

# Cancer Letters

## Immunomodulatory potential of Toll Like Receptor 4 in Triple Negative breast cancer

--Manuscript Draft--

<b>Manuscript Number:</b>	
<b>Article Type:</b>	Original Research Article
<b>Keywords:</b>	Triple negative breast cancer; Pattern recognition receptor; Toll Like Receptor 4; Immunomodulation; IFNY
<b>Corresponding Author:</b>	Nupur Mukherjee, PhD ICMR - National Institute for Research in Reproductive and Child Health Mumbai, Maharashtra INDIA
<b>First Author:</b>	Mayur Jondhale, MSc
<b>Order of Authors:</b>	Mayur Jondhale, MSc Rushigandha Salunke, MSc Elveera Saldhana, MSc Amit Singh, PhD Bipin Bandre, MSc Vainav Patel, PhD Shalaka Joshi, MBBS, DNB, MS Ayushi Sahay, MD Pathology Pratik Chandrani, PhD Nupur Mukherjee, PhD
<b>Abstract:</b>	<p>Background</p> <p>Triple Negative Breast Cancer (TNBC) is a hormone-receptor-negative (ER/PR/Her2)-subtype of breast cancer which has an immunogenic character and shows extensive molecular heterogeneity. TLRs (Toll like receptors) are a class of pattern recognition receptors regulating innate immune response. In the present study, we want to envisage the immunomodulatory role of TLR4 in TNBC.</p> <p>Method</p> <p>The TLR expression was assessed in TNBC tumors using qPCR/flowcytometry/multiplex immunofluorescence imaging. Also, the same was compared using TNBC-TCGA datasets. Also, the correlation of TLR expression with distinct cytokines was compared in TNBC tumors using ELISA and by analysis of TNBC-TCGA transcriptome datasets.</p> <p>Result</p> <p>A variable pattern of TLR expression (TLR 3/4/6/9) was observed in TNBC tumors with comparably higher expression in TIL (Tumor-infiltrating lymphocyte)-enriched TNBC tumors than TIL-low tumors. Interestingly, TLR4 expression was observed to be higher in tumor cells (CDH1+) compared to that in stromal cells (CDH1-) within TNBC tumors. The TLR4 expression was found to significantly correlate with IL10/IFNY cytokine expression in TNBC tumors. Analysis of TNBC-TCGA datasets predicted longer survival probability in TIL-enriched TNBC patients with high TLR 4 and IFNY expression.</p> <p>Conclusion</p> <p>This study suggests that targeting the TLR4 and IFNY signaling could be a potential therapeutic target in TNBC.</p>

<b>Suggested Reviewers:</b>	<p>Denis Collins, PhD National Institute for Cellular Biotechnology <a href="mailto:denis.collins@dcu.ie">denis.collins@dcu.ie</a> Dr Collins is a translational research scientist with expertise in Cancer Biotherapeutics. He has recent publications on development of immunotherapeutic and chemotherapy strategies in breast cancer.</p> <p>Max Kullberg, PhD Asst Prof, University of Alaska Anchorage <a href="mailto:mpkullberg@alaska.edu.in">mpkullberg@alaska.edu.in</a> Dr Kullberg has peer review publications highlighting his expertise in medical therapeutics in cancer</p>
-----------------------------	--



## Review

# Targeting molecular cross-talk between tumor cells and tumor associated macrophage as therapeutic strategy in triple negative breast cancer

Anusha Shettigar<sup>1</sup>, Rushigandha Salunke<sup>1</sup>, Deepak Modi, Nupur Mukherjee\*

*Department of Molecular and Cellular Biology, National Institute for Research in Reproductive and Child Health, Mumbai, India*

## ARTICLE INFO

**Keywords:**

Triple-negative breast cancer  
Tumor-associated macrophages  
Molecular cross-talk  
Therapeutic potential

## ABSTRACT

Triple-negative Breast cancer (TNBC) is a subtype of breast cancer (BC) that lacks expression for ER/PR/Her2 receptors and is associated with aggressive disease pathogenesis and the worst prognosis among other subtypes of BC. Accumulating evidence-based studies indicate the high immunogenic ability of TNBC tumors and the applicability of immunotherapeutic strategies to overcome therapy resistance and tumor recurrence in TNBC patients. However, not all TNBC patients respond equally well to current immunotherapies that mainly target the adaptive immune system for tumor rejection. Recent studies are contemplating the efficacy of tumor-associated macrophage (TAM) targeted therapies since these subpopulations of cells comprise one of the major components of tumor-infiltrating immune cells (TIIs) in the TNBC tumor microenvironment (TME) and play an essential role in priming the adaptive immune response mediators towards both antitumorigenic and pro-tumorigenic response facilitated by intercellular cross-talk between tumor cells and TAM populations present within TNBC-TME. The present review discusses these molecular mechanisms and their consequence on the progression of TNBC tumors. Also, the therapeutic strategies targeting candidate genes/pathways involved in molecular cross-talk between TAM-TNBC cells and their impact on the development and progression of TNBC tumors are also discussed.

## 1. Introduction

### 1.1. Triple negative breast cancer

Triple negative breast cancer (TNBC), a subtype of breast cancer (BC) that lacks expression for ER/PR/Her2 is associated with more aggressive disease pathogenesis and associated with poor prognosis [1]. This subtype of breast cancer is insensitive to endocrine therapy or molecular targeted therapies. Chemotherapy still remains the mainstay systemic treatment and is frequently associated with poor chemotherapy response and tumor recurrence [1,2].

Accumulating studies indicate that the tumor immune microenvironment significantly contributes to tumor progression, overall survival, and prognosis of TNBC patients [3]. Compared to hormone receptor positive subtypes of breast cancer, the TNBC tumor shows remarkably elevated immunogenicity due to high immune cell infiltration, genomic instability, PD-L1 expression, and high mutation rate which makes immunotherapy effective for the treatment of TNBC patients [4,5]. The successful application of immune checkpoint inhibitors (ICIs) has

highlighted the promising role of immunotherapy in treating TNBC patients [6]. Currently, the immunotherapeutic strategies are mostly T cell centric affecting the adaptive immune responses in the TNBC tumor microenvironment (TME) [7,8]. However multiple studies have shown only limited efficacy of such strategies across different TNBC patients [6,9,10]. Therefore, alternate immunotherapeutic strategies are warranted for treating TNBC patients [11].

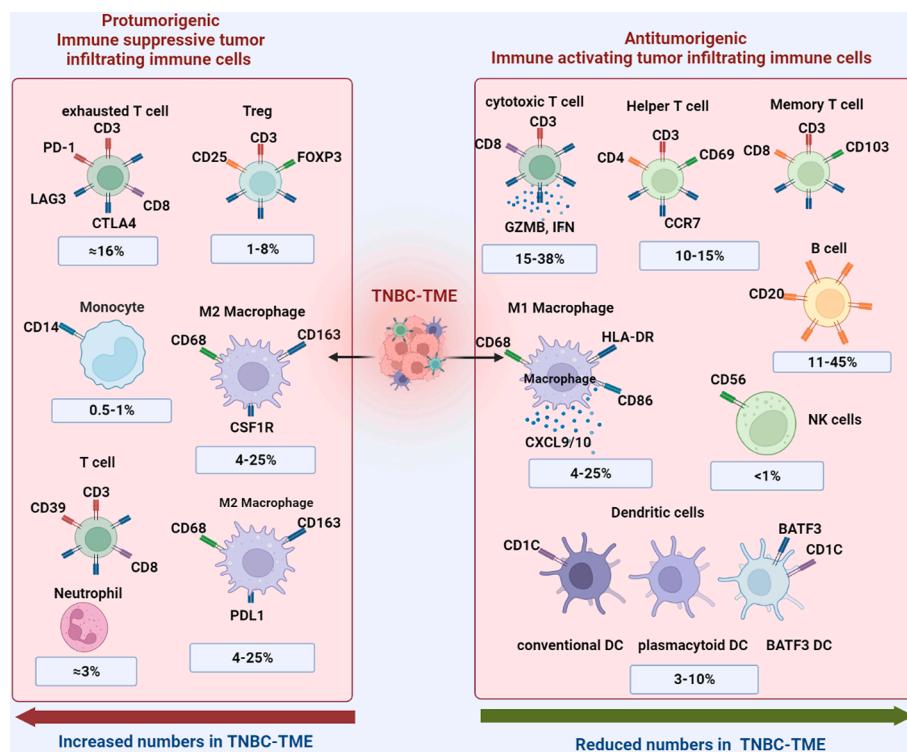
Recently several studies have highlighted the importance of the activation of innate immune responses in addition to adaptive immune responses in the pathogenesis of TNBC [12]. A number of studies have attempted to evaluate therapeutic potential of targeting distinct innate immune cells (dendritic cells, monocytes/macrophages, natural killer cells, and neutrophils) as a potential therapeutic strategy for treating TNBC patients [13–16].

Interestingly amongst the infiltrating immune cells, the tumor associated macrophages (TAMs) constitute a major fraction of the TNBC TME [17]. TAMs are innate immune cells of myeloid lineage that are either derived from circulating monocytes that infiltrate the tumors via blood or from tissue resident macrophages that migrate from the

\* Corresponding author at: Department of Molecular and Cellular Biology, ICMR-National Institute for Research on Reproductive Health, JM Street, Parel, Mumbai, India.

E-mail address: [mukherjeen@nirch.res.in](mailto:mukherjeen@nirch.res.in) (N. Mukherjee).

<sup>1</sup> The authors have contributed equally.



**Fig. 1.** TILs infiltrating TNBC tumors regulating protumorigenic/antitumorigenic responses in tumor TME. References: [20,22,23,25,26,30,57,66,69,70,74,78-90].

surrounding tissue [14]. Certain subsets of TAMs have been frequently associated with protumorigenic responses in TNBC via induction of immunosuppression, invasion, angiogenesis, metastasis, and chemotherapeutic/ drug resistance [14,18-20].

Multiple signalling cues received from distinct cell types present in the tumor TME are known to influence the recruitment and immuno-modulatory function of TAMs present in TNBC TME. A lot of emphasis has been given to the role of interactive molecular cross-talk between tumor cells and TAMs present in TNBC TME to understand its subsequent consequence on the pathogenesis of TNBC [21-23]. Some of these studies have indicated that therapies targeting tumor cells and TAM cross-talk could be promising therapeutic strategies for treating TNBC [24-26].

The present review delineates these molecular mechanisms involved in the cross-talk of tumor cells and TAMs within the TME, its impact on the TNBC tumor progression, and how this knowledge will further help to develop better therapeutic strategies for the treatment of TNBC patients.

## 2. Discussion

### 2.1. Composition of tumor infiltrating immune cells in TNBC tumors

Accumulating evidence suggests that the presence of tumor-infiltrating immune cells (TIIs) within the TNBC tumor can regulate both protumorigenic and antitumorigenic responses (Fig. 1) [27]. The composition of TIIs includes both innate and adaptive immune response mediators such as Monocytes, Macrophages, Dendritic cells, NK cells, Neutrophils, B cells, and T cells. (Fig. 1).

The subpopulation of immune cells infiltrating TNBC tumor includes (i) CD3 + CD8 + T-cells (10–38%), CD3 + CD4 + CCR7 + T-cells (10–34%), CD8 + CD103 + memory T cells, CD20 + B cells (11–45%) constituting the adaptive immune response involved in tumor clearance (ii) PD-1+/ CTLA-4+/LAG3+/TIGIT + T cells (~16-30%), CD4+/FOXP3 + Tregs (1–8%), constituting the adaptive immune system resulting in immune suppression and (iii) macrophages (M2-type

macrophage and M1-type macrophage) (4–50%), different subtypes of dendritic cells (cDC/pDC/BATF3 + DCs) (3–10%), neutrophils (3%), NK cells (<1%), CD14 + myeloid cells (0.5–1.0%) mediating innate immune response promoting both protumorigenic and antitumorigenic response in tumor-TME [17,28-31].

Interestingly, in TNBC tumors, monocytes/TAMs constitute a major fraction of tumor-infiltrating immune cells [17,32] that significantly alter the tumor progression and chemotherapy/immunotherapy response [32 33].

### 2.2. Tumor associated macrophages (TAM) in TNBC

#### 2.2.1. Subtypes of TAMs

The TAMs, are an important class of innate immune response mediators that have been associated with protumorigenic and antitumorigenic responses. The TAMs are known to contribute to the induction of antitumor immunity via phagocytosis of tumor cells and the presentation of tumor-specific antigens for induction of adaptive immune response [34]. Depending on the signaling cues received in different tissues, macrophages can undergo differentiation into two broad subtypes, also known as polarization states: classical M1 type macrophages (M1) or alternatively activated M2 type macrophages (M2) [32,35]. The M1 macrophages are known to express high levels of proinflammatory cytokines, such as INF $\gamma$  and TNF $\alpha$ , and are associated with activation of type 1 helper T cells (Th1) response leading to tumor growth inhibition [36]. While alternatively activated M2-macrophages are known to promote tumorigenesis by diverse mechanisms. This includes secretion of anti-inflammatory cytokines, angiogenic factors, reduction of effector functions of tumor infiltrating lymphocytes (TILs), and recruitment of regulatory T cells (Treg) in tumor tissue [35,36]. With the advent of single-cell omics, a further sub-classification of TAMs is now being proposed to describe the molecular heterogeneity in TAM subpopulations, reviewed in detail elsewhere by Ma et al [37]. Distinct sets of markers are expressed by M1- and M2- TAMs. The CD80 and CD86 expressed on M1-type macrophages have been reported to have the potential to co-stimulate T cell proliferation, IL-2, and IFN- $\gamma$

**Table 1**

. Prognostic relevance of M1 and M2 macrophages (TAMs) in TNBC patients.

