landscape.

Key Words: Cancer, Tumor associated macrophages (TAMs), Tumor microenvironment (TME) and Precision medicine

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INTRODUCTION:

Cancer is a multistep process, resulting more commonly with the accumulation of genetic and epigenetic mutations, traversing a number of molecular pathways. In the year 2020 alone, it was responsible for about 10 million deaths worldwide, second only to the ischemic heart disease.[1] Following decades of research, leading to effective screening programs, improved diagnostic modalities and evolving therapeutic strategies, This has led to decreasing mortality associated with, for example, lung, breast, and colorectal cancers. On the flip side, these very cancers still top the yearly incidence, globally.[2] During the last two decades to address this burgeoning challenge, attention has turned toward precision medicine. Tailored to specific molecular attributes of a patient's tumor, focus of cancer therapeutics has shifted from cytotoxic chemotherapy alone to a complex mix of targeted therapies, enabling immunomodulation and apoptosis. Thus, leading to much improved rate of cure and patient survival.[3]

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Defining the role of tumor associated macrophages (TAMs) in cancer biology and its effect on patients' survival, is among the pathways explored by researchers to expand the repertoire of targeted therapies. TAMs are a specialized class of macrophages integrated into the microenvironment of a solid tumor, and through their regulatory molecules like chemokines, cytokines, growth factors and effects on immune checkpoint proteins in the tumor tissue modulate it.[4] The discourse on TAMs has revealed that their varied distribution, and relative densities correlate with cancer prognosis and patients' survival. We also know that biologically TAMs represent an array of subtypes that are modulated by external effector molecular signals. Two of the predominant types, M1 and M2 for instance, have paradoxical antitumor and protumor properties, respectively. The distribution, density and proportion of TAMs and its subtypes evolve with the tumor progression, tipping the balance toward poorer differentiation of cellular and stromal elements. This pathological construct has been validated by several studies demonstrating poor prognosis with the change in TAMs density and increasing M2 proportion in different cancers including breast[5,6], esophageal[7], gastric[8] and colorectal malignancies.[9-11] We have also learned through theoretical modelling and experimental studies, that it is possible to manipulate TAMs, for example by reverting from M2 to M1 state.[12] This has potential implications for efficient management of cancer and represents the focus of the current review.

Coursing through the biology of the tumor microenvironment,

seaming in the role of TAMs in the milieu, we have explored the potential targets these offer and the current state of clinically relevant therapies in the following sections.

The Tumor Microenvironment (TME)

A formative event in the initiation and progression of cancers is the aberrant cellular differentiation that bypasses immunological defenses by modifying the molecular signals and receptors. This tumor cell differentiation is a product of multiple genetic mutations and epigenetic influences like hypermethylation of CpG islands, for instance, in colorectal cancers (CRC). This may result in the mutation into oncogenes or inactivation of tumor suppressor genes, kick-starting the evolution into undifferentiated cancer cells. This is rapidly checked by the tumor microenvironment, a complex interplay of cellular and non-cellular elements surrounding the tumor, in most cases. In a smaller subset it evolves adversely to actually propagate the tumor. We need to look more closely to understand this switch.

The tumor microenvironment's (TME) cellular component includes pericytes, dendritic cells, macrophages, fibroblast and specialized lymphocytes[13], duly alerted by the stromal chemokines and cytokines.[14] The extracellular matrix (ECM) or the non-cellular elements are the structural proteins like collagens and elastin, along with a complex meshwork of glycoproteins like fibronectin and laminins, and the proteoglycans[15] that influence cellular adhesion, as well as modulate cell proliferation, and intercellular communication.[16] Moreover, it harbors humoral elements like transforming growth factor- β (TGF-β), tumor necrosis factor- α (TNF-α) and interleukins (IL-6)[16] that are released on stromal disruption and in turn activate tissue immune cells to attack the abnormal tumor cell. So, the successful tumor progression can only occur if multiple elements (structural, cellular and humoral) of the tumor micro-environment, are abnormally altered. One of the pathways this evolution occurs is through the extracellular vesicles (EVs) like the exosomes, that are released by all the cells of the body, including the tumor cells. Their uptake is receptor dependent and thus directed toward specific cells. Exosomes are endosomal in origin and can transport lipids, proteins as well as RNAs. In the TME, the exosomes secreted by the tumor and immune cells facilitate their effects on each other. Tumor cell-derived exosomes cause inhibition of natural killer and T cells, promote angiogenesis, metastasis and polarization of macrophages and neutrophils to TAMs and tumor associated neutrophils (TANs). This polarization refers to the process induced by various stimuli that transform specific cell lines into distinct functional phenotypes.[17] The transferring of RNA species can reprogram the recipient cell as well. On the other hand, exosomes released by the immune cells can lead to tumor cell apoptosis. A flurry of other effectors work to bypass tissues immune defenses against cancer progression eventually surpassing it and turning it instead in to a tumor promoting microenvironment.

