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Review

Current status of lectin-based cancer diagnosis and therapy

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Abstract: Lectins are carbohydrate recognizing proteins originating from diverse origins in nature, including animals, plants, viruses, bacteria and fungus. Due to their exceptional glycan recognition property, they have found many applications in analytical chemistry, biotechnology and surface chemistry. This manuscript explores the current use of lectins for cancer diagnosis and therapy. Moreover, novel drug delivery strategies aiming at improving lectin's stability, reducing their undesired toxicity and controlling their non-specific binding interactions are discussed. We also explore the nanotechnology application of lectins for cancer targeting and imaging. Although many investigations are being conducted in the field of lectinology, there is still a limited clinical translation of the major findings reported due to lectins stability and toxicity concerns. Therefore, new investigations of safe and effective drug delivery system strategies for lectins are warranted in order to take full advantage of these proteins.

Keywords: lectins; cancer; glycans; carbohydrates; diagnosis; therapeutics

Abbreviations:

AAL: *Aleuria aurantia* lectin
AAL-2: *Agrocybe aegerita* lectin
ABL: *Agaricus bisporus* lectin
ACA: *Amaranthus caudatus* agglutinin
AEL: *Aspidistra elatior* lectin
AFP-L3: Alpha-fetoprotein-L3
AHA: *Arachis hypogea* agglutinin
AIA: *Artocarpus integrifolia* agglutinin
ALSA: Antibody-lectin sandwich array
AML: *Amaranthus mantegazzianus* lectin
AOL: *Aspergillus oryzae* lectin
BNIP3: BCL2/adenovirus E1B 19 kDa protein-interacting protein 3
BPL: *Bauhinia purpurea* lectin
CA125: Cancer antigen 125

CMP-SA: Cytidine monophosphate-sialic acid
Con A: Concanavalin A
Cra: *Cratylia mollis* lectin
DISC: Death-inducing signaling complex
DL: Dalton's lymphoma
EAC: Ehrlich ascites carcinoma
ERK: Extracellular signal-regulated kinase
FUT8/FucT: fucosyltransferase
GAGs: Glycosaminoglycans
GDP: Guanosine diphosphate
GlcNAcT-V: N-glycan GlcNAc transferase V
GMA: *Glycine max* agglutinin
GNA: *Galanthus nivalis* agglutinin
GSA I: *Griffonia simplicifolia* agglutinin I
GSLs: Glycosphingolipids
HCC: Hepatocellular carcinoma
HddSBL: *Haliotis discus discus* sialic acid binding lectin
HE4: Human epididymis protein 4
HIV: Human immunodeficiency virus
JNK: c-Jun N-terminal protein kinase
LC3-II: Microtubule-associated protein 1A/1B-light chain 3
LCA: *Lens culinaris* agglutinin
LTA: *Lotus tetragonolobus* agglutinin
MAA-II: *Maackia amurensis* agglutinin/*Maackia amurensis* Lectin-II
MCL: *Momordica charantia* seeds
MLs: Mistletoe lectins
NF-κB: Nuclear factor-kappa B
PHA: Phytohemagglutinin lectin
PNA: Peanut agglutinin
PSA: Prostate specific antigen
PTL: *Pinellia ternata* lectin
RCA/RCA I: *Ricinus communis* Agglutinin/Ricin
RLL: *Russula lepida* lectin
SFL: *Sophora flavescens* lectin
Siglecs: Sialic acid binding Ig-like lectins
SNA: *Sambucus nigra* agglutinin/Elderberry lectin
STL-S and *STL-D*: *Solanum tuberosum* lectin
TF: Thomsen-Friedenreich
Tg: Thyroglobulin
Anti-Tg-Ab: *Thyroglobulin* antibody
TJA-I: *Trichosanthes japonica* agglutinin-I
UDP-Gal: Uridine diphosphate galactose
UDP-GlcNAc: Uridine diphosphate-N-acetylglucosamine
UEA I: *Ulex europaeus* agglutinin I
VVL: *Vicia villosa* lectin
WFA: *Wisteria floribunda* agglutinin
WGA: Wheat germ agglutinin

1. Introduction

Since the term “lectin” was first coined in 1954 by Boyd and Shapleigh to define a group of plant agglutinins, lectins have received a lot of attention, partly due to their exceptional sugar binding property, as well as their therapeutic and biotechnological potentials [1,2]. Subsequent studies will later show that lectins are not limited to plants but originate instead from a wider range of sources. In fact, lectins are found in plants, animals and microorganism such as fungi, algae and bacteria [3-5]. According to Harold and Hans [6], regardless of its origin, to be considered a lectin, a protein should fulfill the following three main criteria: (i) bind carbohydrate, (ii) be different from immunoglobulins and (iii) not biochemically modify the carbohydrates which they bind.

These criteria allow the exclusion of tannins, certain lipids, carbohydrate specific antibodies, glycosyltransferases, glycosidases and other enzymes that bind and modify carbohydrates. Lectins have been classified based on their origin, sequence and structural homology, nature of glycan-lectin interactions, multivalency, type of natural ligands, biosynthesis and trafficking [7-10]. More recently, Kumar et al. have divided lectins into two modes of classification [11]. The first mode of classification is essentially based on lectin’s ligand preference and is divided into four different subgroups: (i)

glucose/mannose binding lectins, (ii) galactose and N-acetyl-D-galactosamine binding lectins, (iii) L-fucose binding lectins and (iv) sialic acids binding lectins. Kumar's second mode of classification is based on lectin-like proteins evolutionary features and contains two major types. The first type is based on structural and evolutionary sequence similarities of lectin-like proteins, the second type is essentially composed of lectin-like proteins without established evolutionary sequence.

Various biological functions have been attributed to lectins. In plants, lectins function can be divided into internal and external activities. Externally, plant lectins are believed to play a critical role in root nodules formation through symbiotic rhizobia binding, and protect from insects and fungi. Internally, plant lectins are mainly involved in sugar transport or carbohydrate storage and often activate enzymatic processes [6]. Animal lectins have been associated with much broader functions, including clearance of sulfated glycoprotein, control of lymphocyte migration, control of glycoproteins biosynthesis, induction of apoptosis, induction of angiogenesis, complement activation, lectinophagocytosis, mitogenic activity, modulation of cell-cell and cell-substrate interactions, modulation of signal transduction by B lymphocytes, neuronal myelination and regeneration, sperm-egg interaction, targeting of glycoproteins to lysosomes and tumor metastasis [12]. In microorganisms, lectins are known for enhancing microbial infectivity, fungal parasitism, host recognition, yeast flocculation, defense mechanism, development and morphogenesis [2,12].

