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REVIEW

Galectin-3 as a biomarker in breast neoplasms: Mechanisms and applications in patient care

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Abstract

Galectin-3 is a member of the lectin family encoded by the *LGALS3* gene on chromosome 14. It is secreted by a wide range of immune cells and mammary tumor cells. Through its activity on the tumor microenvironment, in particular on tumor-infiltrating leukocytes, galectin-3 improves the proliferation, survival, and colonizing ability of mammary neoplastic cells. Consequently, galectin-3 expression in the tumor microenvironment could worsen therapeutic outcomes of breast neoplasms and become a biomarker and a therapeutic target in combined immunotherapy in breast neoplasms. There is a limited amount of information that is available on galectin-3 in breast cancer in Africa. In this review, we analyze how galectin-3 influences the tumor microenvironment and its potential as a biomarker and therapeutic target in breast neoplasms. We aim to emphasize the significance of investigating galectin-3 in breast neoplasms in Africa based on the results of studies conducted elsewhere.

KEYWORDS
immunotherapy, M2 macrophage, NK cell, TILs, tumor microenvironment

1 | INTRODUCTION

Lectins are proteins that are able to bind to specific carbohydrate structures; galectins are 1 among the 4 lectin families.[1] This family is defined by an extensive shared amino acid sequence that has an affinity for β-galactosides (i.e., lactose).[2,3] All galectins have a highly conserved core sequence of 130 amino acid residues.[4] In this core sequence, the carbohydrate recognition domain (CRD) of galectins occurs within the domain of residues 30–90. The CRD is implicated in interactions with β-galactosides and forms a part of the biologic functions of galectins.[5] Another interesting feature of galectins is their lack of a signal peptide for secretion. Their di- or multivalence provides galectins with an ability to crosslink ligands, which is the origin of their multiple biologic functions.[6]

Previously known as carbohydrate binding protein (CBP)-35, Mac-P, IgEBP, CBP-30, RL-29, L-29, L-31, L-34, LBL (and other names), galectin-3 is the only known chimeric galectin.[2,7] Since its discovery in 1971, galectin-3 has become the most studied galectin.[8] The role of galectin-3 has been implicated in a great number of human pathologic processes, including neoplasia and has been reviewed elsewhere,[8] yet only a few of the papers on galectin-3 extracted from PubMed referred to breast neoplasms (U.S. National Library of Medicine, Medical Institutes of Health). Moreover, despite the unequal distribution of the galectin-3 variants depending on ethnicity,[9–11] there is a dearth of published data about galectin-3 in cancers in Africa.

Abbreviations: AIP-1, ALG-2 interacting protein-1; ALG-2, apoptosis-linked gene-2; ALIX, ALG-2 Linked protein X; Bcl2, B Cell Lymphoma 2; CA, carbohydrate antigen; CBP, carbohydrate binding protein; CD, cluster of differentiation; CRD, carbohydrate recognition domain; Gal-3, galectin-3; MCP, modified citrus pectin; MDSC, myeloid-derived suppressor cell; NWGR, asparagine-tryptophan-glycine-arginine; PD-1, program death-1; PD-L1, program death-ligand 1; pRB, retinoblastoma protein; SNV, single nucleotide variation; TAM, tumor-associated macrophage; TIL, tumor-infiltrating lymphocyte.

TABLE 1 Galectin-3 allele frequencies depending on ethnicity[29]

SNV[a]	Alleles[b]	Galectin-3 gene allele frequencies					Clinical significance[c]
		European	African	American	East Asian	South Asian	
rs10148371	g.18484G	0.999	0.880	0.991	1.000	1.000	Yes (benign)
rs11125	g.20905A	0.921	0.999	0.921	0.994	0.908	Not reported
rs189994172	g.19269G	0.997	0.999	1.000	1.000	0.998	Not reported
rs4644	g.14001C	0.592	0.747	0.722	0.812	0.651	Not reported
rs4652	g.14102A	0.561	0.054	0.611	0.587	0.515	Not reported
rs556975452	g.14099G	0.999	0.995	0.999	1.000	0.998	Not reported
rs571941262	g.19231G	1.000	0.995	1.000	1.000	1.000	Not reported
rs6573005	g.19308C	0.557	0.286	0.666	0.749	0.556	Not reported

Note: Bold entries: examples of single nucleotide variation with frequency variability depending on the ethnicity.
[a]SNV, single nucleotide variation.
[b]g.18484G refers to a guanine at the position 18484 in the *LGALS3* gene.
[c]Implication in disease reported in CLINVAR database (NCBI).

While in literature, high galectin-3 expression in breast neoplasms has been related to low receptor positivity, aggressive disease, and higher mortality rate, the same disease patterns have been reported to be frequent in African Americans and African women.[12–15] These disease patterns in Africa might be a demographic-driven phenomenon however, galectin-3 involvement should be investigated because of its potential as a biomarker and a therapeutic target.[16,17]

In this review, we describe how galectin-3 influences the tumor microenvironment through regulation of a wide range of biologic processes and discuss its potential as a biomarker and a therapeutic target in breast neoplasms so as to emphasize the need for greater investigation in Africa.

