Tumor Associated Carbohydrate Antigens in Targeted Therapy

[1]Somdutta Saha, Ph.D.*

[1]Computational Biology, R&D, GlaxoSmithKline,
1250 South Collegeville Road, Collegeville. Pennsylavnia 19426.
*Email: somdutta.x.saha@gsk.com

Abstract: Tumor associated carbohydrate antigens (TACAs) are a class of glycans with important structural and signaling functions playing a major role in cell proliferation, differentiation, and apoptosis relevant to oncology. Tumor cells expressing TACAs influence prognosis and survival of cancer patients. Careful consideration must be given to structural aspects during rational design of small molecule therapeutics that mimic the molecular topology of different classes of TACAs even though they are chemically dissimilar but functionally equivalent molecular structures.

Keywords: carbohydrate antigens, targeted therapy, small molecule

Introduction

Structural analyses of receptors/targets provide us with an effective tool for the development of disease therapeutics. Targeted therapies are expected to be more effective than current treatments and less harmful to normal cells. The advent of therapies based on mechanisms that target critical molecular pathways of tumors has evoked considerable interest (Green 2004). There is a growing number of FDA approved monoclonal antibodies and small molecules targeting specific types of cancer suggestive of the growing relevance of this therapeutic approach. Targeted cancer therapies, also referred to as "Personalized Medicine", are being studied for use alone, in combination with other targeted therapies, and in combination with chemotherapy (Joo et al. 2013). Targeted therapy blocks the growth of cancer cells by interfering with specific targeted molecules needed for carcinogenesis and tumor growth, rather than by simply interfering with all rapidly dividing cells.

Tumor Associated Carbohydrate Antigens (TACAs) are perceived to be viable targets for cancer therapeutics because of their

expression profile on normal vs. cancer cells, their multivalent presentation which impacts thermodynamics of binding of molecules and historical context of the innate immune system, whereby antibodies and other pattern-recognition receptors 'see' carbohydrates on bacteria (Beutler and Poltorak 2001). Because of similarities of carbohydrates on cancer cells and on bacteria, the pathophysiology of infection and neoplasia might be regulated because of the commonalities in carbohydrate structure (Beutler and Poltorak 2001; Liu and Ye 2012).

Interest in the carbohydrate moieties of glycol-conjugates has greatly augmented in the last decade since molecular biologists and protein chemists have been forced to acknowledge the vital role played by oligosaccharides as receptors on cell surfaces and as structural components essential for the correct conformation and biological functioning of soluble glycoproteins (Watkins, W.M. 1991). The general principles concerning structural patterns of the sugars, their method of attachment to protein or ceramide, the existence of heterogeneity of the oligosaccharide chains and their mechanisms of biosynthesis have been long established (MOSER and KARNOVSKY 1958). The discovery of a new type of O-glycosidic linkage involved in the glycosylation of nuclear and cytoplasmic components (Hart et al. 1989) and the finding that certain proteins in mammalian cells are anchored in the membrane via glycosyl-phosphatidylinositol structures (Ferguson, M.A.J. et. al. 1988). However, much of the progress made today is in the definition of the biological function of the glycol-conjugates and the introduction of refined methodologies that enable ever smaller quantities of materials to be subjected to structural analysis.

These advances provide details that permit more accurate models that define their geometries and realistic attempts made to determine the nature of the molecular interactions between carbohydrate and protein (Carver, J.P.et al 1989), or carbohydrate and carbohydrate (Kamiya et al. 2011). Gradually, as more knowledge is being gained concerning the enzymes, the glycosyltransferases, involved in the biosynthesis of the oligosaccharide chains, this is enabling approaches to be made to isolate the genes encoding these enzymes and to use these probes to gain further understanding of the genetic regulation of

oligosaccharide synthesis (Paulson and Colley 1989). This aspect is definitely one that has to be understood if we are to have the ability to ensure the correct glycosylation patterns of recombinant glycoprotein products and to interpret the changes that occur in cell surface carbohydrates in differentiation and malignancy.