Lectins carbohydrate specificity is the underlying basis for their multitude biological functions. Consequently, lectins have been investigated for a variety of new applications which has given rise to an emerging field of science known as "lectinology" or the science of lectins [13]. In fact, lectin-bound stationary phases are commercially available for glycoproteins or glycopeptides enrichment [14]. Furthermore, antibody-lectin sandwich array (ALSA) technology has been proposed to address limitations and sensitivity issues, high-throughput sample processing problems and high consumption of clinical samples associated with conventional bioanalytical methods [15-17]. Lectins have also found a wide range of applications in cytochemistry, histochemistry and immunochemistry for the detection and characterization of glycosylated residues and different glycoconjugates in human or animal cells and tissue surfaces [18]. Owing to their potent anti-insect properties, plant lectins have particularly received increasing attentions for their potential in pest management in the field of agriculture [19,20]. This use of lectins in agriculture is expected to address as much as 27% of the worldwide crop loss caused by plant pathogens and related diseases, estimated to US$1350 billion each year [21]. Perhaps, the single field of science where lectins have found an explosion of applications is in surface chemistry. Lectin functionalized surface technologies offer scientists the ease to design homemade and cost effective sensors tailored to unique applications with desired specifications (sensitivity, selectivity) [22-24].

Not surprisingly, lectins ability to specifically and selectively target diver glycosylated biological molecules with good sensitivity has found very promising applications in the field of pharmaceutical sciences. In the past decades, there has been an exponential increase in the use of lectins to address some of the most challenging questions in health sciences [25-28]. This manuscript aims at reviewing the current trend in the use of lectins for cancer diagnosis and therapy.

2. Glycan alterations in cancer

The glycocalyx, a distinctive carbohydrate coating of most vertebrate and bacteria cells' membrane, is composed of glycoproteins, glycolipids, and glycosaminoglycans. Typically the

glycocalyx contains N-linked and O-linked glycans attached to glycoproteins, proteoglycans and glycosphingolipids (GSLs) [29]. Glycosaminoglycans (GAGs), which are also found in the glycocalyx, are O-linked to proteins core [30]. Changes in the glycocalyx structure from physiological state to pathological state are the underlying principle for various lectins-based disease diagnosis. Although poorly understood in the early 1970s (when this phenomenon was first described), recent evolution in glycoscience, genomic, proteomics and mass spectrometry enable precise distinctions in the glycan structure and composition between disease and normal states. In cancer, cell surface glycans alterations following malignant transformation, tumor cell differentiation, and metastasis have been widely documented [31,32]. Common alterations include loss of expression or overexpression of certain glycan structures, the appearance of incomplete, truncated or novel structures and the accumulation of precursors [33]. Kumamoto et al. reported a significant overexpression in the amount of mRNA for uridine diphosphate galactose (UDP-Gal) transporter in colon cancer tissues compared to nonmalignant mucosa tissues [34]. More importantly, UDP-Gal transporter mRNA increase was associated with an enhanced expression of cancer-associated carbohydrate markers such as Thomsen-Friedenreich (TF) and sialyl Lewis A/X antigens in colon cancers. Although this study did not find any significant difference in the mRNA level of uridine diphosphate-N-acetylglucosamine (UDP-GlcNAc) and cytidine monophosphate-sialic acid (CMP-SA) between malignant and nonmalignant colon tissues; numerous other studies have associated UDP-GlcNAc with cancer progression. In fact, cell lines with increased expression of N-glycan GlcNAc transferase V (GlcNAcT-V), an enzyme responsible for β1–6 branching of N-glycans, showed an increased frequency of metastasis in animal models due to an impairment of cell adhesion which consequently promoted tumor cell invasion [35]. Furthermore, the loss of GlcNAcT-V enzyme activity correlates with a loss of the metastatic phenotype [36]. In addition, fucosylation or the transfer of fucose residues to oligosaccharides linked to proteins or lipids, is known to be one of the most prominent glycan modification involved in cancer. Fucosylation, generally increases during carcinogenesis, and is primarily regulated by fucosyltransferases, guanosine diphosphate (GDP)-fucose synthetic enzymes, and GDP-fucose transporter(s) [37]. Recently, Chen et al. reported that an upregulation in the expression of fucosyltransferase 8 (FUT8) in non-small cell lung cancer (NSCLC) correlates with tumor metastasis, disease recurrence, and poor survival rate in patients. This study also determined that knocking down FUT8 in aggressive lung cancer cell lines significantly inhibit cancer cell proliferation, metastasis and tumor growth [38]. Moreover, α-L-fucose has been found to be overexpressed in a variety of cancers, including thyroid carcinoma [39], leukemia [40], lung cancer [41], ovarian carcinoma [42], colorectal adenocarcinoma [43], and brain tumor [44]. In human hepatocellular carcinoma, fucosylation biosynthesis was found to be regulated by a high expression of GDP-L-fucose synthase (FX protein) followed by an increase in GDP-L-fucose, as well as an enhancement in α1-6 fucosyltransferase (α1-6 FucT) expression [45,46]. Consequently, defucosylation was proposed by Listinsky et al. [47] as a selective ablation therapeutic strategy against many human malignancies. This hypothesis was further tested by Yuan et al. by treating human breast cancer MDA-MB-231 with α-L-fucosidase, a glycosidase that specifically removes α-L-fucose. Although MDA-MB-231 cells proliferation and viability was unaffected by fucosidase treatment, the cancer cells invasion was significantly decreased due to a downregulation of cell surface CD44 and CD15 [48] levels. Conversely, Zhao et al. found that the core fucosylation (the addition of a fucose residue in α 1,6 linkage to the first GlcNAc of the oligosaccharide core during posttranslational modifications) is down regulated in gastric cancer and proposed that an upregulation of core fucosylation could effectively inhibit the proliferation of human gastric cancer cells [49]. More specifically, it was

proposed that α-L-fucose might be essential in the malignancy and metastatic phenotype development of many human breast cancers [50]. Furthermore, sialylation, which is essentially the transfer of sialic acid, a nine-carbon acidic monosaccharides, into terminal position of glycan chains, is known to be a prominent alteration in most cancers [51]. It was hypothesized that the high degree of sialylation in cancer may favor disease progression by protecting cancer cells from apoptosis, promoting metastasis, and by conferring a resistance to therapy [52,53]. The increase in sialylation is generally manifested as a specific increase in 2-6-linked sialic acids attached to outer *N-acetyllactosamine* (Galβ1-4GlcNAc) units or to inner GalNAc-α1-O-Ser/Thr units on O-glycans [33]. This specific alteration in sialylation is known to correlate with human cancer progression, metastatic spread, and poor prognosis [54]. Nonetheless, α2-3-sialic acid has also been shown to play an important role in cancer progression. In fact, Cui et al. established that the highest expression level of α2-3-sialic acid residues in breast cancer is associated with metastatic potential [55]. Table 1 is a summary of the main glycans alterations observed in cancer that are discussed in this manuscript.

