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Review

Macrophages in the microenvironment of head and neck cancer: potential targets for cancer therapy

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ABSTRACT

The microenvironment of solid tumors has become a promising target for future therapies modulating immune cells. Patients with advanced head and neck cancer, which still portends a poor outcome, are particularly in need of innovative approaches. In oral squamous cell carcinoma, high density of tumor-associated macrophages (TAMs) appears consistently associated with poor prognosis, whereas data are currently limited for other head and neck sites. Several approaches to block TAMs have been investigated, including TAMs inactivation by means of the colony stimulating factor 1 (CSF-1)/CSF-1 receptor (CSF-1R) inhibitors or strategies to reprogram TAMs from M2 protumoral phenotype toward M1 antitumoral phenotype. This review focuses on both prognostic and therapeutic aspects related to TAMs in head and neck carcinomas.

Introduction

According to recent estimates, cancers of the lip, oral cavity, pharynx, and larynx account for almost 700,000 new cases and 400,000 cancer deaths worldwide every year, thus ranking seventh in both the incidence and mortality, and fifth in the 5-year prevalence among all reported cancers [1]. Despite advances in treatment, prevention, and disease biology understanding, prognosis in patients with oral cavity or pharynx cancer remains dismal. Although 5-year overall survival is about 84% in localized stages, it decreases to 64% and 39% in advanced setting with regional and distant spread, respectively [2].

The microenvironment of solid tumors has been increasingly regarded as a new therapeutic target. The immune cells, including macrophages, represent a major component of tumor microenvironment, that constitute a link between the innate and adaptative responses [3]. Macrophages can be classified into two subgroups: M1 phenotype, which is considered proinflammatory and antitumoral; as opposed to M2 phenotype, possessing immunosuppressive and protumoral effects [4–6]. Under physiological conditions or induced by interferon-γ lipopolysaccharides (LPS) and tumor necrosis factor α (TNFα), macrophages are polarized into M1 macrophages. The M1 macrophages produce high levels of interleukin (IL)- 1β, IL-6, CXC ligand 10 (CXCL10), TNFα and inducible nitric oxide synthase (iNOS) and human leukocyte antigen (HLA)-DR that have antitumoral effects (Fig. 1). On the opposite way, tumor cells via several pathways (CCL-2, IL6, CSF-1, PD-1/PD-L1, CD47/SIRPα) activate or switch macrophages to M2 phenotype (Fig. 2). The M2 macrophages express high levels of IL-10, IL-4 tumor growth factor β (TGFβ), CC ligands, vascular endothelial growth factors (VEGFs) and matrix metalloproteinases (MMPs) to directly or indirectly increase local inflammation, promote tumor progression and metastasis, and induce treatment resistance. Tumor-promoting function of macrophages is related to their capacity of secreting proangiogenic and growth factors and suppressing T-cell effector function by releasing immunosuppressive cytokines and by affecting

Abbreviations: CD68+, CD68-positive; CD163+, CD163-positive; HPV, human papillomavirus; HPV+, HPV-positive; HPV-, HPV-negative; OSCC, oral squamous cell carcinoma; TAMs, Tumor-associated macrophages; TNM, tumor-node-metastasis

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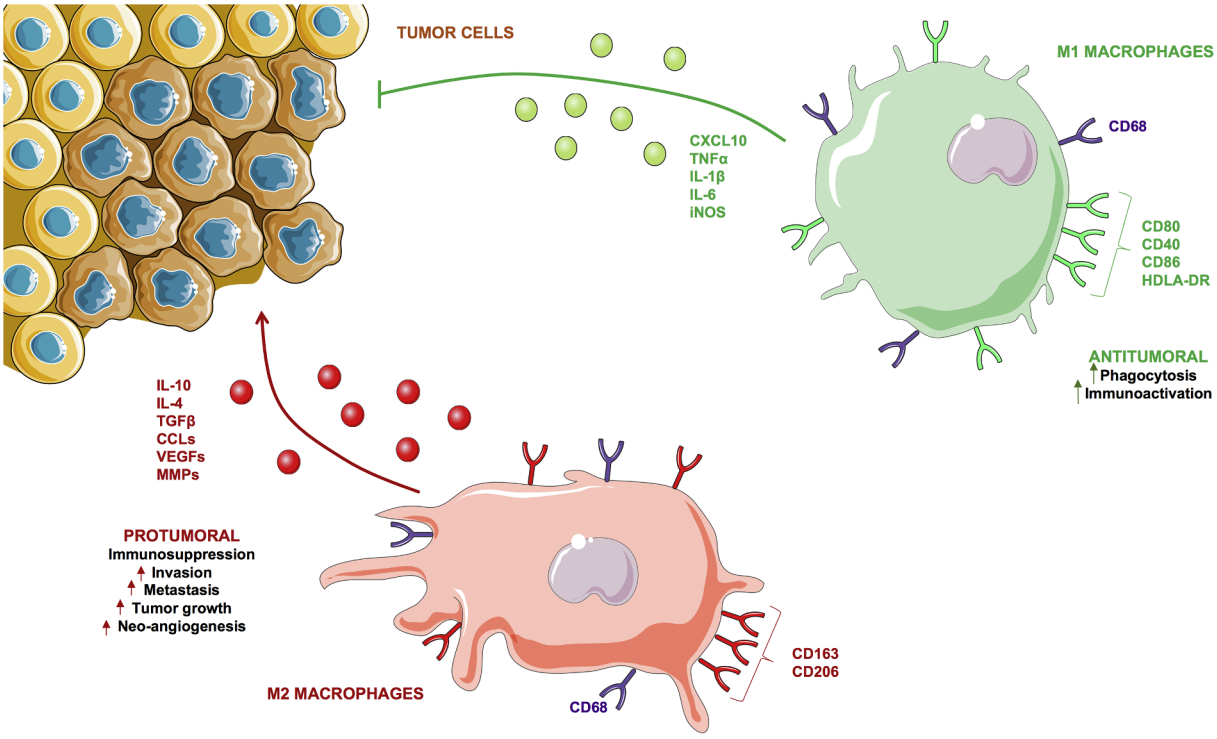

Fig. 1. Phenotypes and functions of M1 and M2 macrophages in tumor microenvironment. Antitumoral M1 macrophages can counteract the tumor by producing antitumoral chemokines (CXCL10, TNFα, IL-1β, IL-6, iNOS) inducing immunoactivation and phagocytosis. Protumoral M2 macrophages produce tumor-promoting factors (IL-10, IL-4, TGFβ, CCLs, VEGFs, MMPs) inducing immunosuppression, invasion, metastasis, tumor growth, and neoangiogenesis. Specific markers to identify each type of macrophage are represented in green and red (CD40, CD80, CD86 and HDL1-DR for M1 macrophages and CD163 and CD206 for M2 macrophages respectively). CD68, represented in purple is a panmarker of macrophages of any phenotype, which is present on both M1 and M2 macrophages. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

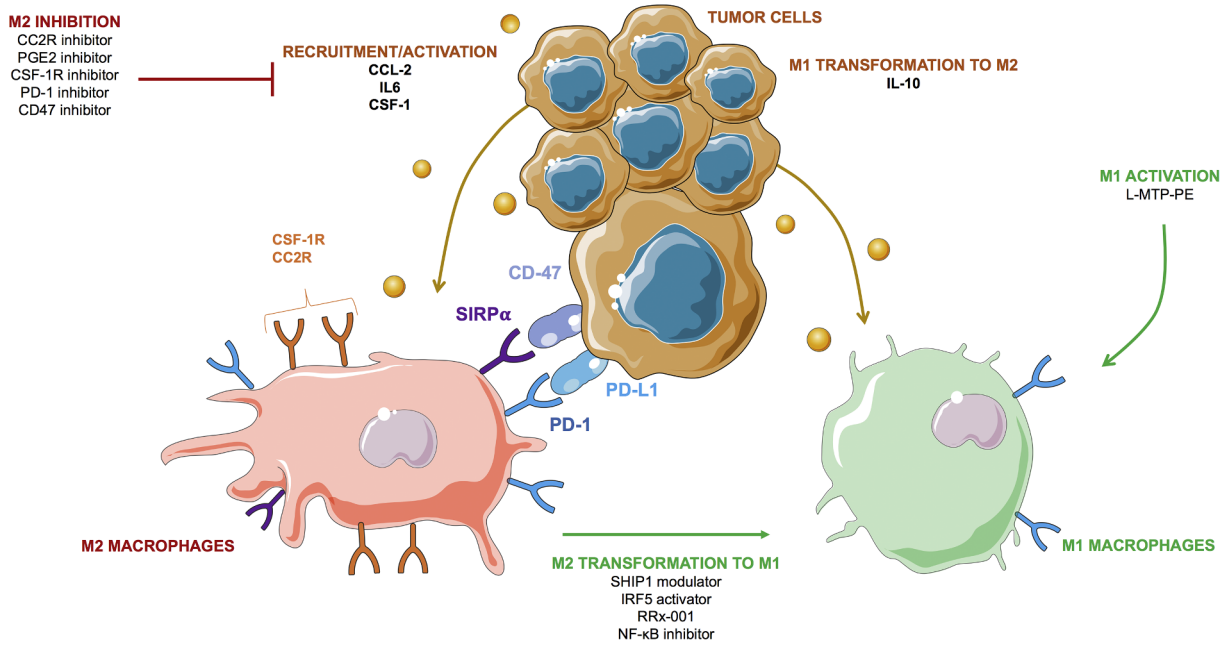

Fig. 2. Targeting macrophages in the tumor microenvironment. Tumor cells actively attract and activate TAMs harboring M2 phenotype, by both direct contact through membrane molecules (PD-1/PD-L1 and CD47/SIRPα) and paracrine loops between macrophages and cancer cells (CCL-2, IL-6, CSF-1). Various strategies to downregulate M2 macrophages or to modulate their functions have been investigated. M2 macrophages can be downregulated by inhibiting CC2R, PGE2, CSF-1R, PD-1 and CD47. M2 macrophages can switch back to antitumor proinflammatory M1 macrophages upon exposure to IRF-5, SIHP1, RRx-001, and NF-κB modulators. M1 macrophages can be activated by L-MTP-PE.

their metabolism [7–10]. However, given a continuum between the M1 and M2 subtypes, the macrophages demonstrate certain plasticity.