TAMs comprise the main bulk of the infiltrating immune cells in TME in comparison to the dendritic cells, the T cells and the other antigens presenting cells.[18] The tumor microenvironment (TME) determines the change in character, for example, from anti-tumor M1 predominant to pro-tumor M2 dominant macrophage polarization. M2 in turn has been shown to promote all the aspects of TME leading to tumor spread, as mentioned earlier.[4,18]

Tumor Associated Macrophages (TAMs)

Macrophages are white blood cells, derived from the peripheral blood monocytes. Their phagocytic properties, responsible for clearing the cellular debris and tumor cells along with the other harmful foreign agents render them a vital component of mononuclear phagocyte immune system.[19,20] Plasticity and adaptability are the two hallmarks of macrophages.[21] On reaching the tumor, influenced by the immune and tumor cells, the various cytokines and chemokines, possibly the lack of oxygen and the resultant increase in lactic acid, these macrophages evolve into tumor associated macrophages (TAMs).[20]

The role of tumor associated macrophages (TAMs) in cancer tissues is central to understanding this approach for improving cancer therapy. As already mentioned earlier, the macrophages can be classified into two major types depending on their polarization states, i.e., classically activated M1 and alternatively activated M2 macrophages.[20] M1 and M2 macrophages are the two extremes of the polarization spectrum with a number of unaccounted subtypes in between.[17,22]

M1 macrophages are considered pro inflammatory and bactericidal. This polarization state is induced by the factors such as, interferon (IFN) ã and lipopolysaccharides (LPS). M1 macrophages secrete a number of Th1 inducing cytokines like, tumor necrosis factor (TNF) α, interleukin 12 (IL-12), IL-6 and IL-18. These cells have high antigen presenting capacity and also produce reactive oxygen species (ROS), thus are responsible for directly killing the tumor cells.[21,23]

M2 macrophages are immunosuppressive, anti-inflammatory and pro tumor. Their polarization is choreographed by IL-4, IL-13 and IL-10. These cells in turn secrete transforming growth factor (TGF)-β and IL-10, which are responsible for the immunosuppressive nature of tumor microenvironment.[17,23]

In the case of tumors, TAMs highly resemble M2 macrophages, as both are activated in response to the similar cytokines and secrete some common factors, while exhibiting few differences as well.[17] A number of growth factors, cytokines, chemokines and enzymes produced by TAMs play an important role in tumor growth and progression. IL-6 increases the chemoresistance by activating the STAT3 pathway and indirectly increasing the anti-apoptotic protein Bcl2 in colorectal and other solid tumors.[24,25] TAMs promote neovascularization by increased secretion of vascular

endothelial growth factor (VEGF), platelet derived growth factor and TGF β.[26] Matrix metalloproteinases (MMPs) promote invasion and metastasis. TGFβ induces epithelial to mesenchymal transition (EMT) in colorectal cancers through smad/snail signaling pathway.[27] Various chemokines like CCL2, CCL5, including other cytokines and enzymes already mentioned can hinder the CD4+ and CD8+ functions and also result in the recruitment of natural Tregs (nTregs), thus resulting in unsuccessful immunosuppressive therapy.[17]

TAMs as Novel Therapeutic Target for Cancer Immunotherapy

Being an important component of TME, tumor associated macrophages are a desirable target for cancer treatment. TAMs provide multiple potential routes for manipulation with the resultant augmentation of their anti-cancer activity.[28] Reducing or blocking monocyte recruitment into tissues, inducing apoptosis of TAMs already present in the tissue, blocking angiogenesis promoting activities through receptor binding, re-educating or repolarizing TAMs from pro-tumor M2 to anti-tumor M1 type, for example, are some pathways (as summarized in Table 1). Each of these translate to either one of the following anti-tumor effects:[29]