Table 1. Glycan, monosaccharides, sugar transporters and enzymes alterations in cancer.

Cancer	Glycan, monosaccharides, sugar transporters and enzymes	References
Colon Cancer	UDP-Gal	[34,56]
	α2-6- sialic acid	
	Thomsen-Friedenreich	
	Sialyl Lewis A/X antigens	
Gastric cancer	α1-6-fucose	[39]
Lung cancer	α-L-fucose	[38,43]
	Fucosyltransferase 8	
Breast Caner	α2-3- sialic acid	[40,55]
	α-L-fucose	
Thyroid carcinoma	α-L-fucose	[41]
Leukemia	α-L-fucose	[42]
Ovarian carcinoma	α-L-fucose	[44]
Colorectal adenocarcinoma	α-L-fucose	[45]
Brain tumor	α-L-fucose	[46]
Hepatocellular carcinoma	GDP-L-fucose synthase	[47,48]
	GDP-L-fucose	
	α1-6 fucosyltransferase	

In colorectal cancer, α2-6-linked sialic acids have been associated with metastasis and therapeutic failure [56]. Sialic acid binding Ig-like lectins (Siglecs) are expressed on most immune cells surface and can transmit immunosuppressive signals upon binding to sialic acid [57]. It has been proposed that altered sialylation of tumor cells may affect interactions with some Siglecs which ultimately promotes

immune system evasion by sending an inhibitory signal to innate immune cells [52]. Similarly, although hypersialylation of the Fas receptor (apoptosis antigen 1) does not affect its agonist binding, this however prevents apoptosis induction in cancer cells. More specifically, α2-6 sialylation of Fas by the sialyltransferase ST6Gal-I prevents the initiation of death-inducing signaling complex (DISC) by hindering the binding of the Fas-associated adaptor molecule (FADD) to the FasR death domain [52,58]. Figure 1 summarizes major glycosylation reactions and glycan structures observed in cancer.

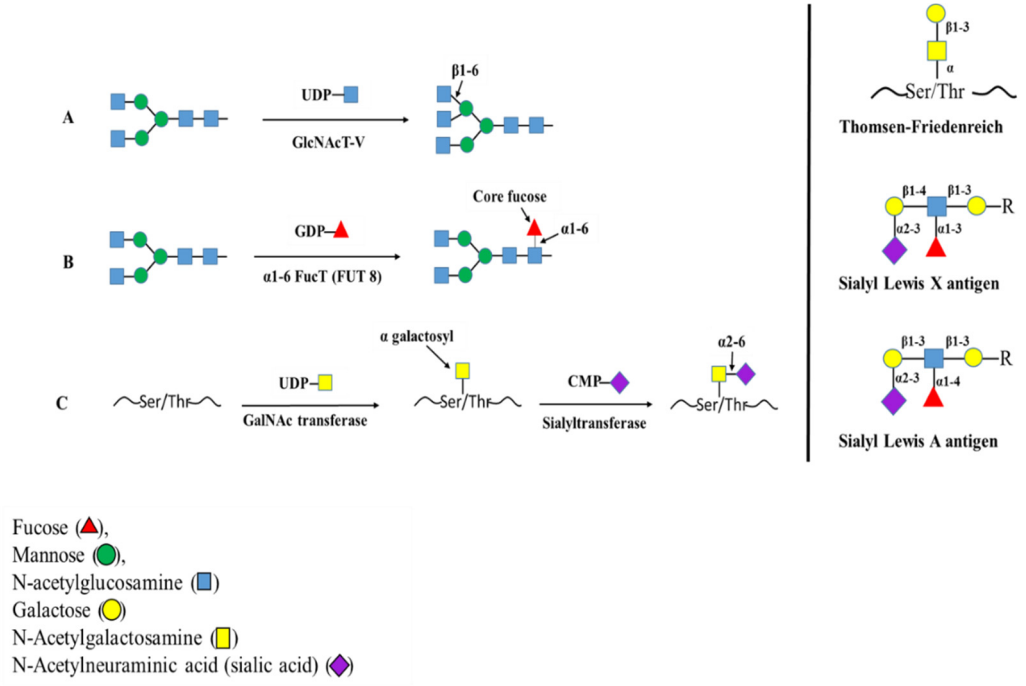

Figure 1. Summary of key glycosylation reactions (A, B and C) and major glycan structures in cancer. Reactions are adapted from [33] and [59].

3. Lectins-based cancer diagnosis

Owing to their high selectivity and specificity for certain glycan structures, lectins have been investigated for their potential in cancer diagnosis. One of the successful clinical translations of lectin use as diagnosis tools is *Lens culinaris* agglutinin (LCA). LCA, a plant lectin extracted from lentil seed, which bind specifically to α1-6 fucose, has been used to diagnose hepatocellular carcinoma (HCC) [60,61]. LCA-based HCC diagnosis relies primarily on a specific affinity of the lectin for Alpha-fetoprotein-L3 (AFP-L3), a malignant tumors specific isoform of AFP glycoprotein. A commercial clinical kit for AFP-L3 serum concentration was subsequently developed for HCC diagnosis [62], which quickly became a valuable clinical alternative to more expensive and sophisticated techniques such as CT scans and MRI imaging [61]. Today, LCA based HCC diagnosis is a FDA approved HCC clinical diagnosis tool covered by the health insurance of the Japanese Medical Service [63] and used by leading cancer treatment centers across the US [64]. LCA/AFP-L3 interaction has also been investigated to diagnose and monitor testicular tumor activity [65].

Lectins have also been investigated for their potential in ovarian cancer diagnosis. Cancer antigen 125 (CA125) and human epididymis protein 4 (HE4) are two FDA approved glycoprotein biomarkers

for ovarian cancer. *Amaranthus caudatus* agglutinin (ACA), *Artocarpus integrifolia* agglutinin (AIA), *Arachis hypogea* agglutinin (AHA), *Vicia villosa* lectin (VVL), *Griffonia simplicifolia* agglutinin I (GSA I) and *Ulex europaeus* agglutinin I (UEA I) are a group of lectins that recognize Thomsen-Friedenreich antigen, Thomsen-nouvelle and sialyl-Thomsen Friedenreich glycan alterations (Figures 1 and 2) of CA125 and HE4 [66-69]. Targeting CA125 glycan alterations with VVL, Chen et al. were able to distinguish benign ovarian neoplasms from invasive epithelial ovarian cancer with a specificity of 61.1% at 90% sensitivity [68]. Other studies have also shown that wheat germ agglutinin (WGA) and *Glycine max* agglutinin (GMA) could potentially be used for ovarian cancer diagnosis [70,71]. Because CA125 glycan alteration include an increase in core-fucosylated bi-antennary monosialylated glycans; LCA and UEA which specifically recognize α1-6 fucose and α1-2 fucose, respectively, have been investigated for their potential in ovarian cancer diagnosis [72]. Furthermore, *Pinellia ternata* lectin (PTL), a lectin recently isolated from mushroom, specifically bind α1-6 fucose residues and could potentially be used in ovarian cancer, breast cancer and pancreatic cancer diagnosis [37].