Tumor-associated macrophages (TAMs) are defined as macrophages located in, or at the close vicinity of the tumor. TAMs are primarily showing characteristics and functions related to M2 protumoral macrophages [11,12]. Several recent preclinical studies demonstrated the protumoral functions of TAMs. In solid tumors (pancreatic, breast, ovarian, gastric, bladder, ovarian and thyroid cancers), the presence of TAMs correlates with poor outcome [13–15]. The relevance of TAMs in solid tumor was well described for some type of cancer, particularly for breast cancers [16]. This observation was also reported in oral squamous cell carcinoma (OSCC) but to a lesser extent in other head and neck cancer subsites [17,18]. Therefore, further improvement of our knowledge of TAMs in head and neck cancer is necessary for an effective development of novel anticancer strategies.

Considering the protumoral functions of TAMs, new therapies against TAMs have recently gained interest. Such treatments, particularly inhibitors of the colony stimulating factor 1 receptor (CSF-1R), are currently evaluated in several clinical trials [19]. CSF-1R inhibitors may also be of interest in head and neck carcinoma (OSCC and other primary sites). In addition, reprogramming TAMs from M2 protumoral phenotype toward M1 antitumoral phenotype seems to be an interesting approach. Another area of research involves combinations with established treatment to enhance tumor response.

In this review, we focused on deciphering the role of TAMs in head and neck cancer and identifying targeted agents relevant for further clinical testing.

Discussion

Characterization of macrophages in tumor specimens from patients

All published studies used immunochemistry to identify TAMs. In these studies, the most frequently used markers to detect TAMs were CD68 and CD163 [20,21]. Immunohistochemistry employing antibodies against CD68 permits to identify all the macrophages regardless their phenotype. The CD68 marker, a sialomucin member of the scavenger receptor supergene family, is expressed by monocytes, macrophages and some others hematopoietic cells (dendritic, neutrophils, basophils and mast cells) [22,23]. The CD163 marker, a hemoglobin-scavenger receptor highly expressed by M2 macrophages, is further used to discriminate between M1 and M2 macrophages (Fig. 3) [21]. The different scoring of CD68 and CD163 immunohistochemistry and the main findings of the different studies concerning head and neck carcinoma are summarized in Table 1 and Table 2, respectively.

Macrophages tumor infiltration using antibody CD68

In a limited cohort of 20 cases of oral and oropharyngeal carcinoma published in 2004, Marcus et al. investigated the presence of macrophages using only CD68 antibody. In this study, macrophages were identified as a prognostic factor in OSCC, since the authors found a strong correlation with lymph node metastases in a univariate analysis ($p < 0.001$) [24].

Lin et al. also used CD68 in 84 cases of laryngeal carcinoma [25]. For every specimen, macrophages were counted in both “intratumoral” (tumor nest) and “peritumoral” (tumor stroma) areas according to Soeda et al. [26]. Three “Hot Spots” of CD68-positive macrophages (CD68+) were selected. Authors classified samples into “low” and “high” groups based on mean number of CD68 + macrophages in each area. Performing a multivariate analysis including other prognostic factors such as tumor-node-metastasis (TNM) stage and histological differentiation, they found that high CD68 + macrophage infiltration was an independent adverse factor for disease free survival, which held true both for peritumoral ($p = 0.016$) and intratumoral CD68 + macrophages ($p = 0.046$).

CD68 marker was also studied by Bagul et al. comparing 30 specimens of OSCC with 10 oral biopsy samples in the control group [27]. In this study, the same quantification as the one used in Lin et al. study was performed. Unlike the previous study, intratumoral and peritumoral areas were not discriminated. Authors divided the tumor samples in four groups defined by proportion of CD68 + cells in three areas of “Hot Spots”. The expression of CD68 marker was significantly higher in the OSCC group than in normal mucosa ($p < 0.001$). However, there was no statistical difference in the expression of CD68 marker between the three grades of differentiation (well, moderately or poorly differentiated) of OSCC.

All these studies restricted their analyses to CD68 + cells. However, this marker is not specific of macrophages and might not be sufficient to characterize TAMs. Since TAMs present often a M2 phenotype, the additional use of a specific marker of M2 such as CD163 could result in better immunochemical characterization of TAMs.

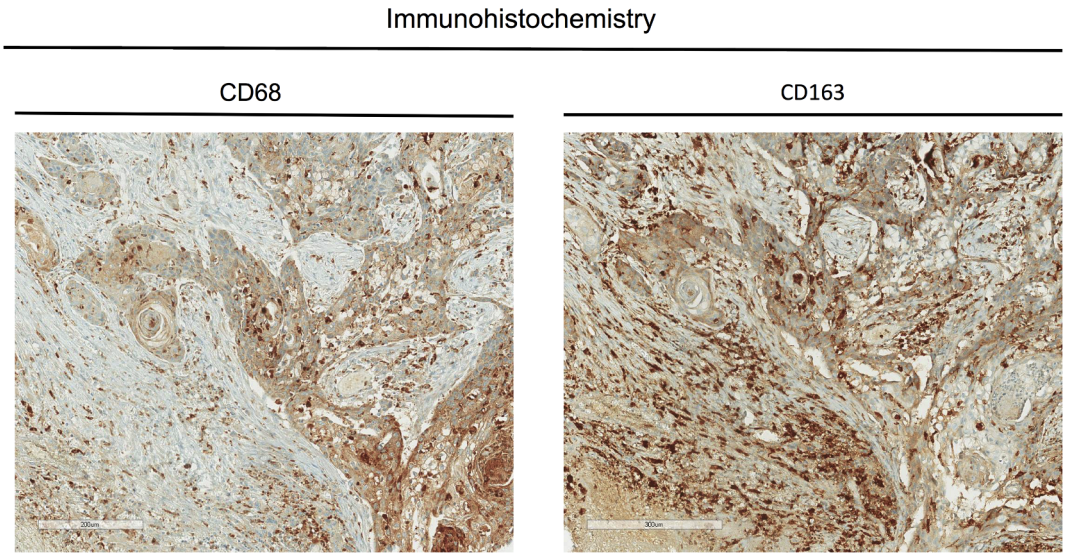

Fig. 3. CD68 and CD163 staining in head and neck carcinoma. CD68-positive macrophages (left panel) and CD163-positive macrophages (right panel) were examined by immunohistochemistry in tumor nest and stroma in a case of hypopharyngeal carcinoma operated in our surgical department. CD68 and CD163 expression was also observed in the cytoplasm of tumor cells.

Table 1
Different types of scoring CD68 + and CD163 + macrophages in head and neck carcinoma.

Reference	Antibody	Location	Method	Specificity	Groups	Cut-off
Lin et al.	CD68	TN and TS	Quantitative	3 Hot spots (X400): mean	Low/High	10 for TN 24 for TS
Bagul et al.	CD68	Not reported	Quantitative	3 Hot spots (X40): proportion	Grade 0,1,2 or 3	< 1, < 10,10–50, > 50%
Hu et al.	CD68/CD163	TN and TS	Quantitative	10 HPF (X400): mean	Neg/Low/High	6/HPF for TN 8/HPF for TS
Sakakura et al.	CD68/CD163	Not reported	Quantitative	> 4 HPF(X400): mean	Low/High	> 200 for CD68 > 90 for CD163

A b b reviations: CD68+, CD68-positive; C163+, CD163-positive; HPF, high power field; TN, tumor nest; TS, tumor stroma.

Combining CD68 and CD163 antibodies to characterize macrophage phenotype

Unspecified head and neck squamous cell carcinoma

The first report was published in 2012 by Fujii et al. demonstrating that CD163-positive (CD163+) M2 macrophages could represent a possible prognostic factors of OSCC [28]. In 2014, He et al. studied 43 OSCC specimens correlating the CD163 marker overexpression with a poor overall survival (OS) ($p < 0.05$) [29].

In the study by Hu et al. [18], 127 primary OSCC specimens and 23 peritumoral tissue samples were analyzed. CD68 + and CD163 + macrophages were found both in tumor nest and stroma. The increase of CD68 + macrophages in tumor nest significantly correlated with mortality ($p = 0.005$), but not in tumor stroma ($p = 0.218$).

In addition, the density of CD163 + macrophages in tumor nest was significantly associated with mortality ($p = 0.044$). In tumor stroma, this density was significantly correlated with recurrence ($p = 0.008$) and mortality ($p = 0.04$). This correlation was also found in the Sakakura's study on 74 OSCC [30]. In the univariate analysis, the infiltration of M2 was correlated with OS ($p = 0.025$) and progression-free survival (PFS) ($p = 0.011$). Analogous correlations were found in a multivariate regression analysis for OS and PFS as well ($p = 0.034$ and $p = 0.023$, respectively) [30].

The *meta*-analysis by Zhang et al. [15] included four studies on head and neck cancer: Fujii's (108 OSCC) [28] and Lin's studies (84 laryngeal carcinomas) [25], which we already referred to, and two further ones (Liu's study with 112 OSCC [31] and Peng's study with 60 nasopharynx carcinomas) [32]. However, the most important results were based mainly on the studies by Fujii and Liu's [28,31]. The correlation between CD68 + macrophages and oral cancer was clearly confirmed. In oral cancer, high density of CD68 + macrophages appeared significantly higher in patients with an advanced (III + IV according to the TNM classification) than in patients with an early tumor stage (I + II) [RR = 1.49 (95%CI, 1.17–1.89)]. This correlation was only found for CD68 + macrophages but not for CD163 + macrophages (evaluated only in the Fujii's study) without any statistical difference between these two groups ($p = 0.50$). Oral cancers showed significant risk ratio (RR) between high CD68 + macrophages infiltration and poor OS (overall survival) [RR = 2.03 (95%CI 1.47–2.80)]. This *meta*-analysis included three out of four studies which analyzed only the CD68 staining. Moreover, this study did not perform multivariate analysis.