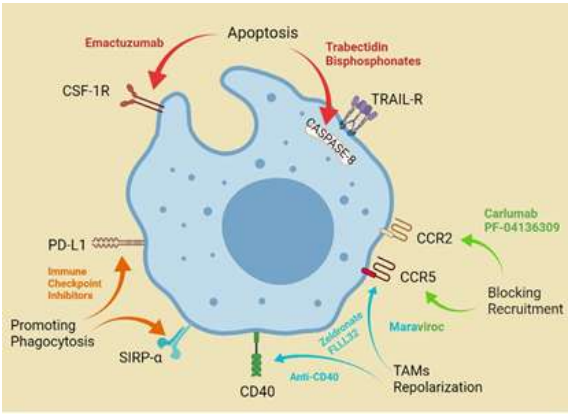
- Direct cellular phagocytosis or cytotoxicity of tumor cells
- Unblocking the cell-death or apoptosis function in tumor cells thus enabling better response to chemotherapeutic agents
- Blocking tumor promoting functions like angiogenesis

In effect this would result in delaying tumor progression or actual tumor regression resulting in improved patient survival. A wholesome volume of research has been directed to these potential targets and its beginning to provide evidence of clinical efficacy.

The therapeutic strategies targeting TAMS, based on experimental research include clearing and inhibiting the activation of TAMS by targeting CSF-1/CSF1R signaling to suppress the tumor growth, promoting the phagocytic activity of macrophages by blocking CD47-SIRPα signaling, limiting monocyte recruitment by targeting CCL2R and inhibition of TAMs by PD-L1 antibody to promote phagocytic activity. Monoclonal antibodies directed against the LILRB1 component of the LILRB1/ MHC class 1 identification mechanism and genetically engineered TAMs lacking the SIRPα and LILRB1 receptors, are few other under trial targets, directed at increasing the phagocytic activity of TAMs.[30] Figure1 graphically demonstrates the currently known agents that target and modulate specific TAMs attributes for potential therapeutic benefit. As promising these treatment options might seem, there is still a long way before they could become a part of regular treatment for solid tumors, as discussed momentarily.

Blocking TAMs Recruitment

Figure1. Specific sites in TAMs that are targeted by various potential agents for therapeutic benefit. For details about these interactions please refer to the related text and table 1.



Blocking TAMs recruitment would theoretically reduce the effect of TAM induced modulation of TME that, although beneficial in initial stages of cancer, are deleterious as tumor progresses. A potential target that triggered significant interest was chemokine CCL2 and its receptor CCR2.[23] A monoclonal antibody against CCL2, carlumab, has been tested in phase I and phase II trials. Although showed encouraging results in the mouse model,[31] and good tolerance in humans, it unfortunately did not translate into therapeutic efficacy as TAM recruitment was not affected among a cohort of prostate cancer patients[32] and a diverse set of solid tumors.[33] Interestingly in combination with FOLFIRINOX (fluorouracil irinotecan plus leucovorin and oxaliplatin) alone, or in combination with CCR2 antagonist as chemotherapy for pancreatic adenocarcinoma,[34] it did lead to TAM depletion in tumors and contributed to partial response in half the patients by hampering tumor growth and metastasis. Among the CRC patients a subset of patients with advanced disease showed encouraging response when Maraviroc, an antagonist to CCR5 receptor of CCL5 chemokine, was used in a preclinical study.[35]

Inducing TAMs depletion

TAMs depletion, already recruited to the tumor, would also potentiate anti-tumor activity in principle, similar to the recruitment blockage paradigm. A wide array of targets and molecules have been employed in basic and clinical research for TAMs depletion.[20] Two of these, namely Bisphosphonates and Trabectidin, are already in clinical use as anti-cancer agents for specific indications. Bisphosphonates are mostly used in patients with bony metastases from solid tumors e.g., breast[36,37] and prostate cancers[38] or against myeloid element in hematological malignancy. As inhibitors of the farnesyl diphosphonate synthase, these tend to accumulate in bone hydroxyapatite where they are taken up by the bone macrophages (osteoclasts), leading to their apoptosis.[39] This macrophage apoptosis is also witnessed non-selectively in non-bony tissues, for example in the liposomal formulation