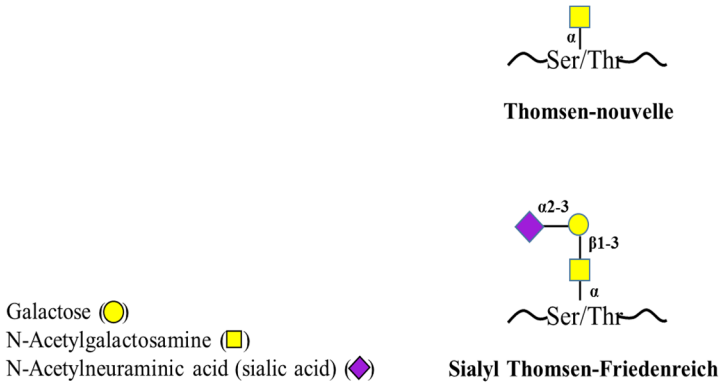

Figure 2. Thomsen-nouvelle and sialyl-Thomsen Friedenreich structures.

Aleuria aurantia lectin (AAL), which specifically recognizes α1-6, α1-3 and α1-4 fucose moieties was successfully used for differential diagnosis and predicted prognosis of pancreatic cancer. In serum samples from pancreatic cancer patients, AAL binds to the highly fucosylated haptoglobin β chain which occurred in 60–80% of patients screened in the study [37]. Moreover, taking advantage of the difference in *sialylation* in pancreatic cancer, Li at al. showed that Alpha-1-β glycoprotein interaction with *Sambucus nigra* agglutinin (SNA) can be used to specifically detect pancreatic cancer with high sensitivity and specificity [73].

Thyroglobulin (Tg) is a FDA approved glycoprotein biomarker for thyroid cancer. Studying the presence of distinct GalNAc termini of N-glycans on human thyroglobulin, Takeya et al. found *Wisteria floribunda* agglutinin (WFA) to have a broad binding activity to different Tg isoforms [74]. This finding might provide additional diagnostic strategies for thyroid cancer using WFA. A different study by Zhao et al. focused instead on the variation of glycosylation in sera anti-Tg-Ab [75]. In fact, to determine the extent of thyroglobulin antibody (anti-Tg-Ab) glycosylation in patients with different thyroid diseases, Zhao et al. applied three different Lectin-ELISAs. In this study, AAL detected the fucose residues, *Ricinus communis* agglutinin I (RCA I) recognized the galactose residues and Elderberry lectin (SNA) specifically bound α2-6-sialic acid in *anti-Tg-Ab*. Among the three lectins investigated, SNA showed a faster binding response to *anti-Tg-Ab* and could potentially serve as a

rapid thyroid cancer detection tool. Likewise, prostate specific antigen (PSA) is the most accurate biomarker approved by the FDA for prostate cancer detection [76]. Using a quartz Crystal Microbalance biosensor, Pihikova et al. determined that SNA has a better affinity for PSA compared to *Maackia amurensis* agglutinin-II (MAA-II) and *Lotus tetragonolobus* agglutinin (LTA), probably due to an increased amount of α2-6-sialic acid glycans in the glycoprotein compared to other glycan types [77]. MAA-II specifically binds α2-3-sialic acid while LTA is known for α-L-fucose detection. This study also explored the potential of electrochemical lectin-based immunosensors in prostate cancer diagnosis. Similarly, in another study reported by Bhanushali et al., SNA and AAL have shown more sensitive detection capabilities (1.58 and 1.45 ng/mL) for PSA glycans compared to *Maackia amurensis* Lectin-II (MAA-II) (1.71 ng/mL) [78]. However, all three lectins showed detection limits significantly lower than the currently used clinical assay cutoff for PSA (4 ng/mL) [67]. This suggests the potential of these lectins to accurately diagnose prostate cancer by detecting PSA glycans levels in serum samples of prostate cancer patients. Furthermore, targeting PSA glycans with Phytohemagglutinin lectin (PHA) from *Phaseolus vulgaris*, Batabyal et al. have demonstrated the ability to differentiate PSA from prostate cancer, benign prostate hyperplasia (BPH) and normal serum [79]. PHA is a plant lectin extracted from the red kidney bean which preferentially recognizes branched N-glycans bearing the β1-6 branched GlcNAcT-V product [80,81]. Similar results were also reported by Basu et al. using the mannose specific lectin Concanavalin A (Con A) [82]. These findings could represent the underlying basis for a new and rapid prostate cancer diagnosis tool which may ultimately reduce unnecessary biopsies in men [83].

Using PHA, Kim et al. successfully identified 26 new colorectal cancer candidate biomarkers that showed 100% specificity and sensitivities greater than 50% [81]. Similarly, using principal component analysis and hierarchical clustering to analyze glycoarrays from five (5) plant lectins, Qui et al. found that except for peanut agglutinin (PNA), all the other lectins tested (Con A, SNA, AAL, MAA-II) successfully separate colorectal cancer samples from normal controls [84]. Although, ConA and SNA differentiated normal controls samples from cancer samples, these two lectins did not show a good separation efficiency between adenoma and cancer samples. On the contrary, in addition of differentiating normal control samples, AAL and MAA-II were better at segregating adenoma from cancer samples. Therefore, AAL and MAA-II could potentially be used for the diagnosis of colorectal cancer but also the study of disease progression. Furthermore, to distinguish metastatic from non-metastatic breast cancer patients, Fry et al. designed lectin microarrays consisting of 45 lectins with different binding preferences. Serum and urine samples were then analyzed for binding differences. Four lectins, *Aspergillus oryzae* lectin (AOL), *Galanthus nivalis* agglutinin (GNA), RCA 120 and *Phaseolus vulgaris* erythroagglutinin (PHA) showed a significant binding difference between sera from metastatic and non-metastatic patients [85]. AOL is a core fucose (α1-6-fucosyl) specific fungus lectin, GNA is a plant lectin that preferentially recognizes mannose rich glycan and RCA 120 is a galactose-binding plant lectin [86-89]. *Trichosanthes japonica* agglutinin-I (TJA-I), RCA 120 and *Bauhinia purpurea* lectin (BPL) also showed significantly higher binding in metastatic compared to non-metastatic urines samples, suggesting that patient urine sample may contain potential glycosylated biomarkers for metastatic breast cancer diagnosis. TJA-I and BPL are two plant lectins that bind specifically α2-6 linked sialic acid and Galβ1-3GalNAc (T-antigen), respectively [90,91]. An overview of different lectins used for cancer diagnosis along with their glycan specificity is provided in Table 2.