TAM/other TIL subpopulations	Method of detection	Sample size	Observation	Study
CD68+/CD163 + TAM	IHC	N = 9 TNBC tumor	<b>Higher numbers in TNBC vs other BC subtypes</b> CD68 + CD163 + TAMs in Tumor stroma were significantly high in TNBC cases compared to HR + cases ( $p = 0.017$ )	[41]
CD68 + CD163 + TAM	IHC	57 TNBC tumor	<b>Higher numbers in TNBC vs other BC subtypes</b> Density of CD163-positive TAMs is significantly higher in both tumor nest and tumor stromal compartments in the TNBC subtype than in HR + subtypes of BC	[42]
CD68 + CD163 + TAM	Multiplexed quantitative IF	N = 160 TNBC tumors	<b>Improved survival</b> High CD163 + TAMs associated with improved Overall survival in TNBC patients ( $P < 0.04$ )	[43]
CD68 + PD-L1 + stromal TAMs	Multiplexed IF	N = 244 TNBC	<b>Improved survival</b> High CD68 + PD-L1 + stromal TAM counts associated with improved prognosis for OS ( $p = 0.030$ ) and BCSS ( $p = 0.018$ ) in TNBC patients	[44]
iNOS + M1 TAM, CD163 + M2-TAM, CD14 + monocytes	TNBC transcriptome or IHC	N = 770 TNBC	<b>M1<sup>high</sup> M2<sup>low</sup> Mono<sup>low</sup>: better prognosis</b> M1 <sup>high</sup> M2 <sup>low</sup> Mono <sup>low</sup> immune phenotype contributed to better 5-year overall survival and disease-free survival of TNBC patients	[45]
CXCL10, IL1B, and TNF + TAMs	TNBC transcriptome datasets	299 TNBC & 1605 non-TNBC patients	<b>Higher expression in TNBC vs other BC subtypes</b>	[46]
CD68 + TAM	IHC	287 TNBC tumor	<b>Unfavourable prognosis</b>	[47]
CD68 + CD163 + TAM	IHC	N = 200 BLBC	<ul style="list-style-type: none"> <li>• High CD68 + TAM infiltration in 39% of TNBC tumors (103/287)</li> <li>• High CD68 + TAMs associated with a higher risk of distant metastases and locoregional recurrence</li> <li>• Higher association of CD68 + TAMs with lympho-vascular invasion</li> </ul> <b>Unfavourable prognosis</b>	[48]
CD68/CD163 + TAM	IHC	107 TNBC tumor	<ul style="list-style-type: none"> <li>• Infiltration of CD163 + and CD68 + macrophages in tumor stroma associated with RFS and OS</li> <li>• Increased stromal infiltration of CD68 + or CD163 + TAMs correlated with larger tumor size, higher histological grade, and higher risk of recurrence and mortality</li> </ul> <b>Unfavourable prognosis</b>	[49]
CD163 + TAM	IHC	N = 91 treatment-naïve TNBC tumor	<b>Unfavorable prognosis</b> Patients with high infiltration of CD163 + TAMs and who failed to achieve pathological complete response (pCR) post-neo-adjuvant chemotherapy had significantly poor OS and RFS	[50]
CD163 + TAM and CDH1 expression	IHC	N = 287 TNBC tumor	<b>Unfavourable prognosis</b> Infiltration of CD163 + TAMs and loss in expression of EMT marker, CDH1 (E-cadherin) significantly associated with recurrence ( $P < 0.001$ ), and poor OS and DFS ( $P < 0.05$ )	[51]

HR: Hormone receptor; TNBC: Triple-negative breast cancer; BC: Breast cancer; BLBC: basal-like BC; TAM: Tumor-associated macrophage; OS, RFS, BCSS: Overall survival, recurrence-free survival and breast cancer-specific survival respectively; IHC: Immunohistochemistry; EMT: Epithelial to mesenchymal transition; IF: Immunofluorescence imaging; TAM: Tumor-associated macrophage; NACT: Neo-adjuvant Chemotherapy treatment.

production [38]. While CD163 transmembrane receptor, expressed on M2-type macrophages is known to be involved in functions like apoptotic cell sequestration, clearance, and inactivation of pro-inflammatory cytokines [39]. The CD206 transmembrane receptor also expressed on M2-type macrophages, is known to be associated with phagocytosis of mannosylated glycoproteins, or receptor-mediated antigen presentation [39]. The stabilin-1, an intracellular sorting receptor, frequently expressed in M2-type TAMs is involved in regulating intracellular trafficking pathways and has been shown to be up-regulated by IL-4 [40].

### 2.2.2. Research status of TAMs in TNBC

A number of recent studies have implicated TAMs in the process of TNBC development and metastasis [32]. Compared to other subtypes of breast cancer higher density of TAMs have been reported in TNBC-TME [41,42]. Distinct subpopulations of TAMs in TNBC-TME have correlated with varied clinical outcomes of TNBC patients (Table 1). In a study by Pelekanou et al [43], a high pro-inflammatory M1-like TAM profile within TNBC tumors was found to be associated with improved survival of TNBC patients. Similarly correlated high M1-like TAM, low M2-TAM, and low monocyte signatures with better overall survival of TNBC patients [44]. While in another study by Wang et al [45] high CD68 + PD-L1 + stromal TAMs were associated with prolonged survival of TNBC

patients.

Interestingly, preclinical studies indicate that in TNBC tumors, both M1 and M2 macrophage phenotypes are induced, which influence tumor pathogenesis and prognosis [46]. For instance, the presence of high numbers of CD68 + TAMs in TNBC-TME has been associated with unfavourable prognosis (higher risk of distant metastasis and locoregional recurrence and higher lymphovascular invasion) of TNBC patients [47,48]. Also, high numbers of immunosuppressive M2-TAMs in tumor-TME have been frequently associated with the induction of tumor progression and poor prognosis of TNBC patients. Multiple studies have correlated the presence of immunosuppressive CD163 + TAMs in TNBC tumors with shorter survival and chemoresistance in TNBC patients [48-53]. Similarly, patients insensitive to immunotherapy involving PD-1 blocking antibodies showed a high density of CD163 + TAMs in TNBC tumors [54].

Distinct subpopulations of TAMs are known to influence protumorigenic effects via their cross-communication with distinct cell types present in TNBC-TME like tumor cells, immune cells, and other stromal cell components, thereby impacting their function and overall pathogenesis of TNBC [36,47,48]. For instance, high CD163 + M2 TAMs have been frequently correlated with a significant reduction in cytotoxic T cell numbers in TNBC tumors [49]. In addition to its interaction with immune cells, the molecular cross-talk between TAMs and TNBC tumor

**Table 2a**

. Molecular mechanisms activated in TAMs in response to cross-talk with TNBC tumor cells.

Molecular events in TAMs (In response to cross-talk with TNBC cells)	Candidate genes involved	Impact on TAMs and TNBC Pathogenesis	Reference
<b>Modulations in cytokines and soluble molecules in TAMs</b>			
Upregulation of cytokines/ chemokines/ growth factors:	CXCL7, MCP-1/3, OPG, IL-1 $\beta$ , LIF, MIG, IL-5, CCL17, IL10, CXCL10, IL-8, CCL2, CCL5, AREG, EGF, S100A4, IGF-1, PDGFB, CCL7, CSF2, IL7, CXCR4, CCL19, IFNG, OPN	<ul style="list-style-type: none"> <li>Polarization of monocytes to M1 and M2 macrophage</li> <li>Increased migration of TAMs</li> <li>Inflammasome activation in TAMs</li> <li>Induction of EMT and tumor metastasis</li> <li>Tumor growth and progression</li> </ul>	[2,57-59]
CCL5-driven TAM reprogramming	CCL7, CSF2, IL-21R, IL7, CXCR4, CCL19, IFNG, OPN	<ul style="list-style-type: none"> <li>CCL5 mediated TNBC-TAM crosstalk-driven tumor metastasis</li> <li>CCL5 regulated recruitment of M1 macrophages, CD8 T cells, CD4 activated T cells, NK activated cells in TNBC-TME</li> </ul>	[59]
Activation of S100A4-driven response:	S100A4, ERK1/2, CD206, CXCL10, IL-8, CCL2, and CCL5	<ul style="list-style-type: none"> <li>Polarization of monocytes to M1 and M2 macrophage</li> <li>Increased secretion of cytokines and chemokines by tumor cell-educated monocytes</li> <li>Fibroblast activation and lung metastasis (ERK1/2 pathway mediated)</li> <li>Increased proliferation, migration, chemoresistance of TNBC cells</li> <li>Breast cancer progression</li> </ul>	[58,60]
IL-1 $\beta$ secretion upregulation	IL-1 $\beta$	<ul style="list-style-type: none"> <li>Polarization and Differentiation of macrophage</li> </ul>	[61]
<b>Modulations in Signalling pathways in TAMs</b>			
Upregulation of genes associated with inflammatory response and tumorigenesis:	ECSCR, ANGPTL4, ITGB4, WISP1, NODAL, DLL4, TGF $\alpha$ , Oncostatin M, FOXQ1, ABCA4, CCL17, IQGAP3, PLAUR, PSMB9	<ul style="list-style-type: none"> <li>TGF-<math>\beta</math>1 secretion from M2-TAMs</li> <li>Increased EMT and cancer stemness in BRCA1-IRIS-overexpressing TNBC cells through TGF-<math>\beta</math>1/T<math>\beta</math>RI/II/AKT mediated cross-talk between M2-TAM and TNBC cells</li> <li>Enrichment of M2-TAMs in TNBC tumors over-expressing BRCA1-IRIS</li> <li>Increased tumor metastasis</li> </ul>	[2]
Activation of SMAD5, p65/NF- $\kappa$ B and/or ERK signalling (via BRCA1-IRIS/AKT/CSF2 signalling in TNBC cells)	CSF2R, SMAD5, p65/NF- $\kappa$ B, ERK, TGF- $\beta$ 1	<ul style="list-style-type: none"> <li>Reduced CD8 + T cells in TNBC-TME</li> <li>Tumor growth and metastasis</li> <li>Activation of cGAS/STING pathway regulated type I and II IFN response in TAMs educated with TNBC tumor/cell lines derived CSF1-loaded-EVs</li> <li>Macrophage differentiation</li> <li>Induction of T cells (memory/effector) and NK cells in TNBC-TME and activation of antitumor immune response</li> <li>Prolonged patient survival</li> <li>Up regulation of TNF-<math>\alpha</math>, IL-6, Nos2, and IL-10 cytokines via modulation of the Soc3/NF-<math>\kappa</math>B pathway in host myeloid cells</li> <li>Enhanced metastasis</li> </ul>	[62]
Activation of JAK2 signal and upregulated PDL-1 expression (in CD169 + TAMs)	G-CSF regulated JAK2 /STAT3/PDL1	<ul style="list-style-type: none"> <li>In response to G-CSF secreted by 4 T1 TNBC cells, increased number of immunesuppressive PDL1 + CD169 + TAMs via activation of JAK2/STAT3 signaling</li> <li>Reduced CD8 + T cells in TNBC-TME</li> <li>Tumor growth and metastasis</li> </ul>	[63]
Activation of cGAS/STING pathway and IFN response mechanisms (in response TNBC cell released CSF-1 containing EVs)	CSF1R(CD115), STING, cGAS, cGAMP, IRF7, CXCL9, CXCL10, ISG	<ul style="list-style-type: none"> <li>Activation of cGAS/STING pathway regulated type I and II IFN response in TAMs educated with TNBC tumor/cell lines derived CSF1-loaded-EVs</li> <li>Macrophage differentiation</li> <li>Induction of T cells (memory/effector) and NK cells in TNBC-TME and activation of antitumor immune response</li> <li>Prolonged patient survival</li> <li>Up regulation of TNF-<math>\alpha</math>, IL-6, Nos2, and IL-10 cytokines via modulation of the Soc3/NF-<math>\kappa</math>B pathway in host myeloid cells</li> <li>Enhanced metastasis</li> </ul>	[55]
Pros1 deletion:	PROS1	<ul style="list-style-type: none"> <li>Enhanced metastasis</li> <li>CCL2 released by TAMs induces EMT and CSC properties in TNBC cells (via activation of AKT/CTNNB1 pathway)</li> <li>Enhanced MAPK signalling along with upregulation of ligands of MAPK pathway (HB-EGF, TGFB3, and TGF-<math>\alpha</math>) in TAM-educated TNBC cells</li> <li>Tumor growth and progression</li> </ul>	[64]
CCL2 released from TAM	PPBP, CXCL3, CCL2/AKT/CTNNB1 signaling	<ul style="list-style-type: none"> <li>Polarization towards M2 subtype of macrophage</li> <li>The maturation process of macrophages</li> </ul>	[2,65]
High number of CD206 + TAMs	HB-EGF, TGFB3, and TGF- $\alpha$ , MAPK pathway	<ul style="list-style-type: none"> <li>Polarization towards M2 subtype of macrophage</li> <li>Regulation of TAM polarization to M1/M2 subtypes</li> </ul>	[66]
<b>Alternate splicing events in TAMs</b>			
Alternative splicing events in genes associated with protein transport-related processes:	Vps33B, SRSF6	<ul style="list-style-type: none"> <li>Polarization towards M2 subtype of macrophage</li> </ul>	[67]
Upregulation in the expression of splicing factors:	• SF3B4, NAA38, SNRPG, ELAVL3 and RBM34	<ul style="list-style-type: none"> <li>Polarization towards M2 subtype of macrophage</li> </ul>	[67]
Modulations in miRNAs involved in TAMs			
Endogenous miRNAs:	<ul style="list-style-type: none"> <li>miRNAs upregulated in TAMs: miRNA-31, miRNA-182</li> <li>miRNAs downregulated in TNBC tumor-derived TAMs: miR-222, miR-146a</li> </ul>	<ul style="list-style-type: none"> <li>Regulation of TAM polarization to M1/M2 subtypes</li> </ul>	[68-72]
Metabolic reprogramming	<ul style="list-style-type: none"> <li>↑citrulline metabolism pathways</li> <li>↓anaerobic glycolysis &amp; Fatty acid catabolism (via PPAR-<math>\alpha</math>/STAT1 signalling)</li> <li>↑HK2, PFKL and ENO1 in TAMs</li> <li>↑NOX2 in TAM</li> <li>↑ADA2 isoenzyme activity</li> </ul>	<ul style="list-style-type: none"> <li>Polarization of monocytes to M2 macrophages</li> <li>Reduced phagocytic functions of TAMs</li> <li>Polarization of monocytes/macrophage to M2-Type</li> <li>increased breast cancer stemness and tumorigenesis</li> </ul>	[2,21,23,73-75]
Upregulation of HSLA in TAMs (in response to lactate production in TNBC cells)	HIF1, Glycolysis pathway, lactate	<ul style="list-style-type: none"> <li>Increased aerobic glycolysis leading to lactate production in TNBC cells</li> </ul>	[25]

ISG: Interferon response genes; TNBC: Triple-negative chemoresistance breast cancer; TAM: Tumor associated macrophage; ROS: Reactive oxygen species.

cells has been suggested to play an important role in regulating the pathogenesis of TNBC [2,55]. For instance, in a mouse model developed with co-inoculation of macrophages and TNBC tumor cells, the TAMs displayed a proinflammatory M1-type phenotype in the early stages of the disease while an anti-inflammatory M2-type phenotype was evident in the late stages of TNBC tumor [56] suggesting a pro-tumorigenic role of TAMs in TNBC. Thus, in the past decade, there has been a surge in studies focusing on delineating the molecular mechanism regulating TAM tumor cell cross-talk and their relevance as potential therapeutic strategies in TNBC. The detailed molecular mechanisms and the therapeutic strategies involved are discussed in the subsequent sections.

