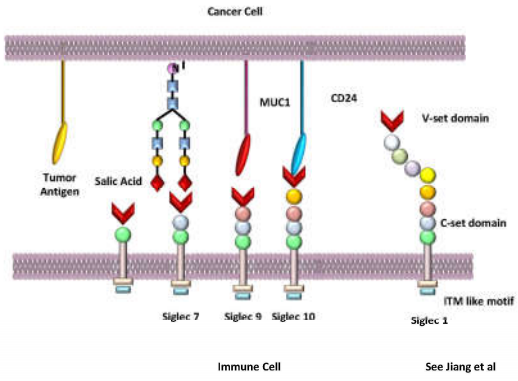

SIALIC ACID: ANOTHER CANCER TARGET OR JUST A RED QUEEN?

TGL 203

ABSTRACT
Sialic acid is a putatively controlling element in glycans on cells. It interacts with Siglecs, lectins, that on surface elements of immune cells. Its presence can accelerate malignant growth. We examine this and therapeutic targets in a broad spectrum of malignancies.

Terrence McGarty
October 2023

NOTICE

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Communications relating to these documents and these should be sent to:
mcgarty@alum.mit.edu.

Contents

1 INTRODUCTION

"The time has come the walrus said to think of many things…"

Cancers are complex entities that have a variety of causes and reasons for proliferation. Many recent developments are single threaded looking at one area in depth. However we know that the complex genomic factors, microenvironmental factors, epigenetic factors are but a few of the drivers that result in a malignancy. Thus there are many rocks to be over-turned and examined. Herein we look at the effects of sialic acid, a common component of cells and its ability to induce a malignancy and also support metastatic behavior.

Sialic acids are a byproduct of glucose metabolism. In turn the sialic acids become bound to glycoproteins, the cell surface proteins that have protruding chains of sugars. The sialic acids often then bind to the terminal end and as a result these surface proteins which can become ligands for attack by the immune system such as T and NK cells are neutralized. Thus when a cell becomes malignant the immune system can be prohibited from attacking this malignant cells due to the blocking presence of the sialic acid molecule.

In this Note we examine some recent studies of various sialic acid action on cancers and primarily prostate and breast. Sialic acid interfaces with immune cells have been noted to reflect the "Red Queen" effect. Namely this is a battle between a pathogen and its host, a malignant cell and an immune cell whereby the challenge is "It takes all the running you can do to keep the same place". Thus as the pathogens change and adapt, the immune cells must do likewise. Malignant cells seem always to keep a step ahead of the immune cells which abate them. Sialic acid modifications and immune cell interfaces present an interesting challenge as well as an opportunity. Herein we attempt to lay out some of the recent efforts in understanding this characteristic. It may present another opportunity to get a step ahead of the "Red Queen"[1].

1.1 PROSTATE CANCER

Prostate cancer is a complex and often heterogeneous malignancy that has challenged the use of various therapeutic methodologies[2]. We have previously shown that in certain individuals a CIS PCa (High grade PIN) may regress when attacked by the immune system. This observation was of limited value because it was just of limited and non-controlled observation. A recent paper by Wen et al:

Sialic acids have been implicated in cancer initiation, progression, and immune evasion in diverse human malignancies. Sialylation of terminal glycans on cell surface and secreted

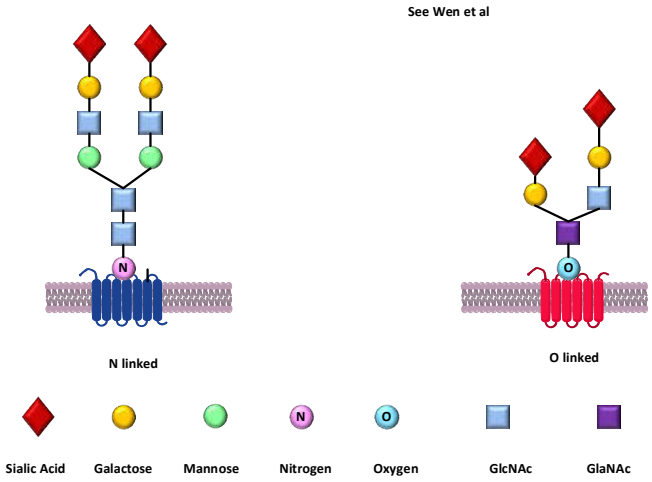
[1] See Varki, *Given the above considerations, it is reasonable to suggest that glycans are particularly prone to Red Queen effects (running to stay in one place). … one can envisage several such effects involving glycan interactions, leading to a delicate balance between preserving endogenous function and evading pathogen attack*

[2] https://www.researchgate.net/publication/264960277_Prostate_Cancer_A_Systems_Approach

glycoproteins is a long recognized feature of cancer cells[3]. Recently, immune checkpoint inhibitor immunotherapy has tremendously improved the outcomes of patients with various cancers. However, available immunotherapy approaches have had limited efficacy in metastatic castration-resistant prostate cancer (CRPC).

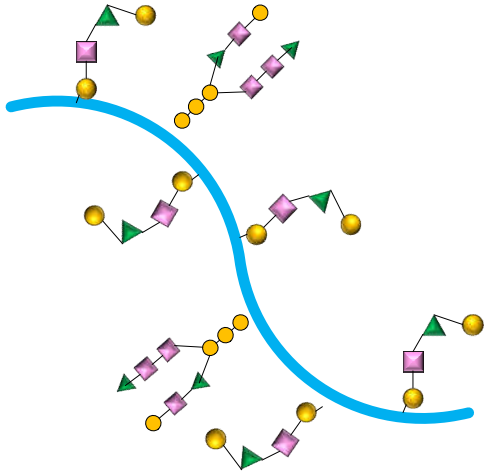
Sialic acid modified glycoproteins in prostate cancers (PCa) and their interaction with Siglec receptors on tumor infiltrating immune cells might underlie immunosuppressive signaling in PCa. Here, we summarize the function of sialic acids and relevant glycosynthetic enzymes in cancer initiation and progression. We also discuss the possible uses of sialic acids as biomarkers in prostate cancer and the potential methods for targeting sialic acid-Siglec interactions for prostate cancer treatment.

An example of some typical sialic structure attached to a cell wall protein are shown below.



In reality the protein strand may look like the one below with multiple glycans and many sialylated.

[3] https://www.researchgate.net/publication/355980410_Glycans_COVID_and_Cancer



1.2 Breast Cancer

In a similar fashion recent work has demonstrated similar influence in breast cancer. In the work by Mereiter et al we have:

Immunotherapies have revolutionized treatment and management of cancers. However, the use of immune checkpoint inhibitors (ICIs) in breast cancer is limited due to lack of efficacy. Sialylation, the modification of glycans with sialic acid, is frequently upregulated in various cancer types and has potent immunoregulatory properties. However, its specific role in breast cancer immune evasion has remained largely elusive. Here, we show that breast cancer sialylation drives the recruitment of polymorphonuclear myeloid suppressor cells to the tumor microenvironment, thus hampering the efficient eradication of tumors by CD8+ T cells.

Notably, abrogation of tumor sialylation, either genetically or pharmacologically, not only facilitated CD8-mediated tumor control but also enabled the recruitment of Tcf7+ memory T cells. Significantly, abrogation of sialylation sensitized PD-1- resistant breast tumors to immunotherapy. Sialylation interference was well-tolerated in mammary development and function. We further demonstrate that hyper-sialylation occurs in over half of human breast cancers and correlates with poor T cell infiltration.

Our results establish sialylation as a central immunoregulator in breast cancer, orchestrating multiple pathways that ultimately culminate in immune evasion. Importantly, our study underscores the potential of targeting this pathway to enhance tumor control, converting immunologically inert tumors into inflamed ones, and prime tumors for synergistic interventions involving immune checkpoint inhibitors.

This observation has powerful corollaries. We will examine some of these in this Note.

1.3 OVERVIEW

Our overall objective in this Note is to present sialic acid and siglecs as an additional element in the use of immunotherapy dealing with cancers. We focused on two types, prostate and breast, because they are the most common in men and women.

As Lubbers et al have noted:

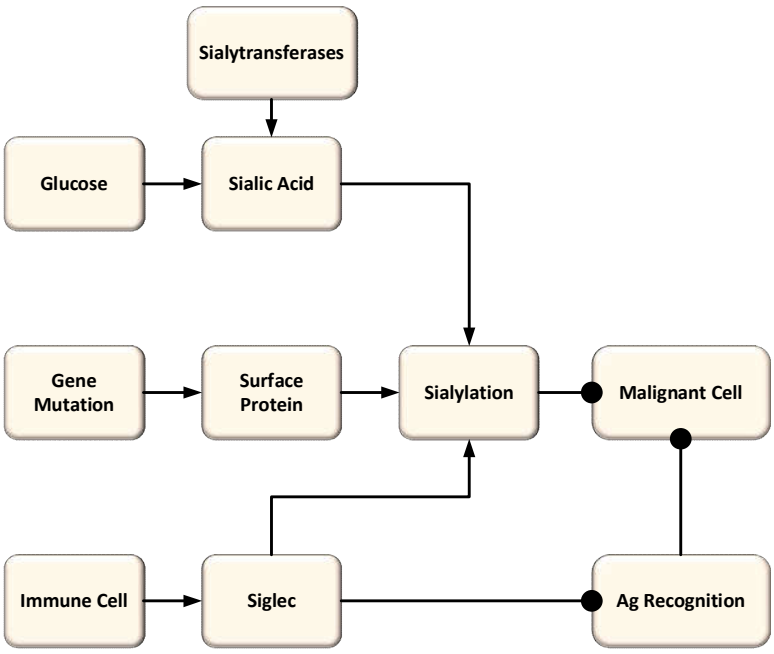
One of the key features of the immune system is its extraordinary capacity to discriminate between self and non-self and to respond accordingly. Several molecular interactions allow the induction of acquired immune responses when a foreign antigen is recognized, while others regulate the resolution of inflammation, or the induction of tolerance to self-antigens.

Post-translational signatures, such as glycans that are part of proteins (glycoproteins) and lipids (glycolipids) of host cells or pathogens, are increasingly appreciated as key molecules in regulating immunity vs. tolerance.

Glycans are sensed by glycan binding receptors expressed on immune cells, such as C-type lectin receptors (CLRs) and Sialic acid binding immunoglobulin type lectins (Siglecs), that respond to specific glycan signatures by triggering tolerogenic or immunogenic signaling pathways.

Glycan signatures present on healthy tissue, inflamed and malignant tissue or pathogens provide signals for "self" or "non-self" recognition. In this review we will focus on sialic acids that serve as "self" molecular pattern ligands for Siglecs. We will emphasize on the function of Siglec-expressing mononuclear phagocytes as sensors for sialic acids in tissue homeostasis and describe how the sialic acid-Siglec axis is exploited by tumors and pathogens for the induction of immune tolerance. Furthermore, we highlight how the sialic acid-Siglec axis can be utilized for clinical applications to induce or inhibit immune tolerance.

Thus the simple model of DNA begets RNA begets protein, albeit grossly correct, fails to account for the multiplicity of secondary factors which often dominate. We have seen such factors as epigenetic ones that delimit expression, as well as a set of other such factors including the tumor micro environment. The flow of the elements in the glycan paradigm is presented below. We shall examine each in some detail. The ultimate goal is to understand how sialic acid can result in a malignancy and metastasis and what therapeutic approaches are possible,



We thus proceed to present the following topics:

1. Sialic acids, their structure, function and generation
2. Glycans, likewise their generation and functions as well as structures
3. Lectins and siglecs, the proteins targeting glycans with sialic acids attached
4. The Immune system and its ligands of siglecs and possible activators or inhibitors
5. The impact on some major cancers; prostate and breast
6. Therapeutic options available to target siglecs and sialic acids

2 GLYCANS

We first start with glycans[4]. Glycans and glycosylation are basically the attachment of sugar molecules, often in linkages of multiple basic sugars, to another molecule, namely a protein. In the case at point, we examine these attached sugars in terms of proteins on the surface of cancer cells.

2.1 INTRODUCTION

We now present a summary of the major glycans and their structures. Varki has presented a significant update regarding glycans. He notes:

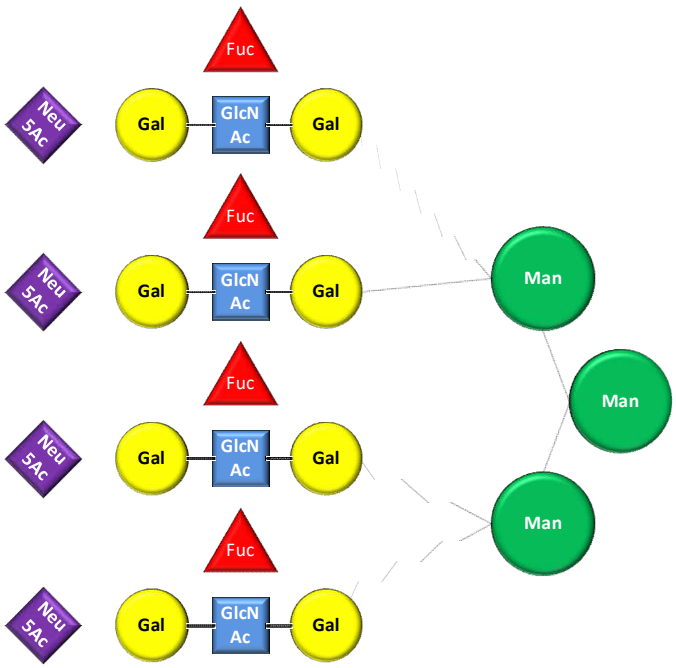
Simple and complex carbohydrates (glycans) have long been known to play major metabolic, structural and physical roles in biological systems. Targeted microbial binding to host glycans has also been studied for decades. But such biological roles can only explain some of the remarkable complexity and organismal diversity of glycans in nature.

Reviewing the subject about two decades ago, one could find very few clear-cut instances of glycan-recognition-specific biological roles of glycans that were of intrinsic value to the organism expressing them. In striking contrast there is now a profusion of examples, such that this updated review cannot be comprehensive. Instead, a historical overview is presented, broad principles outlined and a few examples cited, representing diverse types of roles, mediated by various glycan classes, in different evolutionary lineages.

What remains unchanged is the fact that while all theories regarding biological roles of glycans are supported by compelling evidence, exceptions to each can be found. In retrospect, this is not surprising. Complex and diverse glycans appear to be ubiquitous to all cells in nature, and essential to all life forms. Thus, >3 billion years of evolution consistently generated organisms that use these molecules for many key biological roles, even while sometimes coopting them for minor functions. In this respect, glycans are no different from other major macromolecular building blocks of life (nucleic acids, proteins and lipids), simply more rapidly evolving and complex. It is time for the diverse functional roles of glycans to be fully incorporated into the mainstream of biological sciences.

Below we show a tree like glycan structure which has the ability to link itself to a protein. There is a mannose base and then four identical branches of linked sugars.

[4] https://www.researchgate.net/publication/355980410_Glycans_COVID_and_Cancer This is and earlier paper where we examined cancers and COVID.

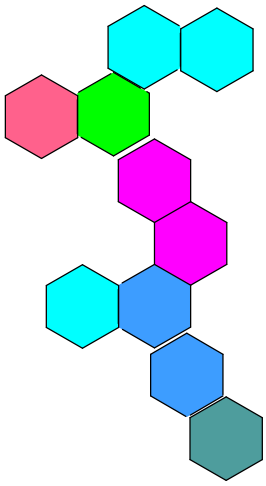


As Bao et al have noted:

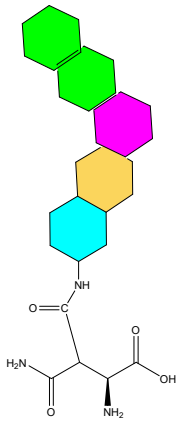
Glycosylation is a complex post-translational modi and it decorates one- proteins. Glycans account for a fifth to one-half of eukaryotic –25% of dry cell mass frcation and have essential functional and pathological roles. Despite their importance, glycans have complex structures that are difficult to study. The complex structures of glycans arise from a context-sensitive biosynthetic network involving dozens of enzymes.

A simple change of a single intermediate glycan or glycosyltransferase will have cascading impacts on the glycans secreted. Unfortunately, current data analysis approaches for glycoprofiling and glycomic data lack the critical systems perspective to decode the interdependence of glycans easily. It is important to understand the network behind the glycoprofiles to understand the behavior of the process better.

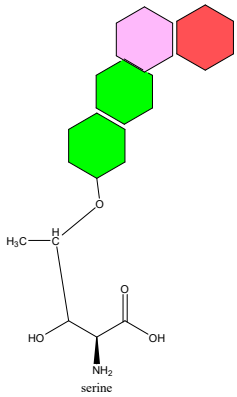
Another view is to see a collection of sugars as shown graphically below. They may be a disparate variety of the ones we have already introduced. They become bound together to effect a complex glycan appendage to a protein.



Now these glycans become attached in two generally specific manners. One is an attachment via the N on the amino acid and the other the O. Then the N and O types bind to the respective amino acids as shown below.

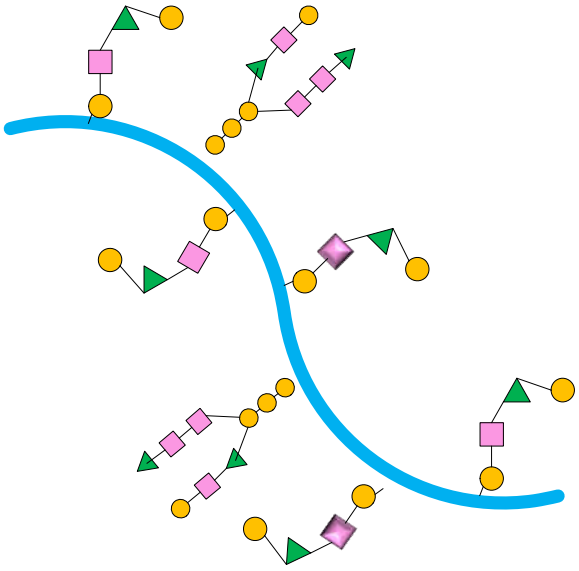


asparagine
N linked



serine
O linked

Subsequently one can imagine a multiplicity of such bindings along the entire length of the protein appearing as “hairs” bristling off the folded protein.



This collection of glycans can dramatically change the binding characteristics of the protein. Recall that all the sugars have a multiplicity of oxygen atoms which can create charge displacements.

Now as Koropatkin et al have noted:

Symbiotic microorganisms that reside in the human intestine are adept at foraging glycans and polysaccharides, including those in dietary plants (starch, hemicellulose and pectin), animal-derived cartilage and tissue (glycosaminoglycans and N-linked glycans), and host mucus (O-linked glycans).

Fluctuations in the abundance of dietary and endogenous glycans, combined with the immense chemical variation among these molecules, create a dynamic and heterogeneous environment in which gut microorganisms proliferate*. In this Review, we describe how glycans shape the composition of the gut microbiota over various periods of time, the mechanisms by which individual microorganisms degrade these glycans, and potential opportunities to intentionally influence this ecosystem for better health and nutrition.*

The above does raise the issue of how the glycans are generated. Are they driven by diet, are they organ specific, are the genetically driven? There has been some reasonable work along the lines of addressing these questions but there still remains a great deal of unknown.

Munkley noted:

Glycosylation is the most common, complex, and dynamic post-translational modification of both membrane-bound and secreted proteins. Glycans are fundamental to many biological processes and play a key role in protein folding, stability, trafficking, and activity, and act as regulators of signalling pathways, cell differentiation, immune recognition, and host–pathogen interactions

[2–4]. Glycans consist of two main classes: O-glycans, initiated in the Golgi apparatus by the initial attachment of GalNAc moieties to serine or threonine residues to form the Tn antigen, and N-glycans, which are initiated in the ER via the addition of an oligosaccharide chain to asparagine residues.

In addition, intracellular proteins can be modified with O-GlcNAc. Glycan chains may be branched or elongated and the cellular glycome is composed of glycans covalently linked to lipids (glycolipids and glycosphingolipids) or proteins (glycoproteins and proteoglycans). The synthesis of glycans is non-templated, meaning that glycan sequences are not directly coded by the genome. Instead, glycans are produced at the tissue level and can respond dynamically to environmental stimuli and signalling molecules via the coordinated activity of biosynthetic enzymes, the trafficking of these enzymes to the endoplasmic reticulum (ER) and Golgi apparatus, and the availability of sugar donors.