Table 2. Lectins for cancer diagnosis.

Cancer	Lectin	Glycan preference	References
Hepatocellular carcinoma	LCA	α1-6 fucose	[60,61]
Testicular cancer	LCA	α1-6 fucose	[65]
Ovarian cancer	ACA	Thomsen-Friedenreich antigen (Galβ1 → 3GalNAcα-O-Ser/Thr); Thomsen-nouvelle (GalNAcα-O-Ser/Thr) and sialyl-Thomsen Friedenreich structures (Neu5Acα2 → 3Galβ1 → 3GalNAcα-O-Ser/Thr)	[37,66-72]
	AIA		
	AHA		
	VVL		
	GSA		
	UEA		
	GMA	GalNAcα1-Ser/Thr and/or GalNAcGalβ1,3GalNAcα1-Ser/Thr)	
	WGA	Sialic acid and N-acetyl glucosamine (GlcNAc)	
	PTL	α1-6 fucose	
	LCA	α1-6 fucose	
	UEA	α1-2 fucose	
Pancreatic cancer	SNA	α2-6-sialic acid	[37,73]
	AAL	α1-3/α1-4 and α1-6 fucosylations	
	PTL	α1-6 fucose	
Breast cancer	GNA	Mannose	[37,85-91]
	RCA	Galactose	
	PHA	GlcNAc	
	AOL	α1-6-fucose	
	TJA-I	α2-6 linked sialic acid	
	PTL	α1-6 fucose	
	BPL	Galβ1-3GalNAc	
Thyroid cancer	RCA I	Galactose	[75]
	SNA	α2-6-sialic acid	
	AAL	α1-6, α1-3 and α1-4 and fucose	
	WFA	GalNAc	
Prostate cancer	SNA	α2-6-sialic acid	[67,77-79,82]
	MAA-II	α2-3-sialic acid	
	LTA	α-L-fucose	
	PHA	Galβ1, 4GlcNAcβ1, 2Man	
	Con A	α-Man > α-Glc > GlcNAc	
Colorectal cancer	PHA	β1-6 branched GlcNAc	[80,81,84]
	AAL	α1-6, α1-3 and α1-4 and fucose	
	Con A	α-Man > α-Glc > GlcNAc	
	SNA	α2-6-sialic acid	
	MAA-II	α2-3-sialic acid	

4. Lectins-based cancer therapy

In addition to their specific glycan recognition, most lectins are also known for their cytotoxicity which makes them ideal anti-cancer therapeutic candidates. Lectins anti-cancer activities generate from diverse mechanisms, including apoptosis, autophagy, and inhibition of tumor growth [92]. Ricin (RCA), one of the first discovered lectins, is an ideal example of how lectins can both specifically target and induce cell death [93]. Extracted from castor plant seeds, RCA is a heterodimer composed of two distinct N-glycosylated polypeptide chains (chain A and chain B) joined by a disulfide bond [94]. Chain A plays the role of an enzyme that irreversibly inactivates mammalian 60S ribosomal subunits rendering them unable to bind the GTP-binding translation elongation factor EF-2, which ultimately prevents protein synthesis [95-97]. Chain B mainly plays the role of a targeting ligand by specifically recognizing galactosyl residues on cell surfaces. Binding of chain B to β1-4-linked galactosyl containing glycoproteins triggers RCA uptake by endocytosis [98]. Recently, Zou and Zhan found that RCA has the potential to selectively kill leukemia (K562) and colon cancer (SW480) tumor cells [99]. RCA induced tumor cell death by apoptosis via caspase-3 activation and DNA fragmentation [100].

Mistletoe lectins (MLs), which also belong to the ribosome inactivating proteins type II family, have been divided into three main types comprised of ML-I, ML-II and ML-III. Similar to RCA, MLs are composed of two polypeptide chains. Chain A inhibits protein synthesis intracellularly by blocking the elongation step of protein biosynthesis through the catalyzed hydrolysis of the N-glycosidic bond at adenine-4324 in the 28S RNA of the 60S ribosomes [101]. Chain B is responsible for the immunomodulatory activity of mistletoe lectin, manifested by enhancing the secretion of cytokines and the activity of natural killer cells [102]. ML-I binds lactose, D-galactose and GalNAc, while ML-II and III bind GalNAc preferably [103-105]. Currently, ML-I is the most studied of the MLs for its potent antitumor and immunomodulatory effects. MLs (mainly ML-I and ML-II) have shown promising antiproliferative activities toward various types of cancer cells, including breast cancer, leukemia, liver cancer, melanoma and lung cancer [106,107]. In fact, Marvibaigi et al. recently published a comprehensive review of the preclinical and clinical effects of mistletoe against breast cancer [108]. MLs induce tumor cell death via an apoptosis pathway by activating several caspases (caspase 8, caspase 9 and caspase 3), inducing a down-regulation in pro-survival protein Bcl-2 and by inhibiting telomerase activity [109-111]. Using a lectin extracted from *Pinellia ternata* (PTL), Zuo et al. have been able to inhibit Sarcoma 180, HeLa (cervical cancer cells) and K562 cells proliferation at a maximum of 85.2, 74.6 and 59.4%, respectively. It was proposed that PTL inhibits cancer cell proliferation by preventing the transition from G_1 to S phase in the cell division cycle, which subsequently induces cells to enter the quiescent G_0 state from G_1, thus cell division cycle arrest. Although cyclophosphamide (a cancer chemotherapy agent) induced a significant tumor size reduction compared to PTL, *in vivo*, tumor growth was still inhibited up to 36% with PTL [112]. Furthermore, a recent study using Con A and *Sophora flavescens* lectin (SFL) established that these lectins display antitumor activities against human breast cancer cells (MCF-7), both *in vitro* and *in vivo* [113]. Tumor cell death, which ultimately led to the decrease in tumor mass volume and weight in MCF-7 bearing nude mice, was also shown to occur by apoptosis. In fact, both ConA and SFL induced an increase in the activities of pro-apoptotic mediator caspase-3, caspase-9 and cytochrome *C* in a dose dependent manner. In addition, an upregulation in pro-apoptotic proteins Bax and Bid and a downregulation in pro-survival protein Bcl-2 and Bcl-X_L levels was associated with the treatment of MCF-7 cells with both lectins. Moreover, Con A reduced the nuclear factor-kappa B (NF-κB), the extracellular signal-