Table 1. Summary of Potential Pathways for Therapeutic Manipulation of TAMS

Target	Agents	Effect	Tumor Targeted	Effect on ATMs
Clearing/Inhibiting TAMs				
CSF1/CSF1R	PLX3397 (Small molecule inhibitor of CSFIR)	Inhibit the expression of CSFR1	Glioblastoma[50]	Reprogramming into less protumoral phenotype/increa-sed phagocytic ability
CSF1 is involved in macrophage recruitment, repolarization and differentiation (20)	Anti CSF1 antibody	CSF1/CSF1R receptor blockade	Pancreatic ductal adenocarcinoma (PDAC)[51]	Decreased number of TAMs+ selective killing of M2 macrophages
Reducing/ blocking monocyte recruitment				
CCL2/CCR2 inhibition (CCL2 is responsible for recruitment of CCR2 positive macrophages)	PF-04136309 (Small molecule CCR2 inhibitor), in combination with FOLFIRINOX	CCR2 inhibitor	Pancreatic ductal adenocarcinoma (PDAC)[34]	TAM reduction
Inducing apoptosis of TAMs already present in the tissues				
Unspecified (macrophages)	Bisphosphonates (zoledronic acid)	Directly effects macrophages	Breast cancer[39]	Effects macrophage polarization, migration, vesicular trafficking, proliferation and survival
Capase-8 activation	Trabectedin (chemo-therapeutic agent)	Activates caspase-8 – dependent apoptosis	Fibrosarcoma [43, 52]	TAM depletion & reduced angiogenesis
CSFIR inhibition	Emactuzumab (anti CSFIR monoclonal antibody)	Blocks CSFIR activation	Diffuse- type giant cell tumor[42]	TAM depletion
Re-educating/repolarizing TAMs from M2 to M1				
	Biphosphonate (zoledronic acid)	Impaired M2 macrophage polarization	Prostate cancer[48]	Impaired M2 polarization without repolarizing to M1
Macrophage repolarization		Inhibit phosphoryl-ation of STAT3 Inhibits NF-kB canonical pathway	Colorectal cancer[11]	Repolarization of M1 to M2
CCL5/CCLR5 inhibition (CCL5 is a T lymphocyte derived chemokine and affects TAMs)	Maraviroc (Viral entry blocking inhibitor for HIV patients)	CCR5 inhibitor	Colorectal cancer[35]	TAMs repolarization (anti-tumor)
Promoting phagocytic activity of macrophages				
	Hu5F9-G4 (anti CD-47 antibody)	Blocks CD- 47	Non- Hodgkin's lymphoma[53]	Tumor cells phagocytosis

Abbreviations: CSF1: Colony stimulating factor-1,CSF1R: Colony stimulating factor-1 receptor, CCL2: Chemokine (C-C motif) ligand 2, STAT3: Signal transducer and activator of transcription factor 3, NF- kB: Nuclear factor kappa light chain enhancer of activated B cells, CCL5: Chemokine (C-C motif) ligand 5, PDL1: Programed cell- death ligand 1, CD47: Cluster of differentiation 47, SIRPá: Signal regulatory protein alpha

of clodronate, and has found efficacy in reducing visceral as well as bony metastasis in patients with breast cancer. Trabectidin is an alkylating chemotherapeutic drug that is approved for treatment of advanced ovarian cancer,[40] liposarcoma, leiomyosarcoma and other soft tissue sarcomas.[41] Along with its direct cytotoxic effect on neoplastic cells, it has been shown to markedly reduce tissue concentration of TAMs by 30-70% through the TRAIL dependent pathway of apoptosis.[42,43]

Another enticing target to induce TAMs apoptosis has been through the Colony Stimulating Factor-1 (CSF-1) and its receptor CSF1R pathway.[44] This pathway has major role in maturation and differentiation of macrophages and monocytes. Antibodies directed against the CSF1R receptor has been shown in murine models to significantly reduce the number of TAMs in tumor tissue that appears to be more selective for M2 macrophages. Emactuzumab, is the more widely used agent that has been utilized in clinical trials of solid tumors[45,46] like breast, prostate and ovarian cancers. The anti-neoplastic agents commonly used in these tumors

tend to upregulate CSF1/CSF1R complex leading to increasing recruitment, activation and differentiation of macrophages to TAMs, and blocking this pathway has resulted in significant reduction of TAMs population in these tumors even in clinical studies. An alternative to antibody approach has been to utilize tyrosine kinase inhibitor like pexidartinib to block the CSF1 receptor. This has found clinical efficacy and approval in enhancing the response in advanced prostate in combination with hormonal therapy. In general, because of the nonspecific response against macrophages throughout the body, these present a lot of side effects and further work is being directed toward agents that would provide selectivity for M2 macrophages in tumor tissues by targeting for example CD-163 receptors for cell selection.