TACAs as essential biomarkers

Broadly speaking, TACAs can be divided into:

1. Glycolipids - GM1, GM2, GD2, and GD3
2. Glycoproteins-Thomsen-Friedenreich (TF or T antigens)
3. Glycoproteins and Glycolipids-Lewis antigens - Le^A, Le^X, and Le^Y

sLe^x, $s\text{-}Le^a$ and sialyl-Thomsen-nouvelle (sTn) are TACAs found in breast cancer . When these antigens are detected at the surface of breast carcinoma cells, they are usually associated with a poor prognosis and a reduced overall survival of the patients (Miles et al. 1994). These glycosylation changes mostly result from the modification of the expression of glycosyltransferase (GT) genes, and the level of expression of sialyltransferases has been proposed as a prognostic marker for the follow-up of breast cancer patients (Recchi et al. 1998) (Table 1).

TACA	**Altered Pathway**	**Comments**
Mucin Type antigens		
Thomsen-Friedenreich	Decrease in Core 2 β6-GlcNAc-T expression, decrease in Core 1 sialylation	Expressed in almost 90% of breast cancers
Lewis antigen		
Sialyl $lewis^x$	Increased expression of FucT (FucT VI and FucT VII)	Higher in metastatic compared to non-metastatic cancers
Sialyl lewis a	Increased expression of FucT (FucT III)	
Lewis Y	Increased expression of FucT	Over-expressed in 60-90% of breast cancers

Gangliosides		
GM3	Increased expression of ST3Gal V	2.8-fold increase compared with normal tissue
GD3	Increased expression of ST8Sia I	1.7-fold increase compared with normal tissue

Table 1: showing the different types of gangliosides, the pathways they affect and their expression level in various types of cancer. Fuc T – α3-fucosyl transferases, ST3Gal V- α-2,3-sialyltransferase (siat9), ST8Sia I - α-2,8-sialyltransferase (Cazet et al. 2010b).

Aberrant glycosylation and the over-expression of certain carbohydrate moieties is a consistent feature of cancers, and tumor-associated oligosaccharides are actively investigated as targets for immunotherapy ((Brooks et al. 2010a) . One of the most common aberrations in glycosylation patterns is the presentation of a single O-linked *N*-acetylgalactosamine on a threonine or serine residue known as the "Tn antigen" (Brooks et al. 2010b; Jensen et al. 1996). Whereas the ubiquitous nature of Tn antigens on cancers has made them a natural choice for vaccine research, such carbohydrate moieties are not always tumor-specific and have been observed on embryonic and nonmalignant adult tissue (Brooks et al. 2010b). Tumors expressing high levels of certain types of TACAs exhibit greater metastasis and progression than those expressing low level of TACAs, as reflected in decreased patient survival rate (Hakomori 2001). Widely studied examples of such TACAs are: (i) H/Le(y)/Le(a) in primary non-small cell lung carcinoma; (ii) sialyl-Le(x) (SLe(x)) and sialyl-Le(a) (SLe(a)) in various types of cancer (Yu et al. 2011); (iii) Tn and sialyl-Tn in colorectal, lung, breast, and many other cancers; (iv) GM2, GD2, and GD3 gangliosides in neuroectodermal tumors (melanoma and neuroblastoma); (v) globo-H in breast, ovarian, and prostate cancer; (vi) disialylgalactosylgloboside in renal cell carcinoma (Hakomori 1996, Hakomori 2001).