### 2.3. Molecular mechanisms involved in tumor-TAMs cross talk in TNBC

#### 2.3.1. Cytokines and soluble molecules mediated TAM - TNBC cell cross-talk

Several cytokines, chemokines, and soluble factors released from TAMs like CCL2, IL6, CXCL10, IL8, IL-1 $\beta$ , EGF, CCL5, amphiregulin, HGF, TGFB or S100A4 [2,57-59] (Table 2a, b, Figs. 2a, b) have been associated with an increase in cell proliferation, cancer stemness, invasion, and tumor metastasis in TNBC. The CSF1 released from TNBC tumor cells has been shown to promote the recruitment of M2-like macrophages and promote tumor progression in TNBC [24]. Also, upregulation of chemokines like CCL5 in TNBC tumor cells has been shown to regulate extracellular vesicles (EV) driven polarization of macrophages via secretion of a variety of factors like CXCL1, CTLA-4, IFNG, OPN, HGF, TGFB, and CCL19 leading to increased metastasis in TNBC [59]. The activation of soluble factors like S100 calcium-binding protein A4 (S100A4) signalling in TNBC cells was shown to promote monocyte-to-macrophage (M2) differentiation along with the increased secretion of pro-inflammatory cytokines such as CCL2, IL6, CXCL10, and IL8 by TAMs thereby promoting tumor progression [60]. Also, the release of soluble CD44 from TNBC cells can promote the polarisation of TAMs towards the M2 subtype thereby causing tumor growth and metastasis via IL1 $\beta$  signalling axis [61].

#### 2.3.2. Signalling pathway mediated TAM -TNBC cell cross-talk

Several signaling pathways are known to interplay between the tumor cells and TAMs, leading to tumor progression in TNBC. For instance, BRAC1-IRIS overexpression in TNBC cells promotes the upregulation of GM-CSF in TNBC cells leading to the recruitment of M2-TAMs in TNBC-TME. The TGF $\beta$ 1 expression by M2 TAMs (in a STAT5, NF- $\kappa$ B, and/or ERK signaling dependent manner) triggered the induction of EMT and stem cell like phenotype in BRCA1-IRIS expressing TNBC cells via TGF- $\beta$ 1/T $\beta$ RI/II/AKT signalling network [62]. In another such study, the upregulation of JAK2 signalling pathway was observed in CD169 + pro-invasive TAMs co-cultured with TNBC tumor cell leading to upregulation of PDL1 expression in TAMs. This mechanism was shown to tumor progression via modulation of the cell cycle and anti-apoptotic pathways in murine TNBC tumours [63]. Besides activation of cGAS/STING pathway regulated type I and II IFN response has been reported in tumor cell educated-TAMs leading to a potent anti-tumorigenic response in TNBC [55]. Also, a recent study showed that PROS1 gene inactivation in TAMs in the premetastatic niche can lead to increased lung metastasis via signal cross-talk between Soc3/NF- $\kappa$ B pathways (in TAMs) and ERK/AKT/ STAT3 pathways (in TNBC tumor cells) [64]. While in another study, the upregulation of MCT-1 gene in TNBC tumor cells has been shown to drive the recruitment and polarization of TAMs toward tumor promoting M2 macrophage phenotype via IL6/IL receptor mediated signalling in TNBC tumors [22]. In addition, the alterations of multiple signalling pathways like mitogen-activated protein kinase (MAPK) pathway, AKT/CTNNB1, TGF- $\beta$ 1/T $\beta$ RI/II/AKT signalling, cancer stemness maintenance pathway (PPBP/CXCL3) have been reported in TNBC cells in response to its cross-talk with TAMs resulting in tumor growth and metastasis [2,65,66] (Table 2a, b, Fig. 2b).

#### 2.3.3. Role of alternate splicing factors in TAM -TNBC cell cross-talk

A number of alternative splicing events in genes associated with DNA repair and DNA damage response pathways along with upregulation in splicing factors like CPSF7, RBM33, SNRNP48, RBM43, and CSTF2 have been reported to occur in TNBC cells co-cultured with monocytes/macrophages. While upregulation in the expression of splicing factors like SF3B4, NAA38, SNRPG, ELAVL3, RBM34, and alternative splicing events in genes associated with protein transport-related pathways (Vps33B and SRSF6) have been reported in TAMs and correlated with maturation processes of macrophages [67]. This highlights the extensive genomic events that are involved in mediating TAM-TNBC cell cross-talk.

#### 2.3.4. miRNA involved in TAM-TNBC cell cross-talk

The altered expression of miRNAs such as miR-33, miR-31, miR-222, miR-146a, miR-33a, miR-130a, miR-182 (specifically upregulated in M2-TAM via activation of TGF $\beta$ -signalling/inactivation of TLR4/NF $\kappa$ B pathway) has been reported to drive the polarization of macrophages in TNBC-TME [62,64,68-72] (Table 2a, Fig. 2a). Also, downregulation in the expression of miRNAs such as miRNA-149 in TNBC tumor cells was shown to inhibit the recruitment and polarization of protumorigenic macrophages by directly targeting CSF1 signalling in TNBC cells thereby regulating downstream EGF/amphiregulin expression in TNBC cell-educated macrophages (Table 2b, Fig. 2b). In TNBC tumors, a low level of miRNA-149 is associated with increased TAM infiltration and reduced patient survival [57].

#### 2.3.5. Metabolic reprogramming mediated TAM TNBC cell cross-talk

The metabolic reprogramming of TAMs and TNBC tumor cells has been shown to regulate the polarization of monocytes/macrophages further leading to a protumorigenic effect on TNBC tumor cells (Table 2a, 2b). Within both TNBC and non-TNBC breast tumors, perturbations in different molecular pathways such as SREBP-1 (sterol regulatory element-binding protein 1) pathway [20], hexokinase-2/PFKL/ENO1 signalling (mediators of aerobic glycolysis), MMR/CCL2 signaling activation and down-regulation of citrulline metabolic pathway (in M2-like TAMs) [2] have been associated with protumorigenic and antitumorigenic functions of macrophages in tumor TME (Table 2a, Fig. 2a).

For instance, in a study by Shin et al. [73] lactate production via activation of glucose metabolic pathway was shown to polarize macrophages to M2-like TAMs thereby promoting tumor growth and metastasis. In another study [74], obesity-driven reprogramming of breast tumor tissue derived TAMs could induce stem-like properties and protumorigenic phenotype in murine and human TNBC cells. This was shown to be mediated by nicotinamide adenine dinucleotide phosphate oxidase 2 (NOX2) signalling pathway (in TAMs) and glyccoprotein 130 (GP130) receptor signalling (in TNBC cells).

In another interesting study, upregulation and release of a lncRNA (HIF-1 $\alpha$ -stabilizing lncRNA, HSLA) from TAMs induced activation of aerobic glycolysis in TNBC cells. The resulting increase in lactate production by TNBC cells, in turn, leads to the upregulation of lncRNA (HSLA) expression in TAMs, thereby forming a feedforward loop promoting tumor progression and chemoresistance [25]. A recent study [75] explored the role of adenosine deaminase (ADA), an enzyme involved in purine metabolism in facilitating TAM-TNBC cell interaction and its impact on tumor progression. In this study, TNBC cells were shown to stimulate increased ADA2 enzyme activity on the surface of monocytes/macrophages. Clinical sample analysis of TNBC patients further revealed a significant correlation of plasma ADA2 activities with soluble pro-tumorigenic M2- type macrophages specific markers and poor prognosis of TNBC patients. Thus, targeting candidate genes associated with extracellular adenosine-dependent pathways might be a beneficial strategy for regulating TAM-induced changes in TNBC.

Thus, accumulating studies indicate that targeting the molecular mechanisms inhibiting the polarization of macrophages towards

**Table 2b**

. Molecular mechanisms activated in TNBC tumor cells in response to cross-talk with TAMs in the tumor TME.

Molecular events in TNBC cells (in response to cross-talk with TAMs)	Candidate genes involved	Impact on TNBC cell functions, on TAMs and breast tumorigenesis	Reference
<b>Modulations of cytokines and soluble molecules in TNBC cells</b>			
Upregulation of cytokines:	IL-23A, CSF-1, IL-8, IL-6, CXCL10, CCL2, CCL5, CSF2, G-CSF, CSF1	<ul style="list-style-type: none"> <li>Recruitment and polarization of tumor-promoting TAMs</li> <li>Increased proliferation and migration and induction of EMT in TNBC cells</li> <li>Release of CSF1 containing EVs from TNBC tumour cells promotes macrophage differentiation and activation of anti-tumorigenic immune response in TNBC tumors along with prolonged patient survival</li> </ul>	[2,24,55,60,62]
Increased CCL5 signalling	CCL5	<ul style="list-style-type: none"> <li>CCL5-regulated Tumor cell-derived Extracellular Vesicle (EV) biogenesis</li> <li>Reprogramming of TAM to pro-invasive phenotype</li> <li>Tumor metastasis</li> <li>Differentiation of monocytes towards the M2-subtype of TAMs</li> </ul>	[59]
<b>Activation of S100A4-BCC-TAM interaction cascade</b>			
Activation of S100A4-BCC-TAM interaction cascade	S100A4, IL-8, IL-6, CXCL10, CCL2, and CCL5	<ul style="list-style-type: none"> <li>Increased secretion of pro-inflammatory cytokines [IL-8, IL-6, CXCL10, CCL2, and CCL]</li> <li>Increased proliferation, acquisition of EMT-like traits, and enhanced migration of TNBC cells promoted by S100A4-treated TNBC cells-educated-M2-like-TAMs</li> <li>Acquisition of chemoresistance to carboplatin drug in TNBC cells exposed to S100A4-treated TNBC cells-educated-M2-like-TAMs</li> <li>Stimulates macrophage driven-IL-1<math>\beta</math> production</li> <li>Tumor growth and metastasis</li> </ul>	[60]
Release of soluble factors (promoted by IL-1 $\beta$ secreting monocytes/TAMs)	solubleCD44	<ul style="list-style-type: none"> <li>Tumor growth and metastasis</li> </ul>	[61]
<b>Modulations in Signalling Pathways in TNBC Cells</b>			
BRCA1-IRIS overexpression:	IRIS (up), NF- $\kappa$ B, CSF2 (up), HIF-1 $\alpha$ (up), AKT1/2 (up), T $\beta$ RI/II (up)	<ul style="list-style-type: none"> <li>Recruitment of CSF2R<math>\alpha</math> + M2-type TAM via activation of HIF-1<math>\alpha</math>/NF-<math>\kappa</math>B/CSF2 signal axis in BRCA-IRIS over-expressing TNBC cells</li> <li>Tumor progression and metastasis</li> </ul>	[62]
Upregulation in MCT-1/IL-6/IL-6R signalling:	EGFR, p-Stat3, Snail, Slug, ZEB1, N-cadherin, PD-L1, Nanog, Sox2, EpCAM and Snail (up), CDH1 (down)	<ul style="list-style-type: none"> <li>Increased EMT</li> <li>Polarization towards M2-TAM subtype</li> <li>Increased breast cancer stemness</li> <li>Increased tumor progression</li> <li>TAMs enhance MAPK pathway signalling in TNBC cells</li> </ul>	[22]
Enhanced MAPK signalling:	HB-EGF, TGFB3, and TGF- $\alpha$ , MAPK pathway		[66]
<b>Modulations of alternate splicing factors in TNBC cells</b>			
Upregulation in the expression of splicing factors:	CPSF7, RBM33, SNRNP48, RBM43, CSTF2, PARP3, EEF1D, MBNL2, ATG12, GCOM1, POLR2M, COPS7B, ZNF507, EEF1D, C17orf70, SAFB, FGFR1, PIK3CD, STAG2, ACADVL, NME3	<ul style="list-style-type: none"> <li>Alternative splicing events in genes associated with DNA repair and DNA damage response pathways</li> <li>Contributes to the proliferation phenotype of TNBC cells</li> </ul>	[67]
Modulation of genes involved in invasion/metastasis/EMT:	SPINK1, LAMC2, IGFBP1, SPP1, SNAI1 (up), CDH1, KRT19, and EPCAM (down), MMP-9 (upregulated in TNBC tumor models)	<ul style="list-style-type: none"> <li>Increased Ki67 + TNBC cell numbers</li> <li>Increased lung and liver metastasis in murine TNBC model developed from co-inoculation of TNBC + TAM</li> <li>Systemic MMP-9 expression increased progressively in murine TNBC tumors developed from co-inoculation with TNBC + macrophage cells</li> </ul>	[2,56]
<b>Modulations in miRNAs in TNBC cells</b>			
Endogenous miRNAs:	miRNAs downregulated in TNBC tumor/tumor cells: miR-149	<ul style="list-style-type: none"> <li>Regulation of recruitment and polarization to TAMs</li> </ul>	[57]
<b>Modulations of metabolic pathways in TNBC cells</b>			
HIF1 mediated upregulated aerobic glycolysis	HISLA/HIF-1 $\alpha$ axis regulated activation of aerobic glycolysis	<ul style="list-style-type: none"> <li>Promotes upregulation of HIF-1<math>\alpha</math>, resulting in activation of aerobic glycolysis and lactate production by TNBC cells</li> <li>Increased chemoresistance</li> </ul>	[25]

BCC: Breast cancer cell; TAM: tumor associated macrophage; TME: Tumor microenvironment; EMT: Epithelial to mesenchymal transition; MCT-1: Monocarboxylate transporter 1; MAPK: Mitogen-activated protein kinase; CCL5: Chemokine ligand 5; BRCA: Breast cancer type 1 gene; PARP: Poly [ADP-ribose] polymerase 1; HISLA: HIF-1 $\alpha$  stabilizing long noncoding RNA.

immunosuppressive M2 phenotype could be tested as an adjunct therapeutic strategy to improve the therapeutic efficacy of administered chemotherapy/immunotherapy drugs in TNBC patients.