Reeducating TAMs: M2 to M1

Utilizing TAMs plasticity i.e., ability of converting to M2 from M1 phenotype and vice versa provide other potential means to influence TME. Reeducating M2 to M1 would the antitumor potential and may potentially improve patient survival.[11,47] Alternatively, preventing M1 conversion to M2 may also achieve this goal by preventing pro-tumoral effects of M2. This construct is still in the realm of experimental or preclinical studies. There are a host of theoretical pathways to achieve that and an increasing number of candidate drugs to modulate these pathways. An example is Zoledronic acid and its effects on TAMs repolarization from M2 to M1.[48] An indirect clinical correlation was provided by Gnant et al., through their clinical trial with addition of Zoledronic Acid to endocrine therapies among 1803 premenopausal breast cancer patients, as part of ABCSG-12 randomized trial, was shown to achieve improved survival.[49] More commonly experimental models focus on STAT3 and NF-êB pathways disruption, using for example antibodies like anti-CD 40 antibody, or designer molecules, e.g., FLLL32, a diketone analogue of curcumin to achieve repolarization.[11]

TAMs Manipulation: other alternatives

Alternative strategies, mentioned earlier, that promote the phagocytic activity of macrophages by blocking CD47-SIRPα signaling, inhibition of TAMs by PD-L1 antibody to promote phagocytic activity, monoclonal antibodies directed against the LILRB1(Leucocyte immunoglobulin like receptor subfamily B member 1) component of the LILRB1/ MHC class 1 identification mechanism and genetically engineered TAMs lacking the SIRPα and LILRB1 receptors, are few other strategies for TAMs manipulation.[30] Most of these are still a long way from clinical utility though and mostly experimental constructs at this time. Table 1 summarizes the pathways, targets and agents that researchers have utilized for therapeutic manipulation of TAMs.

CONCLUSION:

Tumor Associated Macrophages represents the most prevalent stromal cell type in the tumor microenvironment. Several studies have demonstrated evolution of their anti-tumoral role as the M1 subtype, polarizing to the other end of the spectrum, the M2 or the protumor subtype, as the cancer progresses. These have multitude of effects on the element of TME like inducing chemoresistance, reducing apoptosis, increasing tumor vascularity, decreasing inter-cellular adhesion, promoting invasiveness and diminishing the response by cytotoxic immune cells. With the advent of targeted therapies in the treatment of cancers, there is increasing focus on targeting TAMs to improve prognosis. Work is ongoing on novel strategies with specific molecular targets that reduce monocyte recruitment, cull TAMs already present in the TME, reeducate TAMs from protumor M2 to antitumor M1 subtype, and promote phagocytosis by TAMs. Some of these have already entered different phases of clinical trials. With a lot of promise and concerted research focus, there is high expectation that TAMs will provide an effective platform to improve cancer therapy in the coming years.

Authors Contribution:
Fouzia Fazal: Conception, Design, Manuscpt.
Muhammad Arsalan Khan: Conception, Design, Manuscpt.
Sumayya Shawana: Critical Analysis
Muhammad Mubarak: Critical Analysis

REFERENCES

1. Mattiuzzi C, Lippi G. Current Cancer Epidemiology. J Epidemiol Glob Health. 2019;9(4):217-222. doi:10.2991/jegh.k.191008.001
2. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71(3):209-249. doi:10.3322/caac.21660
3. Erickson AW, Ghodrati F, Habbous S, et al. HER2-targeted therapy prolongs survival in patients with HER2-positive breast cancer and intracranial metastatic disease: a systematic review and meta-analysis. Neuro-Oncology Advances. 2020;2(1)doi:10.1093/noajnl/vdaa136
4. Lin Y, Xu J, Lan H. Tumor-associated macrophages in tumor metastasis: biological roles and clinical therapeutic applications. J Hematol Oncol. 2019;12(1):76. doi:10.1186/s13045-019-0760-3
5. Jeong H, Hwang I, Kang SH, Shin HC, Kwon SY. Tumor-Associated Macrophages as Potential Prognostic Biomarkers of Invasive Breast Cancer. J Breast Cancer. Mar 2019;22(1):38-51. doi:10.4048/jbc.2019.22.e5
6. Yang M, Li Z, Ren M, et al. Stromal Infiltration of Tumor-Associated Macrophages Conferring Poor Prognosis of Patients with Basal-Like Breast Carcinoma. J Cancer. 2018;9(13):2308-2316. doi:10.7150/jca.25155
7. Hu JM, Liu K, Liu JH, et al. CD163 as a marker of M2 macrophage, contribute to predicte aggressiveness and prognosis of Kazakh esophageal squamous cell carcinoma. Oncotarget. 2017;8(13):21526-21538. doi:10.18632/ oncotarget .15630
8. Yin S, Huang J, Li Z, et al. The Prognostic and Clinico-pathological Significance of Tumor-Associated Macrophages in Patients with Gastric Cancer: A Meta-Analysis. PLoS One. 2017;12(1):e0170042. doi:10.1371/journal.pone.0170042