This correlation between macrophages and poor prognostic could be explained, as least in part, by the reported role of TAMs in the epithelial to mesenchymal transition [33]. In Hu's report, the HN4, HN6, and SCC9 cultured cancer cell lines from larynx and tongue were analyzed by immunofluorescence and PCR: after the incubation of TAMs-conditioned medium, cancer cells took fibroblast-like appearance. The decrease of E-cadherin and the increase of vimentin were also notified. From all these results, authors concluded that the mutual interaction between TAMs and cancer cells promoted the epithelial to mesenchymal transition of cancer cells, thereby leading to an increase of invasion and metastatic properties of cancer cells [18].

In summary, data published on M2 macrophages appears consistently to favor tumor progression. However, activity of M1 macrophages in the tumor could also be relevant [34–36]. Among CD68-positive cells, C163 marker stain specifically M2 macrophages, but M1 macrophages cannot be precisely currently identified by a specific marker. Moreover, the immunohistochemistry analysis provides only a snapshot of the density and composition of the tumor microenvironment. This technique cannot provide temporal distribution of macrophages according to the plasticity of tumor microenvironment [37].

HPV-related head and neck carcinomas

Besides OSCC, TAM incidence using both CD68 and CD163 was sparsely investigated in other primary sites of head and neck cancer. Moreover, only one study focused on CD68 + cells in oropharyngeal cancer according to human papillomavirus (HPV) status of tumors [38]. In the tumor area, authors found more CD68 + cells in HPV-positive (HPV+) as compared to HPV-negative (HPV-) carcinomas ($p = 0.01$). No such difference was found in the stromal area ($p = 0.1$). In a recently published study reporting 110 cases of carcinomas, most of them emerging from oral cavity or oropharynx, Seminerio et al. found

Table 2
Characteristics of studies reporting TAMs frequency and prognostic value in head and neck cancer.

Reference	Year	Tumor type (# of cases)	Markers	HPV status report
Marcus et al.	2004	OSCC/OPSCC (20)	CD68	No
Peng et al.	2006	NPC (60)	CD68	No
Liu et al.	2008	OSCC (1 1 2)	CD68	No
Lin et al.	2011	LC (84)	CD68	No
Fujii et al.	2012	OSCC (1 0 8)	CD68 and CD163	No
Zhang et al.	2012	OSCC (2 2 0)	CD68 and CD163	No
He et al.	2014	OSCC (1 2 7)	CD68 and CD163	No
Hu et al.	2014	OSCC (43)	CD68 and CD163	No
Bagul et al.	2016	OSCC (30)	CD68	No
Sakakura et al.	2016	OSCC (74)	CD68 and CD163	No
Oguejiofor et al.	2017	OPSCC (1 2 4)	CD68	Yes
Seminerio et al.	2018	SCCHN (1 1 0)	CD68	Yes

A b b reviations: LC, laryngeal carcinoma; NPC, nasopharyngeal carcinoma; OSCC, oral squamous cell carcinoma; OPSCC, oropharyngeal squamous cell carcinoma; SCCHN, squamous cell carcinoma of the head and neck.

Table 3
Summary of preclinical studies targeting TAMs.

Strategy	Pathway	Drug	Tumor type	Outcome	Reference	Year
Inhibiting TAM recruitment	CCL-2/CCR2	PF-04136309	Pancreatic	GI and RM	Sanford et al.	2013
Preventing TAM differentiation	COX-2	–	CRC	*	Brown et al.	2005
Blocking TAM activation	CSF-1R/CSF-1	BLZ945	Cervical and mammary tumor	GI	Strachan et al.	2013
		BLZ945	Glioma	GI/IS	Pyonteck et al.	2013
	CD47/SIRP α	Hu5F9-G4	AML/NHL	GI/IS/WT	Liu et al.	2015
		biAbs	HCCL	WT	Dheilly et al.	2017
		anti mPD1	HNSCC	GI/WT	Yu et al.	2015
Reprogramming TAMs to M1	SHIP1		Cancer cell lines	PI3K pathway inhibition	Ong et al.	2007

Abbreviations: AMIST, advanced malignant incurable solid tumor; AML, acute myeloid leukemia; CMT, cervical and mammary tumor; CRC, colorectal cancer; GI, growth inhibition; HCCL, human cancer cell lines; HNSCC, head and neck squamous cell carcinoma; IS, improved survival; NHL, non-Hodgkin's lymphoma; RM, reduction of metastases; TAMs, tumor associated macrophages; WT, well tolerated.
* Review of some pre-clinical studies.

consistent results with the aforementioned study concerning HPV status: CD68 + macrophage numbers were statistically higher in the intratumoral compartment of HPV+/p16 + tumors than in HPV +/p16 − and HPV- tumors (p = 0.003) [39]. Finally, another study primarily focused on interactions between PD-L1 blockade and CD47/SIRPα axis, Yu et al. mentioned briefly that the expression of CD68, CD163, CD11b, and CD33 was not related to the HPV + status [40].

For other types of HPV-related carcinogenesis, such as cervical carcinoma (cervix uteri), Chen et al. demonstrated a strong association between the malignant transformation of the cervix and an increase in the number of CD163 + and CD68 + macrophages [41].

Chronic inflammation of virus-related tumors is characterized by the infiltration of various leukocyte populations including activated macrophages [42]. As reported for hepatitis B infection [43], several studies suggest that HPV infection promotes macrophage attraction/activation, as well as polarization toward M2 phenotype. M2 macrophages are associated with type 2 helper T lymphocyte (Th2) response, while M1 macrophages induce type 1 helper T lymphocytes (Th1) which favors HPV clearance.

HPV itself hampers host immune responses, downregulating M1 activation via IFN-γ and redirecting them toward M2 phenotype via TGF-β [44,45]. Consistently, Lepique et al. shown that depletion of TAMs from an HPV16-positive tumor model inhibits tumor growth, by increasing of lymphocytes in the tumor tissue [46].

The interaction between TAMs and tumor cells according to HPV status is still not fully elucidated. In addition, their potential role in prognosis is unknown and needs to be further explored to improve targeted approaches dedicated to HPV-defined head and neck squamous cells carcinoma subtypes.

Specificity of TAMs distribution

Spatial distribution of TAMs in tumor tissue, sparsely described for solid tumors, has not been better analyzed for head and neck carcinoma. This distribution could play an important role because it has been reported co-localized with PD-L1 expression and could influence the response to immunotherapies.

In HNSCC, PD-L1-positive macrophages have been reported to be localized at the interface between the tumor periphery and the surrounding inflammatory stroma [47]. Therefore, PD-L1-positive TAMs could create a PD-L1 immunoprotective "barrier" around the tumor nests and contribute to adaptive resistance in PD-L1-positive tumors [47]. This distribution could explain the "induced pattern" of PD-L1 expression described by Scognamiglio et Chen [48]. This spatial distribution could be correlated with some clinical characteristics (HPV status, tumor stage) or with the prognostic. Moreover, this "barrier" could be play a role in the poor response to PD-1 inhibitors.

The others studies about TAMs in HNSCC did not describe particularly the spatial distribution of macrophages. Lin et al. reported several patterns of macrophages in laryngeal carcinomas, including "diffuse" or "aggregative" repartition; macrophages presenting either oval or irregular shapes [25]. For Hu et al., CD68 + and CD163 + macrophages were detected both in tumor nest and stroma, whereas only CD68 + macrophages were found in peritumoral stroma [18].

Further investigation is needed to evaluate the role and the prognostic value of this distribution in HNSCC.

Investigating CD47/SIRPα using antibody CD47

TAMs express other markers, such as the signal-regulatory protein-α (SIRPα). When SIRPα is downregulated, this marker contributes to the tumor promotion [50]. SIRPα is a ligand of CD47 receptor which is expressed by tumor cells [51]. The interaction between CD47 and SIRPα acts as a 'don't eat me' signal to inhibit the phagocytic activity of TAMs [30,50,52–54] and their interaction contribute also to cell migration [49]. The axis CD47/SIRPα is a crucial checkpoint in the TAMs activation.

In head and neck cancer, CD47 expression on tumor was studied in 74 OSCC by Sakakura et al [30]. The expression of CD47 was not significantly correlated with CD63 and CD68 expressions, but was correlated with poor OS (p = 0.015) by univariate analysis. CD47 could represent an independent prognostic factor. However, this correlation was not confirmed by multivariate regression analysis. The relationship between CD47-positive tumor cells and TAMs stays unclear. Further studies are needed in order to determine the relationship between CD47 marker and macrophages.

TAMs: a new therapeutic target

Various strategies and compounds modulating macrophage function and signaling have been investigated in the field of cancer research. All published preclinical and clinical studies about treatment modalities targeting TAMs are summarized in Table 3 and Table 4, respectively. Main therapeutic strategies aiming at modulating TAMs are summarized in Fig. 2.

Targeting macrophage recruitment and differentiation

Recruitment of TAMs is now well characterized [55]. CC chemokine ligand 2 (CCL-2) produced by tumor cells and tumor-associated cells shapes myeloid cells in the bone marrow and attracts them to the peripheral blood where they migrate to the tumor tissue to become immunosuppressive TAMs.

Sanford et al. have previously shown that the CCL-2/CCR-2 chemokine axis has a prognostic significance in human pancreatic ductal adenocarcinoma [56]. Blocking CCL-2 or its receptor CCR-2 reduced the number of tumor-infiltrating macrophages in preclinical studies [16,57,58]. The CCR-2 inhibitor PF-04136309 was tested in a phase Ib

Table 4
Summary of clinical studies targeting TAMs.