Glycans can be conjugated to proteins and lipids, or they can be secreted without conjugation to other macromolecules. In human cells, glycans are primarily constructed from ten monosaccharides: glucose (Glc), galactose (Gal), N-acetylgalactosamine (GalNAc), N-acetylglucosamine (GlcNAc), fucose (Fuc), sialic acid (Neu5Ac), mannose (Man), xylose (Xyl), glucuronic acid (GlcA), and iduronic acid (IdoA). These monosaccharides are assembled into glycans by biosynthetic enzymes in the Golgi apparatus and the ER, and additional complexity can arise from further modifications by sulfation, phosphorylation, methylation, and acetylation. In addition to glycosylation being an intracellular event, recent studies have demonstrated that glycans can undergo further modification by extracellular enzymes, further revealing the complexity of the dynamic glycome.

2.2 N LINKED

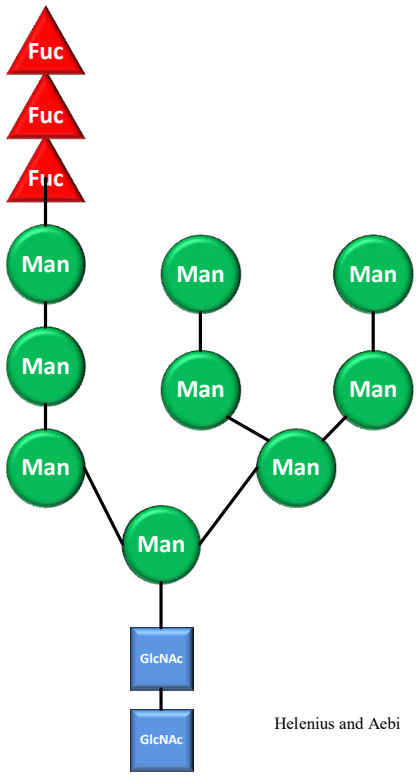
The N linked versions attach specifically to **asparagine**. We have shown this previously. As Helenius and Aebi have noted:

N-linked oligosaccharides arise when blocks of 14 sugars are added cotranslationally to newly synthesized polypeptides in the endoplasmic reticulum (ER). These glycans are then subjected to extensive modification as the glycoproteins mature and move through the ER via the Golgi complex to their final destinations inside and outside the cell. In the ER and in the early secretory pathway, where the repertoire of oligosaccharide structures is still rather small, the glycans play a pivotal role in protein folding, oligomerization, quality control, sorting, and transport.

The above describes the intracellular processes involved in the generation of the glycan associated proteins.

They are used as universal "tags" that allow specific lectins and modifying enzymes to establish order among the diversity of maturing glycoproteins. In the Golgi complex, the glycans acquire more complex structures and a new set of functions. The division of synthesis and processing between the ER (endoplasmic reticulum) and the Golgi complex represents an evolutionary adaptation that allows efficient exploitation of the potential of oligosaccharides.

The authors (Helenius and Aebi) demonstrate several forms and a specific N linked core is presented as follows:



Helenius and Aebi

Note the significant complex structure of this glycan. It is important to also note that such complexity could dramatically change the protein binding and conformation. Where they note:

The N-linked core oligosaccharide. N-linked glycans are added to proteins in the ER as "core oligosaccharides" that have the structure shown. ***These are bound to the polypeptide chain through an N-glycosidic bond with the side chain of an asparagine that is part of the Asn-X-Ser/Thr consensus sequence****. Terminal glucose and mannose residues are removed in the ER by glucosidases and mannosidases.*

The authors continue:

In mature glycoproteins, N-linked glycan moieties are structurally diverse. The sugar composition and the number and size of branches in the sugar tree varies among glycans bound to a protein, among glycoproteins, and among cell types, tissues, and species. However, when initially added in the ER to growing nascent polypeptides, the glycans do not display such heterogeneity. The "core glycans" are homogeneous and relatively simple.

Indeed the complexity of the associated glycans is a driving factor for their impact. Moreover the actual process of creating the complex glycan mix appears as of yet undetermined.

The trimming and processing that the glycans undergo when the glycoprotein is still in the ER introduce only limited additional diversity, because the alterations are shared by all glycoproteins. Thus, the spectrum of glycoforms remains rather uniform until the glycoproteins reach the medial stacks of the Golgi apparatus, where structural diversification is introduced through a series of nonuniform modifications.

Particularly in vertebrate and plant cells, it is the terminal glycosylation in the Golgi complex that gives rise to the tremendous diversity seen in glycoconjugates that reach the cell surface. The switch from structural uniformity in the ER to diversification in the Golgi complex coincides with a marked change in glycan function. In the early secretory pathway, the glycans have a common role in promoting protein folding, quality control, and certain sorting events.

Later, Golgi enzymes prepare them for the spectrum of novel functions that the sugars display in the mature proteins. Here, we mainly address events in the early secretory pathway. We focus on observations that are starting to unmask the logic of the various early trimming and modification events. We also discuss glycan structure and function in light of fundamental differences between the two biosynthetic organelles, the ER and the Golgi complex.

Thus, the oligosaccharide as an N linked version would then attach to an asparagine. As Pearce notes:

N-glycosylation follows a strictly ordered assembly, and the site of modification is predictable to ***asparagine residues*** *(N) of a peptide/ protein only when an NXT/S sequon is present (where X is any residue accept proline). There are two major changes that can occur to the core N-glycan structure, which are increased frequency of a bisecting GlcNAc, or β1,6 and β1,4 branching to the core pentasaccharide.*

Other notable changes occur to the epitopes of secondary structures that are attached to the core N-glycan structure, namely the N-acetyllactosamine units and their further functionalizations (discussed under Cancer epitopes within structures common to different core glycans).

Additionally, whilst O-glycan epitopes are usually discussed as distinct disease specific epitopes, N-glycans tend to be discussed in terms of a change to the pattern of the N-glycome.

In other words, the structures identified are synthesized in normal tissues, but the pattern is altered in disease. Below the bisecting GlcNAc and branching core N-glycome patterns are discussed, followed by other changes in the pattern of the N-glycome in various cancers. Specific epitope changes to N-acetyllactosamines on N-glycans are covered later, as mentioned earlier

N linked glycans play a significant role in receptor functions. However due to their significant diversity and presence understanding that is a challenge.

2.3 O LINKED

Now we briefly examine the other glycan, O linked glycans. As Pearce notes:

O-GalNAc glycans are attached to proteins at ***serine or threonine*** *sites. Although there is no defined sequon where an O-glycan is attached, they are often found within variable number of tandem repeat (VNTR) domains, which are high in serine and threonine repeats.*

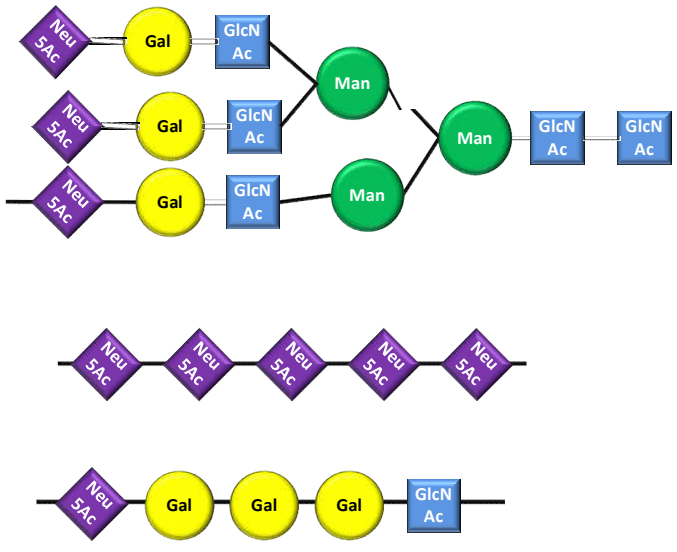
On a mucin, hundreds of O-glycan's can be present within the VNTR regions, expressed in a variety of glycoforms. Mucins are produced primarily by epithelial cells on the surfaces of various membranes, and secreted into the extracellular space. In healthy cells mucins are presented on the apical surface, but cells loose this polarization during malignant transformation, which supports an invasive phenotype (for background information and further reading on this phenomenon see elsewhere.

For example, membrane type I matrix metalloproteinase (MT1-MMP) polarization in malignant transformation is lost on the apical surface of epithelial cells and is found to concentrate in specific membrane structures that sit close to the basement membrane, and aid invasion through degradation of the basement membrane, and activation of other MMPs which are capable of degrading the collagen rich extracellular matrix that often surrounds malignant cells.

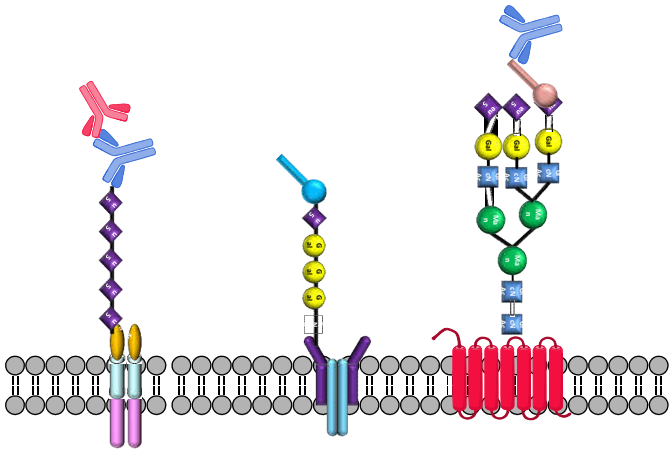
The first step in O-glycan (O-GalNAc) synthesis is UDPGalNAc transferred to a Ser/Thr by ppGalNAcTs, a family of enzymes consisting of ~20 member. O-glycan's are characterized across eight core structures.

Overexpression and/or aberrant expression of mucins by carcinomas has been known for many years. In general, mucins act as anti-adhesins, and therefore aid displacement of malignant cells during metastasis.

The last observation is important since it focuses on the metastatic behavior. We often look at surface proteins such as E cadherin and its change to N cadherin as a driving metastatic factor. However, the glycan function and the mucin production add an additional element worthy of note. Thus understanding glycans in the context of a malignant lesion appears to be as critical as almost all other factors.



The graphic below from Zhou et al depicts various glycans with sialic acid terminators and the bonding to various other proteins.

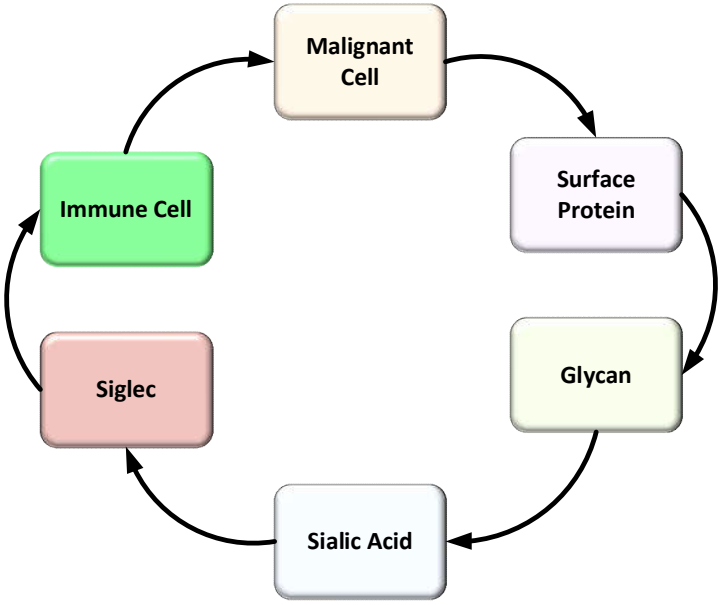


See Zhou et al

3 SIALIC ACID AND SIGLECS

We begin the interaction process with a discussion of sialic acid. We will also provide an initial overview of the other elements in the interaction with sialic acid. Sialic acid is produced within a cell and can become attached to the glycans which in turn are attached to proteins on the cell surface. The presence of sialic acid on the glycans and dramatically change the receptor state of the protein and in turn can dramatically change the control of the immune system on aberrant cells.

The paradigm we will examine is shown below.



As the above notes:

1. A malignant cells produces surface proteins
2. The proteins are covered with glycans
3. The glycans get sialic acids attached
4. Lectins, targeting sialic acids called siglecs, are surface proteins on immune cells
5. The immune cells can attack the malignant cell

However, blockage of this process can result in a metastatic process. We shall examine each step and provide some insight in how a therapeutic process can be provided. We shall discuss each of these elements in turn. We initially focus on sialic acid and briefly review the other key players.

3.1 SIALIC STRUCTURES

We first examine the structure of sialic acid. As Zhou et al note:

Sialic acids, a subset of nine carbon acidic sugars, often exist as the terminal sugars of glycans on either glycoproteins or glycolipids on the cell surface.

Sialic acids play important roles in many physiological and pathological processes via carbohydrate-protein interactions, including cell–cell communication, bacterial and viral infections.

In particular, hypersialylation in tumors, as well as their roles in tumor growth and metastasis, have been widely described. Recent studies have indicated that the aberrant sialylation is a vital way for tumor cells to escape immune surveillance and keep malignance.

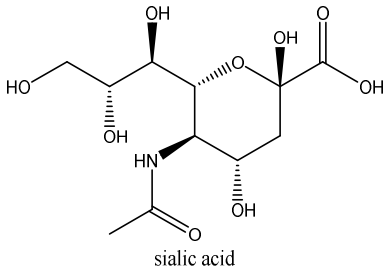
The above observation is key to what we will examine herein. The more one understands about the elements of a cell the more one sees the complexity of what occurs in the process of malignant progression. The observation also delimits the effect of sialic acid to growth and metastasis. It currently does not control the initial steps in a malignant process such as gene mutations or other such factors.

The term "sialic acid" first appeared in 1952 to describe N-acetylneuraminic acid, a major product released by mild acid hydrolysis of glycolipids in the brain or salivary mucins. Sialic acids are a subset of nine carbon acidic sugars that contain approximately fifty derivatives of neuraminic acids.

The most common sialic acid derivatives found in mammals are N-acetylneuraminic acid (Neu5Ac) and N-glycolylneuraminic acid (Neu5Gc).

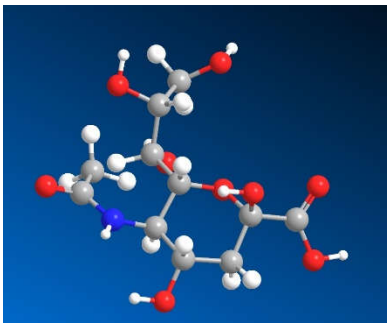
Neu5Ac has an acetyl group on the fifth carbon atom (C5) while Neu5Gc has a glycolyl group instead. Interestingly, humans lack Neu5Gc caused by the mutation of the cytidine monophosphate N-acetylneuraminic acid hydroxylase (CMAH) gene that codes the enzyme transforming CMP-Neu5Ac to CMP-Neu5Gc. However, Neu5Gc is still found in the human glycome as it can be obtained through dietary sources.

The structure below is the base form of sialic acid.

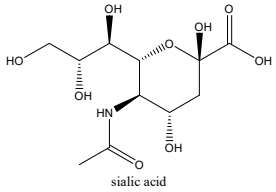


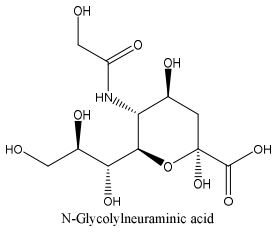
sialic acid

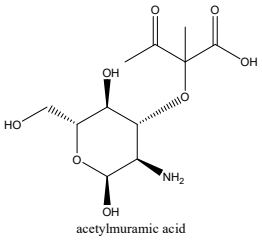
The three dimension presentation of sialic acid base structure is shown below.



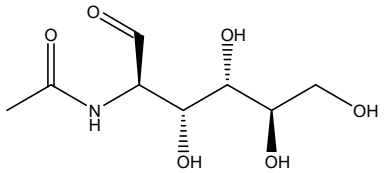
Basic sialic acid is shown below with the two variants we are considering shown below the basic structure.



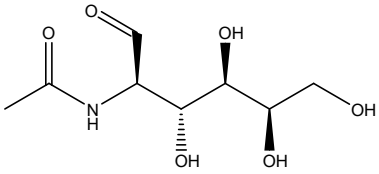




The two amine structure we have noted are shown below. Note the slight difference in conformation on the OH inserts.

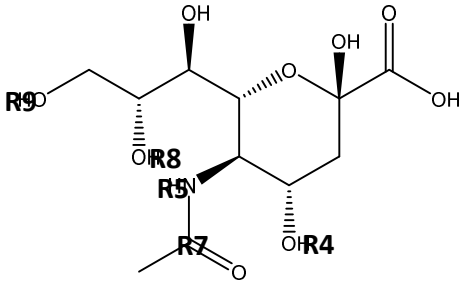


N-Acetyl-D-glucosamine



N-Acetyl-D-galactosamine

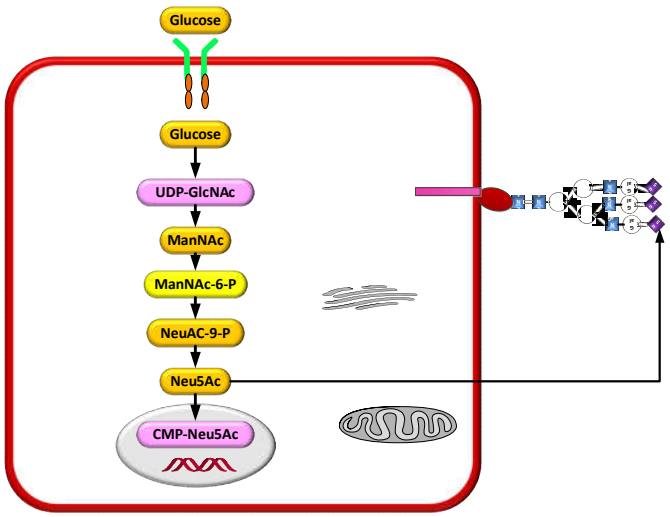
Now the generalized form of sialic acid is shown below with multiple Rs allowing for a significant variety of this molecule.



sialic acid

3.2 SIALIC PATHWAYS

We now consider the production of the sialic acid and its movement to the glycan on a surface protein. We show the pathway below. Glucose enters the cell and the cell can then process it via a multiplicity of internal enzymes creating in the process Neu5Ac, a sialic acid, that results in attaching itself to the end of a glycan which is attached to a surface protein. The key observation is that glucose is a main driver.



The process above may have some significance as regards to the progression of a malignant cell after the initial changes. Namely glucose may indirectly feed the cancer via the blocking of immune cells.

3.3 SIGLECS

We now provide a brief introduction to siglecs. Siglecs are lectin proteins which have an immunoglobulin super family structure, 7 beta sheets, and are on immune cells and which bind to the sialic acid sites on glycans in the target cells. Specifically Siglecs are "**S**ialic-acid-binding **I**mmuno**g**lobulin **LEC**tins" and lectins are the protein class.

As Wen et al note:

Sialic acid is recognized by sialic acid-binding immunoglobulin-type lectins (Siglecs), a family of cell surface receptors that can be divided into two subtypes based on sequence similarity and evolutionary conservation.

CD33-related Siglecs have high sequence homology and contain conserved tyrosine-based signaling motifs. Siglecs with low homology include Siglec-1 (sialoadhesion), Siglec-22 (CD22), Siglec-4 (MAG) and siglec-15.

We shall see that CD22 and CD23 will have a recurrence and are therapeutic targets[5].

[5] From NCBI, *CD22 Predicted to enable CD4 receptor binding activity; protein phosphatase binding activity; and sialic acid binding activity. Involved in B cell activation; negative regulation of B cell receptor signaling pathway; and regulation of endocytosis. Located in early endosome and recycling endosome. And CD33 Enables protein phosphatase binding activity and sialic acid binding activity. Involved in several processes, including negative regulation of cytokine production; negative regulation of monocyte activation; and positive regulation of protein tyrosine phosphatase activity. Located in several cellular components, including Golgi apparatus; external side of plasma membrane; and peroxisome.*

Siglecs are primarily expressed on immune cells and have been implicated in mediating adhesion, cell signaling, endocytosis*, and in modulating immune cell surveillance. Increased sialylation of cancer cell surface glycoproteins was observed years ago, and a large body of evidence implicates sialic acid binding to Siglecs in tumor infiltrating immune cells with immune evasion.*

For instance, sialylated glycoproteins expressed in pancreatic ductal adenocarcinomas (PDAC) interacts with Siglec-7 and Siglec-9 leads to differentiation of monocytes into immune-suppressive macrophages by increasing the expression levels of PD-L1, IL-6, IL-10, and CD206. Moreover, increased levels of sialic acid, sialyltransferases, and Siglec ligands have also been identified in tumor associated stromal cells resulting in immunosuppression. Removal of sialic acid residues in stromal cells with the sialyltransferase inhibitor 3FaxNeu5Ac reversed immune T cell suppression and exhaustion in colon cancer and myeloma cells. Therefore, targeting cancer cell and/or cancer-associated stromal cell sialylation may represent a novel immune checkpoint to reactivate anti-tumor immunity.