regulated kinase (ERK), and the c-Jun N-terminal protein kinase (JNK) levels, and increased tumor suppressor protein p53 and the cyclin-dependent kinase inhibitor p21 levels while SFL only caused similar changes in NF-κB, ERK, p53, and p21 levels and did not affect JNK expression level. Similar to NF-κB, ERK and JNK control many cell processes, including growth, differentiation, transformation and apoptosis [114-118] while p21 is known to regulate cell division cycle progression at G1 and S phase and mediate cellular senescence [119,120]. Similar results were previously reported by Chang et al. when Con A was tested on hepatoma cells [121]. However, Con A was found to preferentially locate in the mitochondria, triggering tumor cell death with microtubule-associated protein 1A/1B-light chain 3 (LC3-II) generation, double-layer vesicle formation, BCL2/adenovirus E1B 19 kDa protein-interacting protein 3 (BNIP3) induction, and acidic vesicular organelle formation following a change in the mitochondria membrane permeability, indicative of an autophagic cell death pathway [121-123].

A galactose-specific lectin (nagaimo lectin), recently purified from the tubers of *Dioscorea opposite*, induced potent antiproliferative activities against breast cancer cells (MCF-7) and liver cancer cells (HepG2) [124]. It was also determined that nagaimo lectin inhibits MCF-7 cells proliferation by apoptosis, potentially through a mitochondrial membrane depolarization. When soybean lectin's (SBL) antitumor effect was tested *in vivo* in Dalton's lymphoma (DL) bearing mice, a strong antiproliferative activity (74.51 ± 3.5 and 82.95 ± 5.8% within ten days of treatment) with selectivity toward tumor cells was observed. SBL-mediated tumor cell death was reported to occur by autophagy, apoptosis and DNA damage through a dose dependent generation of reactive oxygen species [125]. Similarly, *Amaranthus mantegazzianus* lectin (AML) and ACA showed promising antiproliferative activities against rat osteosarcoma-derived cells. *Amaranthus* lectins-induced cell death was also reported to be through an apoptosis pathway [126]. Furthermore, two lectins extracted from *Solanum tuberosum* tubers (*STL-S* and *STL-D*) were recently tested on Ehrlich ascites carcinoma (EAC) in Swiss albino mice and inhibited tumor growth by 79.84 and 83.04%, respectively. Although a mechanism of STLs-induced tumor cell death is yet to be determined, Hasan et al. suggested that STLs might regulate their antiproliferative activity via apoptosis [127]. In addition, a D-galactose-specific lectin extracted from *Momordica charantia* seeds (MCL) showed a strong growth inhibition against EAC (up to 75%). MCL induced EAC cell cycle arrest at the G0/G1 phase suggesting an apoptosis mediated cancer cell death [128]. Moreover, a significant *in vitro* antiproliferative activity of *Aspidistra elatior* lectin (AEL), purified from the rhizomes of *Aspidistra elatior* Blume, has been reported toward Bre-04, Lu-04, HepG2, and Pro-01 tumor cell lines [129]. AEL promotes tumor cells entry in the sub-G1 phase, indicative of a programmed cell death [130]. Leczyme, also known as sialic acid-binding lectin and isolated from oocytes of bullfrog (*Rana catesbeiana*), has previously been reported for its ribonuclease and antitumor activities [131]. Until recently, leczyme-induced tumor cell death was not well understood. Tatsuta et al. showed that leczyme induced tumor cell death via apoptosis by activating the initiator caspases (8 and 9) and the effector caspase (3) [132,133]. Similarly, MLL-2, a lectin purified from the *Musca domestica* Linnaeus fly's larvae, has been shown to inhibit breast cancer cells (MCF-7) proliferation. Cao et al. suggested a mitochondrial pathway to be responsible for the apoptosis mediated tumor cell death observed with MLL-2 [134].

Lectins extracted from various genus and species of mushroom have also shown significant antiproliferative activities both *in-vitro* and *in-vivo* [135,136]. Yu et al. reported that *Agaricus bisporus* lectin (ABL), a Thomsen-Friedenreich antigen binding lectin, is a reversible noncytotoxic inhibitor of epithelial cell proliferation [137]. In fact, a strong inhibition (up to 87%) of [3H]-thymidine incorporation, which correlates directly with the inhibition of cell proliferation, was observed on

human colorectal adenocarcinoma cells (HT29) proliferation. Fifty percent (50%) inhibition of MCF-7 breast cancer cells proliferation was also reported in the same conditions. Although a mechanism for ABL induced anticancer activity was not investigated by Yu et al., in this report, the lack of cytotoxcicity observed with this lectin suggested a different mechanism than the irreversible mechanism by which lectins, such as ricin, inhibit protein biosynthesis through ribosome subunit inactivation. It was later shown that the anti-proliferative effect of ABL is a consequence of the lectin blocking the nuclear localization sequence-dependent protein uptake into the nucleus [138]. Similarly, *Russula lepida* lectin (RLL), an inulin and O-nitrophenyl-β-D-galacto-pyranoside binding lectin, inhibited HepG2 and MCF-7 tumor cells. Furthermore, RLL induced a 67.6% reduction in tumor size *in-vivo*, in male white Kunming mice bearing S-180 tumor [139]. To date, the mechanism of RLL induced anticancer activity is still not well understood. In a separate study, Zhao et al. showed that *Agrocybe aegerita* lectin (AAL-2), a Thomsen-Friedenreich antigen binding lectin, can inhibit various cancer cells proliferation including HeLa, SW480, gastric cancer cells (SGC-7901, MGC80-3, BGC-823), acute promyelocytic leukemia cell HL-60 and mouse sarcoma S-180. AAL-2 also demonstrated a significant inhibition of S-180 tumor in BALB/c mice [140,141]. AAL-2 antitumor activity was shown to occur mainly via apoptosis and DNase activity. A brief summary of lectins discussed in this manuscript for cancer therapy and their carbohydrate preference is presented in Table 3.