2 | GALECTIN-3: GENE, EXPRESSION, AND BIOLOGIC FUNCTIONS

Galectin-3 is encoded by the *LGALS3* gene with chromosomal location 14q21-q22.[18] Currently, 292 coding SNVs of galectin-3 have been described in the literature. Table 1 shows the frequency of some galectin-3 SNVs depending on ethnicity.[11] Table 1 was obtained from the Ensembl variation (SNP) database filtered according to an appreciable percentage of minor allele frequency in Africans. Correspondingly, some alleles show frequency variability depending on ethnicity. For example, Balan and colleagues[10] have reported a higher frequency of the galectin-3 variant rs4644 NM_002306.4:c.191C > A(p.Pro64Hist) in Caucasians compared with Asian women, which is potentially associated with a higher risk of breast neoplasms. Furthermore, rs10148371 NM_0023064.4:c.548G > A(p.Arg183Lys), located in the antideath motif of galectin-3 gene, has been investigated by Patrima Nangia-Makker and colleagues[9] and estimated at frequency of 22% in the normal population of African Americans versus less than 1% in Caucasians and Asians. In summation, the alternative alleles of the rs4652 and rs10148371 are more frequent in Africans whereas the alternative allele of the rs4644 is more frequent in Caucasians. The rs10148371 and rs4644 could be associated respectively with poor prognosis in colon and breast neoplasms.[9,10] *LGALS3* gene can be transcribed in 3 protein-coding splice variants. In adults, the main variant (UNIPROT database accession number: P17931) was detected in numerous cell and tissue types including epithelial cells of organs (e.g., small intestine, colon, cornea, ear, kidneys, lungs, thymus, breast, prostate).[5,19] Galectin-3 was also expressed in immune cells such as macrophages,[20] monocytes,[21] dendritic cells (DCs),[22] eosinophils,[23] mast cells,[24] and NK cells.[25] Normally, T and B lymphocytes do not express galectin-3 even if its expression can be induced by different stimuli.[5,26]

Although a large body of reported data about galectin-3 expression is available in the literature, its mechanisms of regulation are still poorly understood.[19,27,28] The promoter region of the *LGALS3* gene contains several regulatory elements.[29,30] Moreover, evidence of 2 p53 binding sites in the promoter region of *LGALS3* has been reported. This protein down-regulates galectin-3 expression[31] and as consequence, p53 mutations in cancers might be related to galectin-3 overexpression. Its expression regulation is also in part achieved by CpG island methylation in the promoter region of the *LGALS3* gene. The known molecular regulations of galectin-3 was detailed in a review by Wang and colleagues.[32]

Galectin-3 is the only vertebrate chimera type galectin identified thus far. Galectin-3 monomers are composed of 2 functionally different domains: an unusual long N-terminal domain (residues 1–137) and a C-terminal domain (residues 118 –264) (Figure 1).[33–36] The N-terminal domain does not bind to carbohydrates, is essential for galectin-3 antiapoptotic activity, and is responsible for galectin-3 protein–protein interactions that is necessary for the additional biologic activities absent in other galectins.[37] Due to its long N-terminal domain, galectin-3 monomers self-aggregate and form a ring-like structure that is necessary to achieve certain unique biologic functions.[38] The C-terminal domain forms a globular structure and accommodates the whole carbohydrate-binding site, thereby making it responsible for the lectin activity of galectin-3.[34,39] The CRD contains a unique amino

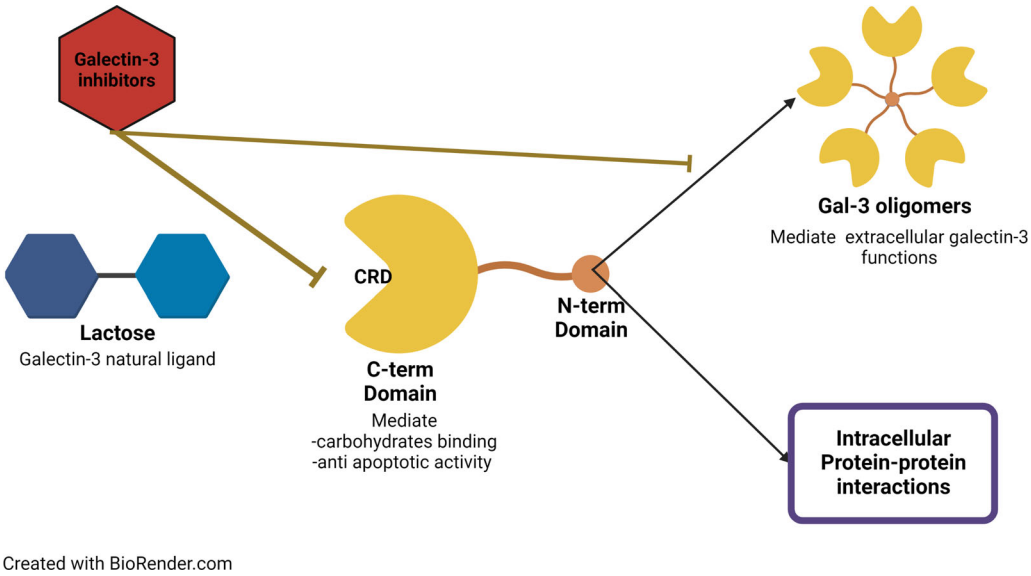

FIGURE 1 Galectin-3 structure, natural ligand, and inhibition mechanism; lactose is the standard ligand of galectin-3; lactose binds to galectin-3 through its carbohydrate recognition domain (CRD) carried by the C-terminal (C-term) domain and induces its oligomerization; N-terminal (N-term) domains crosslink galectin-3 monomers; the CRD participates in galectin-3 antiapoptotic activity; the N-terminal domain mediates intracellular protein–protein interactions while galectin-3 oligomers carry the most of the extracellular galectin-3 functions; galectin-3 inhibitors block the CRD and inhibit galectin-3′s oligomerization