#### 2.4. Therapies targeting tumor-TAM cross-talk in TNBC

Thus, a substantial effort has been focused on developing therapeutic strategies (adjunct/monotherapies) targeting TAMs in different cancers. The strategies employed to target tumor-TAM cross-talk in TNBC include depletion, inhibition, or reprogramming of TAM or recruitment of

immunostimulatory TAMs within tumor-TME. Some of these strategies are discussed in the subsequent section (Table 3, Fig. 3).

##### 2.4.1. Antibodies and inhibitory molecules targeting depletion of protumorigenic TAMs

Inhibition of CSF1R signaling through antagonist antibodies or small molecule inhibitors has been widely used as an effective immunotherapeutic strategy to suppress or deplete TAMs/monocytes in TNBC-TME [76]. In-vitro studies have shown that blockade of CSF-1 signalling with different molecules like miR-149 in TNBC tumor cells inhibits

recruitment of human monocyte / macrophages to tumor TME [57]. Also, in-vivo studies have demonstrated that blockade of CSF1-Receptor signaling using BLZ945, a highly selective small molecule inhibitor of CSF1R, greatly decreases the number of tumor promoting TAMs and increases cytotoxic CD8 + T cells within breast TME [77]. In another study, combining olaparib (PARP inhibitor) with CSFR-1 blocking antibody reduced immune-suppressive macrophages and overcame PARP inhibitor resistance in BRCA-deficient TNBC tumors [20] thereby indicating the relevance of this pathway in regulating TAM functions in TNBC.

Besides, TAM depletion with clodronate liposomes in combination with MEK inhibitor (selumetinib) restricted immuno-suppressive macrophage driven TNBC cell proliferation and tumor growth in mice TNBC tumors [66]. Many clinical trials have evaluated the therapeutic efficacy of either CSF-1 receptor (CSF1R) inhibition with monoclonal antibodies such as LY3022855, IMC-CS4, Emactuzumab, Axatilimab (NCT02265536, NCT01346358, NCT02718911, NCT03238027) or CSF-1 inhibition with mAb like MCS110 (NCT02807844) as an adjunct immunotherapeutic strategy in multiple solid tumors including breast cancer. However, this strategy is promising for a limited number of cancer patients [76] thus, evaluation of alternative immunotherapeutic strategies to target TAM-TNBC cross-talk is required.

Another approach adopted to deplete tumor-promoting TAMs from TNBC TME can be through inhibition of CD47-signaling in TAMs via targeted antibodies. In one such study, combination treatment with CD47-targetted antibodies and chemotherapy drug cabazitaxel led to increased clearance of TNBC cells via activation of immunostimulatory macrophages [30]. Besides targeting oncogenic signalling pathways such as MCT-1/IL6 signalling axis via inactivation of MCT-1 gene in TNBC tumor cells and inhibition of IL6R signalling with antagonist antibody, tocilizumab, synergistically inhibited tumor progression via reprogramming of TAMs towards M1-like phenotype [22].

#### 2.4.2. Nanoparticles and EV-mediated therapies targeting TNBC-derived TAMs

Numerous investigations have demonstrated the therapeutic potential of Nanoparticles/EVs/exosomes as a medium to specifically target and deliver specific drugs/molecules (miRNAs, siRNAs, chemotherapy drugs) to promote the reprogramming of TAMs towards M1-like phenotype in TNBC tumors. In a recent preclinical study by Lepland et al [78], a peptide-targeted nanoformulation of Doxorubicin drug exclusively eliminated immunosuppressive CD206 + TAMs present in murine TNBC model, generating antitumor response without off-target side effects (a drawback of several TAM-depleting agents currently under clinical study). In another study, a group of investigators has shown that blocking monocyte recruitment by inhibiting CCL2-CCR2 pathway with siCCR2-encapsulated nanoparticle (CNP/siCCR2) significantly reduced tumor progression and metastasis in murine TNBC model [79]. In an interesting study by Leonard et.al [80], the authors used a mathematical model to predict how TNBC cells in conjunction with M1/M2 TAMs influence response to nanotherapy with mesoporous particles loaded with albumin-bound paclitaxel (MSV-nab-PTX) in TNBC. The study showed that MSV-nab-PTX nanoparticles promote macrophage polarization towards M1-like subtype in an in-vitro breast cancer model. However, the study also suggests that the presence of both M1 and M2 subtypes of TAMs is necessary for generating effective responses to nanoparticle-mediated chemotherapy in breast tumors.

While in another study, the investigators showed that TNBC cells co-incubated with ferumoxytol, a Food and Drug Administration (FDA)-approved iron supplement, in presence of tumor-associated macrophages, showed increased cell death in TNBC cells while inducing pro-inflammatory M1-like phenotype in TAMs resulting in reduced tumor growth and metastasis in TNBC mice model [81]. Besides, treatment of TNBC tumor cells and TAM co-culture with EVs loaded with miR-33/antagomiR-182 resulted in the reprogramming of M2 to M1 phenotype and induced antitumor effects on TNBC tumor cells and breast cancer

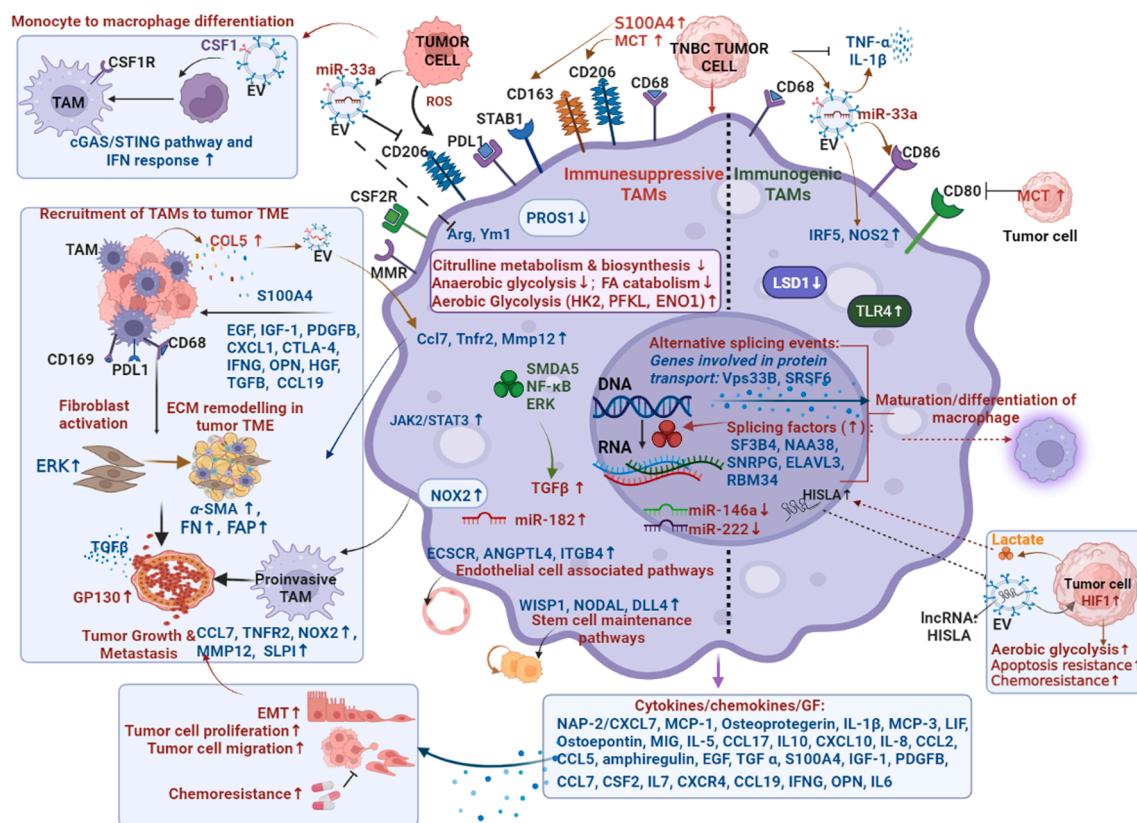


Fig. 2a. . Molecular mechanisms altered in TAM induced in response to cross-talk with TNBC tumor cells. References: [17,27-33].

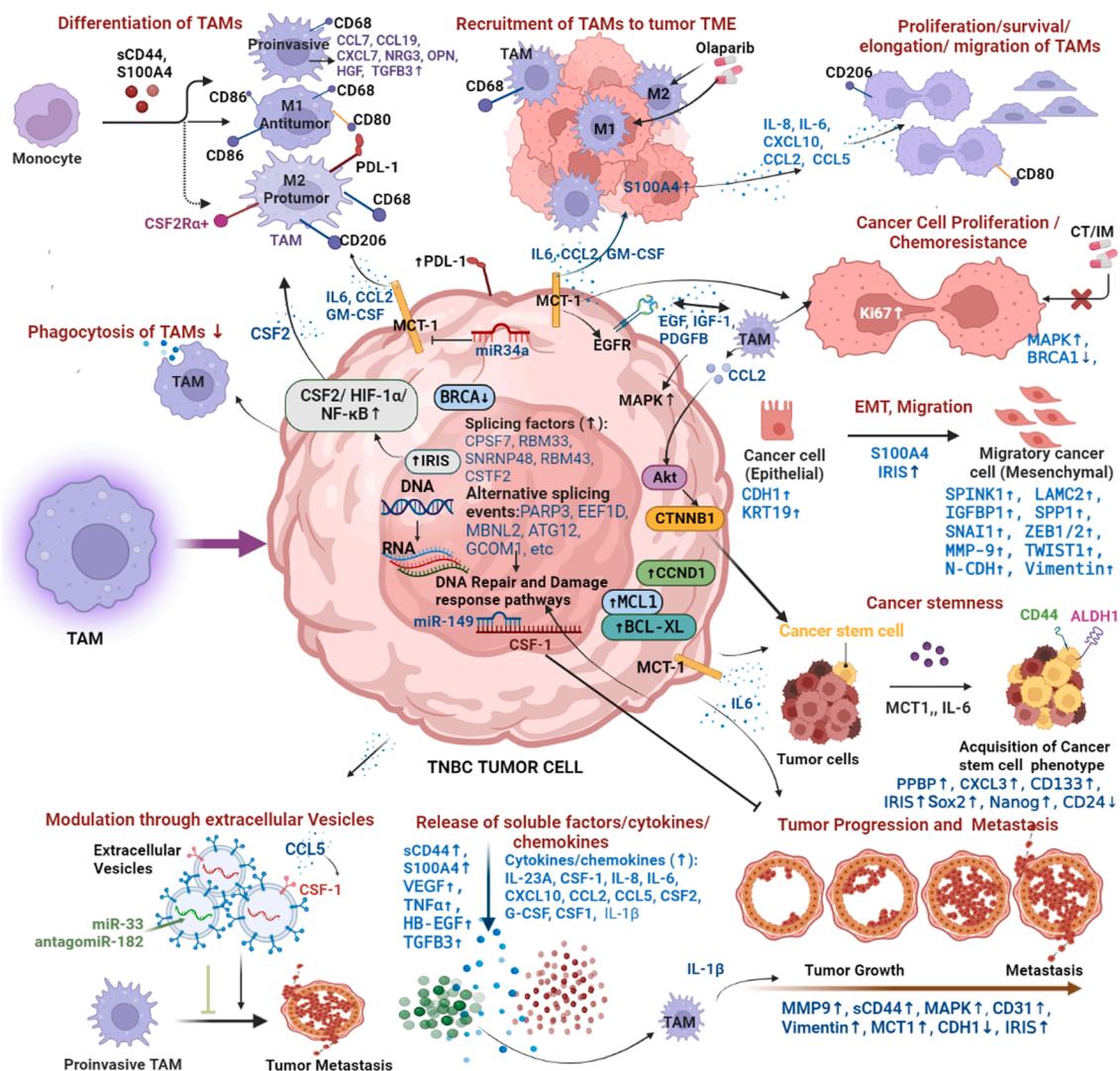


Fig. 2b. Molecular mechanisms altered in TNBC tumor cells in response to cross-talk with TAMs. References: [2,22,24,25,55-57,59-62,66,67].

regression [69,70].