Strategy	Pathway	Drug	Tumor type	Outcome	Reference	Year	Type of study
Inhibiting TAM recruitment	CCL-2/CCR2	PF-04136309	Pancreatic	Tolerance/OR	Nywening et al.	2016	IB
Preventing TAM differentiation	COX-2	Celecoxib	NSCLC	No clinical benefit	Edelman et al.	2017	III
	IL-6	Siltuximab	Ovarian	Partial response	Coward et al.	2011	II
Blocking TAM activation	CSF-1R/CSF-1	PLX-3397	TGCT	OR	Tap et al.	2015	II
		PLX-3397	Glioma	Tolerance/no OR	Butowski et al.	2016	II
		AMG820	AST	Tolerance/no OR	Papadopoulos et al.	2017	I
		RG-7155	DTGCT	Tolerance/OR	Ries et al.	2014	I
Reprogramming TAMs to M1	G6PD	RRx-001	AST	Tolerance	Reid et al.	2015	I
		RRx-001	AST	Tolerance/OR	Oronsky et al.	2017	I and II
Activating M1		L-MTP-PE	Osteosarcoma	OR	Uehara et al.	2015	IV

Abbreviations: AST, advanced solid tumor; DTGCT, diffuse-type giant cell tumor; FFS, failure free survival; L-MTP-PE, liposomal mifamurtide; NSCLC, non-small-cell lung cancer; OR, objective response; PFS, progression free survival; PK, pharmacokinetic; TAMs, tumor-associated macrophages; TGCT, tenosynovial giant-cell tumor.

clinical trial in combination with chemotherapy for patients with pancreatic ductal adenocarcinoma [59]. This therapy was well tolerated. Treatment-related toxicity was comparable in both groups (FOLFIRINOX plus the CCR-2 inhibitor PF-04136309 versus FOLFIRINOX alone). Definitive interruption due to treatment-related toxicity was reported in 2 out of 39 patients (5.1%), and in 1 out of 6 patients (16.7%) in the FOLFIRINOX plus PF-04136309 versus the in FOLFIRINOX group respectively. Concerning treatment response, changes in tumor size from baseline by RECIST for all evaluable patients receiving FOLFIRINOX plus PF-04136309 (n = 33) reached a partial response in 16 out of 33 patients (48.5% [95% CI: 30.80 to 66.54]). This was significantly higher than the pre-specified expected partial response rate of 25% with FOLFIRINOX alone (p = 0.006).

Concerning myeloid cells/macrophage differentiation, a number of in vitro studies demonstrated the predominant role of prostaglandin E2 (PGE2), interleukin 6 (IL-6), and signal transducer and activator of transcription 3 (STAT3) activation loop. When monocytes or immature dendritic cells are exposed to tumor cells, their differentiation into M2 macrophages depends on IL-6, which acts in synergy with other factors. PGE2, IL-6, and STAT3 activation loop seems to be a good target to block the differentiation of macrophages. The inhibition of PGE2 production by blocking the *cyclo*-oxygénase 2 (COX-2) enzyme [60,61] and an anti-IL-6 antibody [62] were tested. In lung cancer, the inhibition of COX-2 by celecoxib could be beneficial in patients with tumors harboring moderate to high COX-2 expression, achieving a better OS and failure-free survival (p = 0.002 and p = 0.018 respectively) [61]. However, the recent phase III clinical trial did not confirm the clinical benefit of this therapeutic [63]. In ovarian cancer, in preclinical and clinical studies, IL-6 antibody can inhibit inflammatory cytokine production, tumor angiogenesis and TAMs infiltration [62]. Other clinical trials are in progress.

Targeting TAMs activation

CSR-1R

Playing a crucial role in tumor microenvironment and displaying protumoral activities, TAMs could represent a potential target for future anticancer therapies. CSF-1 and its receptor are currently the most studied TAMs-related targets.

The CSF-1R is a tyrosine kinase transmembrane receptor. CSF-1R is involved in survival, proliferation, differentiation, recruitment, and other functions of mononuclear phagocytes, particularly macrophages. CSF-1R and its ligands regulate development, morphology, survival, and functioning of TAMs [64–66]. Therefore, CSF-1 pathway seems to be an appropriate target for future anticancer treatments. The development of new CSF-1R/CSF-1 pathway inhibitors started with a few phase I/II clinical trials, without being yet investigated these types of therapies in head and neck carcinoma.

The two main possibilities to block the CSF-1R are an inhibition of the tyrosine kinase activity of the CSF-1R or targeting the CSF-1R by human monoclonal antibodies. The review of Peyraud et al. lists different clinical trials completed or in progress using such inhibitors in pigmented villonodular synovitis (PVNS) or solid tumors [19].

Regarding tyrosine kinase inhibitors, two agents have entered clinical development [19]: PLX-3397 and BLZ-945.

PLX-3397 (pexidartinib) was clinically investigated in two types of solid tumors (tenosynovial giant-cell tumor [67] and glioblastoma [68]). In tenosynovial giant-cell tumors, 83% of patients (95% CI, 67 to 98) had partial response or stable disease. In glioblastoma, no objective response was observed. This treatment seemed well tolerated with some side effects including asthenia, hair color changes, nausea, periorbital edema, rash, and decreased appetite.

BLZ-945 is also a selective inhibitor of CSF-1R [69,70]. The phase I/II studies exploring this agent are still in progress in several advanced solid tumors, including pancreatic cancer, triple-negative breast cancer, and recurrent glioblastoma.

Concerning monoclonal antibodies, FPA-008 (cabiralizumab), RG-7155, IMC-CS4, AMG-820, and PLX-73086 have entered clinical trials as well.

RG-7155 appears particularly promising. In contrast to other antibodies, RG-7155 blocks both ligand-dependent and -independent receptor activation [71]. In a study by Ries et al., RG-7155 depleted macrophages expressing CD163 and CSF-1 in vitro and in vivo [71]. In a phase I clinical trial with RG-7155, Ries et al. observed a clinical benefit in patients diagnosed with Diffuse-Type Giant Cell Tumors (Dt-GCT). In addition, they showed a decrease of TAMs and an increase of CD8/CD4 T cell ratio in other advanced solid tumors [71].

Papadopoulos et al. [72] studied the effect of the antibody AMG-820 in 25 patients with advanced solid tumors, who had relapsed or were refractory to standard treatment. One patient with a liver metastasis of a paraganglioma had a partial response with 40% reduction in tumor size and 6 patients had stable disease.

The conclusion of Peyraud et al. review was that recent clinical trials testing CSF-1R inhibitors as monotherapies have shown encouraging results in the management of PVNS but disappointing outcomes for the treatment of solid tumors [19]. Authors proposed to explore combination therapies, which could represent a more appropriate approach.

Targeting CD47/SIRPα axis

When CD47/SIRPα axis is inhibited, the incidence of TAMs in the tumor is decreased and their immune suppression is reversed [40]. Therefore CD47 inhibitors, bispecific antibodies [73] and other compounds [74], appear as an attractive approach for future immunotherapies. The most potent drugs blocking CD47 are monoclonal antibodies such as Hu5F9-G4 and CC-90002, or TTI-621, a soluble

SIRPα-Fc fusion protein (SIRPαFc). A number of phase I clinical trials in hematologic and solid cancers are ongoing (NCT02678338, NCT02641002, NCT02367196, and NCT02663518) [75–77].

Moreover, Yu's data suggested that CD47/SIRPα axis was attenuated by PD-1 blockade [40]. The efficient anti PD1/PD-L1 therapies could also modulate the TAMs by this axis.

Limiting TAMs immunosuppressive functions

The other effect of the CSF-1R inhibitors is to increase CD8 + T-cells and natural killer cells in tumors, which could antagonize immunosuppressive function of TAMs [70,71]. Moreover, Ries et al. demonstrated that targeting of CSF-1R resulted in a higher CD8/CD4 T-cell ratio in tumor lesions but these results were still not fully explained [71]. The immunostimulation induced by anti-CSF-1R provide rationale for future testing of various combinations of these inhibitors with currently available immunostimulatory therapies, such as sipuleucel-T and anti-CTLA-4 [78]. Based on this rationale, clinical trials assessing anti-CSF-1R agents in combination with immunotherapy are in progress [19].

Futhermore, Zhu et al. demonstrated synergistic effects of CSF-1R inhibitors and immunotherapy in pancreatic cancer models [79]. Indeed, the therapeutic effect of immunotherapy was limited by the induction of T-cell checkpoint molecules, including PD-L1 on tumor cells and CTLA-4 on T-cells. The addition of the anti-CSF-1R agent improved the efficacy of anti-PD- 1 and anti-CTLA-4 checkpoint immunotherapies. Therefore, CSF-1R inhibitors might enhance the efficacy of already used immunotherapies.

Reversing TAMs into antitumor macrophages

Another option for anticancer treatment is to reverse TAMs with M2 phenotype into M1 phenotype macrophages, which have an antitumoral function. Fully polarized M2 macrophages can be redirected in vitro towards the opposite functional phenotype after treatment with cytokines [80]

For example, forced expression of IRF-5 in TAMs, which have a M2 phenotype, switches them to M1 macrophages [21]. M1 cells highly express IRF-5, a transcription factor stimulating the production of type 1 interferon and repressing the production of IL-10.

The SHIP1 phosphatase was also shown to play a predominant role in reprogramming TAMs [81]. Pharmacological modulators of SHIP1 have been developed and are currently under evaluation [82]

RRx-001, a systemically non-toxic potent inhibitor of G6PD, was known to play a prominent role in TAMs conversion into M1 phenotype. RRx-001 was tested in Phase II clinical trials and displayed potent antitumor activity as well as its chemo- and radio-protective properties [83,84]. Red blood cells, which have assimilated RRx-001, are phagocytized by TAMs, which are changed into M1 macrophages.

Targeting NF-κB activation is another way to regulate the phenotype of macrophages [85]. This option was analyzed in the course of progression of oral preneoplasia and malignancies by Ye et al., who studied the SIRPα expressed on macrophages [86]. When SIRPα is down-regulated, particularly by the CD47 of the tumor cells, it could inhibit the phagocytosis ability of macrophages. On the other side, when the SIRPα is up-regulated, it could inhibit the activation of NF-κB and promote the antitumoral M1 macrophage reprogramming [86].