acid sequence among galectins, which is shared with the Bcl2 protein family, the Asn-Trp-Gly-Arg. This motif is in part responsible for the antiapoptotic activity of both galectin-3 and Bcl2.[40] Galectin-3 biologic functions are related to its subcellular location that may differ depending on the cell type[5,26] and can be found both in the cytoplasm and the nucleus of cells.

Biologic functions of galectin-3 are triggered by binding to a wide variety of ligands through lectin-carbohydrate or protein–protein interactions. N-acetyllactosamine is the preferential ligand for the galectin-3 CRD.[5] In the extracellular matrix, laminin, fibronectin, and tenascin are its ligands[41–46] that are overexpressed in breast neoplasms.[47] In the cytoplasm, galectin-3 has proven to bind to cytokeratins, CBP-70, Chrp, geminin-4, ALIX, AIP-1, Bcl2, and MCF7.[48–52] In the nucleus, galectin-3 known counterparts are: geminin-4 (implicated in spliceosome assembly), ALG-2 linked protein (ALIX), and ALG-2 interacting protein-1 (AIP-1) that are part of a cell death pathway.[50,51]

Various biologic functions were ascribed to galectin-3 and exhaustively reviewed by Dumic and colleagues.[19] Depending on the ligand localization, galectin-3 regulates different biologic functions.[53,54] Cytosolic galectin-3 participates in apoptosis inhibition through interactions with Bcl2 and other proteins involved in apoptotic signaling such as cluster of differentiation (CD)95, Nucling, and ALIX/AIP-1.[40,51,53,55] Additionally, galectin-3 abolishes apoptosis by binding with cytosolic synexin in human epithelial cells.[56] Furthermore, galectin-3 not only blocks apoptosis but also regulates cell proliferation and survival through interactions with K-Ras and Akt proteins.[57–60] The extracellular galectin-3 mediates numerous autocrine/paracrine effects (cell adhesion, cell activation) and acts as a chemoattractant for some cells such as macrophages and neutrophils. In this way, galectin-3 involvement in several biologic processes has been reported in cellular homeostasis, immune reactions, organogenesis, and angiogenesis.[54,61,62] Despite its proinflammatory activity reported in numerous studies, galectin-3 seems to dampen immune responses in processes such as wound healing and fetus tolerance during pregnancy.[63–66]

Knowing the regulation of the expression and the functional properties of galectin-3 reported above, this lectin could play a key role in cancer, particularly in breast neoplasms where tumor cells could hijack galectin-3 biologic functions to proliferate, spread, and escape from the immune response.

3 | GALECTIN-3 INFLUENCE ON TUMOR MICROENVIRONMENT

Galectin-3 is implicated in a wide range of biologic processes linked to cancer. This lectin was reported to be secreted by tumor cells and other cells in the tumor microenvironment such as fibroblasts and leukocytes.[8,67] Despite the experimental evidence of the overexpression of galectin-3 in breast neoplasms and its potential involvement in the worse outcome of the disease, the precise role of galectin-3 is still under investigation.[67] Nevertheless, through its influence in a myriad of cancer-related processes, galectin-3 has the potential to promote survival, growth, proliferation, and metastasis of breast tumor cells.[19] Hence, galectin-3 could protect breast neoplasms from therapy and body defenses by acting as a "guardian of tumor microenvironment" due to direct activity in tumor cells and an indirect activity on the tumor microenvironment (extracellular matrix, stromal cells, and immune cells) (Figure 2).[68]