#### 2.4.3. Epigenetic modulators as regulators of TAM-TNBC tumor cross-talk

Epigenetic reprogramming of macrophages is yet another strategy that has been evaluated by some investigators to tweak the phenotype of TAMs towards antitumor immunogenic response in TNBC tumors. For instance, low expression of LSD1, a histone demethylase, has been known to promote the polarisation of macrophages towards the M1 phenotype [82]. And a combination of Phenelzine (LSD1 inhibitor) with Abraxane/PD1-Based Immunotherapy increased the number of M1-type macrophages compared to M2-type macrophages in murine TNBC tumors accompanied with tumor regression [83]. In another study, class IIa HDAC inhibition with a synthetic inhibitor TIMP-3, promoted the recruitment of phagocytic and immunostimulatory TAMs within the tumor microenvironment, thereby improving chemotherapy (carboplatin/paclitaxel) and immunotherapy (anti-PD1) response in murine breast cancer model [26].

#### 2.4.4. Targeting the metabolic pathways regulating TAM-TNBC tumor cross-talk

Metabolic reprogramming within the tumor microenvironment is another major regulator of pro-/anti-inflammatory states of TAMs [23,74,84] and thus presents a good therapeutic strategy for regulating the pathogenesis of TNBC. For instance, inhibition of the Nox2 pathway

in TAMs or GP130 in TNBC cells results in decreased cancer stemness in TNBC cells [74]. Targeting glutamine metabolism is another strategy to regulate the reprogramming of TAMs. For instance, inhibiting glutamine metabolism with a glutamine antagonist prodrug (JHU083), promoted differentiation/polarization of myeloid cells towards antitumorigenic M1-like TAMs and enhanced the efficacy of immune-checkpoint blockade in immunotherapy-resistant murine TNBC model [84]. Treatment with fenofibrate (an agonist for the PPAR- $\alpha$  gene involved in fatty acid beta-oxidation) enhanced fatty acid metabolism in TAM, inhibiting tumor progression in the murine TNBC model. [23]. Interestingly fenofibrate treatment of TNBC tumor cells was shown to induce apoptosis in TNBC cells via PPAR- $\alpha$  independent pathway [85] thereby indicating the capability of fenofibrate to distinctly influence tumor cells and TAMs in TNBC-TME. In another study, blocking a lncRNA (HIF-1 $\alpha$ -stabilizing long noncoding RNA) regulated signal transduction between TAMs and TNBC tumor cells significantly reduced chemoresistance in TAM-educated TNBC cells [25].

#### 2.4.5. Targeting the Tumor-TAM cross-talk through natural compounds

In recent years, a number of preclinical studies have tried to explore the therapeutic efficacy of traditional herbal extracts or their derivatives and other natural compounds in modulating the TAM-regulated progression of TNBC tumors [86-90] (Table 3, Fig. 3). One such study [86] showed that the XIAOPI formula, a traditional Chinese medicine

**Table 3**

. Therapeutic strategies used to target TAM-tumor cell cross-talk in TNBC.

Treatment strategy	Experimental system	Effect on TNBC tumor response	Reference
<b>Antibodies and inhibitory molecules targeting TAMs</b>			
miR-149 regulated inhibition of CSF1/EGF signaling between TAM-TNBC cells	In vitro co-culture system, clinical TNBC samples, and in-vivo TNBC model	<u>Recruitment of TAMs</u> <ul style="list-style-type: none"> <li>miR-149 mediated blockade of CSF1 signalling in TNBC cells</li> <li>Inhibition of recruitment of M2-type macrophages to TNBC tumors</li> <li>Reduced lung metastases</li> </ul>	[57]
anti-CSF-1R Ab + Olaparib (PARP inhibitor)	In-vivo TNBC model	<u>TAM depletion</u> <ul style="list-style-type: none"> <li>Enhanced innate and adaptive anti-tumor immunity</li> <li>Prolonged survival in BRCA-deficient TNBC tumor-bearing mice.</li> </ul>	[20]
TAM depletion with clodronate liposomes + MEK inhibitor (selumetinib):	In-vitro co-culture and in-vivo TNBC model	<u>TAM depletion</u> <ul style="list-style-type: none"> <li>Inhibition of CD206 + M2-macrophage-driven-TNBC cell proliferation</li> <li>Suppressed tumor growth</li> <li>Enhanced T lymphocyte infiltration in murine TNBC tumors</li> </ul>	[66]
TAM depletion with CD47 blockade and cabazitaxel treatment	In vitro and In-vivo TNBC model	<u>TAM depletion</u> <ul style="list-style-type: none"> <li>Increased phagocytosis of TNBC cells by macrophages</li> <li>Polarization towards M1 macrophage phenotype and tumor regression</li> </ul>	[30]
Blockade of MCT-1/IL6R signal by MCT-1 knockdown and IL6R antagonist antibody (tocilizumab) treatment	In vitro and In-vivo TNBC model	<u>Polarization of M2-to M1 TAM</u> <ul style="list-style-type: none"> <li>Polarization of TAMs to M1-subtype</li> <li>Reduced TNBC cell stemness</li> </ul>	[22]
<b>Nanoparticles and EV-mediated therapies targeting TAMs</b>			
TAM-depleting agent ("OximUNO"): star-shaped polyglutamate (St-PGA) loaded with CD206-targeting peptide mUNO (targeting CD206 + TAM) and chemotherapeutic drug doxorubicin	In-vivo TNBC model	<u>TAM depletion</u> <ul style="list-style-type: none"> <li>Diminished the number of CD206 + TAMs and enhanced antitumor immune response, with minimum off target effects of treatment</li> </ul>	[78]
siCCR2-encapsulated cationic nanoparticle targeting CCL2-CCR2 pathway in Inflammatory Monocytes	In-vivo TNBC model	<u>TAM depletion</u> <ul style="list-style-type: none"> <li>Suppressed tumor growth</li> <li>Reduced abundance of monocytes/TAMs in TNBC tumor</li> </ul>	[79]
Combination treatment with MSV-nab-PTX and CRISPR- RICTOR-Liposome system targeting TAMs	3D co-culture system	<u>TAM polarization</u> <ul style="list-style-type: none"> <li>Tumor regression in paclitaxel chemo resistant TNBC tumors</li> </ul>	[80]
Treatment with Ferumoxytol (FDA-approved iron supplement)	In-vitro co-culture and in-vivo BC model	<u>TAM polarization</u> <ul style="list-style-type: none"> <li>Upregulation of M1 specific marker/cytokines and downregulation in M2 specific markers /cytokines in TAMs</li> <li>Increased inflammatory Th1-type responses</li> <li>Increased caspase-3 activity in pre-malignant mammary carcinoma</li> <li>Tumour growth inhibition</li> </ul>	[81]
miR-33/antagomiR-182 loaded extracellular vesicles (EVs) mediated transfer to TAM	In vitro co-culture system	<u>TAM polarization</u> <ul style="list-style-type: none"> <li>Reduced proliferation, invasion, and migration of TAM-educated TNBC cells</li> <li>Therapeutic delivery of antagomiR-182 with cationized mannan-modified EVs inhibits polarization of TAMs to M2-subtype and induces tumour growth inhibition</li> </ul>	[69,70]
<b>Epigenetic modulators targeting TAMs</b>			
Combination of Phenelzine (LSD1 inhibitor) with Abraxane/PD1-Based Immunotherapy	TNBC murine model developed from co-inoculation of TNBC tumor cells and Monocytes	<u>Reprogramming of TAMs</u> <ul style="list-style-type: none"> <li>Epigenetic reprogramming of M2-TAMs towards M1 like TAM in TNBC-TME</li> </ul>	[82,83]
TMP195 (Class IIa HDAC inhibitor) in combination with carboplatin/paclitaxel or anti-PD1 therapy	In-vivo BC model	<u>Recruitment of anti-tumorigenic TAMs</u> <ul style="list-style-type: none"> <li>Recruitment and differentiation of highly phagocytic and stimulatory TAMs in breast tumors</li> <li>Reduced tumor growth and metastases</li> <li>Increased recruitment of Granzyme B + CD8 + T cells in breast tumors</li> <li>Improved chemosensitivity to carboplatin/paclitaxel or checkpoint blockade (anti-PD1) in murine breast cancer model</li> </ul>	[26]

(continued on next page)

**Table 3 (continued)**

Treatment strategy	Experimental system	Effect on TNBC tumor response	Reference
<b>Targeting the metabolic pathways regulating TAMs</b>			
Inhibition of the Nox2 gene in TAMs/GP130 in TNBC cells	Murine TNBC model	<u>Metabolic reprogramming of TAM/TNBC cells</u>	[74]
• Decreased cancer stemness in TNBC cells			
<u>Metabolic reprogramming of TNBC cells</u>			
Blocking a lncRNA signal transduction in TAMs	In-vitro co-culture system, TNBC mice model, and TNBC patient Samples	• Reduced chemoresistance in TAM-educated TNBC cells	[25]
<u>Reprogramming of TAMs</u>			
Blocking Glutamine metabolism in TAMs with JHU083 (glutamine antagonist prodrug)	Murine TNBC model	• Reprogramming of TAMs from a suppressive to a proinflammatory M1-phenotype	[84]
• Inhibits tumor growth			
<u>Reprogramming of TAMs</u>			
Treatment with fenofibrate (agonist for the PPAR- $\alpha$ gene)	TNBC Murine Xenograft model	• Reprogramming of FA catabolism in TAMs	[23,85]
• Enhanced antitumor activity of TAMs			
• Induces apoptosis in TNBC cells			
• Combination of fenofibrate and anti-CD47 therapy significantly inhibited tumor growth TNBC mouse model			
<b>Targeting the Tumor-TAM cross-talk with Natural compounds</b>			
Traditional herbal formulations: XIAOPI formula	In-vitro co-culture system and TNBC mice model	<u>Reprogramming of TAMs</u>	[86]
• Reduced pre-metastatic niche formation in breast cancer via suppressing TAM-secreted CXCL1 signaling			
• Reduced TNBC cell proliferation			
• Reduced tumor growth & metastasis			
<u>Reprogramming of TAMs</u>			
Natural compound: Anemoside A3 (A3), an active compound from <i>Pulsatilla saponins</i>	In vitro macrophage-cancer cell co-culture system and in-vivo TNBC model	• Activation of TLR4-dependent polarization of macrophages towards M1-phenotype	[87]
• Reduced breast tumor growth and angiogenesis			
Traditional herbal formulation: <i>Cordyceps sinensis</i> : a natural compound	In-vivo mice model of TNBC and 3D co-culture assay	<u>Reprogramming of TAMs</u>	[88]
• Inhibition of tumor growth via promoting macrophage polarization towards M1 subtype via activation of NF- $\kappa$ B signaling			
Traditional herbal medicine: <i>Taraxacum mongolicum</i> extract	In vitro co-culture system	<u>Reprogramming of TAMs</u>	[89]
• Induction of polarization of macrophages from M2 to M1 phenotype			
• Reduced proliferation, migration, and invasion capabilities in TAM-educated TNBC cells via down-regulation of IL-10/STAT3/PD-L1 signaling axis			
<u>Reprogramming of TAMs</u>			
Natural compound: Protein-Bound Polysaccharides from Coriolus Versicolor Fungus (PBP)	In vitro co-culture system	• Reduced cell growth and migration of TNBC cells educated with PBP-treated TAMs	[90]
• Decreased secretion of pro-angiogenic factors and upregulated the production of pro-inflammatory cytokines in TNBC cells educated with PBP-treated TAMs			
• M2 to M1 polarization of macrophages			