Some glycosylations and TACAs suppress invasiveness and metastatic potential. Well-documented examples are: (i) blood group A

antigen in primary lung carcinoma; (ii) bisecting β1 -> 4GlcNAc of N-linked structures in melanoma and other cancers (Yamamoto et al. 2000); (iii) galactosylgloboside (GalGb4) in seminoma (Gu and Taniguchi 2008). The biochemical mechanisms by which the above glycosylation changes promote or suppress tumor metastasis and invasion are mostly unknown. A few exceptional cases in which we have some knowledge are: (i) SLe(x) and SLe (a) function as E-selectin epitopes promoting tumor cell interaction with endothelial cells (Cavanna et al. 2001); (ii) some tumor cells interact through binding of TACA to specific proteins other than selectin (Parajuli et al. 2001), or to specific carbohydrate expressed on endothelial cells or other target cells (carbohydrate-carbohydrate interaction) (Monzavi-Karbassi et al. 2001) (iii) functional modification of adhesive receptor (integrin, cadherin, CD44) by glycosylation (Hakomori 1985; Hakomori 2001). So far, a few successful cases of anti-cancer vaccine in clinical trials have been reported, employing TACAs whose expression enhances malignancy (Brezicka et al. 2000; Kim et al. 2000). Examples are STn for suppression of breast cancer, GM2 and GD3 for melanoma, and globo-H for prostate cancer (Hakomori 2001).

Aberrant glycosylation has been found in all tumor cells examined and the expression of TACA, the result of this process, influences the prognosis and survival of cancer patients in a manner that is proportional to the degree of expression (Hakomori 2001). TACA are present in tumors more frequently than oncogene products (e.g. myc, ras, HER2/neu) and their association with tumor progression is stronger than the deletion or inactivation of tumor-suppressing genes (e.g. p53, p16) (Hakomori 2001) . In fact, TACA are not only tumor markers, but constitute part of the machinery that is essential for inducing metastasis and invasiveness (Muramatsu 1993). The modification of cellular glycosylation is indeed a common phenotypic change of cancer cells that mainly affects the outer part of glycans, leading to the expression of tumor-associated carbohydrate antigens (TACAs) (Cazet et al. 2010a). Increased β1->6 branching, increased sLe^x or sialyl-$Lewis^a$ (sLe^a) antigens, or the general increase in sialylation are commonly observed in *N*-linked and *O*-linked glycans of carcinoma cells and are associated with grade, invasion, metastasis and poor

prognosis (Cazet et al. 2010b). These changes in glycosylation often reflect the deregulation of glycosyl transferase expression at the transcriptional level, and several examples have shown that glycosyltransferase genes, including *ST6GAL1* (Le et al. 1992) and *MGAT5* (Buckhaults et al. 1997), are regulated by oncogenes.

Development of therapeutics

The development of vaccines can also be extended using other TACAs, with the following criteria for success: (i) the antigen is expressed highly on tumor cells; (ii) high antibody production depending on two factors: (a) clustering of antigen used in vaccine; (b) choice of appropriate carrier protein or lipid; (iii) high T cell response depending on choice of appropriate carrier protein or lipid; (iv) expression of the same antigen in normal epithelial tissues (e.g., renal, intestinal, colorectal) may not pose a major obstacle, i.e., these tissues are not damaged during immune response (Liao et al. 2013). Idiotypic anti-carbohydrate antibodies that mimic the surface profile of carbohydrate antigens, when administered to patients, elicit anti-carbohydrate antibody response, thus providing an effect similar to that of TACAs for suppression of tumor progression. An extension of this idea is the use of peptide mimetics of TACAs, based on phage display random peptide library (Pashov et al. 2007). Even though examples of using such "mimotopes" as immunogens are highly limited, these mimotopes may overcome the weak immunogenicity of TACAs in general (Pashov et al. 2005; Xu et al. 2005).

In this context, inducing IgM reactive antibodies to TACA expressed on tumor cells is similar to the role played by the body's first line immune surveillance- the circulating, natural IgM antibodies (Saha et al, 2012). But even though carbohydrate reactive IgMs possess these features, they are not considered promising from a therapeutic potential due to their transient nature (Saha et. al. 2012, Kieber-Emmons et al. 2012). The functions of IgM glycan binding antibodies depend on the nature of the target but also on the density of the target. Thus, specific targeting of tumor cells is due in part to over-expression of the carbohydrate antigen on tumor cells, which basically compensates for the low affinity of the antibodies cross-reactive to carbohydrate antigens (Zuckier et al. 2000).TACAs represent a wide range of anti-tumor targets as they are collectively implicated in tumor

cell survival, cell signaling and communication, adhesion and migration (Wilson and Danishefsky 2013). Targeting TACAs will ensure many cell surface proteins or glycans on tumor cells to be targeted all at once, thus ruling out developing individual anti-tumor regimens. Developing mimetic peptides against TACAs help induce wide spectrum of TACA-reactive antibodies. One such carbohydrate mimetic peptide, popularly known as P10s was successfully tested in Phase I clinical trial of stage IV breast cancer patients by the Kieber-Emmons group (Makhoul et al. 2015) .