More specific are provided by Jiang et al who have noted:

Sialic acid-binding receptors are expressed on the surfaces of a variety of immune cells and have complex and diverse immunoregulatory functions in health and diseases.

Recent studies have shown that Siglecs could play diverse immune and nonimmune regulatory roles in the tumor microenvironment (TME) and participate in tumor progression through various mechanisms, such as regulating tumor growth and metastasis, mediating the inflammatory response, and promoting tumor immune escape, thereby affecting the prognoses and outcomes of patients.

However, depending on the cell type in which they are expressed, each Siglec member binds to corresponding ligands in the microenvironment milieu to drive diverse cell physiological and pathological processes in tumors.

Therefore, we herein summarize the expression spectra and functions of the Siglec family in human diseases, particularly cancer, and highlight the possibility of therapeutic interventions targeting the TME in the future …

Siglecs are type I immunoglobulin-like transmembrane proteins consisting of an extracellular structural domain, a transmembrane structural domain, and an intracellular structural domain*.*

This is an important factor. The Ig superfamily includes the classic antibodies. Siglecs have similar characteristics to these antibodies being in the Ig SF.

The intracellular domain is divided into a short lysine-containing tail and an extracellular structural domain consisting of an N-terminal binding Ig domain and a variable number of C2-type structural domains.

Siglec members can exert activating or inhibitory effects depending on the specific motifs within each molecule*, including the immunoreceptor tyrosine-based activation motif (ITAM) and immunoreceptor tyrosine-based inhibition motif (ITIM).*

Inhibitory Siglecs include Siglec-3, Siglec-5, Siglec-7, Siglec-9, and Siglec-10, and their intracellular regions contain ITIM- and ITIM-like domains, which transduce inhibitory signals by recruiting tyrosine phosphatases (SH2 domain-containing protein tyrosine phosphatases, SHPs), such as SHP-1 and SHP-2.

Siglecs can also be classified based on their ability to generate activated intracellular signals depending on the positively charged residue in the transmembrane region*, which can interact with DAP12 carrying the ITAM domain. Human Siglec-4, Siglec-14, Siglec-15, Siglec-16 and mouse Siglec-H belong to this classification*

We can classify the functions of siglecs as follows:

1. The nonimmune regulatory functions of Siglecs
    a. Siglecs induce apoptosis
    b. Siglecs promote tumor angiogenesis
2. The immune regulation of Siglecs
    a. Siglecs mediate antigen presentation
    b. Siglecs inhibit the proliferation and activation of tumor-associated T cells
    c. Siglecs inhibit the killing effect of NK cells
    d. Siglecs affects TAM function
    e. Siglecs weaken the killing effect of tumor-associated neutrophils

We shall discuss these further.

3.4 BINDING

Lectins are a class of glycan binding proteins, GBP. Namely they are proteins which adhere to glycans and thus can effect actions. Siglecs are a class of lectins, specifically they are IgSF, the immunoglobulin super family. We shall discuss the details later. Thus GBP are a superclass which includes siglecs. From Varki et al:

GBPs function in communication between cells in multicellular organisms and in interactions between microbes and hosts and can also be involved in binding growth factors, chemokines and cytokines.

These interactions can take various forms, resulting in movement of molecules, cells, and information.

Trafficking, Targeting, and Clearance of Proteins: Directing movement of glycoproteins within and between cells is a common function for lectins in many organisms. In eukaryotic cells, including yeast as well as "higher" eukaryotes, several groups of lectins are important in glycoprotein biosynthesis and intracellular movement.

In the endoplasmic reticulum (ER), two lectins—calnexin and calreticulin—bind monoglucosylated high-mannose glycans present on newly synthesized glycoproteins, forming part of a quality control system for protein folding.

This binding keeps proteins in the ER until they are correctly folded. Other groups of lectins in the ER, including M-type lectins and proteins containing mannose 6-phosphate receptor homology domains, take part in the process of ER-associated glycoprotein degradation (ERAD), binding partially processed high-mannose glycans on terminally misfolded glycoproteins, causing them to be retrotranslocated into the cytoplasm for deglycosylation, followed by degradation in the proteasome.

One of the best characterized functions of GBPs is in delivery of newly synthesized lysosomal enzymes from the trans-Golgi to lysosomes. P-type lectins recognize mannose 6-phosphate residues that have been added to N-glycans on lysosomal enzymes in the Golgi apparatus, targeting them to endosomes for fusion with lysosomes.

Once released from cells, glycoproteins can also be taken up for degradation in lysosomes. As noted above, the ASGPR on mammalian hepatocytes controls turnover of many serum glycoproteins by recognition of terminal Gal or GalNAc residues. Similarly, the mannose receptor on macrophages and sinusoidal cells of the liver binds and clears glycoproteins with oligomannose N-glycans that are released from cells during inflammation and tissue damage. Not all lectin-mediated targeting leads to degradation.

Glycan-binding subunits of secreted bacterial and plant toxins typically target them to glycolipids on cell surfaces and facilitate entry of the toxins into cells. Many enzymes contain glycan-binding domains that bring another domain with enzyme activity into close proximity with its substrates. One notable group includes bacterial cellulases in which cellulose-binding modules position the enzymatic domain for optimal degradation of cellulose fibers. Following a similar principle, GalNAc- binding domains in polypeptide-N-acetylgalactosaminyltransferases that initiate O-linked glycosylation in animals position these enzymes to add further GalNAc residues to regions of polypeptides that already bear O-glycans.

Cell Adhesion Distinctive glycans on the surfaces of different eukaryotic and prokaryotic cells make them targets for GBPs.

Binding of glycans on the surface of one cell by GBPs on an adjacent cell can induce recognition and adhesion, whereas cross-linking glycans on different cells by multivalent soluble lectins provides an alternative mechanism. Such interactions are exploited in specialized situations exemplified by transient contacts between moving cells.

The selectins—three receptors that function in interactions between white blood cells, platelets, and endothelia—provide some of the best characterized examples of lectin-glycan interactions in cell-cell adhesion. For example, L-selectin on lymphocytes binds glycans on specialized endothelial cells of lymph nodes to induce lymphocyte homing, wherein circulating lymphocytes leave the bloodstream and enter the lymph node.

Other mammalian GBPs that mediate binding of cells to each other or that recognize ligands on the same cell surface include Siglecs and galectins. Lectins in multicellular organisms also mediate interactions between cells and the extracellular matrix and support the organization of matrix components. For example, proteins containing link modules that bind specifically to hyaluronan in cartilage (and other tissues) are essential for structuring the extracellular matrix, and other extracellular proteins bind to sulfated GAGs to organize cell-cell and cell-matrix interactions.

Many bacteria also use lectins to adhere to glycans on host cells, often keeping them from getting washed away. These adhesins are usually present at the ends of long structures called pili or fimbriae that project from the surface of the bacteria. Adhesion can be part of the infection process. For example, a mannose-specific adhesin on pathogenic strains of Escherichia coli that cause urinary infections binds to epithelial cells of the urinary tract. Other glycan-protein interactions between host cells and bacteria provide a mechanism for coexistence.

Several bacterial species that are part of the normal gut flora, including nonpathogenic E. coli, use adhesins to bind to glycolipids present on cells lining the large intestine. Immunity and Infection Many lectins are involved in immune responses in invertebrates as well as in "lower" vertebrates and mammals. Differences between glycans on host and microbial cell surfaces are commonly the basis for innate immune responses. Phagocytosis is a common outcome of the binding

3.4.1 Protein-Glycan Recognition:

We now discuss the recognition process in the binding. Varki et al also note:

A tremendous variety of GBPs are known …

GBPs differ in the types of glycans they recognize and in their binding affinity and kinetics. The underlying structural basis by which a GBP binds with specificity and high affinity to a very limited number of glycans (or even a single glycan) among the many thousands that are produced ….

A wide variety of physical techniques are used to identify and quantify protein-glycan interactions. Differential affinities of glycans for different GBPs revealed by these approaches provide insight into the biological roles of glycans and their cognate GBPs. ***Characterization of protein-glycan recognition using such techniques, in combination with structural studies by nuclear magnetic resonance (NMR) and crystallography, is useful to identify novel antagonists or inhibitors of GBPs.*** *Such approaches are being used, for example, to develop*

inhibitors of neuraminidases to treat influenza virus infections and to screen for high-affinity inhibitors of selectins for the treatment of inflammatory disorders.

The actual details of the binding are yet to be fully understood. Yet like any protein-protein binding, the folds and charge locations dominate.

3.4.2 Historical Background:

Varki et al present a historical perspective:

Much of the initial work on understanding protein-glycan interactions arose from studies on the combining sites of plant lectins and antibodies against specific blood group antigens. These studies led to the development of quantitative assays using glycans to inhibit binding interactions detected by cell agglutination or precipitation of targets, which provided early evidence for the importance of specific sugar structures in biological recognition events. Studies of protein-glycan interactions were instrumental in the development of techniques such as equilibrium dialysis and isothermal titration calorimetry, which are now widely used to analyze protein binding to a variety of types of ligands. On the other hand, methods used to study other types of protein-ligand interactions often need to be adapted to accommodate the specific properties of glycans and the proteins that interact with them. Valency of GBP Interactions Because many GBPs are oligomeric, with each subunit typically having a single carbohydrate-binding domain (carbohydrate-recognition domain [CRD]), many GBPs exhibit multivalent interactions with glycan ligands.

Thus, although the CRD within a GBP may have a particular affinity for a ligand, the multivalent feature enhances binding through increased avidity and allows ligand cross-linking. Although of macrophage lectins to nonhost glycans on bacteria, parasites, and fungi, but many of these macrophage lectins, like DC-SIGN, also recognize host glycans on viruses for phagocytosis. Other lectins circulating in the blood, such as serum mannose- binding protein and ficolins, bind to pathogen cell surfaces and activate the complement cascade, leading to complement-mediated killing.

Binding of glycans to lectins on immune cells can also trigger intracellular signaling that activates or suppresses cellular responses. Receptors that recognize self-glycans such as sialic acid, as well as several that are specific for glycans characteristic of microorganisms, can initiate such signaling. For example, binding of a2-6 linked Sia to CD22, a member of the Siglec family of vertebrate lectins found on B-lymphocytes, initiates signaling that inhibits activation to prevent self-reactivity.

The interesting observation is that most presentations of cancer genomics fail to include sialics and the siglecs. Their roles are often dominant but neglected. For example, in the therapeutics for ALL where CD22 and CD33 inhibitors are used it is often understood that they are targets, say like CD19. Instead they are critical factors in sialyation. They continue:

In contrast, binding of trehalose dimycolate, a glycolipid found in the cell wall of Mycobacterium tuberculosis by the macrophage C-type lectin Mincle, induces a signaling

pathway that causes the macrophage to secrete proinflammatory cytokines. Finally, viruses often use their own GBPs to attach to host cells during infection.

Proteins on virus surfaces, including those on influenza virus, reovirus, Sendai virus, and polyomavirus, bind to sialic acids. In addition to bringing the virus into contact with their cell targets, these hemagglutinins typically induce membrane fusion, facilitating virus entry and delivery of nucleic acids into the cytosol.

This fusion process is a critical one for viruses, since they do not require a specific receptor but can enter via this process.

Glycan- binding receptors on viruses are often highly specific for a particular linkage; human influenza viruses preferentially bind to sialic acids a2-6- linked to Gal, whereas bird influenza viruses prefer a2-3-linked sialic acid. Other viruses, such as herpes simplex virus, have adhesins that bind to heparin surfaces.

We now move to the lectins, the proteins which bind to the glycans also called siglecs. Laubli and Varki note:

Lectins are proteins that bind to glycan ligands through a carbohydrate recognition domain (CRD). Relatively few mammalian Sia-binding lectins have been discovered. Selectins that are vascular cell adhesion molecules mediating trafficking and tethering of leukocytes during vascular extravasation processes bind to a selective set of ligands (selective lectins). …

Depending on their evolutionary history, Siglecs can be divided into conserved Siglecs with orthologues in different species; and, a rapidly evolving CD33-related Siglecs (CD33rSiglecs) that do not always have clear orthologues in all mammalian species. This is also why most CD33rSiglecs have no numbers in mice but are assigned letters. Siglecs are single-pass type I transmembrane proteins belonging to the immunoglobulin superfamily of proteins. Their extracellular domains consist of the V-set domain that recognizes siaologlycans and has high similarity to the variable domain of immunoglobulins.

The V-set domain contains the CRD of Siglecs, followed by a different number of C2-set Iglike domains. While most CD33rSiglecs have intracellular domains with inhibitory ITIM or ITIM-like motifs, the transmembrane domain bears a positively charged amino acid in the less common activating Siglecs. Siglec-1 is a special case with many C2 domains and no intracellular signaling domain. While conserved Siglecs are distributed across different chromosomes in humans, the rapidly evolving CD33rSiglecs are located largely in a cluster on human chromosome 19.

Siglec diversification goes back to early mammals probably due to a 'Red Queen' effect resulting from interactions between hosts and pathogens.

3.5 SIALYLTRANSFERASES

The next element is sialytransferases, enzymes that facilitate expression on glycans. The

dynamics of moving the sialic acid to the glycans is accomplished via a set of enzymes called sialyitransferases. These enzymes may become another targetable set for therapeutics. From Pietrobono and Stecca:

The family of human sialyltransferases consists of 20 enzymes that transfer sialic acid from cytidine monosphosphate N-acetylneuraminic acid (CMP-Neu5Ac) to the terminal glycosyl group of various glycoproteins and glycolipids.

Sialic acid plays a crucial role in several cellular interactions, including with the extracellular matrix, epithelial cells, immune cells, antibodies and other intercellular processes. The attachment of sialic acid to the underlying glycan chain can occur through different glycosidic linkages (α-2,3, α-2,6, α-2,8).

Sialyltransferases can be classified into four main groups depending on the types of glycosidic bonds: ST3GAL1-6 (α-2,3-sialyltransferases), ST6GAL1-2 and ST6GALNAC1-6 (α-2,6-sialyltransferases) and ST8SIA1-6 (α-2,8-sialyltransferases). Sialyltransferases are type II transmembrane glycoproteins usually located in the Golgi apparatus. STs are expressed in a tissue-specific manner and each presents substrate specificity, although with some degree of redundancies.

STs share also a conserved protein structure, that consists of a short N-terminal cytoplasmic domain, a transmembrane domain (TMD), a stem region of variable length and a catalytic domain. The latter contains four conserved sialylmotifs, namely 'L' (long), 'S' (short), 'III' (third position in sequence) and 'VS' (very small), which are involved in recognition of donor and acceptor substrates and catalytic activity …

Sialyltransferase expression appears to be regulated mainly at the level of transcription by a number of factors, including oncogenes, transcription factors (TF), miRNAs, long noncoding RNAs (lncRNA), hormones and natural compounds. In the ST3GAL family, gene transcription results in distinct mRNAs, which are generated by alternative splicing and alternative promoter usage, resulting in tissue-specific expression. The human ST3GAL1 gene contains nine exons.

Results of site-directed mutagenesis indicated that the Sp1 binding sites and an upstream stimulatory factor 1 (USF) binding site in the promoter are involved in the transcriptional regulation of human ST3GAL1.

Human ST3GAL2 has two isoforms regulated by promoters P1 and P2. Human ST3GAL3 has only one mRNA and an Sp1 element, whereas several transcripts and promoters have been described for human ST3GAL4, ST3GAL5 and ST3GAL6. The proto-oncogene c-Myc has been reported to regulate transcription of ST3GAL1, 2 and 5 in colon cancer cells.

In hormone-sensitive prostate cancer (PC) cells, androgens regulate ST3GAL2 transcription by inducing promoter demethylation and increasing GD1a expression, a sialogangloside associated with tumor progression.

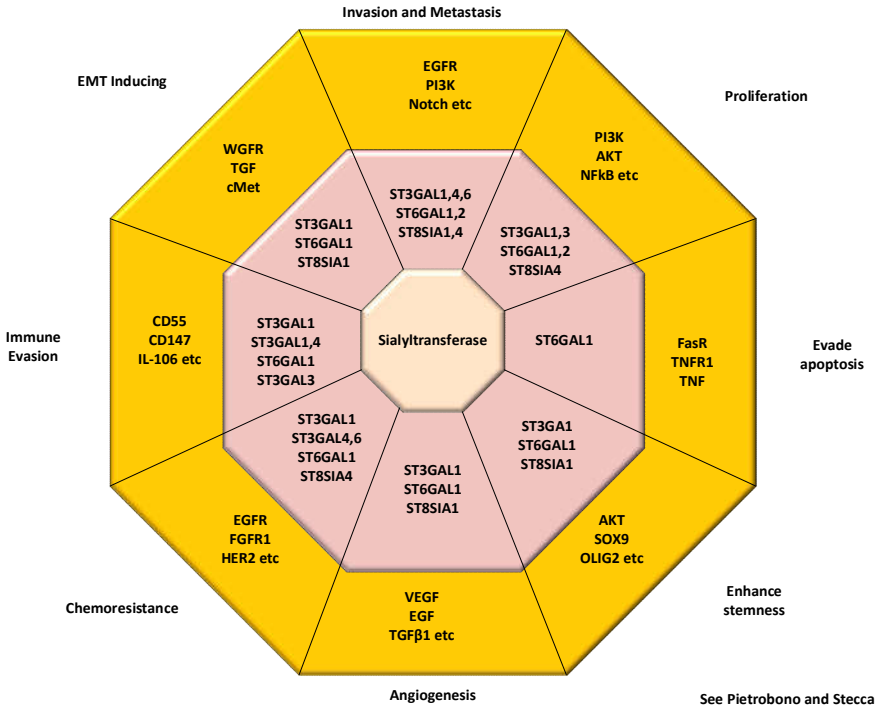
In breast cancer cells, prostaglandin E2 (PGE2), one of the final products of the cyclooxygenase-2 (COX-2) pathway, can induce ST3GAL1 expression in both ER-positive and ER-negative cell lines.

In hepatocellular carcinoma (HCC) cell lines, the tumor suppressors miR-26a, miR-548l and miR-34a have been shown to negatively regulate the expression of ST3GAL5. In addition, miR-26a negatively regulated ST3GAL6, inducing the suppression of HCC cell proliferation, migration, and invasion in vitro.

They summarize the roles of sialytransferases as follows:

1. Sustaining Proliferation and Tumor Growth
2. Activating Invasion and Metastasis, and EMT Inducing Events
3. Promoting Immune Evasion
4. Evading Apoptosis and Cell Death
5. Inducing Angiogenesis
6. Promoting Chemoresistance
7. Enhancing Stemness

The authors present these functions graphically below showing function, gene drivers, specific enzymes.



From Wen et al:

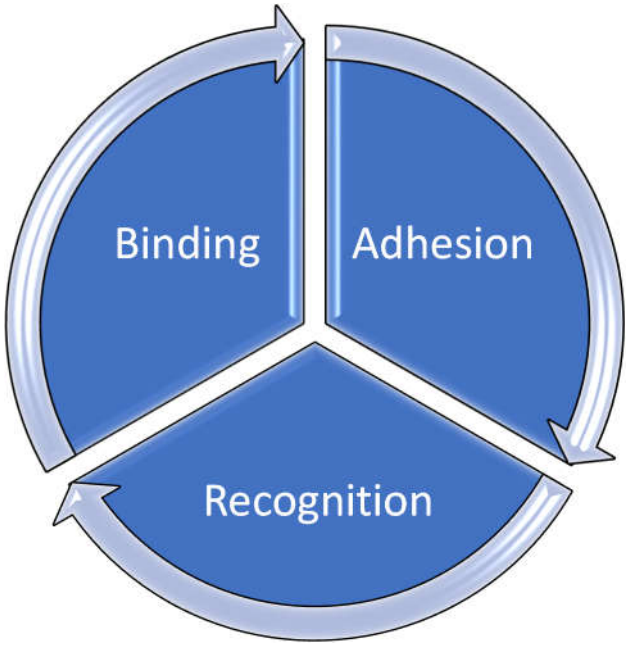
Sialyltransferases comprise a family of 20 enzymes that can be divided into four major categories based on the resulting sialic acid linkages in glycan structure. Specifically, 6 family members catalyze α2-3-linkages of sialic acid to an underlying galactose (Gal) residue, 2 catalyze α2-6-linkages and 6 additional members transfer sialic acids to terminal residues via α2- 8 linkage It is worth noting that out of the 20 sialyltransferases, only a few have been investigated for their roles in PCa development and progression

The Table from Wen et al (as modified) is shown below:

Sialyltransferases	***Expression level***	***Model***	***Function***
ST3GAL1	Increased expression level	LNCaP PC3 DU145	Overexpression of ST3Gal1 blocked O-glycan elongation and reduced LNCaP cell susceptibility to galectin-1–induced cell death.
ST3GAL2	Increased expression level	PC3 DU145 cells	Expression of ST3Gal2 isregulated by NF-κB. ST3Gal2 is required for the synthesis ofGD1a in hormone-sensitive PCa.
ST3GAL3,4,5	Decreased expression level	DU145	Not reported
ST3GAL6	Increased expression level	DU145	Not reported
ST6GAL1	Elevated or high expression level	PC3 DU145 patient tissues	ST6Gal-I expression was positively correlated with PCa grade and poor knockdown decreased proliferation, growth, migration and invasion through PI3K/Akt/GSK-3β/β-catenin
ST8SIA1,2	High expression	DU145	Not reported
ST8SIA4	Moderate expression level	DU145	ST8SAI4 was decreased by alginate ligosaccharide treatment
ST8SIA3,5,6	Low expression level	DU145	Not reported

4 IG SUPERFAMILY

Immunoglobulins are a complex family of proteins having a common assembly profile. The immunoglobulin super family is a family of a large number or proteins that function as adhesion, recognition and binding of cells. The family included the classic IgG etc molecules in antibodies, KIR proteins in NK cells, MHC molecules, PDGFR, and a wide variety of other such proteins. When looking at the sialic acid binding we see that they are effected by Ig super family proteins. The IgSF is characterized by the 7 beta sheets typical of the many proteins in this class.