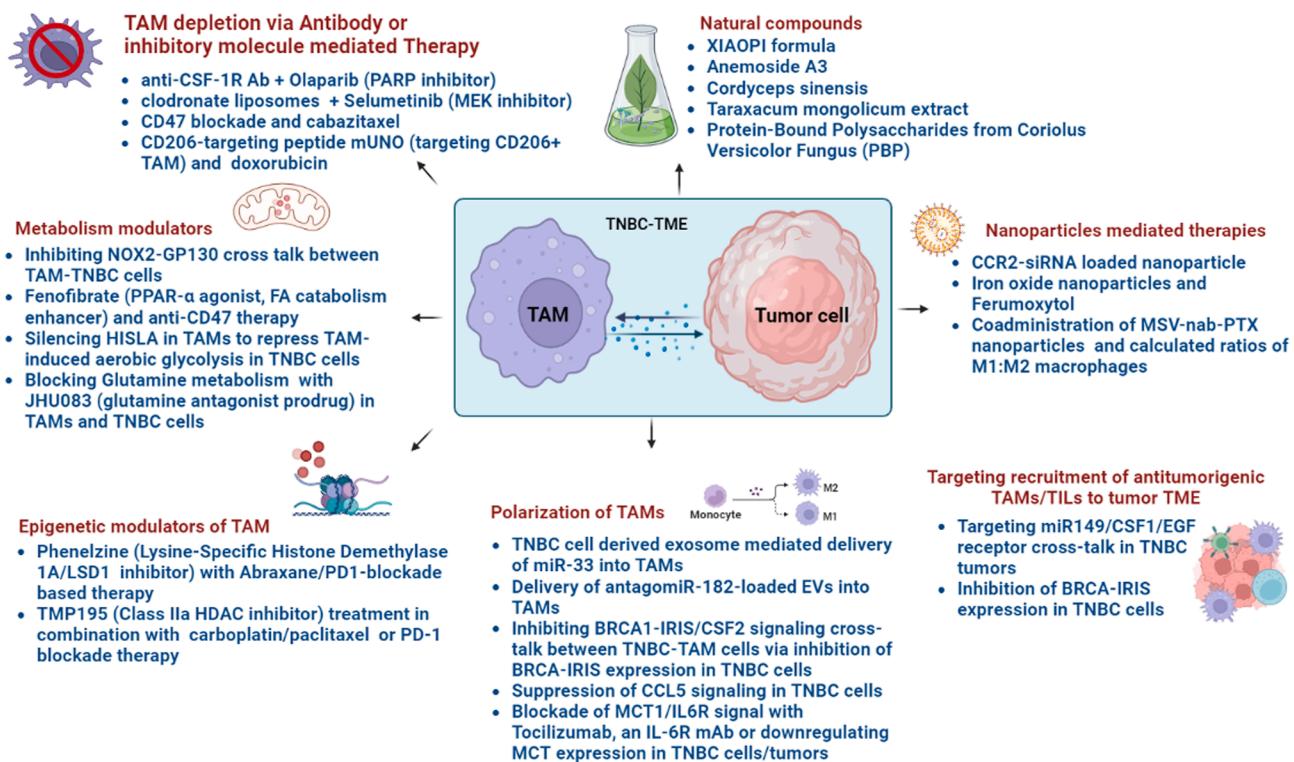
CSF-R1: The receptor of the colony-stimulating factor-1; PARP: Poly (ADP-ribose) polymerase; MEK: MAP kinase; CCR2: C–C chemokine receptor type 2; MSV-nab-PTX: Nano therapy with mesoporous particles loaded with albumin-bound paclitaxel; PTX: paclitaxel; MMPs: matrix metalloproteases; TME: Tumor microenvironment; TNBC: triple negative Breast cancer; BC: Breast cancer; TAM: Tumor-associated macrophage; HDAC: Histone deacetylases; TME: Tumor microenvironment; FDA: Food and Drug Administration.

composed of 10 herbs, could inhibit the proliferation and polarization of M2-type macrophages in TAMs which leads to the inhibition of lung metastasis in murine TNBC tumors. Another preclinical study showed that Anemoside A3, an active compound could block the cross-talk between TNBC cells and macrophages resulting in reduced TNBC cell growth and migration through decreased secretion of pro-angiogenic factors like VEGF, MCP-1, and polarization of TAMs from the M2 to M1 phenotype [87]. In similar studies, different natural compounds like *Cordyceps sinensis* extract [88], *Taraxacum mongolicum* extract [89] or protein-bound polysaccharides (isolated from the *Coriolus versicolor* fungus) [90] were shown to reduce TNBC tumor progression by switching M2-TAM to M1-TAM phenotype and inhibiting TAM-TNBC cross-talk. The outcome of such studies can help develop chemopreventive strategies to prevent TNBC progression and therefore

warrant further study.

### 3. Conclusion

Accumulating evidence indicates that TAMs, which form a major component of tumor-infiltrating immune cells within the TNBC tumors, have the capability to regulate both antitumor and pro-tumorigenic responses in the TNBC TME. Several molecular pathways have been speculated to be involved in mediating the tumor-immune cell cross-talk in the TNBC-TME (Table 2a, b; Figs. 2a, b). Different preclinical and clinical studies have suggested diverse therapeutic strategies to overcome TAM-induced chemotherapy/immunotherapy resistance in TNBC patients (Fig. 3). Therapeutic strategies to either deplete or inhibit the recruitment of TAM to the TNBC-TME have met with some success using



**Fig. 3.** . Therapeutic strategies tested to regulate TAM-tumor cell cross-talk in TNBC. References: [20,22,23,25,26,30,57,66,69,70,74,78-90].

animal and human preclinical models that mimic TNBC progression. Besides, strategies targeting molecular pathways such as mitogen-activated protein kinase (MAPK) pathway, CSF1 signaling, AKT/CTNNB1, TGF- $\beta$ 1/T $\beta$ RI/II/AKT signaling, MCT1/IL6/IL6R signal axis, CSF/EGF receptor cross-talk have been implicated as potential candidates to inhibit TNBC-TAM driven progression of TNBC. Also, treatment strategies targeting epigenetic or metabolic reprogramming of TAMs are promising strategies to arrest tumor growth and metastases in TNBC (Table 3). In addition, pre-clinical studies involving natural compounds or their derivatives have also been observed to reduce tumor burden by inducing recruitment or polarization of antitumorigenic TAMs in the TNBC-TME.

Thus, all these data indicate that therapies targeting TAM-tumor cross-talk are a promising strategy for elevating the treatment responses of TNBC patients. Some of these therapeutic strategies have even translated to phase 1 or phase 2 clinical trials of solid cancer including breast cancer patients with varied clinical responses in patients [91]. Hence, in the future, combining TAM-targeted therapies with conventional chemotherapy, immunotherapy, and adjuvant therapy may become an effective therapeutic strategy to improve the survival and treatment outcome of TNBC patients.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Data availability

No data was used for the research described in the article.

#### Acknowledgments

The authors thank the Director, ICMR-NIRRCH for providing resources and financial assistance through institute intramural funds.

#### References

- [1] L. Yin, J.-J. Duan, X.-W. Bian, S. Yu, Triple-negative breast cancer molecular subtyping and treatment progress, *Breast Cancer Res.* 22 (2020) 61, <https://doi.org/10.1186/s13058-020-01296-5>.
- [2] M. Hollmén, F. Roudnicki, S. Karaman, M. Detmar, Characterization of macrophage - cancer cell crosstalk in estrogen receptor positive and triple-negative breast cancer, *Sci Rep.* 5 (2015) 9188, <https://doi.org/10.1038/srep09188>.
- [3] M. Oishi, M. Asaoka, Y. Tokumaru, L. Yan, R. Matsuyama, T. Ishikawa, I. Endo, K. Takabe, CD8 T Cell Score as a Prognostic Biomarker for Triple Negative Breast Cancer, *Int J Mol Sci.* 21 (2020) 6968, <https://doi.org/10.3390/ijms21186968>.
- [4] T. Yu, G. Di, Role of tumor microenvironment in triple-negative breast cancer and its prognostic significance, *Chinese J. CancerResearch.* 29 (2017) 237–252. <https://doi.org/10.21147/j.issn.1000-9604.2017.03.10>.
- [5] G.A. Valencia, P. Rioja, Z. Morante, R. Ruiz, H. Fuentes, C.A. Castaneda, T. Vidaurre, S. Neciosup, H.L. Gomez, Immunotherapy in triple-negative breast cancer: A literature review and new advances, *World J Clin Oncol.* 13 (2022) 219–236, <https://doi.org/10.5306/wjco.v13.i3.219>.
- [6] L. Li, F. Zhang, Z. Liu, Z. Fan, Immunotherapy for Triple-Negative Breast Cancer: Combination Strategies to Improve Outcome, *Cancers (Basel)* 15 (1) (2023) 321, <https://doi.org/10.3390/cancers15010321>.
- [7] J. Cortés, F. André, A. Gonçalves, S. Kümmel, M. Martín, P. Schmid, F. Schuetz, S. M. Swain, V. Easton, E. Pollex, R. Deurloo, R. Dent, IMpassion132 Phase III trial: atezolizumab and chemotherapy in early relapsing metastatic triple-negative breast cancer, *Future Oncol.* 15 (2019) 1951–1961, <https://doi.org/10.2217/fon-2019-0059>.
- [8] A. Mantovani, F. Marchesi, A. Malesci, L. Laghi, P. Allavena, Tumour-associated macrophages as treatment targets in oncology, *Nat Rev Clin Oncol.* 14 (2017) 399–416, <https://doi.org/10.1038/nrclinonc.2016.217>.
- [9] B.D. Lehmann, J.A. Pienpol, Identification and use of biomarkers in treatment strategies for triple-negative breast cancer subtypes, *J Pathol.* 232 (2014) 142–150, <https://doi.org/10.1002/path.4280>.
- [10] G. Jerusalem, J. Collignon, H. Schroeder, L. Lousberg, Triple-negative breast cancer: treatment challenges and solutions, *Breast Cancer: Targets and Therapy.* (2016) 93. <https://doi.org/10.2147/BCTT.S69488>.
- [11] M. Maqbool, F. Bekele, G. Fekadu, Treatment Strategies Against Triple-Negative Breast Cancer: An Updated Review, *Breast Cancer: Targets and Therapy.* 14 (2022) 15–24, <https://doi.org/10.2147/BCTT.S348060>.
- [12] Y. Ye, C. Xu, F. Chen, Q. Liu, N. Cheng, Targeting Innate Immunity in Breast Cancer Therapy: A Narrative Review, *Front Immunol.* 12 (2021), <https://doi.org/10.3389/fimmu.2021.771201>.
- [13] M. Tang, Y. Liu, Q. Zhang, P. Zhang, J. Wu, J. Wang, Y. Ruan, Y. Huang, Antitumor efficacy of the Runx2-dendritic cell vaccine in triple-negative breast cancer invitro, *Oncol Lett.* (2018), <https://doi.org/10.3892/ol.2018.9001>.
- [14] R. Hirano, K. Okamoto, M. Shinke, M. Sato, S. Watanabe, H. Watanabe, G. Kondoh, T. Kadonosono, S. Kizaka-Kondoh, Tissue-resident macrophages are major tumor-