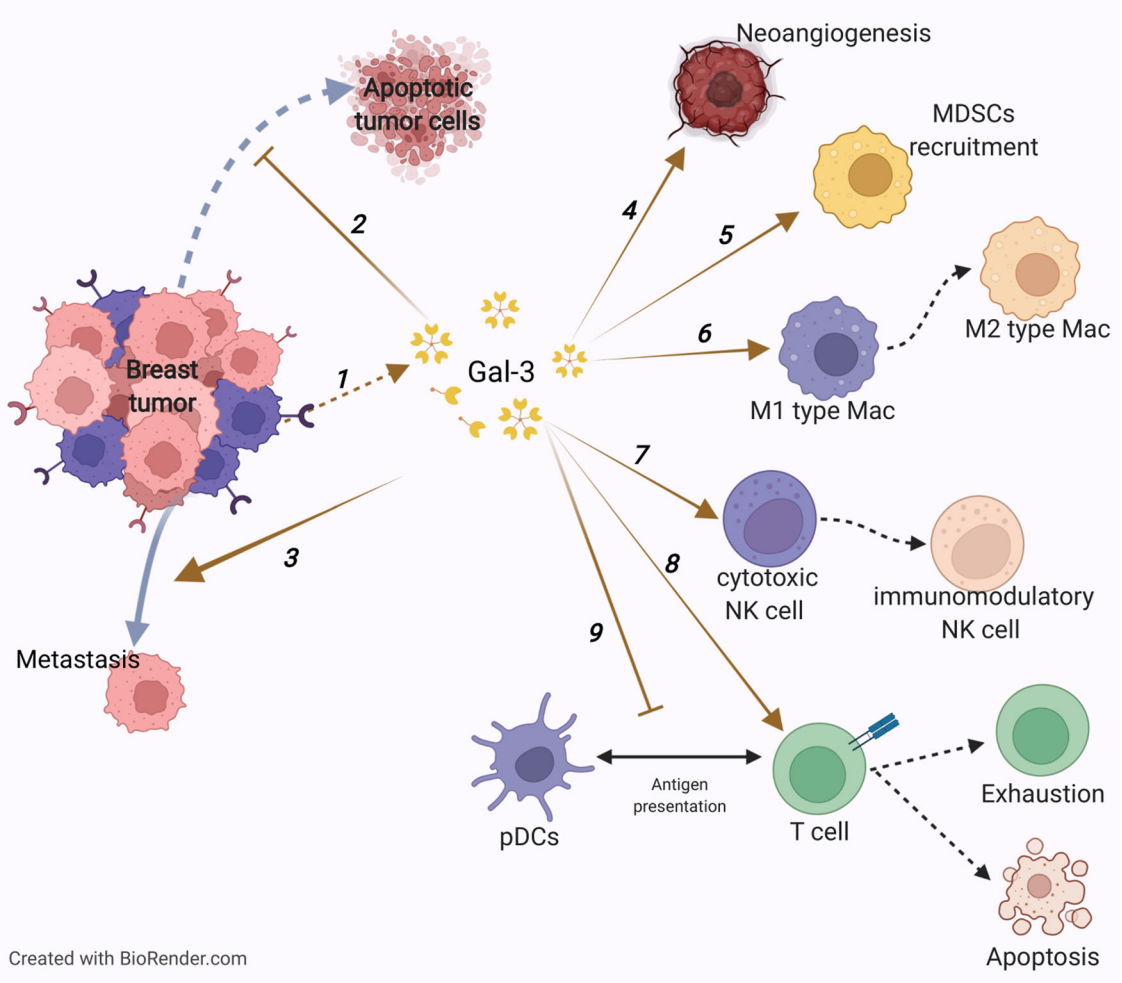

FIGURE 2 Galectin-3 promotes tumor growth, metastasis, and escape from immune system; ***1***: gal-3 is secreted in the tumor microenvironment by tumor cells; ***2***: gal-3 inhibits tumor cells apoptosis; ***3***: gal-3 promotes metastasis; ***4***: gal-3 favors neo-angiogenesis; ***5***: gal-3 recruits MDSCs; ***6***: gal-3 enhances switch of macrophages from M1 to M2 phenotypes; ***7***: gal-3 modifies NK cells effector activity from cytotoxicity to immunomodulation; ***8***: gal-3 induces T cell exhaustion and apoptosis; ***9***: gal-3 depletes pDCs and alters antigen presentation; Gal-3, galectin-3; MDSCs, myeloid derived suppressor cells; cNK, cytotoxic NK; iNK, immunomodulatory NK; Exh, exhausted; Ap, apoptotic; pDCs, plasmacytoid dendritic cells

3.1 | Galectin-3 immortalizes breast tumor cells

Intracellular galectin-3 protects breast tumor cells from drugs and cell-to-cell-contact-mediated apoptosis (Figure 2). Indeed, an in vitro experiment using the human breast carcinoma BT549 cell line has shown the galectin-3 protective effect against cis-diamminedichloroplatinium (cis-platin)-induced apoptosis.[69] However, the wild-type BT549 cells do not express galectin-3. In this experiment, when these cells were transfected with the galectin-3 gene, they became more resistant to cisplatin-induced apoptosis.[69] Through mutagenesis study, the Asn-Trp-Gly-Arg motif of galectin-3 CRD was demonstrated to be necessary for this antiapoptotic activity.[52] Intracellular galectin-3 not only prevents drug-mediated cell death, but also anoikis—the apoptosis of epithelial cells induced by a loss of adhesion to the extracellular matrix.[70] This mechanism could prevent breast carcinoma dissemination and metastasis. In BT549 cells, overexpression of galectin-3 was associated with a cell cycle arrest in the G1 phase that is insensitive to anoikis. In a more recent in vitro study about the consequences of galectin-3 overexpression in Evsa-T, another breast cancer cell line, it has been shown that galectin-3 could also protect cells from anoikis by preventing loss of adherence.[71] The mechanism underlying this is a galectin-3-mediated up-regulation of integrins such as $\alpha 4\beta 7$ in breast tumor cells. It increases the adhesion capacity to extracellular matrix components such as laminin, fibronectin, and vitronectin resulting in breast tumor cells becoming less sensitive to other apoptosis stimuli such as cytokines and radiations. Taken together, these in vitro studies suggest that intracellular galectin-3 protects breast tumor cells from apoptosis induced by different stimuli and lectin does not induce detachment of metastatic cells from the tumor mass but keeps them alive thereafter.[71] Inversely, a study using another breast cancer cell line (MDA-MB-231 human breast carcinoma cells) has reported that intracellular galectin-3 acts synergistically with phosphocaveolin-1 to