In developing therapeutics, the entropic and enthalpic contribution of antibody-antigen complexes plays an important role (Thorpe and Brooks, III 2007). Rational drug design is normally based on the knowledge of the three-dimensional structure of the protein-ligand complex and the thermodynamics of ligand binding involving both enthalpy and entropy components that drives ligand binding (del et al. 2012). The role of conformational entropy and its relative contribution to the free energy of ligand binding to the carbohydrate recognition has been observed in many cases. There is a subtle interplay between structure and conformational fluctuations in the different complexes that fine-tunes the affinity. The change in conformational entropy is comparable in magnitude to the binding enthalpy, demonstrating that it contributes favorably and significantly to ligand binding (del et al. 2012).

It has been suggested that cancer treatment lies in a combination of patient-tailored and multi-targeting strategies. –We believe that, because of their profile of expression and their pro-aggressive function, TACAS will be considered as potent molecular targets (Cazet et al. 2010b). Tumor antigens are often tissue-specific and do not share antigenic patterns, meaning that it is very difficult to generate preventive vaccines for a large population (Xu et al. 2005). Therefore, a close examination of the core structures for TACAs reveal that the these are repeated in the extended oligosaccharides – a concept that can be utilized in developing therapeutics against this class of glycans. TACA-directed approaches not only enforce a natural anti-tumor antibody response, but also conform to a novel view of immunotherapy as it controls complex immune processes related more to the interventions in autoimmunity as opposed to a purely vaccinological view of

tumor vaccine (Saha et al. 2014). A better understanding of the immunoregulatory aspect of anti-TACA responses, for example, will help us to understand the link between TACA immunization, innate immunity, and cellular immunity. The development of therapeutics for cancer necessitates understanding the structural nature of the target and the structural nature of the therapeutic. Advances in genomics and genetics reveal new genes and pathways that are altered in cancer. This information translates into molecular targets for which therapeutics might be developed. Such a concept forms the basis for translational studies, bringing a laboratory finding to the clinic.

References

Beutler, B. and Poltorak, A. (2001) Sepsis and evolution of the innate immune response. Crit Care Med. 29, S2-S6. http://dx.doi.org/10.1097/00003246-200107001-00002
PMid:11445725

Brezicka, T., Bergman, B., Olling, S. and Fredman, P. (2000) Reactivity of monoclonal antibodies with ganglioside antigens in human small cell lung cancer tissues. Lung Cancer 28, 29-36. http://dx.doi.org/10.1016/S0169-5002(99)00107-5

Brooks, C.L., Schietinger, A., Borisova, S.N., Kufer, P., Okon, M., Hirama, T., Mackenzie, C.R., Wang, L.X., Schreiber, H. and Evans, S.V. (2010a) Antibody recognition of a unique tumor-specific glycopeptide antigen. Proc. Natl. Acad. Sci. U. S. A 107, 10056-10061. http://dx.doi.org/10.1073/pnas.0915176107
PMid:20479270 PMCid:PMC2890472

Brooks, C.L., Schietinger, A., Borisova, S.N., Kufer, P., Okon, M., Hirama, T., Mackenzie, C.R., Wang, L.X., Schreiber, H. and Evans, S.V. (2010b) Antibody recognition of a unique tumor-specific glycopeptide antigen. Proc. Natl. Acad. Sci. U. S. A 107, 10056-10061. http://dx.doi.org/10.1073/pnas.0915176107
PMid:20479270 PMCid:PMC2890472