- associated macrophage resources, contributing to early TNBC development, recurrence, and metastases, *Commun Biol.* 6 (2023) 144, <https://doi.org/10.1038/s42003-023-04525-7>.
- [15] X. Yang, D. Weng, Q. Pan, T. Xiang, C. Yang, Z. Wu, M. Li, S. Xie, Y. Tang, J. Xia, J. Zhao, Adjuvant alternative cytokine-induced killer cell combined with natural killer cell immunotherapy improves the prognosis of post-mastectomy breast cancer, *Front Immunol.* 13 (2022), <https://doi.org/10.3389/fimmu.2022.974487>.
- [16] D.P. Saraiha, B.F. Correia, R. Salvador, N. de Sousa, A. Jacinto, S. Braga, M. G. Cabral, Circulating low density neutrophils of breast cancer patients are associated with their worse prognosis due to the impairment of T cell responses, *Oncotarget* 12 (2021) 2388–2403, <https://doi.org/10.18632/oncotarget.28135>.
- [17] L. Keren, M. Bosse, D. Marquez, R. Angoshtari, S. Jain, S. Varma, S.-R. Yang, A. Kurian, D. Van Valen, R. West, S.C. Bendall, M. Angelo, A Structured Tumor-Immune Microenvironment in Triple Negative Breast Cancer Revealed by Multiplexed Ion Beam Imaging, *Cell* 174 (2018) 1373–1387.e19, <https://doi.org/10.1016/j.cell.2018.08.039>.
- [18] A.J. Petty, Y. Yang, Tumor-associated macrophages: implications in cancer immunotherapy, *Immunotherapy* 9 (2017) 289–302, <https://doi.org/10.2217/intm-2016-0135>.
- [19] Y. Tan, M. Wang, Y. Zhang, S. Ge, F. Zhong, G. Xia, C. Sun, Tumor-Associated Macrophages: A Potential Target for Cancer Therapy, *Front Oncol.* 11 (2021), <https://doi.org/10.3389/fonc.2021.693517>.
- [20] A.K. Mehta, E.M. Cheney, C.A. Hartl, C. Pantelidou, M. Oliwa, J.A. Castrillon, J.-R. Lin, K.E. Hurst, M. de Oliveira Taveira, N.T. Johnson, W.M. Oldham, M. Kalocsay, M.J. Berberich, S.A. Boswell, A. Kothari, S. Johnson, D.A. Dillon, M. Lipschitz, S. Rodig, S. Santagata, J.E. Garber, N. Tung, J. Yélamos, J.E. Thaxton, E.A. Mittendorf, P.K. Sorger, G.I. Shapiro, J.L. Guerrero, Targeting immunosuppressive macrophages overcomes PARP inhibitor resistance in BRCA1-associated triple-negative breast cancer, *Nat Cancer* 2 (2020) 66–82, <https://doi.org/10.1038/s43018-020-00148-7>.
- [21] D. Liu, C. Chang, N. Lu, X. Wang, Q. Lu, X. Ren, P. Ren, D. Zhao, L. Wang, Y. Zhu, F. He, L. Tang, Comprehensive Proteomics Analysis Reveals Metabolic Reprogramming of Tumor-Associated Macrophages Stimulated by the Tumor Microenvironment, *J Proteome Res.* 16 (2017) 288–297, <https://doi.org/10.1021/acs.jproteome.6b00604>.
- [22] Y.-S. Weng, H.-Y. Tseng, Y.-A. Chen, P.-C. Shen, A.T. Al Haq, L.-M. Chen, Y.-C. Tung, H.-L. Hsu, MCT-1/miR-34a/IL-6/IL-6R signaling axis promotes EMT progression, cancer stemness and M2 macrophage polarization in triple-negative breast cancer, *Mol Cancer* 18 (2019) 42, <https://doi.org/10.1186/s12943-019-0988-0>.
- [23] Y. Gu, X. Niu, L. Yin, Y. Wang, Y. Yang, X. Yang, Q. Zhang, H. Ji, Enhancing Fatty Acid Catabolism of Macrophages Within Aberrant Breast Cancer Tumor Microenvironment Can Re-establish Antitumor Function, *Front Cell Dev Biol.* 9 (2021), <https://doi.org/10.3389/fcell.2021.665869>.
- [24] J. Ding, C. Guo, P. Hu, J. Chen, Q. Liu, X. Wu, Y. Cao, J. Wu, CSF1 is involved in breast cancer progression through inducing monocyte differentiation and homing, *Int J Oncol.* 49 (2016) 2064–2074, <https://doi.org/10.3892/ijo.2016.3680>.
- [25] F. Chen, J. Chen, L. Yang, J. Liu, X. Zhang, Y. Zhang, Q. Tu, D. Yin, D. Lin, P.-P. Wong, D. Huang, Y. Xing, J. Zhao, M. Li, Q. Liu, F. Su, S. Su, E. Song, Extracellular vesicle-packaged HIF-1 $\alpha$ -stabilizing lncRNA from tumour-associated macrophages regulates aerobic glycolysis of breast cancer cells, *Nat Cell Biol.* 21 (2019) 498–510, <https://doi.org/10.1038/s41556-019-0299-0>.
- [26] J.L. Guerrero, A. Sotayo, H.E. Ponichtera, J.A. Castrillon, A.L. Pourzia, S. Schad, S. F. Johnson, R.D. Carrasco, S. Lazo, R.T. Bronson, S.P. Davis, M. Lobera, M. A. Nolan, A. Letai, Class IIa HDAC inhibition reduces breast tumours and metastases through anti-tumour macrophages, *Nature* 543 (2017) 428–432, <https://doi.org/10.1038/nature21409>.
- [27] M.E. Gatti-Mays, J.M. Balko, S.R. Gameiro, H.D. Bear, S. Prabhakaran, J. Fukui, M. L. Disis, R. Nanda, J.L. Gulley, K. Kalinsky, H. Abdul Sater, J.A. Sparano, D. Cescon, D.B. Page, H. McArthur, S. Adams, E.A. Mittendorf, If we build it they will come: targeting the immune response to breast cancer, *NPJ Breast Cancer* 5 (2019) 37, <https://doi.org/10.1038/s41523-019-0133-7>.
- [28] M.C. Agahozo, M.R. van Bockstal, F.H. Groenendijk, T.P.P. van den Bosch, P. J. Westenend, C.H.M. van Deurzen, Ductal carcinoma in situ of the breast: immune cell composition according to subtype, *Mod. Pathol.* 33 (2020) 196–205, <https://doi.org/10.1038/s41379-019-0331-8>.
- [29] H. Du, Z. Yi, L. Wang, Z. Li, B. Niu, G. Ren, The co-expression characteristics of LAG3 and PD-1 on the T cells of patients with breast cancer reveal a new therapeutic strategy, *Int Immunopharmacol.* 78 (2020), 106113, <https://doi.org/10.1016/j.intimp.2019.106113>.
- [30] X. Cao, B. Li, J. Chen, J. Dang, S. Chen, E.G. Gunes, B. Xu, L. Tian, S. Muend, M. Raoof, C. Querfeld, J. Yu, S.T. Rosen, Y. Wang, M. Feng, Effect of cabazitaxel on macrophages improves CD47-targeted immunotherapy for triple-negative breast cancer, *J Immunother. Cancer.* 9 (2021) e00222.
- [31] J. Deng, A. Thennavan, S. Shah, E. Bagdatlioglu, N. Klar, A. Heguy, C. Marier, P. Meyn, Y. Zhang, K. Labbe, C. Almonte, M. Krosgaard, C.M. Perou, K.-K. Wong, S. Adams, Serial single-cell profiling analysis of metastatic TNBC during Nab-paclitaxel and pembrolizumab treatment, *Breast Cancer Res Treat.* 185 (2021) 85–94, <https://doi.org/10.1007/s10549-020-05936-4>.
- [32] X. Qiu, T. Zhao, R. Luo, R. Qiu, Z. Li, Tumor-Associated Macrophages: Key Players in Triple-Negative Breast Cancer, *Front Oncol.* 12 (2022), <https://doi.org/10.3389/fonc.2022.772615>.
- [33] S. Singh, N. Lee, D.A. Pedroza, I.L. Bado, C. Hamor, L. Zhang, S. Aguirre, J. Hu, Y. Shen, Y. Xu, Y. Gao, N. Zhao, S.-H. Chen, Y.-W. Wan, Z. Liu, J.T. Chang, D. Hollern, C.M. Perou, X.H.F. Zhang, J.M. Rosen, Chemotherapy Coupled to Macrophage Inhibition Induces T-cell and B-cell Infiltration and Durable Regression in Triple-Negative Breast Cancer, *Cancer Res.* 82 (2022) 2281–2297, <https://doi.org/10.1158/0008-5472.CAN-21-3714>.
- [34] X. Zhou, X. Liu, L. Huang, Macrophage-Mediated Tumor Cell Phagocytosis: Opportunity for Nanomedicine Intervention, *Adv Funct Mater.* 31 (2021) 2006220, <https://doi.org/10.1002/adfm.202006220>.
- [35] M. Santoni, E. Romagnoli, T. Saladino, L. Foghini, S. Guarino, M. Capponi, M. Giannini, P.D. Cognini, G. Ferrara, N. Battelli, Triple negative breast cancer: Key role of Tumor-Associated Macrophages in regulating the activity of anti-PD-1/PD-L1 agents, *Biochimica et Biophysica Acta (BBA) - Reviews on Cancer* 1869 (2018) 78–84, <https://doi.org/10.1016/j.bbcan.2017.10.007>.
- [36] C. Medrek, F. Pontén, K. Jirström, K. Leandersson, The presence of tumor associated macrophages in tumor stroma as a prognostic marker for breast cancer patients, *BMC Cancer* 12 (2012) 306, <https://doi.org/10.1186/1471-2407-12-306>.
- [37] R.-Y. Ma, A. Black, B.-Z. Qian, Macrophage diversity in cancer revisited in the era of single-cell omics, *Trends Immunol.* 43 (2022) 546–563, <https://doi.org/10.1016/j.it.2022.04.008>.
- [38] N. Halliday, C. Williams, A. Kennedy, E. Waters, A.M. Pesenacker, B. Soskic, C. Hinze, T.Z. Hou, B. Rowshanravan, D. Janman, L.S.K. Walker, D.M. Sansom, CD86 Is a Selective CD28 Ligand Supporting FoxP3+ Regulatory T Cell Homeostasis in the Presence of High Levels of CTLA-4, *Front Immunol.* 11 (2020), <https://doi.org/10.3389/fimmu.2020.600000>.
- [39] Q. Taban, P.T. Mumtaz, K.Z. Masoodi, E. Haq, S.M. Ahmad, Scavenger receptors in host defense: from functional aspects to mode of action, *Cell Communication and Signaling.* 20 (2022) 2, <https://doi.org/10.1186/s12964-021-00812-0>.
- [40] J. Kzhyshkowska, S. Mamidi, A. Gratchev, E. Kremmer, C. Schmutzmaier, L. Krusell, G. Haus, J. Utikal, K. Schledzewski, J. Scholtze, S. Goerdt, Novel stabilin-1 interacting chitinase-like protein (SI-CLP) is up-regulated in alternatively activated macrophages and secreted via lysosomal pathway, *Blood* 107 (2006) 3221–3228, <https://doi.org/10.1182/blood-2005-07-2843>.
- [41] S.E. Mwafy, D.M. El-Guindy, Pathologic assessment of tumor-associated macrophages and their histologic localization in invasive breast carcinoma, *J Egypt Natl Canc Inst.* 32 (2020) 6, <https://doi.org/10.1186/s43046-020-0018-8>.
- [42] H. Jeong, I. Hwang, S.H. Kang, H.C. Shin, S.Y. Kwon, Tumor-Associated Macrophages as Potential Prognostic Biomarkers of Invasive Breast Cancer, *J Breast Cancer.* 22 (2019) 38, <https://doi.org/10.4048/jbc.2019.22.e5>.
- [43] V. Pelekano, F. Villarroel-Espindola, K.A. Schalper, L. Pusztai, D.L. Rimm, CD68, CD163, and matrix metalloproteinase 9 (MMP-9) co-localization in breast tumor microenvironment predicts survival differently in ER-positive and -negative cancers, *Breast Cancer Res.* 20 (2018) 154, <https://doi.org/10.1186/s13058-018-1076-x>.
- [44] M. Li, L. He, J. Zhu, P. Zhang, S. Liang, Targeting tumor-associated macrophages for cancer treatment, *Cell Biosci.* 12 (2022) 85, <https://doi.org/10.1186/s13578-022-00823-5>.
- [45] J. Wang, L. Browne, I. Slapetova, F. Shang, K. Lee, J. Lynch, J. Beretov, R. Whan, P. H. Graham, E.K.A. Millar, Multiplexed immunofluorescence identifies high stromal CD68+PD-L1+ macrophages as a predictor of improved survival in triple negative breast cancer, *Sci Rep.* 11 (2021) 21608, <https://doi.org/10.1038/s41598-021-01116-6>.
- [46] K.C.S. Pe, R. Saetung, V. Yodsurang, C. Chaotham, K. Suppapat, P. Chanvorachote, S. Tawinwung, Triple-negative breast cancer influences a mixed M1/M2 macrophage phenotype associated with tumor aggressiveness, *PLoS One* 17 (2022) e0273044.
- [47] Z.-Y. Yuan, R.-Z. Luo, R.-J. Peng, S.-S. Wang, C. Xue, High infiltration of tumor-associated macrophages in triple-negative breast cancer is associated with a higher risk of distant metastasis, *Onco Targets Ther.* (2014) 1475. <https://doi.org/10.2147/OTT.S61838>.
- [48] M. Yang, Z. Li, M. Ren, S. Li, L. Zhang, X. Zhang, F. Liu, Stromal Infiltration of Tumor-Associated Macrophages Conferring Poor Prognosis of Patients with Basal-Like Breast Carcinoma, *J Cancer.* 9 (2018) 2308–2316, <https://doi.org/10.7150/jca.25155>.
- [49] T. Jamiani, H. Kuroda, R. Yamaguchi, A. Abe, M. Hayashi, CD68- and CD163-positive tumor-associated macrophages in triple negative cancer of the breast, *Virchows Arch.* 477 (2020) 767–775, <https://doi.org/10.1007/s00428-020-02855-z>.
- [50] J. Ye, X. Wang, J. Shi, X. Yin, C. Chen, Y. Chen, H.-Y. Wu, S. Jiong, Q. sun, M. Zhang, X. Shi, G. Zhou, S. Hassan, J. Feng, X. Xu, W. Zhang, Tumor-associated macrophages are associated with response to neoadjuvant chemotherapy and poor outcomes in patients with triple-negative breast cancer, *J Cancer.* 12 (2021) 2886–2892, <https://doi.org/10.7150/jca.47566>.
- [51] W. Zhang, X. Wang, S. Gao, C. Chen, X. Xu, Q. sun, Z. Zhou, G. Wu, Q. Yu, G. Xu, Y.-Z. Yao, W. Guan, Tumor-associated macrophages correlate with phenomenon of epithelial-mesenchymal transition and contribute to poor prognosis in triple-negative breast cancer patients, *Journal of Surgical Research.* 222 (2018) 93–101. <https://doi.org/10.1016/j.jss.2017.09.035>.
- [52] V. Arole, H. Nitta, L. Wei, T. Shen, A.V. Parwani, Z. Li, M2 tumor-associated macrophages play important role in predicting response to neoadjuvant chemotherapy in triple-negative breast carcinoma, *Breast Cancer Res Treat.* 188 (2021) 37–42, <https://doi.org/10.1007/s10549-021-06260-1>.
- [53] H. Kuroda, T. Jamiani, R. Yamaguchi, A. Kakimoto, A. Abe, O. Harada, A. Masunaga, Tumor microenvironment in triple-negative breast cancer: the correlation of tumor-associated macrophages and tumor-infiltrating lymphocytes, *Clinical and Translational Oncology.* 23 (2021) 2513–2525, <https://doi.org/10.1007/s12094-021-02652-3>.
- [54] D. Hammerl, J.W.M. Martens, M. Timmermans, M. Smid, A.M. Trapman-Jansen, R. Foekens, O.I. Isaeva, L. Voorwerk, H.E. Balciooglu, R. Wijers, I. Nederlof, R. Salgado, H. Horlings, M. Kok, R. Debets, Spatial immunophenotypes predict