promote integrin-dependent matrix remodeling and cell migration.[72] Moreover, in a xenograft model, unsecreted galectin-3 on the breast tumor cellular membranes was shown to be up-regulated proximal to the stroma. This localized expression might facilitate tumor–stromal interactions and consequently improve endothelial cells adhesion, resulting in invasion and metastatic progression.[73] Knowing that galectin-3 activity differs depending on the cell types and its subcellular localization, one can surmise that both cell surface and intracellular galectin-3 enhance breast tumor cells motility and metastasis.

Extracellular galectin-3 could also play a key role in breast tumor cells survival and metastasis. Galectin-3 localization in the extracellular *milieu* has been proven by evidence of its expression and secretion by tumor cells, stromal cells, and leukocytes such as macrophages present in the tumor microenvironment.[20,67] Moreover, other studies reported increased plasmatic levels of galectin-3 in breast and other cancer patients.[74–76] In vitro studies have highlighted the potential role of extracellular galectin-3 in tumor microenvironment. Lagana and colleagues[77] showed that the addition of galectin-3 in mammary cells carcinoma culture stimulates $\alpha 5\beta 1$ integrin activation, thus enhancing fibronectin-dependent tumor cell spreading and motility. A more recent investigation revealed that extracellular galectin-3 could favor breast tumor metastasis through enhancement of N-cadherin turnover, thereby increasing motility in murine mammary cancer cells.[78] The study cited above emphasizes the potential protective role of intracellular galectin-3 against apoptosis and the prometastatic activity of extracellular galectin-3 in breast neoplasms.

In the tumor niche, galectin-3 favors tumor cells adaptation to a hostile tumor microenvironment that could be hypoxic, deprived of nutrients, and contain cytotoxic reactive oxygen species (ROS).[79,80] First, hypoxia has been reported to induce galectin-3 overexpression. It is responsible for the transformation of cell metabolism from oxidative phosphorylation to glycolysis and increases resistance against hypoxia and nutrient deprivation. Second, lectin promotes angiogenesis that improves the homeostasis of the hypoxic microenvironment and nutrient deficient tissues.[81–83] Galectin-3-induced angiogenesis is a consequence of 2 mechanisms: (i) direct activity on differentiation of bone marrow mesenchymal stem cells toward endothelial cells; (ii) enhancement of the proangiogenic activity of tumor-associated macrophages (TAMs).[84] Third, galectin-3 makes tumor cells more resistant to ROS-induced apoptosis. Galectin-3 expression decreases ROS production by increasing glutathione-S-transferase expression,[85] it has been shown to make breast cancer cell line BT549 fully resistant to nitric oxide-induced apoptosis.[86,87] Thus, galectin-3 might keep breast tumor cells alive while they are benefiting from consequences of oxidative stress that are protumor/genomic/metabolic changes in neoplastic cells.[80]

3.2 | Galectin-3 manipulates tumor-infiltrating leukocytes

Galectin-3 also helps tumor cells to escape from the immune response by interacting with both innate and adaptive tumor-infiltrating leukocytes (Figure 2).[88,89] In vitro and in vivo findings from several studies have brought a convergent body of evidence about galectin-3′s role in breast tumor escape to the immune response. Galectin-3 influences 5 immune cell behaviors: DCs, macrophages, myeloid-derived suppressor cells (MDSCs), NK cells, and T lymphocytes.

3.2.1 | Macrophages

An important component of solid tumor mass are TAMs.[90] TAMs are a macrophage population recruited and educated by tumors that are closely related to M2 type-macrophages also known as alternatively activated macrophages.[91,92] In contrast to M1 type macrophages that secrete inflammatory cytokines, M2 type macrophages prefer performing trophic and immunosuppressive tasks.[93,94] In a large cohort study of breast primary tumors (562 tissue microarray samples), Sousa and colleagues[95] reported that positive M2 macrophage cells were strongly associated with fast proliferation, poor differentiation, and histologic ductal types. Some of the tumor characteristics associated with high infiltration levels of M2 macrophages seemed to overlap characteristics of tumors that highly expressed galectin-3.[12] In this study, authors demonstrated that among the 3 breast cancer cell lines, the galectin-3 expressing breast tumor cell line MDA-MB231 was the only cell line able to yield a macrophage differentiation toward the M2 type.[95,96] In this study, it was suspected that a secretion of high levels of M-CSF was the driving mechanism of the M2 phenotype acquisition by macrophages. However, galectin-3 may have played a role in this process. In a study using galectin-3 knock-out mice inoculated with tumor allograft expressing galectin-3, the authors showed that the tumor cells created a galectin-3 gradient that recruits M2 type macrophages.[88] The study found that galectin-3 not only recruits M2 type macrophages but is also essential for their activation. Either the blockade of galectin-3 membrane receptor CD98 or inhibition of its signaling pathway and the use of an inhibitor of the galectin-3 CRF led to the same consequences on alternative macrophages activation.[97] This conclusion was supported by a subsequent experiment of a knock-out of galectin-3 in mice that abrogates the IL-4-mediated alternative macrophages activation.[97] Finally, an in vitro study has shown that under hypoxic conditions, M2 type macrophages secrete a high amount of galectin-3 that contributes to breast neoplasm progression.[98]