5. Clinical translation of anticancer lectins

Although lectins antitumor potential is widely studied and likely to continue to be reported, there is however a slow rate of clinical translation of these proteins into cancer therapeutics. To date, only Mistletoe lectins (MLs) have been extensively studied in clinical trials (Table 4) to assess their anticancer potentials [142]. The lack of clinical trials for many other potentially effective lectins has pushed some scientists to call for an increase and diversity in anticancer lectins clinical trials [143]. Although not approved by the food and drug administration for commercialization in the US, mistletoe extracts are widely used in European countries for the treatment of various cancers, including breast cancer, pancreatic cancer, lung cancer and colon cancer [144-147]. MLs extracts are marketed under various trade names, such as Iscador®, Helixor®, Eurixor®, Lektinol® and Isorel® [148]. Among their many advantages, MLs extracts dramatically increase cancer patients' survival rate and quality of life, prolong relapse intervals, and reduce side effects associated with chemotherapy treatments [108]. More specifically, in a nonrandomized matched-pair study, Iscador® increased patients' mean survival time by 40% (4.23 years) compared to the untreated control groups (3.05 years; $P < 0.001$) [144]. Furthermore, Helixor® was shown to significantly increase cell surface glycoprotein CD107a expression in Natural Killer (NK) cells [147]. CD107a is widely accepted as a functional marker for the identification of natural killer cell activity [149]. Similarly, Lektinol® showed a strong effect on survival ratio, inhibition of primary bladder tumors growth and the formation of multiple metastases when administered at 3–30 ng/0.1 mL/kg animal [150]. Recently, few clinical trials have also emerged to explore the effects of mushroom extracts as anti-cancer therapeutics (Table 4) [151-153]. Lectins are believed to be part of a group of active components responsible for conferring anti-cancer potential in mushrooms, including lentinan, krestin, hispolon, calcaelin and Hericium polysaccharide A and B (HPA and HPB) [154]. Mushrooms extracts have shown promises in various cancers, including breast cancer, pancreatic cancer, liver cancer, oral cancer, prostate cancer, colon cancer, gastric cancer, leukemia, lung cancer and against some forms of malignancies, such as estrogen receptor negative human breast cancer, where chemotherapy has failed.

Table 3. Example of lectins used in cancer therapy.

Lectin	Monosaccharide/glycan preference	Cancer	References
RCA	Galactose	Leukemia (K562)	[99,100]
		Colon Cancer (SW480)	
ML-I, ML-II	Lactose, D-galactose, GalNAc	Breast Cancer	[101-107]
		Leukemia	
		Liver Cancer	
		Melanoma	
		Lung Cancer	
PTL	α1-6 fucose	Sarcoma (180)	[112]
		Cervical Cancer (Hela)	
		Chronic Myelogenous Leukemia (K562)	
Con A	α-Man > α-Glc > GlcNAc	Breast Cancer (MCF-7)	[113,121-123]
		Hepatoma Cells	
SFL	Mannose	Breast Cancer (MCF-7)	[113]
Nagaimo lectin	Galactose	Breast Cancer Cells (MCF-7)	[124]
		Liver Cancer Cells (HepG2)	
SBL	Galactose	Dalton's Lymphoma	[125]
AMM, ACA	Galβ1-3GalNAcα-O-	Osteosarcoma	[126]
STL-S, STL-D	N-Acetylglucosamine	Ehrlich Ascites Carcinoma	[127]
MCL	D-galactose	Ehrlich Ascites Carcinoma	[128]
AEL	D-mannose	Breast Cancer (Bre-04)	[129,130]
		Lung Cancer (Lu-04)	
		Liver Cancer Cells (HepG2)	
		Prostate Cancer (Pro-01)	
Leczyme	Sialic acid	Mesothelioma	[131-133]
		Leukemia	
		Breast	
		Carcinoma	
		Hepatoma Cells	
MLL-2	D-galactose	Breast Cancer Cells (MCF-7)	[134]
ABL	Thomsen-Friedenreich antigen	Colon cancer cells (HT29),	[137]
		Breast Cancer Cells (MCF-7)	
RLL	inulin and O-nitrophenyl-β-D-galacto-pyranoside	Breast Cancer Cells (MCF-7)	[139]
		Liver Cancer Cells (HepG2)	
AAL-2	Thomsen-Friedenreich antigen	HeLa, SW480, SGC-7901, MGC80-3, BGC-823, HL-60 and S-180 cells	[140,141]

Table 4. Example of Mistletoes and Mushroom lectins clinical trials.

	Product tested	Study design	Participant	Major findings	References
MLs	Eurixor®	Pilot trial (phase I/II study)	16	Stabilization of patient quality of life	[145]
	Iscador®	Nonrandomized and randomized matched-pair studies nested within a cohort study	10,226	Prolonged survival time, stimulated self-regulation	[144]
	Viscum album (L.)	randomized clinical trial	220	Prolongation of overall survival	[155]
	Helixor®	Randomized controlled clinical trial.	233	Improved quality of life, reduced chemotherapy side-effects	[156]
	Aviscumine (recombinant mistletoe lectin-I)	Phase I trial	41	Stimulated the immune system with a release of cytokines, stabilized disease	[157]
	Iscador®	Randomized phase II study	72	Chemotherapy dose reductions, less severe side-effects and hospitalizations	[158]
	Lektinol® (PS76A2)	Randomized, placebo-controlled, double-blind, multicenter clinical trial.	352	Safe and effective, improved quality of life	[159]
	lectin-standardized mistletoe extract	Controlled epidemiological multicentric retrolective cohort study	1248	Improved quality of life, prolonged relapse-free intervals	[160]
	Iscador® M spezial	Prospective open 2-armed non-randomized study	33	Lower frequency of nausea/vomiting; low systemic therapy side effects.	[161]
	Abnoba VISCUM(®) Mali	Noninterventional and prospective trial	270	Improved health related quality of life	[162]
Mushroom	*Trametes versicolor*	Phase I	11	Safe and tolerable, improved immune status	[153]
	White button mushroom	Phase I	36	Decreased PSA levels, decreased immunosuppressive factors	[152]

6. Advanced lectins formulations for cancer targeting and therapy

Potential challenges related to lectins successful clinical translation might generate from their poor stability, unspecific binding interactions and difficulties in production and purification [163,164]. To address some of these challenges, Andrade et al. investigated a nanoparticle formulation of *Cratylia mollis* lectin (Cra). Liposomal formulation of Cra, a mannose and glucose binding lectin, was investigated against sarcoma 180 in Swiss mice. The nanoparticle formulation, which ultimately improved the protein stability and delivery, showed a significant tumor inhibition (71%) with minimal tissue toxicity compared to free Cra solution (41%) [165]. Similarly, Lyu et al. reported an alginate-chitosan microparticle formulation of mistletoe lectin which offered an excellent stability of the lectin in acidic conditions with desired drug release profiles, suggesting the potential for MLs oral delivery [166]. Furthermore, a gene therapy approach for lectin drug delivery, which may ultimately provide an anti-cancer genes reservoir, was explored. In fact, a replication-deficient adenovirus-carrying gene encoding *Haliotis discus discus* sialic acid binding lectin (HddSBL) showed significant antiproliferation activities against hepatocellular carcinoma cell line Hep3B and lung cancer cell lines A549 and H1299 [167].