9. Yang C, Wei C, Wang S, et al. Elevated CD163(+)/CD68(+) Ratio at Tumor Invasive Front is Closely Associated with Aggressive Phenotype and Poor Prognosis in Colorectal Cancer. Int J Biol Sci. 2019;15(5):984-998. doi:10.7150/ijbs.29836
10. Krijgsman D, De Vries NL, Andersen MN, et al. CD163 as a Biomarker in Colorectal Cancer: The Expression on Circulating Monocytes and Tumor-Associated Macrophages, and the Soluble Form in the Blood. Int J Mol Sci. 2020;21(16) doi:10.3390/ijms21165925
11. Zhao Y, Ge X, Xu X, Yu S, Wang J, Sun L. Prognostic value and clinicopathological roles of phenotypes of tumour-associated macrophages in colorectal cancer. J Cancer Res Clin Oncol. 2019;145(12):3005-3019. doi:10.1007/s00432-019-03041-8
12. Yahaya MAF, Lila MAM, Ismail S, Zainol M, Afizan N. Tumour-Associated Macrophages (TAMs) in Colon Cancer and How to Reeducate Them. J Immunol Res. 2019;2019: 2368249. doi:10.1155/2019/2368249
13. Labani-Motlagh A, Ashja-Mahdavi M, Loskog A. The Tumor Microenvironment: A Milieu Hindering and Obstructing Antitumor Immune Responses. Front Immunol. 2020;11:940. doi:10.3389/fimmu.2020.00940
14. Yuan Y, Jiang Y-C, Sun C-K, Chen Q-M. Role of the tumor microenvironment in tumor progression and the clinical applications (Review). Oncol Rep. 2016/05/01 2016;35(5): 2499-2515. doi:10.3892/or.2016.4660
15. Wei R, Liu S, Zhang S, Min L, Zhu S. Cellular and Extracellular Components in Tumor Microenvironment and Their Application in Early Diagnosis of Cancers. Analytical Cellular Pathology. 2020/01/08 2020;2020:6283796. doi:10.1155 /2020 /6283796
16. Arneth B. Tumor Microenvironment. Medicina (Kaunas). 2019;56(1)doi:10.3390/medicina56010015
17. Baghban R, Roshangar L, Jahanban-Esfahlan R, et al. Tumor microenvironment complexity and therapeutic implications at a glance. Cell Communication and Signaling. 2020/04/07 2020;18(1): 59. doi:10.1186/s12964-020-0530-4
18. Chen Y, Song Y, Du W, Gong L, Chang H, Zou Z. Tumor-associated macrophages: an accomplice in solid tumor progression. J Biomed Sci. 2019;26(1):78. doi:10.1186/s12929-019-0568-z
19. Yona S, Gordon S. From the Reticuloendothelial to Mononuclear Phagocyte System – The Unaccounted Years. Review. Frontiers in Immunology. 2015-July-01 2015;6(328)doi:10.3389/fimmu.2015.00328
20. Zhou J, Tang Z, Gao S, Li C, Feng Y, Zhou X. Tumor-Associated Macrophages: Recent Insights and Therapies. Front Oncol. 2020;10:188. doi:10.3389/fonc.2020.00188
21. Guttman O, C. Lewis E. M2-like macrophages and tumor-associated macrophages: overlapping and distinguishing properties en route to a safe therapeutic potential. Integrative Cancer Science and Therapeutics. 2016;3(5) doi:10. 15761 /icst.1000204
22. Jayasingam SD, Citartan M, Thang TH, Mat Zin AA, Ang KC, Ch'ng ES. Evaluating the Polarization of Tumor-Associated Macrophages Into M1 and M2 Phenotypes in Human Cancer Tissue: Technicalities and Challenges in Routine Clinical Practice. Front Oncol. 2019;9:1512. doi:10.3389/fonc.2019.01512