3.2.2 | Granulocytes

Macrophages are not the only phagocytic cells recruited by galectin-3. Lectin has also proven to have an ability to induce neutrophils recruitment and activation.[86,99] Neutrophils facilitate breast tumor cells metastasis by inducing immunosuppression in an IL-17A dependent manner.[100] Consequently, galectin-3 could indirectly promote breast neoplasm metastasis.

3.2.3 | MDSCs

MDSCs are immunosuppressive and are another cell type influenced by galectin-3.[101,102] They are switched into harmful effector cells under

influence of certain molecular microenvironments that induce impairment antitumor functions mainly through the production of arginase-1. MDSC also promotes tumor expansion with additional immune mechanisms (mediated by PD-L1 and TGF-β) and nonimmune mechanisms that support cancer stemness, tumor invasion, and metastasis. Biologic and protumor roles in breast neoplasms of MDSCs have been reviewed in other studies.[103,104,105] In a mice model, galectin-3 were shown to recruit MDSCs in the tumor microenvironment.[106] Moreover, Sturgill and colleagues,[107] using a mice model injected with breast tumor cell line 4t1, demonstrated that a galectin-3 inhibitor combined with anti-OX40 therapy is able to reduce tumor-infiltrating MDSCs rehabilitating in this way the CD8 T cell antitumor response.

3.2.4 | Innate lymphocytes

Breast tumor cells may also manipulate innate lymphocytes such as NK cells by secreting galectin-3. NK cells play a key role in antibreast tumor immunity, proof of its role in this process has been emphasized using NOD/SCID mice (which lack adaptive immunity) and NOD/SCID/γ-c^{null} mice (which lack adaptive and NK cells). When transplanted with a breast tumor cell line, the first type of mice developed localized tumors, whereas the second underwent a metastatic disease, thus providing further evidence of the role of NK cells in metastasis prevention.[108] In accordance, NK cells are able to target and kill breast tumor cells that down-regulate human leukocyte antigen-I expression.[109] Several gene expression studies show that a better outcome is associated with a strong cytotoxic infiltrate containing NK cells[110–112] and potential implication of NK cells in the response to immunotherapy have been reported.[113] Breast neoplasms impair NK cells' antitumor functions. The neoplastic mammary cells appear to cause down-regulation of peripheral blood NK cell activation receptors (NKp30, NKG2D, DNAM-1, 2B4, and CD16) while eliciting NK cells inhibitory receptors were up-regulated in patients with invasive and metastatic diseases. In this study, tumor-infiltrating NK cells carried more of the immunomodulatory phenotype (CD56^{high}, CD16I^{ow}) than the cytotoxic (CD56^{dim}, CD16^{high}).[114] NK cells functional impairment in breast neoplasms could be attributed to the release of galectin-3 by neoplastic mammary cells and M2 type macrophages in the tumor microenvironment.[98] To date, all innate lymphoid cells known among which NK cells are potent in expressing the receptor NKp30. After binding with its activator ligands, NKp30 triggers the NK cells' cytotoxicity and the release of IFN-γ.[115,116] Galectin-3 binds to NKp30 and abrogates NK cells' cytotoxicity and IFN-γ release.[116] This is further supported by an in vitro study where NK cells cytotoxicity against MDA-MB-435 breast cancer cells was restored using galectin-3 inhibitors.[117]

In summary, galectin-3 can be involved in several breast tumor escape strategies from the innate immune response. NK cells present within the tumor mass belong to tumor-infiltrating lymphocytes (TILs). In mammary tumors, NK cells account for approximately 5% of the TILs. The other lymphoid cells within the tumor mass are mainly T lymphocytes whose antitumor functions might also be altered by galectin-3.[118]

3.2.5 | T lymphocytes

Several studies have reported a suppressive effect of galectin-3 on TILs antitumor functions.[89,118,119] TILs count was positively correlated with the survival period of breast neoplasm patients. Among these TILs, CD8$^+$ T cells were the lymphocytes type that correlated better with overall patient clinical outcomes.[118] Moreover, patients with a high CD8$^+$ TILs percentage had a better response to immunotherapy. In immunotherapy efficiency, the involvement of this T lymphocyte subset has been reviewed elsewhere.[118] Galectin-3 could prevent TILs killing of breast tumor cells through several ways such as (i) disruption of tumor antigens presentation to TILs; (ii) inhibition of TILs activation; (iii) enhancement of TILs apoptosis and phagocytosis by macrophages; (iv) reduction of T cells recruitment in the tumor microenvironment.