Other strategies focusing on the use of lectins as cancer targeting ligands have also been investigated. Although lectins may exert beneficial anticancer properties in such drug delivery systems; they are typically conjugated onto nanoparticles surface only for the so called "lectin direct targeting". Mo and Lim successfully developed a novel WGA-conjugated isopropyl myristate (IPM)-incorporated PLGA nanoparticle for local delivery of paclitaxel to the lung. This nanoparticle formulation showed a superior *in vitro* cytotoxicity against A549 and H1299 cells compared to the clinical paclitaxel formulation, due to a more efficient cellular uptake via WGA-receptors [168,169]. A similar formulation tested against colon cancer cells (Caco-2 and HT-29 cells) yielded an increased intracellular retention of paclitaxel and an enhanced antiproliferative activity [170]. The intracellular transport profile of lectin-functionalized nanoparticles in Caco-2 cells was further studied by Gao et al. using quantum dots-loaded WGA-PEG nanoparticle. This study demonstrated that WGA-functionalized PEG nanoparticle cellular uptake begins with the binding of WGA to its receptor, onto the cell surface, followed by particle uptake by clathrin and caveolae-mediated endocytosis mechanisms [171]. RCA-conjugated gold nanoparticle was shown to strongly accumulate onto HeLa cells, suggesting the use of such systems for cervical cancer selective targeting [172]. More recently, a gold nanoparticle formulation bearing hydrophobic zinc phthalocyanine photosensitizers and PEG conjugated jacalin (a Thomsen-Friedenreich antigen binding lectin) moieties has been reported for photodynamic therapy. The strong phototoxicity observed in HT-29 cancer cells (95–98%) was mainly due to the specific interactions between jacalin and the antigen expressed onto the cancer cell surface [173]. "Reverse lectin targeting" systems, in which carbohydrate moieties are conjugated to a drug delivery system to target endogenous lectins, have also been proposed. In a phase I clinical trial, such a system (PK2) composed of a polymer bearing doxorubicin with galactosamine has demonstrated liver-specific delivery of doxorubicin when administered by infusion [174].

Furthermore, lectins carbohydrate recognition was proven to be remarkably useful in cancer detection and imaging. In fact, SNA-tagged fluorescent polymeric nanoparticles were engineered to specifically target sialic acid moieties expressed on cancer cell surface (MCF-7 and HeLa) [175]. This study further confirmed the difference in sialic acid expression between cancerous and non-cancerous cells. A similar strategy was investigated for colorectal cancer cell imaging for potential application in colonoscopy. Thus, PNA immobilized onto fluorescent nanospheres showed high affinity and

Table 5. Example of novel drug delivery systems (DDS) for therapeutic lectins.

DDS	Lectin/ monosaccharide	Glycan preference/ lectin targeted	Cancer	Ref.
Liposome	Cra	Mannose, glucose	Sarcoma (180)	[165]
Alginate-Chitosan Microparticle	MLs	Lactose, D-galactose, GalNAc	Liver cancer (SK-Hep10)	[166]
Gene Therapy (Adenovirus)	HddSBL	Sialic acid	Hepatocellular carcinoma (Hep3B), lung cancer (A549, H1299)	[167]
WGA-IPM-PLGA nanoparticle	WGA	Sialic acid and N-acetyl glucosamine (GlcNAc)	Lung cancer (A549 and H1299)	[168, 169]
WGA-PEG nanoparticles	WGA	Sialic acid and N-acetyl glucosamine (GlcNAc)	colon cancer (Caco-2 and HT-29)	[170, 171]
RCA-conjugated gold nanoparticle	RCA	Galactose	Cervical cancer (HeLa)	[172]
Jacalin conjugated PEG-gold nanoparticles bearing hydrophobic zinc phthalocyanine	Jacalin	Thomsen-Friedenreich antigen	colon cancer (HT-29)	[173]
Polymer bearing doxorubicin with galactosamine (PK2)	galactosamine	Hepatic lectin [asialoglycoprotein receptor (ASGPR)]	Liver cancer	[174]
SNA-tagged fluorescent polymeric nanoparticles	SNA	α2-6-sialic acid	Cervical cancer (HeLa) Breast Cancer (MCF-7)	[175]
PNA immobilized onto fluorescent nanospheres	PNA	Thomsen-Friedenreich antigen	Colorectal adenocarcinoma (HT-29, HCT-116, and LS174T)	[176]
lectin conjugated paclitaxel loaded magnetic nanoparticle	Not specified	Human C-type lectin like molecules-1	Chronic myelogenous leukemia (K562)	[177]

specificity for human colorectal adenocarcinoma cell lines (HT-29, HCT-116 and LS174T) implanted on the cecal serosa of immune-deficient mice [176]. The latest investigations in this field are now focusing on the theranostic application of lectin conjugated nanoparticles drug delivery systems. This implies the ability of those systems to diagnose cancer, deliver an anticancer drug and monitor the therapeutic response, all at once. Singh at al. investigated a lectin conjugated paclitaxel loaded magnetic nanoparticle for leukemia theranostic application [177]. The nanoparticle formulation

showed a significantly higher efficacy (~67%) against chronic myelogenous leukemia cells (K562) compared to that of the native paclitaxel. A much longer circulation time ($T_{1/2}$ = 15 h) of this lectin coupled with paclitaxel nanoparticle compared to the native paclitaxel ($T_{1/2}$ = 5 h) was also reported in rats, in the same study. Table 5 is a summary of major lectin-based nanotechnology strategies discussed.

7. Conclusion

In summary, the use of lectins for cancer diagnosis, imaging and treatment has received a lot of attention among researchers. Although the clinical translation of these findings is still a major hurdle, the field of lectinology is expected to grow at an even faster pace in the coming years. Nevertheless, new investigations will probably have to explore safe and effective drug delivery system strategies for lectins, in order to maximize their use and increase the likelihood for their clinical translation. In fact, lectins-induced inflammation, toxicity and their resistance to digestive enzyme are some of the major arguments against these potent proteins [178-181]. In addition to cancer, lectins are being investigated for their role and/or therapeutic potential in other diseases, such as HIV [182-185], rheumatic heart disease [186,187], obesity-induced adipose tissue fibrosis [188] and diabetes [189-191]. Furthermore, understanding the function of lectin in ocular surfaces may provide new avenues for treating certain ocular diseases [192-194].

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Conflict of Interest

All authors declare no conflicts of interest in this manuscript.

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