4.1 STRUCTURE

The structure of the Ig superfamily, IgSF, is of some importance when examining the binding of siglecs. Wong et al have noted:

With over 765 members, the IgSF is one of the largest and most diverse families of proteins in the body.

Members of the IgSF include major histocompatibility complex class I and II molecules, proteins of the T cell receptor complex, virus receptors, and cell surface glycoproteins.

The definitive characteristic of the IgSF members is the presence of one or more immunoglobulin- (Ig-) like domains, which have a characteristic sandwich structure composed of two opposing antiparallel β-pleated sheets, stabilized by a disulphide bridge.

Most of the IgSF members are type I transmembrane proteins, which typically consist of an extracellular domain (which contains one or more Ig-like domains), a single transmembrane domain, and a cytoplasmic tail. IgSF members mediate calcium-independent adhesion through

their N-terminal Ig-like domains, which commonly bind other Ig-like domains of the same structure on an opposing cell surface (homophilic adhesion) but may also interact with integrins and carbohydrates (heterophilic adhesion). The C-terminal intracellular domains of IgSF members often interact with cytoskeletal or adaptor proteins. In this way, the extracellular interactions of IgSF CAMs can lead to signaling within the cell, enabling these proteins to function in a wide range of normal biological processes, as well as pathological events such as tumourigenesis

As Natarajan et al note:

The immunoglobulin (Ig) superfamily (IgSF) consists of a group of proteins that exploit the structural robustness and fundamental stability of the Ig fold for a wide assortment of functions across a broad span of evolutionary time, extending from microorganisms to humans. Several classification schemes, based on three-dimensional structure or amino acid sequence patterns, have been developed to assist in understanding functional and evolutionary relationships.

Although the best-understood activities of IgSF members are related to immunological recognition, a number of related molecules participate in developmental and homeostatic phenomena. Manipulation of the IgSF by recombinant methodologies including bacteriophage and yeast display offers in vitro approaches to increase the available diversity of novel structures for almost limitless functions. …

In general, the definition of the Ig fold is based on two β-sheets with a total of seven β-strands, folded roughly into a 'Greek key' motif.

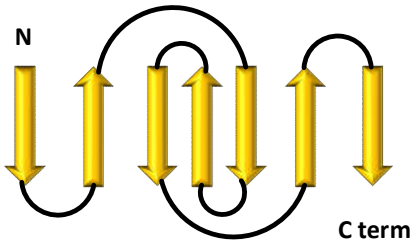
An early classification recognised a core of four β-strands, b, c, e and f, common to all Ig folds, augmented by three to five additional strands. The three-dimensional structures of the fundamental C (constant) Ig folds, designated C1 and C2, ...

These two variants differ in the sheet association of the β-strands. Strands of two anti-parallel β-pleated sheets, the first consisting of strands A, B, E and D and the second of strands G, F and C, form the core of the Ig fold.

Each strand consists of 5–10 amino acids with the side chains of hydrophobic amino acids facing the interior of the sandwich, and those of hydrophilic amino acids facing outwards. In addition to hydrophobic interactions in the interior, a single conserved disulphide bond connecting β-strands B and F enhances the stability of the Ig fo*ld. Surface-exposed amino acid loops connect the β-strands. Both the hydrophilic exterior surfaces of the core and the loops can serve as sites of interaction with other molecules.*

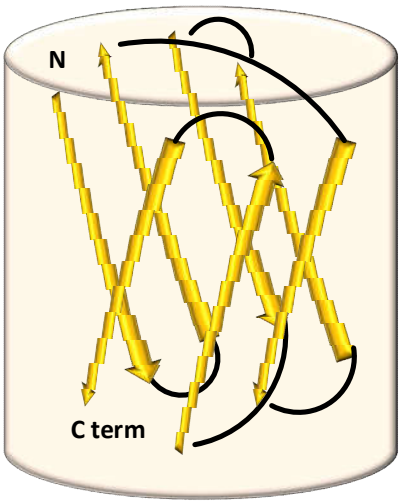
The Ig family is comprised of proteins with the β-folds as shown below[6]. They are then interconnected with di-sulfide bonds such as in the IgG etc immunoglobulins.

[6] See Kessel and Ben-Tar pp 136-137



See Natarajan et al

The two dimensional structure then is shown below as the three dimensional cylindrical structure. The Ig superfamily is thus constructed along these lines.



See Natarajan et al

The authors then proceed to discuss the functions and structures:

Antigen recognition by antibodies and T-cell receptors: *The hallmark of the adaptive immune system is the ability to generate millions of unique antibody combining sites in order to bind specifically to an equally diverse set of antigens. These combining sites are formed and displayed by V domains of both the heavy and the light chain subunits at the amino end. The V-type Ig fold is the scaffold upon which diversity is generated.*

Gene rearrangements in developing B cells produce functional V-gene exons encoding V domains of approximately 110 amino acids. Imprecision at the joining sites of the rearrangement coupled with somatic hypermutation in preferred regions of the rearranged V gene result in highly variable amino acid sequences in a region spatially constrained to the antibody combining site.

Cell adhesion*: Cellular adhesion mediated by IgSF molecules is an important step in the extravasation of leukocytes across endothelial barriers, in axonal growth and development and*

in the triggering of T cells' immune responses by antigen presenting cells. The binding interactions are classified as either homophilic or heterophilic, depending on whether they are based on interactions between similar or distinct cell types.

***Calcium dependence is another distinguishing feature**. Cellular adhesion mediated by IgSF members involves more than mere attachment and is an active process that generates intracellular signals resulting in tyrosine phosphorylation, cytokine secretion and/or cytoskeletal rearrangements. In some cases such as those involving some integrins, adhesion reflects allosteric differences that contribute to measurable differences in binding kinetics and affinity*

4.2 METASTASIS

Metastasis is basically a loss of place. Cells no longer have a structural integrity and the adhesion that keeps cells in place is gone. A key element in this area is the IgSF and its binding. As Wong et al have noted:

Metastasis is a major clinical problem and results in a poor prognosis for most cancers. The metastatic pathway describes the process by which cancer cells give rise to a metastatic lesion in a new tissue or organ. It consists of interconnecting steps all of which must be successfully completed to result in a metastasis.

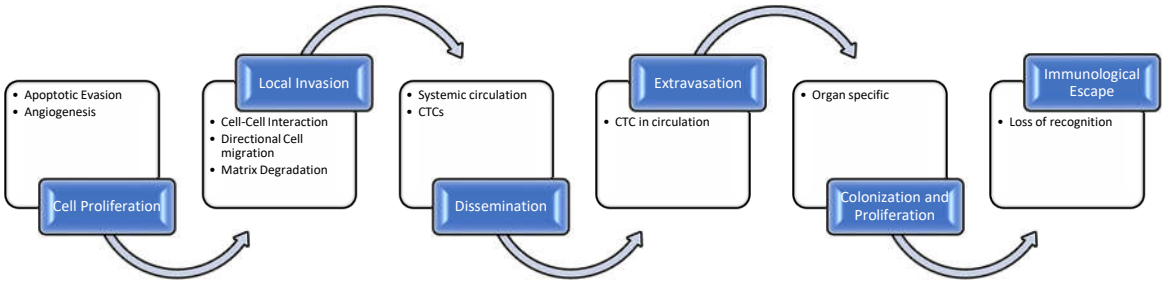
***Cell-cell adhesion is a key aspect of many of these steps**. Adhesion molecules belonging to the immunoglobulin superfamily (Ig-SF) commonly play a central role in cell-cell adhesion, and a number of these molecules have been associated with cancer progression and a metastatic phenotype. Surprisingly, the contribution of Ig-SF members to metastasis has not received the attention afforded other cell adhesion molecules (CAMs) such as the integrins.*

Here we examine the steps in the metastatic pathway focusing on how the Ig-SF members, melanoma cell adhesion molecule (MCAM), L1CAM, neural CAM (NCAM), leukocyte CAM (ALCAM), intercellular CAM-1 (ICAM-1) and platelet endothelial CAM-1 (PECAM-1) could play a role. Although much remains to be understood, this review aims to raise the profile of Ig-SF members in metastasis formation and prompt further research that could lead to useful clinical outcomes. …

A number of IgSF members have been identified as biomarkers for cancer progression. For example, MCAM (also called CD146, Mel-Cam, Muc18, and S-Endo1) has been implicated in the progression of melanoma, as well as in breast and prostate cancer.

Similarly, IgSF members such as L1CAM (CD171), NCAM (CD56), PECAM-1 (CD31), ALCAM (CD166), and ICAM-1 (CD54) have been associated with metastatic progression in a range of cancers including melanoma, glioma, breast, ovarian, endometrial, prostate, and colon cancer…

They then discuss each of the steps which we have graphically presented below.



The authors then conclude:

The metastatic cascade is very complex and most research in this area has focused on the role of integrins and cadherins in cell migration and invasion, using carcinoma as a model system.

The classic case is in melanoma where the melanocytes lose E-cadherin and it is converted to N-cadherin, thus allowing the melanocyte to "wander". Melanoma CIS starts this was with melanocytes "wandering" upward in the epidermis and then ultimately downward. The authors continue:

In writing this paper, our goal was to examine the ***potential role of a selection of IgSF*** *members in the metastatic pathway in different types of cancer,* ***including carcinoma, melanoma, and sarcoma****. Although most of these molecules have been described as tumour biomarkers, the extent and nature of their contribution to the metastatic pathway has not been clear. We have examined aspects of each step in the pathway and have suggested ways in which one or more of the six IgSF members could contribute. Much of this is conjecture based on what is known about the behaviour of these proteins in nontumour systems.*

However, as tumours commonly use existing molecular interactions in inappropriate or aberrant ways, we feel our conclusions indicate some interesting possibilities for further research. Performing these studies, however, will not be easy because of the difficulties of accurately dissecting a system as complex as the metastatic cascade in vivo and the limitations of the in vitro assays used to support in vivo conclusions.

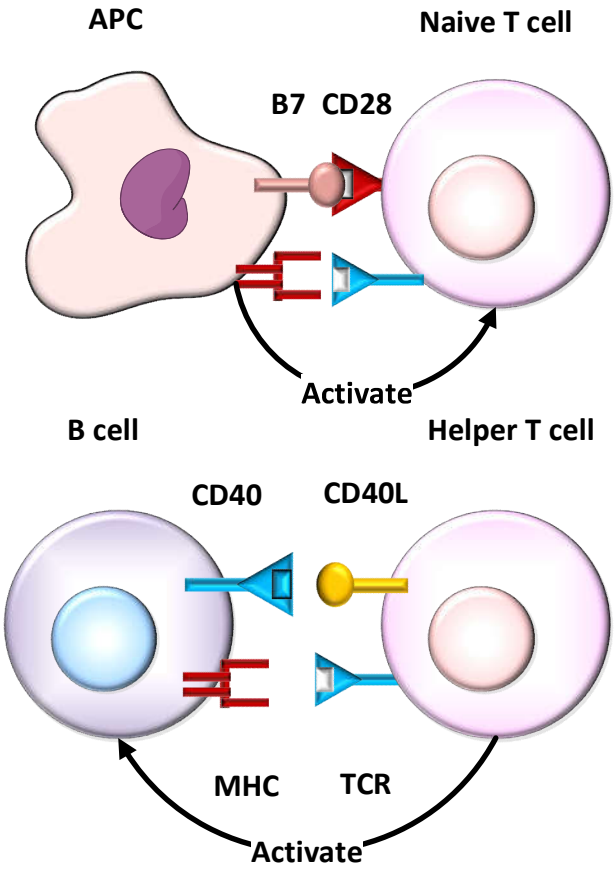
It is for these reasons that much remains to be understood, particularly about the role of IgSF members in the metastatic cascade. Yet the need to understand metastasis is high because most patients that succumb to cancer succumb to metastasis or the complications of its treatment.

5 IMMUNE SYSTEM INTERACTIONS

We now examine several basic immune system interactions as relates to the interaction between sialic acid elements and siglecs.

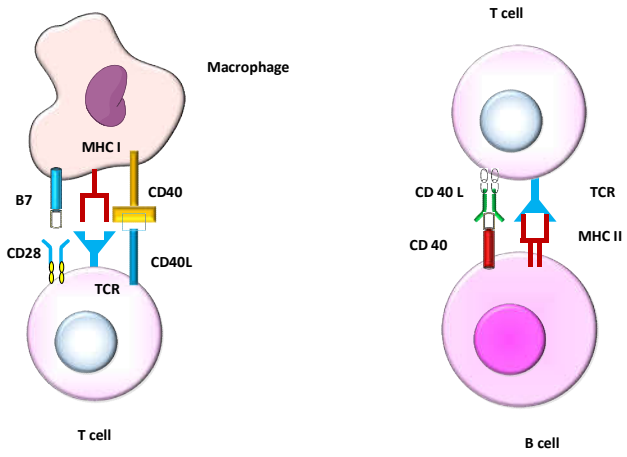
5.1 BASIC IMMUNOTHERAPY

First a brief review of immunotherapy[7]. Antigen presenting cells, such as a macrophage interacts with a naïve T cell with a connection of an MHC. It then becomes activated. A helper T cell can also be activated The T cell receptor is a key element in this process.

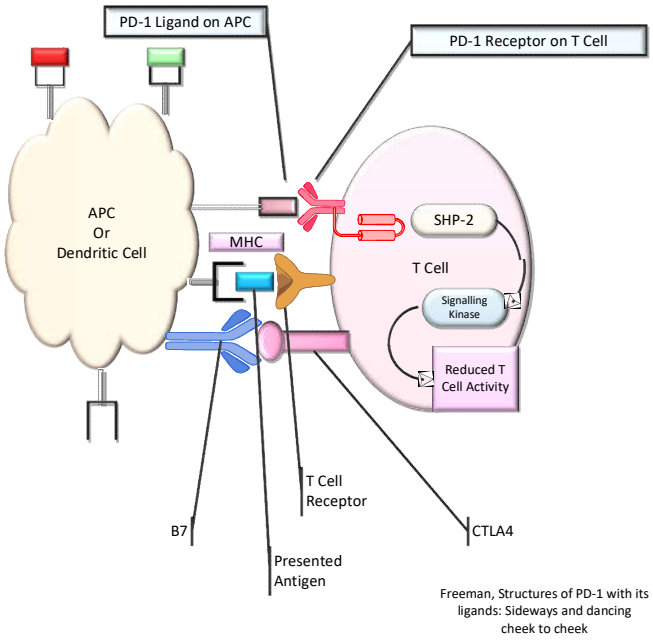


The interaction between a macrophage and T cell and then the T cell and B cell is shown below. The process allows for the development of antibodies.

[7] https://www.researchgate.net/publication/314090163_Cancer_Immunotherapy_A_Systems_Approach



A more complete description is shown below. Here we show the PD-1 and CTLA4 receptors. These are two targets that

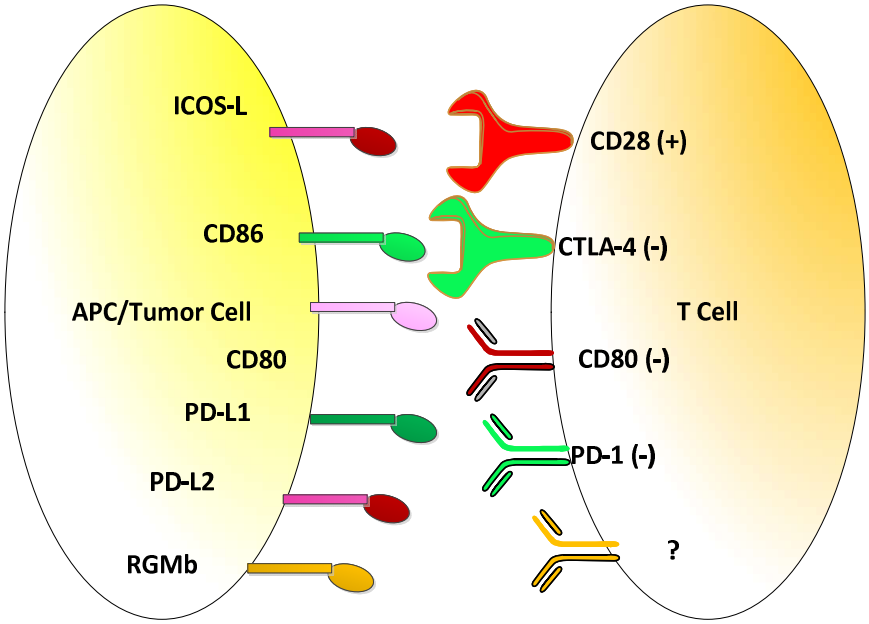


Thus immunotherapy relies on the activation and interactions of the immune cells with the target cells, in this case the cancer cells. Cancer cells often have the ability to suppress the steps we see above. This is especially the case with sialic acids and lectins. The receptors can be turned off and it is essential to reactivate them. Thus the end goal in reactivating sialic acid disrupted cells is to turn the effect of sialic acid off. We now discuss the first step in this process.

5.2 Receptors: Checkpoint Inhibitors

The above characterizations of the operation of the immune system is but a first step. There are a multiplicity of ligands and receptors which can enhance the process or inhibit the process. The inhibitory one are checkpoints and therapy addressing these inhibitory functions are termed

checkpoint blockade. We briefly depict some of the current and somewhat well-known ones. A warning should be noted. It seems to be common place amongst immune therapies that as one barrier is climbed others soon appear. Thus, this may very well be merely a first step in an ever continuing understanding of the complexities of the immune systems.



5.2.1 CTLA-4

CTLA-4 is a checkpoint inhibitor. It has the potential to inhibit the actions of the immune cells to the cell expression this. As Topalian et al state:

The conventional wisdom underlying our vision of how CTLA-4 blockade mediates tumor regression is that it systemically activates T cells that are encountering antigens.

CTLA-4 represents the paradigm for regulatory feedback inhibition. Its engagement down-modulates the amplitude of T cell responses, largely by inhibiting co-stimulation by CD28, with which it shares the ligands CD80 and CD86. As a ‘‘master T cell co-stimulator,’’ CD28 engagement amplifies TCR signaling when the T cell receptor (TCR) is also engaged by cognate peptide-major histocompatibility complex (MHC).

However, CTLA-4 has a much higher affinity for both CD80 and CD86 compared with CD28, so its expression on activated T cells dampens CD28 co-stimulation by out-competing CD28 binding and, possibly, also via depletion of CD80 and CD86 via ‘‘trans-endocytosis’’. Because CD80 and CD86 are expressed on antigen-presenting cells (APCs; e.g., dendritic cells and monocytes) but not on non-hematologic tumor cells, CTLA-4’s suppression of anti-tumor immunity has been viewed to reside primarily in secondary lymphoid organs where T cell activation occurs rather than within the tumor microenvironment (TME).

Furthermore, CTLA-4 is predominantly expressed on CD4+ ‘‘helper’’ and not CD8+ ‘‘killer’’ T cells. Therefore, heightened CD8 responses in anti-CTLA-4-treated patients likely occur

indirectly through increased activation of CD4+ cells. Of note, a few studies suggest that CTLA-4 can act as a direct inhibitory receptor of CD8 T cells, although this role in down-modulating anti-tumor CD8 T cell responses remains to be directly demonstrated. The specific signaling pathways by which CTLA-4 inhibits T cell activation are still under investigation, although activation of the phosphatases SHP2 and PP2A appears to be important in counteracting both tyrosine and serine/threonine kinase signals induced by TCR and CD28.

CTLA-4 engagement also interferes with the ''TCR stop signal,'' which maintains the immunological synapse long enough for extended or serial interactions between TCR and its peptide-MHC ligand. Naive and resting memory T cells express CD28, but not CTLA-4, on the cell surface, allowing costimulation to dominate upon antigen recognition.