Activating antitumoral M1 macrophages

Finally, M1 macrophages and other antitumoral immune cells (e.g. Natural Killer cells) can be activated by immunotherapeutic drugs. One compound seems to be very interesting. The liposomal mifamurtide (L-MTP-PE) is a specific well-defined liposome-encapsulated formulation of the active pharmaceutical ingredient. The active substance, mifamurtide, is incorporated as an integral component of the liposomal bilayer. Mifamurtide is a fully synthetic lipophilic derivative of MDP (muramyl dipeptide), a naturally occurring component of bacterial cell, which enhances the immune activity [87].

The encapsulation of mifamurtide into liposomes has been shown in vitro to enhance the activation of murine macrophages and human monocytes by 100-fold compared with free MDP [88], and to decrease the drug toxicity [89].

The liposomes are selectively taken up by macrophages and progressively broken down within the cell. The tumoricidal activity of mifamurtide-activated macrophages is linked to both direct and indirect effects, including contact-mediated tumor cytotoxicity and release of tumoricidal factors [90]. These effects lead to an activation of M1 macrophages and to an indirect increase of interstitial natural killer cell activity [91], which have a critical role in the anti-tumor immunity. The L-MTP-PE has been approved in Europe for the treatment of advanced or metastatic osteosarcomas in combination with chemotherapy [92]. In the other tumors, only pre-clinical animals studies or pilot clinical studies were published in melanoma, angiosarcoma, ovarian and mammary carcinoma [93–97]. Results were not considered as conclusive in the majority of these studies (Table S1).

Combination with standard therapies

De Palma et al. proved that radiotherapy, DNA damage, cell death, and increased hypoxia in tumors lead to an increase in macrophage recruitment and promote tumor progression in animal models [98]. The increase of TAMs recruitment after local irradiation was also reported in prostate cancer [99]. In addition, Kioi et al. showed that the hypoxia-inducible factor-1 (HIF-1) pathway was stimulated by radiation-induced tumor hypoxia and that inhibiting HIF-1 could result in a decreased infiltration of myeloid cells in tumors [100].

Moreover, TAMs have been shown to blunt chemotherapy-induced antitumor responses by secreting chemoprotective factors such as MMP-9 and cathepsins [98,101]. This could explain that the inhibitors of CSF-1R have been shown to increase the efficacy of chemotherapy for pancreatic tumors [57] and lung cancer [102].

The combination of TAMs-targeting agents with standard therapies could potentiate and enhance tumor response to radiotherapy and/or chemotherapy. Therefore, in ongoing studies, CSF-1R inhibitors combined with the standard therapies (surgery, chemotherapy, and radiation) are evaluated.

An increasing number of studies evaluated possible correlation between the PD-1/PD-L1 axis and TAMs in various cancer types [103,104], including OSCC [40,105]. TAMs could express both PD-1 and PD-L1 markers. PD-1-positive TAMs show less phagocytosis, as compared to their PD-1 negative counterparts [106]. Therefore PD-1 inhibitors may increase macrophage phagocytosis and reduces tumor growth in a macrophage-dependent fashion [106]. Moreover, PD-1 inhibitors demonstrated efficacy in blocking the CD47/SIRPα axis [40].

On the other hand, PD-L1-positive TAMs have been reported to be localized at the interface between the tumor periphery and the surrounding inflammatory stroma. Therefore PD-L1 positive TAMs could create a PD-L1 immunoprotective "barrier" around the tumor nests and contribute to adaptive resistance [47].

Therefore, combining anti-PD-1/PD-L1 with drugs targeting TAMs warrants further investigations.

Conclusion

In OSCC, high density of TAMs is associated with a poor prognosis [15,18]. However, the prognostic value was sparsely studied in other primary sites of head and neck carcinoma such as laryngeal and hypopharyngeal carcinomas. Using only the CD68 marker without a specific marker of M2 macrophages, one study analyzed the macrophages in laryngeal carcinoma [25]. It would be interesting to evaluate the occurrence of TAMs in laryngeal or hypopharyngeal subtypes and the correlation between the density of TAMs and survival parameters.

Intriguingly, in colorectal cancer, high density of TAMs seems to be associated with a better OS in contrast to the majority of other solid tumors [15]. In addition, in the *meta*-analysis of Zhang et al., no significant correlation between high density of TAMs and tumor stage or OS was found in a subgroup of esophageal cancer patients [15]. Larynx and hypopharynx cancer belong to head and neck carcinoma, but they are not similar to OSCC, due to their localization and carcinogenesis [107,108]. Thus, it would be interesting to know if the results concerning TAMs in larynx and hypopharynx cancer are comparable to those in OSCC.

Moreover, in HPV-induced cervical cancer, the correlation between prognosis and the density of TAMs is unclear [109,110]. Only two studies compared the incidence of CD68 + cells between HPV + and HPV- head and neck squamous cell carcinomas [39,40]. These analyses were restricted to the CD68 status of tumors, therefore information on TAMs is lacking since no staining with CD163 was performed. Yu et al. did not find a correlation between CD68 or CD163 and HPV status [40]. However, a correlation between HPV status and macrophages was not the primary objective of their analysis. Hence, it would be interesting to further study the frequency of TAMs in HPV + and HPV- subgroups.

The mutual interaction between TAMs and cancer cells plays a crucial role in tumor progression. Therefore, inhibitors of TAMs seem to be a viable target for future drug investigations. Different strategies are used to exploit TAMs as a therapeutic target in cancer [111]. Currently, the most promising approaches include inhibitors of the CSF-1/CSF-1R and SIRPα/CD47 pathways. The SIRPα/CD47 axis seems to be an interesting molecular cascade with several options being tested for targeting thereof [73,74]. Moreover, the SIRPα/CD47 axis could be blocked by PD-1 inhibitors already used in clinical practice [40]. Recent clinical trials testing CSF-1R inhibitors used as single agent, unfortunately, showed disappointing outcomes in solid tumors. These unsatisfactory results of CSF-1R inhibitors were partly explained by the presence of GM-CSF inhibiting anti-CSF-1R-mediated cell death [69,71]. Combining CSF-1R inhibitors with other compounds such as immunotherapy, chemotherapy, or targeted therapies could represent the next step to investigate [19].

Further research activities aim at reprogramming TAMs into anti-tumoral M1 macrophages, decreasing thus protumoral versus anti-tumoral effects. In this regard, different targets have been tested including IRF-5, SHIP1 phosphatase, RRx-001, and SIRPα.

Various imaging modalities can be used to visualize TAMs. The development of fluorescent proteins allows subcellular-resolution molecular imaging of cell-cell interactions between tumor cells and TAMs [112,113]. In clinical practice, new imaging protocols revealing details on macrophage infiltration (e.g. ferumoxytol) can be considered in the future to observe TAMs activity by magnetic resonance or positron emission tomography [114,115].

In conclusion, TAMs appear to be an attractive target in head and neck carcinoma. Compounds aiming at modulating macrophage proprieties, along with TAMs inhibitors, are currently investigated alone or in combination with standard therapies in several solid tumors.This strategy represents an innovative anticancer approach in head and neck carcinoma.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.oraloncology.2018.10.040.