Buckhaults, P., Chen, L., Fregien, N. and Pierce, M. (1997) Transcriptional regulation of N-acetylglucosaminyltransferase V by the src oncogene. J. Biol. Chem. 272, 19575-19581. http://dx.doi.org/10.1074/jbc.272.31.19575
PMid:9235963

Cavanna, B., Jiang, H., Allaria, S., Carpo, M., Scarlato, G. and Nobile-Orazio, E. (2001) Anti-GM(2) IgM antibody-induced complement-mediated cytotoxicity in patients with dysimmune neuropathies. J. Neuroimmunol. 114, 226-231.

http://dx.doi.org/10.1016/S0165-5728(00)00461-6

Cazet, A., Julien, S., Bobowski, M., Burchell, J. and Delannoy, P. (2010a) Tumour-associated carbohydrate antigens in breast cancer. Breast Cancer Res. 12, 204. http://dx.doi.org/10.1186/bcr2577 PMid:20550729 PMCid:PMC2917018

Cazet, A., Lefebvre, J., Adriaenssens, E., Julien, S., Bobowski, M., Grigoriadis, A., Tutt, A., Tulasne, D., Le, B., X and Delannoy, P. (2010b) GD(3) synthase expression enhances proliferation and tumor growth of MDA-MB-231 breast cancer cells through c-Met activation. Mol. Cancer Res. 8, 1526-1535. http://dx.doi.org/10.1158/1541-7786.MCR-10-0302 PMid:20889649

del, C.F.-A., Diaz, D., Berbis, M.A., Marcelo, F., Canada, J. and Jimenez-Barbero, J. (2012) Protein-carbohydrate interactions studied by NMR: from molecular recognition to drug design. Curr. Protein Pept. Sci. 13, 816-830. http://dx.doi.org/10.2174/138920312804871175 PMid:23305367 PMCid:PMC3706953

Green, M.R. (2004) Targeting targeted therapy. N. Engl. J. Med. 350, 2191-2193. http://dx.doi.org/10.1056/NEJMe048101 PMid:15118072

Gu, J. and Taniguchi, N. (2008) Potential of N-glycan in cell adhesion and migration as either a positive or negative regulator. Cell Adh. Migr. 2, 243-245. http://dx.doi.org/10.4161/cam.2.4.6748

Hakomori, S. (1985) Aberrant glycosylation in cancer cell membranes as focused on glycolipids: overview and perspectives. Cancer Res. 45, 2405-2414. PMid:3886132

Hakomori, S. (2001) Tumor-associated carbohydrate antigens defining tumor malignancy: basis for development of anti-cancer vaccines. Adv. Exp. Med. Biol. 491, 369-402. http://dx.doi.org/10.1007/978-1-4615-1267-7_24

Hart, G.W., Haltiwanger, R.S., Holt, G.D. and Kelly, W.G. (1989) Glycosylation in the nucleus and cytoplasm. Annu. Rev. Biochem. 58, 841-874. http://dx.doi.org/10.1146/annurev.bi.58.070189.004205 PMid:2673024

Jensen, T., Galli-Stampino, L., Mouritsen, S., Frische, K., Peters, S., Meldal, M. and Werdelin, O. (1996) T cell recognition of Tn-glycosylated peptide antigens. Eur. J. Immunol. 26, 1342-1349. http://dx.doi.org/10.1002/eji.1830260625 PMid:8647215

Joo, W.D., Visintin, I. and Mor, G. (2013) Targeted cancer therapy--are the days of systemic chemotherapy numbered? Maturitas 76, 308-314. http://dx.doi.org/10.1016/j.maturitas.2013.0

9.008
PMid:24128673 PMCid:PMC4610026

Kamiya, Y., Yagi-Utsumi, M., Yagi, H. and Kato, K. (2011) Structural and molecular basis of carbohydrate-protein interaction systems as potential therapeutic targets. Curr. Pharm. Des 17, 1672-1684. http://dx.doi.org/10.2174/138161211796355074
PMid:21619528