5.2.2 PD-1

In a similar manner to CTLA-4, PD-1 is also an inhibitor. As Topalian et al state:

The PD-1 system of immune modulation bears similarities to CTLA-4 as well as key distinctions. Similar to CTLA-4, PD-1 is absent on resting naive and memory T cells and is expressed upon TCR engagement. However, in contrast to CTLA-4, PD-1 expression on the surface of activated T cells requires transcriptional activation and is therefore delayed.

Also in contrast to CTLA-4, PD-1 contains a conventional immunoreceptor tyrosine inhibitory motif (ITIM) as well as an immunoreceptor tyrosine switch motif (ITSM). PD-1's ITIM and ITSM bind the inhibitory phosphatase SHP-2. PD-1 engagement can also activate the inhibitory phosphatase PP2A. PD-1 engagement directly inhibits TCR-mediated effector functions and increases T cell migration within tissues, thereby limiting the time that a T cell has to survey the surface of interacting cells for the presence of cognate peptide-MHC complexes.

Therefore, T cells may ''pass over'' target cells expressing lower levels of peptide-MHC complexes. In contrast to CTLA-4, PD-1 blockade is viewed to work predominantly within the TME, where its ligands are commonly overexpressed by tumor cells as well as infiltrating leukocytes. This mechanism is thought to reflect its important physiologic role in restraining collateral tissue damage during T cell responses to infection. In addition, tumor-infiltrating lymphocytes (TILs) commonly express heightened levels of PD-1 and are thought to be ''exhausted'' because of chronic stimulation by tumor antigens, analogous to the exhausted phenotype seen in murine models of chronic viral infection, which is partially reversible by PD-1 pathway blockade.

Importantly, the phenotypes of murine knockouts of PD-1 and its two known ligands are very mild, consisting of late-onset organ-specific inflammation, particularly when crossed to autoimmune- prone mouse strains. This contrasts sharply with the Ctla-4 knockout phenotype and highlights the importance of the PD-1 pathway in restricting peripheral tissue inflammation. Furthermore, it is consistent with clinical observations that autoimmune side effects of anti-PD-1 drugs are generally milder and less frequent than with anti- CTLA-4. Despite the conventional wisdom that CTLA-4 acts early in T cell activation in secondary lymphoid tissues whereas PD-1

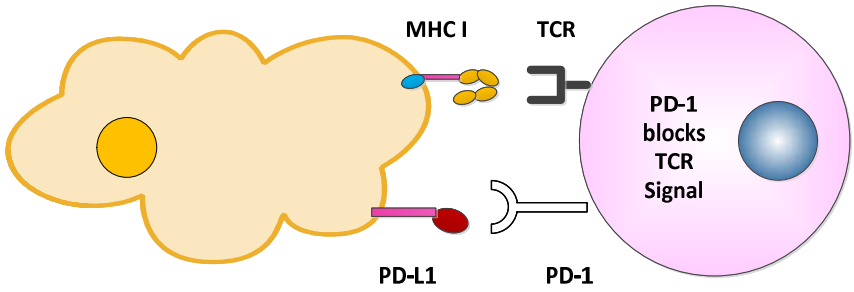
inhibits execution of effector T cell responses in tissue and tumors, this distinction is not absolute.

Beyond its role in dampening activation of effector T cells, CTLA-4 plays a major role in driving the suppressive function of T regulatory (Treg) cells. Tregs, which broadly inhibit effector T cell responses, are typically concentrated in tumor tissues and are thought to locally inhibit anti-tumor immunity.

Therefore, CTLA-4 blockade may affect intratumoral immune responses by inactivating tumor-infiltrating Treg cells. Recent evidence has demonstrated anti-tumor effects from CTLA-4 blockade even when S1P inhibitors block lymphocyte egress from lymph nodes, indicating that this checkpoint exerts at least some effects directly in the TME as opposed to secondary lymphoid tissues.

Conversely, PD-1 has been shown to play a role in early fate decisions of T cells recognizing antigens presented in the lymph node. In particular, PD-1 engagement limits the initial ‘‘burst size’’ of T cells upon antigen exposure and can partially convert T cell tolerance induction to effector differentiation.

The authors present a graphic regarding how this blocking or checkpoint functions. We depict this below.



Oncogenic pathway in tumor cell uses AKT for gene amplification allowing PD-L1 expression

As Freeman states:

T cell activation requires a TCR mediated signal, but the strength, course, and duration are directed by costimulatory molecules and cytokines from the antigen-presenting cell (APC). An unexpected finding was that some molecular pairs attenuate the strength of the TCR signal, a process termed coinhibition. The threshold for the initiation of an immune response is set very high, with a requirement for both antigen recognition and costimulatory signals from innate immune recognition of ‘‘danger’’ signals.

Paradoxically, T cell activation also induces expression of coinhibitory receptors such as programmed death-1 (PD-1). Cytokines produced after T cell activation such as INF- and IL-4 up-regulate PD-1 ligands, establishing a feedback loop that attenuates immune responses and

limits the extent of immune-mediated tissue damage unless overridden by strong costimulatory signals. PD-1 is a CD28 family member expressed on activated T cells, B cells, and myeloid cells. In proximity to the TCR signaling complex, PD-1 delivers a coinhibitory signal upon binding to either of its two ligands, PD-L1 or PD-L2.

Engagement of ligand results in tyrosine phosphorylation of the PD-1 cytoplasmic domain and recruitment of phosphatases, particularly SHP2. This results in dephosphorylation of TCR proximal signaling molecules including ZAP70, PKC, and CD3, leading to attenuation of the TCR/CD28 signal.

The role of the PD-1 pathway in peripheral T cell tolerance and its role in immune evasion by tumors and chronic infections make the PD-1 pathway a promising therapeutic target.

5.2.3 KIR

Killer inhibitory receptors, KIRs, are another class of immune activators.

Abbas et define KIRs as follows:

Killer cell Ig-like receptors (KIRs) *Ig superfamily receptors* ***expressed by NK cells*** *that recognize different alleles of HLA-A, HLA-B, and HLA-C molecules. Some KIRs have signaling components with ITIMs in their cytoplasmic tails, and these deliver inhibitory signals to inactivate the NK cells. Some members of the KIR family have short cytoplasmic tails without ITIMs but associate with other ITAM-containing polypeptides and function as activating receptors.*

In a more detailed presentation Topalian et al state:

NK cells are a population of innate immune cells with well documented roles in infectious and tumor immunity. Like activated CD8 T cells, NK cells mediate target cell apoptosis via secretion of preformed granules containing perforin and granzymes. However, unlike CD8 T cells, NK cells do not recognize unique peptides in the context of classical MHC I molecules.

Instead, NK function is controlled by the complex interplay of a series of activating receptors and killer inhibitory receptors (KIRs) and their ligands. In humans, KIR molecules are polymorphic and bind to certain MHC I alleles, and not all KIR/ ligand pairs are equally capable of inhibiting NK cell function.

Indeed, bone marrow transplants in which donor NK cells lack the ability to be inhibited by host KIR ligands have been shown to result in lower relapse rates and improved OS, supporting the importance of this cell type in cancer immunity. The relative importance of NK cells in murine models of cancer immunotherapy has been documented by multiple studies but is especially highlighted by studies in which NK cell activation via IL-15 can eradicate fairly advanced tumors in the absence of CD8 T cells. So, in a sense, KIRs can be thought of as immune checkpoint molecules, and blocking KIRs on NK cells could be exploited to augment anti-tumor immunity.

To that end, a fully human anti-KIR mAb has entered clinical testing. This antibody (initially IPH-2101, Innate Pharma; now lirilumab, Bristol-Myers Squibb) binds to the human KIR molecules KIR2DL-1, KIR2DL-2, and KIR2DL-3 as well as to KIR2DS-1 and KIR2DA-2, preventing their binding to HLA-C MHC I molecules. A phase I trial of anti-KIR in acute myelogenous leukemia has been completed. Several studies in hematologic and solid cancers are ongoing, but of particular interest are trials in which lirilumab is being combined with anti-PD-1 (nivolumab) or with anti-CTLA-4 (ipilimumab). These trials are important in that each seeks to combine innate immune activation via anti-KIR with activation of the adaptive immune system, therefore offering the potential for additive or synergistic anti-tumor efficacy.

In addition NCBI notes[8]:

Killer cell immunoglobulin-like receptors (KIRs) are transmembrane glycoproteins expressed by natural killer cells and subsets of T cells. The KIR genes are polymorphic and highly homologous and they are found in a cluster on chromosome 19q13.4 within the 1 Mb leukocyte receptor complex (LRC). The gene content of the KIR gene cluster varies among haplotypes, although several "framework" genes are found in all haplotypes (KIR3DL3, KIR3DP1, KIR3DL4, KIR3DL2).

The KIR proteins are classified by the number of extracellular immunoglobulin domains (2D or 3D) and by whether they have a long (L) or short (S) cytoplasmic domain. KIR proteins with the long cytoplasmic domain transduce inhibitory signals upon ligand binding via an immune tyrosine-based inhibitory motif (ITIM), while KIR proteins with the short cytoplasmic domain lack the ITIM motif and instead associate with the TYRO protein tyrosine kinase binding protein to transduce activating signals. The ligands for several KIR proteins are subsets of HLA class I molecules; thus, KIR proteins are thought to play an important role in regulation of the immune response.

5.2.4 Toll Like Receptors

The Toll Like Receptors, "toll" means weird or strange in German, and they play a significant role in the innate system. The TLR play a significant but complex role in implementing immune responses. As Travis notes:

At the heart of this protection are proteins, called Toll-like receptors (TLRs), on cells of the innate immune system. Over the past decade, it has become clear that TLRs are the long-sought cell-surface receptors that recognize common microbial features such as bacterial wall components or the distinctive DNA sequences of a virus.

This role could date back to the earliest multicellular organisms, as humans and some of the most evolutionarily primitive animals share TLRs and the molecules involved in the TLR signaling cascade.

[8] https://www.ncbi.nlm.nih.gov/gene/3811

Abbas et al define them as follows:

Toll-like receptors: A family of pattern recognition receptors of the innate immune system that are expressed by many cell types and recognize microbial structures, such as flagellin, lipopolysaccharide, peptidoglycan, double-stranded RNA, and CpG DNA. TLRs transduce signals that lead to the expression of inflammatory and antiviral genes. There are 10 human TLRs, 7 of which are expressed on the plasma membrane of cells and 3 are located in endosomal membranes.

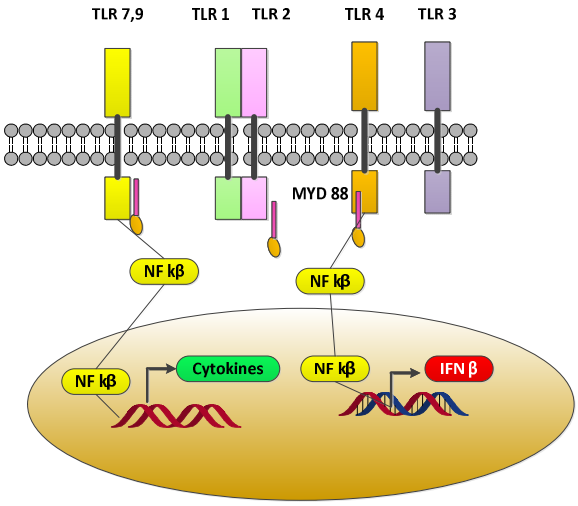
Takeda and Ashira note:

Toll receptor was originally identified in Drosophila as an essential receptor for the establishment of the dorso-ventral pattern in developing embryos [1]. In 1996, Hoffmann and colleagues demonstrated that Toll-mutant flies were highly susceptible to fungal infection [2]. This study made us aware that the immune system, particularly the innate immune system, has a skillful means of detecting invasion by microorganisms.

Subsequently, mammalian homologues of Toll receptor were identified one after another, and designated as Toll-like receptors (TLRs). Functional analysis of mammalian TLRs has revealed that they recognize specific patterns of microbial components that are conserved among pathogens, but are not found in mammals. In signaling pathways via TLRs, a common adaptor, MyD88, was first characterized as an essential component for the activation of innate immunity by all the TLRs.

However, accumulating evidence indicates that individual TLRs exhibit specific responses. Furthermore, they have their own signaling molecules to manifest these specific responses. In this review, we will focus on the recent advances in our understanding of the mechanism of TLR-mediated signaling pathways.

Now following their analysis, we can depict the TLR functions as shown below.

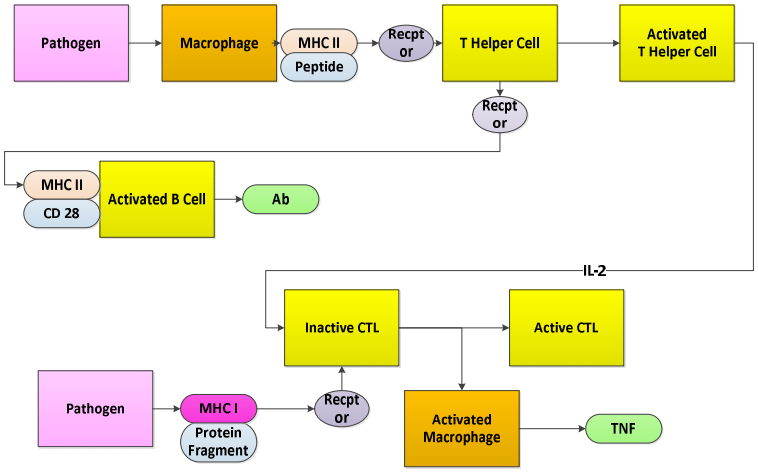


We will see more from these TLR as we proceed.

5.3 The Immune System as Process

The following is, at a very high level, a complete set of interactions of the immune system. We have not addressed specific receptors nor have we detailed control mechanism. But generally, the system functions as described below. The innate and the adaptive systems function somewhat hand in glove and then what we have below is primarily the adaptive system.

Pathogens activate the system and depending on where and how they are initiated various elements take over. The checkpoints we described previously can arise and inhibit this process. The checkpoints arise as a result of intracellular pathway aberrations. These aberrations can be met by immunotherapeutic approaches and/or by therapeutics dealing with the pathway itself. Melanoma therapeutics is an example of this approach.



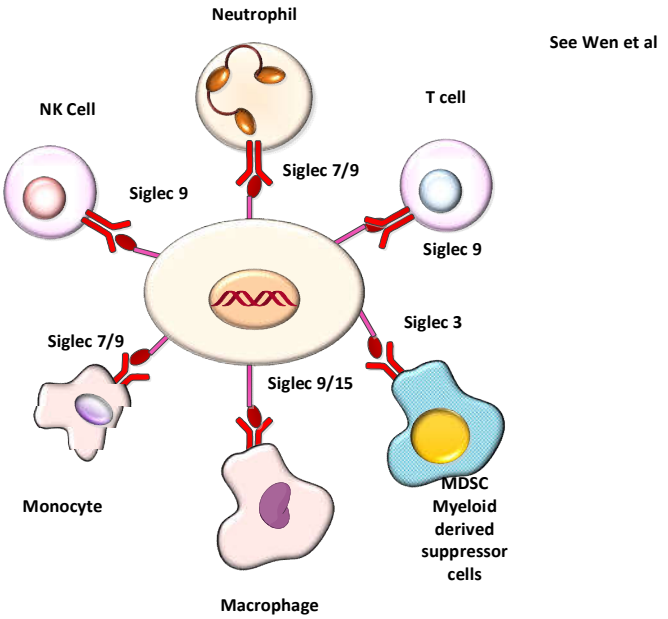
This simplified diagram above depicts a high-level understanding of the adaptive immune system. The key observation in this section is not just the high-level elements, but as we noted with checkpoints, the ever-evolving complexities that throttle the immune system.

The importance of this diagram is that like so many models of gene interaction in cancer cells, this is a model of immune system interaction. It is in a simple manner the beginning of an engineering approach to understanding and utilizing the immune system. It combines the grammar, namely the differing elements, and the logic, how these elements interplay to effect something. The rhetorical side, namely applying these to address a pathology, is what we will examine next.

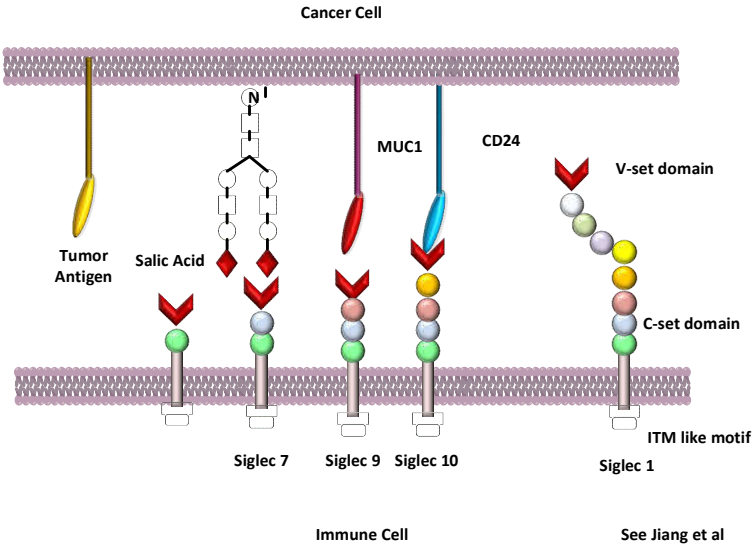
5.4 Siglecs and Immunity

As we previously noted, Siglecs are "**S**ialic-acid-binding **I**mmuno**g**lobulin **LEC**tins" Lectins are proteins that bind to glycans and the structure of the lectin is immunoglobulin like. Siglecs have strong effects on proteins and their interactions especially as regards to the immune system. They can enhance or defeat the immune responses. They are factors that may play a strong role in controlling the immunotherapeutic approaches which work well with certain malignancies and poorly with others. They also may become targetable to improve immunotherapy.

From Wen et al:



We now show below the interaction on typical cancer cells.



As Geijtenbeek and Gringhuis have noted:

Dendritic cells (DCs) are located throughout the body to capture and internalize invading pathogens, and subsequently process and present antigen on MHC class I and class II molecules to CD8+ and CD4+ T cells, respectively.

Antigen presentation by DCs is in itself not sufficient to induce effective T cell responses against pathogens.

CD4+ T cells need to differentiate into distinct T helper (TH) cell subsets depending on the type of infection;

TH1 cells secrete interferon-γ (IFNγ), which activates macrophages to fight intracellular micro- organisms,

TH2 cells secrete interleukin-4 (IL-4), IL-5 and IL-13 to induce humoral immune responses against helminths, and

IL-17-secreting TH17 cells mobilize phagocytes to clear extracellular fungi and bacteria1.

Furthermore, regulatory T cells are needed to control the activity of effector TH cells. Thus, DCs need to translate information about the invading pathogen into a cytokine gene expression profile that directs the correct TH cell differentiation pathway. Pathogen recognition is central to the induction of T cell differentiation. Although the variety of pathogens is immense, groups of pathogens share similar structures known as pathogen-associated molecular patterns (PAMPs), which enable their recognition2.

DCs express numerous pattern recognition receptors (PRRs) that interact with PAMPs to induce cytokine expression. PRRs include the archetypical Toll-like receptors (TLRs), as well as non-TLRs such as intracellular nucleotide- binding domain and leucine-rich-repeat-containing family (NLRs), retinoic acid-inducible gene I (RIG-I)- like receptors and C-type lectin receptors (CLRs) …

CLRs expressed by DCs interact with pathogens primarily through the recognition of mannose, fucose and glucan carbohydrate structures. Together, these CLRs recognize most classes of human pathogens; mannose specificity allows the recognition of viruses, fungi and mycobacteria, fucose structures are more specifically expressed by certain bacteria and helminths and glycan structures are present on mycobacteria and fungi8,9.

Recognition by CLRs leads to the internalization of the pathogen, its degradation and subsequent antigen presentation9 (BOX 1). These properties are important for vaccine design1. However, an even more powerful application has remained largely unexplored: targeting of the signalling pathways downstream of CLRs to tailor immune responses to break tumour-induced immuno-suppression, to induce TH1-type responses against virus infections or to redirect allergic TH2 cell responses to protective TH1 cell responses

As Crocker et al note:

Cell surfaces in the immune system are richly equipped with a complex mixture of glycans, which can be recognized by diverse glycan-binding proteins. The Siglecs are a family of sialic-acid-binding immunoglobulin-like lectins that are thought to promote cell–cell interactions and regulate the functions of cells in the innate and adaptive immune systems through glycan recognition.