References

[1] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015;65:87–108. https://doi.org/10.3322/caac.21262.
[2] Howlader N, Noone A, Krapcho M. SEER Cancer Statistics Review, 1975–2014. National Cancer Inst 2017http://Seer.cancer.gov/Csr/1975_2014/.
[3] Lewis CE, Pollard JW. Distinct role of macrophages in different tumor microenvironments. Cancer Res 2006;66:605–12. https://doi.org/10.1158/0008-5472.CAN-05-4005.
[4] Galdiero MR, Garlanda C, Jaillon S, Marone G, Mantovani A. Tumor associated macrophages and neutrophils in tumor progression. J Cell Physiol 2013;228:1404–12. https://doi.org/10.1002/jcp.24260.
[5] Ruffell B, Affara NI, Coussens LM. Differential macrophage programming in the tumor microenvironment. Trends Immunol 2012;33:119–26. https://doi.org/10.1016/j.it.2011.12.001.
[6] Murray PJ, Allen JE, Biswas SK, Fisher EA, Gilroy DW, Goerdt S, et al. Macrophage activation and polarization: nomenclature and experimental guidelines. Immunity 2014;41:14–20. https://doi.org/10.1016/j.immuni.2014.06.008.
[7] Wynn TA, Chawla A, Pollard JW. Macrophage biology in development, homeostasis and disease. Nature 2013;496:445–55. https://doi.org/10.1038/nature12034.
[8] Biswas SK, Mantovani A. Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm. Nat Immunol 2010;11:889–96. https://doi.org/10.1038/ni.1937.
[9] Gordon S, Martinez FO. Alternative activation of macrophages: mechanism and functions. Immunity 2010;32:593–604. https://doi.org/10.1016/j.immuni.2010.05.007.
[10] Hoves S, Sutton VR, Haynes NM, Hawkins ED, Fernández Ruiz D, Baschuk N, et al. A critical role for granzymes in antigen cross-presentation through regulating phagocytosis of killed tumor cells. J Immunol Baltim Md 1950;2011(187):1166–75. https://doi.org/10.4049/jimmunol.1001670.
[11] Mantovani A, Marchesi F, Malesci A, Laghi L, Allavena P. Tumour-associated macrophages as treatment targets in oncology. Nat Rev Clin Oncol 2017;14:399–416. https://doi.org/10.1038/nrclinonc.2016.217.
[12] Mantovani A, Sica A. Macrophages, innate immunity and cancer: balance, tolerance, and diversity. Curr Opin Immunol 2010;22:231–7. https://doi.org/10.1016/j.coi.2010.01.009.
[13] Kurahara H, Shinchi H, Mataki Y, Maemura K, Noma H, Kubo F, et al. Significance of M2-polarized tumor-associated macrophage in pancreatic cancer. J Surg Res 2011;167:e211–9. https://doi.org/10.1016/j.jss.2009.05.026.
[14] Shabo I, Stål O, Olsson H, Doré S, Svanvik J. Breast cancer expression of CD163, a macrophage scavenger receptor, is related to early distant recurrence and reduced patient survival. Int J Cancer 2008;123:780–6. https://doi.org/10.1002/ijc.23527.
[15] Zhang Q, Liu L, Gong C, Shi H, Zeng Y, Wang X, et al. Prognostic significance of tumor-associated macrophages in solid tumor: a meta-analysis of the literature. PloS One 2012;7:e50946. https://doi.org/10.1371/journal.pone.0050946.
[16] Qian B-Z, Li J, Zhang H, Kitamura T, Zhang J, Campion LR, et al. CCL2 recruits inflammatory monocytes to facilitate breast tumor metastasis. Nature 2011;475:222–5. https://doi.org/10.1038/nature10138.
[17] Lee C-H, Liu S-Y, Chou K-C, Yeh C-T, Shiah S-G, Huang R-Y, et al. Tumor-associated macrophages promote oral cancer progression through activation of the Axl signaling pathway. Ann Surg Oncol 2014;21:1031–7. https://doi.org/10.1245/s10434-013-3400-0.
[18] Hu Y, He M-Y, Zhu L-F, Yang C-C, Zhou M-L, Wang Q, et al. Tumor-associated macrophages correlate with the clinicopathological features and poor outcomes via inducing epithelial to mesenchymal transition in oral squamous cell carcinoma. J Exp Clin Cancer Res CR 2016;35:12. https://doi.org/10.1186/s13046-015-0281-z.
[19] Peyraud F, Cousin S, Italiano A. CSF-1R inhibitor development: current clinical status. Curr Oncol Rep 2017;19:70. https://doi.org/10.1007/s11912-017-0634-1.
[20] Alves AM, Diel LF, Lamers ML. Macrophages and prognosis of oral squamous cell carcinoma: a systematic review. J Oral Pathol Med Off Publ Int Assoc Oral Pathol Am Acad Oral Pathol 2017. https://doi.org/10.1111/jop.12643.
[21] Heusinkveld M, van der Burg SH. Identification and manipulation of tumor associated macrophages in human cancers. J Transl Med 2011;9:216. https://doi.org/10.1186/1479-5876-9-216.
[22] Martin AW. Chapter 6 - Immunohistology of Non-Hodgkin Lymphoma. In: Dabbs DJ, editor. Diagn. Immunohistochem. Third Ed.Philadelphia: W.B. Saunders; 2011. p. 156–88. https://doi.org/10.1016/B978-1-4160-5766-6.00010-8.
[23] Naeim F, Nagesh Rao P, Song SX, Phan RT. Chapter 2 - Principles of Immunophenotyping. In: Naeim F, Nagesh Rao P, Song SX, Phan RT, editors. Atlas Hematop. Second Ed.Academic Press; 2018. p. 29–56. https://doi.org/10.1016/B978-0-12-809843-1.00002-4.
[24] Marcus B, Arenberg D, Lee J, Kleer C, Chepeha DB, Schmalbach CE, et al. Prognostic factors in oral cavity and oropharyngeal squamous cell carcinoma. Cancer 2004;101:2779–87. https://doi.org/10.1002/cncr.20701.
[25] Lin J-Y, Li X-Y, Tadashi N, Dong P. Clinical significance of tumor-associated macrophage infiltration in supraglottic laryngeal carcinoma. Chin J Cancer 2011;30:280–6.
[26] Soeda S, Nakamura N, Ozeki T, Nishiyama H, Hojo H, Yamada H, et al. Tumor-associated macrophages correlate with vascular space invasion and myometrial invasion in endometrial carcinoma. Gynecol Oncol 2008;109:122–8. https://doi.org/10.1016/j.ygyno.2007.12.033.

[27] Bagul N, Roy S, Ganjre A, Kathariya R, Meher A, Singh P. Quantitative assessment of tumor associated macrophages in head and neck squamous cell carcinoma using CD68 marker. An Immunohistochem Study J Clin Diagn Res JCDR 2016;10:ZC81-84. https://doi.org/10.7860/JCDR/2016/13924.7670.

[28] Fujii N, Shomori K, Shiomi T, Nakabayashi M, Takeda C, Ryoke K, et al. Cancer-associated fibroblasts and CD163-positive macrophages in oral squamous cell carcinoma: their clinicopathological and prognostic significance. J Oral Pathol Med Off Publ Int Assoc Oral Pathol Am Acad Oral Pathol 2012;41:444–51. https://doi.org/10.1111/j.1600-0714.2012.01127.x.

[29] He K-F, Zhang L, Huang C-F, Ma S-R, Wang Y-F, Wang W-M, et al. CD163+ tumor-associated macrophages correlated with poor prognosis and cancer stem cells in oral squamous cell carcinoma. BioMed Res Int 2014;2014:838632. https://doi.org/10.1155/2014/838632.

[30] Sakakura K, Takahashi H, Kaira K, Toyoda M, Murata T, Ohnishi H, et al. Relationship between tumor-associated macrophage subsets and CD47 expression in squamous cell carcinoma of the head and neck in the tumor microenvironment. Lab Investig J Tech Methods Pathol 2016;96:994–1003. https://doi.org/10.1038/labinvest.2016.70.

[31] Liu S-Y, Chang L-C, Pan L-F, Hung Y-J, Lee C-H, Shieh Y-S. Clinicopathologic significance of tumor cell-lined vessel and microenvironment in oral squamous cell carcinoma. Oral Oncol 2008;44:277–85. https://doi.org/10.1016/j.oraloncology.2007.02.007.

[32] Peng J, Ding T, Zheng L-M, Shao J-Y. Influence of tumor-associated macrophages on progression and prognosis of nasopharyngeal carcinoma. Ai Zheng Aizheng Chin J Cancer 2006;25:1340–5.

[33] Lee C-C, Lin J-C, Hwang W-L, Kuo Y-J, Chen H-K, Tai S-K, et al. Macrophage-secreted interleukin-35 regulates cancer cell plasticity to facilitate metastatic colonization. Nat Commun 2018;9:3763. https://doi.org/10.1038/s41467-018-06268-0.

[34] Ma J, Liu L, Che G, Yu N, Dai F, You Z. The M1 form of tumor-associated macrophages in non-small cell lung cancer is positively associated with survival time. BMC Cancer 2010;10:112. https://doi.org/10.1186/1471-2407-10-112.

[35] Ong S-M, Tan Y-C, Beretta O, Jiang D, Yeap W-H, Tai JJY, et al. Macrophages in human colorectal cancer are pro-inflammatory and prime T cells towards an anti-tumour type-1 inflammatory response. Eur J Immunol 2012;42:89–100. https://doi.org/10.1002/eji.201141825.

[36] 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. https://doi.org/10.1183/09031936.00065708.

[37] Mantovani A, Sica A, Locati M. New vistas on macrophage differentiation and activation. Eur J Immunol 2007;37:14–6. https://doi.org/10.1002/eji.200636910.

[38] Oguejiofor K, Galletta-Williams H, Dovedi SJ, Roberts DL, Stern PL, West CML. Distinct patterns of infiltrating CD8+ T cells in HPV+ and CD68 macrophages in HPV- oropharyngeal squamous cell carcinomas are associated with better clinical outcome but PD-L1 expression is not prognostic. Oncotarget 2017;8:14416–27. https://doi.org/10.18632/oncotarget.14796.

[39] Seminerio I, Kindt N, Descamps G, Bellier J, Lechien JR, Mat Q, et al. High infiltration of CD68+ macrophages is associated with poor prognoses of head and neck squamous cell carcinoma patients and is influenced by human papillomavirus. Oncotarget 2018;9:11046–59. https://doi.org/10.18632/oncotarget.24306.

[40] Yu G-T, Bu L-L, Huang C-F, Zhang W-F, Chen W-J, Gutkind JS, et al. PD-1 blockade attenuates immunosuppressive myeloid cells due to inhibition of CD47/SIRPα axis in HPV negative head and neck squamous cell carcinoma. Oncotarget 2015;6:42067–80. https://doi.org/10.18632/oncotarget.5955.

[41] Chen X-J, Han L-F, Wu X-G, Wei W-F, Wu L-F, Yi H-Y, et al. Clinical significance of CD163+ and CD68+ tumor-associated macrophages in high-risk HPV-related cervical cancer. J Cancer 2017;8:3868–75. https://doi.org/10.7150/jca.21444.

[42] Mantovani A, Romero P, Palucka AK, Marincola FM. Tumour immunity: effector response to tumour and role of the microenvironment. LancetLond Engl 2008;371:771–83. https://doi.org/10.1016/S0140-6736(08)60241-X.

[43] Bility MT, Cheng L, Zhang Z, Luan Y, Li F, Chi L, et al. Virus Infection and Immunopathogenesis in a Humanized Mouse Model: Induction of Human-Specific Liver Fibrosis and M2-Like Macrophages. PLoS Pathog 2014;10. https://doi.org/10.1371/journal.ppat.1004032.

[44] Deligeoroglou E, Giannouli A, Athanasopoulos N, Karountzos V, Vatopoulou A, Dimopoulos K, et al. HPV infection: immunological aspects and their utility in future therapy. Infect Dis Obstet Gynecol 2013;2013:540850https://doi.org/10.1155/2013/540850.

[45] Smola S. Immunopathogenesis of HPV-associated cancers and prospects for immunotherapy. Viruses 2017;9. https://doi.org/10.3390/v9090254.

[46] Lepique AP, Daghastanli KRP, Cuccovia IM, Villa LL. HPV16 tumor associated macrophages suppress antitumor T cell responses. Clin Cancer Res Off J Am Assoc Cancer Res 2009;15:4391–400. https://doi.org/10.1158/1078-0432.CCR-09-0489.

[47] Lyford-Pike S, Peng S, Young GD, Taube JM, Westra WH, Akpeng B, et al. Evidence for a role of the PD-1:PD-L1 pathway in immune resistance of HPV-associated head and neck squamous cell carcinoma. Cancer Res 2013;73:1733–41. https://doi.org/10.1158/0008-5472.CAN-12-2384.