Galectin-3 reduces the number of circulating plasmacytoid dendritic cells (pDCs) that are superior to conventional DCs in CD8+ T cell activation. This hypothesis was confirmed in a mice model study in which galectin-3 knock-out increased the number of circulating pDCs.[119] The study found that intracellular galectin-3 could inhibit antigen presentation to CD4$^+$ T lymphocytes because of its ability to mediate translocation of ALIX from the cytoplasm to the immunologic synapse. Thereby galectin-3 reduced the secretion of IFN-γ.[89] Even if the antigen presentation to T cells succeeds, galectin-3 is still able to prevent antitumor effector functions of TILs through their exhaustion. Several studies have shown that galectin-3 antagonists and antigalectin-3 antibodies were potent in restoring cytotoxicity and IFN-γ release of exhausted CD8$^+$ T cells.[119–124] This emphasizes the potential of combining galectin-3 blockade with adoptive T cell therapy in tumors. Mechanisms are proposed for explaining galectin-3 mediation of T lymphocytes exhaustion for example, the impairment of T lymphocytes' antitumor function either by the prevention of TCR aggregation or through the binding and activation of LAG-3 on CD8$^+$ T cells membrane.[60,119]

Furthermore, extracellular galectin-3 released by tumor cells induces T cell death. Stowell and colleagues[125] reported that galectin-3 increases the expression of phosphatidylserine on the T cell surface. The consequences of this phosphatidylserine expression are either apoptosis of T cells or making them targets for macrophages phagocytosis.[125] Additionally, when binding to CD29 and CD7, galectin-3 induces T cell death by triggering the intrinsic apoptosis pathway.[126] Finally, galectin-3 crosslinks IFN-γ with the extracellular matrix and inhibits IFN-γ secretion by CD4$^+$ T cells.[89,127] In this fashion, galectin-3 prevents the IFN-γ-dependent recruitment of CD8$^+$ T cells in the tumor microenvironment.[127]

To summarize, galectin-3 could favor tumor progression and metastasis through several pathways. The aforementioned findings emphasize the potential key role of galectin-3 in breast neoplasms, patient

TABLE 2 Galectin-3 a candidate biomarker in a wide variety of neoplasms

Pathologies	Type of biomarker	Applications	References
Colorectal cancer	Serum and tumor	Prognosis	149
Nonsmall cell lung cancer	Tumor	Prognosis an follow up	150
Lung and prostate cancer	Tumor	Theranostics	151
Cervical cancer	Tumor	Theranostics	152
Thyroid cancer	Tumor	Diagnosis	153
Glioma tumor	Tumor	Diagnosis	154
Breast cancer	Tumor	Prognosis	155

outcomes, and therapeutic responses. Galectin-3 should both be considered for the development of new biomarkers and targeted therapies in breast neoplasms. Its highly immunosuppressive functions, described in several studies, makes galectin-3 blockade therapy an interesting option for combination with currently validated immunotherapy.

4 | GALECTIN-3 AS BIOMARKER IN BREAST CANCER

Galectin-3 is involved in several pathologic processes with numerous studies reporting that it is detectable and quantifiable on the cell surface and in biologic fluids such as serum and urine. These studies have provided evidence of the potential of galectin-3 as a sensitive diagnostic or prognostic biomarker in several diseases.[17,128–131] Since variations in galectin-3 expression seems not to be influenced by age, body mass index (BMI), or sex, it can be considered as a stable biomarker for a wide range of diseases including neoplasms.[132]

Galectin-3 could become a biomarker in several neoplastic diseases (Table 2)[133] of which there is a limited amount of data from Africa. In our previous investigation, we demonstrated that galectin-3 plasmatic concentrations were higher in breast cancer patients compared with healthy controls and were positively correlated with breast tumor sizes.[76] Significantly higher levels of this lectin were observed in breast tumors compared with precancerous tissues as well as the triple-negative breast cancer molecular subtype has the greatest galectin-3 expression.[134] In a study of Zhang and colleagues,[12] galectin-3 was consistently expressed in triple-negative breast carcinoma associated with specific clinicopathologic and immunohistochemical features, thus making it a novel subtype-specific marker in breast cancer and a potential target for overcoming chemo-resistance. Another study focusing on serum galectin-3 reported that circulating galectin-3 rates were significantly higher in breast cancer patients compared with healthy controls and were associated with metastasis occurrence. Therefore, galectin-3 might be a promising therapeutic target for the development of effective agents to reduce metastasis.[135] However, there are conflicting data concerning galectin-3 overexpression and patient prognosis. Two studies demonstrated that low levels of galectin-3 expression in breast cancer were significantly associated with increased tumor vascular invasion and reduced disease-free survival and long-term overall survival.[136,137]

In samples from the same tissue, galectin-3 protein signature was significantly divergent from the mRNA signatures obtained from in silico analyses.[67] A specific galectin-3 protein expression signature was shown to contribute to the phenotypic heterogeneity of aggressive breast cancer subtypes and in particular triple-negative and HER2 subtypes.[67] Additionally, the stromal and tumor expression of certain galectins, particularly galectin-3, were associated with a poor prognosis. The study concluded that although protein expression and mRNA expression signature seems not redundant, galectin-3 expression signatures in tumor tissues could become a prognostic biomarker for breast cancer.[67] Moreover, during breast tissue neoplastic transformation and progression, galectin-3 protein and mRNA expression signatures are under dynamic changes and could be useful during follow-up of breast cancer patients.[73] However, galectin-3 levels are modified by clinical features in patients such as disease stage, inflammation, autoimmunity, and viral infections. Thus, galectin-3 could lack specificity as a diagnostic biomarker in breast cancer and its use should be limited to prognostic and theragnostic purposes.[8] New therapeutic strategies based on galectin-3 inhibition are currently designed in other neoplastic diseases and could be extended to breast cancer.