In this Review, we describe recent studies on signalling mechanisms and discuss the potential role of Siglecs in triggering endocytosis and in pathogen recognition. Finally, we discuss the

postulated functions of the recently discovered CD33-related Siglecs and consider the factors that seem to be driving their rapid evolution …

The immunoglobulin domain is a highly versatile fold that can be used to bind an almost infinite array of molecular structures, as illustrated by antibodies and T-cell receptors. The 'immuno globulin-type' (I-type) lectins are a discrete subset of the IgSF that exploit the remarkable structural diversity of glycans in their recognition functions. The Siglecs (sialic-acid-binding immunoglobulin-like lectins) are the best characterized I-type lectins2–9. They are type 1 membrane proteins displaying an amino-terminal V-set immunoglobulin domain that binds sialic acid and variable numbers (16 in the case of sialoadhesin) of C2-set immunoglobulin domains.

They are categorized into two subsets on the basis of their sequence similarity and evolutionary conservation. Sialoadhesin (also known as Siglec-1 and CD169), CD22 (also known as Siglec-2), myelin-associated glycoprotein (MAG; also known as Siglec-4) and the recently discovered Siglec-15 are quite distantly related (~25–30% sequence identity) and have clear orthologues in all mammalian species examined. In comparison, the CD33-related Siglecs share ~50–99% identity but seem to be evolving rapidly by multiple processes, including gene duplication, exon shuffling, exon loss and gene conversion.

This has resulted in important differences in the repertoires of CD33-related Siglecs among mammalian species. Initial analyses of the genomes of fish, amphibians and birds indicate that, whereas typical CD33-related Siglecs are absent, a clear orthologue of MAG is present in all three taxa11. In humans, there are nine CD33-related Siglecs and one Siglec-like protein, whereas in mice there are five CD33-related Siglecs. So, it is difficult to assign orthologues, which has required the use of different numbering systems for the human and mouse CD33-related Siglecs

Crocker et al discuss several CD surface proteins that are operable and CD22 and CD33 are discussed as follows:

5.4.1 CD22

CD22 is a well-documented regulator of B-cell signalling, homeostasis and survival*.*

This Siglec is best known for helping to set a threshold for antigen-induced activation of B cells, an activity that involves as many as six tyrosine-based motifs in the cytoplasmic domain of CD22, including three ITIMs. B-cell receptor (BCR) ligation leads to increased phosphorylation of the ITIMs of CD22 by the SRC-family kinase LYN, which results in the recruitment of SHP1 (SRC homology 2 (SH2)-domain containing protein tyrosine phosphatase 1) and the downregulation of BCR signalling. However, this oversimplifies the complexity of CD22 signalling, as it can also recruit positive effectors of cell activation, including GRB2 (growth-factor-receptor-bound protein 2),

SHC (SH2- domain-containing transforming protein C), PI3K (phosphoinositide 3-kinase) and PLCγ2 (phospholipase Cγ2). This results in activation of alternative signalling pathways that contribute to the regulation of B-cell activation. The impact of CD22 on these pathways

probably depends on the manner of B-cell activation. Ligation of the BCR with either antigen or IgM-specific antibody, or simultaneous ligation of the BCR and CD40 (with IgM-specific and CD40-specific antibodies) result in the differential phosphorylation of CD22 tyrosine-based motifs both quantitatively and qualitatively.

Furthermore, CD22 does not seem to affect B-cell signalling when activated by ligation of cell-surface IgG83.

A detailed molecular understanding of the role of CD22 will continue to evolve as B-cell signalling pathways become better defined. Recent work on CD22 has provided important insights into how sialic-acid recognition can modulate its signalling functions. B cells of CD22-deficient mice exhibit hyperimmune responses in vitro and in vivo8,79, consistent with the loss of negative regulation by ITIMs of CD22.

Several CD22 functions, including BCR-dependent proliferation and B-cell turnover rates, depend on the ligand-binding function of CD22, as shown using mice that carry knock-in mutations of CD22 that ablate its ability to bind sialic acid.

In contrast to CD22-deficient mice, ST6GAL1-deficient mice (which lack CD22 ligands) exhibit hypoimmune responses. B cells from mice that are deficient in both CD22 and ST6GAL1 behave similarly to those from CD22-deficient mice, which indicates that the immuno deficiency of ST6GAL1-deficient mice depends on the presence of CD22.

Following BCR ligation in vitro, the immunodeficiency caused by the absence of cis ligands in ST6GAL1-deficient mice is manifest by reduced B-cell proliferation and calcium flux, and increased CD22 phosphorylation and recruitment of SHP1

5.4.2 CD33

The CD33-related Siglecs are mainly expressed by mature cells of the innate immune system, such as neutrophils, eosinophils, monocytes, macrophages, NK cells, DCs and mast cells.

CD33 itself is well known as a marker of myeloid progenitor cells, indicating a potential role for CD33 in the regulation of cellular proliferation and/or differentiation.

Other CD33-related Siglecs seem to be expressed at later stages of haematopoiesis. Numerous studies point to important roles of CD33-related Siglecs in modulating leukocyte behaviour, including inhibition of cellular proliferation, induction of apoptosis, inhibition of cellular activation, induction of proinflammatory cytokine secretion and, in the case of Siglec-H on plasmacytoid DCs (pDCs), suppression of interferon-α (IFNα) production.

In general, these functions have been defined using selected Siglecs and the extent to which they can be extrapolated to the other CD33-related Siglecs is unknown. The signalling pathways are poorly understood but in most cases are assumed to involve the ITIM and ITIM-like motifs and recruitment of tyrosine phosphatases …

5.5 GLYCANS AND SIGLECS AND CANCER

We now consider the collection of factors and their relationship in malignancies. As Varki et al noted:

SLex and SLea epitopes were first identified as tumor antigens on glycosphingolipids. Expression of these antigens by epithelial carcinomas correlates with metastatic potential in mice and with tumor progression, metastatic spread, and poor prognosis in humans.

SLex and SLea epitopes on glycoprotein ligands are key recognition determinants of the selectins. Indeed, selectin ligands are expressed on carcinoma cells, and mucin-like tumor antigens carrying SLex and SLea are found in the blood of carcinoma patients. Transgenic overexpression of E-selectin in mouse liver causes carcinoma cells that would normally metastasize to the lung to be redirected toward colonization of the liver, supporting the concept that SLe/selectin interactions are important mediators of metastasis. Furthermore, metastasis is attenuated in mice lacking P-selectin or L-selectin or by administering heparin, which blocks binding by these selectins.

Selectin interactions also help explain the classic observation that cancer cells entering the bloodstream form thromboemboli with platelets and leukocytes, which facilitate arrest in the vasculature, assist extravasation through the endothelium, and help in evasion of the immune system. Similar interactions involving soluble cancer mucins may contribute to hypercoagulability (Trousseau's syndrome), a condition responsive to heparin treatment. Because of the prominent role of selectins in cancer progression, these receptors are major therapeutic targets.

Certain sialyl-Lewis-related structures may also influence carcinoma progression by interacting with Siglecs, which generally have immunosuppressive functions. For example, disialyl-Lea and sialyl-6-sulfo-Lex structures may protect against early carcinogenic events by binding to Siglec-7 on macrophages.

This interaction suppresses macrophage production of the pro-oncogenic inflammatory mediator, Cox2, thereby exerting an anti-oncogenic function. However, during cancer development, many other sialylated glycan ligands for Siglecs are increased, and these bind to inhibitory Siglecs on various immune cell populations to induce immunosuppression and facilitate tumor progression….

Cancer stem cells, or tumor-initiating cells, constitute a small subpopulation of cancer cells that has tumor-initiating capability.

Several glycans that are specific markers for embryonic stem cells (stage-specific embryonic antigen-3 [SSEA-3], SSEA-3 with fucose [Globo H], and SSEA-4) are also expressed by cancer stem cells. SSEA-1, an embryonic stem cell marker in mice, is found in cancer stem cells in human gliomas.

Thus the expression of these glycans appears to be associated with the "sternness" of cells. Other cancer stem cell markers include the glycoproteins, CD 133 (prominin-1), CD24, and CD44, all of which are regulated by their glycosylation status. Among these receptors, the activation of CD44 by its ligand, hyaluronan, is particularly important for maintaining cancer stem cell features. Cancer stem cells are often investigated in the context of EMT.

Cells that have undergone EMT are highly similar to cancer stem cells[9]*. EMT is a critical event in tumor progression that prepares cancer cells for metastasis. It is governed by several well-defined transcription factors such as SNAIL and ZEB. EMT induces the differential expression of a subset of glycosyltransferases. Cancer cells with a mesenchymal phenotype up-regulate ST6GAL1 and MGAT5, while down-regulating MGAT3.*

The corresponding changes in N-glycan branching and sialylation affect the stability and/or activity of many target molecules central to the process of EMT, such as cadherins and integrins. Other EMT-associated glycan modifications include the GDI ganglioside, and decreased expression of Gg4 and GM2 glycolipids, along with increases in SLex and SLea. EMT is typified by alterations in cell adhesion and invasiveness; however, marked changes in cellular metabolism also occur, many of which are directed by O-GlcNAcylation. The addition of O-GlcNAc to E-cadherin, Snail, and other EMT- related proteins modulates protein stability or trafficking, leading ultimately to dysregulated expression of metabolic genes.

As Jiang et al note:

Siglec family members are specifically expressed on a variety of immune cells, including human macrophages, T cells, B cells, dendritic cells (DCs), and natural killer (NK) cells, and are often involved in many important physiological processes, including the initial activation, proliferation, and apoptosis of immune cells.

Siglecs play important regulatory roles in the immune response by mediating cell-to-cell or pathogen-to-cell interactions through recognition of the monosaccharide sialic acid (Sia) on the surface of tumor cells. In tumors, the glycosylation of Sia on the cell surface is likely altered, thus promoting the formation of tumor associated carbohydrates recognized by individual Siglec members, which can transmit inhibitory signals, accelerate the progression of pathological processes and promote the immune escape of tumor cells.

The Sia–Siglec axis exerts different physiological functions in humans, as it modulates the balance between self and nonself recognition and mediates cell adhesion, cell signaling, and the uptake of sialylated pathogens.

The binding between a carboxyl group of sialylated glycoconjugates and a Siglec molecule reduces the inflammatory response, inhibits phagocytosis and reduces cellular activation.

In addition, the Sia-Siglec axis is involved in the capture and presentation of antigens by antigen-presenting cells and affects the functions of antigen-presenting cells. During immune

[9] https://www.researchgate.net/publication/333704252_EMT_lncRNA_TGF_SMAD_and_Cancers

activation, Siglecs counter regulate overresponsive immune reactions upon immune stimulation by damage-associated molecular patterns (DAMPs) to aid in host immune evasion, potentially leading to cancer progression.

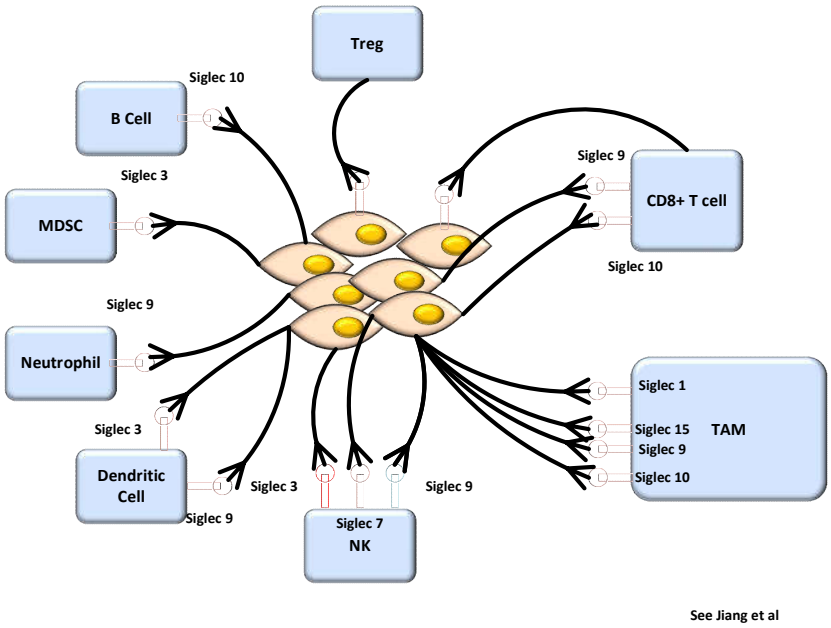
The tumor microenvironment (TME) also promotes abnormal secretion of Sia from tumor cells, which in turn stimulates the upregulation of Siglec expression in infiltrating immune cells.

Siglecs can promote tumor immune escape by inducing M2-type macrophage polarization and altering the direction of T-cell differentiation and NK-cell activity.

Thus, dysregulation of the Sia-Siglec axis in tumors might contribute to immunosuppressive cell signal transduction to facilitate the formation of an immune-negative microenvironment, thereby promoting tumor growth and assisting in the immune escape of tumor cells.

Nevertheless, some Siglec molecules can deliver activation signals to promote antitumor immune responses and enhance antitumor function in the host. In recent years, an increasing number of therapeutic agents targeting Siglecs and their ligands have been developed and used in clinical trials and represent a promising immunotherapeutic approach for tumors.

The authors summarize the controls via the following graphic:



The following Table is from Lubbers et al (as modified):

Cell	1	2	3	5	7	8	9	10	11	15	16
Monocyte	x		x		x		x	x			
moDC	x		x		x		x	x			
moMφ	x		x		x		x	x			
Conventional DC		x	x		x		x			x	
Plasmatoid DC	x			x							
Mφ	x		x			x	x		x	x	x

As Lubbers et al have noted:

Aberrant glycosylation of multiple cancers and its influence on cancer progression and metastasis are well-known. ***Increased sialylation, α2,3; α2,6, and α2,8 linked sialic acids, has been demonstrated in multiple tumor tissues like renal cell carcinoma, prostate cancer, colon cancer, breast cancer, head and neck squamous cell carcinoma and oral cancer.*** *This aberrant sialylation can also be detected in serum serving as potential biomarkers for cancer detection, progression and treatment responses.*

In a mouse model for melanoma, hyper sialylation of B16 melanoma cells leads to increased tumor growth, associated with an enhanced T regulatory/T effector balance and reduced NK cell activity within the tumor and secondary lymphoid organs.

DCs that interacted and sampled sialylated antigens via Siglec-E (murine homologue of human Siglec-7 and Siglec- 9) induced regulatory T cells and inhibited effector T cell function in-vivo. These findings revealed that tumor sialylation impedes T cell-mediated anti-tumor immune responses, while promoting tumor-associated regulatory T cells. Blocking the inhibitory effects of sialic acids with a sialic acid blocking glycomimetic in a B16-OVA mouse model revealed reduced tumor growth, enhanced tumor killing by ovalbumin specific CD8+ T cells and inhibition of metastasis.

In breast cancer a specific glycoform of transmembrane mucin 1, MUC1-T is sialylated, creating MUC1-sT. The MUC1-sT can interact with Siglec-9 on monocytes and thereby induce secretion of IL-6, M-CSF and chemokines associated with tumor progression.

Binding of MUC1-sT to Siglec- 9 on macrophages induces a tumor-associated macrophage (TAM) phenotype, that inhibits CD8+ T cell proliferation and results in the upregulation of IDO, CD163 and PDL1 in-vivo. Another specific mucin glycoform, called MUC2-sT, has been shown to increase apoptosis of immature moDCs. Together, this points toward a broad immunological suppression by tumor-produced sialylated mucins. Antibodies against Siglecs are explored for the treatment of different cancer types.

For Acute Lymphoblastic Lymphoma (ALL) the FDA approved Inotuzumab Ozogamicin (Besponsa R), a monoclonal antibody against Siglec-2 coupled to the toxic agent calicheamicin is used.

This antibody targets Siglec-2 positive Blymphoblasts and causes cell death of these cells through the toxic agent. Trials with this antibody revealed that an enhanced number of patients reached complete remission and had an increased overall progression free survival. However, serious adverse effects were seen like myeloid suppression, which could be due to the presence of Siglec-2 on DC subsets. Another Siglec that is targeted for the treatment of AML.

Munkley notes:

Aberrant glycosylation in cancer was first described more than fifty years ago. Since then, changes to glycans have been identified in every type of cancer, and altered glycosylation has been linked to all of the cancer hallmarks.

Many of the first cancer-specific antibodies detect oncofoetal antigens present on embryonic and cancer cells but not in adult healthy tissue, and numerous ***FDA-approved tumour markers, including CEA, CA125, and PSA****, are glycan antigens or glycoproteins. Common changes to the tumour glycome include aberrant sialylation, fucosylation, truncated O-glycans and alterations to O- and N-glycan branching.*

A dense layer of tumour-associated glycans coats the cell surface of cancer cells and is a driving force behind tumour growth, metastasis and immune evasion.

Aberrant glycosylation can interfere with cell adhesion molecules such as cadherins and integrins and alter the function of receptor tyrosine kinases (RTKs). *Tumour-associated glycans can also bind to lectins, including galectins, sialic acid-binding immunoglobulin-type lectins (Siglecs) and Selectins. Glycans have functional roles in regulating cell proliferation, cell signalling, cell adhesion, extracellular matrix interactions and proximal and distal communication.*

These biological processes play a critical role in cancer biology, and it has become evident that tumour glycosylation can have a major impact on cancer progression, tumour immunity, and clinical outcome.

Sialic acid is a key monosaccharide building block of mammalian cell-surface glycans and in humans, the most common sialic acid is N-acetylneuraminic acid (Neu5Ac). Sialic acid residues are present at the tip of glycans, positioning them at the forefront of crucial biological processes.

One common feature of cancer cells is increased cell-surface sialylation. The 'sialome' is a subclass of the glycome, and has been described as a dense forest coating the cell surface in a complex array of sialylated structures that has far-reaching consequences for cancer .

In this review, I discuss the mechanisms behind how cancer cells become hypersialylated, how increased sialylation is advantageous to cancer cells and tumours, and highlight emerging strategies to target aberrant sialylation to develop new cancer therapeutics.

6 BREAST CANCER

Breast cancer, BCa, is a common female malignancy which is currently classified by surface markers such as estrogen receptors, ER, and HER2 receptors, or none of the two classes. HER2+ BCa were originally the most aggressive but with the introduction of various monocloanal antibody (MAb) approaches it is become controllable. Unlike prostate cancer, PCa, BCa has received great attention for a long period and there are well accepted therapeutic regimens available. However the use of immunotherapeutic approaches have been unsuccessful.

6.1 IMMUNOTHERAPY

Now as Mereiter et al have noted:

Immune checkpoint inhibitors (ICIs) have rapidly become a standard of care for multiple cancers, harnessing the patient's immune system to combat the disease. However, the currently approved ICIs have shown limited efficacy in breast cancer (BC). Nevertheless, BCs often exhibit abundant tumor-infiltrating lymphocytes, albeit rarely expressing programmed death-ligand 1 (PD-L1). This suggests that alternative pathways of immunoregulation are in place that could be exploited for BC immunotherapy. Sialylation, the addition of sialic acid to glycoproteins, glycolipids, or glyco-RNA, is altered in multiple cancers. Sialic acids, negatively charged nine-carbon sugars that cap glycan structures, play an important role in various cell-cell and cell-matrix interactions.

In recent years, sialylation has garnered attention due to its emerging importance in immunoregulation including anti-tumor immune responses. Sialylation is therefore a promising candidate as a targetable immunoregulatory pathway. Despite this promise, our current understanding of the role of sialylation in breast cancer and whether sialylation might be mechanistically involved in the resistance of breast cancer to ICI is largely incomplete. ...

The transformation of sialic acids into the activated donor-substrate CMP-Sialic acid is mediated through the enzyme CMAS (Cytidine Monophosphate N-Acetylneuraminic Acid Synthetase) and is an essential step of sialylation. Subsequently, CMP-Sialic acids are transported to the Golgi apparatus, where various sialyltransferases utilize them as donor substrates to add sialic acid and modify glycans.

To genetically assess the role of sialylation in breast cancer, we used CRIPSR/Cas9 to knock out Cmas in 4T1, EMT6 and E0771 murine mammary cancer cells and in MDA-MB-231 and BT-474 human breast cancer cells. Importantly, the changes in surface glycosylation induced by Cmas KO were similar in mouse and human cell lines. The loss of sialylation (assessed through the loss of SNA and MAL2 binding) triggered a shift in the glycome, characterized by an increase in terminal galactose, which can be determined using the lectins ECL, PNA and GSL1.

These changes were confirmed by structural N-glycomic analysis using mass spectrometry, which shows a shift in both mouse and human cell models from terminal sialylated to terminal galactosylated N-glycans. Subsequently, we performed comprehensive glycoproteomic analysis

using our in-house developed SugarQb pipeline . This analysis enabled the quantitative assessment of 4859 and 2771 unique glycopeptides, from 424 and 384 glycoproteins, for mouse and human breast cancer cell lines, respectively, providing, to our knowledge, the most extensive glycoproteome map of breast cancer cells to date.

Interestingly, the GO-term analysis revealed that through Cmas KO affected glycoproteins in human breast cancer cell lines were largely involved in immune response pathways, a feature most closely reflected by the 4T1 murine mammary cancer cell line.