[48] Scognamiglio T, Chen Y-T. Beyond the percentages of PD-L1-positive tumor cells: induced versus constitutive PD-L1 expression in primary and metastatic head and neck squamous cell carcinoma. Head Neck Pathol 2018;12:221–9. https://doi.org/10.1007/s12105-017-0857-3.

[49] Zhang Y, Sime W, Juhas M, Sjölander A. Crosstalk between colon cancer cells and macrophages via inflammatory mediators and CD47 promotes tumour cell migration. Eur J Cancer Oxf Engl 1990;2013(49):3320–34. https://doi.org/10.1016/j.ejca.2013.06.005.

[50] Oldenborg PA, Zheleznyak A, Fang YF, Lagenaur CF, Gresham HD, Lindberg FP. Role of CD47 as a marker of self on red blood cells. Science 2000;288:2051–4.

[51] Willingham SB, Volkmer J-P, Gentles AJ, Sahoo D, Dalerba P, Mitra SS, et al. The CD47-signal regulatory protein alpha (SIRPa) interaction is a therapeutic target for human solid tumors. Proc Natl Acad Sci U S A 2012;109:6662–7. https://doi.org/10.1073/pnas.1121623109.

[52] Brown EJ, Frazier WA. Integrin-associated protein (CD47) and its ligands. Trends Cell Biol 2001;11:130–5.

[53] Jaiswal S, Jamieson CHM, Pang WW, Park CY, Chao MP, Majeti R, et al. CD47 is upregulated on circulating hematopoietic stem cells and leukemia cells to avoid phagocytosis. Cell 2009;138:271–85. https://doi.org/10.1016/j.cell.2009.05.046.

[54] Majeti R, Chao MP, Alizadeh AA, Pang WW, Jaiswal S, Gibbs KD, et al. CD47 is an adverse prognostic factor and therapeutic antibody target on human acute myeloid leukemia stem cells. Cell 2009;138:286–99. https://doi.org/10.1016/j.cell.2009.05.045.

[55] Murdoch C, Giannoudis A, Lewis CE. Mechanisms regulating the recruitment of macrophages into hypoxic areas of tumors and other ischemic tissues. Blood 2004;104:2224–34. https://doi.org/10.1182/blood-2004-03-1109.

[56] Sanford DE, Belt BA, Panni RZ, Mayer A, Deshpande AD, Carpenter D, et al. Inflammatory monocyte mobilization decreases patient survival in pancreatic cancer: a role for targeting the CCL2/CCR2 axis. Clin Cancer Res Off J Am Assoc Cancer Res 2013;19:3404–15. https://doi.org/10.1158/1078-0432.CCR-13-0525.

[57] Mitchem JB, Brennan DJ, Knolhoff BL, Belt BA, Zhu Y, Sanford DE, et al. Targeting tumor-infiltrating macrophages decreases tumor-initiating cells, relieves immunosuppression, and improves chemotherapeutic responses. Cancer Res 2013;73:1128–41. https://doi.org/10.1158/0008-5472.CAN-12-2731.

[58] Zhao L, Lim SY, Gordon-Weeks AN, Tapmeier TT, Im JH, Cao Y, et al. Recruitment of a myeloid cell subset (CD11b/Gr1 mid) via CCL2/CCR2 promotes the development of colorectal cancer liver metastasis. Hepatol Baltim Md 2013;57:829–39. https://doi.org/10.1002/hep.26094.

[59] Nywening TM, Wang-Gillam A, Sanford DE, Belt BA, Panni RZ, Cusworth BM, et al. Phase 1b study targeting tumour associated macrophages with CCR2 inhibition plus FOLFIRINOX in locally advanced and borderline resectable pancreatic cancer. Lancet Oncol 2016;17:651–62. https://doi.org/10.1016/S1470-2045(16)00078-4.

[60] Brown JR, DuBois RN. COX-2: a molecular target for colorectal cancer prevention. J Clin Oncol Off J Am Soc Clin Oncol 2005;23:2840–55. https://doi.org/10.1200/JCO.2005.09.051.

[61] Edelman MJ, Watson D, Wang X, Morrison C, Kratzke RA, Jewell S, et al. Eicosanoid modulation in advanced lung cancer: cyclooxygenase-2 expression is a positive predictive factor for celecoxib + chemotherapy–cancer and Leukemia group B Trial 30203. J Clin Oncol Off J Am Soc Clin Oncol 2008;26:848–55. https://doi.org/10.1200/JCO.2007.13.8081.

[62] Coward J, Kulbe H, Chakravarty P, Leader D, Vassileva V, Leinster DA, et al. Interleukin-6 as a therapeutic target in human ovarian cancer. Clin Cancer Res Off J Am Assoc Cancer Res 2011;17:6083–96. https://doi.org/10.1158/1078-0432.CCR-11-0945.

[63] Edelman MJ, Wang X, Hodgson L, Cheney RT, Baggstrom MQ, Thomas SP, et al. Phase III randomized, placebo-controlled, double-blind trial of celecoxib in addition to standard chemotherapy for advanced non-small-cell lung cancer with cyclooxygenase-2 overexpression: CALGB 30801 (Alliance). J Clin Oncol Off J Am Soc Clin Oncol 2017;35:2184–92. https://doi.org/10.1200/JCO.2016.71.3743.

[64] Hume DA, MacDonald KPA. Therapeutic applications of macrophage colony-stimulating factor-1 (CSF-1) and antagonists of CSF-1 receptor (CSF-1R) signaling. Blood 2012;119:1810–20. https://doi.org/10.1182/blood-2011-09-379214.

[65] Hamilton JA. Colony-stimulating factors in inflammation and autoimmunity. Nat Rev Immunol 2008;8:533–44. https://doi.org/10.1038/nri2356.

[66] Chitu V, Stanley ER. Colony-stimulating factor-1 in immunity and inflammation. Curr Opin Immunol 2006;18:39–48. https://doi.org/10.1016/j.coi.2005.11.006.

[67] Tap WD, Wainberg ZA, Anthony SP, Ibrahim PN, Zhang C, Healey JH, et al. Structure-guided blockade of CSF1R kinase in tenosynovial giant-cell tumor. N Engl J Med 2015;373:428–37. https://doi.org/10.1056/NEJMoa1411366.

[68] Butowski N, Colman H, De Groot JF, Omuro AM, Nayak L, Wen PY, et al. Orally administered colony stimulating factor 1 receptor inhibitor PLX3397 in recurrent glioblastoma: an Ivy foundation early phase clinical trials consortium phase II study. Neuro-Oncol 2016;18:557–64. https://doi.org/10.1093/neuonc/nov245.

[69] Pyonteck SM, Akkari L, Schuhmacher AJ, Bowman RL, Sevenich L, Quail DF, et al. CSF-1R inhibition alters macrophage polarization and blocks glioma progression. Nat Med 2013;19:1264–72. https://doi.org/10.1038/nm.3337.

[70] Strachan DC, Ruffell B, Oei Y, Bissell MJ, Coussens LM, Pryer N, et al. CSF1R inhibition delays cervical and mammary tumor growth in murine models by attenuating the turnover of tumor-associated macrophages and enhancing infiltration by CD8+ T cells. Oncoimmunology 2013;2:e26968. https://doi.org/10.4161/onci.26968.

[71] Ries CH, Cannarile MA, Hoves S, Benz J, Wartha K, Runza V, et al. Targeting tumor-associated macrophages with anti-CSF-1R antibody reveals a strategy for cancer therapy. Cancer Cell 2014;25:846–59. https://doi.org/10.1016/j.ccr.2014.05.016.

[72] Papadopoulos KP, Gluck L, Martin LP, Olszanski AJ, Tolcher AW, Ngarmchamnanrith G, et al. First-in-human study of amg 820, a monoclonal anti-colony-stimulating factor 1 receptor antibody, in patients with advanced solid tumors. Clin Cancer Res Off J Am Assoc Cancer Res 2017;23:5703–10. https://doi.org/10.1158/1078-0432.CCR-16-3261.

[73] Dheilly E, Moine V, Broyer L, Salgado-Pires S, Johnson Z, Papaioannou A, et al. Selective blockade of the ubiquitous checkpoint receptor CD47 is enabled by dual-targeting bispecific antibodies. Mol Ther 2017;25:523–33. https://doi.org/10.