Kieber-Emmons, T., et al. (2012). "The promise of the anti-idiotype concept." Front Oncol 2: 196. http://dx.doi.org/10.3389/fonc.2012.00196
PMid:23267437 PMCid:PMC3526099

Kim, S.K., Ragupathi, G., Cappello, S., Kagan, E. and Livingston, P.O. (2000) Effect of immunological adjuvant combinations on the antibody and T-cell response to vaccination with MUC1-KLH and GD3-KLH conjugates. Vaccine 19, 530-537. http://dx.doi.org/10.1016/S0264-410X(00)00195-X

Le, M.N., Laudet, V., Svensson, E.C., Cazlaris, H., Van, H.B., Lagrou, C., Stehelin, D., Montreuil, J., Verbert, A. and Delannoy, P. (1992) The c-Ha-ras oncogene induces increased expression of beta-galactoside alpha-2, 6-sialyltransferase in rat fibroblast (FR3T3) cells. Glycobiology 2, 49-56. http://dx.doi.org/10.1093/glycob/2.1.49

Liao, S.F., Liang, C.H., Ho, M.Y., Hsu, T.L., Tsai, T.I., Hsieh, Y.S., Tsai, C.M., Li, S.T., Cheng, Y.Y., Tsao, S.M., Lin, T.Y., Lin, Z.Y., Yang, W.B., Ren, C.T., Lin, K.I., Khoo, K.H., Lin, C.H., Hsu, H.Y., Wu, C.Y. and Wong, C.H. (2013) Immunization of fucose-containing polysaccharides from Reishi mushroom induces antibodies to tumor-associated Globo H-series epitopes. Proc. Natl. Acad. Sci. U. S. A 110, 13809-13814. http://dx.doi.org/10.1073/pnas.1312457110
PMid:23908400 PMCid:PMC3752246

Liu, C.C. and Ye, X.S. (2012) Carbohydrate-based cancer vaccines: target cancer with sugar bullets. Glycoconj. J. 29, 259-271. http://dx.doi.org/10.1007/s10719-012-9399-9

Makhoul, I., Hutchins, L., Emanuel, P.D., Pennisi, A., Siegel, E., Jousheghany, F., Monzavi-Karbassi, B. and Kieber-Emmons, T. (2015) Moving a Carbohydrate Mimetic Peptide into the clinic. Hum. Vaccin. Immunother. 11, 37-44. http://dx.doi.org/10.4161/hv.34300
PMid:25483513 PMCid:PMC4514369

Miles, D.W., Happerfield, L.C., Smith, P., Gillibrand, R., Bobrow, L.G., Gregory, W.M. and Rubens, R.D. (1994) Expression of sialyl-Tn predicts the effect of adjuvant chemotherapy in node-positive breast cancer. Br. J. Cancer 70, 1272-1275. http://dx.doi.org/10.1038/bjc.1994.486

Monzavi-Karbassi, B., Cunto-Amesty, G., Luo, P., Shamloo, S., Blaszcyk-Thurin, M.

and Kieber-Emmons, T. (2001) Immunization with a carbohydrate mimicking peptide augments tumor-specific cellular responses. Int. Immunol. 13, 1361-1371.
http://dx.doi.org/10.1093/intimm/13.11.1361
PMid:11675368

MOSER, H. and KARNOVSKY, M.L. (1958) Studies on the biosynthesis of cerebroside galactose. Neurology 8, 81-83.
http://dx.doi.org/10.1212/WNL.8.Suppl_1.81
PMid:13541617

Muramatsu, T. (1993) Carbohydrate signals in metastasis and prognosis of human carcinomas. Glycobiology 3, 291-296.
http://dx.doi.org/10.1093/glycob/3.4.291
PMid:8400544

Parajuli, P., Yanagawa, H., Hanibuchi, M., Takeuchi, E., Miki, T., Yano, S. and Sone, S. (2001) Humanized anti-ganglioside GM2 antibody is effective to induce antibody-dependent cell-mediated cytotoxicity in mononuclear cells from lung cancer patients. Cancer Lett. 165, 179-184.
http://dx.doi.org/10.1016/S0304-3835(01)00427-X