23. Pinto ML, Rios E, Duraes C, et al. The Two Faces of Tumor-Associated Macrophages and Their Clinical Significance in Colorectal Cancer. Front Immunol. 2019;10:1875. doi:10 .3389/fimmu.2019.01875
24. Xu X, Ye J, Huang C, Yan Y, Li J. M2 macrophage-derived IL6 mediates resistance of breast cancer cells to hedgehog inhibition. Toxicol Appl Pharmacol. 2019;364:77-82. doi:10. 1016/j.taap.2018.12.013
25. Yin Y, Yao S, Hu Y, et al. The Immune-microenvironment Confers Chemoresistance of Colorectal Cancer through Macrophage-Derived IL6. Clinical Cancer Research. 2017;23(23):7375-7387. doi:10.1158/1078-0432.Ccr-17-1283
26. Tamura R, Tanaka T, Yamamoto Y, Akasaki Y, Sasaki H. Dual role of macrophage in tumor immunity. Immunotherapy. Aug 2018;10(10):899-909. doi:10.2217/imt-2018-0006
27. Cai J, Xia L, Li J, Ni S, Song H, Wu X. Tumor-Associated Macrophages Derived TGF-â-Induced Epithelial to Mesenchymal Transition in Colorectal Cancer Cells through Smad2,3-4/Snail Signaling Pathway. Cancer Res Treat. Jan 2019;51(1):252-266. doi:10.4143/crt.2017.613
28. Yang L, Zhang Y. Tumor-associated macrophages: from basic research to clinical application. Journal of Hematology & Oncology. 2017/02/28 2017;10(1):58. doi:10.1186/s13045-017-0430-2
29. Anfray C, Ummarino A, Andón FT, Allavena P. Current Strategies to Target Tumor-Associated-Macrophages to Improve Anti-Tumor Immune Responses. Cells. 2019;9(1) doi:10.3390/cells9010046
30. Barkal AA, Weiskopf K, Kao KS, et al. Engagement of MHC class I by the inhibitory receptor LILRB1 suppresses macrophages and is a target of cancer immunotherapy. Nat Immunol.2018;19(1):76-84.doi:10.1038/s41590-017-0004-z
31. Sanford DE, Belt BA, Panni RZ, et al. Inflammatory Monocyte Mobilization Decreases Patient Survival in Pancreatic Cancer: A Role for Targeting the CCL2/CCR2 Axis. Clinical Cancer Research. 2013;19(13):3404-3415. doi:10.1158/1078-0432.Ccr-13-0525
32. Pienta KJ, Machiels J-P, Schrijvers D, et al. Phase 2 study of carlumab (CNTO 888), a human monoclonal antibody against CC-chemokine ligand 2 (CCL2), in metastatic castration-resistant prostate cancer. Investigational New Drugs. 2013/ 06/01 2013;31(3):760-768. doi:10.1007/s10637-012-9869-8
33. Brana I, Calles A, LoRusso PM, et al. Carlumab, an anti-C-C chemokine ligand 2 monoclonal antibody, in combination with four chemotherapy regimens for the treatment of patients with solid tumors: an open-label, multicenter phase 1b study. Target Oncol. 2015;10(1):111-23. doi:10.1007/s11523-014-0320-2
34. Nywening TM, Wang-Gillam A, Sanford DE, et al. Targeting tumour-associated macrophages with CCR2 inhibition in combination with FOLFIRINOX in patients with borderline resectable and locally advanced pancreatic cancer: a single-centre, open-label, dose-finding, non-randomised, phase 1b trial. The Lancet Oncology. 2016;17(5):651-662. doi:10.1016 /s1470-2045(16)00078-4
35. Halama N, Zoernig I, Berthel A, et al. Tumoral Immune Cell Exploitation in Colorectal Cancer Metastases Can Be Targeted Effectively by Anti-CCR5 Therapy in Cancer Patients. Cancer Cell. 2016;29(4):587-601. doi:10.1016/j.ccell.2016.03.005