1016/j.ymthe.2016.11.006.
[74] Weiskopf K. Cancer immunotherapy targeting the CD47/SIRPα axis. Eur J Cancer Oxf Engl 1990;2017(76):100–9. https://doi.org/10.1016/j.ejca.2017.02.013.
[75] Liu J, Wang L, Zhao F, Tseng S, Narayanan C, Shura L, et al. Pre-clinical development of a humanized anti-CD47 antibody with anti-cancer therapeutic potential. PloS One 2015;10:e0137345. https://doi.org/10.1371/journal.pone.0137345.
[76] Matlung HL, Szilagyi K, Barclay NA, van den Berg TK. The CD47-SIRPα signaling axis as an innate immune checkpoint in cancer. Immunol Rev 2017;276:145–64. https://doi.org/10.1111/imr.12527.
[77] Zeng D, Sun Q, Chen A, Fan J, Yang X, Xu L, et al. A fully human anti-CD47 blocking antibody with therapeutic potential for cancer. Oncotarget 2016;7:83040–50. https://doi.org/10.18632/oncotarget.13349.
[78] Chen TH, Chen CH, Shyu JF, Wu CW, Lui WY, Liu JC. Distribution of the superficial temporal artery in the Chinese adult. Plast Reconstr Surg 1999;104:1276–9.
[79] Zhu Y, Knolhoff BL, Meyer MA, Nywening TM, West BL, Luo J, et al. CSF1/CSF1R blockade reprograms tumor-infiltrating macrophages and improves response to T-cell checkpoint immunotherapy in pancreatic cancer models. Cancer Res 2014;74:5057–69. https://doi.org/10.1158/0008-5472.CAN-13-3723.
[80] Stout RD, Jiang C, Matta B, Tietzel I, Watkins SK, Suttles J. Macrophages sequentially change their functional phenotype in response to changes in microenvironmental influences. J Immunol Baltim Md 1950;2005(175):342–9.
[81] Rauh MJ, Sly LM, Kalesnikoff J, Hughes MR, Cao L-P, Lam V, et al. The role of SHIP1 in macrophage programming and activation. Biochem Soc Trans 2004;32:785–8. https://doi.org/10.1042/BST0320785.
[82] Ong CJ, Ming-Lum A, Nodwell M, Ghanipour A, Yang L, Williams DE, et al. Small-molecule agonists of SHIP1 inhibit the phosphoinositide 3-kinase pathway in hematopoietic cells. Blood 2007;110:1942–9. https://doi.org/10.1182/blood-2007-03-079699.
[83] Oronsky B, Paulmurugan R, Foygel K, Scicinski J, Knox SJ, Peehl D, et al. RRx-001: a systemically non-toxic M2-to-M1 macrophage stimulating and prosensitizing agent in Phase II clinical trials. Expert Opin Investig Drugs 2017;26:109–19. https://doi.org/10.1080/13543784.2017.1268600.
[84] Reid T, Oronsky B, Scicinski J, Scribner CL, Knox SJ, Ning S, et al. Safety and activity of RRx-001 in patients with advanced cancer: a first-in-human, open-label, dose-escalation phase 1 study. Lancet Oncol 2015;16:1133–42. https://doi.org/10.1016/S1470-2045(15)00089-3.
[85] Li E, Xu Z, Zhao H, Sun Z, Wang L, Guo Z, et al. Macrophages promote benzopyrene-induced tumor transformation of human bronchial epithelial cells by activation of NF-κB and STAT3 signaling in a bionic airway chip culture and in animal models. Oncotarget 2015;6:8900–13. https://doi.org/10.18632/oncotarget.3561.
[86] Ye X, Zhang J, Lu R, Zhou G. Signal regulatory protein α associated with the progression of oral leukoplakia and oral squamous cell carcinoma regulates phenotype switch of macrophages. Oncotarget 2016;7:81305–21. https://doi.org/10.18632/oncotarget.12874.
[87] Sugimoto M, Germain RN, Chedid L, Benacerraf B. Enhancement of carrier-specific helper T cell function by the synthetic adjuvant, N-acetyl muramyl-L-alanyl-D-isoglutamine (MDP). J Immunol Baltim Md 1950;1978(120):980–2.
[88] Sone S, Mutsuura S, Ogawara M, Tsubura E. Potentiating effect of muramyl dipeptide and its lipophilic analog encapsulated in liposomes on tumor cell killing by human monocytes. J Immunol Baltim Md 1950;1984(132):2105–10.
[89] Fidler IJ, Brown NO, Hart IR. Species variability for toxicity of free and liposome-encapsulated muramyl peptides administered intravenously. J Biol Response Mod 1985;4:298–309.
[90] Nardin A, Lefebvre M-L, Labroquère K, Faure O, Abastado J-P. Liposomal muramyl tripeptide phosphatidylethanolamine: Targeting and activating macrophages for adjuvant treatment of osteosarcoma. Curr Cancer Drug Targets 2006;6:123–33.
[91] Talmadge JE, Schneider M, Collins M, Phillips H, Herberman RB, Wiltrout RH. Augmentation of NK cell activity in tissue specific sites by liposomes incorporating MTP-PE. J Immunol Baltim Md 1950;1985(135):1477–83.
[92] Uehara T, Fujiwara T, Takeda K, Kunisada T, Ozaki T, Udono H. Immunotherapy for bone and soft tissue sarcomas. BioMed Res Int 2015;2015.. https://doi.org/10.1155/2015/820813.
[93] Teske E, Rutteman GR, vd Ingh TS, van Noort R, Misdorp W. Liposome-encapsulated muramyl tripeptide phosphatidylethanolamine (L-MTP-PE): a randomized clinical trial in dogs with mammary carcinoma. Anticancer Res 1998;18:1015–9.
[94] Vail DM, MacEwen EG, Kurzman ID, Dubielzig RR, Helfand SC, Kisseberth WC, et al. Liposome-encapsulated muramyl tripeptide phosphatidylethanolamine adjuvant immunotherapy for splenic hemangiosarcoma in the dog: a randomized multi-institutional clinical trial. Clin Cancer Res Off J Am Assoc Cancer Res 1995;1:1165–70.
[95] MacEwen EG, Kurzman ID, Vail DM, Dubielzig RR, Everlith K, Madewell BR, et al. Adjuvant therapy for melanoma in dogs: results of randomized clinical trials using surgery, liposome-encapsulated muramyl tripeptide, and granulocyte macrophage colony-stimulating factor. Clin Cancer Res Off J Am Assoc Cancer Res 1999;5:4249–58.
[96] Klimp AH, De Vries EG, Scherphof GL, Daemen T. Chemo-immunotherapy of ovarian cancer in a murine tumour model. Anticancer Res 2000;20:2585–92.
[97] Gianan MA, Kleinerman ES. Liposomal muramyl tripeptide (CGP 19835A lipid) therapy for resectable melanoma in patients who were at high risk for relapse: an update. CancerBiother Radiopharm 1998;13:363–8. https://doi.org/10.1089/cbr.1998.13.363.
[98] De Palma M, Lewis CE. Macrophage regulation of tumor responses to anticancer therapies. Cancer Cell 2013;23:277–86. https://doi.org/10.1016/j.ccr.2013.02.013.
[99] Xu J, Escamilla J, Mok S, David J, Priceman S, West B, et al. CSF1R signaling blockade stanches tumor-infiltrating myeloid cells and improves the efficacy of radiotherapy in prostate cancer. Cancer Res 2013;73:2782–94. https://doi.org/10.1158/0008-5472.CAN-12-3981.
[100] Kioi M, Vogel H, Schultz G, Hoffman RM, Harsh GR, Brown JM. Inhibition of vasculogenesis, but not angiogenesis, prevents the recurrence of glioblastoma after irradiation in mice. J Clin Invest 2010;120:694–705. https://doi.org/10.1172/JCI40283.
[101] Shree T, Olson OC, Elie BT, Kester JC, Garfall AL, Simpson K, et al. Macrophages and cathepsin proteases blunt chemotherapeutic response in breast cancer. Genes Dev 2011;25:2465–79. https://doi.org/10.1101/gad.180331.111.
[102] Pass HI, Lavilla C, Canino C, Goparaju C, Preiss J, Noreen S, et al. Inhibition of the colony-stimulating-factor-1 receptor affects the resistance of lung cancer cells to cisplatin. Oncotarget 2016;7:56408–21. https://doi.org/10.18632/oncotarget.10895.
[103] Kostine M, Briaire-de Bruijn IH, Cleven AHG, Vervat C, Corver WE, Schilham MW, et al. Increased infiltration of M2-macrophages, T-cells and PD-L1 expression in high grade leiomyosarcomas supports immunotherapeutic strategies. Oncoimmunology 2018;7:e1386828. https://doi.org/10.1080/2162402X.2017.1386828.
[104] Chen J, Li G, Meng H, Fan Y, Song Y, Wang S, et al. Upregulation of B7–H1 expression is associated with macrophage infiltration in hepatocellular carcinomas. CancerImmunol Immunother CII 2012;61:101–8. https://doi.org/10.1007/s00262-011-1094-3.
[105] Mattox AK, Lee J, Westra WH, Pierce RH, Ghossein R, Faquin WC, et al. PD-1 expression in head and neck squamous cell carcinomas derives primarily from functionally anergic CD4+ TILs in the presence of PD-L1+ TAMs. Cancer Res 2017;77:6365–74. https://doi.org/10.1158/0008-5472.CAN-16-3453.
[106] Gordon SR, Maute RL, Dulken BW, Hutter G, George BM, McCracken MN, et al. PD-1 expression by tumour-associated macrophages inhibits phagocytosis and tumour immunity. Nature 2017;545:495–9. https://doi.org/10.1038/nature22396.
[107] Shiga K, Ogawa T, Katagiri K, Yoshida F, Tateda M, Matsuura K, et al. Differences between oral cancer and cancers of the pharynx and larynx on a molecular level. Oncol Lett 2012;3:238–43. https://doi.org/10.3892/ol.2011.451.
[108] Shiga K, Matsuura K, Tateda M, Saijo S, Ogawa T, Miyagi T, et al. Allelic loss correlated with tissue specificity in head and neck squamous cell carcinomas and the clinical features of patients. Tohoku J Exp Med 2004;204:163–72.
[109] Zijlmans HJMAA, Fleuren GJ, Baelde HJ, Eilers PHC, 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. https://doi.org/10.1002/cncr.22428.
[110] Hammes LS, Tekmal RR, Naud P, Edelweiss MI, Kirma N, Valente PT, et al. Macrophages, inflammation and risk of cervical intraepithelial neoplasia (CIN) progression–clinicopathological correlation. Gynecol Oncol 2007;105:157–65. https://doi.org/10.1016/j.ygyno.2006.11.023.
[111] Yang L, Zhang Y. Tumor-associated macrophages, potential targets for cancer treatment. Biomark Res 2017;5:25. https://doi.org/10.1186/s40364-017-0106-7.
[112] Yamauchi K, Tome Y, Yamamoto N, Hayashi K, Kimura H, Tsuchiya H, et al. Color-coded real-time subcellular fluorescence imaging of the interaction between cancer and host cells in live mice. Anticancer Res 2012;32:39–43.
[113] Dovas A, Patsialou A, Harney AS, Condeelis J, Cox D. Imaging interactions between macrophages and tumour cells that are involved in metastasis in vivo and in vitro. J Microsc 2013;251:261–9. https://doi.org/10.1111/j.1365-2818.2012.03667.x.
[114] Tassa C, Shaw SY, Weissleder R. Dextran-coated iron oxide nanoparticles: a versatile platform for targeted molecular imaging, molecular diagnostics, and therapy. Acc Chem Res 2011;44:842–52. https://doi.org/10.1021/ar200084x.
[115] Weissleder R, Nahrendorf M, Pittet MJ. Imaging macrophages with nanoparticles. Nat Mater 2014;13:125–38. https://doi.org/10.1038/nmat3780.