Overall, these data show that compared to other mammary cancer cell lines, the 4T1 cells presents a high degree of sialylation while retaining an overall lectin-interactome close to that of healthy mammary glands, and upon desialylation affected pathways closely resemble those observed in human breast cancer cells.

The key results are as follows:

1. ***Functional and structural glycan characterization of the murine breast cancer models.***
2. ***Sialylation deficiency licenses cytotoxic T cells to control mammary tumors***
3. ***Loss of sialylation sensitizes mammary tumors to immunotherapy.***
4. ***CD8+ T cell-mediated tumor control relies on MHC-I and PD-L1 surface expression***
5. ***Pharmacological inhibition of sialylation phenocopies Cmas genetic ablation for cancer immunosurveillance***
6. ***Sialylation deficiency increases cancer immunosurveillance in autochthonous mammary tumors and sensitizes to PD-1 blockade***
7. ***Sialylation negatively correlates with CD8+ T cell infiltration in human breast cancer***

They conclude:

The unique immune privilege of the breast, which constitutes a strong barrier against autoimmune attacks, allows marked morphological and molecular changes during development and pregnancy. Intriguingly, the immunoregulatory part of the Siglec (Sialic acid-binding immunoglobulin-type lectins) family has expanded in mammals together with the evolution of the mammary glands. We therefore speculated that among all organs, the mammary epithelium, which is the evolutionary youngest and defining organ of mammals, may particularly capitalize on the sialic acid immunoregulatory circuits.

Surprisingly, the loss of sialylation showed only minor effects on the development and function of mammary glands in mice, suggesting that sialic acids are not crucial components of cellular functions and local immunoregulation of the mammary glands under unchallenged conditions. This is in line with previous studies that have shown that sialylation is not essential for early embryonic development and organogenesis in mice. In contrast, our data indicates that the absence of sialylation appears to have beneficial effects, as it protects against the spontaneous formation of mammary tumors upon progestin and carcinogen treatment.

However, it is likely that sialylation of mammary glands plays a beneficial role in unexplored processes, such as infection clearance or prevention of autoimmune diseases. Importantly, the lack of evident pathology in conditional knockout mice suggests that local interference with mammary gland sialylation may be well-tolerated and non-hazardous to the organ in breast cancer patients.

Similarly, the loss of sialylation did not affect the in vitro growth of any of the examined murine and human BC cell lines, and the overall proteome remained largely unchanged. This provides support for the non-essential role of sialylation in both healthy and transformed mammary cells. In the 4T1 mammary cancer cell line, which is widely utilized in murine BC studies, the impact of sialylation interference appears to be exclusively due to immunoregulation. This is evident from the absence of growth disparities when compared to sialylation-competent tumor cells in immunocompromised mice.

Moreover, it is conceivable that the removal of sialylation also prevents metastasis, as previously described. Our comprehensive investigation, which included lectin binding, structural glycomics, and quantitative and structural glycoproteomics analyses, demonstrates the dual nature of sialylation interference, encompassing both loss-of-function and gain-of-function. Future exploration is warranted to determine whether the upregulated glycan epitopes resulting from loss of sialylation, such as increased terminal galactosylation, contribute to the observed phenotypes and potentially unveil new vulnerabilities for targeted interventions.

Mechanistically and using single cell transcriptomics, we report that sialylation in breast tumors drives the recruitment of PMN-MDSCs while hindering the efficient eradication of tumors by CD8+ T cells.

Consequently, abrogation of tumor sialylation facilitated CD8+ mediated tumor control and the recruitment of Tcf7+ CD8+ memory T cells within the TME. In multiple studies, Tcf7+ CD8+ memory T cells have been linked to ICI responses. The interference with sialylation therefore resulted not only in prolonged control of the breast tumors but, most importantly, also culminated in breaking the resistance of breast tumors to anti-PD1 therapy. Previous studies have shown that other innate immune components, such as tumorassociated macrophages, can contribute to improved CD8+ T cell-mediated tumor control upon partial desialylation of colorectal cancer and melanoma models. This phenotype was functionally linked to the actions of Siglecs.

In contrast, complete desialylation interference in a colorectal tumor model resulted in immune-dependent accelerated tumor growth. These observations are likely consequences of the intricate sialylation-immune axis and suggest that, similar to other immunotherapies, the outcome might depend on tumor-intrinsic properties.

Thus, it is crucial to define key determinants that can serve as predictive markers for treatment outcomes following sialylation interference.

For breast cancer, our data, along with findings from other studies, consistently demonstrate that sialylation contributes to a suppressed immune response. This is likely attributable not only to sialylation-driven recruitment and polarization of immune-dampening innate immune cells but also, as we observed in our experiments, to a sialylation-dependent increase in surface expression of MHC-I and decreased cell surface expression of PD-L1.

Previous reports have shown that increased MHC-I surface retention can also be observed in desialylated dendritic cells. Collectively, sialylation in breast cancer cells appears to affect various cellular pathways and immune cells, thereby promoting immune escape of the tumor cells. In this study, using two preclinical models of immunologically "cold" and ICI-unresponsive mammary cancers, we have demonstrated that sialylation interference can sensitize tumors to combination therapy with anti-PD1 immune checkpoint inhibition.

This intervention represents, to our knowledge, the first successful preclinical strategy to render aggressive MPA/DMBA mammary tumors amenable to immunotherapy. This data underscores the potential of sialylation interference strategies for future clinical combination therapies, including those currently being assessed in clinical trials (NCT05259696).

Finally, the high frequency with which we observed hyper-sialylation in breast tumors of patients, along with its significant negative correlation with tumor-infiltrating T lymphocytes, suggests that a substantial number of breast cancer patients could benefit from therapeutic interventions targeting sialylation.

6.2 TARGETING

As Crocker et al note:

Taken together, it is clear that Siglecs in the immune system have the potential to mediate both cell–cell interactions and signalling functions.

However, defining their precise functions and determining which ligands are biologically relevant pose an important challenge. This is beginning to be tackled using a combination of experimental approaches, including the production of genetically manipulated mice, biochemical analyses of ligand recognition and dissection of signalling pathways.

In particular, several recent studies using mice that lack CD22, CD22 ligands or both, as well as mice expressing mutant forms of CD22 that cannot bind sialylated glycans, have begun to shed light on the complex factors involved. These have also provided a conceptual framework for understanding how the less well-characterized CD33-related Siglecs may contribute to regulation of leukocyte functions, as revealed in a recent study of Siglec-F-deficient mice. Sialoadhesin (recognized by the antibody MOMA-1) is well known as a macrophage-specific marker and adhesion molecule but its biological functions have remained enigmatic.

However, several recent studies of sialoadhesin-deficient mice have shown an unexpected role of this receptor in modulating immune and inflammatory responses. New data are available on the endocytic functions of Siglecs and their interactions with various sialylated pathogens that could

be important in both host defence and pathogenicity. Finally, there is emerging evidence that CD33-related Siglecs have undergone significant changes during human evolution. In this Review, we discuss how these recent advances have significantly furthered our understanding of the roles of Siglecs in the immune system and wherever possible we attempt to relate these functions to glycan recognition and physiology. Sialic-acid recognition by Siglecs The mammalian glycome contains numerous sialylated glycans that can be potentially recognized as ligands by Siglecs. It is assumed that this recognition is important for modulating the functions of Siglecs as regulators of adhesion, cell signalling and endocytosis.

In general, Siglecs show low affinity (a Kd of 0.1–3 mM) for the sialic acid N-acetylneuraminic acid (Neu5Ac) α2–3 and α2–6 linkages to galactose (Neu5Acα2–3Gal and Neu5Acα2–6Gal) that are commonly found as terminal sequences on glycans of glycoproteins and glycolipids of most mammalian cells, and Siglecs have an overlapping specificity for such sialosides (sialic-acid-containing glycans).

However, when examined for their ability to recognize a diverse set of natural sialoside structures found in mammalian species, each Siglec shows a characteristic specificity profile. CD22 is unique in having a strong preference for Neu5Acα2–6Gal and Neu5Gcα2–6Gal structures. Siglec-7 and Siglec-11 prefer sialosides with the Neu5Ac(α2–8)Neu5Ac structure. Several Siglecs (such as Siglec-8 and Siglec-9) have differential specificity for sialosides that contain both sialic acid and sulphate, with the position of the sulphate being an important determinant of specificity.

In addition, Siglecs have different specificities for the many sialic acid species found in nature. Of particular interest is the evolutionary loss of N-glycolylneuraminic acid (Neu5Gc) in humans, as Neu5Gc is the preferred…

7 PROSTATE CANCER

Now significant work has been done on many of the above and more[10]. We now focus on prostate cancer, PCa, and some specific targeting of glycan impact. As Scott and Munkley had noted:

Prostate cancer is the most commonly diagnosed malignancy in men, claiming over 350,000 lives worldwide annually. Current diagnosis relies on prostate-specific antigen (PSA) testing, but this misses some aggressive tumours, and leads to the overtreatment of non-harmful disease. Hence, there is an urgent unmet clinical need to identify new diagnostic and prognostic biomarkers. As prostate cancer is a heterogeneous and multifocal disease, it is likely that multiple biomarkers will be needed to guide clinical decisions.

Fluid-based biomarkers would be ideal, and attention is now turning to minimally invasive liquid biopsies, which enable the analysis of tumour components in patient blood or urine. Effective diagnostics using liquid biopsies will require a multifaceted approach, and a recent high-profile review discussed combining multiple analytes, including changes to the tumour transcriptome, epigenome, proteome, and metabolome.

However, the concentration on genomics-based paramaters for analysing liquid biopsies is potentially missing a goldmine. Glycans have shown huge promise as disease biomarkers, and data suggests that integrating biomarkers across multi-omic platforms (including changes to the glycome) can improve the stratification of patients with prostate cancer.

A wide range of alterations to glycans have been observed in prostate cancer, including changes to PSA glycosylation, increased sialylation and core fucosylation, increased O-GlcNacylation, the emergence of cryptic and branched N-glyans, and changes to galectins and proteoglycans. In this review, we discuss the huge potential to exploit glycans as diagnostic and prognostic biomarkers for prostate cancer, and argue that the inclusion of glycans in a multi-analyte liquid biopsy test for prostate cancer will help maximise clinical utility

7.1 ROLE OF GLYCANS

As Gilgunn et al note :

The diagnosis and treatment of prostate cancer (PCa) is a major health-care concern worldwide. This cancer can manifest itself in many distinct forms and the transition from clinically indolent PCa to the more invasive aggressive form remains poorly understood. It is now universally accepted that glycan expression patterns change with the cellular modifications that accompany the onset of tumorigenesis.

The aim of this study was to investigate if differential glycosylation patterns could distinguish between indolent, significant, and aggressive PCa. Whole serum N-glycan profiling was carried

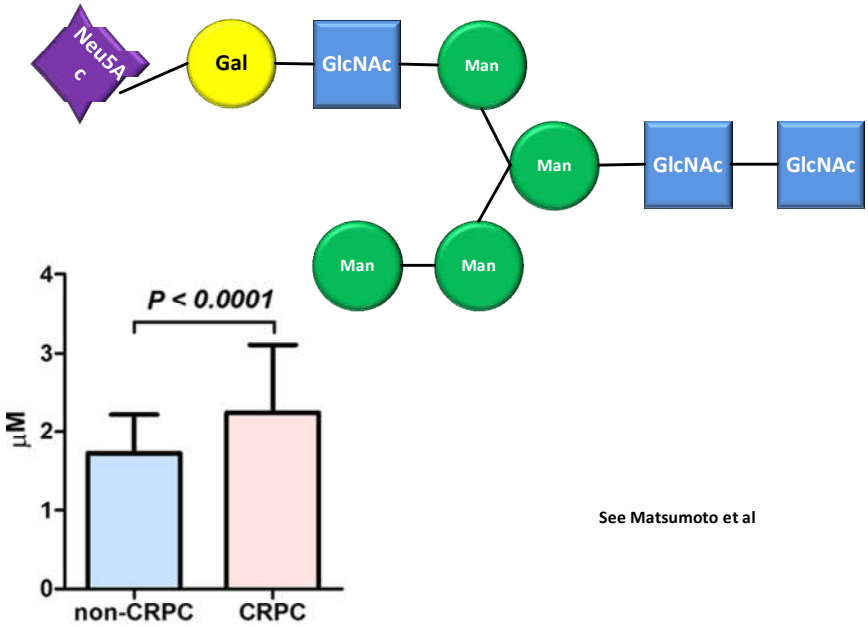
[10] https://www.researchgate.net/publication/264960277_Prostate_Cancer_A_Systems_Approach

out on 117 prostate cancer patients' serum using our automated, high-throughput analysis platform for glycan-profiling which utilizes ultra-performance liquid chromatography (UPLC) to obtain high resolution separation of N-linked glycans released from the serum glycoproteins.

We observed increases in hybrid, oligomannose, and biantennary digalactosylated monosialylated glycans (M5A1G1S1, M8, and A2G2S1), bisecting glycans (A2B, A2(6)BG1) and monoantennary glycans (A1), and decreases in triantennary trigalactosylated trisialylated glycans with and without core fucose (A3G3S3 and FA3G3S3) with PCa progression from indolent through significant and aggressive disease.

These changes give us an insight into the disease pathogenesis and identify potential biomarkers for monitoring the PCa progression, however these need further confirmation studies.

From Matsumoto et al the authors present a set of glycans and their prevalence in non-CRPC and CRPC. The example below is just one example. It is interesting to see the complexity of glycans in such a profile.



See Matsumoto et al

7.2 BIOMARKERS

As Wen et al noted:

Total sialic acid (TSA) levels are significantly elevated in serum samples from PCa patients, but are comparable to serum levels in patients with benign prostatic hyperplasia (BPH). However, patients with a PSA in the range of 4–10 ng/ml, a grey zone for PCa diagnosis, TSA was found to be significantly elevated in patients with PCa compared to patients with BPH with a sensitivity of 86% and specificity of 84% in diagnosing malignancy, suggesting TSA can improve selection

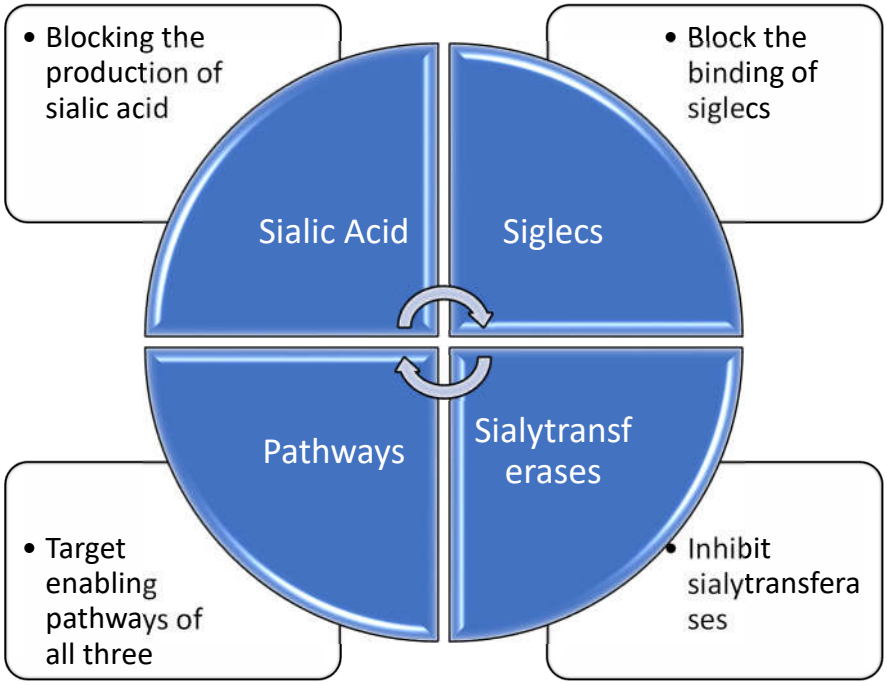
of patients for biopsy with elevated PSA levels. In addition, TSA levels are significantly higher in patients with distant metastases compared to those with localized disease and healthy controls. A study of 408 patients with PCa and 132 patients with BPH has confirmed elevated TSA levels in patients with PCa and bone metastasis. PCa cell lines carry different sialic acids on their cell surfaces at different ratios. For example, DU145 cells express 5 times more α2,6-salic acid than α2,3-sialic acid, while PC3 cells express 4 times more α2,3-salic acid than α2,6-salic acid. Removal of these salic acids from cell surface by sialidase treatment impaired the ability of PC3 and DU145 cells to form avascular multicellular prostaspheres, a common in vitro assay to measure the amount of cancer stem cells within a tumor/cancer cell line that is correlated with cancer metastasis and aggressiveness. Differential expression of sialoglycoproteins involved in cell motility, migration, and invasion is also observed on cell surfaces between non-metastatic and highly metastatic sublines derived from PC3 cells. …

Sialic acids and sialoglycoconjugates likely have critical roles in cancer development and progression and is a rapidly developing field of study.

Although more than 80 sialic acid types and 20 human sialyltransferases responsible for their biosynthesis have been investigated in cancer generally, only a few have been evaluated in PCa. Much additional work is necessary to understand the roles of sialyltransferases in driving prostate cancer progression and potentially allowing immune evasion in PCa. In addition, application of novel glycoproteomic approaches to study sialylation in PCa could help identify potential biomarkers for diagnosis and prognosis, as well as therapeutic targets. Finally, strategies to disrupt sialic acid-Siglec interactions in prostate cancer should be explored as a potential checkpoint-based immunotherapy in PCa.

8 THERAPEUTICS

There are a multiplicity of approaches for therapeutics for cancers. BCa had achieved some success, albeit not using immunotherapy. PCa has less success and the closest is Sepuleucil using dendritic cells targeting the cancer. This has limited success. We briefly review the current status of PCa and then look at sialic related approaches.



8.1 CURRENT TECHNIQUES

In a recent review paper by Siridan et al the authors note:

Cancer immunotherapy has gained traction in recent years owing to remarkable tumor clearance in some patients. Despite the notable success of immune checkpoint blockade (ICB) in multiple malignancies, engagement of the immune system for targeted prostate cancer (PCa) therapy is still in its infancy. Multiple factors contribute to limited response, including the heterogeneity of PCa, the cold tumor microenvironment, and a low number of neoantigens. Significant effort is being invested in improving immune-based PCa therapies. This review is a summary of the status of immunotherapy in treating PCa, with a discussion of multiple immune modalities, including vaccines, adoptively transferred T cells, and bispecific T cell engagers, some of which are undergoing clinical trials.

In addition, this review also focuses on emerging mechanism-based small-molecule tyrosine kinase inhibitors with immune modulatory properties that, either as single agents or in combination with other immunotherapies, have the potential to improve clinical outcomes … the existing and emerging strategies for PCa immunotherapy.

1. *The classic immune checkpoint blockade (ICB) therapy uses monoclonal antibodies against PD-1/PD-L1/CTLA-4. ICB in combination with other therapies is currently being tested to maximize efficacy.*

2. *Several DNA/RNA peptide vaccines have shown promise in inhibiting PCa growth.*

3. *Generation and testing of novel fusion proteins and nucleic acid formulations are underway.*

4. *T cell engagers and bispecific antibodies (BiTEs) that create cancer-destroying contact between immune cells and cancer cells is another proficient immunotherapy strategy gaining prominence.*

5. *Profiling of circulating T cells (CTCs) to screen treatment-induced antigenic alterations and novel marker detection is emerging as an effective strategy for personalized therapeutic intervention ensuring generation of targeted antibodies with improved clinical efficiency.*

6. *Multiple immune cell types bearing chimeric antigen receptors (CARs) are being engineered and tested for therapeutic efficacy against PCa.*

7. *Targeting tyrosine kinases of molecular significance contributing to immunosuppression using small-molecule inhibitors and their combination with other immunotherapies also hold promise in improving existing PCa treatment regimens.*

What has not been discussed in this recent review is any of the elements we have discussed herein. Namely trying to work around the blockage of immune cells by the molecules we have discussed.

8.2 SIALIC APPROACHES

Mereiter et al have recently present results on managing breast cancer via a therapeutic approach suppressing sialic acids. They observe the following:

1. *Sialylation deficiency licenses cytotoxic T cells to control mammary tumors.*
2. *Loss of sialylation sensitizes mammary tumors to immunotherapy.*
3. *CD8+ T cell-mediated tumor control relies on MHC-I and PD-L1 surface expression.*
4. *Pharmacological inhibition of sialylation phenocopies Cmas genetic ablation for cancer immunosurveillance*[11].
5. *Deletion of Cmas in the mouse mammary gland*
6. *Sialylation deficiency increases cancer immunosurveillance in autochthonous mammary tumors and sensitizes to PD-1 blockade*
7. *Sialylation negatively correlates with CD8+ T cell infiltration in human breast cancer.*

[11] *The transformation of sialic acids into the activated donor-substrate **CMP-Sialic** acid is mediated through the enzyme **CMAS (Cytidine Monophosphate N-Acetylneuraminic Acid Synthetase)** and is an essential step of sialylation*

Specifically they noted:

Having demonstrated the potential of interfering with sialylation through genetic ablation, our next objective was to investigate whether similar effects could be achieved in cancer treatment through pharmacologic intervention.