Pashov, A., Monzavi-Karbassi, B., Raghava, G. and Kieber-Emmons, T. (2007) Peptide mimotopes as prototypic templates of broad-spectrum surrogates of carbohydrate antigens for cancer vaccination. Crit Rev. Immunol. 27, 247-270.
http://dx.doi.org/10.1615/CritRevImmunol.v27.i3.50
PMid:18197820

Pashov, A., Perry, M., Dyar, M., Chow, M. and Kieber-Emmons, T. (2005) Carbohydrate mimotopes in the rational design of cancer vaccines. Curr. Top. Med. Chem. 5, 1171-1185.
http://dx.doi.org/10.2174/15680260577437 0928
PMid:16248790

Paulson, J.C. and Colley, K.J. (1989) Glycosyltransferases. Structure, localization, and control of cell type-specific glycosylation. J. Biol. Chem. 264, 17615-17618.
PMid:2681181

Recchi, M.A., Hebbar, M., Hornez, L., Harduin-Lepers, A., Peyrat, J.P. and Delannoy, P. (1998) Multiplex reverse transcription polymerase chain reaction assessment of sialyltransferase expression in human breast cancer. Cancer Res. 58, 4066-4070.
PMid:9751611

Saha, S., Pashov, A. et al. (2012). "Carbohydrate Mimetic Peptide vaccines" In: Anticarbohydrate Antibodies- From Molecular Basis to Clinical Application (Ed: P Kosma and S. Müller-Loennies). 229-254.
http://dx.doi.org/10.1007/978-3-7091-0870-3_10

Saha, S., Pashov, A., Siegel, E.R., Murali,

R. and Kieber-Emmons, T. (2014) Defining the recognition elements of Lewis Y-reactive antibodies. PLoS. One. 9, e104208. http://dx.doi.org/10.1371/journal.pone.0104208

Thorpe, I.F. and Brooks, C.L., III. (2007) Molecular evolution of affinity and flexibility in the immune system. Proc. Natl. Acad. Sci. U. S. A 104, 8821-8826. http://dx.doi.org/10.1073/pnas.0610064104 PMid:17488816 PMCid:PMC1885586

Wilson, R.M. and Danishefsky, S.J. (2013) A vision for vaccines built from fully synthetic tumor-associated antigens: from the laboratory to the clinic. J. Am. Chem. Soc. 135, 14462-14472. http://dx.doi.org/10.1021/ja405932r

Xu, Y., Sette, A., Sidney, J., Gendler, S.J. and Franco, A. (2005) Tumor-associated carbohydrate antigens: a possible avenue for cancer prevention. Immunol. Cell Biol. 83, 440-448. http://dx.doi.org/10.1111/j.1440-1711.2005.01347.x

Yamamoto, H., Swoger, J., Greene, S., Saito, T., Hurh, J., Sweeley, C., Leestma, J., Mkrdichian, E., Cerullo, L., Nishikawa, A., Ihara, Y., Taniguchi, N. and Moskal, J.R. (2000) Beta1,6-N-acetylglucosamine-bearing N-glycans in human gliomas: implications for a role in regulating invasivity. Cancer Res. 60, 134-142. PMid:10646865

Yu, S., Wang, Q., Zhang, J., Wu, Q. and Guo, Z. (2011) Synthesis and Evaluation of Protein Conjugates of GM3 Derivatives Carrying Modified Sialic Acids as Highly Immunogenic Cancer Vaccine Candidates. Medchemcomm. 2, 524-530. http://dx.doi.org/10.1039/c1md00033k PMid:21927709 PMCid:PMC3172705

Zuckier, L.S., Berkowitz, E.Z., Sattenberg, R.J., Zhao, Q.H., Deng, H.F. and Scharff, M.D. (2000) Influence of affinity and antigen density on antibody localization in a modifiable tumor targeting model. Cancer Res. 60, 7008-7013. PMid:11156404