- response to anti-PD1 treatment and capture distinct paths of T cell evasion in triple negative breast cancer, *Nat Commun.* 12 (2021) 5668, <https://doi.org/10.1038/s41467-021-25962-0>.
- [55] M. Tkach, J. Thalmensi, E. Timperi, P. Gueguen, N. Névo, E. Grisard, P. Sirven, F. Cocozza, A. Gouronne, L. Martin-Jaular, M. Jouve, F. Delisle, N. Manel, D.C. Rookhuizen, C.L. Guerin, V. Soumelis, E. Romano, E. Segura, C. Théry, Extracellular vesicles from triple negative breast cancer promote pro-inflammatory macrophages associated with better clinical outcome, *Proceedings of the National Academy of Sciences.* 119 (2022). <https://doi.org/10.1073/pnas.2107394119>.
- [56] J. Steenbrugge, K. Breyne, K. Demeyere, O. De Wever, N.N. Sanders, W. Van Den Broeck, C. Colpaert, P. Vermeulen, S. Van Laere, E. Meyer, Anti-inflammatory signaling by mammary tumor cells mediates prometastatic macrophage polarization in an innovative intraductal mouse model for triple-negative breast cancer, *J. Experim. Clin. Cancer Res.* 37 (2018) 191, <https://doi.org/10.1186/s13046-018-0860-x>.
- [57] I. Sánchez-González, A. Bobien, C. Molnar, S. Schmid, M. Strotbek, M. Boerries, H. Busch, M.A. Olaiyoye, miR-149 Suppresses Breast Cancer Metastasis by Blocking Paracrine Interactions with Macrophages, *Cancer Res.* 80 (2020) 1330–1341, <https://doi.org/10.1158/0008-5472.CAN-19-1934>.
- [58] Y. Qi, T. Zhao, R. Li, M. Han, Macrophage-Secreted S100A4 Supports Breast Cancer Metastasis by Remodeling the Extracellular Matrix in the Premetastatic Niche, *Biomed Res Int.* 2022 (2022) 1–14, <https://doi.org/10.1155/2022/9895504>.
- [59] D.C. Rabe, N.D. Walker, F.D. Rustandy, J. Wallace, J. Lee, S.L. Stott, M.R. Rosner, Tumor Extracellular Vesicles Regulate Macrophage-Driven Metastasis through CCL5, *Cancers (Basel).* 13 (2021) 3459, <https://doi.org/10.3390/cancers13143459>.
- [60] L. Prasmickaita, E.M. Tenstad, S. Pettersen, S. Jabeen, E.V. Egeland, S. Nord, A. Pandya, M.H. Haugen, V.N. Kristensen, A. Børresen-Dale, O. Engebråten, G. Mælandsmo, Basal-like breast cancer engages tumor-supportive macrophages via secreted factors induced by extracellular S100A4, *Mol Oncol.* 12 (2018) 1540–1558, <https://doi.org/10.1002/1878-0261.12319>.
- [61] J.-H. Jang, D.-H. Kim, J.M. Lim, J.W. Lee, S.J. Jeong, K.P. Kim, Y.-J. Surh, Breast Cancer Cell-Derived Soluble CD44 Promotes Tumor Progression by Triggering Macrophage IL1 $\beta$  Production, *Cancer Res.* 80 (2020) 1342–1356, <https://doi.org/10.1158/0008-5472.CAN-19-2288>.
- [62] E. Sami, B.T. Paul, J.A. Koziol, W.M. ElShamy, The Immunosuppressive Microenvironment in BRCA1-IRIS-Overexpressing TNBC Tumors Is Induced by Bidirectional Interaction with Tumor-Associated Macrophages, *Cancer Res.* 80 (2020) 1102–1117, <https://doi.org/10.1158/0008-5472.CAN-19-2374>.
- [63] W. Jing, X. Guo, G. Wang, Y. Bi, L. Han, Q. Zhu, C. Qiu, M. Tanaka, Y. Zhao, Breast cancer cells promote CD169+ macrophage-associated immunosuppression through JAK2-mediated PD-L1 upregulation on macrophages, *Int Immunopharmacol.* 78 (2020), 106012, <https://doi.org/10.1016/j.intimp.2019.106012>.
- [64] A. Maimon, V. Levi-Yahid, K. Ben-Meir, A. Halpern, Z. Talmi, S. Priya, G. Mizraji, S. Mistrieli-Zerbib, M. Berger, M. Baniyash, S. Loges, T. Bustyn-Cohen, Myeloid cell-derived PROS1 inhibits tumor metastasis by regulating inflammatory and immune responses via IL-10, *J. Clin. Invest.* 131 (2021), <https://doi.org/10.1172/JCI126089>.
- [65] X. Chen, M. Yang, J. Yin, P. Li, S. Zeng, G. Zheng, Z. He, H. Liu, Q. Wang, F. Zhang, D. Chen, Tumor-associated macrophages promote epithelial–mesenchymal transition and the cancer stem cell properties in triple-negative breast cancer through CCL2/AKT/β-catenin signaling, *Cell Commun. Signal.* 20 (2022) 92, <https://doi.org/10.1186/s12964-022-00888-2>.
- [66] Q. Zhang, K. Le, M. Xu, J. Zhou, Y. Xiao, W. Yang, Y. Jiang, Z. Xi, T. Huang, Combined MEK inhibition and tumor-associated macrophages depletion suppresses tumor growth in a triple-negative breast cancer mouse model, *Int Immunopharmacol.* 76 (2019), 105864, <https://doi.org/10.1016/j.intimp.2019.105864>.
- [67] W. Ding, D. Li, P. Zhang, L. Shi, H. Dai, Y. Li, X. Bao, Y. Wang, H. Zhang, L. Deng, Mutual editing of alternative splicing between breast cancer cells and macrophages, *Oncol Rep.* 42 (2) (2019) 629–656, <https://doi.org/10.3892/or.2019.7200>.
- [68] Z. Weihua, Z. Guorong, C. Xiaolong, L. Weizhan, MiR-33a functions as a tumor suppressor in triple-negative breast cancer by targeting EZH2, *Cancer Cell Int.* 20 (2020) 85, <https://doi.org/10.1186/s12935-020-1160-z>.
- [69] M. Moradi-Chaleshtori, M. Bandehpour, N. Heidari, S. Mohammadi-Yeganeh, S. Mahmoud Hashemi, Exosome-mediated miR-33 transfer induces M1 polarization in mouse macrophages and exerts antitumor effect in 4T1 breast cancer cell line, *Int Immunopharmacol.* 90 (2021), 107198, <https://doi.org/10.1016/j.intimp.2020.107198>.
- [70] C. Ma, D. He, P. Tian, Y. Wang, Y. He, Q. Wu, Z. Jia, X. Zhang, P. Zhang, H. Ying, Z.-B. Jin, G. Hu, miR-182 targeting reprograms tumor-associated macrophages and limits breast cancer progression, *Proceedings of the National Academy of Sciences.* 119 (2022). <https://doi.org/10.1073/pnas.2114006119>.
- [71] Y. Li, L. Zhao, B. Shi, S. Ma, Z. Xu, Y. Ge, Y. Liu, D. Zheng, J. Shi, Functions of miR-146a and miR-222 in Tumor-associated Macrophages in Breast Cancer, *Sci Rep.* 5 (2015) 18648, <https://doi.org/10.1038/srep18648>.
- [72] X. Wang, H. Qiu, R. Tang, H. Song, H. Pan, Z. Feng, L. Chen, miR-30a inhibits epithelial–mesenchymal transition and metastasis in triple-negative breast cancer by targeting ROR1, *Oncol Rep.* (2018), <https://doi.org/10.3892/or.2018.6379>.
- [73] E. Shin, J.S. Koo, Glucose Metabolism and Glucose Transporters in Breast Cancer, *Front Cell Dev Biol.* 9 (2021), <https://doi.org/10.3389/fcell.2021.728759>.
- [74] P. Tiwari, A. Blank, C. Cui, K.Q. Schoenfelt, G. Zhou, Y. Xu, G. Khramtsova, F. Olopade, A.M. Shah, S.A. Khan, M.R. Rosner, L. Becker, Metabolically activated adipose tissue macrophages link obesity to triple-negative breast cancer, *J. Experim. Med.* 216 (2019) 1345–1358, <https://doi.org/10.1084/jem.20181616>.
- [75] B. Kuttryb-Zajac, G. Harasim, A. Jedrzejewska, O. Krol, A. Braczko, P. Jablonska, P. Mierzejewska, J. Zieliński, E.M. Slominska, T. Ryszard, Smolenski, Macrophage-Derived Adenosine Deaminase 2 Correlates with M2 Macrophage Phenotype in Triple Negative Breast Cancer, *Int J Mol Sci.* 22 (2021) 3764, <https://doi.org/10.3390/ijms22073764>.
- [76] S.A. O'Brien, J. Orf, K.M. Skrzypczynska, H. Tan, J. Kim, J. DeVoss, B. Belmontes, J.G. Egen, Activity of tumor-associated macrophage depletion by CSF1R blockade is highly dependent on the tumor model and timing of treatment, *Cancer Immunol Immunother.* 70 (2021) 2401–2410, <https://doi.org/10.1007/s00262-021-02861-3>.
- [77] D.C. Strachan, B. Ruffell, Y. Oei, M.J. Bissell, L.M. Coussens, N. Pryer, D. Daniel, 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, *Oncioimmunology.* 2 (2013) e26968.
- [78] A. Lepland, A. Malfanti, U. Haljasorg, E.K. Asciutto, M. Pickholz, M. Bringas, S. Dordić, L. Salumäe, P. Peterson, T. Teesalu, M.J. Vicent, P. Scodeller, Depletion of Mannose Receptor-Positive Tumor-associated Macrophages via a Peptide-targeted Star-shaped Polyglutamate Inhibits Breast Cancer Progression in Mice, *Cancer Res. Commun.* 2 (2022) 533–551, <https://doi.org/10.1158/2767-9764.CRC-22-0043>.
- [79] S. Shen, Y. Zhang, K.-G. Chen, Y.-L. Luo, J. Wang, Cationic Polymeric Nanoparticle Delivering CCR2 siRNA to Inflammatory Monocytes for Tumor Microenvironment Modification and Cancer Therapy, *Mol Pharm.* 15 (2018) 3642–3653, <https://doi.org/10.1021/acs.molpharmaceut.7b00997>.
- [80] F. Leonard, L.T. Curtis, A.R. Hamed, C. Zhang, E. Chau, D. Sieving, B. Godin, H. B. Frieboes, Nonlinear response to cancer nanotherapy due to macrophage interactions revealed by mathematical modeling and evaluated in a murine model via CRISPR-modulated macrophage polarization, *Cancer Immunol Immunother.* 69 (2020) 731–744, <https://doi.org/10.1007/s00262-020-02504-z>.
- [81] S. Zanganeh, G. Hutter, R. Spitzer, O. Lenkov, M. Mahmoudi, A. Shaw, J. S. Pajarinen, H. Nejadnik, S. Goodman, M. Moseley, L.M. Coussens, H.E. Daldrup-Link, Iron oxide nanoparticles inhibit tumour growth by inducing pro-inflammatory macrophage polarization in tumour tissues, *Nat Nanotechnol.* 11 (2016) 986–994, <https://doi.org/10.1038/nano.2016.168>.
- [82] T. Boulding, R.D. McCuaig, A. Tan, K. Hardy, F. Wu, J. Dunn, M. Kalimutho, C. R. Sutton, J.K. Forwood, A.G. Bert, G.J. Goodall, L. Malik, D. Yip, J.E. Dahlstrom, A. Zafar, K.K. Khanna, S. Rao, LSD1 activation promotes inducible EMT programs and modulates the tumour microenvironment in breast cancer, *Sci Rep.* 8 (2018) 73, <https://doi.org/10.1038/s41598-017-17913-x>.
- [83] A.H.Y. Tan, W. Tu, R. McCuaig, K. Hardy, T. Donovan, S. Tsimbalyuk, J. K. Forwood, S. Rao, Lysine-Specific Histone Demethylase 1A Regulates Macrophage Polarization and Checkpoint Molecules in the Tumor Microenvironment of Triple-Negative Breast Cancer, *Front Immunol.* 10 (2019), <https://doi.org/10.3389/fimmu.2019.01351>.
- [84] M.H. Oh, I.H. Sun, L. Zhao, R.D. Leone, I.M. Sun, W. Xu, S.L. Collins, A.J. Tam, R. L. Blosser, C.H. Patel, J.M. Englert, M.L. Arwood, J. Wen, Y. Chan-Li, L. Tenora, P. Majer, R. Rais, B.S. Slusher, M.R. Horton, J.D. Powell, Targeting glutamine metabolism enhances tumor-specific immunity by modulating suppressive myeloid cells, *J Clin Invest.* 130 (7) (2020) 3865–3884, <https://doi.org/10.1172/JCI131859>.
- [85] T. Li, Q. Zhang, J. Zhang, G. Yang, Z. Shao, J. Luo, M. Fan, C. Ni, Z. Wu, X. Hu, Fenofibrate induces apoptosis of triple-negative breast cancer cells via activation of NF-κB pathway, *BMC Cancer.* 14 (2014) 96, <https://doi.org/10.1186/1471-2407-14-96>.
- [86] Y. Zheng, N. Wang, S. Wang, B. Yang, H. Situ, L. Zhong, Y. Lin, Z. Wang, XIAOPI formula inhibits the pre-metastatic niche formation in breast cancer via suppressing TAMs/CXCL1 signaling, *Cell Commun Signal.* 18 (2020) 48, <https://doi.org/10.1186/s12964-020-0520-6>.
- [87] L. Yin, Z. Fan, P. Liu, L. Chen, Z. Guan, Y. Liu, Y. Luo, Anemoside A3 activates TLR4-dependent M1-phenotype macrophage polarization to represses breast tumor growth and angiogenesis, *Toxicol Appl Pharmacol.* 432 (2021), 115755, <https://doi.org/10.1016/j.taap.2021.115755>.
- [88] J. Li, H. Cai, H. Sun, J. Qu, B. Zhao, X. Hu, W. Li, Z. Qian, X. Yu, F. Kang, W. Wang, Z. Zou, B. Gu, K. Xu, Extracts of Cordyceps sinensis inhibit breast cancer growth through promoting M1 macrophage polarization via NF-κB pathway activation, *J Ethnopharmacol.* 260 (2020), 112969, <https://doi.org/10.1016/j.jep.2020.112969>.
- [89] X.-X. Deng, Y.-N. Jiao, H.-F. Hao, D. Xue, C.-C. Bai, S.-Y. Han, Taraxacum mongolicum extract inhibited malignant phenotype of triple-negative breast cancer cells in tumor-associated macrophages microenvironment through suppressing IL-10 / STAT3 / PD-L1 signaling pathways, *J Ethnopharmacol.* 274 (2021), 113978, <https://doi.org/10.1016/j.jep.2021.113978>.
- [90] Protein-Bound Polysaccharides from Coriolus Versicolor Fungus Disrupt the Crosstalk Between Breast Cancer Cells and Macrophages through Inhibition of Angiogenic Cytokines Production and Shifting Tumour-Associated Macrophages from the M2 to M1 Subtype, *Cellular Physiology and Biochemistry.* 54 (2020) 615–628, <https://doi.org/10.33594/000000244>.
- [91] A.S. Franzén, M.J. Raftery, G. Pecher, Implications for Immunotherapy of Breast Cancer by Understanding the Microenvironment of a Solid Tumor, *Cancers (Basel).* 14 (2022) 3178, <https://doi.org/10.3390/cancers14133178>.