36. Diel IJ, Solomayer EF, Costa SD, et al. Reduction in new metastases in breast cancer with adjuvant clodronate treatment. N Engl J Med. 1998;339(6):357-63. doi:10.1056/ nejm199808063390601
37. Powles T, Paterson S, Kanis JA, et al. Randomized, Placebo-Controlled Trial of Clodronate in Patients With Primary Operable Breast Cancer. Journal of Clinical Oncology. 2002;20 (15):3219-3224. doi:10.1200/jco.2002.11.080
38. Macherey S, Monsef I, Jahn F, et al. Bisphosphonates for advanced prostate cancer. Cochrane Database Syst Rev. Dec 26 2017;12(12):Cd006250. doi:10.1002/ 14651858. CD006 250.pub2
39. Junankar S, Shay G, Jurczyluk J, et al. Real-time intravital imaging establishes tumor-associated macrophages as the extraskeletal target of bisphosphonate action in cancer. Cancer Discov. 2015;5(1):35-42. doi:10.1158/2159-8290.Cd-14-0621
40. Ventriglia J, Paciolla I, Cecere SC, et al. Trabectedin in Ovarian Cancer: is it now a Standard of Care? Clin Oncol (R Coll Radiol). Aug 2018;30(8):498-503. doi:10. 1016/j.clon. 2018.01.008
41. Gordon EM, Sankhala KK, Chawla N, Chawla SP. Trabectedin for Soft Tissue Sarcoma: Current Status and Future Perspectives. Adv Ther. 2016;33(7):1055-71. doi:10.1007/ s12325-016-0344-3
42. Ries CH, Cannarile MA, Hoves S, et al. Targeting tumor-associated macrophages with anti-CSF-1R antibody reveals a strategy for cancer therapy. Cancer Cell. 2014;25(6):846-59. doi:10.1016/j.ccr.2014.05.016
43. Germano G, Frapolli R, Belgiovine C, et al. Role of macrophage targeting in the antitumor activity of trabectedin. Cancer Cell. 2013;23(2):249-62. doi:10.1016/j.ccr.2013.01.008
44. Mantovani A, Marchesi F, Malesci A, Laghi L, Allavena P. Tumour-associated macrophages as treatment targets in oncology. Nat Rev Clin Oncol. 2017;14(7):399-416. doi:10. 1038/nrclinonc.2016.217
45. Machiels J-P, Gomez-Roca C, Michot J-M, et al. Phase Ib study of anti-CSF-1R antibody emactuzumab in combination with CD40 agonist selicrelumab in advanced solid tumor patients. Journal for ImmunoTherapy of Cancer. 2020;8(2): e001153. doi:10.1136/jitc-2020-001153
46. Gomez-Roca CA, Italiano A, Le Tourneau C, et al. Phase I study of emactuzumab single agent or in combination with paclitaxel in patients with advanced/metastatic solid tumors reveals depletion of immunosuppressive M2-like macrophages. Ann Oncol. 2019;30(8):1381-1392. doi:10.1093/ annonc/ mdz163
47. van Dalen FJ, van Stevendaal M, Fennemann FL, Verdoes M, Ilina O. Molecular Repolarisation of Tumour-Associated Macrophages. Molecules. 2018;24(1)doi: 10.3390/ molecules24010009
48. Giuseppina Comito1 CPS, Maria Letizia Taddei1, Michele Lanciotti3 Sergio Serni3, AM, Paola Chiarugi1,2 , Elisa Giannoni1. Zoledronic acid impairs stromal reactivity by inhibiting M2-macrophages polarization and prostate cancer-associated fibroblasts. Oncotarget, 2017, Vol 8, (No 1), pp: 118-132. 2016;
49. Gnant M, Mlineritsch B, Stoeger H, et al. Adjuvant endocrine therapy plus zoledronic acid in premenopausal women with early-stage breast cancer: 62-month follow-up from the ABCSG-12 randomised trial. The Lancet Oncology. 2011;12(7):631-641. doi:10.1016/s1470-2045(11)70122-x
50. Yan D, Kowal J, Akkari L, et al. Inhibition of colony stimulating factor-1 receptor abrogates microenvironment-mediated therapeutic resistance in gliomas. Oncogene. 2017; 36(43):6049-6058. doi:10.1038/onc.2017.261
51. Zhu Y, Knolhoff BL, Meyer MA, et al. CSF1/CSF1R blockade reprograms tumor-infiltrating macrophages and improves response to T-cell checkpoint immunotherapy in pancreatic cancer models. Cancer Res. 2014;74(18):5057-69. doi:10. 1158/0008-5472.Can-13-3723
52. Liguori M. BC, Pasqualini F., Bergomas F., Pesce S., Sironi M., Grizzi F., Mantovani A., Belgiovine C., Allavena P. Functional TRAIL receptors in monocytes and tumor-associated macrophages: . Functional TRAIL receptors in monocytes and tumor-associated macrophages: A possible targeting pathway in the tumor microenvironment. Oncotarget. 2016; 7: 41662-41676.
53. Advani R, Flinn I, Popplewell L, et al. CD47 Blockade by Hu5F9-G4 and Rituximab in Non-Hodgkin's Lymphoma. N Engl J Med. 2018;379(18):1711-1721. doi:10.1056/ NEJMoa 1807315
54. Gordon SR, Maute RL, Dulken BW, et al. PD-1 expression by tumour-associated macrophages inhibits phagocytosis and tumour immunity. Nature. 2017;545(7655):495-499. doi:10.1038/nature22396