5 | ANTIGALECTIN-3 THERAPIES

Immunotherapy development has possibly been the most important innovation in the field of anticancer therapy. However, due to tumor resistance mechanisms, particularly the highly immunosuppressive tumor microenvironment, some patients poorly or do not respond to immunotherapy depending on the cancer types.[138,139] Hence, it is extremely critical that new therapeutic approaches, for increasing the effectiveness of existing therapies, be developed to target immunosuppressive components such as galectin-3 of the tumor microenvironment.[140]

The multiplicity and diversity of its biologic and pathologic functions have made galectin-3 an emerging research attractor in medicine and therapy development.[8,17,19,141] In breast neoplasms, the in vitro/vivo evidence and its association with poor patient outcomes, particularly in triple-negative breast neoplasms, make galectin-3 an interesting

target for the development of therapeutic strategies in combination with chemotherapy, radiation or immunotherapy.[8,67] Both galectin-3 and its cellular ligands could be targeted to inhibit its protumor activity. For example, in vitro targeting of the galectin-3 ligands LAG3 has significantly enhanced proliferation and activation of T cells from multiple myeloma patients.[140]

Globally, 3 different approaches have been taken in developing drugs that inhibit the galectin-3 molecules. The first approach is based on the use of disaccharides or modified saccharides such as lactose, N-acetyl-lactosamine, TD139, and 2-deoxy-D-glucose.[121,124,142] Recently, a C-3 substituted N,N'-diacetyllactosamine glycomimetics was developed. This glycan exhibited up to 43-fold stronger inhibitory potency to galectin-3 than the standard lactose. Coupling this molecule to human serum albumin gave rise to a complex with up to 4209-fold increase in inhibitory potency per glycan compared with lactose. These neo-glycoproteins primarily act as scavengers of exogenous cancer cells secreted galectin-3 that protect T-lymphocytes against galectin-3 induced apoptosis.[143] Modified citrus pectin (MCP), the second approach, uses large natural polysaccharides that contain galactose. Examples of such MCP are: GR-MD-02, GM-CT-01, Pectasol, and GCS-100.[144–146] Both modified saccharides and MCP block galectin-3 by binding to its CRD (Figure 1).[121] The third approach consists of the development of antigalectin-3 monoclonal antibodies such as 14D11 antibody (blocks the CRD) and B2C10 (binds to the N-terminal domain of galectin-3 and inhibits its oligomerization).[121,147]

At present, there is no published data from clinical trials of galectin-3 blockade therapy in breast neoplasms. Galectin-3 inhibition based-therapy has been tested in other cancers and the use of the combination of galectin-3 inhibitor the GR-MD-02 with anti-PD-1 pembrolizumab has shown encouraging results in patients with melanoma compared with the standalone use of pembrolizumab.[148] Regarding the promising results in preclinical breast cancer models, the same strategies could be adopted in breast neoplasms by combining galectin-3 inhibitors with existing immunotherapy. Several in vitro and mice model studies highlight the potential benefits of targeting galectin-3 in breast neoplasms. For example, Pectasol was able to inhibit growth and induce apoptosis of the MDA-MB-231 breast cancer cells.[145] Moreover, GM-CT-01, GCS-100, and B210 antigalectin-3 antibody were able to remove galectin-3 from T lymphocyte membranes, thus reestablishing antitumor functions of $CD8^+$ and $CD4^+$ T cells.[121] The 2-deoxyglucose therapy diminished the immunosuppression of T cells by tumor secreting galectin-3.[124] Consequently, we hypothesize that galectin-3 blockade therapy could increase the efficacy of adoptive T cell-based therapy.

6 | CONCLUDING REMARKS

This review represents an attempt to emphasize the necessity of studying galectin-3 role in breast cancer in African patients. The unequal allele frequencies of its polymorphic variants suggests that this lectin involvement in breast neoplasms may vary depending on ethnicity (Table 1). The overlapping characteristics of the main breast tumor types in Africa (triple negative subtype) with those of breast neoplasms that highly express galectin-3 suggests its role in the aggressiveness of the disease in Africans. Thus, galectin-3 could play a major role in the prognosis and care strategies of breast neoplasms in African countries. Additionally, several in vitro studies with breast cancer cell lines or mice models of breast neoplasms support galectin-3 as a candidate for development of targeted therapy. However, caution should be taken in extrapolating data from galectin-3 activity in vitro and in vivo models to clinical situations.

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AUTHORSHIP

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DISCLOSURE

The authors declare no conflict of interest.

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