To accomplish this, we administered 3FaxPeracetyl N-Acetylneuraminic acid, a sialyltransferase inhibitor (STI), to 4T1 tumor bearing mice[12].

Treatment with STI resulted in a significant reduction in tumor growth. Additionally, STI licensed the tumor bearing host to better respond to anti-PD-1-based immunotherapy, leading to prolonged tumor control and a doubling of the overall survival. Importantly, similar to genetic ablation of sialylation, pharmacological interference was dependent on an intact adaptive immune system since no spontaneous nor immunotherapy-induced tumoricidal activity was observed in Rag2-/- γc-/- mice.

Of note, in vitro experiments demonstrated that STI did not exhibit any direct cytotoxicity in 4T1 cells. Time course immune profiling revealed modulations of the tumor immune landscape upon STI treatment, consistent with those described in Cmas KO 4T1 tumors.

Thus, STI induced a drop in PMN-MDSCs, concomitant to a rise in tumor-infiltrating memory CD8+ T cells on day 8 following tumor induction. On day 14 post tumor induction, significantly fewer PD-1 expressing T cells were present in tumors treated with both STI and anti-PD-1. The increased infiltration of CD8+ T cells persisted until the endpoint of the study.

Overall, pharmacologic ablation of sialylation recapitulated the effects observed with our Cmas genetic ablation experiments, emphasizing the potential of sialylation interference as a therapeutic approach in future clinical settings. …

The unique immune privilege of the breast, which constitutes a strong barrier against autoimmune attacks, allows marked morphological and molecular changes during development and pregnancy. Intriguingly, the immunoregulatory part of the Siglec (Sialic acid-binding immunoglobulin-type lectins) family has expanded in mammals together with the evolution of the mammary glands.

We therefore speculated that among all organs, Surprisingly, the loss of sialylation showed only minor effects on the development and function of mammary glands in mice, suggesting that sialic acids are not crucial components of cellular functions and local immunoregulation of the mammary glands under unchallenged conditions. This is in line with previous studies that have

[12] https://www.emdmillipore.com/US/en/product/Sialyltransferase-Inhibitor-3Fax-Peracetyl-Neu5Ac-Calbiochem,EMD_BIO-566224?ReferrerURL=https%3A%2F%2Fwww.google.com%2F&bd=1 *A cell-permeable sialylic acid analog that upon cellular uptake is transformed into a CMP-Neu5Ac (Cat. No. 233264) mimetic bearing a C3 fluorine substituent at the axial position, effectively* ***inhibiting sialyltransferase*** *in a donor substrate CMP-Neu5Ac-competitive manner. Shown to effectively abloishes HL-60 cell surface SLeX expression (by >95%; 200 µM for 5 days), resulting in dramatic reductions in cell surface E-selectin and P-selectin binding (by >95% and >80%, respectively), without affecting cell viability or proliferation.*

shown that sialylation is not essential for early embryonic development and organogenesis in mice.

In contrast, our data indicates that the absence of sialylation appears to have beneficial effects, as it protects against the spontaneous formation of mammary tumors upon progestin and carcinogen treatment. However, it is likely that sialylation of mammary glands plays a beneficial role in unexplored processes, such as infection clearance or prevention of autoimmune diseases. Importantly, the lack of evident pathology in conditional knockout mice suggests that local interference with mammary gland sialylation may be well-tolerated and non-hazardous to the organ in breast cancer patients.

Similarly, the loss of sialylation did not affect the in vitro growth of any of the examined murine and human BC cell lines, and the overall proteome remained largely unchanged. This provides support for the non-essential role of sialylation in both healthy and transformed mammary cells. In the 4T1 mammary cancer cell line, which is widely utilized in murine BC studies, the impact of sialylation interference appears to be exclusively due to immunoregulation.

This is evident from the absence of growth disparities when compared to sialylation-competent tumor cells in immunocompromised mice. Moreover, it is conceivable that the removal of sialylation also prevents metastasis, as previously described. Our comprehensive investigation, which included lectin binding, structural glycomics, and quantitative and structural glycoproteomics analyses, demonstrates the dual nature of sialylation interference, encompassing both loss-of-function and gain-of-function. Future exploration is warranted to determine whether the upregulated glycan epitopes resulting from loss of sialylation, such as increased terminal galactosylation, contribute to the observed phenotypes and potentially unveil new vulnerabilities for targeted interventions.

In multiple studies, Tcf7+ CD8+ memory T cells have been linked to ICI responses. The interference with sialylation therefore resulted not only in prolonged control of the breast tumors but, most importantly, also culminated in breaking the resistance of breast tumors to anti-PD1 therapy. Previous studies have shown that other innate immune components, such as tumor associated macrophages, can contribute to improved CD8+ T cell-mediated tumor control upon partial desialylation of colorectal cancer and melanoma models. This phenotype was functionally linked to the actions of Siglecs.

In contrast, complete desialylation interference in a colorectal tumor model resulted in immune-dependent accelerated tumor growth.

These observations are likely consequences of the intricate sialylation-immune axis and suggest that, similar to other immunotherapies, the outcome might depend on tumor-intrinsic properties. Thus, it is crucial to define key determinants that can serve as predictive markers for treatment outcomes following sialylation interference.

For breast cancer, our data, along with findings from other studies, consistently demonstrate that sialylation contributes to a suppressed immune response.

This is likely attributable not only to sialylation-driven recruitment and polarization of immune-dampening innate immune cells but also, as we observed in our experiments, to a sialylation-dependent increase in surface expression of MHC-I and decreased cell surface expression of PD-L1.

Previous reports have shown that increased MHC-I surface retention can also be observed in desialylated dendritic cells.

Collectively, sialylation in breast cancer cells appears to affect various cellular pathways and immune cells, thereby promoting immune escape of the tumor cells. In this study, using two preclinical models of immunologically "cold" and ICI-unresponsive mammary cancers, we have demonstrated that sialylation interference can sensitize tumors to combination therapy with anti-PD1 immune checkpoint inhibition.

This intervention represents, to our knowledge, the first successful preclinical strategy to render aggressive MPA/DMBA mammary tumors amenable to immunotherapy. This data underscores the potential of sialylation interference strategies for future clinical combination therapies, including those currently being assessed in clinical trials (NCT05259696).

One fragment targets a marker on the surface of T cells, CD3, and the other targets a tumor associated antigen. When simultaneous binding occurs, it allows the T cell to come into contact and kill tumor cells.

The first BiTE to gain FDA approval was ***blinatumomab (Blincyto),*** *a CD3/CD19 BiTE, which is indicated for Philadelphia negative relapsed or refractory Bcell progenitor acute lymphoblastic leukemia*[13].

Since then, many other BiTEs have been developed and are being evaluated for the treatment for hematologic malignancies. For example, AMG330, a CD3/ CD33 BiTE that is being evaluated as a potential treatment for AML*[14]*.

In mouse models, AMG330 treatment reduces tumor growth, but additional preclinical studies are needed to optimize effector-to-target ratio as the data showed there was insufficient target cell lysis in samples that had low initial effector-to-target ratios.

CAR T cells provide highly potent and specific targeting, albeit with systemic immune toxicity requiring careful management. Clinical studies of Siglec-targeting CAR T cells are all in early

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[13] https://www.cancer.gov/news-events/cancer-currents-blog/2023/blincyto-leukemia-minimal-residual-disease *Blinatumomab attaches to T cells and cancer cells, enabling T cells to find and destroy cancer cells (bottom right). During this process, T cells are activated, creating more killer T cells*

[14] https://ashpublications.org/blood/article/132/Supplement%201/25/264250/A-Phase-1-First-in-Human-Study-of-AMG-330-an-Anti *Current treatment options for R/R AML are highly inadequate. CD33 is expressed in >99% of AML cases. BiTE®s have been effective in R/R Acute Lymphoblastic Leukemia. AMG 330 is a BiTE® that binds CD33 and CD3 on T cells, facilitating T-cell destruction of CD33+ cells. The objectives of this ongoing study are to evaluate the safety, pharmacokinetics, and pharmacodynamics of AMG 330 in R/R AML and to estimate the maximum tolerated dose.*

phases and data are currently insufficient to establish whether enhanced efficacy or an improved therapeutic index can be achieved compared with the ADC strategy.

In nonclinical studies, the efficacy of Siglec-2–targeting CAR T cells may be influenced by the epitope targeted by the CAR; however, the mechanism for this effect is not fully understood.

As with Siglec-2 targeting, CAR T cells created against Siglec-3 have shown activity in preclinical studies, but because Siglec-3 is expressed on myeloid precursor cells more broadly, treatment also led to hematopoietic toxicity.

One option to potentially mitigate this liability could be to create modified CAR T-cell therapies that can be switched off to prevent long-term, life-threatening immune suppression.

One of the more recently developed CAR T-cell therapies targets Siglec-6, which is commonly expressed on AML cell lines but not on normal hematopoietic stem and progenitor cells (HSPC). tumors by CD8+ T cells.

Consequently, abrogation of tumor sialylation facilitated CD8+ mediated tumor control and the recruitment of Tcf7+ CD8+ memory T cells within the TME. In multiple studies, Tcf7+ CD8+ memory T cells have been linked to ICI responses. The interference with sialylation therefore resulted not only in prolonged control of the breast tumors but, most importantly, also culminated in breaking the resistance of breast tumors to anti-PD1 therapy. Previous studies have shown that other innate immune components, such as tumor associated macrophages, can contribute to improved CD8+ T cell-mediated tumor control upon partial desialylation of colorectal cancer and melanoma models. This phenotype was functionally linked to the actions of Siglecs. In contrast, complete desialylation interference in a colorectal tumor model resulted in immune-dependent accelerated tumor growth.

These observations are likely consequences of the intricate sialylation-immune axis and suggest that, similar to other immunotherapies, the outcome might depend on tumor-intrinsic properties. Thus, it is crucial to define key determinants that can serve as predictive markers for treatment outcomes following sialylation interference. For breast cancer, our data, along with findings from other studies, consistently demonstrate that sialylation contributes to a suppressed immune response.

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This data underscores the potential of sialylation interference strategies for future clinical combination therapies, including those currently being assessed in clinical trials.

8.3 SIGLECS

As Laubli et al have noted:

Targeting Siglecs and Sialoglycan– Siglec Immunoregulatory Interactions The consistent and specific expression of Siglecs on subsets of immune cells provides a substantial opportunity to target Siglecs and the sialic acid glycocalyx for cancer treatment; broadly, two approaches are being pursued.

The first approach uses agents that target Siglecs on immune cells that have transformed into malignant cells to localize a cytotoxic payload to a particular immune cell type. Agents that use this approach have already been approved, yet significant therapeutic opportunity remains[15].

The second approach uses agents that target the immunoregulatory interaction between Siglecs and their sialic acid ligands to reprogram immune cells for an immunologic attack.

Therapeutics Targeting the Siglecs as Tumor-Associated Markers:

Because Siglecs are cell surface receptors, antibody-based therapeutics represent an effective approach to target malignant immune cells retaining Siglec expression as lineage markers. Antibody-tethered cytotoxic function can take many forms, including antibody–drug conjugates(ADC), anti-Siglec bispecific T-cell engagers(BiTE), and chimeric antigen receptor (CAR) T-cell therapies.

Although antibody-dependent cellular cytotoxicity (ADCC) may be considered favorable because of the potential for increased safety over ADCs, naked Siglec-targeted antibodies have not demonstrated sufficient activity in the cancer setting. ADCs Siglecs are endocytosed after binding to a ligand, with internalization of tethered molecules, making them excellent targets for ADC therapies, especially in cases where the toxin must be delivered within specific subsets of immune cells.

In general, Siglec-3 is highly expressed, with relative specificity on myeloid cells, and can serve as a lineage marker for myeloid cells. It also is enriched on acute myeloid leukemia (AML) cells. The first ADC to gain FDA approval was gemtuzumab ozogamicin (Mylotarg),

[15] https://mylotarg.pfizerpro.com/ *CD33 is widely expressed in AML and is present in nearly all patients. It is expressed on leukemic blasts in approximately 90% of patients, regardless of cytogenetic or molecular abnormalities*

which targets Siglec-3 (CD33). It is indicated for the treatment of adults and children who have CD33-positive AML.

Siglec-2 is expressed primarily on B cells. Thus, anti–Siglec-2 ADCs have been used for B-cell leukemias and lymphomas.

Inotuzumab ozogamicin *(Besponsa) is an ADC comprising an anti–Siglec-2 antibody linked to a small-molecule toxin, calicheamicin, which induces DNA damage, it is indicated for the treatment of adults with relapsed or refractory B-cell precursor acute lymphoblastic leukemia*[16].

Moxetumomab pasudotox *(Lumoxiti) is not an ADC, but a protein construct that is a fusion between an anti–Siglec-2 monobody and PE38, a fragment of a Pseudomonas toxin*[17]*. It is approved for the treatment of hairy cell leukemia, a rare type of slow-growing leukemia that arises in B cells. The demonstration of effective agents targeting Siglecs has solidified their relevance as targets for cancer treatment.*

On the basis of the precedent of targeting Siglec-2 (CD22) and Siglec-3 (CD33) with ADCs and related therapeutics, clinical development has been progressing for Siglec-targting agents that employ BiTE or CAR T-cell technology.

BiTEs represent a mechanism for efficiently engaging cytotoxic T cells to kill cancer cells. They are composed of two single-chain variable fragments designed to target two antigens, with the mammary epithelium, which is the evolutionary youngest and defining organ of mammals, may particularly capitalize on the sialic acid immunoregulatory circuits[18].

Jiang et al note:

Therapeutic targeting of the Sia-Siglec axis is promising for the treatment of tumors because Siglecs are mostly expressed in immune cells and affect the TME. Currently, monoclonal antibodies (mAbs) targeting Siglecs are applied to deplete tumor cells via passive immunotherapy. Most mAbs specifically bind a target antigen and neutralize or stimulate its activity; however, newer therapeutic strategies, such as immune checkpoint inhibition, and T-cell engaging therapies, such as bispecific T-cell engaging (BiTE) single-chain antibody constructs

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[16] https://besponsa.pfizerpro.com/?gclid=Cj0KCQjwjt-oBhDKARIsABVRB0wpCIFhHFYw-BvoWtV75uVRlryqaifk3c7S-9qB-rhjbPu3R6v_i48aAoE_EALw_wcB&gclsrc=aw.ds *BESPONSA combines the specificity of an anti-CD22 humanized monoclonal antibody with the potent antitumor activity of calicheamicin. Nonclinical data suggest BESPONSA delivers calicheamicin to CD22-expressing cells, inducing DNA damage and apoptosis*

[17] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6323103/ *Moxetumomab pasudotox-tdfk (LUMOXITI™), an anti CD22 recombinant immunotoxin, has been developed by MedImmune and its parent company AstraZeneca for the treatment of hairy cell leukaemia. The product, discovered at the National Cancer Institute, is an optimised version of immunotoxin CAT-3888. Moxetumomab pasudotox is composed of the Fv fragment of an anti-CD22 monoclonal antibody fused to a 38 kDa fragment of Pseudomonas exotoxin A, PE38. The Fv portion of moxetumomab pasudotox binds to CD22, a cell surface receptor expressed on a variety of malignant B-cells, thereby delivering the toxin moiety PE38 directly to tumour cells.*

[18] https://www.researchgate.net/publication/346245151_Poly-specific_Antibodies

and chimeric antigen receptor (CAR) T cells, have shown remarkable efficacy in clinical trials. Here, we discuss drugs targeting Siglecs and their progress in their clinical application in tumor therapy.

8.4 SIALYITRANSFERASES

As we noted previously the enzymes moving the sialyic acid to the glycan, sialyitransferase, is another therapeutic target. As Pietrobono and Stecca have noted:

In the last two decades, several sialyltransferase inhibitors have been identified by design, in natural products or microbial metabolites, and from high-throughput screening methods. Most of these inhibitors can be classified into: (i) acceptor analogues, (ii) donor analogues, based on the structure of CMP-Neu5Ac, (iii) bisubstrate analogues, and (iv) transition-state analogues, whereas others can be obtained from natural products. Whilst many of these inhibitors are not good candidates for therapeutic intervention due to their high polarity and charge that counteract cell absorption, some of them showed encouraging effects in vivo.

1. *Acceptor Analogues: Sialyltransferases catalyze the transfer of a sialic acid residue from a sugar nucleotide donor to a glycoconjugated acceptor. Both the nature of the nucleotide donor and the terminal structure of the glycan portion of the sugar acceptor determine the specificity of each sialyltransferase. However, reports on acceptor-type inhibitors are limited*

2. *Donor Analogues: Over the last decade, several groups focused on the design and synthesis of CMP-sialic acid analogues (donor analogs), able to prevent sialic acid transfer by competing with the natural donor CMP-Neu5Ac in the binding to the active site of sialyltransferases. Including:*

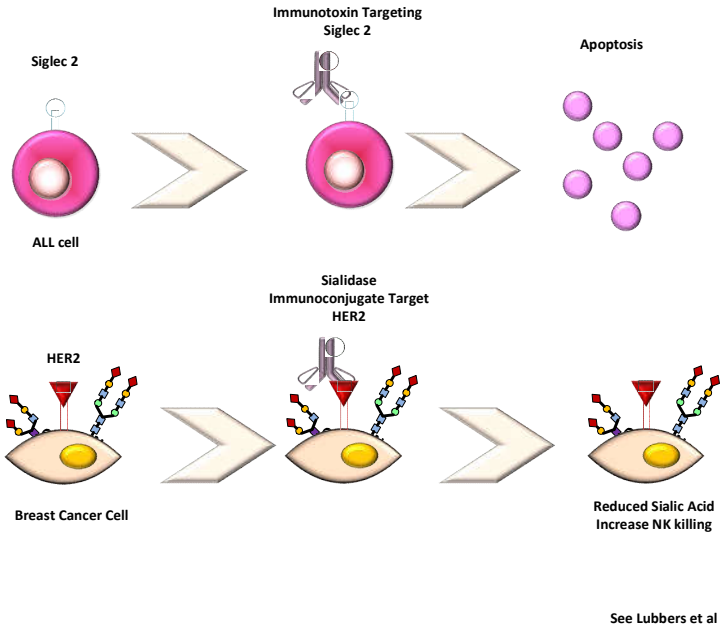
    a. *Nucleoside Fragment (Cytidine Analogues)*
    b. *Sugar Fragment (Sialic Acid Analogues)*

3. *Bisubstrate Analogues: Inhibitors of the bisubstrate analogue type are designed to mimic donor and acceptor substrates, containing motifs of both donor and acceptor that are covalently bound to each other.*

4. *Transition-State Analogues: The mechanism of sialyltransferases involves the nucleophilic attack of a deprotonated hydroxyl of an acceptor on the anomeric carbon of Neu5Ac, which generates an oxocarbenium-like transition-state (TS), with the CMP moiety acting as a leaving group. Previous studies proposed TS analogues as potent sialyltransferase inhibitors*

8.5 SUMMARY

Lubbers et al have presented a summary of the discussed two therapeutic techniques. We present them graphically below:



Lubbers et al discusses several others. Clearly there is now a multiplicity of possible therapeutics as well as targets.

9 OBSERVATIONS

We now make several observations which may extend the presentation in this Note.

9.1 How does the totality of the tumor micro environment play a role in suppressing immunotherapy?

The TME often has a protective role in keep the tumor mass isolated from any immune attack. This of the sialic elements can allow for metastatic expansion, will the TME inhibit any such attempts.

9.2 Can targeting of sialic acids or even siglecs be specific enough?

One issue is the common presence of sialic acid in glycans, even in benign cells. Will there be collateral damage to benign cells as a result of a therapeutic attack?

9.3 Sugars appear to be the principal driver for sialic acid production. Does that infer that insulin impaired individuals are at greater risk?

Recently we examine the use of metformin in PCa[19]. An earlier paper first examined the efficacy of glucose suppression in PCa[20]. Will glucose suppression be an adjunct to treatment?

9.4 What are the adverse effects of these targeting mechanisms?

As with any therapeutic, one must address the adverse effects. Give the pan-cellular approach, can one estimate what these may be?

9.5 Can we obtain improved targeting with polyspecific Ab?

With polyspecific Ab, can we improve the targeting of the malignant cells and reduce any adverse events?

[19] https://www.researchgate.net/publication/351051261_Metformin_Prostate_Cancer_and_Efficacy April 2021

[20] https://www.researchgate.net/publication/351034816_Metformin_and_Statins_in_PCa February 2015

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